REPUBLIKA HRVATSKA
SVEUČILIŠTE JOSIPA JURJA STROSSMAYERA U OSIJEKU

FAKULTET AGROBIOTEHNIČKIH ZNANOSTI OSIJEK

Elena Petrović, univ. mag. ing. agr.

IDENTIFIKACIJA PATOGENIH GLJIVA I ANTIFUNGALNI UTJECAJ ETERIČNIH ULJA I BILJNIH VODA NA PATOGENE MASLINE

DOKTORSKI RAD

Osijek, 2025.

REPUBLIKA HRVATSKA
SVEUČILIŠTE JOSIPA JURJA STROSSMAYERA U OSIJEKU

FAKULTET AGROBIOTEHNIČKIH ZNANOSTI OSIJEK

Elena Petrović, univ. mag. ing. agr.

IDENTIFIKACIJA PATOGENIH GLJIVA I ANTIFUNGALNI UTJECAJ ETERIČNIH ULJA I BILJNIH VODA NA PATOGENE MASLINE

- Doktorski rad-

Osijek, 2025.

REPUBLIKA HRVATSKA
SVEUČILIŠTE JOSIPA JURJA STROSSMAYERA U OSIJEKU

FAKULTET AGROBIOTEHNIČKIH ZNANOSTI OSIJEK

Elena Petrović, univ. mag. ing. agr.

IDENTIFIKACIJA PATOGENIH GLJIVA I ANTIFUNGALNI UTJECAJ ETERIČNIH ULJA I BILJNIH VODA NA PATOGENE MASLINE

- Doktorski rad-

Mentor: prof. dr. sc. Karolina Vrandečić

Komentor: dr. sc. Sara Godena

Povjerenstvo za ocjenu:

prof. dr. sc. Jasenka Ćosić, redoviti profesor u trajnom zvanju, Fakultet agrobiotehničkih znanosti Osijek, predsjednik
prof. dr. sc. Renata Baličević, redoviti profesor u trajnom zvanju, Fakultet agrobiotehničkih znanosti Osijek, član
dr. sc. Tomislav Duvnjak, znanstveni savjetnik u trajnom izboru, Poljoprivredni institut Osijek, član

Osijek, 2025.

REPUBLIKA HRVATSKA
SVEUČILIŠTE JOSIPA JURJA STROSSMAYERA U OSIJEKU

FAKULTET AGROBIOTEHNIČKIH ZNANOSTI OSIJEK

Elena Petrović, univ. mag. ing. agr.

IDENTIFIKACIJA PATOGENIH GLJIVA I ANTIFUNGALNI UTJECAJ ETERIČNIH ULJA I BILJNIH VODA NA PATOGENE MASLINE

- Doktorska disertacija -

Mentor: prof. dr. sc. Karolina Vrandečić

Komentor: dr. sc. Sara Godena

Javna obrana doktorske disertacije održana je _____ godine pred Povjerenstvom za obranu:

prof. dr. sc. Jasenka Ćosić, redoviti profesor u trajnom zvanju, Fakultet agrobiotehničkih znanosti Osijek, predsjednik
prof. dr. sc. Renata Baličević, redoviti profesor u trajnom zvanju, Fakultet agrobiotehničkih znanosti Osijek, član
dr. sc. Tomislav Duvnjak, znanstveni savjetnik u trajnom izboru, Poljoprivredni institut Osijek, član

Osijek, 2025.

TEMELJNA DOKUMENTACIJSKA KARTICA

Sveučilište Josipa Jurja Strossmayera u Osijeku **Doktorska disertacija**
Fakultet agrobiotehničkih znanosti Osijek
Poslijediplomski sveučilišni (doktorski) studij: Poljoprivredne znanosti
Smjer: Zaštita bilja

UDK: 632.4+631.84:633.11
Znanstveno područje: Biotehničke znanosti
Znanstveno polje: Poljoprivreda
Grana: Fitomedicina

Identifikacija patogenih gljiva i antifungalni utjecaj eteričnih ulja i biljnih voda na patogene masline

Elena Petrović, univ. mag. ing. agr.

Disertacija je izrađena na Institutu za poljoprivredu i turizam, Poreč i Fakultetu agrobiotehničkih znanosti Osijek, Sveučilište Josipa Jurja Strossmayera u Osijeku

Mentor: prof. dr. sc. Karolina Vrandečić
Komentor: dr. sc. Sara Godena

Gljive su iznimno važni organizmi, s ključnom ulogom u ekosustavima. U poljoprivrednoj proizvodnji je čak 70–80% biljnih bolesti prouzročeno gljivama. Precizna identifikacija gljiva ključna je zbog njihove raznolikosti, pri čemu se koristi kombinacija morfoloških i molekularnih metoda. Klimatske promjene i intenzivna poljoprivreda pogoduju širenju fitopatogena, a Mediteran se smatra osobito ranjivim. Maslina je pogođena sve većim brojem bolesti koje uzrokuju patogene gljive. Ovo istraživanje je za ciljeve imalo identifikaciju gljiva s masline, ispitivanje njihove patogenosti, procjenu antifungalnog učinka eteričnih ulja (EtU) i otpadnih biljnih voda masline (OBVM) te utvrđivanje osjetljivosti različitih sorata maslina na patogene. Hipoteze su uključivale mogućnost pronalaska novih patogena, varijacije u patogenosti gljiva i osjetljivosti sorti te učinkovitosti alternativnih tretmana poput EtU i OBVM u suzbijanju patogena. Terenskim istraživanjem u Istri i na Kvarneru, Hrvatska, prikupljeni su uzorci zaraženih maslina. Iz njih su izolirane i identificirane različite vrste gljiva, pri čemu su korištene morfološke i molekularne metode. Ukupno je identificirano šest vrsta iz porodice Botryosphaeriaceae i osam vrsta iz razreda Sordariomycetes, od kojih je pet prvi put zabilježeno na maslini u Hrvatskoj uz dodatnih šest koje su prvi put zabilježene na maslini u svijetu. Testovi patogenosti pokazali su da su svi izolati gljiva patogeni za maslinu, dok su testovi otpornosti sorata pokazali da sorte pokazuju različite razine otpornosti na zaraze. Antifungalni testovi s EtU pokazali su da su EtU kineskog cimeta, origana i njihove glavne komponente (e-cinamaldehid i karvakrol) iznimno učinkoviti protiv svih testiranih gljiva. S druge strane, EtU limuna i paprene metvice, kao i njihove komponente (limonen i mentol), pokazali su slabije djelovanje. EtU su u mnogim slučajevima pokazala veću učinkovitost od komercijalnih fungicida. Analizom OBVM utvrđeno je da se one, nakon određene obrade, mogu koristiti kao održiva alternativa kemijskim sredstvima. Također, OBVM mogu se koristiti za izolaciju fenola i mikroorganizama. Zaključno, ovo istraživanje ističe važnost kombinirane primjene morfoloških i molekularnih metoda za identifikaciju gljiva, potrebu za razvojem održivih metoda zaštite bilja i važnost istraživanja otpornosti sorata.

Broj stranica: 356
Broj slika i grafikona: 21
Broj tablica: 5
Broj literaturnih navoda**:** 92
Jezik izvornika: hrvatski (znanstveni radovi objavljeni su na engleskom jeziku)
Ključne riječi: antagonizam, biološka kontrola, prvi izvještaj, valorizacija otpada*, Olea europea* L.

Datum obrane:

Povjerenstvo za obranu:
prof. dr. sc. Jasenka Ćosić, predsjednik
prof. dr. sc. Renata Baličević, član
dr. sc. Tomislav Duvnjak, član

Disertacija je pohranjena u: Nacionalna i sveučilišna knjižnica u Zagrebu, Sveučilište Josipa Jurja Strossmayera u Osijeku, Sveučilište u Zagrebu, Sveučilište u Rijeci, Sveučilište u Splitu

BASIC DOCUMENTATION CARD

University of Josip Juraj Strossmayer in Osijek **PhD thesis**
Faculty of Agrobiotechnical Sciences Osijek
Postgraduate university study: Agricultural sciences
Course: Plant Protection

UDK: 632.4+631.84:633.11
Scientific Area: Biotechnical Sciences
Scientific Field: Agriculture
Branch: Phytomedicine

Identification of pathogenic fungi and the antifungal effect of essential oils and olive mill wastewaters on olive pathogens

Elena Petrović, univ. mag. ing. agr.

Thesis performed at Institute of Agriculture and Tourism, Poreč, and Faculty of Agrobiotechnical Sciences Osijek, University of Josip Juraj Strossmayer in Osijek

Supervisor: Prof. Karolina Vrandečić, PhD
Co-supervisor: Sara Godena, PhD

Fungi are extremely important organisms, playing key role in ecosystems. In agriculture, as much as 70–80% of plant diseases are caused by fungi. Accurate identification of fungi is crucial due to their diversity, employing a combination of morphological and molecular methods. Climate change and intensive agriculture favor the spread of phytopathogens, with the Mediterranean region considered particularly vulnerable. Olive trees are increasingly affected by diseases caused by pathogenic fungi. This research had several main objectives: to identify fungi associated with olives, to examine their pathogenicity, to assess the antifungal effects of essential oils (EOs) and olive mill wastewaters (OMWWs), and to determine the susceptibility of different olive cultivars to pathogens. The hypotheses included the possibility of discovering new phytopathogens, variations in fungal pathogenicity and varietal sensitivity, and the effectiveness of alternative treatments such as EOs and OMWWs in controlling pathogens. Field surveys in Istria and Kvarner, Croatia, led to the collection of infected olive samples. Various fungal species were isolated and identified using morphological and molecular methods. A total of six species from the family Botryosphaeriaceae and eight species from the class Sordariomycetes were identified, five of which were recorded on olive in Croatia for the first time, along with an additional six species recorded on olive for the first time worldwide. Pathogenicity tests showed that all fungal isolates were pathogenic to olives, while cultivar resistance tests revealed that different cultivars exhibited varying levels of resistance to infection. Antifungal tests with EOs demonstrated that Chinese cinnamon and oregano EOs, along with their main components (e-cinnamaldehyde and carvacrol), were highly effective against all tested fungi. Conversely, lemon and peppermint EOs, as well as their components (limonene and menthol), showed weaker activity. In many cases, EOs exhibited greater efficacy than commercial fungicides. Analysis of OMWWs indicated that, after specific treatment, it can serve as a sustainable alternative to chemical agents. Additionally, OMWWs can be used for the extraction of phenols and microorganisms. In conclusion, this research highlights the importance of combining morphological and molecular methods for fungal identification, the need to develop sustainable plant protection methods, and the significance of studying indigenous cultivars for their disease resistance.

Number of pages: 356
Number of figures: 21
Number of tables: 5
Number of references: 92
Original in: croatian (scientific papers in english)
Key words: antagonism, biological control, first report, waste valorisation, *Olea europea* L.

Date of the thesis defense:

Reviewers:
Prof. Jasenka Ćosić, PhD, President
Prof. Renata Baličević, PhD, Member
Tomislav Duvnjak, PhD, Member

Thesis deposited in: National and University Library in Zagreb, University of Josip Juraj Strossmayer in Osijek, University of Zagreb; University of Rijeka; University of Split

Istraživanje ove doktorske disertacije provedeno je u okviru projekata Hrvatske zaklade za znanost „Prirodni bioaktivni spojevi kao izvor potencijalnih antimikrobnih tvari u suzbijanju bakterijskih i drugih gljivičnih patogena masline“, Anti-Mikrobi-OL (AMO), UIP-2020-02-7413 i “Projekt razvoja karijera mladih istraživača - izobrazba novih doktora znanosti” DOK-2021-02-2882.

Infrastrukturnu, administrativnu i tehničku potporu za izradu ovog doktorskog rada pružila je matična Organizacija, Institut za poljoprivredu i turizam, Poreč.

KAZALO

1. UVOD

Procjenjuje se da na Zemlji postoji između 2,2 i 3,8 milijuna vrsta gljiva, no znanstveno je opisano tek oko 150 tisuća vrsta, što znači da je veliki dio ove skupine organizama neistražen (Hawksworth i Lücking, 2017). Gljive imaju ključnu ulogu u ekosustavima i ljudskom životu, tj. sudjeluju u razgradnji organskih tvari, ulaze u korisne simbioze s biljkama, proizvode farmakološki značajne spojeve, ali istovremeno mogu uzrokovati bolesti kod biljaka, ljudi i životinja (Case i sur., 2022). Procjenjuje se da je čak 70–80% svih biljnih bolesti uzrokovano gljivama (Zeilinger i sur., 2016). Zbog njihove raznolikosti i ekološke važnosti, točna identifikacija gljiva ključan je korak u mikologiji. Mikologija je iznimno kompleksno i interdisciplinarno područje koje uključuje znanja iz biologije, bioinformatike, ekologije, agronomije i molekularne biologije. Danas se gljive identificiraju kombinacijom morfoloških i molekularnih metoda. Morfološka analiza uključuje mikroskopsko promatranje struktura i analizu kulturalnih karakteristika dok molekularne metode koriste metode poput PCR-a tj. lančane reakcije polimerazom (engl. *Polymerase Chain Reaction*), elektroforeze i sekvenciranja. Kaliterna (2013) ističe da su pogreške u identifikaciji gljiva kroz povijest dovele do velikog broja sinonima i pogrešnih klasifikacija. Međutim, iako su molekularni alati značajno unaprijedili identifikaciju vrsta, oni i dalje nisu dovoljni, u mnogim slučajevima nije moguće precizno odrediti vrstu bez kombinacije ovih tehnika (Hyde i sur., 2024). Jedan od glavnih problema u identifikaciji gljiva je preklapanje morfoloških karakteristika među vrstama, što može dovesti do pogrešnih identifikacija. Također, morfologija gljiva može varirati ovisno o uvjetima okoliša, poput temperature, vlage ili dostupnosti hranjivih tvari. S druge strane, baze podataka, poput GenBank-a (National Center for Biotechnology Information, NCBI, Maryland, SAD), još uvijek ne sadrže dovoljno genetičkih sekvenci za mnoge vrste gljiva, što otežava molekularnu analizu i zahtijeva daljnja istraživanja (Petrović i sur., 2024a). Hyde i sur. (2024) upozoravaju da je broj dostupnih sekvenci gljiva daleko manji u usporedbi s bakterijama, što naglašava potrebu za proširenjem baza podataka. Gljive predstavljaju jednu od najvećih znanstvenih nepoznanica, s obzirom na njihov veliki broj i značaj u ekosustavima. Njihova identifikacija i istraživanje ključni su za zaštitu bilja, zdravlje ljudi i održivu poljoprivredu. Pretpostavke su da će u budućnosti, s obzirom na klimatske promjene i porast globalne trgovine, istraživanja gljivičnih patogena igrati još važniju ulogu u sigurnosti hrane i zaštiti ekosustava.

2. PREGLED LITERATURE

Biljni patogeni, uključujući gljive, uzrokuju značajne ekonomske gubitke u poljoprivredi. Brojne gljive proizvode mikotoksine koji imaju štetan učinak na zdravlje ljudi i životinja (Havranek i sur., 2014). Zbog toga je pravovremena dijagnostika gljivičnih bolesti ključna za njihovo učinkovito suzbijanje. Klimatske promjene i intenzivna poljoprivreda dodatno doprinose širenju biljnih bolesti i pojavi patogenih vrsta gljiva. Na Mediteranu, zbog specifičnih klimatskih uvjeta i visoke bioraznolikosti, posljednjih godina bilježimo povećanje broja novih fitopatogenih gljiva. Kim i sur. (2019) ističu da je Mediteran jedna od najranjivijih regija na svijetu kada je riječ o klimatskim promjenama, što sugerira da će broj biljnih bolesti u budućnosti nastaviti rasti. Nadalje, stalna interakcija između biljaka i patogena potaknula je brzu evoluciju gljivičnih vrsta, što rezultira pojavom novih, virulentnijih sojeva (Möller i Stukenbrock, 2017).

Maslina (*Olea europaea* L.) je jedna od najvažniji kultura koje se uzgajaju na Mediteranu. Prema službenim podacima Državnog zavoda za statistiku proizvodnja maslina u Republici Hrvatskoj za 2024. godinu iznosila je oko 54,4 tisuće t, a u svijetu se na godišnjoj razini proizvede oko 20,2 milijuna t maslina (FAO, 2025). Fitopatogene gljive uvelike otežavaju uzgoj maslina. Određene vrste, koje su od ranije poznate kao uzročnici bolesti maslina, sve se češće pojavljuju, dok su pojedine vrste bile od sekundarne važnosti ili nikada prije nisu prijavljene kao uzročnici bolesti maslina (Nigro i sur., 2018.). U Hrvatskoj je do sada opisano tek nekoliko patogenih gljiva na maslini i do sada je proveden vrlo mali broj istraživanja. Među najznačajnije vrste gljiva koje napadaju maslinu navode se vrste iz porodice Botryosphaeriaceae (Moral i sur., 2010., Hernández-Rodríguez i sur., 2022.). Prvi izvještaj o vrsti *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & De Not na maslini, uzročniku patule ili "dalmatinske bolesti", datira još iz 1883. godine te je prvi put zapažena upravo u Hrvatskoj, na stablima u Dalmaciji.

Za kontrolu gljivičnih bolesti biljaka i dalje se najčešće upotrebljavaju kemijski fungicidi. Međutim, upotreba fungicida nosi dva velika izazova: fungicidi mogu sadržavati štetne tvari koje negativno utječu na okoliš i zdravlje ljudi (Petrović i sur., 2024b) te gljive razvijaju otpornost na fungicide, što otežava njihovu kontrolu (Barber i sur., 2020). Ovaj problem dodatno se pogoršava činjenicom da se u antifungalnim lijekovima za ljude koriste slične kemijske tvari kao u fungicidima za biljke, što može potaknuti otpornost patogenih gljiva na lijekove koji se koriste u liječenju ljudi (Hyde i sur., 2024). Poljoprivredna praksa danas se sve

više usmjerava prema održivim metodama zaštite nasada, koje smanjuju štetan utjecaj kemijskih sredstava na okoliš. Europska unija donijela je dokumente "European Green Deal" (Europski zeleni plan) i "Strategiju bioraznolikosti", s ciljem smanjenja uporabe pesticida i fungicida za 50% do 2030. godine (Bažok, 2020). Ove strategije potiču istraživanja o alternativnim metodama zaštite bilja, uključujući biološku kontrolu patogena, genetsku otpornost biljaka i optimizaciju uzgojnih uvjeta. Prirodni biljni spojevi i ekstrakti, poput eteričnih ulja (EtU), sve se češće ispituju kao održiva alternativa kemijskim sredstvima za zaštitu bilja (Sarkhosh i sur., 2018.; Sun i sur., 2022.).

EtU predstavljaju sekundarne metabolite biljaka s izraženim antimikrobnim svojstvima. Prema definiciji Međunarodne organizacije za normizaciju (ISO/D1S9235.2), EtU su proizvodi dobiveni procesima poput destilacije vodom ili parom, mehaničke obrade ili suhe destilacije različitih biljnih materijala. U usporedbi s biljnim uljima, razlikuju se po kemijskom sastavu, što im omogućava prepoznatljive mirisne karakteristike; lako su zapaljiva i brzo hlape na temperaturama između 40 i 80 °C (Bowles, 2012). Dosad je poznato oko 3 000 različitih EtU (Burt i Reinders, 2003.), koje biljkama često služe kao obrambeni mehanizmi protiv štetočinja. Najzastupljenije tvari u njihovom sastavu uključuju terpene i terpenoide, dok se rjeđe pojavljuju spojevi s dušikom ili sumporom, kumarini i fenilpropanoidi (Niu i Gilbert, 2004.; Hyldgaard i sur., 2012.). Obično sadrže više desetaka različitih komponenti, od kojih su dvije do tri prisutne u značajno većim količinama (Bakkali i sur., 2008.). Zanimljivo je da čak i kada potječu od iste biljne vrste, pa čak i klonova biljaka, EtU mogu imati bitno različit kemijski sastav ako se biljke uzgajaju u različitim klimatskim ili geografskim uvjetima (Bowles, 2012).

U novije vrijeme sve veću pozornost u istraživanjima privlače biljne otpadne vode nastale tijekom prerade maslina (OBVM), koje se razmatraju kao moguća i ekološki prihvatljiva zamjena za kemijska sredstva u poljoprivredi. OBVM se pojavljuju kao nusprodukt procesa proizvodnje maslinovog ulja. Ovisno o korištenoj tehnologiji, bilo da se radi o klasičnom prešanju, trofaznom ili dvofaznom centrifugalnom sustavu, uz maslinovo ulje nastaju i različite količine krutih i tekućih ostataka. Kod prve dvije metode dobivaju se tri proizvoda: ulje, čvrsti ostatak (komina) i tekući otpad, dok dvofazni sustav smanjuje količinu tekućeg otpada jer rezultira samo uljem i vlažnom kominom (Klen i Vodopivec, 2011; Yakhlef i sur., 2018). Međutim, iako se radi o nusproizvodu, velike količine OBVM predstavljaju značajan ekološki izazov, budući da sadrže visoku razinu fitotoksičnih tvari koje mogu negativno djelovati na vodene organizme i mikrobiološku ravnotežu tla (European Commission, 2009). Zbog takvog kemijskog sastava, ne preporučuje se njihova neposredna primjena u navodnjavanju ili ispuštanje u prirodne vodotoke bez prethodne obrade (Cibelli i sur., 2017). Upravo zato,

pronalazak održivih načina za njihovu ponovnu uporabu, osobito u poljoprivredi, postaje ključan za zaštitu okoliša i zdravlja ljudi (Cibelli i sur., 2017). Jedan od glavnih problema povezanih s OBVM jest način njihova zbrinjavanja, budući da se često odlažu bez ikakve prethodne prerade, čime značajno doprinose zagađenju okoliša (Ghilardi i sur., 2022.; Bouhia i sur., 2022.). Ipak, neka istraživanja pokazuju da OBVM, zahvaljujući sadržaju organskih i mineralnih sastojaka, mogu imati i pozitivan učinak na razvoj biljaka, pod uvjetom da se prethodno adekvatno obrade, primjerice filtriranjem, termičkom obradom ili centrifugiranjem (Shabir i sur., 2023.). Osim toga, dokazano je da prerađene OBVM mogu imati i antimikrobna svojstva te djelovati inhibitorno na rast određenih patogenih gljiva i bakterija (Yangui i sur., 2008.; Cibelli i sur., 2017.; Yakhlef i sur., 2018).

Kao jedna od glavnih preventivnih mjera nastanka gljivičnih bolesti preporučuje se izbor otpornih sorti. Pretpostavlja se da se na području Mediterana uzgaja više od 1 000 različitih sorata i tipova maslina (Pribetić, 2006), dok Rugini i sur. (2005) navode da broj kultiviranih sorata može doseći i oko 2 500. Na području istarskih maslinika, nešto više od trećine stabala pripada autohtonim sortama, među kojima su najzastupljenije: Buža (50,69 %), Istarska bjelica (30,22 %), Rosinjola (5,72 %) i Crnica, poznata i kao Karbonera (5,60 %), dok ostale sorte čine preostalih 7,77 %. Noviji nasadi često se podižu s introduciranim, uglavnom talijanskim sortama poput Leccina, Frantoia i Pendolina (Bertoša, 2005).

3. CILJEVI I HIPOTEZE ISTRAŽIVANJA

Ciljevi ovog istraživanja su:

1. Identificirati i okarakterizirati gljive izolirane s masline.
2. U *in vivo* i *in vitro* uvjetima utvrditi patogenost identificiranih gljiva s masline.
3. U *in vitro* uvjetima utvrditi antifungalno djelovanje eteričnih ulja, njihovih glavnih komponenti i biljnih voda i komponente hidroksitirosol na rast micelija fitopatogenih gljiva.
4. Utvrditi razlike u antifungalnom djelovanju između eteričnih ulja i njihovih komponenti, biljnih voda i komponente hidroksitirosol i komercijalnih fungicida na rast micelija.
5. U *in vivo* uvjetima utvrditi osjetljivost odabranih sorti maslina na identificirane gljive.

Glavne hipoteze ovog istraživanja su:

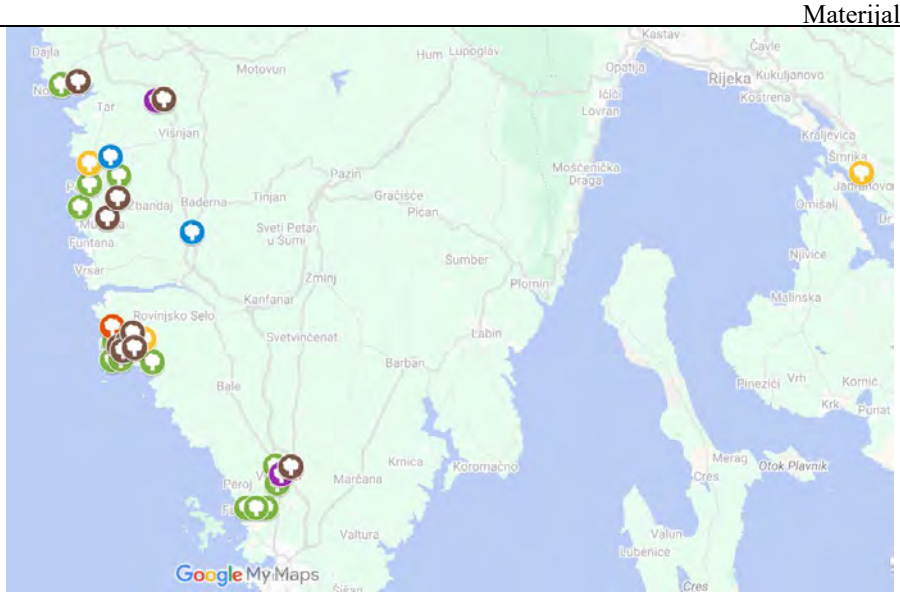
1. Na maslini su prisutne nove vrste gljiva koje do sada nisu utvrđene u Hrvatskoj.
2. Postoje razlike u patogenosti gljiva izoliranih s masline.
3. Eterična ulja, njihove glavne komponente i biljne vode i komponenta hidroksitirosol međusobno se razlikuju u utjecaju na rast micelija fitopatogenih gljiva.
4. Neka eterična ulja, njihove glavne komponente i biljne vode i komponente hidroksitirosol imaju jednaku učinkovitost kao i komercijalni fungicid.
5. Odabrane sorte masline značajno se razlikuju u otpornosti na ispitivane gljive.

4. MATERIJALI I METODE RADA

4.1. Obilazak terena i izolacija gljiva iz biljnog materijala

Za potrebe istraživanja, 2021. i 2022. godine provedeno je terensko istraživanje u Istri i na Kvarneru u Hrvatskoj. Prikupljali su se uzorci biljnog tkiva maslina koje su pokazivale simptome gljivičnog oboljenja, uključivši sušenje grana i izbojaka ili mladica, sušenje i otpadanje listova i plodova, pucanje kore grana i debla, savijanje grana, promjenu boje kore, nekroze tkiva i pojave rak rana. Ukupno su prikupljeni uzorci sa 26 lokacija, odnosno 112 stabala, a po stablu je prikupljeno u prosjeku 5 do 10 uzoraka. Uzorci su prikupljeni s dijelova biljke gdje je prijelaz između zdravih i zaraženih dijelova bio vidljivo izražen. Uzorci su stavljeni u čiste crne plastične vrećice, označeni su i pohranjeni u prijenosni hladnjak na +4 °C. Uzorci su fotografirani, nakon čega su isprani vodom iz slavine te su obrađeni ovisno o tome je li uzorak bio grana, list, kora ili plod masline. Pomoću sterilnog kirurškog skalpela uklonjena je kora s grana, a zatim su uzorci usitnjeni voćarskim škarama. Komadići grana potopljeni su u 70% etanol, nakon čega su isprani sterilnom destiliranom vodom. Ukoliko se radilo o debljim granama, iste su potopljene u 10 % otopinu natrijevog hipoklorita (varikine) te isprane u sterilnoj destiliranoj vodi. Potom su uzorci raspoređeni na sterilni ubrus papir unutar laminarnog protočnog kabineta (Nüve LN 090, Ankara, Turska) kako bi se omogućilo sušenje. Za površinsku sterilizaciju, listovi su isprani pod vodom iz slavine, zatim su potopljeni u 70 % etanol, isprani sterilnom destiliranom vodom i postavljeni na sterilni ubrus papir u laminarnom protočnom kabinetu. Nakon sušenja, uzorci su nacijepljeni na krumpir-dekstroza agar (KDA) s dodatkom antibiotika (35 mg/L penicilina ili 25 mg/L streptomicina) i inkubirani na 25 °C u uvjetima potpune tame. Nakon što su se gljive razvile, iste su se precijepile na čistu KDA kako bi se dobila čista kultura gljive. Nakon dobivanja čiste kulture, iste su se precijepile na podloge ovisno o vrsti gljive i zahtjevima koje imaju za razvoj i sporulaciju.

S obzirom na veliki broj gljiva koji je izoliran iz uzoraka biljnog materijala, za potrebe izrade doktorske disertacije odabrane su vrste iz porodice Botryosphaeriaceae, kao jedne od najagresivnijih uzročnika gljivičnih bolesti maslina te pojedine vrste iz razreda Sordariomycetes, koje do sada nisu bile izolirane iz maslina u Hrvatskoj i/ili svijetu. Lokacije prikupljenih uzoraka prikazane su na Slici 1.



Slika 1. Lokacije na kojima su pronađene vrste iz porodice Botryosphaeriaceae označene su na karti zelenom bojom, *Bicogniauxia* spp. smeđom bojom, *Cytospora* sp. ljubičastom bojom, *Nigrospora* spp. žutom bojom, *Phaeoacremonium* sp. narančastom bojom i *Sordaria* sp. plavom bojom. Slika je izrađena primjenom alata Google Maps.

4.2. Morfološka karakterizacija

Nakon što su se gljive razvile na KDA, provedena je morfološka karakterizacija gljiva te usporedba rezultata s rezultatima molekularne dijagnostike. Za detaljniju morfološku karakterizaciju gljive su nacijepljene na podloge koje su omogućile razvoj spora i struktura potrebnih za morfološku karakterizaciju. Za vrste iz porodice Botryosphaeriaceae koristio se vodeni agar (VA) s dodatkom borovih iglica (*Pinus* L.) te inkubacija na 25 ºC. Za *Cytospora* sp. koristio se KDA i temperatura inkubacije od 25 ºC. Za vrste *Biscogniauxia* spp. i *Sordaria* sp. koristili su se KDA, maltno-ekstraktni agar (MEA) i VA te temperatura inkubacije od 25 ºC. Za vrste *Nigrospora* spp. koristila se podloga KDA i temperatura inkubacije od 28 ºC, dok je za izolat JA20 NP, kasnije identificiran kao *Nigrospora osmanthi* Mei Wang & L. Cai isprobano nekoliko različitih podloga s ciljem poticanja izolata na sporulaciju, uključujući inkubaciju na KDA, VA i MEA pri temperaturama 22 °C, 25 °C, 28 °C, 30 °C; 1/2 jačine KDA, VA + borove iglice, uzgoj na tkivu biljke domaćina, uzgoj na mikroskopskom stakalcu, izlaganje ultraljubičastom svjetlu (12 h dan / 12 h noć), inokulacija na kori banane i KDA + banana pri 28 ºC. Podloga KDA + banana je potaknula sporulaciju izolata. Detaljna morfološka karakterizacija uključivala je temeljitu procjenu značajki kolonije, obuhvaćajući karakteristike poput boje, oblika, uzdignutosti, ruba, površine i prozirnosti. Nadalje, analizirane su karakteristike spora, uključujući boju, oblik, prisutnost ili odsutnost pregrada (septuma) te njihove dimenzije. Dodatno, provedeno je određivanje brzine rasta i kardinalnih temperatura

za rast micelija kod vrsta iz porodice Botryosphaeriaceae, prema Kaliterna (2013).

4.3. Molekularna dijagnostika

Za molekularnu dijagnostiku izolata koristile su se čiste kulture gljiva. Za ekstrakciju ukupne genomske DNK korišteni su komercijalni kitovi, prema uputama proizvođača. Za provedbu PCR reakcije koristili su se parovi početnica odabrani prema relevantnoj literaturi, za ITS regiju genoma prema White i sur. (1990), za TUB2 prema Glass i Donaldson (1995) i Woudenbreg i sur. (2009) i za TEF1-α prema Carbone i Kohn (1995). Popis korištenih početnica za vrste prikazani su u Tablici 1.

Tablica 1. Popis korištenih regija genoma i odgovarajućih početnica za amplifikaciju.

Regija genoma i korištene početnice	**Vrsta gljive**					
	Botryosphaeriaceae	*Biscogniauxia* spp.	*Cytospora* sp.	*Nigrospora* spp.	*Phaeoacremonium sp.*	*Sordaria sp.*
ITS						
ITS1 (5′ TCCGTAGGTGAACCTGCGG 3′)	x	x	x	x	x	x
ITS5 (5′ GGAAGTAAAAGTCGTAACAAGG 3′)				x		
ITS4 (5′ TCCTCCGCTTATTGATATGC3′)	x	x	x	x	x	x
TUB2						
Bt2a (5′ GGTAACCAAATCGGTGCTGCTTTC 3′)	x	x	x		x	x
Bt2b (5′ ACCCTCAGTGTAGTGACCCTTGGC 3′)	x	x	x		x	x
Btub2Fd (5′ AACATGCGTGAGATTGTAAGT 3′)				x		
Btub4Rd (5′ TAGTGACCCTTGGCCCAGTTG 3′)				x		
TEF1-α						
EF1-728F (5′ CATCGAGAAGTTCGAGAAGG 3′)	x	x		x	x	x
EF1-986R (5′ TACTTGAAGGAACCCTTACC3′)	x	x		x	x	x

Amplifikacija za vrste iz porodice Botryosphaeriaceae i vrstu *Sordaria* sp. provedena je prema metodi Slippers i sur. (2004), za *Biscogniauxia* spp. prema Mazzaglia i sur. (2001), dok je za *Cytospora* sp. korišten protokol prema Fan i sur. (2020). Za *Nigrospora* spp. PCR amplifikacija

ITS (parovi početnica ITS1 i ITS4) i TEF1-α regija izvedena je prema White i sur. (1990), dok je za ITS (parovi početnica ITS5 i ITS4) i TUB2 regije korišten protokol prema Hao i sur. (2020). Amplifikacija za *Phaeoacremonium* sp. provedena je prema Alves i sur. (2006). Nakon provedene PCR reakcije, izvršena je elektroforeza na 1% agaroznom gelu te vizualizacija PCR produkata radi određivanja veličine produkta i uspješnosti reakcije. Sekvenciranje PCR produkata provedeno je putem Macrogen Europe sekvencijskog servisa (Amsterdam, Nizozemska). Sekvenciranje je provedeno u oba smjera, koristeći iste početnice kao i za PCR amplifikaciju. Dobivene nukleotidne sekvence očitane su i uređene pomoću softvera Sequencher® (Gene Codes Corporation, Michigan, SAD). Usporedna analiza sekvenci provedena je s postojećim sekvencama dostupnim u GenBank bazi podataka Nacionalnog centra za biotehnološke informacije (NCBI, Maryland, SAD). Konsenzus sekvence dobivene ovim istraživanjem pohranjene su u GenBank (NCBI, Maryland, SAD) bazu podataka. Filogenetske analize provedene su zasebno za pojedinačne genske regije kao i za kombinirane regije. Poravnanje sekvenci izvršeno je pomoću softvera ClustalX2 (Saitou i sur., 1987). Evolucijska povijest rekonstruirana je metodom najbližeg susjeda (eng. Neighbour-Joining Method), pri čemu je prikazano optimalno stablo. Korištena je bootstrap analiza s 1000 ponavljanja koja prikazuje postotak ponovljenih stabala u kojima su se povezane taksonomske jedinice grupirale zajedno, što je prikazano uz odgovarajuće grane (Felstenstein i sur., 1985). Evolucijske udaljenosti izračunate su metodom maksimalne složene vjerojatnosti (eng. Maximum Composite Likelihood) i prikazane kao broj zamjena baza po lokaciji (Tamura i sur., 2004). Za izvođenje evolucijske analize korišten je softver MEGA11 (Tamura i sur., 2021).

4.4. Testovi patogenosti

Testovi patogenosti provedeni su u uvjetima *in vivo* i/ili *in vitro*. Za provedbu testova korišten je jedan reprezentativan izolat svake vrste. Ukupno je testirana patogenost 14 izolata gljiva. Za vrste iz porodice Botryosphaeriaceae, *Biscogniauxia mediterranea* (De Not.) Kuntze i *Cytospora pruinosa* Défago, koje su utvrđene u ovom istraživanju, patogenost je već prethodno dokazana u istraživanjima, dok za ostale vrste nije bila ispitivana. Stoga je, na temelju proučene literature i simptoma koje uzrokuju na drugim biljnim vrstama, odabran biljni dio na kojem se vršila umjetna zaraza. Odnosno, za sve vrste osim *Nigrospora* spp., zaraza sadnica i/ili biljnih dijelova provedena je na drvenom dijelu biljke (granama). Primjenom bušača micelija u ranu na grani, na kojoj je prethodno uklonjen sloj kore, umetnut je disk micelija istog promjera kao rana, a rana je zatim zatvorena vazelinom i parafilmom. Kao kontrola koristio se disk čistog

KDA. Za *Nigrospora* spp. zaraza je izvršena injektiranjem homogeniziranog samljevenog micelija u peteljku lista, s obzirom na to da su navedene gljive poznate kao uzročnici bolesti listova (pjegavosti ili točkastog oboljenja lista). Kao kontrola korištena je sterilna destilirana voda. Sadnice i biljni dijelovi držani su u kontroliranim uvjetima (u plasteniku i u laboratoriju) do pojave simptoma. Tijekom tog perioda bilježeni su simptomi koji su se razvijali. Nakon razdoblja inkubacije, zaraženi biljni dijelovi prikupljeni su u vrećice. U slučaju zaraze na granama, mjerene su nekrotične promjene iznad i ispod mjesta inokulacije. Radi potvrde patogenosti, proveden je Kochov postulat, mali dio nekrotičnog tkiva s perifernog dijela lezije inokuliran je na KDA kako bi se ponovno izolirala gljiva korištena za inokulaciju.

4.5. Test osjetljivosti sorti

S obzirom na to da su vrste iz porodice Botryosphaeriaceae poznate kao izrazito česti i među najagresivnijim patogenima masline, u ovom istraživanju ispitana je osjetljivost najzastupljenijih sorata maslina koje se uzgajaju na području Istre. Poseban naglasak stavljen je na procjenu reakcije sorata koje su od gospodarskog značaja, kako bi se utvrdile eventualne razlike u stupnju osjetljivosti pojedinih genotipova. Od hrvatskih autohtonih sorata odabrane su Istarska bjelica, Buža i Rosinjola, dok je od introduciranih sorata odabrana sorta Leccino.

4.6. Antifungalno djelovanje eteričnih ulja

Za ispitivanje antifungalnog djelovanja EtU korišteno je šest komercijalnih EtU, zajedno s najzastupljenijom komponentom svakog EtU. Korištena EtU dobivena su iz sljedećih biljaka: kineski cimet (*Cinnamomum aromaticum* Nees), limun (*Citrus* × *limon*), paprena metvica (*Mentha* × *piperita* L.), bosiljak (*Ocimum tenuiflorum* L.), origano (*Origanum compactum* Benth) i timijan (*Thymus vulgaris* L.). Sva EtU, osim EtU limuna, nabavljena su od tvrtke Pranarōm International Ltd. (Ath, Belgija), dok je EtU limuna nabavljeno od tvrtke Fagron Hrvatska d.o.o. (Donja Zelina, Hrvatska). Sva EtU analizirana su plinskom kromatografijom – masenom spektrometrijom (GC-MS) u laboratoriju proizvođača. Komponente karvakrol, eugenol i mentol nabavljene su od tvrtke Thermo Fisher Scientific Inc. (Fair Lawn, NJ, SAD), e-cinamaldehid i limonen od Thermo Fisher GmbH (Kandel, Njemačka), a timol od VWR International (Leuven, Belgija).

EtU testirana su u pet koncentracija: 10 µL/10 mL, 20 µL/10 mL, 50 µL/10 mL, 75 µL/10 mL i 100 µL/10 mL, što odgovara volumnim omjerima od 0,1 %, 0,2 %, 0,5 %, 0,75 % i 1,0 % u podlozi. Testirana koncentracija predominantne komponente izračunata je na temelju njenog

postotka unutar EtU za svaku ispitivanu koncentraciju EtU (Edris i sur., 2003; Palfi, 2017). Čisti KDA korišten je kao pozitivna kontrola. Kao negativna kontrola korištena su dva komercijalna fungicida koja se uobičajeno primjenjuju za suzbijanje fitopatogenih gljiva u uzgoju maslina: Nativo 75WG (Bayer d.o.o., Zagreb, Hrvatska) i Cabrio TOP (BASF Croatia, Zagreb, Hrvatska). Fungicidi su razrijeđeni do radne koncentracije preporučene za tretiranje maslina prema uputama proizvođača. Koncentracija za Nativo 75WG iznosila je 20 g/100 L, a za Cabrio TOP 200 g/100 L. Umjesto vode, korištena je ista količina KDA (Palfi, 2017). EtU i njihove komponente korišteni su za procjenu kontaktnog učinka na rast micelija gljiva. KDA pripremljen je prema uputama proizvođača, a temperatura cijele podloge praćena je dok se nije ohladila na približno 50 °C. Nakon hlađenja, 10 mL KDA ulijevano je u sterilne Falcon epruvete. U epruvete je potom pipetiran odgovarajući volumen EtU/komponente/fungicida te je smjesa miješana staklenim štapićem i lagano izmiješana u vorteksu dok se nije postigla homogena otopina. Otopina je izlivena u sterilne Petrijeve zdjelice promjera 90 mm. Nakon što se podloga stvrdnula, micelijski disk promjera 4 mm aktivno rastuće kulture gljive izrezan je pomoću bušača micelija i inokuliran u središte KDA podloge sterilnom laboratorijskom iglom, pazeći da gornja strana diska bude okrenuta prema podlozi. Petrijeve zdjelice zatvorene su parafilmom i inkubirane pod odgovarajućim uvjetima. Cijeli postupak proveden je u laminarnom protočnom kabinetu. Za vrste iz porodice Botryosphaeriaceae, *Biscogniauxia* spp., *Cytospora* sp., *Phaeoacremonium* sp. i *Sordaria* sp. temperatura inkubacije postavljena je na 25 °C, dok je za *Nigrospora* spp. postavljena na 28 °C. Eksperiment je proveden u tri ponavljanja za svaki tretman (EtU/komponente/fungicidi) i koncentraciju. Rast micelija mjerio se nakon 2, 4 i 10 dana od inokulacije. Za izolate kod kojih nije zabilježen rast gljiva pri konačnom mjerenju, polovica diska prenesena je sterilnom laboratorijskom iglom na novu sterilnu Petrijevu zdjelicu s KDA. Uzorci su inkubirani pod istim uvjetima kao i tijekom prvotne inokulacije. Ako se rast gljive nastavio nakon inkubacije, tretman je klasificiran kao fungistatičan, dok je u slučaju izostanka micelijskog rasta tretman smatran fungicidnim. Najniža koncentracija EtU/ komponente/ fungicida koja je rezultirala potpunom inhibicijom rasta micelija zabilježena je kao minimalna inhibicijska koncentracija (MIC), a najniža koncentracija koja je pokazala fungicidno djelovanje zabilježena je kao minimalna fungicidna koncentracija (MFC).

4.7. Antifungalno djelovanje otpadnih biljnih voda masline

OBVM prikupljena je izravno od prerađivača maslina u Istarskoj županiji, 2021. godine.

Vrijeme između berbe i prerade maslina iznosilo je četiri sata, a temperatura tijesta tijekom prerade održavana je na 24 °C. Proces malaksacije odvijao se uz kontinuirano hlađenje vodom temperature 12 °C. Maslinovo ulje ekstrahirano je centrifugiranjem, korištenjem dvofaznog Pieralisi sustava s mlinom čekićarem. OBVM je izdvojena iz hrvatskih sorti maslina Buža, Buža Puntoža, Istarska bjelica i Rosinjola te talijanske sorte Leccino. Nakon prikupljanja uzoraka OBVM iz uljara, isti su pohranjeni u hladnjaku na +4 °C tijekom devet dana (Russo i sur., 2022). U početku je određena boja uzoraka, a zatim su uzorci filtrirani pomoću vakuum filtra s filter papirom visokog protoka. Nakon filtracije, uzorci su centrifugirani pri 4000 okretaja/minuti tijekom 10 minuta na +4 °C pomoću centrifuge Hettich 320 R (Tuttlingen, Njemačka). pH vrijednost OBVM uzoraka određena je na sobnoj temperaturi pomoću pH metra, koji je prethodno kalibriran s certificiranim pH puferima (Mettler-Toledo GmbH, Greifensee, Švicarska). Uzorci su potom podijeljeni u dvije frakcije. U jednu frakciju dodana je klorovodična kiselina (HCl) kako bi se pH smanjio na 2, s ciljem sprječavanja oksidacije i očuvanja fenolnih spojeva (Klen i Vodopivec, 2011; Klen i sur., 2015; Yakhlef i sur., 2018), dok je druga frakcija ostavljena u prirodnom stanju bez dodataka. Svaka frakcija dodatno je podijeljena, pri čemu je jedan dio pohranjen na -20 °C, a drugi na sobnoj temperaturi.

4.7.1. Određivanje fizikalnih i kemijskih parametara

OBVM (300 µL) pomiješana je s 1 200 µL metanola kako bi se postigao omjer razrjeđenja 1:5. Smjesa je intenzivno izmiješana (vorteksirana) i centrifugirana pri 16 000×g tijekom 5 minuta na 25 °C pomoću centrifuge. Nakon centrifugiranja, 300 µL supernatanta je liofilizirano i rekonstituirano u 600 µL početne mobilne faze koja se sastojala od vode s 2 % metanola i 0,1 % octene kiseline. Rekonstituirana otopina vorteksirana je 30 sekundi i prebačena u bočice za HPLC analizu. Fenolni profil određen je korištenjem LC-MS/MS sustava. Separacija je provedena na C18 core-shell koloni (2,1 mm × 150 mm, 2,7 µm, Advanced Materials Technology) na 37 °C. Ubrizgano je 1 µL uzorka, a elucija je provedena linearnim gradijentom s mobilnim fazama A (voda / 0,1 % octena kiselina) i B (metanol / 0,1 % octena kiselina) protokom od 0,35 mL/minuti. Polifenolni spojevi identificirani su i kvantificirani pomoću analitičkih standarda. Svaki uzorak analiziran je u četiri ponavljanja, a sadržaj fenola izražen je kao srednja vrijednost ± standardna devijacija.

Određivanje koncentracije šećera provedeno je prema metodi DuBois i sur. (1956), pomoću spektrofotometra na valnim duljinama od 480 nm i 490 nm. Apsorbancija je mjerena u kvarcnim kivetama od 1 cm nakon dodatka DuBois reagensa. Mjerenja su provedena na sobnoj temperaturi, a koncentracija šećera izračunata je na temelju kalibracijske krivulje izrađene

pomoću glukoze (0–100 mg/L). Svaki uzorak analiziran je u tri ponavljanja, a rezultati su izraženi kao srednja vrijednost ± standardna devijacija.

Za određivanje udjela suhe tvari i vode, 3 mL svakog uzorka stavljeno je u staklenu čašu za vaganje. Uzorci su sušeni u sušioniku na 103 °C tijekom 24 h. Masa prazne čaše, uzorka prije i nakon sušenja, određene su analitičkom vagom. Masa suhe tvari izračunata je formulom: $mdm = m3 - m1$, gdje je mdm masa suhe tvari, $m1$ masa prazne čaše, $m2$ masa čaše s uzorkom prije sušenja, a $m3$ masa nakon sušenja. Početna masa uzorka izračunata je kao $m\text{wet} = m2 - m1$. Udio vode izračunat je prema $WH2O = 100\ \% - Wdm$, gdje je $Wdm = (mdm/m\text{wet}) \times 100\ \%$. Svaki uzorak analiziran je u tri ponavljanja, a rezultati su izraženi kao srednja vrijednost ± standardna devijacija.

Ukupni sadržaj ugljika i dušika istovremeno je analiziran pomoću analizatora TOC-L-CPH/TNM-L-ROHS. Uzorak od 3 mL pomiješan je s 27 mL 0,2 % HCl. Nakon homogenizacije, analiziran je primjenom uređaja. Svaki uzorak analiziran je u tri ponavljanja, a rezultati su izraženi kao srednja vrijednost ± standardna devijacija.

4.7.2. Antifungalno djelovanje OBVM i fenola

Za procjenu učinaka OBVM i fenola korišteni su reprezentativni izolati fitopatogenih gljiva izoliranih s maslina, koji su prethodno korišteni i za određivanje antifungalnog djelovanja EtU. Za testiranje antifungalnog djelovanja korišteno je pet različitih OBVM, kao i fenoli vanilinska kiselina, koja je bila najzastupljenija ili među najzastupljenijima u analiziranim uzorcima te hidroksitirozol, koji je, prema literaturi, identificiran kao dominantan spoj u OBVM s jakim antimikrobnim djelovanjem (Krid i sur., 2011; Yakhlef i sur., 2018). Vanilinska kiselina i hidroksitirozol nabavljeni su od Sigma Aldrich (Merck KGaA, Darmstadt, Njemačka). OBVM je testirana u pet koncentracija: 0,2 %, 0,5 %, 2 %, 6 % i 10 % (volumno u podlozi), dok su hidroksitirozol i vanilinska kiselina testirani u koncentracijama od 0,1 % i 0,5 %. Čista KDA korištena je kao pozitivna kontrola, dok je fungicid Nativo 75WG (Bayer d.o.o., Zagreb, Hrvatska), uobičajeno korišten u maslinarstvu, korišten kao negativna kontrola. Fungicid je razrijeđen prema preporuci proizvođača na 20 g/100 L. KDA je korišten umjesto vode (Palfi, 2017). KDA je pripremljen prema uputama proizvođača, a temperatura podloge praćena je do hlađenja na 45 °C (Yangui i sur., 2008). Potom je 10 ml KDA preneseno u sterilne Falcon epruvete. Dodana je odgovarajuća količina OBVM, spoja ili fungicida. Otopina je promiješana staklenim štapićem i lagano izmiješana (vorteksirana) do homogenosti. Otopina je ulivena u sterilne Petrijeve zdjelice (90 mm). Nakon skrućivanja podloge, disk micelija gljive promjera 4-mm prenesen je sterilnom iglom u središte podloge, s površinom diska okrenutom prema

podlozi. Zdjelice su zatvorene parafilmom i inkubirane u mraku na 25 °C, za sve vrste osim *Nigrospora* spp., za koju je korišteno 28 °C. Svaka varijanta i koncentracija izvedena je u tri ponavljanja. Rast gljiva praćen je drugog i sedmog dana nakon inokulacije. Za izolirane vrste kod kojih nije uočen rast do kraja pokusa, polovica diska micelija prenesena je na svježi KDA radi provjere održivosti, kao i kod testova s EtU.

4.7.3. Antagonistička aktivnost mikroorganizama izoliranih iz OBVM

Uzorci OBVM su protreseni i homogenizirani pomoću vorteks uređaja. Zatim je 100 µL neobrađene OBVM pipetirano na tri različite hranjive podloge: KDA, MEA i hranjivi agar (HA). Pripremljena su tri ponavljanja za svaku podlogu. Nakon sedmodnevne inkubacije na sobnoj temperaturi (23 °C), razvijeni mikroorganizmi preneseni su na čiste podloge. Za izolaciju gljiva korišten je KDA + 25 mg/l streptomicina. Za pročišćavanje bakterija, uzorci su stavljeni u sterilni PBS (fosfatno-puferska otopina), vorteksirani i centrifugirani pri 1 000×g tijekom 5 minuta, a zatim inokulirani na čisti HA. Postupci su ponavljani do dobivanja čistih kultura. Ukupno su izolirana tri izolata kvasaca, jedan izolat bakterije i tri izolata gljiva.

Za identifikaciju kvasaca i bakterija korištena je MALDI-TOF MS analiza. Kvasci su uzgajani na KDA + 25 mg/l streptomicina, a bakterije na HA tijekom četiri dana na 23 °C. Uzorci kolonija analizirani su pomoću uređaja Bruker Microflex LT MS (Billerica, Massachusetts, SAD), koristeći brzu ekstrakciju pomoću FA i HCCA matrice. Spektri su uspoređeni s referentnom bazom podataka Bruker Biotyper v3.1. Za identifikaciju plijesni korištena je PCR metoda, koristeći istu metodologiju za identifikaciju izolata gljiva prikupljenih na terenu. Za amplifikaciju korištena je ITS regija genoma, primjenom para početnica ITS1 i ITS4 (White et al., 1990).

Antagonistički pokus proveden je metodom dvostruke kulture (Fokkema, 1978; Živković i sur., 2010). Izolati su prethodno inkubirani sedam dana na 25 °C u mraku. U sterilne Petrijeve zdjelice uliveno je 10 ml KDA. Na jednu stranu zdjelice postavljen je disk micelija patogene gljive, a na suprotnu stranu inokuliran je izolat bakterije/kvasca (1 µL), udaljen 3 cm od patogena. KDA inokulirana samo s patogenom služila je kao kontrola. Pokus je postavljen u tri ponavljanja. Kod pokusa s *Penicillium* sp. korišten je 4-mm micelijski disk. Postotak inhibicije rasta (PGI) izračunat je prema formuli:

PGI (%) = (KR − R1) / KR × 100, gdje je KR udaljenost od inokulacije do ruba kolonije u kontroli, a R1 udaljenost prema antagonistu.

PGI vrijednosti kategorizirane su prema ljestvici:

0=bez inhibicije, 1=1–25 %, 2=26–50 %, 3=51–75 %, 4=76–100 % inhibicije.

Zona inhibicije mjerena je kao udaljenost između patogena i antagonista sedmog dana inkubacije.

4.7.4. Statistička analiza

Za određivanje kardinalnih temperatura za rast gljiva iz porodice Botryosphaeriaceae izračunate su prosječne vrijednosti. Empirijsko matematičko modeliranje, primjenom metode najmanjih kvadrata prema opisu Sánchez i sur. (2003), provedeno je u okviru odgovarajućih polinomnih regresija trećeg, četvrtog, petog i šestog stupnja, koristeći program Microsoft Office Excel. Budući da se jednadžba šestog stupnja najbolje uklapala u ispitivane podatke i najvjernije je pratila točke određenih brzina rasta, ona je korištena za daljnje određivanje kardinalnih temperatura. Kako bi se iz jednadžbe dobile minimalne, maksimalne i optimalne kardinalne temperature, opisano empirijsko matematičko modeliranje primjenom metode najmanjih kvadrata provedeno je pomoću matematičkog programa Wolfram Alpha LLC (Wolfram Research, Inc.,Champaign, Illinois, SAD).

Podaci dobiveni testiranjem osjetljivosti sorata analizirani su jednofaktorskom analizom varijance (ANOVA), nakon čega je primijenjen Tukeyjev test radi utvrđivanja statistički značajnih razlika između srednjih vrijednosti na razini značajnosti od 5 %. Statistička obrada podataka provedena je pomoću statističkog softvera SAS Enterprise Guide 8.4. (SAS Institute, Cary, North Carolina, SAD). Podaci su prikazani kao aritmetičke sredine, standardne devijacije i 95 %-tni intervali pouzdanosti za srednju vrijednost.

Podaci dobiveni testiranjem antifungalnog djelovanja EtU analizirani su primjenom dvofaktorske ANOVA analize, uzimajući u obzir sva dostupna mjerenja, tretmane i koncentracije, koristeći Python 3.8.10 (Python Software Foundation, Wilmington, DE, SAD), kako bi se utvrdilo postoje li statistički značajne razlike između primijenjenih tretmana te procijenile interakcije između svih analiziranih skupina. Potom, provedena je jednofaktorska ANOVA te Tukeyjev test višestrukih usporedbi, koristeći SAS Enterprise Guide 8.4. (SAS Institute, Cary, North Carolina, SAD). Podaci su prikazani kao aritmetičke sredine, standardne devijacije i 95 %-tni intervali pouzdanosti za srednju vrijednost. U određenim slučajevima, kada rezultati jednofaktorske ANOVA analize nisu pokazali statistički značajne razlike između tretmana, ali su vizualno uočene razlike u rezultatima, dodatno su provedeni T-testovi za usporedbu određenih parova, također pomoću SAS Enterprise Guide 8.4 (SAS Institute, Cary, North Carolina, SAD). Inhibicijski učinak EtU i njihovih komponenti na rast micelija fitopatogenih gljiva izračunat je u programu Microsoft Office Excel prema formuli Wu i sur.

(2013): I (%)=C−TC−0,4×100, gdje je I (%) postotak inhibicije rasta micelija izazvane ispitivanom tvari, C je promjer rasta gljive kod kontrole, a T promjer rasta gljive na tretiranom KDA. Za OBVM statistička analiza kemijskog profila, izračuni povezani s antagonističkim učinkom, izračun postotne inhibicije, vrijednosti MIC i MFC, kao i izrada stupčastih dijagrama za prikaz antagonističkog učinka, provedeni su pomoću programa Microsoft Office Excel. Grafički prikazi (toplinske karte) izrađeni su koristeći Python 3.10.12. (Python Software Foundation, Wilmington, DE, SAD). Antifungalna učinkovitost OBVM, komponenti i fungicida procijenjena je pomoću SAS Enterprise Guide 8.4 (SAS Institute, Cary, North Carolina, SAD). Podaci su prikazani kao aritmetičke sredine, standardne devijacije i 95 %-tni intervali pouzdanosti za srednju vrijednost.

5. REZULTATI ISTRAŽIVANJA S RASPRAVOM

5.1. Simptomi gljivičnog oboljenja zamijećeni na terenu

Tijekom terenskih istraživanja na zaraženim stablima maslina opažen je širok spektar simptoma, uključujući sušenje i propadanje grana i izbojaka, venuće i otpadanje listova, trulež i otpadanje plodova, pucanje i crvenkasto-smeđe obojenje kore, kao i pojava nekrotičnih lezija (Slika 2). Nakon laboratorijskih analiza utvrđeno je da su ti simptomi povezani sa zarazama uzrokovanim različitim vrstama gljiva.

Simptomi zaraze gljivama iz porodice Botryosphaeriaceae uključivali su sušenje i odumiranje grana i izbojaka, otpadanje listova, pucanje kore te pojavu nekroza na kori. Ovi simptomi u skladu su s onima prethodno zabilježenima u drugim istraživanjima (Kaliterna i sur., 2012; Hernández-Rodríguez i sur., 2022).

Kod određenih stabala uočen je kompleks simptoma uključujući stvaranje rak-rana, intenzivno sušenje i pucanje kore, unutarnje obojenje tkiva ispod kore u nijansama od crvenkastosmeđe do gotovo crne, što je povezano sa zarazom vrstama roda *Biscogniauxia*. Do sada je *B. mediterranea* jedina vrsta ovog roda potvrđena kao patogen na maslini, s utvrđenim simptomima nekroze kore, njenog odvajanja i sušenja grana (Gharbi i sur., 2020).

Simptomi sušenja grana i grančica, smeđe unutarnje nekroze u granama i ispod kore te propadanje plodova povezani su sa zarazom s vrstom *Cytospora* sp. Ove nalaze potvrđuju i Moral i sur. (2017), koji navode *Cytospora* spp. kao uzročnike sušenja grana masline.

Simptomi pjegavosti listova, gdje su listovi bili suhi, žućkastosmeđe do tamnosmeđe boje sa smeđim pjegama, a otpali kod jače izraženih zaraza, uočeni su kod zaraza vrstama *Nigrospora* spp. Ovi simptomi obično su zahvaćali samo dijelove krošnje. Iako *Nigrospora* spp. do sada nisu bile zabilježene kao patogeni masline, postoje raniji nalazi izolacije vrste *N. oryzae* s maslina bez potvrđene patogenosti (Fisher i sur., 1992). Međutim, *Nigrospora* spp. su poznati uzročnici bolesti lista kod drugih biljnih vrsta (Raza i sur., 2019; Luo i sur., 2020).

Zaraza gljivom roda *Phaeoacremonium* bila je praćena sušenjem bočnih grana, osobito s jedne strane stabla te pojavom unutarnjih nekroza smeđe boje koje su se širile ispod kore. Vrste ovog roda poznate su kao patogeni masline, uzrokujući simptome sušenja grana i nekroza (Carlucci i sur., 2013; Úrbez-Torres i sur., 2013).

Kod pojedinih stabala opaženi su simptomi sušenja grana i listova te promjene boje kore povezane sa zarazom vrstom *Sordaria* sp.. Međutim, prema dostupnoj literaturi, ova gljiva do

sada nije zabilježena kao uzročnik bolesti masline.

Slika 2. Simptomi gljivičnog oboljenja na maslini uzrokovani vrstama: A) *Botryosphaeria dothidea*, B) *Neofusicoccum parvum*, C) *Nigrospora philosophiae-doctoris*, D) *Biscogniauxia mediterranea*, E) *Cytospora pruinosa*, F) *Nigrospora gorlenkoana*, G) *Phaeoacremonium iranianum*, H) *Sordaria fimicola*.

5.2. Molekularna i morfološka identifikacija gljiva

Phillips i sur. (2013) navode da su morfološke karakteristike same po sebi nedovoljne za definiranje rodova ili identifikaciju vrsta, s obzirom na njihovu varijabilnost tijekom razvoja te neizbježno preklapanje karakteristika. Autori navode kako se najtočnija identifikacija gljiva postiže kombinacijom morfoloških karakteristika i molekularnih metoda. Stoga, na temelju morfološke karakterizacije te molekularne i filogenetske identifikacije, ukupno je identificirano 13 izolata gljiva iz porodice Botryosphaeriaceae. Među njima su identificirana četiri izolata

vrste *B. dothidea*, po jedan izolat vrsta *Diplodia mutila* (Fr.) Fr., *D. seriata* De Notaris i *Dothiorella iberica* A.J.L. Phillips, J. Luque & A. Alves, dva izolata vrste *Do. sarmentorum* (Fr.) A.J.L. Phillips, Alves & Luque te četiri izolata vrste *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips.

Također, identificirano je deset izolata iz roda *Biscogniauxia*, od kojih je devet identificirano kao *B. mediterranea*, a jedan kao *B. nummularia* (Bull.) Kuntze. Nadalje, identificirana su dva izolata vrste *C. pruinosa*, tri izolata vrsta iz roda *Nigrospora*, identificirana kao *N. gorlenkoana* Novobr., *N. osmanthi* i *N. philosophiae-doctoris* M. Raza, Qian Chen & L. Cai, jedan izolat vrste *Phaeoacremonium iranianum* L. Mostert, Grafenhan, W. Gams & Crous te dva izolata vrste *Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not. Popis svih izolata, uključujući sorte maslina s koje su prikupljeni i odgovarajući GenBank pristupni brojevi prikazani su u Tablici 2. Detaljni opisi morfoloških karakteristika te rezultati određivanja kardinalnih temperatura za rast micelija kod vrsta iz porodica Botryosphaeriaceae dostupni su u cjelovitim radovima.

Tablica 2. Oznake prikupljenih izolata, identificirane vrste, sorte s kojih su izolati prikupljeni te GenBank pristupni brojevi.

Izolat	**Vrsta**	**Sorta masline**	**GenBank pristupni brojevi**		
	Botryosphaeriaceae		**ITS**	**TUB2**	**TEF1-α**
R8 NP	*Botryosphaeria dothidea*	Nepoznata	OQ338370	OQ348378	OQ348385
PL1 NP		Nepoznata	OQ352832	OQ361692	OQ553927
N17 BJA3		Istarska bjelica	OQ353073	OQ361697	OQ553926
R19 F		Frantoio	OQ354201	OQ361700	OQ361701
IKB9 B2II	*Diplodia mutila*	Buža	OQ338569	OQ348379	OQ348386
V16 K2II	*Diplodia seriata*	Karbonaca	OQ352870	OQ361695	OQ361696
V16 BI	*Dothiorella iberica*	Buža	OQ339205	OQ348381	OQ348388
V12 PEN	*Dothiorella sarmentorum*	Pendolino	OQ339150	OQ348380	OQ348387
R18 PEN1		Pendolino	OQ341230	OQ348383	OQ348390
IMK9 IBVI	*Neofusicoccum parvum*	Istarska bjelica	OQ352837	OQ361693	OQ361694
V16 K1		Karbonaca	OQ341191	OQ348382	OQ348389
R18 B1		Buža	OQ353087	OQ361698	OQ361699
V21 B5I		Buža	OQ341428	OQ348384	OQ553928

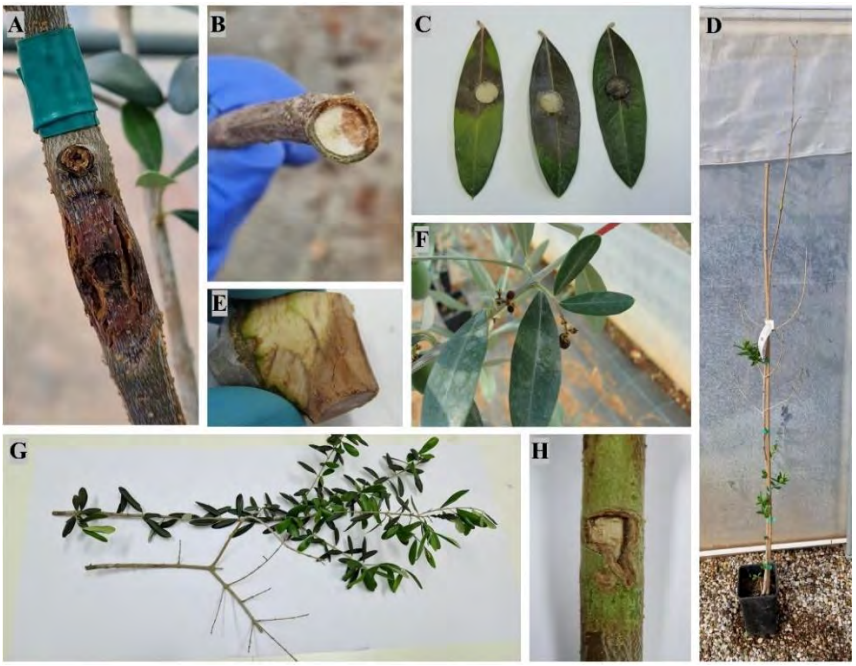
	Sordariomycetes				
SL2 PRI	*Biscogniauxia mediterranea*	Porečka rosulja	OQ734646	OQ744688	OQ754165
N17 BJA1		Istarska bjelica	OQ733307	OQ744682	OQ744689
R18 B3I		Buža	OQ733361	OQ744684	OQ744691
R18 LECII		Leccino	OQ746452	OQ744687	OQ744694
R18 LEC1		Leccino	OQ733486	OQ744686	OQ744693
R19 B1		Buža	OQ733324	OQ744683	OQ744690
ISN9 LDC3I		Leccio del Corno	OQ729816	OQ942633	OQ744681
IMK9 36II		Nepoznata	OQ646787	OQ725012	OQ725013
R18 BII		Buža	OQ933231	OQ744685	OQ744682
V16 B3	*Biscogniauxia nummularia*	Buža	OQ743781	OQ754166	OQ754167
SL2 PRIV	*Cytospora pruinosa*	Porečka rosulja	OQ642321	OQ652101	/
V16 BIII		Buža	OQ644501	OQ694815	/
P13 LECIII	*Nigrospora gorlenkoana*	Leccino	OP999642	OQ286068	OQ286069
JA20 NP	*Nigrospora osmanthi*	Nepoznata	OP999639	OQ275027	OQ275028
R18 BI	*Nigrospora philosophiae-doctoris*	Buža	OP999644	OQ286067	OQ286066
SL1 NP2	*Sordaria fimicola*	Nepoznata	OQ828656	OQ835632	OQ835629
ISN9 PEN		Pendolino	OQ828658	OQ835630	OQ83563
R18 B4	*Phaeoacremonium iranianum*	Buža	OP627795	OP684932	OP684933

5.3. Test patogenosti

Svih 14 testiranih izolata pokazali su se kao patogeni na maslini. Nakon provedene inokulacije sadnica i biljnih dijelova masline zabilježeni su simptomi slični i/ili isti onima opaženima u prirodnim uvjetima zaraze, uključujući stvaranje rak-rana, pojavu nekroza, sušenje i opadanje

listova, promjene boje kore i druge znakove propadanja tkiva (Slika 3).

Na temelju provedenih istraživanja, identificirano je ukupno šest različitih vrsta kao uzročnika botriosferijskog sušenja maslina u Hrvatskoj, tj. *B. dothidea*, *D. mutila*, *D. seriata*, *Do. iberica*, *Do. sarmentorum* i *N. parvum*. Prisutnost vrsta *D. mutila*, *Do. iberica* i *Do. sarmentorum* na stablima masline u Hrvatskoj do sada nije bila dokumentirana, što ovo istraživanje čini prvim izvješćem o njihovoj pojavi. Nadalje, ovo istraživanje predstavlja prvo dokumentirano izvješće o prisutnosti gljive *B. mediterranea* i *C. pruinosa* kao uzročnika bolesti masline u Hrvatskoj. Također, po prvi put u svijetu potvrđeno je da *B. nummularia*, *S. fimicola, P. iranianum* te tri identificirane vrste roda *Nigrospora* spp. uzrokuju bolesti maslina, uključujući prvo zabilježeno uzrokovanje biljne bolesti od strane vrste *N. philosophiae-doctoris*.



Slika 3. Rezultati testova patogenosti: A) *Diplodia mutila*, B) *Dothiorella iberica*, C) s lijeva na desno: *Nigrospora philosophiae-doctoris*, *N. gorlenkoana*, *N. osmanthi*, D) *Biscogniauxia mediterranea*, E) *Phaeoacremonium iranianum*, F) *Cytospora pruinosa*, G) *Biscogniauxia nummularia*, H) *Sordaria fimicola*.

5.4. Test osjetljivosti sorti

Sorta masline Buža pokazala je najveću otpornost na *Do. sarmentorum*, sa statistički značajnom razlikom u usporedbi s ostalim sortama (Tablica 3). Slijede po otpornosti, silaznim redom: *Do. iberica*, *D. seriata* i *B. dothidea*. S druge strane, sorta Buža pokazala je najveću osjetljivost na *D. mutila* i *N. parvum*.

Sorta Istarska bjelica pokazala je najveću otpornost na *Do. iberica* i *Do. sarmentorum*, zatim na *D. seriata* i *B. dothidea*. Najveća osjetljivost zabilježena je prema *N. parvum*, sa statistički značajnom razlikom u usporedbi s ostalim vrstama. Istarska bjelica također je pokazala izraženu osjetljivost na *D. mutila*.

Sorta Leccino imala je najveću otpornost na *Do. iberica* i *D. seriata*. Najosjetljivija je bila na *B. dothidea*, zatim na *Do. sarmentorum* i *N. parvum*, a značajna osjetljivost zabilježena je i na *D. mutila*.

Sorta Rosinjola pokazala je najveću otpornost na *Do. sarmentorum* i *Do. iberica*, a zatim na *D. seriata* i *B. dothidea*. S druge strane, pokazala je značajnu osjetljivost na *N. parvum* i *D. mutila*.

Tablica 3. Rezultati testa patogenosti /testa otpornosti sorata s prosječnim vrijednostima duljine nekrotičnih promjena (srednja vrijednost ± standardna devijacija, u mm) za šest izolata gljiva iz porodice Botryosphaeriaceae na sadnicama maslina, sa sterilnim KDA kao negativnom kontrolom.

Vrsta	**Sorta**							
	Buža		Istarska bjelica		Leccino		Rosinjola	
Botryosphaeria dothidea	9,30 ± 3,69	b	31,75 ± 11,88	c	65,41 ± 17,82	a	12,87 ± 6,26	b
Diplodia mutila	19,16 ± 6,27	a	84,33 ± 33,28	b	44,95 ± 20,75	b	22,25 ± 6,36	a
Diplodia seriata	5,50 ± 1,85	b	11,15 ± 5,25	c	5,10 ± 2,32	c	10,05 ± 5,41	bc
Dothiorella iberica	5,50 ± 1,03	b	3,65 ± 1,93	c	3,75 ± 2,02	c	4,95 ± 2,85	cd
Dothiorella sarmentorum	4,70 ± 1,92	bc	4,55 ± 1,42	c	53,99 ± 20,04	ab	4,85 ± 0,75	cd
Neofusicoccum parvum	16,15 ± 5,32	a	320,75 ± 87,39	a	48,45 ± 18,69	ab	24,11 ± 7,87	a
Kontrola	0,0 ± 0,0	c	0,0 ± 0,0	c	0,0 ± 0,0	c	0,0 ± 0,0	d
LSD	4,87		48,62		19,99		6,90	

***** Srednje vrijednosti u istom retku koje imaju ista slova nisu značajno različite prema Tukeyjevom testu značajnosti ($p < 0,05$).

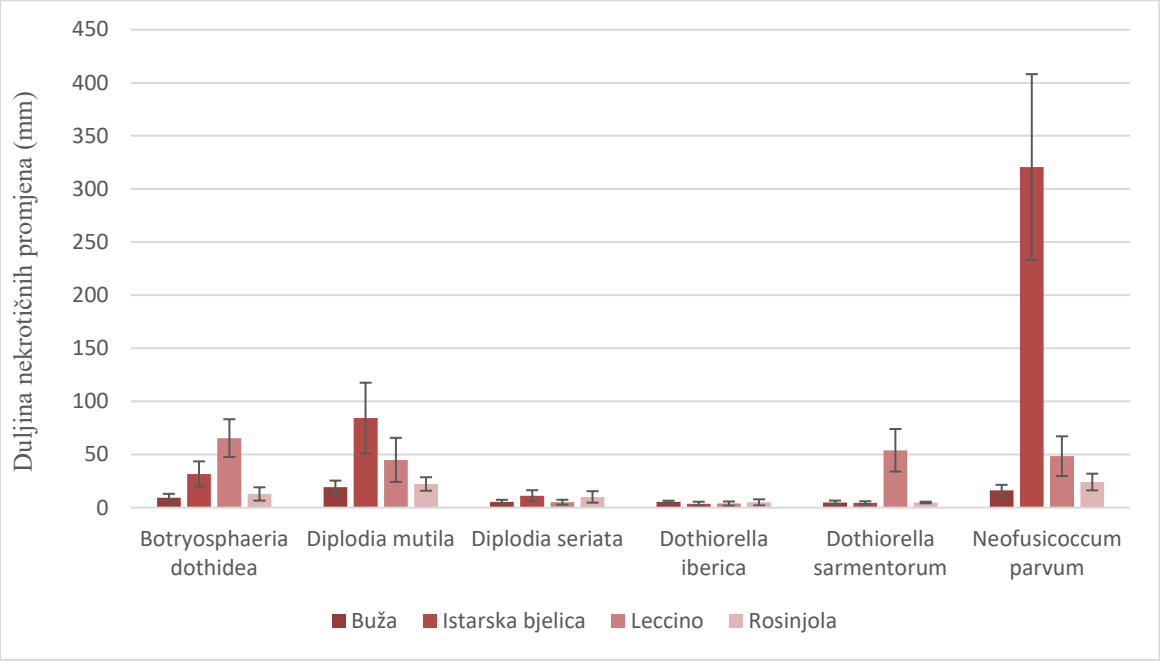
Pri evaluaciji otpornosti sorti na patogene gljive (Slika 4), Buža je pokazala najveću otpornost prema *B. dothidea* (9,30 ± 3,69), slijedila je Rosinjola (12,87 ± 6,26) te Istarska bjelica (31,75 ± 11,88), dok je Leccino bila najosjetljivija (65,41 ± 17,82). Slično tome, za *D. mutila,* Buža je ponovno pokazala najvišu otpornost (19,16 ± 6,27), zatim Rosinjola (22,25 ± 6,36) i Leccino (44,95 ± 20,75), dok je Istarska bjelica bila najosjetljivija (84,33 ± 33,28).

Za *D. seriata*, Leccino je pokazala najvišu otpornost (5,10 ± 2,32), potom Buža (5,50 ± 1,85) i Rosinjola (10,05 ± 5,41), dok je Istarska bjelica pokazala umjerenu osjetljivost (11,15 ± 5,25).

U slučaju *Do. iberica*, Istarska bjelica bila je najotpornija (3,65 ± 1,93), zatim Leccino (3,75 ± 2,02) i Rosinjola (4,95 ± 2,85), dok je Buža bila najosjetljivija (5,50 ± 1,03).

Za *Do. sarmentorum*, Istarska bjelica također je pokazala najvišu otpornost (4,55 ± 1,42), slijede Buža (4,70 ± 1,92) i Rosinjola (4,85 ± 0,75), dok je Leccino bila znatno osjetljivija (53,99 ± 20,04).

U slučaju zaraze s *N. parvum*, Buža je ponovno pokazala najveću otpornost (16,15 ± 5,32), zatim Rosinjola (24,11 ± 7,87) i Leccino (48,45 ± 18,69), dok je Istarska bjelica bila izrazito osjetljiva (320,75 ± 87,39).



Slika 4. Rezultati testa osjetljivosti sorata. Prikazane su prosječne vrijednosti duljine nekrotičnih promjena (mm) po pojedinoj sorti, prikazane stupcima različitih nijansi crvene boje – od svijetlocrvene do tamnocrvene. Svaki stupac predstavlja srednju vrijednost 10 mjerenja. Okomite crne linije prikazuju standardnu devijaciju.

Otpornost sorti maslina na bolesti predstavlja ekonomski isplativu alternativu kemijskoj zaštiti, s minimalnim utjecajem na okoliš (Moral i sur., 2017). Latinović i sur. (2013) i Moral i sur. (2017) također su utvrdili razlike u otpornosti sorti na pojedine vrste iz porodice Botryosphaeriaceae. Kako navode Latinović i sur. (2013), identifikacija otpornih sorti mogla bi predstavljati temeljni element ekonomičnog suzbijanja bolesti, osobito za brojne male proizvođače koji si ne mogu priuštiti tretiranje velikih stabala pesticidima.

5.5. Antifungalno djelovanje eteričnih ulja i komponenti

Rezultati dvofaktorske ANOVA analize, provedene za svaku gljivu, uzimajući u obzir sve čimbenike, tj. primijenjene tretmane, dane mjerenja i koncentracije, pokazali su da kombinacija svih triju čimbenika značajno utječe na rast micelija. Pri analizi interakcije između tretmana (eterična ulja/komponente/fungicid) i koncentracije utvrđena je visoko značajna interakcija, što ukazuje na to da učinak koncentracije na rast micelija ovisi o vrsti tretmana. Slično tome, analiza interakcije između tretmana i dana mjerenja također je pokazala visoko značajnu interakciju, što ukazuje da se učinci tretmana mijenjaju tijekom vremena. Nadalje, pri procjeni interakcije između primijenjenih koncentracija i dana mjerenja utvrđena je značajna interakcija, što ukazuje na to da učinak koncentracije ovisi o danu mjerenja.

Također, pri zajedničkom promatranju sva tri čimbenika, tj. tretmana, primijenjenih koncentracija i dana mjerenja, zabilježena je visoko značajna trostruka interakcija. Navedeno implicira da optimalna učinkovitost tretmana ovisi o specifičnoj kombinaciji vrste EtU/komponente/fungicida i koncentracije te da se učinkovitost pojedinih tretmana smanjuje tijekom vremena.

Najbolji učinak u inhibiciji rasta micelija gljiva korištenih u ovom istraživanju postignut je primjenom EtU kineskog cimeta i origana, kao i njihovih najzastupljenijih komponenti, e-cinamaldehida i karvakrola, koji su potpuno inhibirali rast micelija svih testiranih gljiva. Nasuprot tome, najslabiji učinak zabilježen je kod tretmana s EtU limuna i paprene metvice, kao i njihovih komponenti limonena i mentola te komponente timol. Posebno se istaknula vrsta *Do. iberica* među predstavnicima porodice Botryosphaeriaceae, koja je pokazala najveću osjetljivost na sve primijenjene tretmane, dok je unutar razreda Sordariomycetes to zabilježeno za vrstu *N. gorlenkoana*. Li i sur. (2021) navode karvakrol kako komponentu s iznimno jakim antifungalnim djelovanjem. Autori ističu utjecaj karvakrola na hife *B. dothidea,* koje su nakon tretmana bile smežurane, deformirane, s vidljivim pukotinama i povećanim razgranavanjem, dok je također došlo do oštećenja stanične membrane, povećanja periplazmatskog prostora, plazmolize i smanjenja sadržaja lipida i ergosterola. Karvakrol povećava propusnost stanične membrane, uzrokuje gubitak staničnih sadržaja i može značajno utjecati na mitohondrijsku aktivnost patogena.

Radi pojednostavljenog prikaza rezultata, interpretacija je temeljena na postotku inhibicije te je prikazana zasebno za vrste iz porodice Botryosphaeriaceae i za vrste iz roda Sordariomycetes. Grafikoni koji prikazuju postotak inhibicije deseti dan nakon početka mjerenja, kao i ANOVA tablica, postotci inhibicije te vrijednosti MIC i MFC, prikazani su u cjelovitom radu.

5.5.1. Botryosphaeriaceae

Gledano po vrsti gljive, za vrstu *B. dothidea*, najučinkovitiji tretmani u inhibiciji rasta micelija imala su EtU kineskog cimeta, origana i timijana, kao i njihove glavne komponente karvakrol i e-cinamaldehid. Svi navedeni tretmani postigli su potpunu inhibiciju rasta micelija pri koncentraciji od 0,1 %, čime su nadmašili učinkovitost komercijalnih fungicida. Fungicid Nativo 75WG pokazao je visoku učinkovitost s potpunom inhibicijom četvrti dan i 95,7 % inhibicije deseti dan. Suprotno tome, Cabrio TOP imao je znatno kraći i slabiji učinak, već drugi dan zabilježeno je 71,7 % inhibicije, dok je do desetog dana učinkovitost pala na svega 9,7 %. Komponenta timol pokazala je djelomičan antifungalni učinak. Iako su više koncentracije rezultirale jačom inhibicijom, učinak nije bio postojan jer je do desetog dana ponovno došlo do znatnog razvoja micelija. EtU svetog bosiljka i njegova glavna komponenta eugenol pokazali su potpunu inhibiciju pri koncentraciji od 0,2 %, dok su pri nižim koncentracijama imali ograničen učinak. EtU paprene metvice počelo je pokazivati fungistatički učinak pri koncentraciji od 0,2 %, no do desetog dana micelij je ponovno potpuno prerastao Petrijevu zdjelicu. S druge strane, EtU limuna te komponente mentol i limonen nisu pokazali antifungalno djelovanje, čak ni pri najvišoj primijenjenoj koncentraciji od 1,0 %. Ammad i sur. (2018) proveli su *in vitro* ispitivanja antifungalne učinkovitosti EtU limuna protiv tri patogene vrste, uključujući *B. dothidea*. Njihovi rezultati pokazali su da je EtU limuna inhibiralo rast *B. dothidea* pri koncentraciji od 0,25 %. Nasuprot tome, u našem istraživanju, ni EtU limuna ni njegov glavni sastojak limonen nisu pokazali inhibicijski učinak na rast micelija *B. dothidea.* Zhang i sur. (2018) testirali su antifungalnu aktivnost 20 čistih spojeva protiv *B. dothidea* pri koncentraciji od 400 µg/mL, gdje je mentol inhibirao rast micelija za približno 40,2 %. Međutim, u našem istraživanju, mentol nije pokazao učinak na rast micelija, čak ni pri najvišoj ispitanoj koncentraciji.

Za vrstu *D. mutila*, najučinkovitiji tretmani bili su EtU kineskog cimeta i origana te komponente karvakrol, e-cinamaldehid i eugenol, koji su pri koncentraciji od 0,1 % potpuno inhibirali rast micelija tijekom cijelog razdoblja ispitivanja. Njihova učinkovitost bila je veća u usporedbi s fungicidima. Fungicid Nativo 75WG postigao je 100 % inhibiciju drugog dana, 90,3 % četvrtog dana i 76,7 % desetog dana. Cabrio TOP bio je manje učinkovit, s postotkom inhibicije 81,2 % drugi dan, 57,8 % četvrti dan i samo 17,1 % deseti dan. EtU svetog bosiljka i timijana pokazala su nešto slabiji učinak pri koncentraciji od 0,1 %; micelij je ponovno rastao nakon četvrtog dana, ali pri 0,2 % oba EtU su potpuno inhibirala rast micelija. Komponenta timol pokazala je dobru učinkovitost pri višim koncentracijama; zabilježena je 100 % inhibicija

drugi i četvrti dan te 78,7 % deseti dan. Paprena metvica i limun imali su slabiji učinak. Inhibicija je bila izraženija na početku, posebice kod EtU paprene metvice, ali do desetog dana micelij je ponovno potpuno prerastao Petrijevku. Njihove komponente, mentol i limonen, pokazale su samo kratkotrajnu i ograničenu inhibiciju.

EtU kineskog cimeta, origana i timijana te komponente karvakrol i trans-cinamaldehid bile su najučinkovitije protiv vrste *D. seriata*, s potpunom inhibicijom micelija na 0,1 % tijekom svih dana ispitivanja. Fungicid Nativo 75WG bio je učinkovitiji od Cabrio TOP-a (71,7 % nasuprot 30,6 % inhibicije deseti dan), no oba su bila slabija od tretmana s EtU. EtU svetog bosiljka i njegova komponenta eugenol pokazali su snažno djelovanje pri 0,2 %, dok su pri 0,1 % imali ograničenu učinkovitost. Timol je pokazao visoku antifungalnu aktivnost pri višim koncentracijama, nadmašivši učinak fungicida Cabrio TOP-a, ali nije nadmašio učinak Nativa 75WG. EtU paprene metvice je bilo vrlo slabo, sa samo 3,1 % inhibicije na 0,2 % drugi dan, a do desetog dana micelij je potpuno prerastao medij. Slično je zabilježeno i kod EtU limuna. Limonen i mentol nisu pokazali antifungalno djelovanje niti pri jednoj ispitivanoj koncentraciji. U literaturi nisu dostupni podaci o učinku EtU na *D. seriata* i *D. mutila*. Štůsková i suradnici (2023) ispitivali su učinak fenolnih spojeva na rast patogena vinove loze, uključujući *D. seriata* i *N. parvum*, gdje su eugenol i timol pokazali snažno inhibicijsko djelovanje, pri čemu je eugenol postigao 100 % inhibiciju rasta pri koncentraciji od 2,5 µL/mL.

Kod vrste *Do. iberica*, potpuna inhibicija rasta micelija postignuta je pri 0,1 % s EtU kineskog cimeta, svetog bosiljka, limuna, origana i timijana te komponentama karvakrol, e-cinamaldehid i eugenol. Oba komercijalna fungicida, Cabrio TOP i Nativo 75WG, pokazala su visoku učinkovitost pri testiranim koncentracijama. EtU timijana bilo je učinkovito u svim koncentracijama, dok je timol pokazao slabiji učinak, iako je inhibicija bila potpuna do četvrtog dana na 0,1 % i 0,2 %, micelij je desetog dana ponovno prerastao medij. Potpuna i dugotrajna inhibicija postignuta je tek na 0,5 %. EtU paprene metvice je pokazalo snažno djelovanje na 0,2 %, no na nižim koncentracijama inhibicija nije bila postojana. Mentol i limonen imali su vrlo ograničen učinak; iako je drugi dan zabilježena djelomična inhibicija rasta, već četvrti dan micelij je u potpunosti ispunio Petrijevu zdjelicu. Mentol je pokazao nešto veću učinkovitost od limonena pri nižim koncentracijama, no ta razlika se izgubila pri koncentraciji od 1,0 %, gdje je limonen zabilježio neznatno višu inhibiciju.

Za vrstu *Do. sarmentorum*, EtU kineskog cimeta i origana te komponente karvakrol, e-cinamaldehid i eugenol pokazali su potpunu inhibiciju rasta micelija već pri koncentraciji od 0,1 %. Oba testirana fungicida bila su učinkovita, iako slabija od navedenih prirodnih tretmana. EtU svetog bosiljka pokazalo je 100 % inhibiciju pri 0,2 %, dok je na 0,1 % učinkovitost bila

ograničena i kratkotrajna. EtU timijana je inhibiralo rast micelija do četvrtog dana, ali do desetog dana micelij je ponovno prerastao medij. Potpuna inhibicija postignuta je tek pri 0,5 %. Komponenta timol bila je učinkovita od 0,2 %, a potpuna inhibicija održana je kroz cijeli period pri 1,0 %. EtU paprene metvice je pokazalo snažnu inhibiciju samo pri koncentraciji od 0,5 %. Na nižim koncentracijama, inhibicija je bila kratkotrajna. Mentol nije pokazao učinak do koncentracije od 0,75 %, gdje je zabilježena slaba inhibicija, dok je do četvrtog dana učinak u potpunosti izostao. EtU limuna i komponenta limonen nisu imali značajan antifungalni učinak. Dosadašnja istraživanja nisu uključivala ispitivanja učinaka EtU na *Do. iberica* i *Do. sarmentorum*.

EtU kineskog cimeta i origana, zajedno s komponentama e-cinamaldehid i karvakrol, pokazala su najvišu učinkovitost protiv vrste *N. parvum*, s potpunom inhibicijom micelijskog rasta pri koncentraciji od 0,1 %. EtU svetog bosiljka, paprene metvice i timijana također su bila vrlo učinkovita, ali pri nešto višoj koncentraciji (0,2 %). Među njima, sveti bosiljak bio je najdjelotvorniji pri nižim koncentracijama. Eugenol je u potpunosti inhibirao rast micelija pri koncentraciji od 0,5 %. Fungicid Nativo 75WG postigao je 92,2 % inhibicije deseti dan, dok je Cabrio TOP imao vrlo slab učinak, inhibicija je pala s 56,6 % drugog dana na svega 1,9 % četvrtog dana, nakon čega je micelij ponovno potpuno prekrio medij. Timol je pokazao antifungalni učinak već pri najnižim koncentracijama. EtU limuna te komponente mentol i limonen nisu pokazali nikakav antifungalni učinak protiv ove gljive pri bilo kojoj testiranoj koncentraciji. U ispitivanju zaštitnih spojeva protiv *N. parvum* na stabljikama borovnice, Latorre i sur. (2013) utvrdili su da je tebukonazol (0,5 %) bio najučinkovitiji fungicid. Twizeyimana i sur. (2013) ispitivali su učinak 12 različitih fungicida na gljive iz porodice Botryosphaeriaceae, uključujući *N. parvum* i *Do. iberica*, pri čemu su najefikasnijima ocijenjeni azoksistrobin, fludioksonil, metkonazol, propikonazol i piraklostrobin. U ovom istraživanju, fungicid Nativo 75WG (kombinacija trifloksistrobina i tebukonazola) pokazao se učinkovitijim u suzbijanju svih ispitivanih vrsta iz porodice Botryosphaeriaceae u usporedbi s fungicidom Cabrio TOP, osim za *Do. iberica* i *Do. sarmentorum*, gdje su oba fungicida bila 100 % učinkovita.

5.5.2. Sordariomycetes

EtU svetog bosiljka, kineskog cimeta, origana i timijana te komponente karvakrol, e-cinamaldehid i eugenol pokazali su najveću učinkovitost u inhibiciji rasta micelija *B. mediterranea.* Već pri koncentraciji od 0,1 % zabilježena je potpuna inhibicija micelijskog rasta kroz sve dane ispitivanja. Komponenta timol i EtU paprene metvice pokazali su 100 %

inhibiciju tek pri koncentraciji od 0,5 %. EtU paprene metvice je pri nižim koncentracijama imalo umjeren učinak. Od fungicida, Nativo 75WG bio je učinkovitiji (86,8 % na deseti dan) u odnosu na Cabrio TOP (75,9 %). EtU limuna te komponente mentol i limonen pokazali su učinak samo drugi dan, čak i pri najvišim koncentracijama. EtU limuna imalo je jači učinak (77,3 %) od komponente limonen (29,9 %).

Slično kao kod prethodne vrste, kod *B. nummularia* najučinkovitija su bila EtU svetog bosiljka, kineskog cimeta, origana i timijana te karvakrol, e-cinamaldehid, eugenol i fungicid Nativo 75WG; svi su postigli potpunu inhibiciju rasta već na 0,1 %. EtU paprene metvice je bilo učinkovito pri 0,5 %, ali na nižim koncentracijama učinak je bio ograničen, primjerice, na 0,1 % zabilježeno je samo 2,7 % inhibicije (drugi dan). Cabrio TOP bio je nešto slabiji od Nativo 75WG, ali svejedno učinkovit. Limun, limonen i mentol pokazali su djelovanje na početku, no micelij je u svim slučajevima potpuno prerastao Petrijevku do desetog dana. Limonen je bio najučinkovitiji (90,3 % na četvrti dan mjerenja), dok je mentol pokazao najslabiji učinak (50 %).

Za *C. pruinosa*, EtU kineskog cimeta i origana te karvakrol, e-cinamaldehid, eugenol i timol pokazali su 100 % inhibiciju već na 0,1 %. Oba fungicida također su bila učinkovita. EtU paprene metvice (0,5 %), timijana (0,75 %) i limuna (1,0 %) inhibirala su micelij, dok su niže koncentracije pokazale djelomičan učinak. Sveti bosiljak i mentol nisu pokazivali rast do četvrtog dana, ali do desetog dana micelij se razvio. Mentol je bio učinkovitiji (95,3 % na 1,0 %) od limonena, koji je bio najslabiji (28,3 %).

Za *N. gorlenkoana*, EtU kineskog cimeta, svetog bosiljka, origana, paprene metvice i timijana te komponente karvakrol, trans-cinamaldehid i eugenol postigli su potpunu inhibiciju rasta na 0,1 % svih dana. Fungicidi su također bili 100 % učinkoviti. Timol je bio učinkovit pr 0,2 %, a na 0,5 % postignuta je potpuna inhibicija. EtU limuna imalo je slabiji učinak, najviša zabilježena inhibicija iznosila je 86,4 % (drugi dan na 1,0 %). Limonen i mentol djelovali su samo na početku i pri višim koncentracijama, bez održivog učinka do desetog dana.

Za *N. osmanthi*, EtU svetog bosiljka, kineskog cimeta, origana i timijana, kao i karvakrol i e-cinamaldehid, pokazali su potpunu inhibiciju micelijskog rasta već na 0,1 %. EtU paprene metvice je bilo učinkovito pri 0,5 %, dok je Nativo 75WG imao slabiju dugoročnu učinkovitost (100 % drugi dan; 60,1 % deseti dan). EtU limuna i limonen nisu u potpunosti inhibirali rast, iako je viša koncentracija povećavala učinak. Eugenol i mentol pokazali su učinak koji nije varirao s koncentracijom. Timol je bio učinkovit na 0,5 %, no do desetog dana micelij se ipak razvio.

EtU kineskog cimeta, svetog bosiljka, origana i timijana te komponente karvakrol, e-

cinamaldehid i eugenol pokazali su potpunu inhibiciju pri 0,1 % na vrstu *N. philosophiae-doctoris*. Fungicid Cabrio TOP bio je jednako učinkovit, dok je Nativo 75WG imao slab učinak, tj. 2,6 % četvrti dan, a potpuni rast je zabilježen deseti dan. EtU paprene metvice, limuna i komponenta timol postigli su 100 % inhibiciju pri koncentraciji 0,5 %, a timol je bio najdjelotvorniji na nižim koncentracijama. Limonen i mentol pokazali su ograničenu inhibiciju samo do četvrtog dana; limonen je bio nešto učinkovitiji (18,2 %) od mentola (14,1 %).

Kineski cimet, sveti bosiljak, origano, timijan, karvakrol, e-cinamaldehid, eugenol i fungicid Cabrio TOP postigli su potpunu inhibiciju rasta gljive *P. iranianum* već pri 0,1 %. EtU paprene metvice je bilo učinkovito tek na 1,0%. Nativo 75WG bio je slabiji (22,2 % drugi dan; 70 % deseti dan), dok je EtU limuna bilo najslabije među ispitivanim EtU (67,5 % deseti dan). Limonen, timol i mentol nisu postigli potpunu inhibiciju ni pri najvišim koncentracijama. Limonen je bio učinkovitiji u početku, ali timol je imao bolji učinak na kraju. Mentol je bio najslabiji (36,3 %).

EtU kineskog cimeta, svetog bosiljka, origana i timijana te karvakrol, trans-cinamaldehid i eugenol, kao i oba fungicida, postigli su potpunu inhibiciju rasta vrste *S. fimicola* pri 0,1 %. Paprena metvica i timol bili su učinkoviti pri koncentraciji ≥0,5 %, pri čemu je timol pokazao veću učinkovitost pri nižim koncentracijama. Limun, limonen i mentol bili su najmanje učinkoviti. Inhibicija je zabilježena samo drugi dan, a najbolji rezultat imao je limun (85,5 % drugi dan pri koncentraciji od 1,0 %).

Trenutačno nisu dostupni podaci o metodama suzbijanja patogena *B. nummularia*, dok istraživanja usmjerena na *B. mediterranea* obuhvaćaju primjenu antagonističkih mikroorganizama (Karami i sur., 2017; Rodrigo i sur., 2017). Yangui i sur. (2017) istraživali su učinkovitost EtU pet različitih vrsta eukaliptusa te pet uzoraka EtU *Myrtus communis* L. Najveći antifungalni učinak pokazala su EtU *Eucalyptus camaldulensis* Dehnh. i *Myrtus* sp. iz regije Zaghouan. Slično tome, za patogene *C. pruinosa* te novootkrivene patogene na maslini, *N. gorlenkoana*, *N. osmanthi* i N. *philosophiae-doctoris*, do danas nisu provedena istraživanja o mogućnostima njihova suzbijanja. U slučaju patogena *P. iranianum*, do danas je provedeno samo jedno istraživanje, koje je uključivalo ispitivanje primjene biočara u svrhu suzbijanja patogena, ali na vinovoj lozi (Idbella i sur., 2024). Za patogena *S. fimicola* dostupni su isključivo stariji podaci iz 1979. godine (Buchenauer i sur., 1979), u kojima je utvrđeno da fungicidi poput triadimefona, triadimenola, fenarimola, nuarimola i imazalila posjeduju toksično djelovanje na ovu vrstu. Međutim, od tada nisu provedena daljnja istraživanja vezana uz primjenu suvremenih fungicida ili alternativnih metoda kontrole.

5.5.3. MIC i MFC vrijednosti za eterična ulja

Na temelju MIC i MFC vrijednosti, među ispitanim EtU, kineski cimet i origano pokazali su najjače fungicidno djelovanje (MFC 0,1 % za sve vrste). Sveti bosiljak također je bio vrlo učinkovit (MFC 0,1–0,2 %), osim na *Do. sarmentorum* (0,5 %). Timijan je imao MFC u rasponu 0,1–0,75 %, dok EtU limuna nije djelovalo na *B. dothidea*, *N. parvum* i *Do. sarmentorum*, a MFC je određen samo za tri vrste. EtU paprene metvice imalo je varijabilne rezultate, s najnižom MFC vrijednošću za *N. gorlenkoana* (0,1 %) i najvišom za *P. iranianum* (1,0 %). Od komponenti, najbolju antifungalnu aktivnost pokazali su karvakrol i e-cinamaldehid. Eugenol je bio učinkovit za većinu vrsta (MFC 0,1–0,5 %), dok limonen i mentol nisu pokazali značajnu učinkovitost, s brojnim vrstama kod kojih nije bilo moguće odrediti MFC. Timol je pokazao dobru učinkovitos (MFC 0,1–0,5 %), ali nije djelovao na više vrsta.

5.6. Antifungalno djelovanje OBVM

5.6.1. Fizikalno-kemijska svojstva OBVM

Boja OBVM razlikovala se među ispitivanim sortama masline, krećući se u rasponu od žute do smeđe boje (Tablica 4). Najtamnija boja zabilježena je kod OBVM sorte Istarska bjelica, dok je najsvjetlija boja utvrđena kod OBVM sorte Buža puntoža. Vrijednosti pH pokazivale su tendenciju prema kiselom, osim kod OBVM sorte Buža puntoža, koja je imala neutralni pH od 7,17. Najveće koncentracije suhe tvari i šećera zabilježene su kod OBVM sorte Istarska bjelica (35,72 mg/mL i 4,05 mg/mL), dok su najniže koncentracije zabilježene kod OBVM sorte Buža puntoža (1,54 mg/mL i 0,17 mg/mL).

Nakon tretmana s HCl-om, OBVM sorte Buža puntoža i dalje je pokazivala najniže koncentracije suhe tvari i šećera (3,07 mg/mL i 0,25 mg/mL), iako su te vrijednosti bile više u odnosu na netretirani uzorak. Iznenađujuće, kod OBVM sorte Istarska bjelica, koja je prije tretmana imala najviši sadržaj suhe tvari i šećera, nakon tretmana s HCl-om zabilježen je nagli pad koncentracije suhe tvari na 4,1 mg/mL, dok je istovremeno zadržana najveća koncentracija šećera među svim ispitivanim tretmanima (5,0 mg/mL). Najviša koncentracija suhe tvari nakon tretmana HCl-om zabilježena je kod OBVM sorte Leccino. Kod svih OBVM, osim one sorte Leccino, koncentracije šećera bile su više u uzorcima tretiranim HCl-om u odnosu na netretirane uzorke.

Što se tiče koncentracija ugljika i dušika u OBVM, najviše vrijednosti zabilježene su kod OBVM sorte Istarska bjelica, dok su najniže koncentracije utvrđene kod OBVM sorte Buža

puntoža. Promatrajući učinak HCl tretmana na sadržaj ugljika, niže koncentracije ugljika zabilježene su u uzorcima s HCl-om kod OBVM sorti Buža, Buža puntoža, Istarska bjelica i Leccino. Suprotan trend zabilježen je kod OBVM sorte Rosinjola, kod koje su koncentracije ugljika bile više u tretiranim uzorcima u usporedbi s netretiranima. Što se tiče sadržaja dušika, veće koncentracije utvrđene su u HCl-tretiranim OBVM sorte Buža, Istarska bjelica i Rosinjola u odnosu na netretirane uzorke, dok su niže koncentracije dušika zabilježene u HCl-tretiranim uzorcima OBVM sorti Buža puntoža i Leccino.

Tablica 4. Rezultati fizikalno-kemijske analize OBVM.

Ispitivani parametar	**Tip OBVM**	**Sorta iz koje je dobivena OBVM**				
		Buža	**Buža puntoža**	**Istarska bjelica**	**Leccino**	**Rosinjola**
Boja	O	žuto-smeđa	svijetlo narančasta-smeđa	tamno narančasta-smeđa	svijetlo siva - bijela	smeđa
pH	O	6	7,17	5,26	6,06	6.66
Suha tvar (mg)	O	5,61 ± 0,19	1,54 ± 0,44	35,72 ± 0,74	19,42 ± 0,29	10,04 ± 0,25
	H-O	5,42 ± 0,15	3,07 ± 0,34	4,1 ± 0,72	10,85 ± 0,24	10,13 ± 0,02
Udio vode (%)	O	99,81 ± 0,01	99,94 ± 0,01	98,79 ± 0,02	99,34 ± 0,01	99,65 ± 0,01
	H-O	99,81 ± 0,01	99,89 ± 0,01	99,86 ± 0,02	99,59 ± 0,07	99,65 ± 0,00
Udio šećera (mg/mL)	O	0,65 ± 0,02	0,17 ± 0,002	4,05 ± 0,20	2,95 ± 0,10	1,66 ± 0,01
	H-O	0,73 ± 0,02	0,25 ± 0,006	5,0 ± 0,1	1,99 ± 0,07	1,82 ± 0,01
Udio ugljika (mg C/L)	O	618,55 ± 0,29	297,30 ± 1,69	4453,50 ± 9,97	2251,50 ± 2,89	1245,50 ± 0,49
	H-O	557,00 ± 2,48	245,40 ± 0,16	4448,50 ± 4,31	1705,50 ± 1,06	1383,50 ± 0,49
Udio dušika (mg N/L)	O	8,37 ± 0,03	7,05 ± 0,01	37,09 ± 0,08	16,83 ± 0,04	12,19 ± 0,02
	H-O	9,15 ± 0,01	6,60 ± 0,01	44,44 ± 0,06	15,42 ± 0,05	14,34 ± 0,01

*O: OBVM bez HCl; H-O: OBVM s HCl.

**Koncentracija šećera izražena je kao srednja vrijednost mjerena na valnoj duljini od 480 nm i 490 nm.

5.6.2. HPLC analiza fenola

Podaci prikazani u ovom istraživanju upućuju na značajan utjecaj zakiseljavanja pomoću HCl na fenolni profil OBVM (Slika 5). Uočeni učinci mogu se svrstati u tri skupine: povećanje koncentracija fenola, smanjenje koncentracija određenih fenola te potpuni nestanak specifičnih fenola nakon tretmana zakiseljavanjem. Na primjer, tretman HCl-om doveo je do povećanja koncentracija najzastupljenijih fenola u OBVM sorti Buža, Buža puntoža i Istarska bjelica, dok

je pad koncentracija zabilježen u OBVM sorti Leccino i Rosinjola. U pojedinim slučajevima, poput OBVM sorte Buža puntoža, klorogenska kiselina nije bila detektirana u uzorku bez tretmana, ali se pojavila nakon tretmana HCl-om. Suprotno tome, spoj quercetin-3,4'-diglukozid bio je prisutan u netretiranom uzorku, ali nije detektiran nakon tretmana HCl-om. Značajne varijacije u koncentracijama izorhamnetina zabilježene su u sva četiri mjerenja za svaku OBVM, što je potvrđeno i standardnom devijacijom. Osim toga, veće oscilacije u koncentracijama između mjerenja uočene su i kod drugih fenola, poput luteolina i kvercetina, osobito u OBVM sorti Buža i Buža puntoža.

U OBVM sorte Buža, bez tretmana HCl-om, najzastupljeniji spojevi bili su vanilinska kiselina, luteolin-7-rutinozid, izorhamnetin, dihidrokvercetin (taksoifolin) i 3,4,5-trihidroksibenzojeva kiselina (galna kiselina). Nakon tretmana HCl-om, najzastupljeniji fenoli bili su luteolin-7-rutinozid, vanilinska kiselina, vanilin-4-glukozid, *p*-kumarska kiselina i 3,4,5-trihidroksibenzojeva kiselina (galna kiselina).

Kod OBVM sorte Buža puntoža, najzastupljeniji spojevi kod tretmana bez HCl bili su vanilinska kiselina, izorhamnetin, vanilin-4-glukozid, 3,4,5-trihidroksibenzojeva kiselina i *p*-kumarska kiselina. Nakon tretmana HCl-om, najzastupljeniji fenoli bili su 3,4-dihidroksibenzojeva kiselina (protokatehuična kiselina), vanilinska kiselina, vanilin-4-glukozid, *p*-kumarska kiselina i luteolin-7-rutinozid.

U OBVM sorte Istarska bjelica, najzastupljeniji fenoli u netretiranom uzorku bili su luteolin-7-rutinozid, vanilin-4-glukozid, vanilinska kiselina, kvercetin-3-rutinozid (rutin) i luteolin-7-glukozid. Nakon tretmana HCl-om, najzastupljeniji fenoli bili su luteolin-7-rutinozid, verbaskozid, vanilin-4-glukozid, kvercetin-3-rutinozid i luteolin-7-glukozid.

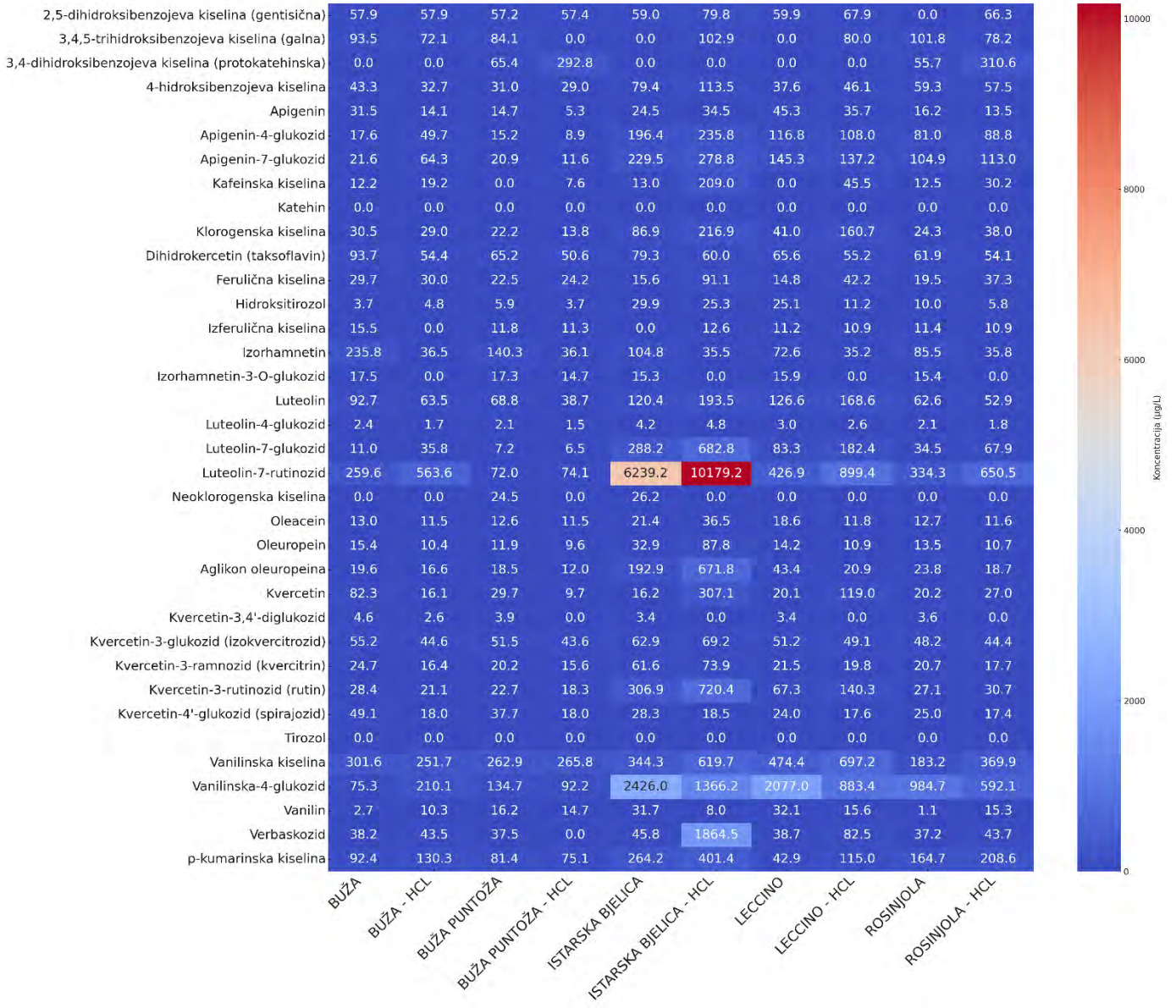
U OBVM sorte Leccino, bez tretmana HCl-om, najzastupljeniji fenoli bili su vanilin-4-glukozid, vanilinska kiselina, luteolin-7-rutinozid, apigenin-7-glukozid i luteolin. Nakon tretmana HCl-om, najzastupljeniji spojevi bili su luteolin-7-rutinozid, vanilin-4-glukozid, vanilinska kiselina, luteolin-7-glukozid i luteolin.

U OBVM sorte Rosinjola, najzastupljeniji fenoli bez HCl tretmana bili su vanilin-4-glukozid, luteolin-7-rutinozid, vanilinska kiselina, *p*-kumarska kiselina i apigenin-7-glukozid. Nakon tretmana HCl-om, najzastupljeniji fenoli bili su luteolin-7-rutinozid, vanilin-4-glukozid, vanilinska kiselina, 3,4-dihidroksibenzojeva kiselina (protokatehuična kiselina) i *p*-kumarska kiselina.

Općenito, vanilinska kiselina, vanilin-4-glukozid i luteolin-7-rutinozid bili su najzastupljeniji fenolni spojevi u OBVM prikupljenima u ovom istraživanju. Najviša ukupna koncentracija fenolnih spojeva zabilježena je u OBVM sorte Istarska bjelica, dok je najniža koncentracija

utvrđena u uzorku sorte Buža puntoža, što je u skladu s nalazima za sadržaj ukupne suhe tvari, koja je također bila najveća kod OBVM sorte Istarska bjelica, a najniža kod OBVM sorte Buža puntoža. Zakiseljavanje HCl-om općenito je rezultiralo višim ukupnim koncentracijama fenola u svim OBVM, osim u slučaju OBVM sorte Buža puntoža.

Istarska maslinova ulja poznata su po relativno visokom udjelu polifenola u usporedbi s vrijednostima zabilježenima u literaturi (Bertoša i Matijašić, 2005). Među njima se posebno ističu ulja sorte Istarska bjelica zbog iznimno visokog sadržaja polifenola (Bertoša i Matijašić, 2005). Slično tome, najviša koncentracija fenolnih spojeva zabilježena je u OBVM sorte Istarska bjelica. To se može povezati i s činjenicom da je ta otpadna voda imala najveći udio suhe tvari, što je bilo u korelaciji s povišenim razinama šećera, dušika i ugljika. Što se tiče zakiseljavanja OBVM pomoću HCl-a i njegova utjecaja na fenole, ono značajno utječe na fenolni sastav OBVM, s izraženim pozitivnim i negativnim učincima. U mnogim slučajevima, zakiseljavanje HCl-om rezultiralo je ili povećanjem ili smanjenjem koncentracije određenih fenola. To sugerira da zakiseljavanje može povećati stabilnost ili oslobađanje tih fenola, moguće razgradnjom glikoziliranih oblika u njihove slobodne fenolne spojeve. S druge strane, smanjenje sadržaja fenola može se pripisati razgradnji osjetljivih fenola pod kiselim uvjetima ili njihovoj transformaciji u druge fenolne derivate koji nisu bili kvantificirani u ovom skupu podataka. Poznato je da su određeni fenoli posebno osjetljivi na razgradnju pod specifičnim uvjetima (Brenes i sur., 2001.; Romero i sur., 2004.).



Slika 5. Toplinska karta prikazuje razlike u koncentracijama fenola u OBVM sorti Buža, Buža puntoža, Istarska bjelica, Leccino i Rosinjola s HCl-om i bez HCl-a.

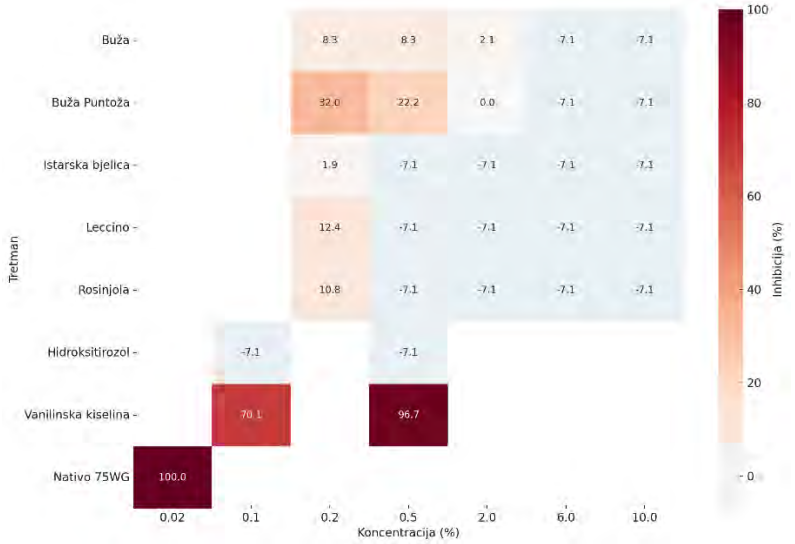
5.6.3. Antifungalno djelovanje OBVM

5.6.3.1. Botryosphaeriaceae

Općenito, među ispitivanim gljivama, *N. parvum* pokazala se kao najotpornija vrsta, pri čemu su samo vanilinska kiselina i fungicid imali inhibitorni učinak na rast micelija gljive. Suprotno tome, *Do. iberica* bila je najosjetljivija vrsta na sve tretmane. Inhibitorni učinci OBVM na rast micelija zabilježeni su isključivo kod vrste *Do. sarmentorum* sedmi dan mjerenja. Kod vrste *D. seriata* svi tretmani s OBVM stimulirali su rast micelija. Kod *D. mutila*, samo najniža

koncentracija svih OBVM imala je inhibitorni učinak, dok su više koncentracije pokazale stimulativni učinak kod svih tretmana. Sličan obrazac zabilježen je kod *B. dothidea*, gdje su niže koncentracije OBVM inhibirale rast micelija, dok su više koncentracije poticale rast. Vanilinska kiselina bila je učinkovitija u usporedbi s komponentom hidroksitirozol, a fungicid Nativo 75WG također je pokazao visoku učinkovitost u inhibiciji rasta micelija gljiva. Među OBVM, najznačajniji učinci uočeni su kod OBVM sorte Leccino, zatim sorte Buža. S obzirom na to da su učinci tretmana bili izraženiji drugog dana mjerenja i da je većina tretmana djelovala fungistatski, postotak inhibicije rasta gljivičnog micelija prikazan je grafički po vrstama gljiva i tretmanima drugog dana mjerenja za vrste iz porodice Botryosphaeriaceae, ali i za vrste iz razreda Sordariomycetes.

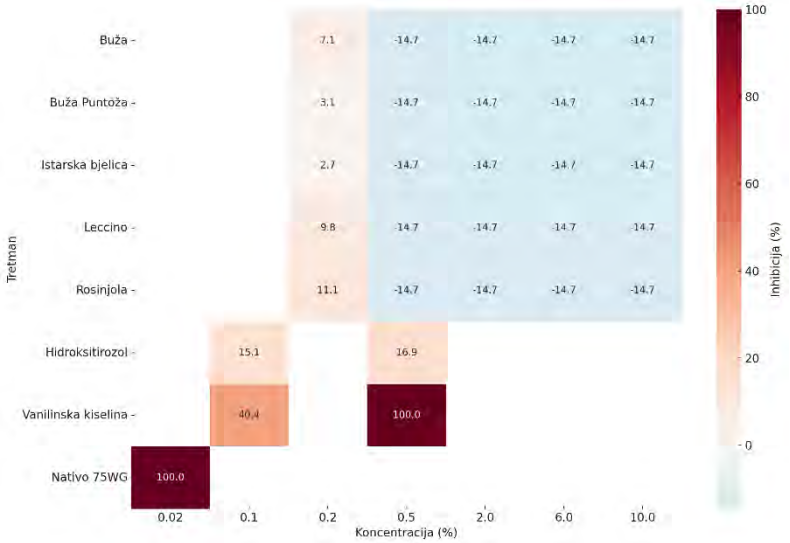
Kod vrste *B. dothidea* OBVM sorte Buža pri koncentracijama od 0,2 % do 2 % pokazala je slab inhibicijski učinak (Slika 6). Pri višim koncentracijama (6 % i 10 %) zabilježen je slab stimulirajući učinak. Kod OBVM sorte Buža puntoža, najveći inhibicijski učinak ostvaren je pri najnižoj ispitivanoj koncentraciji, dok je s povećanjem koncentracije zabilježen stimulirajući učinak. Sedmi dan mjerenja, kod svih tretmana s OBVM nije zabilježen nikakav učinak. Kod sorte Istarska bjelica, pri najnižoj koncentraciji zabilježen je minimalni inhibicijski učinak (1,87 %), dok su pri višim koncentracijama uočeni stimulirajući učinci. Sličan obrazac je zabilježen i kod tretmana s OBVM sorte Leccino, gdje je inhibicija pri najnižoj koncentraciji iznosila 12,44 %, dok su više koncentracije rezultirale stimulacijom rasta micelija. Kod tretmana s OBVM sorte Rosinjola, inhibicijski učinak na najnižoj koncentraciji iznosio je 10,78 %. Hidroksitirozol je tijekom drugog dana mjerenja pokazao isključivo stimulirajući učinak pri obje ispitivane koncentracije. Nasuprot tome, vanilinska kiselina pokazala je izraženiji inhibicijski učinak pri višoj koncentraciji oba dana mjerenja, pri čemu je sedmi dan inhibicija iznosila 12,01 % i 77,13 %. Nativo 75WG je inhibirao rast micelija za 100 %.



Slika 6. Inhibicijski učinak različitih tretmana (OBVM, fenoli i fungicid) pri različitim koncentracijama, drugi dan mjerenja, na *Botryosphaeria dothidea*. Negativne vrijednosti ukazuju na stimulirajući učinak tretmana.

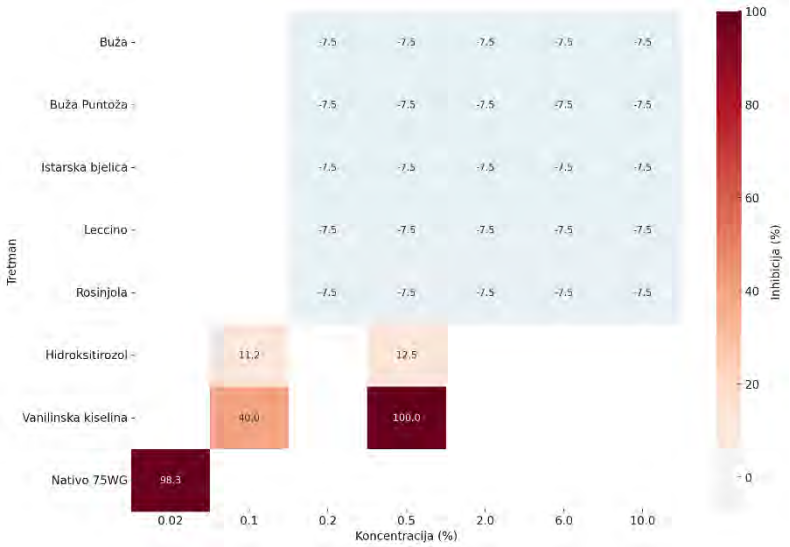
Kod vrste *D. mutila* (Slika 7), svi tretmani s OBVM pokazali su inhibicijski učinak isključivo pri najnižoj ispitivanoj koncentraciji od 0,2 %. Drugi dan mjerenja najveći inhibicijski učinak zabilježen je kod sorte Rosinjola i iznosio je 11,11%. Sve ostale koncentracije (0,5 % do 10 %) imale su stimulirajući učinak, s prosječnim povećanjem rasta od 14,67 % drugi dan mjerenja. Hidroksitirozol je pokazao slab inhibicijski učinak, pri čemu drugi dan mjerenja inhibicija iznosila 15,11 % i 16,89 % pri koncentracijama od 0,2 i 0,5 %, dok je deseti dan izostao inhibicijski učinak.

Vanilinska kiselina je pri višoj koncentraciji inhibirala rast gljive u potpunosti (100 %) oba dana mjerenja, kao i fungicid Nativo 75WG.



Slika 7. Inhibicijski učinak različitih tretmana (OBVM, fenoli i fungicid) pri različitim koncentracijama, drugi dan mjerenja, na *Diplodia mutila*. Negativne vrijednosti ukazuju na stimulirajući učinak tretmana.

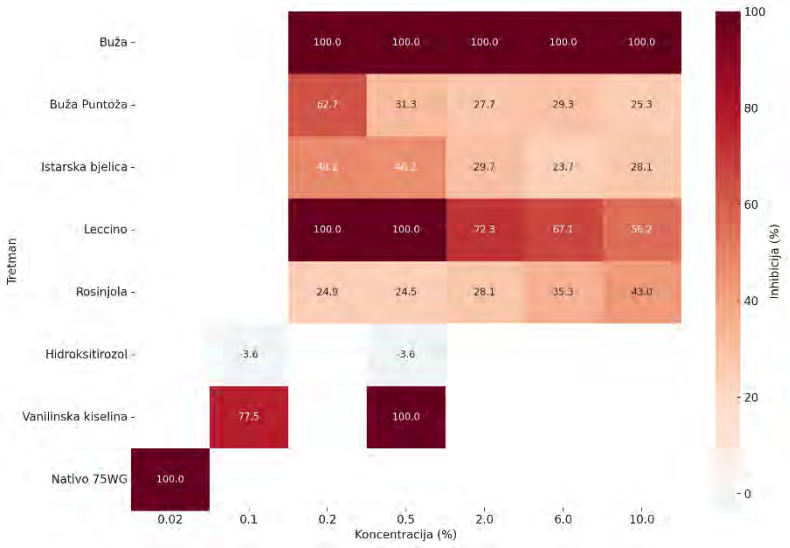
Kod vrste *D. seriata* svi tretmani s OBVM pokazali su stimulirajući učinak pri svim ispitivanim koncentracijama drugi dan mjerenja (Slika 8). Sedmi dan mjerenja nije zabilježen nikakav utjecaj na inhibiciju rasta micelija. Hidroksitirozol je drugi dana mjerenja pokazao slab inhibicijski učinak pri obje ispitivane koncentracije, dok je vanilinska kiselina potpuno inhibirala rast micelija (100 %) oba dana mjerenja pri 0,5 %. Nativo 75WG inhibirao je rast za 98,33 % drugi dan mjerenja.



Slika 8. Inhibicijski učinak različitih tretmana (OBVM, fenoli i fungicid) pri različitim koncentracijama, drugi dan mjerenja, na *Diplodia seriata*. Negativne vrijednosti ukazuju na stimulirajući učinak tretmana.

Kod vrste *Do. iberica*, OBVM sorte Buža pokazala je snažan inhibicijski učinak, pri čemu je drugi dana mjerenja u potpunosti inhibiran rast gljive (100 %) (Slika 9), dok sedmog dana nije zabilježen inhibicijski učinak. Kod OBVM sorte Buža puntoža, zabilježen je smanjen inhibicijski učinak s porastom koncentracije, pri čemu je najviša inhibicija ostvarena pri koncentraciji od 0,2 %. Zanimljivo, pri koncentraciji od 2 % inhibicijski učinak bio je slabiji nego pri 0,5 % i 6 %. Slično, kod OBVM sorte Istarska bjelica s porastom koncentracije uočen je pad inhibicijskog učinka. Najveći učinak zabilježen je pri 0,2 %. Za razliku od toga, OBVM sorte Leccino pokazala je maksimalan inhibicijski učinak (100 %) pri koncentracijama od 0,2 % i 0,5 % drugi dan mjerenja, dok sedmi dan nije uočen učinak. OBVM sorte Rosinjola pokazao je suprotan trend, inhibicijski učinak se povećavao s porastom koncentracije, a najveći učinak (42,97 %) zabilježen je pri koncentraciji od 10 % drugi dan mjerenja. Hidroksitirozol je pri obje ispitivane koncentracije imao blago stimulirajući učinak, dok je vanilinska kiselina pokazala 100% inhibiciju pri koncentraciji od 0,5 %. Nativo 75WG inhibirao je rast za 100% oba dana.

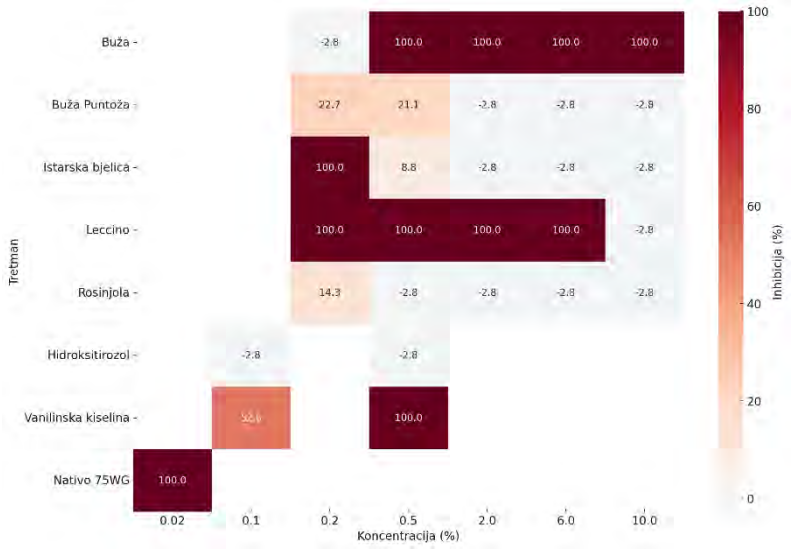
.



Slika 9. Inhibicijski učinak različitih tretmana (OBVM, fenoli i fungicid) pri različitim koncentracijama, drugi dan mjerenja, na *Dothiorella iberica*. Negativne vrijednosti ukazuju na stimulirajući učinak tretmana.

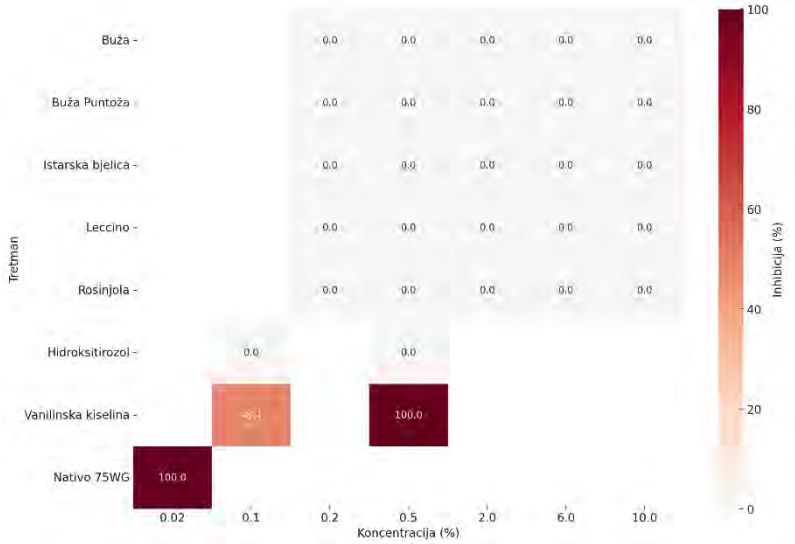
Kod vrste *Do. sarmentorum*, OBVM sorte Buža pokazala je blagi stimulirajući učinak pri koncentraciji od 0,2 % (2,79 %), dok su koncentracije od 0,5 % i 10 % rezultirale potpunom inhibicijom rasta micelija na oba dana mjerenja. OBVM sorte Buža puntoža imala je inhibicijski učinak pri koncentracijama od 0,2 % i 0,5 % (Slika 10). Međutim, pri višim

koncentracijama zabilježen je blagi stimulirajući učinak (2,79 %). Kod OBVM sorte Istarska bjelica, pri najnižoj koncentraciji (0,2 %) zabilježena je potpuna inhibicija rasta (100 %), dok je pri 0,5% inhibicija iznosila tek 8,76 %. Od koncentracije 2 % na više, zabilježeni su isključivo stimulirajući učinci na oba dana mjerenja. Slično je uočeno i kod OBVM sorte Rosinjola, gdje je pri koncentraciji od 0,2 % zabilježen slab inhibicijski učinak (14,34 %), dok su više koncentracije imale stimulirajući utjecaj. OBVM sorte Leccino je pri koncentracijama od 0,2 do 6 % inhibirala rast za 100 %, dok je pri 10 % imala stimularijuće djelovanje. Vanilinska kiselina pokazala je potpuni inhibicijski učinak pri koncentraciji od 0,5 %, kao i fungicid Nativo 75WG pri primijenjenoj koncentraciji.



Slika 10. Inhibicijski učinak različitih tretmana (OBVM, fenoli i fungicid) pri različitim koncentracijama, drugi dan mjerenja, na *Dothiorella sarmentorum*. Negativne vrijednosti ukazuju na stimulirajući učinak tretmana.

Kod vrste *N. parvum*, svi tretmani s OBVM, uključujući i hidroksitirozol, nisu pokazali nikakav inhibicijski učinak na rast micelija niti drugi (Slika 11) niti sedmi dan mjerenja. Nasuprot tome, vanilinska kiselina je pri koncentraciji od 0,5 % pokazala izrazito snažan antifungalni učinak, inhibirajući rast micelija u potpunosti (100 %) oba dana mjerenja. Nativo 75WG inhibirao je rast *N. parvum* s 100 % učinkovitosti drugog dana te 93,41 % sedmog dana mjerenja.



Slika 11. Inhibicijski učinak različitih tretmana (OBVM, fenoli i fungicid) pri različitim koncentracijama, drugi dan mjerenja, na *Neofusicoccum parvum*. Negativne vrijednosti ukazuju na stimulirajući učinak tretmana.

Iako je koncentracija fenolnih spojeva u OBVM sorte Istarska bjelica bila najviša, a antimikrobna aktivnost OBVM često se povezuje s prisutnošću fenola (Alfano et al., 2011; Muzzalupo et al., 2020), ova OBVM nije pokazala najsnažniju inhibiciju rasta micelija gljiva. Što se tiče hidroksitirozola, koji se često navodi kao fenol s najjačim antimikrobnim djelovanjem, njegova učinkovitost bila je ograničena na vrste *D. mutila* i *D. seriata*. Nije imao učinka na *N. parvum*, dok je na B. *dothidea*, *Do. iberica* i *Do. sarmentorum* imao blago stimulativno djelovanje. Ipak, ovaj stimulativni učinak treba tumačiti s oprezom, s obzirom na osjetljivost i varijabilnost bioloških ispitivanja. Vanilinska kiselina pokazala je daleko bolje rezultate u inhibiciji rasta micelija gljiva.

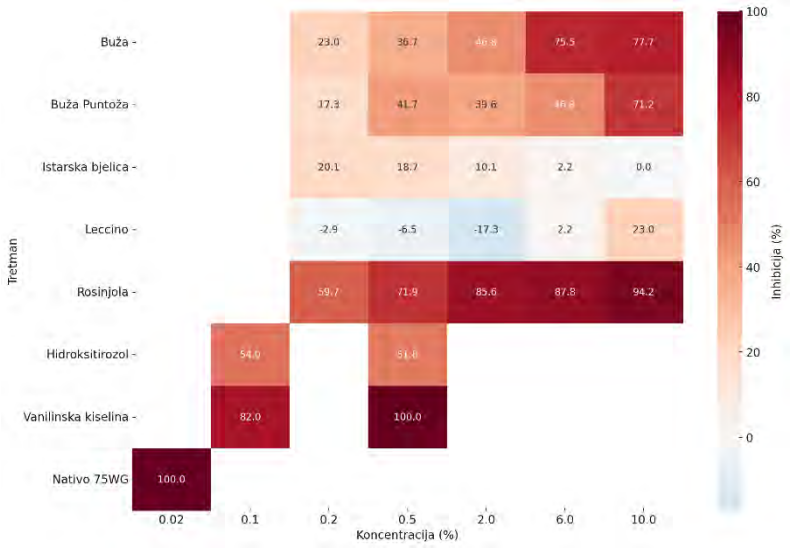
U vezi s vrijednostima MIC i MFC, fungicid je, kako se i očekivalo, pokazao najbolje rezultate, pokazujući fungicidni učinak na sve ispitivane gljive osim na *D. seriata*, kod koje je zabilježen samo fungistatski učinak. Među ostalim tretmanima, MFC vrijednosti su zabilježene samo za određene vrste u tretmanima s OBVM sorti Buža, Leccino i vanilinskom kiselinom.

5.6.3.2. Sordariomycetes

Testirane gljive predstavljaju relativno nove i/ili nove patogene koji pogađaju stabla maslina te za većinu navedenih vrsta nisu provedena ispitivanja fungicida niti evaluacije prirodnih sredstava za zaštitu bilja, unatoč tome što su neke vrste ranije opisane kao patogeni na drugim biljnim vrstama.

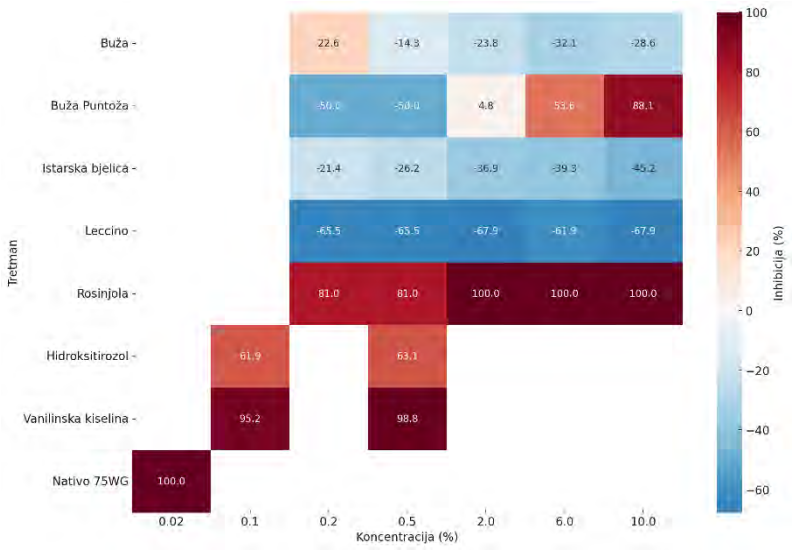
Antifungalna učinkovitost primijenjenih tretmana značajno se razlikovala ovisno o koncentraciji i vrsti gljive, međutim OBVM sorte Rosinjola pokazala je najveću učinkovitost u inhibiciji rasta micelija gljiva, dok je OBVM sorte Leccino pokazala najslabije rezultate. Među ispitivanim gljivama, *N. philosophiae-doctoris* bila je najosjetljivija na sve tretmane, dok je *N. gorlenkoana* pokazala stimulativni odgovor na sve OBVM.

Vezano za vrstu *B. mediterranea*, inhibicijski učinak OBVM sorte Buža pri najnižoj koncentraciji zabilježen je isključivo drugog dana mjerenja (Slika 12). Sličan trend opažen je i kod OBVM sorte Buža puntoža. Suprotno tome, tretmani s OBVM sorte Istarska bjelica pokazali su izraženiji učinak pri nižim koncentracijama, ali isključivo drugi dan, dok je sedmi dana micelij u potpunosti ispunio Petrijevu zdjelicu. Pri najvišoj koncentraciji nije zabilježen inhibicijski učinak. OBVM sorte Leccino pri 0,2 do 2 % pokazala je stimulativni učinak, dok su koncentracije od 6 % i 10 % imale inhibicijski učinak samo drugog dana. Nasuprot tome, OBVM sorte Rosinjola pokazala je inhibicijske učinke oba dana, s inhibicijom koja je rasla s porastom koncentracije. Od dva ispitivana fenola, vanilinska kiselina pokazala je najveću učinkovitost, potpuno inhibirajući rast micelija (100 %) oba dana pri koncentraciji od 0,5 %. Hidroksitirozol je imao sličan učinak pri obje koncentracije (53,95 % i 51,79 %), ali isključivo drugi dan. Fungicid Nativo 75WG bio je drugi najučinkovitiji tretman nakon vanilinske kiseline.



Slika 12. Inhibicijski učinak različitih tretmana (OBVM, fenoli i fungicid) pri različitim koncentracijama, drugi dan mjerenja. na *Biscogniauxia mediterranea*. Negativne vrijednosti ukazuju na stimulirajući učinak tretmana.

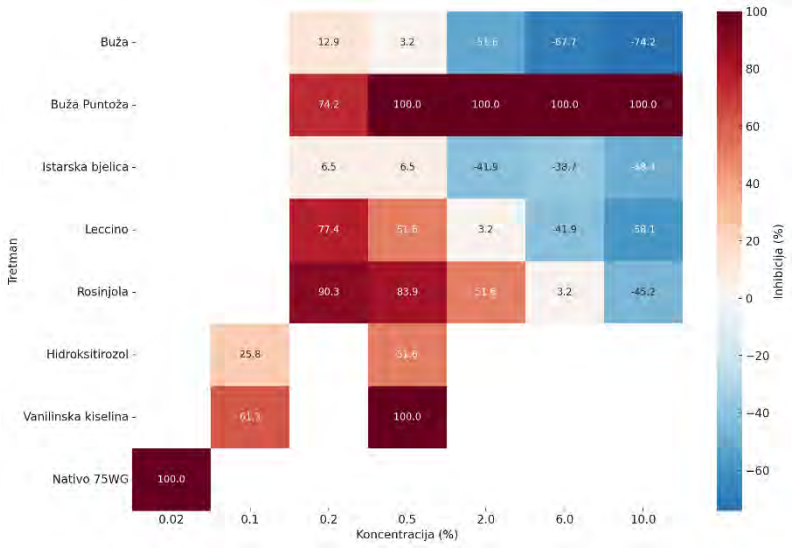
Na vrstu *B. nummularia* OBVM sorte Buža imala je stimulativni učinak (Slika 13) koji je rastao s koncentracijom, iako je pri koncentraciji od 10 % inhibicija bila nešto manja nego pri 6 % (za 3,57 %). OBVM sorte Buža puntoža je pri koncentracijama od 0,2 % i 0,5 % imala stimulativan učinak drugi dan. Koncentracije od 2 % do 10 % pokazale su inhibicijski učinak oba dana. OBVM sorti Istarska bjelica i Leccino imale su stimulativan učinak koji je rastao s koncentracijom, osim kod OBVM sorte Leccino pri 6 %, gdje je zabilježen manji postotak inhibicije u odnosu na niže koncentracije i višu koncentraciju. OBVM sorte Rosinjola pokazala je visok inhibicijski učinak, dosegnuvši 100 % inhibicije pri koncentracijama od 2 do 10 % oba dana, a značajna inhibicija zabilježena je i pri nižim koncentracijama. Kao i kod vrste *B. mediterranea*, vanilinska kiselina bila je učinkovitija od hidroksitirozola, pri čemu je pri koncentraciji od 0,5 % inhibirala rast micelija za 98,81 % i 93,02 % drugi i sedmi dan. Nativo 75WG bio je najučinkovitiji tretman, potpuno inhibirajući rast (100 %) oba dana.



Slika 13. Inhibicijski učinak različitih tretmana (OBVM, fenoli i fungicid) pri različitim koncentracijama, drugi dan mjerenja, na *Biscogniauxia nummularia*. Negativne vrijednosti ukazuju na stimulirajući učinak tretmana.

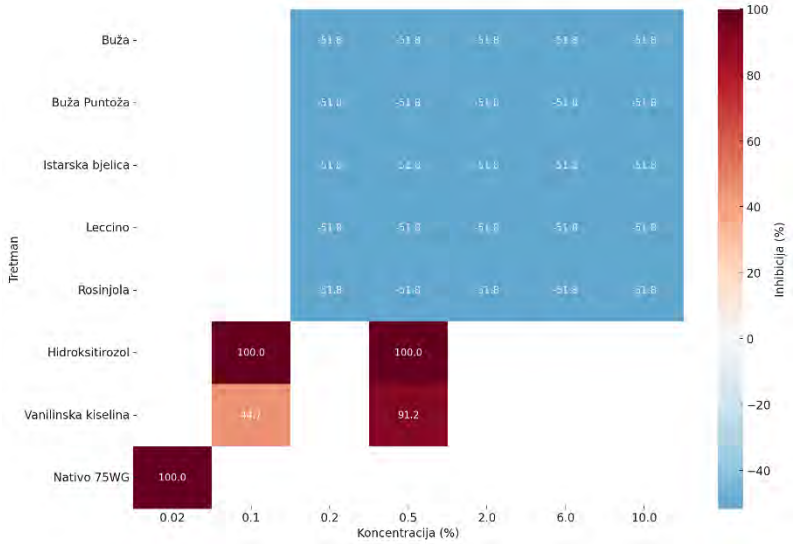
Na vrstu *C. pruinosa* OBVM sorte Buža imala je slab inhibicijski učinak, zabilježen samo drugi dan pri koncentracijama od 0,2 % i 0,5 % (Slika 14), dok je sedmi dan zabilježen stimulativni učinak. Koncentracije iznad 2 % pokazale su stimulirajući učinak oba dana, koji je rastao proporcionalno s koncentracijom. OBVM sorte Buža puntoža imala je snažan inhibicijski učinak, sa 100 % inhibicijom pri koncentracijama iznad 0,5 % drugi dan te pri 6 % i 10 % oba dana. OBVM sorte Istarska bjelica pri 0,2 % i 0,5 % pokazala je slab inhibicijski učinak drugi dan, dok je sedmi dan zabilježen stimulativni učinak. Koncentracije od 2 % do 10 % imale su

stimulativan učinak oba dana. OBVM sorte Leccino pri koncentracijama od 0,2 % do 2 % imale su inhibicijski učinak drugi dan, dok je sedmi dan zabilježen stimulativni učinak. Pri koncentracijama od 6 % i 10 %, zabilježen je stimulativni učinak oba dana. OBVM sorte Rosinjola pri koncentracijama od 0,2 % do 6 % pokazala je opadajući inhibicijski učinak s porastom koncentracije drugog dana, dok je pri 10 % zabilježen stimulativni učinak oba dana. Hidroksitirozol je imao jači učinak pri višim koncentracijama, dok je vanilinska kiselina ponovno bila najučinkovitija, postižući 100 % inhibiciju oba dana pri koncentraciji od 0,5 %, jednako kao i fungicid Nativo 75WG.



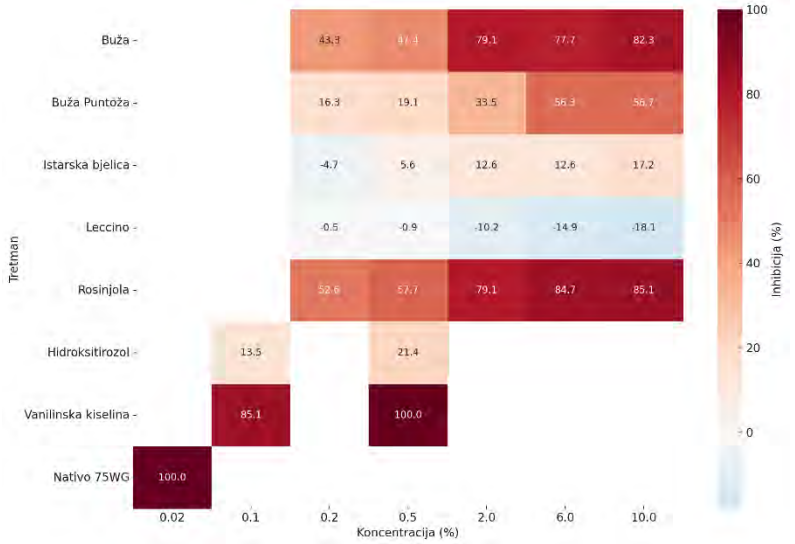
Slika 14. Inhibicijski učinak različitih tretmana (OBVM, fenoli i fungicid) pri različitim koncentracijama, drugi dan mjerenja, na *Cytospora pruinosa*. Negativne vrijednosti ukazuju na stimulirajući učinak tretmana.

Vezano za vrstu *N. gorlenkoana*, svi tretmani OBVM pokazali su ujednačen stimulirajući učinak (Slika 15). Hidroksitirozol je inhibirao rast micelija (100 %) pri obje koncentracije, ali samo drugi dan mjerenja, dok je do sedmog dana micelij u potpunosti ispunio Petrijevu zdjelicu. Vanilinska kiselina pokazala je bolju inhibiciju pri višoj koncentraciji (91,18 % i 83,33 %), dok je Nativo 75WG bio najučinkovitiji, postigavši 100 % inhibicije oba dana.



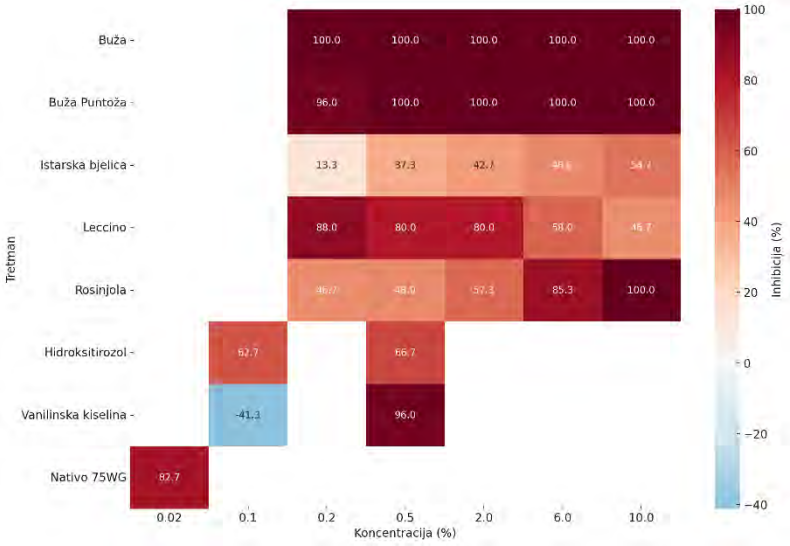
Slika 15. Inhibicijski učinak različitih tretmana (OBVM, fenoli i fungicid) pri različitim koncentracijama, drugi dan mjerenja, na *Nigrospora gorlenkoana*. Negativne vrijednosti ukazuju na stimulirajući učinak tretmana.

Na *N. osmanthi* OBVM sorte Buža pokazala je inhibicijski učinak koji se povećavao s koncentracijom, dosegnuvši 82,33 % i 79,85 % inhibicije pri 10 %. Sličan trend uočen je i kod OBVM sorte Buža puntoža (Slika 16), iako je pri koncentracijama 0,2 % i 0,5 % inhibicija zabilježena samo drugi dan, dok je pri 10% iznosila 56,74 % i 56,20 % drugi, odnosno sedmi dan. OBVM sorte Istarska bjelica pri 0,2 % imala je blagi stimulirajući učinak, dok su više koncentracije pokazale inhibicijski učinak samo drugi dan, s inhibicijom koja se povećavala s koncentracijom. OBVM sorte Leccino pokazala je stimulirajući učinak koji se povećavao s koncentracijom. OBVM sorte Rosinjola pokazala je inhibicijski učinak oba dana, s povećanjem inhibicije pri višim koncentracijama. Hidroksitirozol je imao jači inhibicijski učinak pri višoj koncentraciji, ali samo drugi dan, dok je vanilinska kiselina pri 0,5 % potpuno inhibirala rast (100%) oba dana. Nativo 75WG je inhibirao rast 100% drugi dan te 75,19 % sedmi dan.



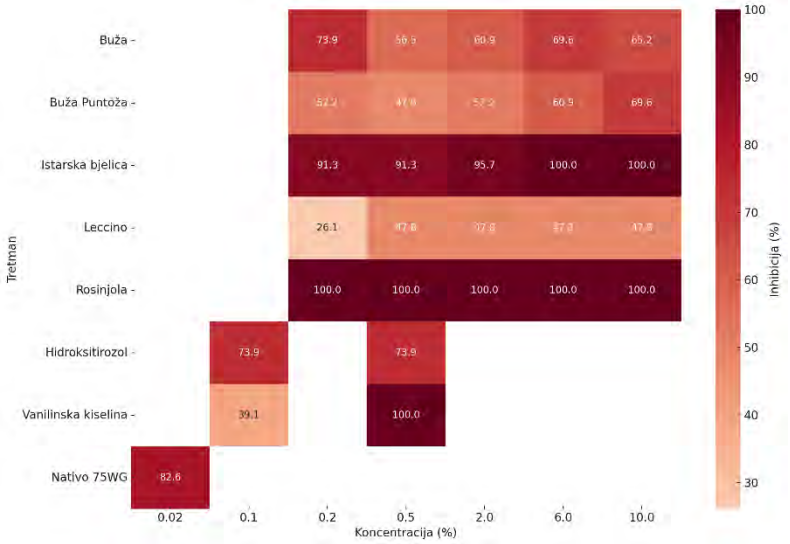
Slika 16. Inhibicijski učinak različitih tretmana (OBVM, fenoli i fungicid) pri različitim koncentracijama, drugi dan mjerenja, na *Nigrospora osmanthi*. Negativne vrijednosti ukazuju na stimulirajući učinak tretmana.

Vezano za vrstu *N. philosophiae-doctoris*, OBVM sorti Buža i Buža puntoža potpuno su inhibirale rast pri svim koncentracijama (Slika 17), osim OBVM sorte Buža puntoža pri 0,2 %, gdje je inhibicija iznosila 96 %. Međutim, do sedmog dana micelij je potpuno ispunio Petrijevu zdjelicu. OBVM sorte Istarska bjelica imala je porast inhibicije s povećanjem koncentracije, dosegnuvši 54,67 % pri 10 %, no do sedmog dana micelij je potpuno izrastao. Kod OBVM sorte Leccino zabilježeno je smanjenje inhibicije s povećanjem koncentracije. OBVM sorte Rosinjola pokazala je jaču inhibiciju s porastom koncentracije, dosegnuvši 100 % pri koncentraciji od 10 %, međutim do sedmog dana micelij je također potpuno izrastao. Hidroksitirozol je pri obje koncentracije drugi dan pokazao sličnu inhibiciju (62,67 % i 66,67 %), dok sedmi dan nije zabilježen inhibicijski učinak. Vanilinska kiselina pri 0,1% imala je stimulirajući učinak, dok je pri 0,5 % pokazala snažnu inhibiciju oba dana. Nativo 75WG inhibirao je rast micelija za 82,67 % drugi dan.



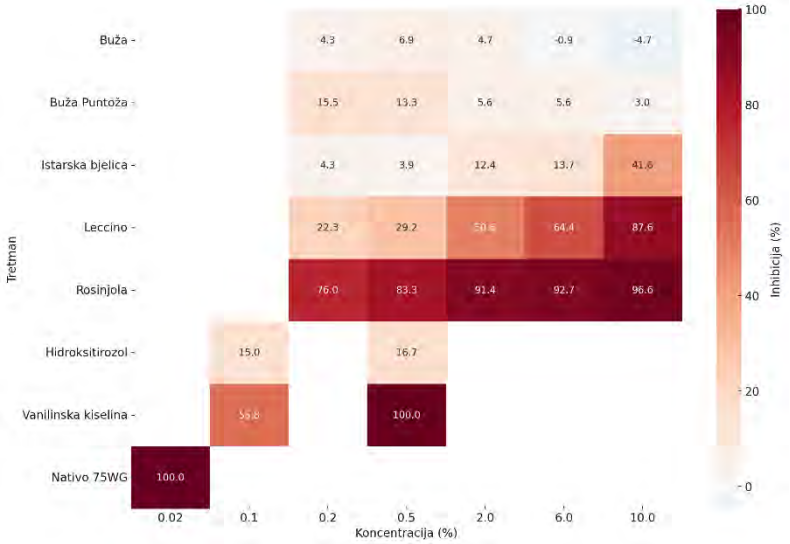
Slika 17. Inhibicijski učinak različitih tretmana (OBVM, fenoli i fungicid) pri različitim koncentracijama, drugi dan mjerenja, na *Nigrospora philosophiae-doctoris*. Negativne vrijednosti ukazuju na stimulirajući učinak tretmana.

Što se tiče vrste *P. iranianum*, OBVM sorte Buža imala je inhibicijski učinak na rast micelija (Slika 18), koji je linearno rastao s povećanjem koncentracije oba dana mjerenja, dosegnuvši 65,21 % i 65,67 % pri najvišoj koncentraciji drugi i sedmi dan. Sličan obrazac uočen je i kod OBVM sorte Buža puntoža, iako je pri 0,5 % zabilježen nešto slabiji učinak nego pri nižim i višim koncentracijama. OBVM sorte Istarska bjelica pokazala je snažan inhibicijski učinak, osobito pri koncentracijama od 6 % i 10 %, gdje je drugi dan postignuta potpuna inhibicija (100 %). Međutim, sedmi dan je inhibicija pri 10 % bila nešto niža nego pri 6 %. OBVM sorte Leccino također je imala inhibicijski učinak koji je ostao konstantan u rasponu od 0,5 % do 10 % drugi dan. OBVM sorte Rosinjola potpuno je inhibirala rast ovog patogena pri svim koncentracijama, oba dana mjerenja. Vanilinska kiselina inhibirala je rast 100 % pri koncentraciji od 0,5 % oba dana. Hidroksitirozol je pokazao isti inhibicijski učinak pri obje koncentracije drugi dan, dok je sedmi dan niža koncentracija bila učinkovitija. Fungicid Nativo 75WG bio je učinkovit, inhibirajući rast micelija za 82,61 % i 77,61 % drugog, odnosno sedmog dana.



Slika 18. Inhibicijski učinak različitih tretmana (OBVM, fenoli i fungicid) pri različitim koncentracijama, drugi dan mjerenja, na *Phaeoacremonium iranianum*. Negativne vrijednosti ukazuju na stimulirajući učinak tretmana.

Vezano za vrstu *S. fimicola*, OBVM sorti Buža, Buža puntoža i Istarska bjelica nisu pokazali inhibicijski učinak sedmi dan. Drugi dan, OBVM sorte Buža je pokazala vrlo slab inhibicijski učinak pri koncentracijama od 0,2 % do 2 %, dok je pri 6 % i 10 % zabilježen blagi stimulirajući učinak (Slika 19). OBVM sorte Buža puntoža imala je najveći inhibicijski učinak (15,45 %) pri najnižoj koncentraciji, koji se smanjivao s povećanjem koncentracije. Nasuprot tome, OBVM sorte Istarska bjelica imala je jači inhibicijski učinak s povećanjem koncentracije, dosegnuvši 41,63 % pri 10 % drugog dana. OBVM sorte Leccino pokazala je porast inhibicije ovisno o koncentraciji, oba dana, dosegnuvši 87,55 % i 86,04 % pri 10 % drugog i sedmog dana. Sličan trend uočen je i kod OBVM sorte Rosinjola, gdje je inhibicija pri 10 % iznosila 96,56 % drugi dan te 69,76 % sedmi dan. Hidroksitirozol je pokazao slabiji učinak na inhibiciju rasta, a sedmi dan nije zabilježen inhibicijski učinak. Vanilinska kiselina pri koncentraciji od 0,5 % i Nativo 75WG postigli su 100 % inhibiciju rasta oba dana mjerenja.



Slika 19. Inhibicijski učinak različitih tretmana (OBVM, fenoli i fungicid) pri različitim koncentracijama na *Sordaria fimicola*. Negativne vrijednosti ukazuju na stimulirajući učinak tretmana.

Što se tiče MIC i MFC vrijednosti, minimalna fungicidna koncentracija (MFC) određena je samo za tretmane s OBVM sorte Buža puntoža za vrste *C. pruinosa*, OBVM sorte Rosinjola na *P. iranianum* te za tretmane vanilinskom kiselinom na *B. mediterranea* i *S. fimicola*. Za fungicide su MFC vrijednosti utvrđene za njihov učinak na *B. nummularia*, *C. pruinosa*, *N. gorlenkoana* i *S. fimicola*. MIC vrijednost nije određena za OBVM sorte Buža na *N. gorlenkoana*, OBVM sorte Buža puntoža na *N. gorlenkoana*, OBVM sorte Istarska bjelica na *B. nummularia* i *N. gorlenkoana*, OBVM sorte Leccino na *B. nummularia*, *N. gorlenkoana* i *N. osmanthi*, kao ni za OBVM sorte Rosinjola na *N. gorlenkoana* i *P. iranianum*. Najčešće utvrđene MIC vrijednosti kretale su se između 0,1 % i 0,2 %.

5.6.4. Antagonistički testovi

Popis svih izoliranih vrsta mikroorganizama iz OBVM prikazan je u Tablici 5. Ukupno je izoliran jedan izolat bakterije, tri izolata kvasca i tri izolata plijesni. Bakterija je pronađena isključivo u OBVM sorte Buža. Iz OBVM sorti Istarska bjelica i Leccino izolirana je samo vrsta *Penicillium crustosum* Thom. Sekvence ITS regije izolata pohranjene su u GenBank bazi podataka Nacionalnog centra za biotehnološke informacije (NCBI, Maryland, SAD) pod pristupnim brojevima PV092539 za izolat R_BB, PQ826427 za izolat BJ_P, PQ826435 za izolat L_P i PQ826436 za izolat BP_P.

Tablica 5. Popis izoliranih mikroorganizama iz OBVM, s oznakom izolata.

OBVM	**Oznaka izolata**	**Izolirani mikroorganizam**
Buža	B_RB	*Rhodotorula mucilaginosa* (A. Jörg.) F.C. Harrison
	B_BB	*Bacillus velezensis* Ruiz-Garcia et al.
Buža puntoža	BP_P	*P. crustosum*
Istarska bjelica	BJ_P	*P. crustosum*
Leccino	L_P	*P. crustosum*
Rosinjola	R_RB	*R. mucilaginosa*
	R_BB	*Nakazawaea molendiniolei* (N. Cadez, B. Turchetti & G. Peter) C. P. Kurtzman & C. J. Robnett

P. crustosum je vrsta koja se često povezuje s kontaminacijom hrane, uzrokujući kvarenje različitih prehrambenih proizvoda. Ova je vrsta ranije zabilježena na maslinama i njihovim nusproizvodima u Španjolskoj (Baffi i sur., 2012). Pokazala je značajan potencijal za buduće industrijske primjene zahvaljujući izraženim enzimskim aktivnostima (Baffi i sur., 2012).

B. velezensis je aerobna, Gram-pozitivna bakterija sposobna za formiranje endospora i poticanje rasta biljaka. Različiti sojevi ove vrste dokumentirani su zbog svoje sposobnosti inhibicije rasta patogena, uključujući gljive, bakterije i nematode (Rabbee i sur., 2019). Soj *B. velezensis* OEE1, izoliran iz endogenog korijenskog tkiva maslina, pokazao je antifungalnu aktivnost *in vitro* protiv *Verticillium dahliae* Kleb., s inhibicijskom stopom većom od 92 %. *In vivo* ispitivanja pokazala su da *B. velezensis* OEE1 značajno smanjuje indeks težine bolesti, postotak uvenulih biljaka te gustoću mikrosklerocija u prirodno zaraženom tlu (Cheffi Azabou i sur., 2020).

R. mucilaginosa je biotehnološki značajan kvasac koji je privukao veliku pažnju zbog svoje sposobnosti korištenja širokog spektra supstrata, iznimne otpornosti na stres i drugih korisnih svojstava. *R. mucilaginosa* smatra se vrlo pogodnim kandidatom za proizvodnju karotenoida, lipida, enzima i drugih vrijednih bioproizvoda, osobito putem biorafiniranja jeftinih poljoprivrednih otpadnih materijala (Li i sur., 2022). Ghilardi i sur. (2020) pokazali su da se supstrati dobiveni iz otpadnih voda maslinarstva mogu učinkovito koristiti za proizvodnju karotenoida pomoću *R. mucilaginosa*. Zanimljivo je da su Jarboui i sur. (2012., 2013.) uočili

da *R. mucilaginosa* CH4 može igrati značajnu ulogu u pročišćavanju OBVM uklanjanjem polifenolnih spojeva, uključujući katehol, galnu kiselinu, p-kumarinsku kiselinu, protokatehuičnu kiselinu, tiroziol, vanilinsku kiselinu i druge.

N. molendiniolei (sin. *Nakazawaea molendini-olei* ili *Candida molendinolei*) prepoznata je po otpornosti na fenolne spojeve te sposobnosti pretvorbe oleuropeina u hidroksitirozol (Ghomari i sur., 2020). Također se koristi kao starter kultura za kontroliranu fermentaciju maslina (Ciafardini i Zullo, 2019). Nadalje, *N. molendiniolei* pokazuje značajne enzimske aktivnosti, poput β-glukozidaze i peroksidaze. Ove aktivnosti doprinose ograničavanju povećanja kiselosti maslinovog ulja tijekom skladištenja, no istovremeno su povezane s povećanjem oksidacijskih parametara, što s vremenom dovodi do smanjenja kvalitete maslinovog ulja (Giavalisco i sur., 2023).

5.6.4.1. Botryosphaeriaceae

Najveći antagonistički učinak protiv fitopatogenih gljiva iz porodice Botryosphaeriaceae zabilježen je kod vrste *Do. iberica*, gdje je pet od osam testiranih izolata antagonista pokazalo snažan antagonistički učinak. Najveći antagonistički učinak na *B. dothidea* pokazao je izolat *P. crustosum* iz OBVM sorte Istarska bjelica (BJ_P) (70,54 %), dok je *P. crustosum* iz OBVM sorte Buža puntoža (BP_P) također bio učinkovit (67,86 %) (Slika 20). Suprotno tome, *N. molendiniolei* (R_BB) pokazao je najslabiji učinak (11,76 %).

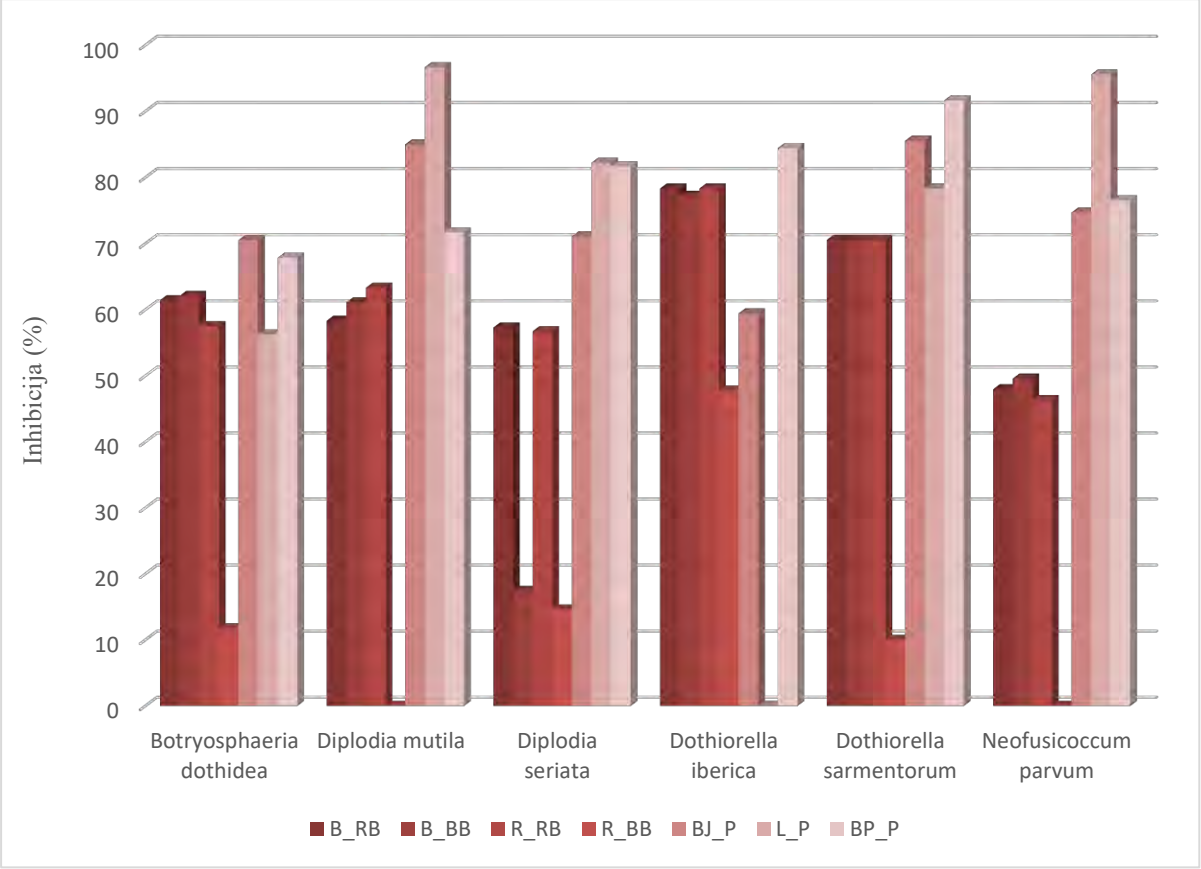
Za *D. mutila*, najveći postotak inhibicije zabilježen je kod izolata *P. crustosum* iz OBVM sorte Leccino (L_P) (96,67 %), a *P. crustosum* iz OBVM sorte Istarska bjelica (BJ_P) također je pokazao značajnu učinkovitost (85 %). Protiv *D. seriata*, *P. crustosum* iz OBVM sorti Leccino (L_P) (82,22 %) i Buža puntoža (BP_P) (81,67 %) pokazali su najveću inhibiciju. Nasuprot tome, *B. velezensis* (B_BB) i *N. molendiniolei* (R_BB) iz OBVM sorte Rosinjola bio je znatno manje učinkovit.

Najbolji učinak na *Do. iberica* imao je *P. crustosum* iz OBVM sorte Buža puntoža (BP_P) (84,44 %), dok je *P. crustosum* iz OBVM Istarska bjelica (BJ_P) (59,44 %) pokazao umjerenu učinkovitost. *P. crustosum* iz OBVM sorti Buža puntoža (BP_P) (91,67 %) i Istarska bjelica (BJ_P) (85,56 %) pokazali su izrazito visoku inhibiciju za vrstu *Do. sarmentorum*, dok je *N. molendiniolei* (R_BB) imao vrlo slab učinak (10 %).

P. crustosum iz OBVM sorte Leccino (L_P) gotovo je potpuno inhibirao rast *N. parvum* (95,57 %), dok su izolati iz OBVM sorti Istarska bjelica (BJ_P) (74,69 %) i Buža puntoža (BP_P) (76,54 %) također bili učinkoviti.

Među testiranim izolatima *Penicilliuma*, L_P se pokazao kao najučinkovitiji antagonistički

organizam, posebno protiv *D. mutila* i *N. parvum*. Najslabiji antagonistički učinak zabilježen je kod izolata R_BB, *N. molendiniolei*, jer nije zabilježena nikakva antagonistička interakcija između tog izolata i *D. mutila* ili *N. parvum*. Također, nije zabilježen antagonistički učinak između izolata L_P i *Do. iberica*. Gharsallah i sur. (2020) izolirali su *P. crustosum* iz kukaca prikupljenih u maslinicima. Autori su dokazali patogenost *P. crustosum* pomoću testova na izrezanim izdancima, gdje je izolacija *P. crustosum* F14 izazvala posmeđivanje kore. Uz to, u studiji su dokumentirane i antagonističke interakcije između ovog izolata i gljivičnih vrsta kao što su *Aspergillus calidoustus* Varga, Houbraken & Samson, *Penicillium chrysogenum* Thom i *Alternaria consortialis* (Thm.) J.W. Groves & S. Hughes. Suprotno tome, kod izolata *P. crustosum* F33, koji je također prikupljen iz kukaca u maslinicima, nisu opaženi antagonistički učinci. Ovo istraživanje također je potvrdilo postojanje razlika u antagonističkom utjecaju između izolata *Penicillium* sp., kao i varijacije u antagonističkim utjecajima istog izolata na različite gljivične vrste. Najjači antagonistički učinak zabilježen je između izolata L_P i vrsta *N. parvum* i *D. mutila*, dok je najslabija interakcija zabilježena između L_P i *Do. iberica*, gdje nije zabilježen antagonistički učinak izolata na patogena.



Slika 20. Antagonistička aktivnost mikroorganizama izoliranih iz OBVM protiv fitopatogenih gljiva. Graf prikazuje postotak inhibicije rasta micelija gljiva uzrokovan djelovanjem mikroorganizama izoliranih iz OBVM. Pojedini izolati testirani su protiv šest fitopatogenih

gljiva: *Botryosphaeria dothidea*, *Diplodia mutila*, *Diplodia seriata*, *Dothiorella iberica*, *Dothiorella sarmentorum* i *Neofusicoccum parvum*. Viši postoci inhibicije ukazuju na jači antagonistički učinak. Oznake predstavljaju: B_RB i R_RB – izolati *Rhodotorula mucilaginosa* izolirani iz, redom, OBVM sorti Buža i Rosinjola, B_BB – izolat *Bacillus velezensis* izolirana iz OBVM sorte Buža, BP_P, BJ_P i L_P – izolati *Penicillium crustosum* izolirani iz, redom, OBVM sorti Buža puntoža, Istarska bjelica i Leccino, R_BB – izolat *Nakazawaea molendiniolei* izoliran iz OBVM sorte Rosinjola.

5.6.4.2. Sordariomycetes

Uočene su značajne varijacije u antagonističkim učincima testiranih izolata na fitopatogene gljive iz roda Sordariomycetes. *B. mediterranea* pokazala se kao najotpornija vrsta na tretmane antagonistima. Ova vrsta poznata je kao agresivni patogen na specifičnim biljnim domaćinima te je široko prepoznata kao uzročnik bolesti raka na šumskim stablima (Patejuk i sur., 2023). *R. mucilaginosa* izolat iz OBVM sorte Buža (B_RB) pokazao je jači antagonistički učinak u usporedbi s onim izoliranim iz OBVM sorte Rosinjola. U slučaju *P. crustosum*, zabilježene su znatne razlike u antagonističkoj aktivnosti među izolatima ove vrste. Ni bakterija ni kvasac nisu pokazali inhibicijski učinak na rast *B. mediterranea*. Međutim, *P. crustosum* pokazao je visok postotak inhibicije rasta micelija, pri čemu je najučinkovitiji bio izolat iz OBVM sorte Istarska bjelica (BJ_P) (Slika 21), s postotkom inhibicije od 82,78 %.

Izolat *P. crustosum* iz OBVM sorte Istarska bjelica (BJ_P) imao je stimulirajući učinak na rast *B. nummularia*. Najviši stupanj inhibicije postignut je izolatom iz OBVM sorte Buža puntoža (BP_P) s 81,94 %, a slijedi izolat BB_B s 80,56 % te su svi ostali antagonisti pokazali inhibiciju veću od 70 %.

Izolat *P. crustosum* (L_P) iz OBVM sorte Leccino imao je stimulirajući učinak na rast *C. pruinosa*. Najjači antagonist bio je *P. crustosum* (BP_P) iz OBVM sorte Buža puntoža, s 81,99 % inhibicije, dok je najslabiji bio *R. mucilaginosa* iz OBVM sorte Rosinjola (R_RB), s inhibicijom od samo 7,78 %.

Izolat *P. crustosum* iz OBVM sorte Buža puntoža (BP_P) nije imao inhibicijski učinak na rast micelija *N. gorlenkoana*. Najjači antagonistički učinak pokazao je izolat iz OBVM sorte Istarska bjelica (BJ_P), s 82,2 % inhibicije, a slijedi bakterija *B. velezensis* (B_BB) s 64,44 % inhibicije.

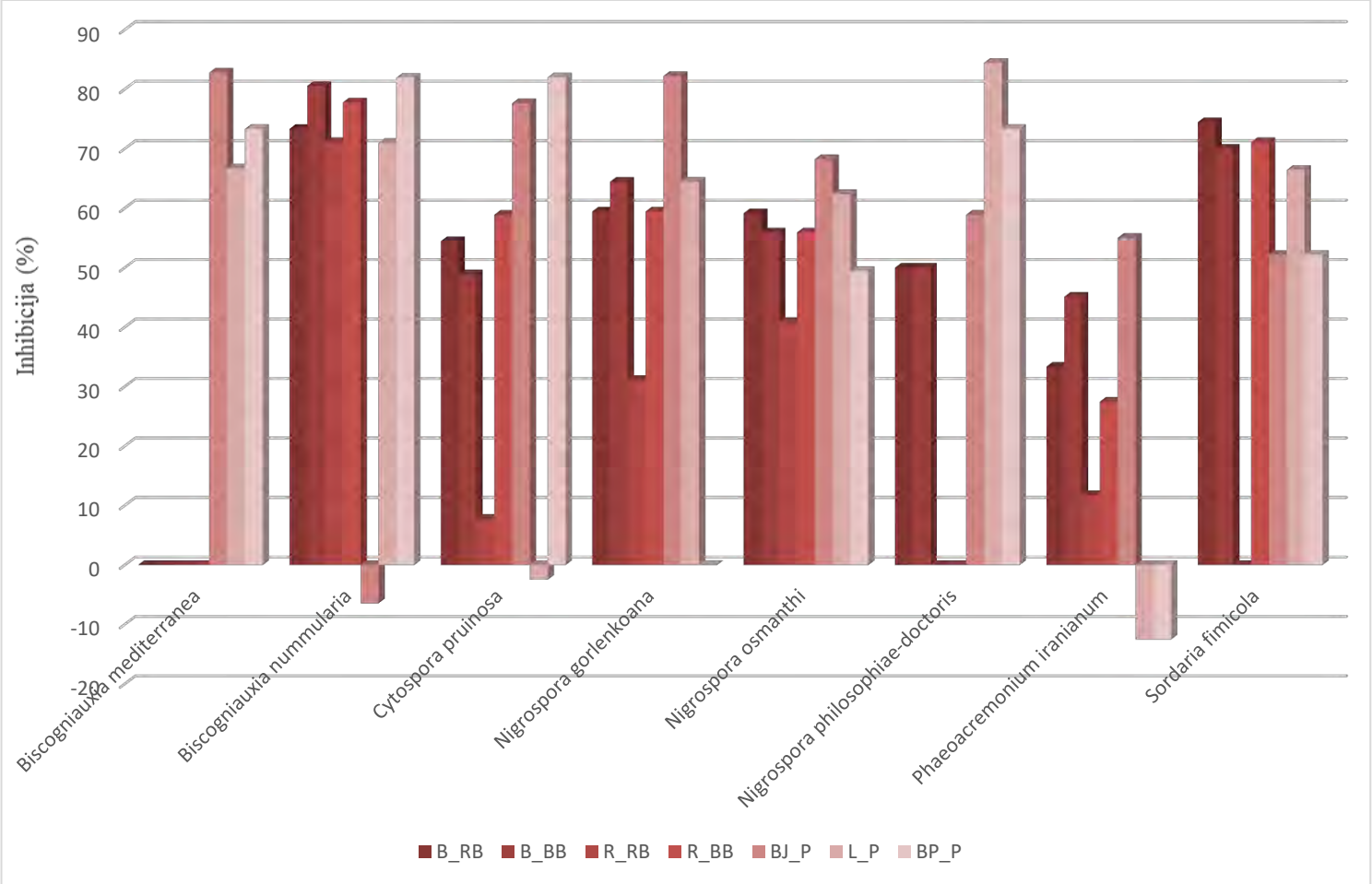
Antagonistički učinci testiranih mikroorganizama na *N. osmanthi* kretali su se između 40 i 70%. Najučinkovitiji je bio izolat *P. crustosum* iz OBVM sorte Istarska bjelica (BJ_P), s 68,24 % inhibicije, dok je najslabiji bio *R. mucilaginosa* iz OBVM sorte Rosinjole (R_RB), s 40,86 %

inhibicije.

Ni *R. mucilaginosa* (R_RB) ni *N. molendiniolei* (R_BB) iz OBVM sorte Rosinjola nisu imali inhibicijski učinak na *N. philosophiae-doctoris*. Najučinkovitiji antagonist bio je *P. crustosum* iz OBVM sorte Leccino (L_P), s 84,44 % inhibicije, a slijedi *P. crustosum* iz OBVM sorte Buža puntoža sa 73,33 % inhibicije.

Izolati *P. crustosum* iz OBVM sorti Buža puntoža (BP_P) i Leccino (L_P) stimulirali su rast micelija *P. iranianum*. Suprotno tome, *P. crustosum* iz OBVM sorte Istarska bjelica (BJ_P) pokazao je najjači antagonistički učinak među svim testiranim izolatima, s inhibicijom od 55 %, dok je najslabiji antagonist bio *R. mucilaginosa* iz OBVM sorte Rosinjola (R_RB), s inhibicijom od samo 11,76 %.

Izolat *R. mucilaginosa* iz OBVM sorte Rosinjola (R_RB) nije imao učinka na inhibiciju rasta micelija *S. fimicola*. Suprotno tome, najučinkovitiji je bio izolat *R. mucilaginosa* iz OBVM sorte Buža (B_RB), s 74,44 % inhibicije, a slijedi *N. molendiniolei* (R_BB) sa 71,11 % inhibicije.



Slika 21. Antagonistička aktivnost mikroorganizama izoliranih iz OBVM protiv fitopatogenih gljiva. Graf prikazuje postotak inhibicije rasta micelija gljiva uzrokovan djelovanjem

mikroorganizama izoliranih iz OBVM. Pojedini izolati testirani su protiv osam vrsta iz razreda Sordariomycetes. Viši postoci inhibicije ukazuju na jači antagonistički učinak. Negativne vrijednosti ukazuju na stimulacijski utjecaj. Oznake predstavljaju: B_RB i R_RB – izolati *Rhodotorula mucilaginosa* izolirani iz, redom, OBVM sorti Buža i Rosinjola, B_BB – izolat *Bacillus velezensis* izoliran iz OBVM sorte Buža, BP_P, BJ_P i L_P – izolati *Penicillium crustosum* izolirani iz, redom, OBVM sorti Buža puntoža, Istarska bjelica i Leccino, R_BB – izolat *Nakazawaea molendiniolei* izoliran iz OBVM sorte Rosinjola.

6. ZAKLJUČCI

- Na maslini su zabilježene nove vrste gljiva koje do sada nisu utvrđene u Hrvatskoj i/ ili svijetu. Prisutnost vrsta *D. mutila*, *Do. iberica*, *Do. sarmentorum, B. mediterranea* i *C. pruinosa* na stablima masline i kao uzročnika bolesti masline u Hrvatskoj do sada nije bila dokumentirana, što ovo istraživanje čini prvim izvješćem o njihovoj pojavi. Nadalje, po prvi put u svijetu utvrđeno je da su *B. nummularia*, *S. fimicola, P. iranianum*, *N. gorlenkoana*, *N. osmanthi* i *N. philosophiae-doctoris* uzročnici bolesti maslina. Također, vrsta *N. philosophiae-doctoris* prvi put je prepoznata kao uzročnik bolesti biljaka.
- Utvrđene su značajne razlike u patogenosti među ispitivanim vrstama gljiva. Dok su neke gljive uzrokovale brzi razvoj simptoma na biljnim tkivima, druge su pokazale slabu ili sporiju patogenost. Ovi rezultati ukazuju da nije svaka gljiva izolirana s masline podjednako opasna te da pojedine vrste mogu djelovati oportunistički ili sekundarno nakon primarnih oštećenja biljke.
- Rezultati pokazuju da postoji značajna varijabilnost u antifungalnom djelovanju prirodnih pripravaka. Neka EtU, primjerice EtU kineskog cimeta i origana te njihovi glavne komponente pokazali su snažno inhibicijsko djelovanje na rast micelija svih testiranih gljiva. S druge strane, pojedina EtU i OBVM imala su slabiji ili selektivan učinak, djelujući samo na određene vrste. Fenol hidroksitirosol imao je nešto slabiji učinak u odnosu na pojedina EtU, OBVM i vanilinsku kiselinu. Ovi rezultati potvrđuju da izbor sredstva ima ključnu ulogu u učinkovitosti biološke kontrole fitopatogenih gljiva.
- U usporednim testovima, određena EtU i komponente, kao i OBVM i fenoli, pokazali su inhibicijsko djelovanje na rast gljiva koje je bilo usporedivo s komercijalnim fungicidima. Posebno su se istaknuli kineski cimet i origano i njihove glavne komponente, koji su, u pojedinim slučajevima, nadmašili učinkovitost kemijskog fungicida. Takvi rezultati otvaraju mogućnost razvoja prirodnih pripravaka kao alternativnih ili dopunskih sredstava u održivoj zaštiti bilja.
- Testiranja otpornosti različitih sorata masline pokazala su razlike u osjetljivosti prema patogenim gljivama. Na temelju provedenih istraživanja može se zaključiti da su vrste *N. parvum* i *D. mutila* pokazale najviši stupanj patogenosti, s prosječnom dužinom lezija većom od 30 mm, dok su vrste iz roda *Dothiorella* pokazale znatno slabiju

patogenost; pritom se sorta Istarska bjelica istaknula kao najosjetljivija na zarazu vrstom *N. parvum*. Ovi nalazi imaju važnu praktičnu vrijednost jer omogućuju odabir otpornijih sorata za sadnju u područjima s visokim rizikom od bolesti.

7. LITERATURA

1. Alfano, G., Lustrato, G., Lima, G., Vitullo, D., Ranalli, G (2011.): Characterization of composted olive mill wastes to predict potential plant disease suppressiveness. Biological Control, 58, 199–207.
2. Alves, A., Correia, A., Phillips, A.J.L. (2006): Multi-gene genealogies and morphological data support *Diplodia cupressi* sp. nov., previouly recognized as *D. pinea* f. sp. *cupressi*, as a distinct species. Fungal Diversity, 23, 1–15.
3. Ammad, F., Moumen, O., Gasem, A., Othmane, S., Hisashi, K.-N., Zebib, B., Merah, O. (2018.): The potency of lemon (*Citrus limon* L.) essential oil to control some fungal diseases of grapevine wood. Comptes Rendus Biologies, 341, 97–101.
4. Baffi, M.A., Romo-Sánchez, S., Úbeda-Iranzo, J., Briones-Pérez, A.I (2012.): Fungi isolated from olive ecosystems and screening of their potential biotechnological use. New Biotechnology, 29, 451–456.
5. Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M (2008.): Biological effects of essential oils—A review. Food and Chemical Toxicology, 46 (2), 446–475.
6. Barber, A.E., Riedel, J., Sae-Ong, T., Kang, L., Brabetz, W., Panagiotou, G., Deising, H.B., Kurzai, O (2020.): Efects of agricultural fungicide use on *Aspergillus fumigatus* abundance, antifungal susceptibility, and population structure. mBio, 11 (6): e02213–20.
7. Bažok, R (2020.): Je li održiva uporaba pesticida doista održiva? Glasilo biljne zaštite, 20 (3), 384-389.
8. Bertoša, M. (2005): Istarska Enciklopedija. Leksikografski zavod Miroslav Krleža, Zagreb, Hrvatska.
9. Bouhia, Y., Hafidi, M., Ouhdouch, Y., El Boukhari, M.E., El Fels, L., Zeroual, Y., Lyamlouli, K (2022.): Microbial community succession and organic pollutants removal during olive mill waste sludge and green waste co-composting. Frontiers in Microbiology, 12, 814553.
10. Bowles, E.J. (2012.): Eterična Ulja. Veble Commerce, Zagreb, Hrvatska.
11. Brenes, M., García, A., García, P., Garrido, A (2001.): Acid hydrolysis of secoiridoid aglycons during storage of virgin olive oil. Journal of Agricultural and Food Chemistry, 49 (11), 5609–5614.
12. Buchenauer, H. (1979.): Comparative studies on the antifungal activity of triadimefon, triadimenol, fenarimol, nuarimol, imazalil and fluotrimazole in vitro. Journal of Plant Disease and Protection, 86, 341–354.
13. Burt, S.A., Reinders, R.D (2003.): Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. Letters in Applied Microbiology, 36, 162–167.
14. Carbone, I., Kohn, L.M. (1995.): A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia, 91, 553–556.
15. Carlucci, A., Raimondo, M.L., Cibelli, F., Phillips, A.J.L., Lops, F. (2013.): *Pleurostomophora richardsiae*, *Neofusicoccum parvum*, and *Phaeoacremonium aleophilum* associated with a decline of olives in southern Italy. Phytopathologia Mediterranea, 52, 517–527.
16. Case, N.T., Berman, J., Blehert, D.S., Cramer, R.A., Cuomo, C., Currie, C.R., Ene, I.V., Fisher, M.C., Fritz-Laylin, L.K., Gerstein, A.C., Glass, N.L., Gow, N.A.R., Gurr, S.J., Hittinger, C.T., Hohl, T.M., Iliev, I.D., James, T.Y., Jin, H., Klein, B.S., Kronstad, J.W., Lorch, J.M., McGovern, V., Mitchell, A.P., Segre, J.A., Shapiro, R.S., Sheppard, D.C., Sil, A., Stajich, J.E., Stukenbrock, E.E., Taylor, J.W., Thompson, D., Wright, G.D., Heitman, J., Cowen, L.E (2022.): The future of fungi: threats and opportunities. G3 (Bethesda), 12 (11), jkac224.
17. Cheffi Azabou, M., Gharbi, Y., Medhioub, I., Ennouri, K., Barham, H., Tounsi, S., Triki, M.A (2020.): The endophytic strain *Bacillus velezensis* OEE1: An efficient biocontrol agent against Verticillium wilt of olive and a potential plant growth-promoting bacteria. Biological Control, 142, 104168.
18. Ciafardini, G., Zullo, B.A (2019.): Use of selected yeast starter cultures in industrial-scale processing of brined Taggiasca black table olives. Food Microbiology, 84, 103250.
19. Cibelli, F., Bevilacqua, A., Raimondo, M.L., Campaniello, D., Carlucci, A., Ciccarone, C., Sinigaglia, M., Corbo, M.R. (2017.): Evaluation of fungal growth on olive-mill wastewaters treated at high temperature and by high-pressure homogenization. Frontiers in Microbiology, 8, 2515.

20. DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F (1956.): Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28 (3), 350–356.
21. Edris, A.E., Farrag, E.S. (2003.): Antifungal activity of peppermint and sweet basil essential oils and their major aroma constituents on some plant pathogenic fungi from the vapor phase. Food/Nahrung, 47, 117–121.
22. European Commission (2025.): Recovery, recycling, resource. Valorisation of olive mill effluents by recovering high added value bio-products. Life Public Database. Dostupno na: LIFE 3.0 - LIFE07 ENV/IT/ (0004.), 21. (pristupljeno: 21 ožujak 2025.).
23. Fan X.L., Bezerra J.D.P., Tian C.M., Crous P.W. (2020.): *Cytospora* (Diaporthales) in China. Persoonia, 45, 1–45.
24. FAO, Food and Agriculture Organization of United Nations (2025.): Crop and Livestock Products. FAOSTAT. Dostupno na: FAOSTAT (pristupljeno: 29 svibanj 2025.).
25. Felsenstein, J (1985.): Confidence limits on phylogenies: an approach using the bootstrap. Evolution, 39 (4), 783–791.
26. Fisher, P.J., Petrini, O., Petrini, L.E., Descals, E. (1992.): A preliminary study of fungi inhabiting xylem and whole stems of *Olea europaea*. Sydowia, 44, 117–121.
27. Fokkema, N.J. (1978.): Fungal antagonism in the phyllosphere. Annals in Applied Biology, 89, 115–117.
28. Gharbi, Y., Ennouri, K., Bouazizi, E., Cheffi, M., Ali Triki, M. (2020.): First report of charcoal disease caused by *Biscogniauxia mediterranea* on *Olea europaea* in Tunisia. Journal of Plant Pathology, 102, 961.
29. Gharsallah, H., Ksentini, I., Naayma, S., Taieb, K.H., Abdelhedi, N., Schuster, C., Triki, M.A., Ksantini, M., Leclerque, A (2020.): Identification of fungi in Tunisian olive orchards: Characterization and biological control potential. BMC Microbiology, 20, 307.
30. Ghilardi, C., Negrete, P.S., Gutierrez, G.R., Monetta, P., Arroyo-Lopez, F.N., Hornero-Mendez, D., Carelli, A.A., Borroni, V (2022.): Influence of olive mill waste phenolic compounds levels on carotenoid production by *Rhodotorula* spp. Process Biochemistry, 120, 275–286.
31. Ghilardi, C., Sanmartin Negrete, P., Carelli, A.A., Borroni, V (2020.): Evaluation of olive mill waste as substrate for carotenoid production by *Rhodotorula mucilaginosa*. Bioresources and Bioprocessing, 7, 52.
32. Ghomari, O., Merzouki, M., Benlemlih, M (2020.): Optimization of bioconversion of oleuropein, of olive leaf extract, to hydroxytyrosol by *Nakazawaea molendini-olei* using HPLC-UV and a method of experimental design. Journal of Microbiological Methods, 176, 106010.
33. Giavalisco, M., Zotta, T., Parente, E., Siesto, G., Capece, A., Ricciardi, A (2023.): Effect of oil-borne yeasts on the quality of extra-virgin olive oils of Basilicata region. International Journal of Food Microbiology, 386, 110041.
34. Glass, N.L., Donaldson, G.C. (1995.): Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology, 61, 1323–1330.
35. Hao, Y., Aluthmuhandiram, J.V.S., Chethana, K.W.T., Manawasinghe, I.S., Li, X., Liu, M., Hyde, D.K., Phillips, A.J.L., Zhang, W. (2020.): *Nigrospora* species associated with various hosts from Shandong peninsula, China. Mycobiology, 48, 169–183.
36. Havranek, J., Tudor Kalit, M., Bažok, R., Đugum, J., Grbeša, D., Hadžiosmanović, M., Ivanković, A., Jakopović, I., Orešković, S., Rupić, V., Samaržija, D (2014.): Sigurnost hrane od polja do stola. M.E.P. d.o.o., Hrvatska.
37. Hawksworth, D.L., Lücking, R (2017.): Fungal diversity revisited: 2.2 to 3.8 million species. Microbiology Spectrum, 5 (4).
38. Hernández-Rodríguez, L., Mondino-Hintz, P., Alaniz-Ferro, S (2022.): Diversity of *Botryosphaeriaceae* species causing stem canker and fruit rot in olive trees in Uruguay. Journal of Phytopathology, 170 (4), 264-277.
39. Hyde, K.D., Baldrian, P., Chen, Y., Chethana, K.W.T., De Hoog, S., Doilom, M., Gomes de Farias, A.R., Gonçalves, M.F.M., Gonhkom, D., Gui, H., Hilário, S., Hu, Y., Jayawardena, R.S., Khyaju, S., Kirk, P.M., Kohout, P., Luangharn, T., Maharachchikumbura, S.S.N., Manawasinghe, I.S., Mortimer, P.E., Niego, A.G.T., Phonemany, M., Sandargo, B., Senanayake, I.C., Stadler, M., Surup, F., Thongklang, N., Wanasinghe, D.N., Bahkali, A.H., Walkter, A (2024.): Current trends, limitations and future research in the fungi? Fungal Diversity, 125, 1–71.
40. Hyldgaard M., Mygind T., Meyer R.L (2012.): Essential oils in food preservation: mode of action, synergies and interactions with food matrix components. Frontiers in Microbiology, 3 (12), 1–24.

41. Idbella, M., Baronti, S., Vaccari, F.P., Abd-ElGawad, A.M., Bonanomi, G. (2024.): Long-Term Application of Biochar Mitigates Negative Plant–Soil Feedback by Shaping Arbuscular Mycorrhizal Fungi and Fungal Pathogens. Microorganisms, 12, 810.
42. Jarboui, R., Baati, H., Fetoui, F., Gargouri, A., Gharsallah, N., Ammar, E (2012.): Yeast performance in wastewater treatment: Case study of *Rhodotorula mucilaginosa*. Environmental Technology, 33, 951–960.
43. Jarboui, R., Magdich, S., Ayadi, R.J., Gargouri, A., Gharsallah, N., Ammar, E (2013.): *Aspergillus niger* P6 and *Rhodotorula mucilaginosa* CH4 used for olive mill wastewater (OMW) biological treatment in single pure and successive cultures. Environmental Technology, 34, 629–636.
44. Kaliterna, J (2013.): Identifikacija, patogenost i rasprostranjenost vrsta gljiva iz porodica Botryosphaeriaceae i Diaporthaceae na vinovoj lozi u Hrvatskoj. Doktorski rad. Sveučilište u Zagrebu, Agronomski fakultet, Hrvatska.
45. Kaliterna, J., Miličević, T., Ivić, D., Benčić, D., Mešić, A. (2012.): First report of *Diplodia seriata* as causal agent of olive dieback in Croatia. Plant Disease, 96, 290.
46. Karami, J., Kavosi, M.R., Babanezhad, M., Kiapasha, K. (2017.): Integrated management of the charcoal disease by silviculture, chemical and biological methods in forest parks. Journal of Sustainable Forestry, 37, 429–444.
47. Kim, G.-U., Chen, D (2019.): Climate change over the Mediterranean and current destruction of marine ecosystem. Scientific Reports, 9, 18813.
48. Klen, T.J., Vodopivec, B.M (2011.): Ultrasonic extraction of phenols from olive mill wastewater: comparison with conventional methods. Journal of Agricultural and Food Chemistry, 59 (24), 12725–12731.
49. Klen, T.J., Wondra, A.G., Vrhovšek, U.,Vodopivec, B.M (2015.): Phenolic profiling of olives and olive oil process-derived matrices using UPLC-DAD-ESI-QTOF-HRMS analysis. Journal of Agricultural and Food Chemistry, 63 (15), 3859–3872.
50. Krid, S., Bouaziz, M., Ali Triki, M., Gargouri, A., Rhouma, A (2011.): Inhibition of olive knot disease by polyphenols extracted from olive mill waste water. Journal of Plant Pathology, 93, 561–568.
51. Latinović, J., Mazzaglia, A., Latinović, N., Ivanović, M., Gleason, M.L. (2013.): Resistance of olive cultivars to *Botryosphaeria dothidea*, causal agent of olive fruit rot in Montenegro. Crop Protection, 48, 35–40.
52. Latorre, B.A., Torres, R., Silva, T., Elfar, K. (2013.): Evaluation of the use of wound-protectant fungicides and biological control agents against stem canker (*Neofusicoccum parvum*) of blueberry. Ciencia e Investigacion Agraria, 40, 547–557.
53. Li, J., Fu, S., Fan, G., Li, D., Yang, S., Peng, L., Pan, S. (2021.): Active compound identification by screening 33 essential oil monomers against *Botryosphaeria dothidea* from postharvest kiwifruit and its potential action mode. Pesticide Biochemistry and Physiology, 179, 104957.
54. Luo, F., Li, W., Zhu, T., Han, S., Qiao, T., Li, S. (2020.): First report of *Nigrospora aurantiaca* causing leaf spot disease of *Castanea mollissima* in China. Plant Disease, 104, 2730.
55. Mazzaglia, A., Anselmi, N., Gasbarri, A., Vannini, A. (2001.): Development of a Polymerase Chain Reaction (PCR) assay for the specific detection of *Biscogniauxia mediterranea* living as an endophyte in oak tissues. Mycological Research,105, 952–956.
56. Möller, M., Stukenbrock, E.H (2017.): Evolution and genome architecture in fungal plant pathogens. Nature Reviews Microbiology, 15, 756–771.
57. Moral, J., Muñoz-Díez, C., González, N., Trapero, A., Michailides, T.J (2010.): Characterization and pathogenicity of Botryosphaeriaceae species collected from olive and other host in Spain and California. Phytopathology, 100 (12), 1340-1351.
58. Moral, J., Agusti-Brisach, C., Pérez-Rodríguez, M., Xaviér, C., Carmen-Raya, M., Rhouma, A., Trapero, A. (2017.): Identification of fungal species associated with branch dieback of olive and resistance of table cultivars to *Neofusicoccum mediterraneum* and *Botryosphaeria dothidea*. Plant Disease, 101, 306–316.
59. Muzzalupo, I., Badolati, G., Chiappetta, A., Picci, N., Muzzalupo, R (2020.): *In vitro* antifungal activity of olive (*Olea europaea*) leaf extracts loaded in chitosan nanoparticles. Frontiers in Bioengineering and Biotechnology, 8, 151.

60. Nigro, F., Antelemi, I., Sion, V (2018.): Integrated control of aerial fungal diseases of olive. ISHS Acta Horticulturae 1199: VIII International Olive Symposium, 327-333.
61. Niu, C., Gilbert, E.S (2004.): Colorimetric method for identifying plant essential oil components that affect biofilm formation and structure. Applied and Environmental Microbiology, 70 (12), 6951–6956.
62. Patejuk, K., Baturo-Cieśniewska, A., Pusz, W., Kaczmarek-Pieńczewska, A. (2022.): Biscogniauxia Charcoal Canker—A New Potential Threat for Mid-European Forests as an Effect of Climate Change. Forests, 13, 89.
63. Palfi, M. (2017.): Antifungalno djelovanje eteričnih ulja i njihovih komponenti na fitopatogene gljivice u in vitro uvjetima. Doktorska disertacija, Sveučilište Josipa Jurja Strossmayera u Osijeku, Fakultet agrobiotehničkih znanosti Osijek, Hrvatska.
64. Petrović, E., Godena, S., Ćosić, J., Vrandečić, K (2024.): Identification and pathogenicity of *Biscogniauxia* and *Sordaria* species isolated from olive trees. Horticultirae, 10 (3), 243.
65. Petrović, E., Vrandečić, K., Ćosić, J., Godena, S (2024.): Chemical control of olive fungal diseases: Strategies and risks. Poljoprivreda, 30 (1), 44-53.
66. Phillips, A.J.L., Alves, A., Abdollahzadeh, J., Slippers, B., Wingfield, M.J., Groenewald, J.Z., Crous, P.W. (2013.): The Botryosphaeriaceae: Genera and species known from culture. Studies in Mycology, 76, 51–167.
67. Pribetić, Đ. (2006.): Sorte Maslina u Istri; MIH d.o.o.: Poreč, Hrvatska.
68. Rabbee, M.F., Ali, M.S., Choi, J., Hwang, B.S., Jeong, S.C., Baek, K.H (2019.): *Bacillus velezensis*: A valuable member of bioactive molecules within plant microbiomes. Molecules 24, 1046. .
69. Raza, M., Zhang, Z.-F., Hyde, K.D., Diao, Y.-Z., Cai, L. (2019.): Culturable plant pathogenic fungi associated with sugarcane in southern China. Fungal Diversity, 99, 1–104.
70. Rodrigo, S., Santamaria, O., Halecker, S., Lledó, S., Stadler, M. (2017.): Antagonism between *Byssochlamys spectabilis* (anamorph *Paecilomyces variotii*) and plant pathogens: Involvement of the bioactive compounds produced by the endophyte. Annals of Applied Biology, 171, 464–476.
71. Romero, C., Brenes, M., García, P., García, A., Garrido, A (2004.): Polyphenol changes during fermentation of naturally black olives. Journal of Agricultural and Food Chemistry, 52 (7), 1973–1979.
72. Rugini, E., Mencuccini, M., Biasi, R., Altamura, M.M. (2005.): Olive (*Olea europea* L.). U: Protocol for Somatic Embryogenesis in Woody Plants; Jain, S.M., Gupta, P.K., Ed.; Foresty Sciences; Springer: Dordrecht, Nizozemska, 77, 345–360.
73. Russo, E., Spallarossa, A., Comite, A., Pagliero, M., Guida, V., Belotti, V., Caviglia, D., Schito, A.M (2022.): Valorization and potential antimicrobial use of olive mill wastewater (OMW) from Italian olive oil production. Antioxidants 11, 903.
74. Saitou, N., Nei, M (1987.): The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4, 406–425.
75. Sánchez, M.E., Venegas, J., Romero, M.A., Phillips, A.J.L., Trapero, A. (2003.): Botryosphaeria and related taxa causing oak canker in southwestern Spain. Plant Disease, 87, 1515–1521.
76. Sarkhosh, A., Schaffer, B., Vargas, A. I., Palmateer, A. J., Lopez, P., Soleymani, A (2018.): *In vitro* evaluation of eight plant essential oils for controlling *Colletotrichum*, *Botryosphaeria*, *Fusarium* and *Phytophthora* fruit rots of avocado, mango and papaya. Plant protection science, 54 (3), 153–162.
77. Shabir, S., Ilyas, N., Saeed, M., Bibi, F., Sayyed, R.Z., Almaki, W.H (2023.): Treatment technologies for olive mill wastewater with impacts on plants. Environmental Research, 216, 114399.
78. Slippers, B., Crous, P.W., Denman, S., Coutinho, T.A., Wingfield, B.D., Wingfield, M.J. (2004.): Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. Mycologia, 96, 83–101.
79. Sun, M., Liu, J., Li, J., Huang, Y (2022.): Endophytic bacterium *Serratia plymuthica* from chinese leek suppressed apple ring rot on postharvest apple fruit. Frontiers in microbiology, 12, 802887.
80. Štůsková, K., Mondello, V., Hakalová, E., Tekielska, D., Fontaine, F., Eichmeier, A. (2023.): Phenolic compounds inhibit viability and infectivity of the grapevine pathogens *Diplodia seriata*, *Eutypa lata*, *Fomitiporia mediterranea*, and *Neofusicoccum parvum*. Phytopathologia Mediterranea, 62, 307–319.

81. Tamura, K., Nei, M., Kumar, S (2004.): Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences U. S. A., 101 (30), 11030–11035.
82. Tamura, K., Stecher, G., Kumar, S (2021.): MEGA11: Molecular Evolutionary Genetics Analysis version 11. Molecular Biology and Evolution, 38 (7), 3022–3027.
83. Twizeyimana, M., McDonald, V., Mayorquin, J.S., Wang, D.H., Na, F., Akgül, D.S., Eskalen, A. (2013.): Effect of fungicide application on the management of avocado branch canker (formerly Dothiorella canker) in California. Plant Disease, 97, 897–902
84. Yakhlef, W., Arhab, R., Romero, C., Brenes, M., de Castro, A., Medina, E (2018.): Phenolic composition and antimicrobial activity of Algerian olive products and by-products. LWT, 93, 323–328.
85. Yangui, T., Rhouma, A., Ali Triki, M., Gargouri, K., Bouzid, J (2008.): Control of damping-off caused by *Rhizoctonia solani* and *Fusarium solani* using olive mill waste water and some of its indigenous bacterial strains. Crop Protection, 27, 189–197.
86. Yangui, I., Boutiti, M.Z., Boussaid, M., Messaoud, C. (2017.): Essential Oils of *Myrtaceae* species growing wild in Tunisia: chemical variability and antifungal activity against *Biscogniauxia mediterranea*, the causative agent of charcoal canker. Chemical Biodiversity, 14, e1700058.
87. Úrbez-Torres, J.R., Peduto, F., Vossen, P.M., Krueger, W.H., Gubler, W.D. (2013.): Olive twig and branch dieback: etiology, incidence, and distribution in California. Plant Disease 97, 231–244.
88. White, T.J., Bruns, T.D., Lee, S.B., Taylor, J.W. (1990.): 38—Amplification and direct sequencing of fungal ribosomal RNA genes forphylogenetics. U: PCR—Protocols and Applications—A Laboratory Manual; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Ed.; Academic Press Inc.: Cambridge, MA, USA, 315–322.
89. Woudenberg, J.H.C., Aveskamp, M.M., de Grutyer, J., Spiers, A.G., Crous, P.W. (2009.): Multiple Didymella teleomorphs are linked to the *Phoma clematidina* morphotype. Persoonia, 22, 56–62.
90. Zeilinger S., Gupta V.K., Dahms T.E.S., Silva R.N., Singh H.B., Upadhyay R.S., Gomes E.V., Tsui C.K.M., Nayak, S.C (2016.): Friends or foes? Emerging insights from fungal interactions with plants. FEMS Microbiology Reviews, 40 (2), 182-207.
91. Zhang, Z., Xie, Y., Hu, X., Shi, H., Wei, M., Lin, Z. Antifungal Activity of Monoterpenes against *Botryosphaeria dothidea*. Natural Product Communications, 13, 1721–1724.
92. Živković, S., Stojanović, S., Ivanović, Ž., Gavrilović, V., Popović, T., Balaž, J (2010.): Screening of antagonistic activity of microorganisms against *Colletotrichum acutatum* and *Colletotrichum gloeosporioides*. Archives of Biological Sciences, 62 (3), 611–623.

Izvorni znanstveni rad broj 1 u obliku i izvornom jeziku na kojem je objavljen u znanstvenom časopisu

Naslova rada: First Report of *Phaeoacremonium iranianum* Causing Olive Twig and Branch Dieback

Autori: Elena Petrović, Karolina Vrandečić, Jasenka Ćosić, Gabriella Kanižai Šarić, Sara Godena

Tip rada: Izvorni znanstveni članak

Časopis: Plants

Kategorija: A1

Impakt faktor: 4,5 (2022.)

Kvartil: Q1

Primljen na recenziju: 15. studenog 2022.

Prihvaćen za objavljivanje: 13. prosinca 2022.

Status: Objavljen

Volumen: 11

Broj: 24

Broj rada: 3578

WOS broj: 000643514400001

plants

MDPI

Communication

First Report of *Phaeoacremonium iranianum* Causing Olive Twig and Branch Dieback

Elena Petrović [1], Karolina Vrandečić [2], Jasenka Ćosić [2], Gabriella Kanižai Šarić [2] and Sara Godena [1,*]

[1] Institute of Agriculture and Tourism, 52440 Poreč, Croatia
[2] Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, 31000 Osijek, Croatia
* Correspondence: sara@iptpo.hr

Abstract: In an olive orchard on the western part of Istria, Croatia, twig and branch dieback was observed on several olive trees. In total, seven samples from symptomatic trees were collected. Samples were analyzed, and four fungal isolates showed morphological similarities to the species *Phaeoacremonium*. One isolate, chosen as a representative, was taken for molecular identification and pathogenicity tests. Based on the DNA sequence data of the ITS, TUB, and EF1α gene regions, the isolate was identified as *P. iranianum*. Pathogenicity tests were conducted on detached olive branches and olive trees in the greenhouse. To the best of our knowledge, this is the first report of twig and branch dieback on olive caused by *Phaeoacremonium iranianum*.

Keywords: *Phaeoacremonium iranianum*; olive; dieback

Citation: Petrović, E.; Vrandečić, K.; Ćosić, J.; Kanižai Šarić, G.; Godena, S. First Report of *Phaeoacremonium iranianum* Causing Olive Twig and Branch Dieback. *Plants* **2022**, *11*, 3578. https://doi.org/10.3390/plants11243578

Academic Editor: Georgios Koubouris

Received: 15 November 2022
Accepted: 13 December 2022
Published: 19 December 2022

1. Introduction

Olive (*Olea europaea* L.) is one of the most important crops in the Mediterranean part of Croatia. According to the latest statistical data, the Croatian national production of olives is approximately 23,000 tones [1]. Olive trees are known to be drought-resistant and hardy but susceptible to several major diseases [2]. Recently, however, olive trees are becoming more susceptible to diseases caused by phytopathogenic fungi. We believe the main reasons for this increased susceptibility are changes in cultivation methods, the planting of infected plant material, increasing resistance of pathogens to fungicides, and climate extremes. In recent years, there have been various occurrences of new diseases in olive trees in Istria that were unknown even to experienced olive growers. Unfortunately, studies of pathogens associated with olive decline in Croatia are few. In order to create a plant protection strategy (within the framework of sustainable olive production) and for proper tillage of the soil before planting (especially if a crop that hosts the same diseases as olive was grown on the plot), the detection of the causal agents of these unusual olive diseases is crucial.

2. Materials and Methods

2.1. Sampling and Fungal Isolation

In 2021, olive trees which showed signs of twig and branch dieback, discoloration of the bark, and necrotic lesions were spotted in an olive orchard on the western side of Istria, Croatia. The area of the orchard was 0.43 ha and contained approximately 70 olive trees. Disease incidence was reaching 40%. Olive trees of the orchard (100% local cultivar 'Buza') were over 30 years old and grown on the soil where grapevine had been grown beforehand. In total, seven samples from seven trees (one sample per tree) of branches from symptomatic trees of 'Buza' were collected and brought to the laboratory for analysis. Small pieces of branches (4 × 4 mm) were rinsed under tap water, surface sterilized in 70% ethanol for one minute, rinsed two times in sterile distilled water, and placed on a sterile paper sheet in a laminar flow cabinet until dry. Pieces of branches were plated on

PDA amended with 35 mg/L of penicillin and incubated. After five days of incubation at 25 °C under dark conditions, isolates were transferred onto the fresh PDA medium for pure culture.

2.2. Morphological and Molecular Identification

After 14 days of incubation at 25 °C in dark conditions, pure fungal cultures were taken for examination. Four isolates showed morphological similarities to the genus *Phaeoacremonium*. One isolate (R18 B4), chosen as representative, was taken for molecular identification. Total DNA from the isolate was extracted with Maxwell® RSC Plant DNA Kit (Promega, Madison, WI, USA). The PCR reaction was performed using ITS1/ITS4 [3], Bt2a/Bt2b [4], and EF1-728F/EF1-986R [5] pair of primers. The PCR reaction mixture was composed of 12,5 µL of EmeraldAmp® GT PCR Master Mix, 0,5 µL of each primer, 6,5 µL of nuclease-free water, and 5 µL of genomic DNA. Polymerase chain reactions (PCR) (Table S1) were conducted in a SureCycler 8800 Thermal Cycler (Agilent Technologies, Santa Clara, CA, USA). The PCR products were visualized on 1% agarose gel light using an iBright CL1000 Imaging System (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). Purification of PCR products was conducted with the GenElute™ PCR Clean-Up Kit (Sigma-Aldrich®, Burlington, MA, USA), and sequencing (with EZ-Seq) of the PCR products was performed by Macrogen Europe (Amsterdam, The Netherlands). Sequences were edited in Sequencher® (Gene Codes Corporation, Ann Arbor, MI, USA) and compared with sequences from GenBank®. Phylogenetic analysis (Figure S4) was performed using ITS sequence data from reference isolate R18 B4 and ITS sequence data of isolates (of species *P. iranianum*, *P. minimum*, and *Botryosphaeria dothidea*) using sequence data from GenBank. The sequences were aligned using ClustalX2 (UCD Dublin, Ireland) software, and a phylogenetic tree was made using MEGA11 (Pennsylvania State University, State College, PA, USA) software. Sequence alignment was generated from neighbor-joining tree.

2.3. Pathogenicity Tests of Isolate

Two pathogenicity tests were conducted to determine pathogenicity of the isolate on the olive tree: one on detached branches from cultivars 'Buza' and 'Rosinjola' in the laboratory and another one on the four-year-old olive tree of the cultivar 'Rosinjola' in the greenhouse. Detached branches were washed with water, surface sterilized in 10% sodium hypochlorite solution for 10 min, rinsed with sterile distilled water for 10 min, and placed in a laminar flow cabinet, on sterile paper, until dry. Branches were inoculated by placing a 4 mm-diameter mycelium plug from a 14-day-old PDA culture of R18 B4 isolate in a wound made with a 4 mm-diameter cork-borer. Wounds were sealed with Vaseline and protected with Parafilm. Ten branches in total, per cultivar, were used. Fungal treatments were compared to the control treatment inoculated only with PDA plugs without mycelia, sealed with Vaseline, and protected with Parafilm. Inoculated branches were kept in laboratory conditions for 20 days.

Branches from olive trees found in the greenhouse were chosen at random and inoculated the same way as previously described for detached branches. Inoculated plants had been kept in a greenhouse, at approximately 25 °C, for three months, from March to July 2022, and were monitored for the presence of symptoms. After incubation, samples were collected, and in an attempt to fulfill Koch's postulate, small pieces of necrotic tissue from the edge of each lesion were cut and placed on PDA to recover inoculated fungus.

3. Results

3.1. Sampling and Fungal Isolation

In the field, the symptoms of the disease on 'Buza' olive trees were the wilting and dieback of twigs and branches, as well as brown internal necrosis (Figure 1). Symptoms such as dieback were observed on lateral branches, on one side of the trees. When the outer layer of bark from the branches was scraped away, it was revealed that the brownish discoloration

had extended on the surrounding tissue. Successful fungal isolation was obtained in four out of seven samples (57.1%). Isolations with saprophytic fungus were discharged.

Figure 1. (**a**,**b**) Disease symptoms on olive branches in an orchard near Rovinj in Istria, Croatia, in the year 2021.

3.2. Morphological and Molecular Identification

Fungal isolates have been identified based on the colony characteristics (color, form, margin, elevation, surface, and opacity) and spore characteristics (color, presence or absence of septum, and shape). The developed fungal colonies were brownish on PDA, reverse darker brown; circular shaped with an entire edge; and with aerial, opaque, and cottony mycelium and branched septate hyphae (Figure 2). The isolate produced hyaline, unseptate, and ovoid conidia. An average conidia body length was (oblong-ellipsoidal) 4.5 × 1.5 µm. These morphological characteristics identified the fungus as *Phaeoacremonium iranianum* L. Mostert, Gräfenhan, W. Gams & Crous, 2006 [6]. For molecular identification, consensus sequences of representative R18 B4 isolate were produced (GenBank accession numbers: OP627795 for ITS, OP684932 for TUB, and OP684933 for the EF1α gene). BLAST analysis of the sequences showed 100% similarity with *P. iranianum* (reference number MG745842 for ITS, KF179086 for TUB, and KF764625 for the EF1α gene).

3.3. Pathogenicity Tests of Isolate

The symptoms of the disease on the olive branches tested in the laboratory and on the branches collected from olives in the greenhouse showed similar symptoms to the branch samples collected from the field survey. Brown streaking in cross-sections was detected (Figure 3), and when the outer layer of bark from the branches was scraped away, brown discoloration (staining) had extended around the affected tissue. The pathogen had been consistently reisolated from affected pieces of wood, while the controls remained healthy. To fulfill Koch's postulate, one isolate chosen as a representative was carried out for molecular identification, as previously described, using an ITS5/ITS4 [7] pair of primers. Purification and sequencing of the PCR products were performed by Macrogen Europe (Amsterdam, Netherlands). BLAST analysis of the ITS5 and ITS4 sequences showed 100% similarity with *P. iranianum* (reference number MG745842).

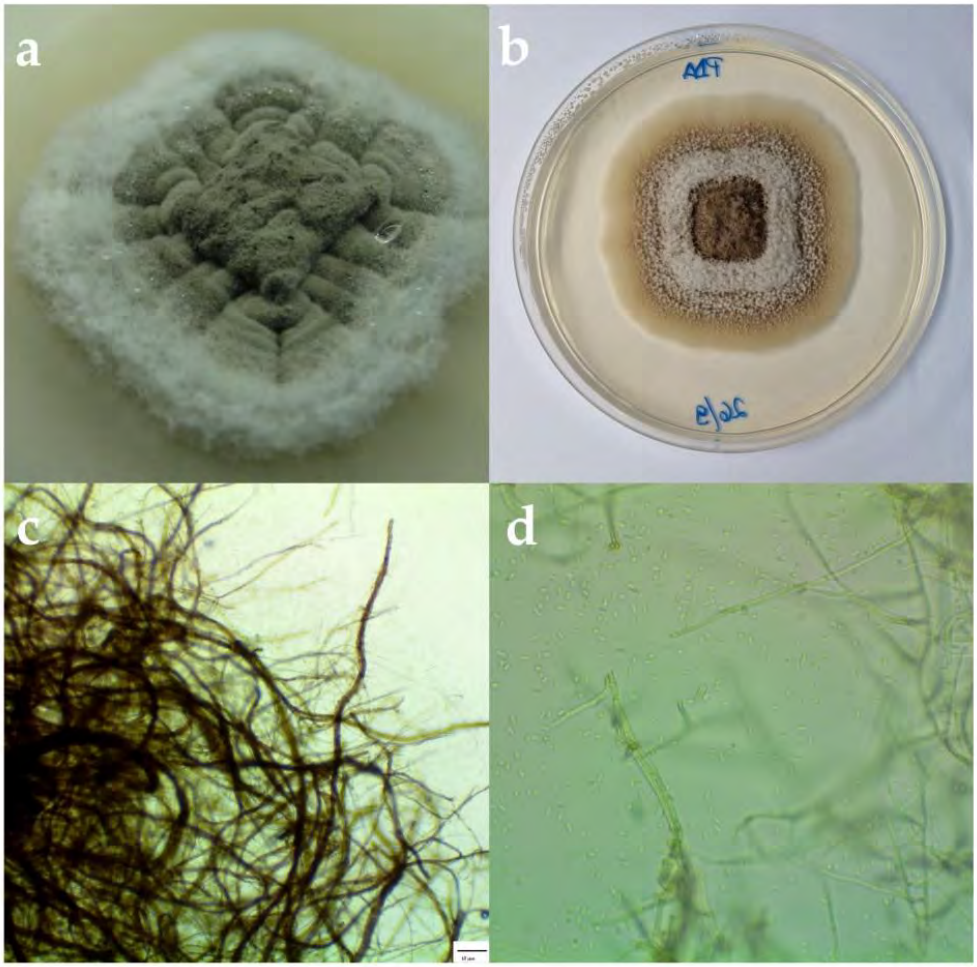


Figure 2. (**a**) *Phaeoacremonium iranianum* colony, on PDA, after two weeks in the dark at 25 °C. (**b**) *P. iranianum* colony, on PDA, after one month. (**c**) Micrographs of *P. iranianum* isolate under the microscope. Scale bar = 10 µm. (**d**) Hyaline, ovoid conidia.

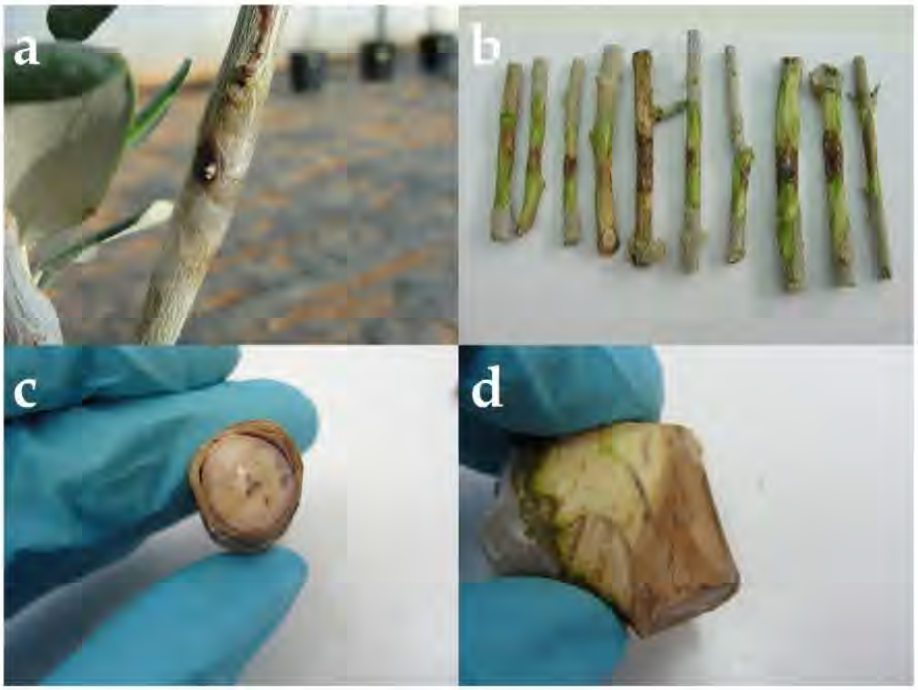


Figure 3. (**a**–**d**) Disease symptoms on branches used in pathogenicity tests from March to July 2022.

4. Discussion

There are several species from the *Phaeoacremonium* genus associated with olive diseases worldwide: *Phaeoacremonium africanum* [8,9], *P. alvesii* [10], *P. italicum* [10–12], *P. minimum* [8–14], *P. oleae* [8,9], *P. parasiticum* [8–11,15], *P. prunicola* [8,9], *P. scolytii* [8–12], *P. spadicum* [8,9], and *P. sicilianum* [10,11]. *P. iranianum* is a species from the family *Togniniaceae* [6]. It was previously described as a plant pathogen on several species of woody plants, including almond trees [16,17], citrus trees [18], cypress trees [19], forest trees [20], grapevine [20–27], pome fruit (apple, quince, hawthorn, pear) [28], and prunus trees [29]. It has most commonly been reported as associated with Petri and Esca diseases, one of the most destructive declining diseases affecting grapevine [22]. As the observed infected olive trees were grown on the ground where grapevines were previously grown, and since olive and grapevine share common pathogens, such as phytopathogenic fungi from the *Botryosphaeriaceae* family, there is a high risk of transmission of *P. iranianum* between grapevines and olive trees [26]. Aerial spores can be dispersed between vineyards that are near each other and those established in close proximity to fruit orchards, ornamental trees, or numerous other woody hosts [26]. This poses a danger to olive trees, especially in the Mediterranean part of Croatia, where vines and olives are often grown together.

Before planting, it is imperative to correctly prepare the soil and to start with the healthiest planting material possible. Baloyi et al. [24] states pruning wounds on grapevine as entry sites for infection by *Phaeoacremonium* species, so it can be said that pruning wounds on olive can act as entry sites for infection, too. Measures such as pruning wound protection (this includes the use of chemicals with different modes of activity) and the disinfection of tools are necessary. Pruning should be carried out during dry weather, as spores are released during the rain. It is necessary to monitor olive orchards, remove infected parts of the tree, and remove them from orchards, as they can be a source of inoculum.

Since there is no data on protection strategies for species of *P. iranianum*, the need for further research (especially of research based on alternative nonchemical or biological control strategies that will enable growers to minimize chemical inputs, within the framework of sustainable olive production) is emphasized.

To the best of our knowledge, this is the first report of *Phaeoacremonium iranianum* causing olive twig and branch dieback on olive trees.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants11243578/s1. ITS4 and ITS5 sequences from Koch's postulate. Table S1: PCR amplification program set according to Alves et al. (2006) [30]. Figure S1: Phylogenetic tree based on internal transcribed spacer (ITS) sequences alignment. The evolutionary history was inferred using the Neighbor-Joining method [31]. The optimal tree is shown. The evolutionary distances were computed using the Maximum Composite Likelihood method [32] and are in the units of the number of base substitutions per site. This analysis involved six nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 596 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [33].

Author Contributions: Conceptualization, E.P. and S.G.; methodology, E.P and S.G.; investigation, E.P. and S.G., writing—original draft preparation, E.P.; writing—review and editing, S.G., K.V., J.Č., and G.K.Š. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Croatian Science Foundation Installation Research Project "Natural bioactive compounds as a source of potential antimicrobial agents in the control of bacterial and other fungal pathogens of olives", Anti-Mikrobi-OL (AMO), UIP-2020-02-7413.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All sequence data for isolate R18 B4 are available in NCBI GenBank following the accession numbers in the manuscript. Sequence data from Koch's postulate, PCR amplification program, and phylogenetic tree are available in the Supplementary Materials.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Croatian Bureau of Statistics. Available online: https://podaci.dzs.hr/2021/hr/10118 (accessed on 25 October 2022).
2. Phillips, A.J.L.; Rumbos, I.C.; Alves, A.; Correia, A. Morphology and phylogeny of *Botryosphaeria dothidea* causing fruit rot of olives. *Mycopathologia* **2005**, *159*, 433–439. [CrossRef] [PubMed]
3. White, T.J.; Bruns, T.D.; Lee, S.B.; Taylor, J.W. 38—Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics. In *PCR—Protocols and Applications—A Laboratory Manual*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press, Inc.: New York, NY, USA, 1990; pp. 315–322.
4. Glass, N.L.; Donaldson, G.C. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl. Environ. Microbiol.* **1995**, *61*, 1323–1330. [CrossRef] [PubMed]
5. Carbone, I.; Kohn, L.M. A Method for Designing Primer Sets for Speciation Studies in Filamentous Ascomycetes. *Mycologia* **1995**, *91*, 553–556. [CrossRef]
6. Mostert, L.; Groenewald, J.Z.; Summerbell, R.C.; Gams, W.; Crous, P.W. Taxonomy and Pathology of *Togninia* (*Diaporthales*) and its *Phaeoacremonium* Anamorphs. *Stud. Mycol.* **2006**, *54*, 1–113. [CrossRef]
7. European and Mediterranean Plant Protection Organization (EPPO). PM 7/129 (1) DNA barcoding as an identification tool for a number of regulated pests. *Bull. OEPP/EPPO Bull.* **2016**, *46*, 501–537. [CrossRef]
8. Van Dyk, M.; Spies, C.F.J.; Mostert, L.; van der Rijst, M.; du Plessis, I.L.; Moyo, P.; van Jaarsveld, W.J.; Halleen, F. Pathogenicity Testing of Fungal Isolates Associated with Olive Trunk Diseases in South Africa. *Plant Dis.* **2022**, *105*, 4060–4073. [CrossRef]
9. Spies, C.F.J.; Mostert, L.; Carlucci, A.; Moyo, P.; van Jaarsveld, W.J.; du Plessis, I.L.; van Dyk, M.; Halleen, F. Dieback and decline pathogens of olive trees in South Africa. *Persoonia* **2020**, *45*, 195–220. [CrossRef] [PubMed]
10. Carlucci, A.; Lops, F.; Cibelli, F.; Raimondo, M.L. *Phaeoacremonium* species associated with olive wilt and decline in southern Italy. *Eur. J. Plant Pathol.* **2015**, *141*, 717–729. [CrossRef]
11. Raimondo, M.L.; Lops, F.; Carlucci, A. First Report of *Phaeoacremonium oleae* and *P. viticola* Associated with Olive Trunk Diseases in Italy. *Plant Dis.* **2022**, *106*, 331. [CrossRef]
12. Agusti-Brisach, C.; Jimenez-Urbano, J.P.; Raya, M.D.; Lopez-Moral, A.; Trapero, A. Vascular Fungi Associated with Branch Dieback of Olive in Super-High-Density Systems in Southern Spain. *Plant Dis.* **2021**, *106*, 797–818. [CrossRef]

13. Carlucci, A.; Raimondo, M.L.; Cibelli, F.; Phillips, A.J.L.; Lops, F. *Pleurostomophora richardsiae*, *Neofusicoccum parvum*, and *Phaeoacremonium aleophilum* associated with a decline of olives in southern Italy. *Phytopathol. Mediterr.* **2013**, *52*, 517–527.
14. Úrbez-Torres, J.R.; Peduto, F.; Vossen, P.M.; Krueger, W.H.; Gubler, W.D. Olive Twig and Branch Dieback: Etiology, Incidence, and Distribution in California. *Plant Dis.* **2013**, *97*, 231–244. [CrossRef] [PubMed]
15. Van Dyk, M.; Spies, C.F.J.; Mostert, L.; Halleen, F. Survey of Trunk Pathogens in South African Olive Nurseries. *Plant Dis.* **2021**, *105*, 1630–1639. [CrossRef] [PubMed]
16. Gramaje, D.; Agustí-Brisach, C.; Pérez-Sierra, A.; Moralejo, E.; Olmo, D.; Mostert, L.; Damm, U.; Armengol, J. Fungal trunk pathogens associated with wood decay of almond trees on Mallorca (Spain). *Persoonia* **2012**, *28*, 1–13. [CrossRef]
17. Olmo, D.; Armengol, J.; Leon, M.; Gramaje, D. Pathogenicity testing of lesser-known fungal trunk pathogens associated with wood decay of almond trees. *Eur. J. Plant Pathol.* **2015**, *143*, 607–611. [CrossRef]
18. Espargham, N.; Mohammadi, H.; Gramaje, D. A Survey of Trunk Disease Pathogens within Citrus Trees in Iran. *Plant* **2020**, *9*, 754. [CrossRef]
19. Mohamadi, H.; Kazemi, S.; Farahmand, H. *Phaeoacremonium* and *Botryosphaeriaceae* species associated with cypress (*Cupressus sempervirens* L.) decline in Kerman province (Iran). *Phytopathol. Mediterr.* **2014**, *53*, 27–39.
20. Kazemzadeh Chakusary, M.; Mohammadi, H.; Khodaparast, S.A. Decline-associated *Phaeoacremonium* species occurring on forest trees in the north of Iran. *For. Pathol.* **2017**, *47*, e12368. [CrossRef]
21. Gramaje, D.; Armengol, J.; Colino, M.I.; Santiago, R.; Moralejo, E.; Olmo, D.; Luque, J.; Mostert, L. First Report of *Phaeoacremonium inflatipes*, *P. iranianum*, and *P. sicilianum* Causing Petri Disease of Grapevine in Spain. *Plant Dis.* **2009**, *93*, 964–965. [CrossRef]
22. White, C.L.; Halleen, F.; Fischer, M.; Mostert, L. Characterisation of the fungi associated with esca diseased grapevines in South Africa. *Phytopahologia Mediterr.* **2011**, *50*, S204–S223.
23. Úrbez-Torres, J.R.; Haag, P.; Bowen, P.; O'Gorman, D.T. Grapevine Trunk Diseases in British Columbia: Incidence and Characterization of the Fungal Pathogens Associated with Esca and Petri Diseases of Grapevine. *Plant Dis.* **2014**, *98*, 469–482. [CrossRef] [PubMed]
24. Baloyi, M.A.; Mostert, L.; Halleen, F. Pathogenicity of ten *Phaeoacremonium* species associated with esca and Petri disease of Grapevine. *Phytopathol. Mediterr.* **2018**, *57*, 538–546.
25. Agusti-Brisach, C.; Lopez-Moral, A.; Raya-Ortega, M.C.; Franco, R.; Roca-Castillo, L.F.; Trapero, A. Occurrence of grapevine trunk diseases affecting the native cultivar Pedro Ximenez in southern Spain. *Eur. J. Plant Pathol.* **2019**, *153*, 599–625. [CrossRef]
26. Halleen, F.; Baloyi, M.A.; Bester, M.C.; Mostert, L. Aerial inoculum patterns of Petri disease pathogens in South African vineyards and rootstock mother blocks. *Phytopathol. Mediterr.* **2020**, *59*, 515–536.
27. Aigoun-Mouhous, W.; Mahamedi, A.E.; León, M.; Chaouia, C.; Zitouni, A.; Barankova, K.; Eichmeier, A.; Armengol, J.; Gramaje, D.; Berraf-Tebbal, A. *Cadophora sabaouae* sp. nov. and *Phaeoacremonium* Species Associated with Petri Disease on Grapevine Propagation Material and Young Grapevines in Algeria. *Plant Dis.* **2021**, *105*, 3657–3668. [CrossRef] [PubMed]
28. Sami, S.; Mohammadi, H.; Heydarnejad, J. *Phaeoacremonium* species associated with necrotic wood of pome fruit trees in Iran. *J. Plant Pathol.* **2014**, *96*, 487–495.
29. Damm, U.; Mostert, L.; Crous, P.W.; Fourie, P.H. Novel *Phaeoacremonium* species associated with necrotic wood of *Prunus* trees. *Persoonia* **2008**, *20*, 87–102. [CrossRef] [PubMed]
30. Alves, A.; Correia, A.; Phillips, A.J.L. Multi-gene genealogies and morphological data support *Diplodia cupressi* sp. nov., previouly recognized as *D. pinea* f. sp. *cupressi*, as a distinct species. *Fungal Divers.* **2006**, *23*, 1–15.
31. Saitou, N.; Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **1987**, *4*, 406–425.
32. Tamura, K.; Nei, M.; Kumar, S. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11030–11035. [CrossRef]
33. Tamura, K.; Stecher, G.; Kumar, S. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [CrossRef] [PubMed]

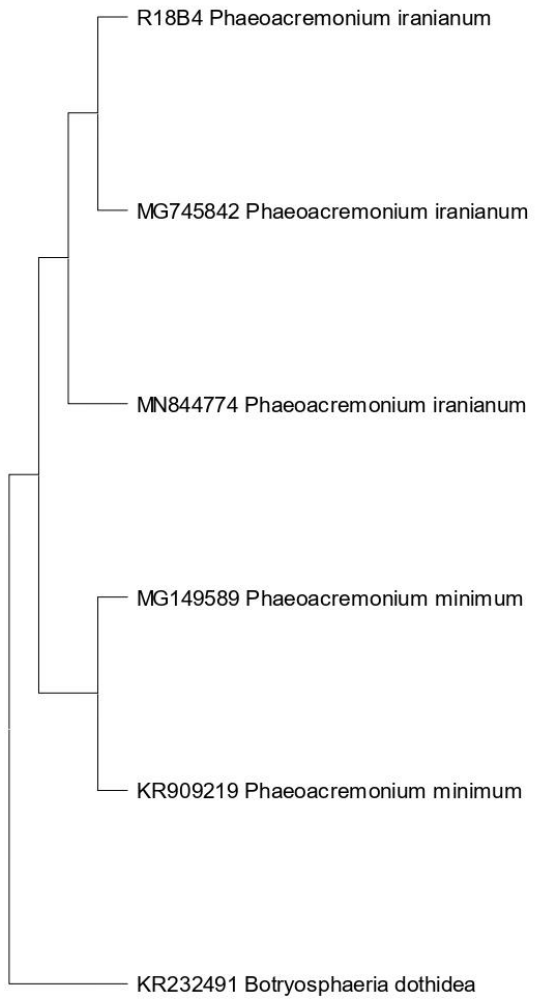


Figure S1. Phylogenetic tree based on internal transcribed spacer (ITS) sequences alignment. The evolutionary history was inferred using the Neighbor-Joining method [31]. The optimal tree is shown. The evolutionary distances were computed using the Maximum Composite Likelihood method [32] and are in the units of the number of base substitutions per site. This analysis involved 6 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 596 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [33].

Table S1. PCR amplification program set according to Alves et al. (2006) [30].

Hot Start 95 °C		Denaturation 94 °C	Annealing 55 °C	Elongation 72 °C		Elongation 72 °C
5 minutes	Start Cycle 30 times	30 seconds	45 seconds	1 minute and 30 seconds	End Cycle	10 minutes

Naslov izvornog znanstvenog rada broj 1: First Report of *Phaeoacremonium iranianum* Causing Olive Twig and Branch Dieback

Prošireni sažetak:

U masliniku smještenom na zapadnom dijelu Istre u Hrvatskoj, tijekom 2021. godine zabilježeni su simptomi sušenja grana na nekoliko stabala maslina. Maslinik, površine 0,43 ha, sadržavao je približno 70 stabala sorte Buža, starih preko 30 godina, posađenih na tlu gdje je prethodno uzgajana vinova loza. Incidencija bolesti dosezala je 40%. Prikupljeni su uzorci sa sedam simptomatičnih stabala, iz kojih su izolirane četiri gljive s morfološkim karakteristikama sličnim rodu *Phaeoacremonium*. Jedan reprezentativni izolat podvrgnut je molekularnoj identifikaciji i testovima patogenosti. Analizom sekvenci ITS, TUB2 i TEF1-α regija genoma izolat je identificiran kao *Phaeoacremonium iranianum* L. Mostert, Grafenhan, W. Gams & Crous. Testovi patogenosti provedeni su na odrezanim granama masline i na mladim stablima u plasteničkim uvjetima. Rezultati su potvrdili sposobnost izolata da izazove simptome sušenja grana, što ukazuje na njegovu patogenost na maslini. Ovo je prvi zabilježeni slučaj u Hrvatskoj, a prema dostupnim podacima i prvi u svijetu, koji potvrđuje *P. iranianum* kao uzročnika sušenja grana masline. Otkriće naglašava potrebu za daljnjim istraživanjima usmjerenim na razvoj strategija zaštite maslina, posebno s obzirom na održivu poljoprivredu i smanjenje upotrebe kemijskih sredstava.

Ključne riječi: *Phaeoacremonium iranianum*; maslina, sušenje

Izvorni znanstveni rad broj 2 u obliku i izvornom jeziku na kojem je objavljen u znanstvenom časopisu

Naslova rada: First Report of Olive Branch Dieback in Croatia Caused by *Cytospora pruinosa* Défago

Autori: Elena Petrović, Karolina Vrandečić, Dario Ivić, Jasenka Ćosić, Sara Godena

Tip rada: Izvorni znanstveni članak

Časopis: Microorganisms

Kategorija: A1

Impakt faktor: 4,1 (2023.)

Kvartil: Q1

Primljen na recenziju: 07. lipnja 2023.

Prihvaćen za objavljivanje: 26. lipnja 2023.

Status: Objavljen

Volumen: 11

Broj: 7

Broj rada: 1679

WOS broj: 001038782000001

microorganisms

MDPI

Article

First Report of Olive Branch Dieback in Croatia Caused by *Cytospora pruinosa* Défago

Elena Petrović [1], Karolina Vrandečić [2], Dario Ivić [3,*], Jasenka Ćosić [2] and Sara Godena [1,*]

[1] Institute of Agriculture and Tourism, Karla Huguesa 8, 52440 Poreč, Croatia; elena@iptpo.hr
[2] Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Vladimira Preloga 1, 31000 Osijek, Croatia; kvrandecic@fazos.hr (K.V.); jcosic@fazos.hr (J.Ć.)
[3] Centre for Plant Protection, Croatian Agency for Agriculture and Food, Gorice 68b, 10000 Zagreb, Croatia
* Correspondence: dario.ivic@hapih.hr (D.I.); sara@iptpo.hr (S.G.)

Abstract: Olive (*Olea europaea* L.) is a very important crop grown in the Mediterranean part of Croatia. Olive branch and fruit dieback symptoms were observed in two olive orchards in Istria, Croatia. The samples from symptomatic trees were collected and brought to the laboratory for analysis. Based on their morphological characterization, isolated fungi were identified as *Cytospora* sp. Two representative isolates (one per orchard) were taken for molecular analysis, and based on DNA sequence data of the ITS and TUB gene regions, and phylogenetic analysis of the sequences, the isolates were identified as *Cytospora pruinosa* Défago. To determine pathogenicity, pathogenicity tests were conducted on detached olive branches and two-year-old olive trees in the greenhouse. This is the first report of *C. pruinosa* causing olive branch and fruit dieback in Croatia.

Keywords: canker; *Cytospora* sp.; fungal disease; *Olea europaea* L.

check for updates

Citation: Petrović, E.; Vrandečić, K.; Ivić, D.; Ćosić, J.; Godena, S. First Report of Olive Branch Dieback in Croatia Caused by *Cytospom pruinosa* Défago. *Microorganisms* **2023**, *11*, 1679. https://doi.org/10.3390/microorganisms11071679

Academic Editors: Tomislav Cernava, Beibei Ge and Kyungseok Park

Received: 7 June 2023
Revised: 23 June 2023
Accepted: 26 June 2023
Published: 28 June 2023

1. Introduction

The olive (*Olea europaea* L.) is a medium-sized evergreen tree from the family *Oleaceae*, which integrates a unique set of morphological and developmental characteristics suited to the conditions of its Mediterranean origin [1]. The Mediterranean climate is characterized by an amount of rainfall ranging from 150 to 800 mm per year, and by the uneven distribution of rains, concentrated above all in winter and spring months [2,3]. The olive tree is very adapted to extreme environmental conditions, such as drought and high temperatures, and it is resistant to decay [2,4].

During the last decade, plantings and production of European olive (*Olea europaea* L.) have increased globally by about 10 and 20% [5]. According to the latest statistical data, worldwide production of olives is approximately 23 million tons, and it is cultivated on approximately 10 million ha [6]. In Croatia, olives are cultivated on almost 20 thousand ha with a production of 23 thousand tons of olives [6]. Olive is, along with vines, the most common crop grown in the Mediterranean part of Croatia [7]. In Istria, olive trees are known for about 2500 years [8]. Hundreds of named cultivars of both types of olives, table and oil, are grown. The most important domesticated and introduced olive cultivars in Istria (Croatia) are 'Bjelica', 'Buža', and 'Leccino'.

Olives are susceptible to different bacterial, viral, and fungal pathogens, which can cause severe diseases of the drupe, leaves, wood, and roots [9]. Trunk pathogens can infect olive trees through wounds, and cause dieback of twigs and branches, which can lead to a reduced fruit-bearing capacity and lifespan of olive trees [9,10]. Consequently, fungal trunk diseases can cause substantial economic losses [5]. Branches affected with cankers can show symptoms such as fruit rot or twig dieback. A number of different pathogens are reported as the causal agents associated with olive cankers and twig dieback [10]. One of the most harmful olive pathogens associated with the fungal canker of olives is species from the *Botryosphaeriaceae* family. The *Botryophaeriaceae* were found to be the most prevalent fungal

family causing olive twig and branch dieback in California, Italy, and Spain [9–11]. Olive diseases are poorly studied in Croatia, with most research focusing on practical aspects of leaf spot, caused by *Venturia oleaginea* (Castagne) Rossman & Crous, and olive knot, caused by *Pseudomonas savastanoi* (Janse 1982) Gardan et al., management [12]. Thus far, the species *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & De Not., *Diaporthe neotheicola* (Sacc.) Udayanga & Castl., *Diplodia seriata* De Notaris, *Neofusicuccoum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, *Phaeoacremonium iranianum* L. Mostert, Gräfenhan, W. Gams & Crous, *Phoma incompta* Sacc. & Martelli (*Comoclathris incompta* (Sacc. & Martelli) Ariyaw. & K.D. Hyde), *Pleurostomophora richardsiae* (Nannf.) Reblova & Jaklitsch, and *Verticillium dahliae* Klebahn have been described as fungal pathogens of olive in Croatia [12–17].

Cytospora spp. (anamorphs of *Valsa* spp.) are common inhabitants of woody plants and they include important stem and branch canker pathogens [18]. Since many different pathogens could be associated with the same syndrome, the correct diagnosis of the diseases is difficult. Diseases associated with *Cytospora* spp. have been referred to as Cytospora-, Leucostoma-, Perennial-, or Valsa canker [5,18–20]. There have been several reports of *Cytospora* spp. on olive trees, but there is a lack of knowledge about the diversity and biology of *Cytospora* spp. affecting olive [5]. *Cytospora* genus belongs to the order *Diaporthales*, and was introduced in 1818 with four species, namely: *C. betulina*, *C. epimyces*, *C. resinae*, and *C. ribis* [21]. The genus *Cytospora* has asexual morphs in *Valsa*, *Leucostoma*, *Valsella*, and *Valseutypella* [19]. Recently, all sexual genera were synonymized with *Valsa* as a subgenus or species [19]. This order is well known to contain endophytes, phytopathogens, and saprobes, with worldwide distribution [22–24]. Currently, there are 3373 described species of *Diaporthales*, in 238 genera and 11 families [25]. Many different names and name changes within *Cytospora* species have caused confusion for plant pathologists. The Index Fungorum lists 672 species of *Cytospora* and 886 species of *Valsa*. Most species are believed to be synonyms [18]. Currently, there are 173 records of *Cytospora* species in GenBank.

In the past, species identification in *Cytospora* was largely based on host affiliation, morphological characters of pycnidia/perithecia, and spore dimensions, with morphological description [26–28]. This morphological species approach is confounded by many morphological characters' overlap among species and by the morphological plasticity of pycnidial locules which are affected by the host bark and cambium characteristics (e.g., stromatal arrangement in the host tissues, locular arrangement within pycnidia, locule division into chambers, independent or shared locular walls) [18,19,29–31]. The first molecular phylogeny of *Cytospora* was inferred from ITS sequences [29], and identifications were performed mostly based on the ITS gene region [19,32,33]. Recently, more *Cytospora* species were recognized when using analysis including multiple protein-coding loci, such as α-actin (ACT), beta-tubulin (TUB2), the RNA polymerase II second largest subunit (RPB2), or translation elongation factor 1-alpha (TEF1-a) [20,34].

Cytospora species are primarily wound pathogens and cause cankers and dieback on many genera of hardwoods and coniferous trees, but rarely on herbaceous plants [18]. On many tree species, these fungi are considered facultative wound parasites that attack weakened trees [18]. Petrini [35] describes *Cytospora* spp. as latent pathogens existing as symptomless endophytic infections. The pathogens infect the inner bark, which is also referred to as the bark periderm [18]. *Cytospora pruinosa* Défago may infect olive trees through pruning wounds and wounds caused by cold. Abiotic stress may also play a role in decline [36]. The climatological conditions and the availability of susceptible hosts may influence the distribution of *Cytospora* species [5]. Plant pathologists are concerned that pathogens such as species of *Cytospora* might move from an introduced host to a native host or vice versa [18]. Because *Cytospora* species cause disease in plants under stress, the movement of a pathogen to a new host may reflect the vulnerability of a host's defensive systems under stress or the severity of the stress [18]. Fruiting bodies of *Cytospora* spp. consist of stromata (conidiomata) that usually contain either labyrinthine chambers or clusters of pycnidia, having filamentous conidiophores and allantoid hyaline conidia [18].

Conidia oozing from pycnidia embedded in dead or dying host cortical tissues during humid or wet conditions are considered infectious propagules potentially initiating new infections. Conidia exude from the fruiting bodies in gelatinous matrices, usually as yellow, orange, red, or pallid tendrils [18]. They are dispersed to new plant tissues by rain-splash, where they germinate and infect the host through cracks and wounds to the bark created by breakage of shade-weakened twigs and branches, insect injuries, leaf scars, pruning wounds, winter-injured buds, twigs, and bark [31,37,38]. As plant pathogens, *Cytospora* species are primarily associated with canker diseases. Symptoms vary with host species and stage of development [26]. *Cytospora* species mainly impact branches, but they can cause more destructive infections in the trunk and larger scaffolds, consequently limiting the productivity of orchards [38,39]. The diseased inner bark and the bark above the infected cambium may appear sunken and yellow, brown, reddish-brown, grey, or black, becoming watery and odorous as the tissues deteriorate. The wood below the cambium is stained brown. Later, these fungi quickly girdle and kill branches and twigs, forming several black sporocarps [26].

The objective of this study was to identify the causal agent of canker disease on olive trees observed in two orchards in Istria, Croatia, based on morphological examination, phylogenetic analysis, and to confirm a pathogen is the cause of a particular disease using Koch's postulates.

2. Materials and Methods

2.1. Sampling and Fungal Isolation

In 2021, olive trees showing signs of dieback were observed in two olive orchards in Istria, Croatia. The first orchard was on the northern side of Istria, in Kaštelir (45°17′30″ N, 13°40′51″ E). The area of the orchard was 6.5 ha, with 44 different olive cultivars. Symptoms were observed on olive trees from the local cultivar 'Porečka rosulja'. The second orchard was on the western side, in Vodnjan (44°56′21″ N, 13°50′18″ E). The area of this orchard was 1 ha and it contained two different olive cultivars. Symptoms were observed on olive trees of the local cultivar 'Buža'. In both orchards, olive trees were between five and 20 years old. In total, five samples (one sample per tree) of branches from symptomatic trees were collected from each orchard and delivered to the Laboratory for Plant Protection at the Institute of Agriculture and Tourism in Poreč (Istria, Croatia) for analysis.

Whole olive fruits and small pieces of branches and leaves (5 × 5 mm) were rinsed under tap water, surface sterilized in 70% ethanol for one minute (two minutes for fruits), rinsed two times in sterile distilled water, and placed on a sterile paper sheet in a laminar flow cabinet until dry. Plant parts were plated on potato-dextrose agar (PDA) and incubated for seven days at 25 °C under laboratory conditions. After incubation, the growing tips of hyphae were transferred aseptically on the fresh PDA medium, for pure culture.

2.2. Morphological Identification

Pure fungal cultures of ten isolates on PDA were taken for examination. Species have been identified based on the spores (color, shape, presence or absence of septa, and dimensions) and colony characteristics (color, form, elevation, margin, surface, and opacity). A study of the fungal structure was performed with a Boeco BM-2000 microscope, a Boeco BCAM10 camera, and a B-View software (Boeckel + Co (GmbH + Co) KG, Hamburg, Germany). Morphometric values were compared with previously published data for the genus [26].

2.3. Molecular Identification and Phylogenetic Analyses

Two representative isolates, SL2 PRIV and V16 BIII (one per orchard), were chosen for molecular identification. To fully characterize the isolates, DNA sequences of internal transcribed spacer (ITS) and beta-tubulin (TUB) were determined. Fresh mycelia of fungal isolates grown on PDA for seven days at 25 °C were scraped with a sterile toothpick from the colony margins and used for genomic DNA extraction. Total genomic DNA from the

isolate was extracted using a Maxwell® RSC Instrument (Promega, Madison, WI, USA) and Maxwell® RSC Plant DNA Kit (Promega, Madison, WI, USA). The nuclear ribosomal DNA repeats were amplified using ITS1 (5′ TCCGTAGGTGAACCTGCGG 3′) and ITS4 (5′ TCCTCCGCTTATTGATATGC 3′) pair of primers [40]. Oligonucleotide primers Bt2a (5′ GGTAACCAAATCGGTGCTGCTTTC 3′) and Bt2b (5′ ACCCTCAGTGTAGTGACC-CTTGGC 3′) were used to amplify a portion of the TUB gene [41]. The polymerase chain reaction (PCR) mixture was composed of 12.5 µL of EmeraldAmp® GT PCR Master Mix, 0.5 µL of each primer, 6.5 µL of nuclease-free water, and 5 µL of genomic DNA. PCR was conducted in a SureCycler 8800 Thermal Cycler (Agilent Technologies, Santa Clara, CA, USA) under the following conditions for both gene regions: initial denaturation step for two minutes at 95 °C followed by 35 cycles for 30 s of denaturation at 95 °C, 30 s for annealing at 48 °C, one minute for extension at 72 °C, and a final extension step of eight minutes at 72 °C [26]. For both isolates, the amplification of the TUB region was not accomplished, so a second PCR was performed using 1 µL of the first PCR amplification as a template. PCR products were visualized on 1% agarose gel light using an iBright CL1000 Imaging System (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). Purification and sequencing of PCR products was conducted by Macrogen Europe services (Amsterdam, The Netherlands). Nucleotide sequences were read and edited in Sequencher® (Gene Codes Corporation, Ann Arbor, MI, USA). Sequences were compared with those of *Cytospora* species from previous studies available in the National Center for Biotechnology Information database GenBank®. Consensus sequences were produced and deposited into GenBank®. Phylogenetic analysis was made using ITS sequence data from isolates used in this study and relevant isolates from GenBank®. Sequences were aligned using ClustalX2 (UCD Dublin, Dublin, Ireland) software, and a phylogenetic tree was made using MEGA11 (Pennsylvania State University, Center, PA, USA) software.

2.4. Pathogenicity Tests

To determine the pathogenicity of fungal species, the same two isolates selected for molecular analysis were chosen for the inoculation of olive branches. Two pathogenicity tests were performed: inoculation on detached branches and inoculation on olive trees.

2.4.1. Pathogenicity on Detached Branches

In total, ten segments of branches per cultivar, 10 cm long, were collected from healthy olive trees of cultivars 'Buža', 'Leccino', and 'Porečka rosulja' grown at the Institute of Agriculture and Tourism in Poreč. Branch segments were rinsed under tap water, surface sterilized in 10% sodium hypochlorite for 10 min, and rinsed with sterile distilled water for 10 min. Segments were placed in a laminar flow cabinet on a sterile paper tissue. After air drying, the branch segments were marked and sealed at both ends with Parafilm to reduce desiccation. Wounds 4 mm in diameter were made in the bark with a cork borer to remove the outer bark but to leave the inner bark intact. A 4 mm diameter mycelium plug from an 8-day-old PDA culture of isolates was placed in each wound. Inoculated wounds were sealed with Vaseline and protected with Parafilm. Two replicates were performed. PDA plugs without mycelium were used as a control. Inoculated branches were placed in a plastic bag on a sterile paper tissue soaked with sterile distilled water, and incubated under laboratory conditions at approximately 21 °C for 10 weeks.

2.4.2. Pathogenicity on Olive Trees

Branches of 2-year-old olive trees of cultivars 'Buža', 'Leccino', and 'Porečka rosulja' were inoculated in a greenhouse at the Institute of Agriculture and Tourism in Poreč. Four branches per replicate, per cultivar were inoculated for each isolate. Branches were disinfected with 70% ethanol, and 4 mm diameter wounds were made in the bark with a cork borer and inoculated the same way as previously described for detached branches. Inoculated plants had been kept in a greenhouse, at approximately 25 °C, for 6 months, from March to September 2022, and monitored for the presence of symptoms. After incubation,

samples were collected. In an attempt to fulfill Koch's postulate, small pieces of necrotic tissue from the edge of the developed lesion were placed on a PDA medium to reisolate the inoculated fungus.

3. Results

3.1. Sampling and Fungal Isolation

The symptoms of the disease in the field can be described as the dieback of twigs and branches (Figure 1), brown internal necrosis on branches and under the bark, leaf necrosis, and fruit dieback. The symptoms were observed on trees from cultivars 'Porečka rosulja' and 'Buža'. Symptoms affect only part of the trees. Morphologically similar fungal colonies were retrieved from all 10 samples.

Figure 1. (**a**) Disease symptoms on olive tree in orchard. (**b**) Branch segment with bark discoloration taken for analysis.

3.2. Morphological Identification

Based on the colony and spore characteristics, 10 fungal isolates have been identified as *Cytospora pruinosa* Défago. Colonies developing on PDA had reached a nine cm diameter after 10 days at 25 °C on PDA. Colonies were irregularly shaped, with aerial, opaque, fluffy mycelium becoming flat with age. Initially, they were white becoming creamy white-beige with age with black pycnidia distributed on the surface (Figure 2). The reverse of the colonies was creamy white with an orange undertone and visible black pycnidia. Hyphae were septate, hyaline, and yellowish. Conidia were elongated, hyaline, aseptate, and smooth, and 5.6–6.8 × 1.1–1.3 µm diameter ($\bar{x}$ = 5.9 × 1.1 µm, n = 30).

3.3. Molecular Identification and Phylogenetic Analyses

Consensus sequences of representative isolates were produced and deposited in GenBank® under accession numbers: OQ642321 and OQ644501 for ITS, and OQ652101 and OQ694815 for the TUB gene region (available in Supplementary Materials). Blast analysis of the sequences from the SL2 PRIV isolate showed 100% similarity for ITS and TUB gene regions with *C. pruinosa*. Blast analysis of the sequence from the V16 BIII isolate showed 100% similarity for ITS and 99.79% similarity for the TUB gene region with *C. pruinosa*. The phylogenetic tree was constructed using the Neighbor-Joining method (Figure 3). DNA sequence analysis and phylogenetic analysis confirm the identity of SL2 PRI and V16 BIII isolates as *C. pruinosa*.

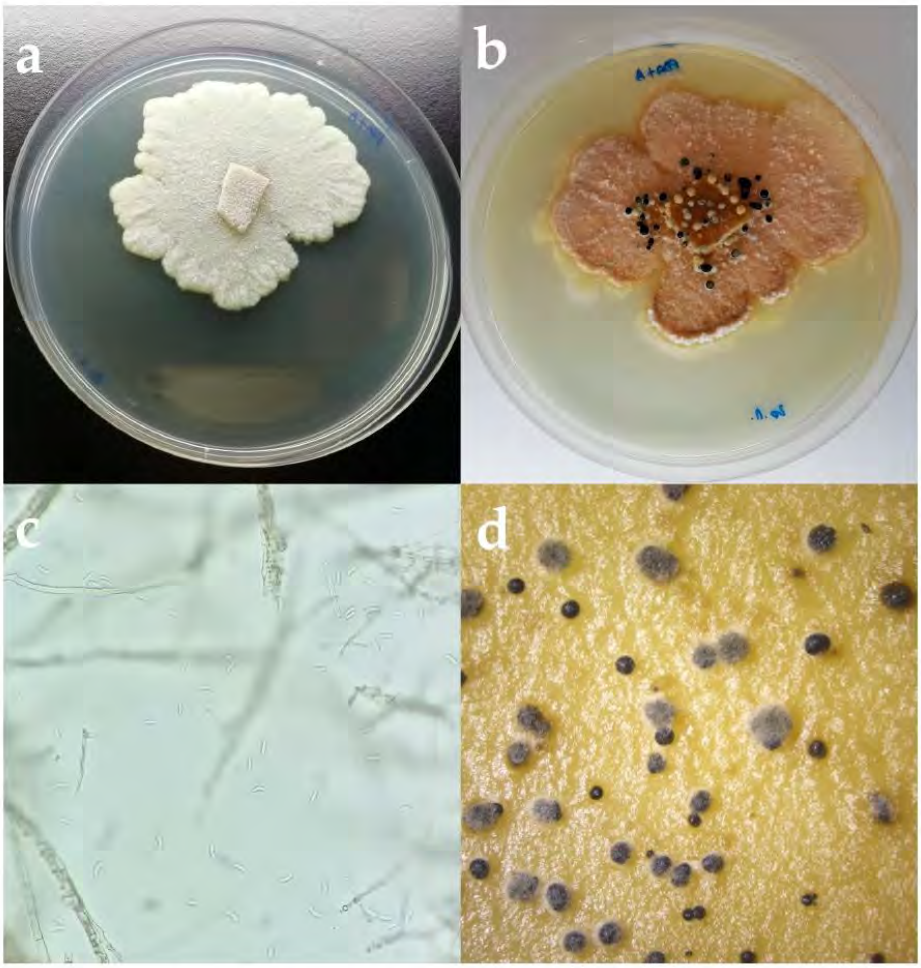


Figure 2. (**a**) *Cytospora pruinosa* colony on PDA after 5 days in the dark at 25 °C. (**b**) *C. pruinosa* colony on PDA after 2 weeks. (**c**) Hyphae and spores of *C. pruinosa* isolate under the microscope. Scale bar = 10 µm. (**d**) Conidiomata formed on PDA.

3.4. Pathogenicity Tests of Isolate

The symptoms of the disease on the olive branches tested in the laboratory and on the olive trees in the greenhouse showed the same symptoms as olive trees observed in the field survey. Dieback of twigs, brown internal necrosis on branches and under the bark, bark discoloration, and fruit collapse were detected (Figure 4). The pathogen had been consistently reisolated from all affected pieces of wood. Only saprobes were isolated from the control branches and trees.

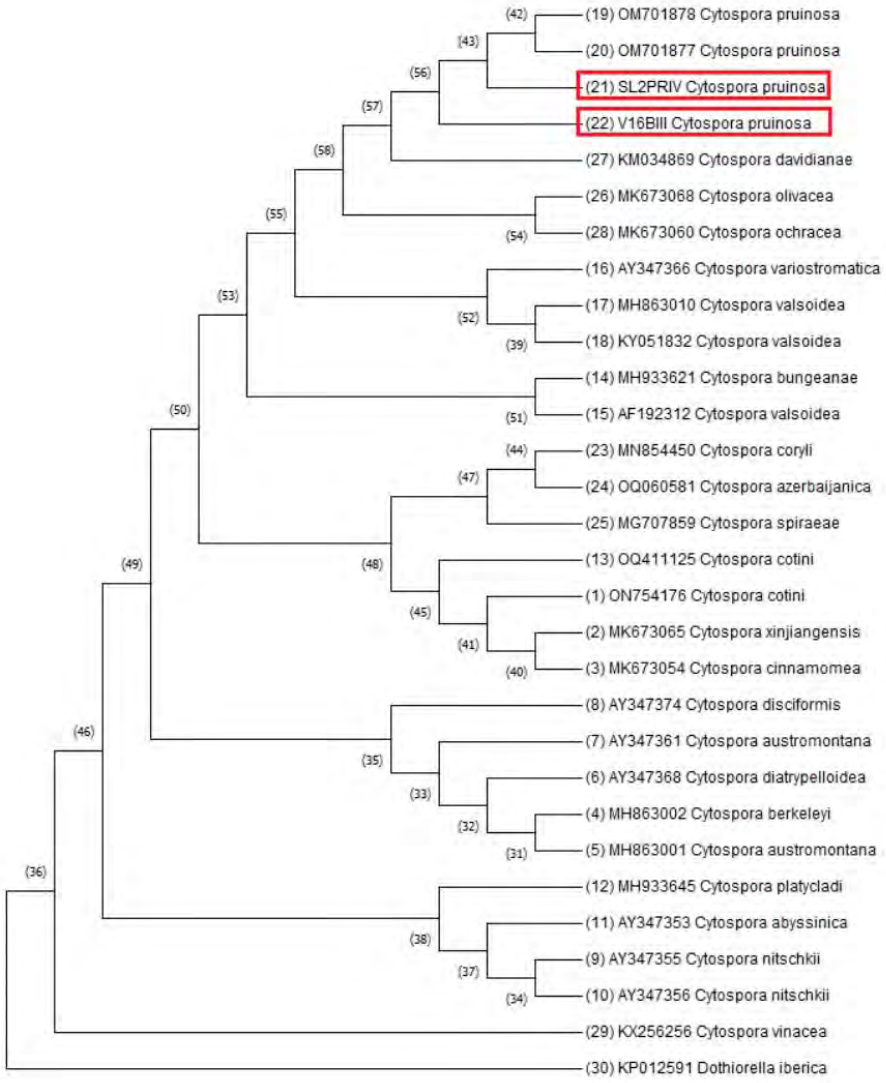


Figure 3. The evolutionary history was inferred using the Neighbor-Joining method [42]. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches [43]. The evolutionary distances were computed using the Maximum Composite Likelihood method [44] and are in the units of the number of base substitutions per site. This analysis involved 30 nucleotide sequences. *Dothiorella iberica* isolate 211 KP012591 was used as an outgroup. Sequences from this research are marked with red rectangles. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 769 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [45].

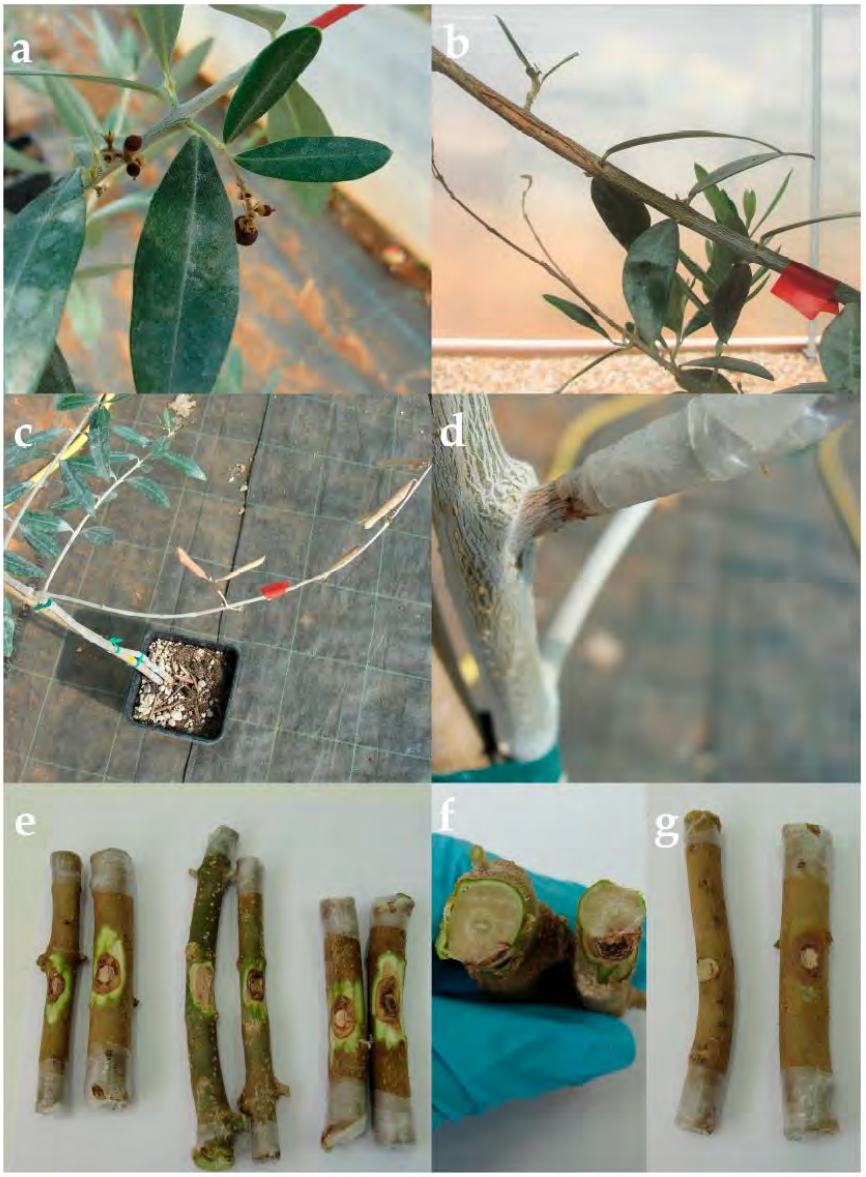


Figure 4. Disease symptoms on olive trees used in pathogenicity tests in the greenhouse after 6 months at 25 °C: (**a**) fruit collapse, (**b**) branch necrosis, (**c**) branch dieback, (**d**) bark discoloration. (**e**,**f**) Disease symptoms on olive branches used in pathogenicity tests in the laboratory: (**g**) Difference between the control branch inoculated with pure PDA plug (left) and the branch inoculated with *C. pruinosa* (right).

4. Discussion

In this research, *C. pruinosa* was identified based on morphological characteristics, molecular data of ITS and TUB gene region, and a phylogenetic tree made based on internal transcribed spacer sequence alignment. Pathogenicity tests were conducted on detached

olive branches and whole plants from three different olive cultivars. Cultivars 'Buža' and 'Porečka rosulja' were chosen for the test because all isolates were derived from those olive cultivars. Cultivar 'Leccino' was chosen as one of the most distributed and resistant olive cultivars in Istria. Lesions appeared on all tested branches and plants, except for the controls. In addition, Koch's postulate was carried out on all infected and control plants. Disease symptoms of infection of olive trees inoculated with *C. pruinosa* comprised reddish-brown discoloration of bark, stained brown discoloration below the cambium, leaf necrosis, branch, fruit, and twig dieback.

Members of *Cytospora* genus are cosmopolitan and occur on a broad host range [26]. The first record of *C. pruinosa* on olives was in 2006 in South Africa [18]. In addition to Africa [18,46] it was found as a pathogen of olives in Spain [11]. Other *Cytospora* species known as olive pathogens are *C. oleicola* D.P. Lawr., L.A. Holland & Trouillas [5,31], *C. oleina* Berl. [47], *C. olivarum* Úrbez-Torr., D.P. Lawr., Peduto, Gubler & Trouillas [5], *C. plurivora* D.P. Lawr., L.A. Holland & Trouillas, and *C. sorbicola* Norphanph., Bulgakov, T.C. Wen & K.D. Hyde [31]. Except dieback of olives, *C. pruinosa* is associated with dieback of ash tree *Fraxinus excelsior* L. *C. pruinosa* can infect ash trees weakened after primary infection by fungus *Hymenoscyphis fraxineus* (T. Kowalski) Baral, Queloz & Hosoya, or emerald ash borer *Agrilus planipennis* Fairmaire [48,49].

Moral et al. [11] reinforce the idea that inoculation in vivo is essential for the characterization of fungal pathogens. In their trial, *C. pruinosa* did not cause symptoms when tested on 5-year-old potted 'Gordal Sevilliana' olive trees in a greenhouse at 25 to 30 °C. Contradictory to their study, *C. pruinosa* formed the largest average lesion length of all the isolates used in a study conducted in 2021 in South Africa [46]. However, of the six tested isolates of *C. pruinosa*, two isolates developed long lesions with values of 41.14 mm and 36.94 mm, one isolate with 10.53 mm, while the rest had values of less than 10 mm. In total, of 58 fungal isolates comprising 38 species, *C. pruinosa* produced not only the longest lesions but also one isolate was on the bottom 13 isolates with an average lesion length of only 3.20 mm [46]. In the research carried out by Úrbez-Torres et al. [5], six months after inoculation of olive branches, *C. oleicola* and *C. olivarum* caused lesions that averaged 26.7 mm in length. All tested isolates of *C. oleina* in spring inoculations resulted in the formation of a necrotic area around the inoculation point 43 to 56 mm long, while inoculation made in autumn resulted in the death of the whole twig [47]. In this trial, six months after inoculation of trees, *C. pruinosa* caused lesions that averaged 10.4 mm on cv. Porečka rosulja, 22.4 mm on cv. Leccino, and 31.36 mm on cv. Buža. Compared to other fungal pathogens, such as species from the *Botryosphaeriaceae* family, *Cytospora* species were shown as less aggressive pathogens.

Inability to form symptoms could be explained by *C. pruinosa* previously being part of a species complex with genetic variances expected to be high. These variances could create implications regarding the variation in virulence-related genes [18,46].

Control of *Cytospora* diseases is difficult and focusing management efforts against the most aggressive encountered *Cytospora* species will be essential [31]. Preventive practices, such as proper pruning and pruning of infected parts, clean tools, removal of infected plant material from orchards, treatment of pruning wounds, and selection of resistant cultivars may be an effective preventative strategy against infection with *Cytospora* species. Mechanical pruning results in multiple cuts on trees, which greatly surpass the number of pruning wounds produced in traditionally farmed low-density orchards, so further research needs to be carried out to investigate the impact of mechanized practices on infection by *Cytospora* spp. [5].

There is no data about protection measures for *C. pruinosa* exclusively; protection measures against other *Cytospora* species have been listed. Regarding fungicides, thiophanate-methyl (alone, amended in 50% latex paint, combined with VitiSeal, and combined with latex paint at 50 and 70%), captan, 50% latex paint, lime sulfur, and VitiSeal combined with lime sulfur has the potential for reducing the species *C. leucostoma*, *C. plurivora*, and *C. sorbicola* [50–52]. In addition, antagonistic fungal species *Trichoderma viride* SC1 provide

excellent pruning wound protection against *C. sorbicola* [51]. Moreover, antagonistic assays in vitro showed that secondary metabolites of *Bacillus pumilus* Meyer and Gottheil strain JK-SX001 extracted using methylbenzene could also suppress the growth of *C. chrysosperma* (Pers.) Fr. [53].

Since there is a lack of information about the biology and pathogenicity of this species, further research needs to be carried out. Urbez-Torres [5] propose research based on spore-trapping studies combined with studies evaluating the effectiveness of pruning wound protectants for development of effective control strategies. *Cytospora* species has not been recorded on other plant species in Croatia so far. Given that spores of some of the *Cytospora* species, such as *C. leucostoma*, can be wind-blown to 76 m from the inoculum source [54], and given that *Cytospora* species are pathogens of numerous plant species, especially woods, the question arises as to the possibilities of their spread on other plants, mostly on vines, because vines and olives are often grown together in these areas.

5. Conclusions

To the best of our knowledge, this is the first report of *Cytospora pruinosa* Défago causing olive twig and branch dieback on olive trees in Croatia.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microorganisms11071679/s1. Flatfile records of the isolates from NCBI.

Author Contributions: Conceptualization, E.P. and S.G.; methodology, E.P. and S.G.; investigation, E.P. and S.G.; writing—original draft preparation, E.P.; writing—review and editing, S.G., D.I., K.V. and J.Ć. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Croatian Science Foundation Installation Research Project "Natural bioactive compounds as a source of potential antimicrobial agents in the control of bacterial and other fungal pathogens of olives", Anti-Mikrobi-OL (AMO), UIP-2020-02-7413, and "Young Researchers' Career Development Project" DOK-2021-02-2882.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All sequence data are available in NCBI GenBank following the accession numbers in the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Rapoport, H.F.; Fabbri, A.; Sebastiani, L. *Olive Biology. The Olive Tree Genome*; Springer: Cham, Switzerland, 2016; pp. 13–25.
2. Fraga, H.; Moriondo, M.; Leolini, L.; Santos, J.A. Mediterranean olive orchards under climate change: A review of future impacts and adaptation strategies. *Agronomy* **2021**, *11*, 56. [CrossRef]
3. Ponti, L.; Gutierrez, A.P.; Ruti, P.M.; Dell'Aquila, A. Fine-scale ecological and economic assessment of climate change on olive in the Mediterranean Basin reveals winners and losers. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 5598–5603. [CrossRef]
4. Mafrica, R.; Piscopo, A.; De Bruno, A.; Poiana, M. Effects of climate on fruit growth and development on olive oil quality in cultivar Carolea. *Agriculture* **2021**, *11*, 147. [CrossRef]
5. Úrbez-Torres, J.R.; Lawrence, D.P.; Hand, F.P.; Trouillas, F.P. Olive twig and branch dieback in California caused by *Cytospora oleicola* and the newly described species *Cytospora olivarum* sp. nov. *Plant Dis.* **2020**, *104*, 1908–1917. [CrossRef]
6. Food and Agriculture Organization of the United Nations. Available online: https://www.fao.org/faostat/en/#data/QCL (accessed on 5 June 2023).
7. Gluhić, D. Jesenska gnojidba vinove loze i masline. *Glas. Zast. Bilja* **2020**, *37*, 97–110. [CrossRef]
8. Žužić, I. *Maslina i Maslinovo Ulje: Sa Posebnim Osvrtom na Istru*; Olea, udruga maslinara Istarske županije; TIPOMAT: Velika Gorica, Croatia, 2008; pp. 1–380.
9. Carlucci, A.; Raimondo, M.L.; Cibelli, F.; Phillips, A.J.L.; Lops, F. *Pleurostomophora richardsiae*, *Neofusicoccum parvum* and *Phaeoacremonium aleophilum* associated with a decline of olives in southern Italy. *Phytopathol. Mediterr.* **2013**, *52*, 517–527.
10. Úrbez-Torres, J.R.; Peduto, F.; Vossen, P.M.; Krueger, W.H.; Gubler, W.D. Olive twig and branch dieback: Etiology, incidence, and distribution in California. *Plant Dis.* **2013**, *97*, 231–244. [CrossRef] [PubMed]

11. Moral, J.; Agusti-Brisach, C.; Pérez-Rodríguez, M.; Xaviér, C.; Carmen-Raya, M.; Rhouma, A.; Trapero, A. Identification of fungal species associated with branch dieback of olive and resistance of table cultivars to *Neofusicoccum mediterraneum* and *Botryosphaeria dothidea*. *Plant Dis.* **2017**, *101*, 306–316. [CrossRef] [PubMed]
12. Ivić, D.; Ivanović, A.; Miličević, T.; Cvjetković, B. Shoot necrosis of olive caused by Phoma incompta, a new disease of olive in Croatia. *Phytopathol. Mediterr.* **2010**, *49*, 414–416.
13. Cvjetković, B. *Mikoze i Pseudomikoze Voćnjaka i Vinove Loze*; Zrinski: Čakovec, Croatia, 2010; pp. 5–534.
14. Godena, S.; Ivić, D.; Goreta Ban, S. *Uzročnici Djelomičnog ili Potpunog Sušenja Stabala Maslina*; Priručnik o rezultatima VIP projekta; Institut za Poljoprivredu i Turizam: Poreč, Croatia, 2019; pp. 1–49.
15. Kaliterna, J.; Miličević, T.; Ivić, D.; Benčić, D.; Mesić, A. First report of Diplodia seriata as causal agent of olive dieback in Croatia. *Plant Dis.* **2012**, *96*, 290. [CrossRef]
16. Petrović, E.; Vrandečić, K.; Ćosić, J.; Kanižai Šarić, G.; Godena, S. First Report of *Phaeoacremonium iranianum* causing olive twig and branch dieback. *Plants* **2022**, *11*, 3578. [CrossRef] [PubMed]
17. Kaliterna, J.; Miličević, T.; Benčić, D.; Mesić, A. First report of Verticillium wilt caused by *Verticillium dahliae* on olive trees in Croatia. *Plant Dis.* **2016**, *100*, 2526. [CrossRef]
18. Adams, G.C.; Roux, J.; Wingfield, M.J. *Cytospora* species (*Ascomycota*, *Diaporthales*, *Valsaceae*): Introduced and native pathogens of trees in South Africa. *Australasian Plant Pathol.* **2006**, *35*, 521–548. [CrossRef]
19. Adams, G.C.; Roux, J.; Wingfield, M.J.; Common, R.; Roux, J. Phylogenetic relationships and morphology of *Cytospora* species and related teleomorphs (*Ascomycota*, *Diaporthales*, *Valsaceae*) from Eucalyptus. *Stud. Mycol.* **2005**, *52*, 1–144.
20. Lawrence, D.P.; Travadon, R.; Pouzoulet, J.; Rolshausen, P.E.; Wilcox, W.F.; Baumgartner, K. Characterization of *Cytospora* isolates from wood cankers of declining grapevine in North America, with the description of two new *Cytospora* species. *Plant Pathol.* **2017**, *66*, 713–725. [CrossRef]
21. Ehrenberg, C.G. *Sylvae Mycologicae Berolinenses*; Formis Theophili Bruschcke: Berlin, Germany, 1818; pp. 1–52.
22. Castlebury, L.A.; Rossman, A.Y.; Jaklitsch, W.J.; Vasilyeva, L.N. A preliminary overview of the *Diaporthales* based on large subunit nuclear ribosomal DNA sequences. *Mycologia* **2002**, *94*, 1017–1031. [CrossRef]
23. Rossman, A.Y.; Farr, D.F.; Castlebury, L.A. A review of the phylogeny and biology of the *Diaporthales*. *Mycoscience* **2007**, *48*, 135–144. [CrossRef]
24. Fan, X.; Bezerra, J.D.P.; Tian, C.M.; Crous, P.W. Families and genera of Diaporthalean fungi associated with canker and dieback of tree hosts. *Persoonia* **2018**, *40*, 119–134. [CrossRef] [PubMed]
25. Encyclopedia of Life. 2023. Available online: https://eol.org/pages/5612 (accessed on 21 April 2023).
26. Fan, X.L.; Bezerra, J.D.P.; Tian, C.M.; Crous, P.W. *Cytospora* (*Diaporthales*) in China. *Persoonia* **2020**, *45*, 1–45. [CrossRef]
27. Grove, W. The British species of *Cytospora*. *Bull. Misc. Inf.* **1923**, *1923*, 1–30. [CrossRef]
28. Spielman, L.J. A monograph of *Valsa* on hardwoods in North America. *Can. J. Bot.* **1985**, *63*, 1355–1378. [CrossRef]
29. Adams, G.C.; Surve-Iyer, R.S.; Iezzoni, A.F. Ribosomal DNA sequence divergence and group I introns within the *Leucostoma* species *L. cinctum*, *L. persoonii*, and *L. parapersoonii* sp. nov., ascomycetes that cause Cytospora canker of fruit trees. *Mycologia* **2002**, *94*, 947–967.
30. Wang, X.; Wei, J.; Huang, L.; Kang, Z. Re-evaluation of pathogens causing *Valsa* canker on apple in China. *Mycologia* **2011**, *103*, 317–324. [CrossRef] [PubMed]
31. Lawrence, D.P.; Holland, L.A.; Nouri, M.T.; Tavadon, R.; Abramians, A.; Michailides, T.J.; Trouillas, F.P. Molecular phylogeny of Cytospora species associated with canker diseases of fruit and nut crops in California, with the descriptions of ten new species and one new combination. *IMA Fungus* **2018**, *9*, 333–369. [CrossRef] [PubMed]
32. Fan, X.; Hyde, K.D.; Liu, M.; Liang, Y.; Tian, C. *Cytospora* species associated with walnut canker disease in China, with description of a new species C. gigalocus. *Fungal Biol.* **2015**, *119*, 310–319. [CrossRef] [PubMed]
33. Zhu, H.Y.; Tian, C.M.; Fan, X. Multigene phylogeny and morphology reveal *Cytospora spiraeae* sp. nov. (*Diaporthales*, *Ascomycota*) in China. *Phytotaxa* **2018**, *338*, 49–62. [CrossRef]
34. Monkai, J.; Tibpromma, S.; Manowong, A.; Mapook, A.; Norphanphoun, C.; Hyde, K.D.; Promputtha, I. Discovery of three novel Cytospora species in Thailand and their antagonistic potential. *Diversity* **2021**, *13*, 488. [CrossRef]
35. Petrini, O. Fungal endophytes of tree leaves. In *Microbial Ecology of Leaves*; Andrews, J.H., Hirano, S.S., Eds.; Springer: New York, NY, USA, 1991; pp. 179–197.
36. Lazarevic, J.; Menkis, A. *Cytospora friesii* and *Sydowia polyspora* are associated with the sudden dieback of *Abies concolor* in Southern Europe. *Plant Prot. Sci.* **2022**, *58*, 258–263. [CrossRef]
37. Tekauz, A.; Patrick, Z. The role of twig infections on the incidence of perennial canker of peach. *Phytopathology* **1974**, *64*, 683–688. [CrossRef]
38. Biggs, A.R. Integrated approach to controlling Leucostoma canker of peach in Ontario. *Plant Dis.* **1989**, *73*, 869–874. [CrossRef]
39. Chang, L.S.; Iezzoni, A.F.; Adams, G.C.; Ewers, F.W. Hydraulic conductance in susceptible versus tolerant peach seedlings infected with *Leucostoma persoonii*. *J. Am. Soc. Hortic. Sci.* **1991**, *116*, 831–834. [CrossRef]
40. White, T.J.; Bruns, T.D.; Lee, S.B.; Taylor, J.W. 38—Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics. In *PCR—Protocols and Applications—A Laboratory Manual*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press: Cambridge, MA, USA, 1990; pp. 315–322.

41. Glass, N.L.; Donaldson, G.C. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl. Environ. Microbiol.* **1995**, *61*, 1323–1330. [CrossRef] [PubMed]
42. Saitou, N.; Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **1987**, *4*, 406–425.
43. Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **1985**, *39*, 783–791. [CrossRef] [PubMed]
44. Tamura, K.; Nei, M.; Kumar, S. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11030–11035. [CrossRef]
45. Tamura, K.; Stecher, G.; Kumar, S. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [CrossRef]
46. van Dyk, M.; Spies, C.F.J.; Mostert, L.; van der Rijst, M.; du Plessis, I.L.; Moyo, P.; van Jaarsveld, W.J.; Haleen, F. Pathogenicity testing of fungal isolates associated with olive trunk diseases in South Africa. *Plant Dis.* **2021**, *105*, 4060–4073. [CrossRef]
47. Rumbos, I.C. *Cytospora oleina* causing canker and dieback of olive in Greece. *Plant Pathol.* **1988**, *37*, 441–444. [CrossRef]
48. Kowalski, T.; Bilanski, P.; Kraj, W. Pathogenicity of fungi associated with ash dieback towards *Fraxinus excelsior*. *Plant Pathol.* **2017**, *66*, 1228–1238. [CrossRef]
49. Rajtar, N.N.; Held, B.W.; Blanchette, R.A. Fungi from galleries of the emerald ash borer produce cankers in ash trees. *Forests* **2021**, *12*, 1509. [CrossRef]
50. Miller, S.T.; Otto, K.L.; Sterle, D.; Minas, I.S.; Stewart, J.E. Preventive fungicidal control of *Cytospora leucostoma* in peach orchards in Colorado. *Plant Dis.* **2019**, *103*, 1138–1147. [CrossRef] [PubMed]
51. Holland, L.A.; Travadon, R.; Lawrence, D.P.; Nouri, M.T.; Trouillas, F. Evaluation of pruning wound protection products for the management of almond canker diseases in California. *Plant Dis.* **2021**, *105*, 3365–3375. [CrossRef] [PubMed]
52. Miller, S.T.; Sterle, D.; Minas, I.S.; Stewart, J.E. Exploring fungicides and sealants for management of *Cytospora plurivora* infections in western Colorado peach production systems. *Crop Prot.* **2021**, *146*, 105654. [CrossRef]
53. Ren, J.H.; Li, H.; Wang, Y.F.; Ye, J.R.; Yan, A.Q.; Wu, X.W. Biocontrol potential of an endophytic *Bacillus pumilus* JK-SX001 against poplar canker. *Biol. Control.* **2013**, *67*, 421–430. [CrossRef]
54. Bertrant, P.F.; English, H. Release and dispersal of conidia of Valsa leucostoma. *Phytopathology* **1976**, *66*, 987–991. [CrossRef]

Naslov izvornog znanstvenog rada broj 2: First Report of Olive Branch Dieback in Croatia Caused by *Cytospora pruinosa* Défago

Proširenи sažetak:

U 2021. godini, u dva maslinika u Istri, Hrvatska, primijećeni su simptomi sušenja grana i plodova maslina. Prvi maslinik, smješten u Kašteliru, prostire se na 6,5 ha i sadrži 44 različite sorte maslina, dok se drugi, u Vodnjanu, prostire na 1 ha i sadrži dvije sorte maslina. Simptomi su zabilježeni na lokalnim sortama Porečka rosulja i Buža. Uzorci simptomatičnih stabala prikupljeni su i analizirani. Izolirane gljive su, na temelju morfoloških karakteristika, identificirane kao pripadnici roda *Cytospora*. Dva reprezentativna izolata, po jedan iz svakog maslinika, podvrgnuta su molekularnoj analizi. Sekvenciranjem ITS i TUB2 regija te filogenetskom analizom, izolati su identificirani kao *Cytospora pruinosa* Défago. Za potvrdu patogenosti, provedeni su testovi na odrezanim granama u laboratoriju i dvogodišnjim stablima maslina u plasteničkim uvjetima. Rezultati su pokazali da *C. pruinosa* uzrokuje simptome sušenja grana i plodova, potvrđujući njezinu patogenost na maslini. Ovo je prvi zabilježeni slučaj u Hrvatskoj koji potvrđuje *C. pruinosa* kao uzročnika sušenja grana i plodova masline.

Ključne riječi: rak; *Cytospora* sp.; gljivična bolest; *Olea europaea* L.

Izvorni znanstveni rad broj 3 u obliku i izvornom jeziku na kojem je objavljen u znanstvenom časopisu

Naslova rada: First Report of *Nigrospora* Species Causing Leaf Spot on Olive (*Olea europaea* L.)

Autori: Elena Petrović, Karolina Vrandečić, Jasenka Ćosić, Edyta Đermić, Sara Godena

Tip rada: Izvorni znanstveni članak

Časopis: Horticulturae

Kategorija: A1

Impakt faktor: 3,1 (2023.)

Kvartil: Q1

Primljen na recenziju: 23. kolovoz 2023.

Prihvaćen za objavljivanje: 19. rujna 2023.

Status: Objavljen

Volumen: 9

Broj: 10

Broj rada: 1067

WOS broj: 001099372900001

horticulturae

MDPI

Article

First Report of *Nigrospora* Species Causing Leaf Spot on Olive (*Olea europaea* L.)

Elena Petrović [1], Karolina Vrandečić [2], Jasenka Ćosić [2], Edyta Đermić [3] and Sara Godena [1,*]

[1] Institute of Agriculture and Tourism, Karla Huguesa 8, 52440 Poreč, Croatia; elena@iptpo.hr
[2] Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Vladimira Preloga 1, 31000 Osijek, Croatia; kvrandecic@fazos.hr (K.V.); jcosic@fazos.hr (J.Ć.)
[3] Faculty of Agriculture, University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia; edermic@agr.hr
* Correspondence: sara@iptpo.hr

Abstract: Leaf spot symptoms were spotted in two olive orchards in Istria and in Kvarner Gulf, Croatia. Fungal species from three representative isolates (P13 LECIII, R18 BI, JA20 NP) have been morphologically characterized based on the colony and conidial characteristics. Several techniques were performed for inducing the sporulation of the JA20 NP isolate. Only PDA + banana medium was successful. PCR was conducted for ITS, TUB, and EF1α gene regions. Phylogenetic analyses were performed using internal transcribed spacer, beta-tubulin, and translation elongation factor 1-alpha sequence data. Three types of tests were conducted: a pathogenicity test on detached leaves, on detached and scratched leaves, and on olive seedlings. Ultimately, from the morphological characterizations, DNA sequence analysis of ITS, TUB, and EF1α gene regions, and phylogenetic analysis, these species were identified as *Nigrospora gorlenkoana* Novobr., *Nigrospora osmanthi* Mei Wang & L. Cai, and *Nigrospora philosophiae-doctoris* M. Raza, Qian Chen & L. Cai. This is the first report of *Nigrospora* species causing leaf spot on olive trees and the first report of *Nigrospora philosophiae-doctoris* as a plant pathogen. Fungal leaf diseases in conditions that are favorable for infection and disease development can lead to a decrease in the yield and olive oil quality. Therefore, it is necessary to conduct further research and the monitoring of fungal leaf diseases.

Keywords: fungal disease; isolate; olive tree; pathogenicity

Citation: Petrović, E.; Vrandečić, K.; Ćosić, J.; Đermić, E.; Godena, S. First Report of *Nigrospora* Species Causing Leaf Spot on Olive (*Olea europaea* L.). *Horticulturae* **2023**, *9*, 1067. https://doi.org/10.3390/horticulturae9101067

Academic Editor: Zhi Li

Received: 23 August 2023
Revised: 18 September 2023
Accepted: 19 September 2023
Published: 22 September 2023

1. Introduction

The olive tree, *Olea europaea* L., is among the world's most important crops. At present, the approximate production levels per year are 23.0 million tons of olives and 3.0 million tons of oil [1]. The crop is indigenous to the Mediterranean region with a mild, rainy winter and a hot, dry summer [2]. In the Republic of Croatia, the production area includes from the north of the Istrian peninsula, the Kvarner Gulf, and the Dalmatia coastal belt with the islands to the south. In recent years, interest in olive oil production has been rising due to certain socio-economic factors, such as a higher demand for olive oil, the possibility of achieving higher prices, and tourism development.

Among all fungal pathogens affecting olives, *Venturia oleaginea* (Castagne) Rossman & Crous and *Pseudocercospora cladosporioides* (Sacc.) U. Braun are two of the most important pathogens causing leaf spots: peacock spot disease (syn. bird's-eye spot, olive leaf scab, olive leaf spot) and cercosporiosis (syn. cercospora leaf spot) [3]. Olive leaf spots caused by *V. oleaginea* and *P. cladosporioides* result in the defoliation of leaves and weakness or death of branches, a reduced fruit set, and a decrease in the oil yield in the following years [4–8].

Other fungal causal agents of leaf spot symptoms on olives are *Alternaria alternata* (Fr.) Keissl. [9], *Colletotrichum acutatum* J.H. Simmonds and *C. gloeosporioides* (Penz.) Penz. & Sacc. [10], *Neofabraea kienholzii* (Seifert, Spotts & Levesque) Spotts, Levesque & Seifert, and *Phylctema vagabunda* Desmazières [11].

Leaf spot usually manifests as small, circular-to-elliptical spots on the leaves. Spots are initially green or yellow-green but gradually turn brown or black as the disease progresses. They often have a dark border and may have a yellow halo around them. In severe cases, the spots can merge, leading to significant defoliation. Leaf spot fungi usually thrive in warm and humid conditions. Rain or overhead irrigation can facilitate the spread of the disease by splashing fungal spores onto healthy leaves. The disease exhibits its highest activity during the wetter months of the year. While leaf spot primarily affects the leaves, a severe infection can weaken the tree and reduce its overall vitality. In severe cases, defoliation can expose fruit to direct sunlight, leading to sunburn and reduced fruit quality. Leaf spot diseases also weaken trees by interrupting the process of photosynthesis. This means the tree produces less energy, which can lead to stunted growth and smaller and less flavorful olives. This can be problematic for olive growers who rely on high-quality fruit for oil production or table olives. Leaf spot diseases can also affect the appearance of olive trees, which may be a concern for growers who want their orchard to be visually appealing. Management practices, such as maintaining good tree spacing and air circulation, pruning, avoiding excessive moisture on the leaves, proper disposal of infected leaves and pruning debris, planting resistant cultivars, etc., can help to maintain the health and productivity of olive orchards.

This study focuses on fungal species from the *Nigrospora* genus, new causal agents of a leaf spot symptom on olives. The *Nigrospora* genus has been introduced in 1902. for *N. panici*, which was isolated as an endophyte from leaves of *Panicum amphibium* in Java, Indonesia [12]. Based on its conidial characteristics, *Nigrospora* was placed in *Dermateaceae* (*Moniliales*) by Barnett and Hunter [13]. Kirk et al. [14] assigned *Nigrospora* and its *Khuskia* sexual morph to *Trichosphaeriaceae* (*Trichosphaeriales*). Wang et al. [15] placed the *Nigrospora* species in the family *Apiosporaceae* based on the phylogenetic analyses of combined ITS, TUB, and EF1-α sequence data of 165 strains from China and Europe [16].

Currently, there are 45 records of *Nigrospora* species in the MycoBank database, namely *Nigrospora aerophila*, *N. arundinacea*, *N. aurantiaca*, *N. bambusae*, *N. brasiliensis*, *N. camelliae-sinensis*, *N. canescens*, *N. chinensis*, *N. cooperae*, *N. covidalis*, *N. endophytica*, *N. falsivesicularis*, *N. gallarum*, *N. globosa*, *N. globospora*, *N. gorlenkoana*, *N. gorlenkoanum*, *N. gossypii*, *N. guangdongensis*, *N. guilinensis*, *N. hainanensis*, *N. javanica*, *N. lacticolonia*, *N. macarangae*, *N. magnoliae*, *N. manihoticola*, *N. maydis*, *N. musae*, *N. oryzae*, *N. osmanthi*, *N. padwickii*, *N. panici*, *N. pernambucoensis*, *N. philosophiae-doctoris*, *N. pyriformis*, *N. rubi*, *N. sacchari*, *N. sacchari-officinarum*, *N. saccharicola*, *N. singularis*, *N. sphaerica*, *N. vesicularifera*, *N. vesicularis*, *N. vietnamensis*, and *N. zimmermanii* [17].

Until now, only *Nigrospora oryzae* was isolated from olive trees [18,19], but it was not described as a pathogen on olives.

The aims of this research were to determine the causal agent of leaf spot symptoms on olive trees in Croatia, to morphologically characterize the fungal species from representative isolates, to molecularly identify the isolates of phytopathogenic fungal species using PCR and DNA sequence analysis of ITS, TUB, and EF1α gene regions, and to determine isolate pathogenicity in pathogenicity tests conducted in the laboratory and in a greenhouse experiment.

2. Materials and Methods

2.1. Collection of Plant Materials and Fungal Isolations

During the summer and autumn of 2021, leaf spot symptoms were spotted on olive trees on the coastal belt in Jadranovo, Kvarner Gulf (45°13′46″ N, 14°36′40″ E), and in olive orchards in Vabriga (45°17′14″ N, 13°36′41″ E) and Španidiga (45°03′02.2″ N, 13°42′43.9″ E) in Istria, Croatia (Figure 1).

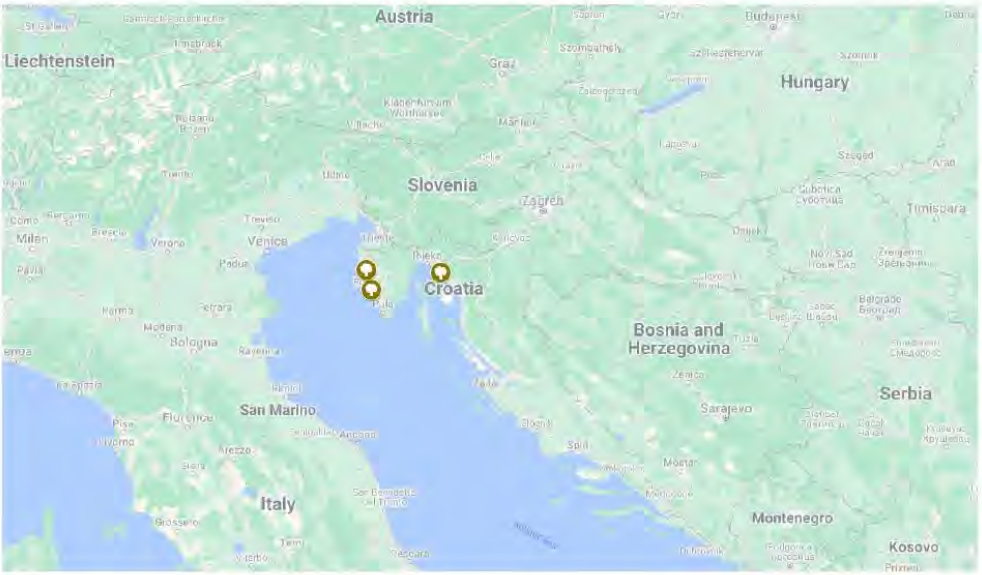


Figure 1. Locations of collected samples: Vabriga, and Špainidiga in Istria, and Jadranovo in Kvarner Gulf.

The symptoms were yellow and brown spots on leaves and defoliation. The samples from symptomatic trees were collected (30 leaves from each tree) in a sterile plastic bag, placed in a portable refrigerator at +4 °C, and immediately brought to the Laboratory for Plant Protection at the Institute of Agriculture and Tourism in Poreč (Croatia) for analysis. Olive varieties on which samples were collected in Istria were 'Buža' and 'Leccino'. The olive variety on which samples were collected in Kvarner Gulfs remains unknown.

Fresh olive leaves were used to isolate the causal agent of yellow and brown spots on leaves. For surface sterilization, leaves were rinsed under tap water for 1 min and transferred to aseptic conditions. Leaves were submerged in 70% ethanol for two minutes, rinsed in sterile distilled water, and placed on a sterile paper sheet in a laminar flow cabinet to surface-dry. Entire leaves were plated on potato dextrose agar (PDA) supplemented with 35 mg/L of penicillin and incubated at 25 °C under dark conditions. After seven days of incubation, the isolates were transferred into the fresh PDA medium for pure culture.

2.2. Morphological Characterization

After 7 and 30 days of incubation at 28 °C in dark conditions, pure fungal cultures were taken for examination. Fungal species, from three representative isolates (P13 LECIII, R18 BI, JA20 NP), have been characterized based on the colony characteristics (color, form, elevation, margin, surface, and opacity) and conidial characteristics (color, shape, presence or absence of septum, dimensions). A Boeco BM-2000 microscope, Boeco BCAM10 camera, and B-View software (Boeckel + Co (GmbH + Co), Hamburg, Germany) were used to capture conidia and hyphae. Isolate JA20 NP did not sporulate on PDA. Several techniques were performed for inducing the sporulation of the JA20 NP isolate. Pine needle medium was prepared according to Su et al. [20]. The banana peel technique was performed based on Kindo et al. [21]. The PDA + banana medium was prepared based on a technique for the pine needle medium described in Su et al. [20], by putting 100 g of fresh banana peel (instead of pine needle) and 20 g of potatoes into 1 L of distilled water, boiling for 30 min, filtrating, and keeping the volume at 1 L by adding distilled water. After filtration, it was amended with 20 g of agar and autoclaved for 20 min at 121 °C.

2.3. DNA Extraction, Amplification, and Sequencing

Fresh fungal mycelia of fungal isolates grown on PDA for 5 days, at 28 °C, under dark conditions, were scraped with a sterile laboratory needle from the colony margins and used for genomic DNA (deoxyribonucleic acid) extraction. Total DNA from the isolate was extracted using the Extract-N-Amp™ Plant PCR kit (Sigma-Aldrich, Merck, Saint Louis, MO, USA) according to manufacturer's protocol. The PCR (polymerase chain reaction) amplification process was performed using ITS1/ITS4 [22], ITS5/ITS4 [23], Btub2Fd/Btub4Rd [24], and EF1-728F/EF1-986R [25] pairs of primers (Table 1). The PCR reaction mixture was composed of 12.5 µL of EmeraldAmp® GT PCR Master Mix, 0.5 µL (10 µM) of each primer, 6.5 µL of nuclease-free water, and 5 µL (4.5 ng/µL) of genomic DNA. The PCR was conducted in a SureCycler 8800 Thermal Cycler (Agilent Technologies, Santa Clara, CA, USA) using different PCR conditions for ITS, TUB, and EF1α gene regions (Table 2). In the case of the R18 BI isolate, amplification of the EF1-alpha region was unsuccessful. Therefore, a second PCR was conducted using 1 µL of the initial PCR amplification as the template. Electrophoresis was performed using 1% agarose gel amended with two drops of GelRed® Nucleic Acid Gel Stain (Biotium, Fremont, CA, USA) at 110 V for 30 min in 1x TAE buffer with a BIO-RAD Power Pac 300 electrophoresis power supply (Agilent, Santa Clara, CA, USA). After electrophoresis, the PCR products were visualized using an iBright CL1000 Imaging System (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). Purification of PCR products was performed with the GenElute™ PCR Clean-Up Kit (Sigma-Aldrich®, Burlington, MA, USA).

Table 1. List of the primers used for PCR and sequencing.

Locus	Primer	Sequence (5′-3′)
Internal transcribed spacer (ITS)	ITS1	5′ TCCGTAGGTGAACCTGCGG 3′
	ITS4	5′ TCCTCCGCTTATTGATATGC 3′
	ITS5	5′ GGAAGTAAAAGTCGTAACAAGG 3′
Beta-tubulin	Btub2Fd	5′ AACATGCGTGAGATTGTAAGT 3′
	Btub4Rd	5′ TAGTGACCCTTGGCCCAGTTG 3′
Translation elongation factor 1-alpha	EF1-728F	5′ CATCGAGAAGTTCGAGAAGG 3′
	EF1-986R	5′ TACTTGAAGGAACCCTTACC 3′

Table 2. PCR amplification program for ITS and EF1α region sets, according to White et al. [22], and for ITS and TUB region sets, according to Hao et al. [26].

ITS1/ITS4 and EF-728F/EF-986R						
HOT START 95 °C	Start Cycle 34 times	Denaturation 95 °C	Annealing 58 °C	Elongation 72 °C	End Cycle	Elongation 72 °C
3 min		30 s	30 s	1 min		10 min
ITS5/ITS4, and Btub2Fd/Btub4Rd						
HOT START 95 °C	Start Cycle 34 times	Denaturation 95 °C	Annealing 58 °C	Elongation 72 °C	End Cycle	Elongation 72 °C
3 min		30 s	30 s	1 min		10 min

2.4. DNA Sequence Assembly and Phylogenetic Analysis

Sequencing of the PCR products was performed by Macrogen Europe (Amsterdam, The Netherlands). Sequences were edited in Sequencher® (Gene Codes Corporation, Ann Arbor, MI, USA) and compared with sequences from GenBank®.

The phylogenetic trees were constructed and the evolutionary history of the isolated fungi was concluded based on the Neighbor-joining method [27]. Phylogenetic analysis was

performed using ITS, TUB, and EF1α sequence data from isolates and relevant sequence data of *Nigrospora* and *Botryosphaeria dothidea* (outgroup) isolates from GenBank® (a list of isolates used is presented in Table 3). The sequences were aligned using ClustalX2 (UCD Dublin, Dublin, Ireland) software, and a phylogenetic tree was made using MEGA11 software (Pennsylvania State University, State College, PA, USA).

Table 3. Genbank accession numbers of isolates used for phylogenetic analysis based on research carried out by Chen et al. [28].

Species	GenBank Accession Number			References
	ITS	TUB	TEF1α	
Botryosphaeria dothidea	AY236949	AY236927	AY236898	[29]
Nigrospora aurantiaca	KX986064	KY019465	KY019295	[15]
	MN215771	MN329935	MN264010	[30]
N. bambusae	KY385307	KY385319	KY385313	[15]
	KY385306	KY385320	KY385314	[15]
N. brasiliensis	KY569629	MK720816	MK753271	[31]
	KY569630	MK720817	MK753272	[31]
N. camelliae-sinensis	KX985986	KY019460	KY019293	[15]
	MN215775	MN329939	MN264014	[30]
N. chinensis	KX986023	KY019462	KY019422	[15]
	KX986026	KY019548	KY019445	[15]
N. covidalis	OK335209	OK431479	OK431485	[28]
	OK335210	OK431480	OK431486	[28]
N. falsivesicularis	MN215778	MN329942	MN264017	[30]
	MN215779	MN329943	MN264018	[30]
N. globospora	OK335211	OK431481	OK431487	[28]
	OK335212	OK431482	OK431488	[28]
N. gorlenkoana	KX986048	KY019456	KY019420	[15]
N. guilinensis	KX985983	KY019459	KY019292	[15]
	KX986063	KY019608	KY019404	[15]
N. hainanensis	KX986091	/	KY019415	[15]
	MN215780	MN329944	MN264019	[30]
N. lacticolonia	KX985978	KY019458	KY019291	[15]
	/	MN329948	MN264023	[30]
N. musae	KX986076	KY019455	KY019419	[15]
	KX986042	KY019567	KY019371	[15]
N. oryzae	KX985931	KY019601	KY019396	[15]
	KX985954	KY019481	KY019307	[15]
N. osmanthi	KX986010	KY019461	KY019421	[15]
	KX986017	KY019540	KY019438	[15]
N. philosophiae-doctoris	OK335213	OK431483	OK431489	[28]
	OK335214	OK431484	OK431490	[28]

Table 3. *Cont.*

Species	GenBank Accession Number			References
	ITS	TUB	TEF1α	
N. pyriformis	KX985940	KY019457	KY019290	[15]
	MN215787	MN329988	MN264026	[30]
N. rubi	KX985948	KY019475	KY019302	[15]
N. sacchari-officinarum	MN215791	MN329954	MN264030	[30]
	MN215792	MN329955	MN264031	[30]
N. saccharicola	MN21578	/	MN264027	[30]
	MN215789	MN329952	MN264028	[30]
N. singularis	MN215793	MN329956	MN264032	[30]
	MN215794	MN329957	MN264033	[30]
N. sphaerica	KX985965	KY019492	KY019318	[15]
	MN215811	MN329974	MN264050	[30]
N. vesicularifera	MN215812	MN329975	MN264051	[30]
	MN215814	MN329977	MN264053	[30]
N. vesicularis	KX986088	KY019463	KY019294	[15]
	KX985939	KY019467	/	[15]
N. zimmermanii	KY385309	KY385317	KY385311	[15]
	MN215824	MN329987	MN264063	[30]

2.5. Pathogenicity Test

Three pathogenicity tests were conducted to determine the isolates' pathogenicity: pathogenicity test on detached leaves, pathogenicity test on detached and scratched leaves, and pathogenicity test on olive seedlings.

2.5.1. Pathogenicity Test on Detached Leaves and Scratched Detached Leaves

In May 2022, healthy olive leaves of the cultivars Leccino and Buža were collected from a collection orchard at the Institute of Agriculture and Tourism in Poreč (Istria, Croatia). Leaves were washed with tap water, surface-sterilized in 1% sodium hypochlorite solution for three minutes, rinsed with sterilized distilled water for one minute, and placed in a laminar flow cabinet, on sterile paper, until dry. After air-drying, ten leaves of each cultivar (per isolate), scratched with a needle, and ten unscratched leaves of each cultivar (per isolate), were inoculated by placing a 5 mm-diameter mycelium plug, taken from the margins of a five-day-old PDA culture of the isolates. The same numbers of scratched and unscratched leaves inoculated using pure PDA agar plugs were used as controls. Leaves were placed on a sterile filter paper, sprayed with sterile distilled water, in a Petri dish, and protected with parafilm. Two replicates were performed. Leaves were incubated at 28 °C, in dark conditions. After nine days, samples were analyzed.

2.5.2. Pathogenicity Test on Olive Seedlings

For the whole plant assay, the fungus was grown on PDA at 28 °C for five days, and mycelia were collected. Approximately one gram of mycelia was grinded in 10 mL of sterilized distilled water. The grinded mycelia were homogenized by mixing with a vortex mixer. In the greenhouse of the Institute of Agriculture and Tourism, the grinded mycelia were injected into the petiole of the leaves of three-year-old olive seedlings of cultivar Rosinjola. Thirty leaves (ten per isolate) were inoculated with a grinded mycelium. An equal number of control leaves were inoculated with sterile distilled water to serve as a

negative control. Two replicates were performed. The inoculated plants were kept in a greenhouse for two weeks. After the incubation period, samples from all tests were collected and, in an effort to adhere to Koch's postulate, small sections of necrotic tissue from the periphery of lesions were excised and placed on PDA to isolate the inoculated fungus.

3. Results

3.1. Field Survey and Disease Symptoms

Symptoms of leaf spot were observed on mature 10- to 38-year-old trees in Istria, Croatia. Leaves were dry and yellowish to chocolate-brown in color (Figure 2). Some of them had brown spots. Defoliation was observed on leaves with expanded spots, and symptoms affected only part of the trees.

Figure 2. Symptoms on olive leaves, (**a**) *Nigrospora gorlenkoana*, (**b**) *Nigrospora osmanthi*, (**c**,**d**) *Nigrospora philosophiae-doctoris*.

3.2. Molecular Phylogenetic Identification

For molecular identification, consensus sequences of isolates were produced and registered in GenBank®. GenBank® accession numbers for each isolate and its genome region are represent in Table 4.

Table 4. GenBank accession numbers of the sequences.

SPECIES	ISOLATE	COLLECTION DATE	Varieties	Genbank Accession Number		
				ITS	TUB	Ef1α
Nigrospora gorlenkoana	P13 LECIII	24 September 2021	Leccino	OP999642	OQ286068	OQ286069
Nigrospora osmanthi	JA20 NP	31 October 2021	Unknown	OP999639	OQ275027	OQ275028
Nigrospora philosophiae-doctoris	R18 BI	14 October 2021	Buža	OP999644	OQ286067	OQ286066

BLAST analysis of the sequences from the P13 LECIII isolate showed 100% similarity for ITS and TUB and 99.27% similarity for the EF1α gene region to *N. gorlenkoana*. BLAST

analysis of the sequences from the JA20 NP isolate showed 100% similarity for ITS, TUB, and EF1α gene regions to *N. osmanthi*. BLAST analysis of the sequences from the R18 BI isolate showed 100% similarity for ITS and TUB and 98.64% similarity for the EF1α gene region to *N. philosophiae-doctoris*. Phylogenetic trees were constructed by aligning ITS, TUB, and EF1α sequences, and the evolutionary history was inferred using the Neighbor-Joining method [27]. Finally, a multilocus tree was created from a combination of ITS, TUB, and EF1α sequence alignments. The optimal trees are displayed in Figures 3–6. Beneath the branches are the percentages of replicate trees where related taxa clustered together in the bootstrap test based on 1000 replicates [32]. The Maximum Composite Likelihood method [33] was used to calculate evolutionary distances, expressed in the units of base substitutions per site. The *Botryosphaeria dothidea* isolate CMW8000 was used as the outgroup. Ambiguous positions were excluded via pairwise detection using MEGA11 software [34].

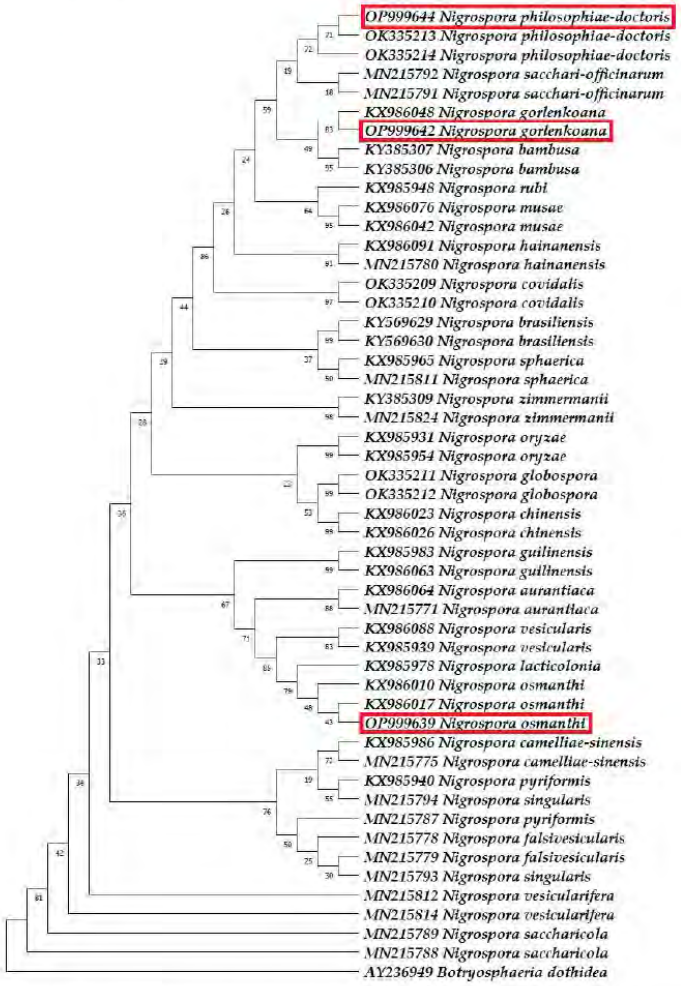


Figure 3. Phylogenetic tree based on internal transcribed spacer sequence alignment. Sequences identified in this research are highlighted with red rectangles. This analysis encompassed 51 nucleotide sequences, resulting in a final dataset comprising 595 positions.

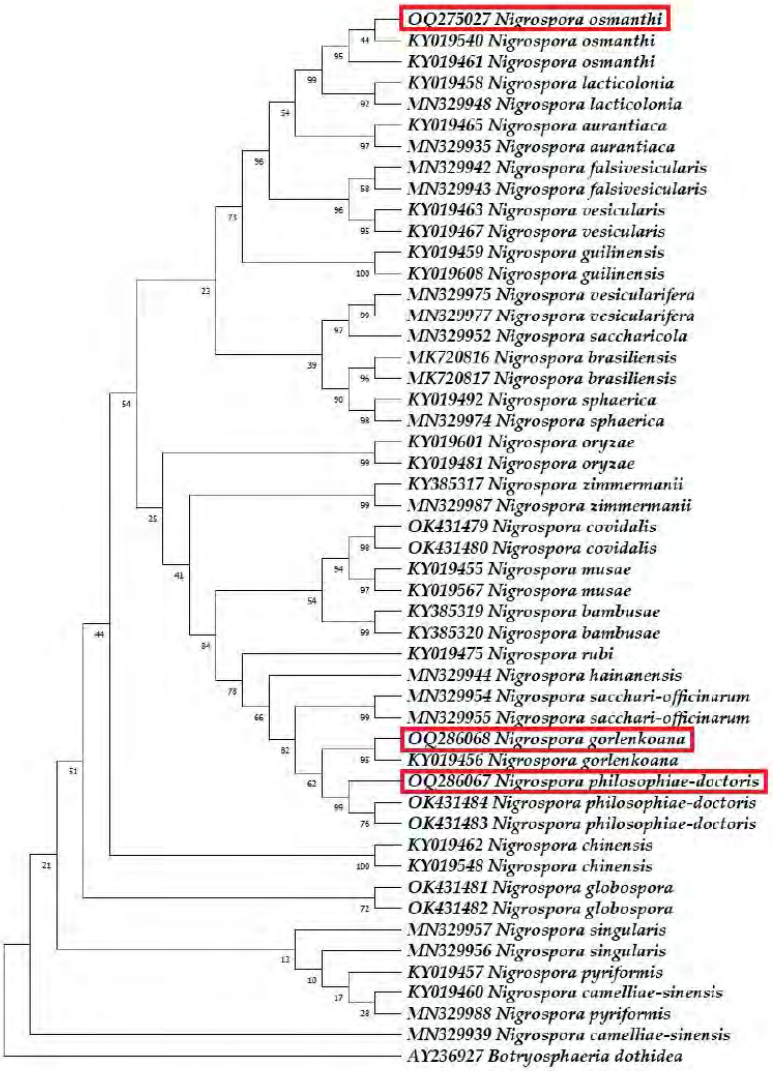


Figure 4. Phylogenetic tree based on beta-tubulin sequence alignment. Sequences identified in this research are highlighted with red rectangles. This analysis encompassed 50 nucleotide sequences, resulting in a final dataset comprising 808 positions.

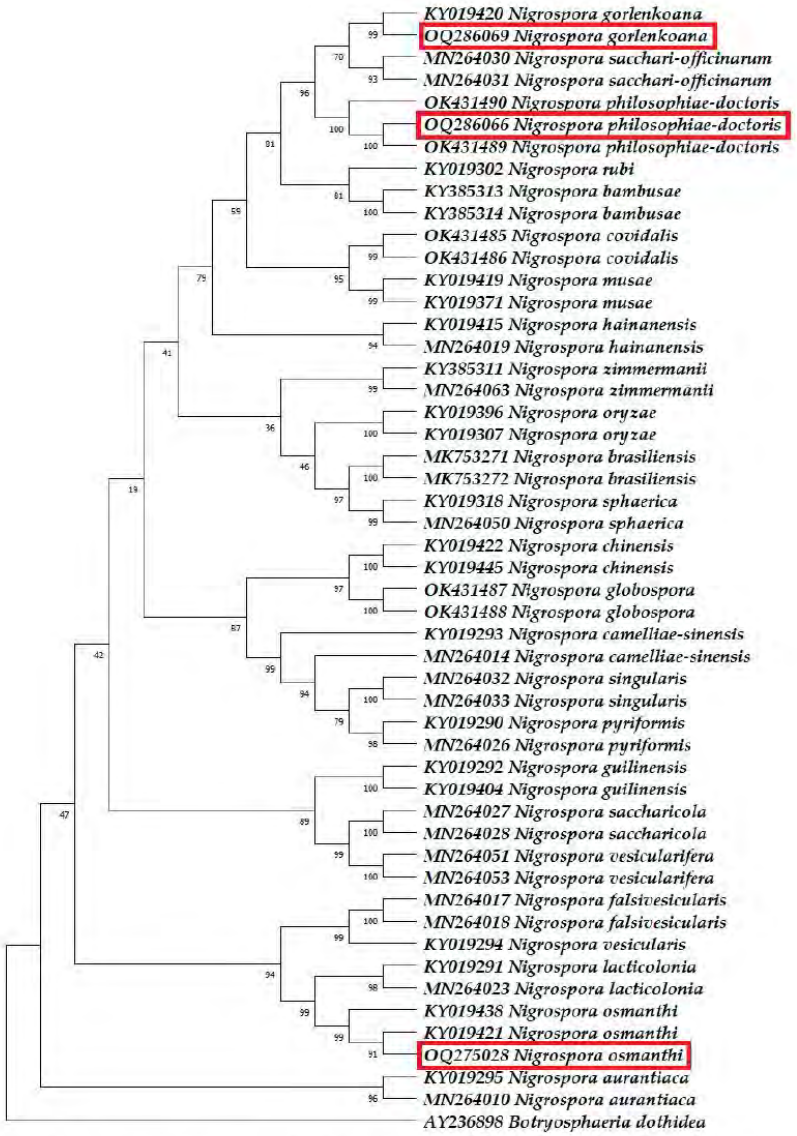


Figure 5. Phylogenetic tree based on translation elongation factor 1-alpha sequence alignment. Sequences identified in this research are highlighted with red rectangles. This analysis encompassed 51 nucleotide sequences, resulting in a final dataset comprising 567 positions.

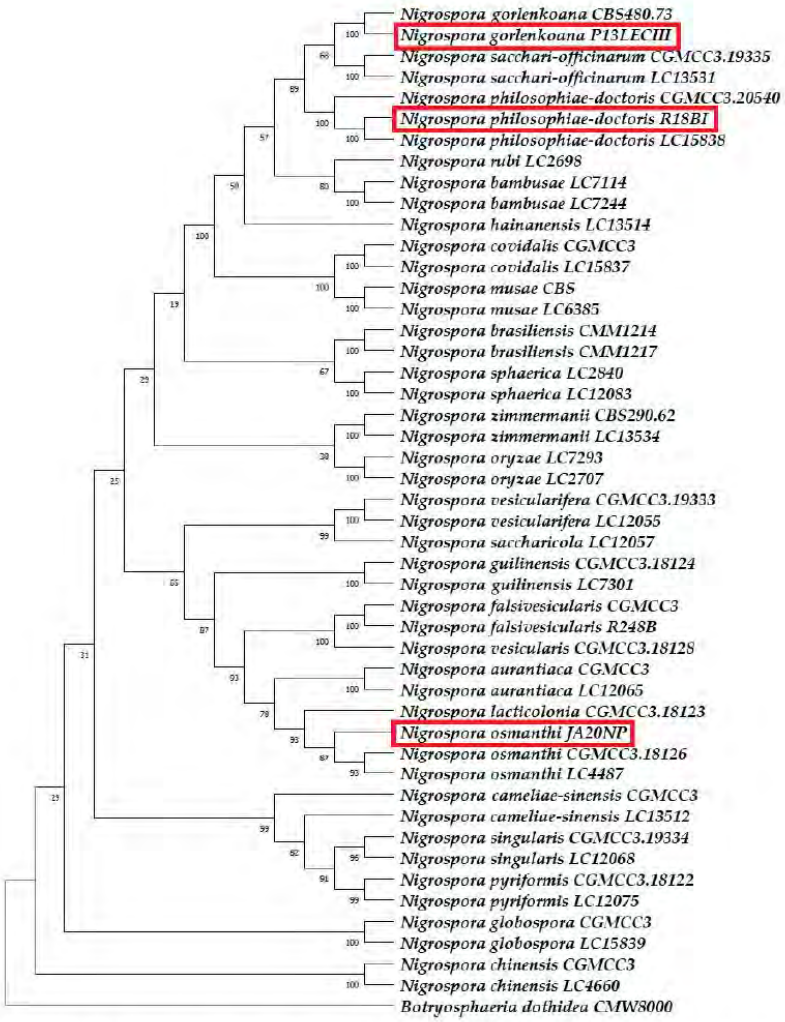


Figure 6. Multilocus tree based on internal transcribed spacer, beta-tubulin, and translation elongation factor 1-alpha sequence alignment. Sequences identified in this research are highlighted with red rectangles. This analysis encompassed 48 nucleotide sequences, resulting in a final dataset comprising 1581 positions.

Ultimately, from the DNA sequence analysis of ITS, TUB, and EF1α gene regions and the phylogenetic analysis, these species were identified as *Nigrospora gorlenkoana* Novobr., *Nigrospora osmanthi* Mei Wang & L. Cai, and *Nigrospora philosophiae-doctoris* M. Raza, Qian Chen & L. Cai.

3.3. Morphological Characterization and Fungal Incidence

Regarding sporulation, successful sporulation of the JA20 NP isolate was observed exclusively when it was cultured on a PDA + banana medium, as shown in Table 5.

Table 5. List of techniques used for inducing sporulation of the JA20 NP isolate and results.

TECHNIQUES									
PDA Temperatures: 22 °C, 25 °C, 28 °C, 30 °C	1/2 strength PDA medium	WA Temperatures: 22 °C, 25 °C, 28 °C, 30 °C	Pine needle extracts + WA	MEA Temperatures: 22 °C, 25 °C, 28 °C, 30 °C	Host tissue	Slide culture	Exposure to near-ultraviolet light (12 h day/12 h night)	Banana peel	PDA + banana medium
x	x	x	x	x	x	x	x	x	✓

x—sporulation not determined, ✓—sporulation recorded; WA—water agar, MEA—malt extract agar.

3.3.1. *Nigrospora gorlenkoana*

Colonies on PDA had reached a nine-centimeter diagram after two days at 28 °C and on WA after five days and sporulated after three days of incubation on PDA medium. Colonies of *N. gorlenkoana* developing on PDA (Figure 7a,b) were circular-shaped with aerial, woolly mycelium, entire-margined, opaque, floccose, raised with fuzzy edges, and growing rapidly; at first, they were white, becoming light grey when they matured and the reverse initially white, becoming darker grey when they matured. Conidia of *N. gorlenkoana* were round-shaped, light-brown-to-black-colored and aseptated, solitary, smooth, and 10.5–13.8 × 13.5–17.3 µm in diameter ($\bar{x}$= 11.9 × 15.1 µm, n = 30). Hyphae were smooth, septate, hyaline, and yellowish. On WA, colonies were white and growing poorly.

3.3.2. *Nigrospora osmanthi*

Colonies on PDA had reached a nine-centimeter diagram after three days at 28 °C and on WA after seven days and sporulated after five days of incubation on PDA + banana medium. Colonies of *N. osmanthi* developing on PDA (Figure 7c,d) were circular-shaped with an aerial, slightly woolly mycelium, entire-margined, opaque, raised a little, filiform, and growing rapidly; they were creamy-white-colored up and the reverse becoming greyish when mature. Conidia of *N. osmanthi* were round-shaped, black-colored, and aseptated, solitary, smooth, and 11.2–12.8 × 12.7–15.4 in diameter ($\bar{x}$ = 12.5 × 15.3 µm, n = 30). Hyphae were smooth, septate, hyaline, and yellowish. On WA, colonies were white and growing poorly.

3.3.3. *Nigrospora philosophiae-doctoris*

Colonies on PDA had reached a nine-centimeter diagram after three days at 28 °C and on WA after nine days and sporulated after four days of incubation on PDA medium. Colonies of *N. philosophiae-doctoris* developing on PDA (Figure 7e,f) were circular-shaped with aerial, woolly mycelium, entire-margined, opaque, floccose, raised with fuzzy edges, and growing rapidly; they were white-to-greyish-colored and reverse initially white, becoming creamy white when mature. Conidia of *N. philosophiae-doctoris* were round-shaped, brown-to-black-colored, aseptated, solitary, smooth, and 10.8–15.6 × 8.4–14.4 in diameter ($\bar{x}$ = 11.6 × 13.2, n = 30). Hyphae were smooth, septate, and hyaline. On WA, colonies were white to greyish and growing poorly.

3.4. Pathogenicity Tests

3.4.1. Pathogenicity Test on Detached Leaves

The symptoms of the disease on olive leaves tested in the laboratory showed similar symptoms as the leaf samples collected from the field survey. All inoculated leaves had yellowish-to-chocolate-brown spots (Figure 8). No symptoms were spotted on the control leaves. The re-isolated fungus from the diseased leaves was identical to the *Nigrospora* species.

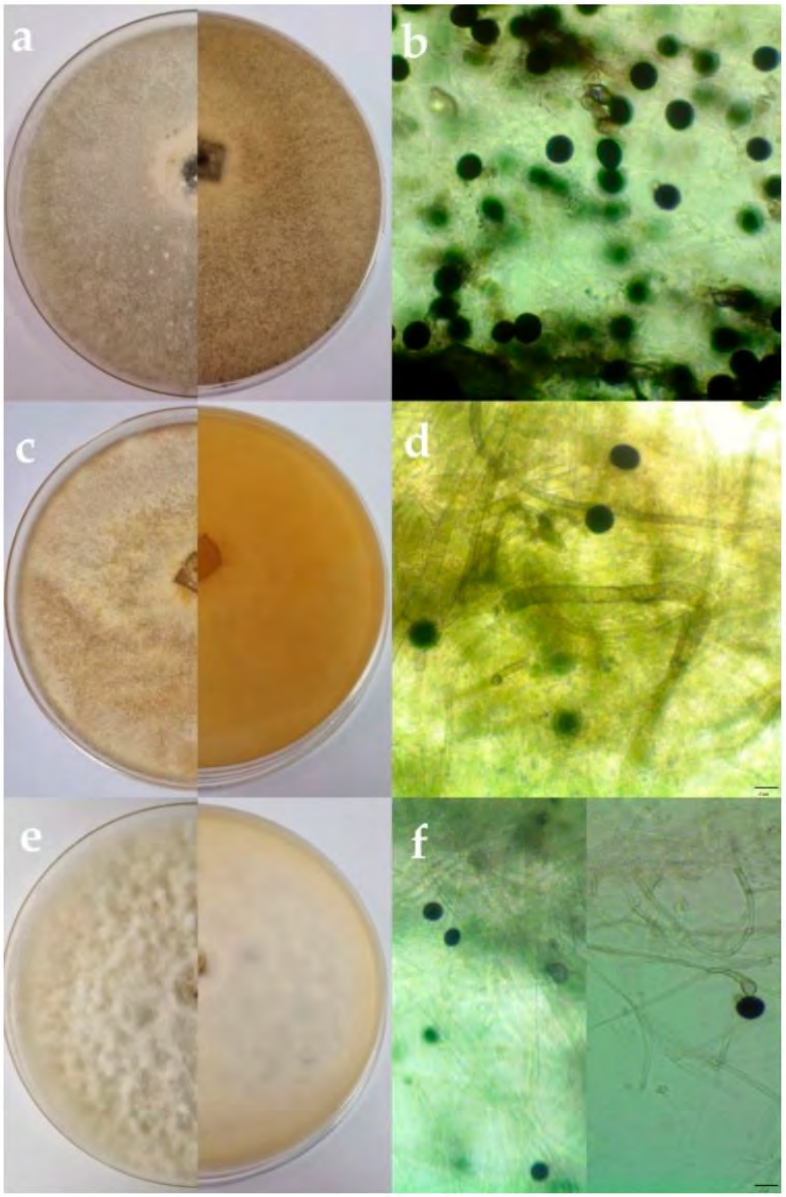


Figure 7. Left: Upper surface and reverse overview of cultures five days after incubation at 28 °C on PDA medium. Right: micrographs of isolates under the microscope with conidia. Scale bar = 10 µm, (**a**,**b**) *Nigrospora gorlenkoana*, (**c**,**d**) *N. osmanthi*, (**e**,**f**) *N. philosophiae-doctoris*.

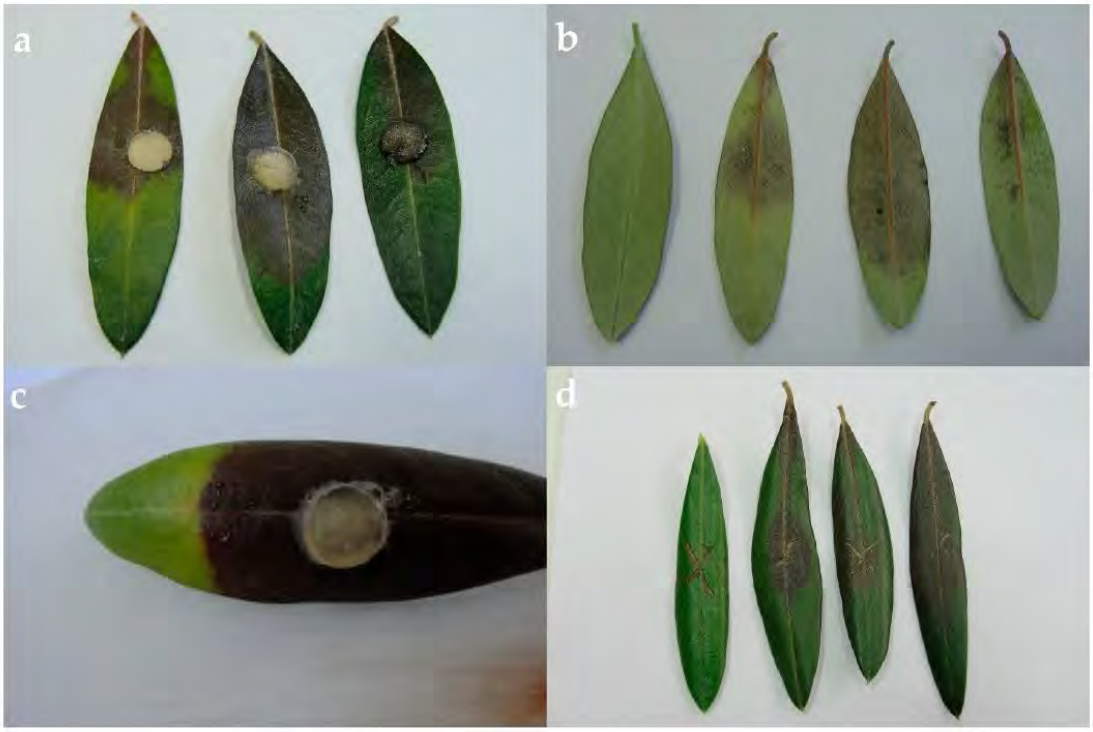


Figure 8. (**a**) Symptoms on unwounded leaves, from left to right: *N. philosophiae-doctoris*, *N. gorlenkoana*, *N. osmanthi*. (**b**) Symptoms on the bottoms of unwounded leaves, top left: control, from control to right: *N. philosophiae-doctoris*, *N. gorlenkoana*, *N. osmanthi*. (**c**) Symptoms on unwounded leaf: *N. philosophiae-doctoris*. (**d**) Symptoms on wounded leaves, from left to right: control, *N. philosophiae-doctoris*, *N. gorlenkoana*, *N. osmanthi*.

3.4.2. *Pathogenicity Test on Olive Seedlings*

The first symptoms were observed three days after inoculation. All inoculated leaves had chocolate-brown spots, similar to those observed in the field. The symptoms progressed until the entire leaf surface was covered (Figure 9). No symptoms were observed on the control leaves. The re-isolated fungus from the diseased leaves was identical to the *Nigrospora* species.

Figure 9. Symptoms on olive seedling leaves (cv. Rosinjola) in the pathogenicity test in the greenhouse, (**a**) *Nigrospora gorlenkoana*, (**b**) *N. osmanthi*, (**c**) *N. philosophiae-doctoris* after 9 days, (**d**) *N. philosophiae-doctoris* after 15 days.

4. Discussion

In this study, three different species of the *Nigrospora* genus, i.e., *N. gorlenkoana*, *N. osmanthi*, and *N. philsophiae-doctoris*, were detected on olive trees in Croatia. These species were the causal agents of leaf spots on olive trees in Istria and in Kvarner Gulf (Croatia).

Nigrospora species are cosmopolitan, filamentous, dematiaceous taxa distributed on various hosts, including crops with economic importance [15,16]. These species were isolated from different hosts around the world, such as *Cirsium setosum*, *Nelumbo* sp., *Oryza sativa*, *Vitis vinifera*, etc. [26]. They are known as plant pathogens, endophytes, and saprobes [35,36]. *Nigrospora* species known as plant pathogens cause many diseases, but

the most common disease is leaf spot. A list of reported *Nigrospora* pathogens, diseases, and distributions is represented in Table 6. *Nigrospora* sp. are also known as contaminants on farm-stored maize [37] and stored wheat [38] and are the causative agent of the post-harvest rot of ginger rhizomes [39] and post-harvest black rot of kiwifruit [40,41]. There are records of *N. oryzae* infecting the roots of 21 plant species [42] and *N. sphaerica* isolated from diseased grapevines [43], but there are no data on the pathogenicity of this fungus on the mentioned plant species.

Based on GenBank® data, *N. philosophiae-doctoris* was first isolated from the plant species *Disporum sessile* (Thunb.) D.Don ex Schult. & Schult.f., but there are no reports of this fungus as a plant pathogen. *N. philosophiae-doctoris* clustered in a well-supported clade closely related to *N. sacchari-officinarum* and *N. gorlenkoana*. *N. philosophiae-doctoris* produces smaller conidiogenous cells, when compared to those in *N. sacchari-officinarum* and *N. gorlenkoana*, and smaller conidia than those of *N. sacchari-officinarum* [28]. The conidial sizes frequently overlap among morphologically similar, but phylogenetically distinct, species of *Nigrospora*, and identification based on molecular and phylogenetic data for this fungal species is crucial [15]. In this research for molecular identification, consensus sequences were made of ITS1, ITS5, and ITS4 sequence data for the ITS gene region, Btub2Fd, and Btub4Rd sequence data for the TUB gene region and EF-728F and EF-986R sequence data for the EF1α gene region. In our research, *Nigrospora* species were distinguished based on morphological and molecular data, four phylogenetic trees were made, and they support three separate species.

Sporulation in fungi usually occurs when the growth rate is reduced and is hampered under conditions that favor rapid mycelial growth [44]. Many techniques are used for inducing the sporulation of fungi, such as slide culture [45], low-nutrient media [46], sporulation on host tissue, etc. In our survey, several techniques were performed for inducing the sporulation of the JA20 NP isolate (Table 5) in order to carry out morphological research. Banana peel culture is used for inducing the sporulation of *Nigrospora sphaerica* [21] but was not effective for the *Nigrospora osmanthi* JA20 NP isolate. Interestingly, this isolate sporulates only on PDA + banana medium. Spore dispersal in *Nigrospora* is aided by the wind, rain splashes, and insect vectors [47], so it can be easily spread around the orchard and create defoliation and economical losses. In some cases, an irregular string of a mucilaginous substance (small hyaline drop) was found to be attached to the spore [48]. It has been hypothesized that this substance facilitates adherence to the host substrate or to a vector as a successful spore-dispersal mechanism [26]. The moth *Sitotroga cerealella* transports the spores of *Nigrospora oryzae*, which adhere to its body [49]. Alfaro [50] has described how the non-gravid females of the mite *Pediculopsis graminum* transport the conidia of *Nigrospora oryzae* in their abdominal sacks. Webster [48] states that the spores can be transmitted while adhered to the body of the mite. A study of airborne fungal spores carried out at nine locations in Nigeria showed that the numbers of *Nigrospora* spores significantly correlate with the relative humidity, light intensity, and temperature [51]. Research conducted on bananas has shown that with an increase in the temperature, the rotting of bananas caused by *Nigrospora* species speeds up, with maximum infection recorded at 30 °C [52]. In our survey, out of the four temperatures at which all three isolates were kept on PDA (represented in Table 5), the fastest growth was recorded at 28 °C. Therefore, this temperature was chosen as the incubation temperature for morphological analysis of the isolates.

Regarding protection measures, for *N. oryzae*, Azoxystrobin (EC50 = 0.0001 mg/L) had the most significant fungal-controlling effect, followed by Prochloraz (copper salt), 15% Difenoconazole + 15% Propiconazole, Difenoconazole, Pyraclostrobin, and Myclobutanil [53]. Lignans, isopicropodophyllone, and dehydropodophyllotoxin, isolated from the leaves of *Podophyllum hexandrum* (Royle) T. S. Ying of Pakistani origin showed strong antifungal activity against *N. oryzae* [54]. *Bacillus thuringiensis* var. *israelensis* has shown inhibitory effects against *Nigrospora* sp. [55]. The mushroom species *Coprinellus disseminates* (isolate 12b), *Marasmiellus palmivorus* (isolate 42b), *Trametes maxima* (isolate 56e), and *Lenti-*

nus sajor-caju (isolate 60a) have potential antagonistic effects on *Nigrospora* species via the production of secondary metabolites and mycoparasitic interactions [56]. Unfortunately, there are no data about protection measures for these three species identified in this study.

Some *Nigrospora* species can act as an antagonist against fungal and bacterial plant pathogens [57,58]. Phomalactone from *Nigrospora sphaerica* exhibits a broad spectrum of antimicrobial activity against human and phytopathogenic bacteria and fungi [59], such as *Phytophthora infestans* (Mont.) de Bary, which causes tomato late blight [60]. An ethyl acetate extract of *Nigrospora sphaerica* affects the cell wall in growing methicillin-resistant *Staphylococcus aureus* Rosenbach and *Klebsiella pneumonia* (Schroeter 1886) Trevisan [61].

Table 6. A list of reported *Nigrospora* pathogens, diseases, and distributions.

SPECIES	HOST (Common Name)	TAXONOMY ID	DISEASE SYMPTOMS	DISTRIBUTION	REFERENCES
Nigrospora aurantiaca	Chinese chestnut	*Castanea mollissima* Blume	Leaf spot	China	[62]
	Pandan rampeh	*Pandanus amaryllifolius* Roxb.	Leaf spot	Malaysia	[63]
	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
	Tobacco	*Nicotiana tabacum* L.	Leaf spot	China	[64]
Nigrospora brasiliensis	Cochineal cactus	*Nopalea cochenillifera* (L.) Salm-Dyck	Brown leaf spot	Brazil	[31]
Nigrospora camelliae-sinensis	Black tea	*Camellia sinensis* L.	Leaf blight	China	[65]
	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
Nigrospora chiensis	Tea-oil plant	*Camellia oleifera* C. Abel	Leaf blight	China	[66]
Nigrospora falsivesicularis	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
Nigrospora guiliensis	Chinese corktree	*Phellodendron chinense* C. K. Schneid.	Leaf spot	China	[67]
Nigrospora hainanensis	Cochineal cactus	*Nopalea cochenillifera* (L.) Salm-Dyck	Brown spot	Brazil	[68]
	Pink wood sorrel	*Oxalis corymbosa* DC.	Leaf spot	China	[69]
	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
Nigrospora lacticolonia	Date palm	*Phoenix dactylifera* L.	Leaf spot	Oman	[70]
	Dragon fruit	*Hylocereus polyrhizus* (F.A.C.Weber) Britton & Rose	Reddish-brown spot	Malaysia	[71]
	Great Bougainvillea	*Bougainvillea spectabilis* Raeusch. Willd.	Leaf spot	China	[72]
	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
Nigrospora oryzae	Aloe-vera	*Aloe vera* var. *chinensis* (Haw.) A.Berger	Leaf spot	China	[73]
			Leaf spot	Pakistan	[74]
			Leaf spot	Bangladesh	[75]
	Asiatic dayflower	*Commelina communis* L.	Leaf spot	China	[76]
	Bayberry	*Morella rubra* Lour.	Twig blight	China	[53]
	Blueberry	*Vaccinium corymbosum* L.	Leaf spot	China	[77]
	Chinese photinia	*Photinia serratifolia* (Desf.) Kalkman (syn. *Photinia serrulata* Lindl.)	Leaf spot	China	[78]
	Cotton	*Gossypium hirsutum* L.	Leaf spot	China	[79]

Table 6. *Cont.*

SPECIES	HOST (Common Name)	TAXONOMY ID	DISEASE SYMPTOMS	DISTRIBUTION	REFERENCES
	Cotton-rose	*Hibiscus mutabilis* Mill.	Black leaf spot	China	[80]
	Crepe-ginger	*Hellenia speciosa* (J.Koenig) Govaerts (syn. *Costus speciosus* (J.Koenig) Sm.)	Leaf spot	China	[36]
	Dendrobium (Shi Hu)	*Dendrobium candidum* Wall. ex Lindl.	Leaf spot	China	[81]
	Dove tree	*Davidia involucrata* Baill.	Leaf blight	China	[82]
	Dryland winter wheat	*Triticum* L.	Crown and rot root	Azerbaijan	[83]
	Giant red	*Arundo donax* L.	Foliar and cane rot	Europe	[84]
	Ginger	*Zingiber officinale* Roscoe	Leaf spot	China	[85]
	Indian lotus	*Nelumbo nucifera* Gaertn.	Leaf spot	China	[86]
	Indian mustard	*Brassica juncea* (L.) Czern.	Stem blight	India	[87]
	Kentucky bluegrass	*Poa pratensis* L.	Leaf spot	Ontario	[88]
	Kidney bean	*Phaseolus vulgaris* L.	Leaf spot	China	[89]
	Kiwifruit	*Actinidia deliciosa* (A.Chev.) C.F.Liang & A.R.Ferguson	Brown/black spot	China	[90]
	Million bells	*Calibrachoa* hybrid cultivar	Leaf spot	Argentina	[91]
	Pearl millet	*Cenchrus americanus* (L.) Morrone (syn. *Pennisetum americanum* (L.) Leeke)	Leaf spot	Iran	[92]
	Peppermint	*Mentha spicata* L.	Brown leaf spot	Iran	[93]
	Poplar	*Populus alba* L. × *P. berolinensis* Dipp. (hybrid poplar)	Leaf blight	China	[94]
	Rice	*Oryza sativa* L.	Sheaths and grains of sheath rot	Bangladesh	[95]
	Tobacco	*Nicotiana tabacum* L.	Leaf spot	China	[96]
	Watermelon	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	Leaf spot	China	[97]
	Wheat	*Triticum aestivum* Vill.	Dark brown to black lesions	Kazahstan	[98]
			Crown and root rot	Kazahstan	[99]
	Wild rice	*Oryza rufipogon* Griff.	Leaf spot	China	[100]
	Zebra leaf aloe	*Aloe zebrina* Baker	Flower malformation	Namibia	[101]
Nigrospora osmanthi	Fiddle-leaf fig	*Ficus pandurata* Hance	Leaf blight	China	[102]
	Java tea	*Orthosiphon stamineus* Benth.	Leaf blight	Malaysia	[103]
	St. Augustine grass	*Stenotaphrum secundatum* (Walter) Kuntze	Leaf blight	China	[104]
	Tartary buckwheat	*Fagopyrum tataricum* (L.) Gaertn.	Leaf spot	China	[105]
Nigrospora panici	Big marigold	*Tagetes erecta* L.	Leaf blight	Bangladesh	[106]
	French marigold	*Tagetes patula* L.	Leaf blight	Bangladesh	[106]
Nigrospora pyriformis	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
	White goosefoot	*Chenopodium album* L.	Leaf spot	China	[107]

Table 6. *Cont.*

SPECIES	HOST (Common Name)	TAXONOMY ID	DISEASE SYMPTOMS	DISTRIBUTION	REFERENCES
Nigrospora sacchari-officinarum	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
Nigrospora saccharicola	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
Nigrospora singularis	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
Nigrospora sp.	Black tea	*Camellia sinensis* L.	Blister blight lesions	India	[108]
	Cochineal cactus	*Nopalea cochenillifera* (L.) Salm-Dyck	Brown spot	Brazil	[68]
	Maize	*Zea mays* L.	Weight/discoloration/necrosis of grains	Brazil	[109]
Nigrospora sphaerica	Balloon flower	*Platycodon grandiflorus* (Jacq.) A. DC.	Necrosis	China	[28]
	Black tea	*Camellia sinensis* L.	Leaf blight	India	[110]
				China	[111]
	Blueberry	*Vaccinium corymbosum* L.	Leaf spot, twig and shoot blight	Argentina	[112]
	Calabash	*Lagenaria siceraria* (Molina) Standl.	Leaf spot	Georgia	[113]
	China fir	*Cunninghamia lanceolata* (Lamb.) Hook.	Leaf blight	China	[114]
	Chinese Wisteria	*Wisteria sinensis* (Sims) DC., 1825	Leaf spot	Turkey	[115]
	Cochineal cactus	*Nopalea cochenillifera* (L.) Salm-Dyck	Brown spot	Brazil	[68]
	Corn mint	*Mentha canadensis* L.	Leaf blight	China	[116]
	Cowpea	*Vigna unguiculata* (L.) Walp.	Leaf spot	India	[117]
	Curcuma	*Curcuma wenyujin* Y.H.Chen & C.Ling	Leaf blight	China	[118]
	Date palm	*Phoenix dactylifera* L.	Not applicable	Iraq	[119]
			Root disease	Oman	[120]
			Leaf spot	Pakistan	[121]
	Devilpepper	*Rauvolfia serpentina* (L.) Benth. ex Kurz	Leaf spot and antracnose	Bangladesh	[122]
	Dragon fruit	*Selenicereus monacanthus* (hort. ex Lem.) D.R.Hunt (syn. *Hylocereus polyrhizus* (F.A.C.Weber) Britton & Rose)	Reddish-brown spot	Malaysia	[71]
	Dragon fruit (pitaya)	*Selenicereus undatus* (Haw.) D.R.Hunt (syn. *Hylocereus undatus* (Haw.) Britton & Rose)	Reddish-brown spot	Philippines	[123]
			Reddish-brown spot	China	[124]
	Elephant grass	*Cenchrus purpureus* (Schumach.) Morrone	Leaf blight	China	[125]
	European nettle tree	*Celtis australis* L., 1753	Leaf spot	India	[126]
	False Daisy	*Eclipta prostrata* (L.) L.	Leaf spot	China	[127]
	Kinnow mandarin	hybrid: *Citrus nobilis* Lour. × *Citrus deliciosa* Ten.	Leaf spot	Pakistan	[128]
	Kiwifruit	*Actinidia deliciosa* (A. Chev.) C.F.Liang & A.R.Ferguson	Leaf spot	China	[129]
	Liqourice	*Glycyrrhiza glabra* L.	Leaf spot	India	[130]

Table 6. *Cont.*

SPECIES	HOST (Common Name)	TAXONOMY ID	DISEASE SYMPTOMS	DISTRIBUTION	REFERENCES
	Mango	*Mangifera indica* L.	Leaf spot	India	[131]
			Twig dieback and leaf spot	Egypt	[132]
	Moonlight cactus	*Selenicereus monacanthus* (hort. ex Lem.) D.R.Hunt (syn. *Hylocereus monacanthus* (hort. ex Lem.) Britton & Rose)	Reddish-brown spot	Philippines	[123]
	Mulberry	*Morus alba* Hort. ex Loudon L.	Shot hole	China	[133]
				India	[134]
	Passion fruit	*Passiflora edulis* Sims	Leaf blight	China	[135]
	Peanut	*Arachis hypogaea* L.	Leaf blight	China	[136]
	Pitaya	*Selenicereus megalanthus* (K.Schum. ex Vaupel) Moran (syn. *Hylocereus megalanthus* (K.Schum. ex Vaupel) Ralf Bauer)	Reddish-brown spot	Philippines	[123]
	Purging nut	*Jatropha curcas* L.	Necrosis, chlorosis	India	[137]
	Qing qian liu	*Cyclocarya paliurus* (Batalin) Iljinsk.	Leaf blight	China	[138]
	Sesame	*Sesamum indicum* L.	Leaf blight	China	[139]
			Leaf blight	Pakistan	[140]
	Sugarcane	*Saccharum* spp.	Leaf blight	China	[141]
	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
	Three-leaf Akebia	*Akebia trifoliata* (Thunb.) Koidz.	Dried-shrink fruit	China	[142]
	Tea-oil plant	*Camellia oleifera* C. Abel	Leaf blight	China	[143]
	Watermelon (wild melon)	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	Leaf spot	Malaysia	[144]
	White moho	*Heliocarpus americanus* L.	Leaf spot	Brazil	[145]
Nigrospora vesicularifera	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
Nigrospora zimmermani	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]

5. Conclusions

This study identified, described and characterized three fungal species that caused leaf spot symptoms on olive trees in Croatia. *Nigrospora* species can be economically significant as plant pathogens, causing crop loses in agriculture. Early detection can help prevent the spreading, so it is important to identify and manage leaf spot promptly to prevent it from causing damage to olive trees. Over time, repeated infections can weaken the olive tree. Weakened trees are more susceptible to other diseases and environmental stressors, which can lead to a decline in the tree's overall health and longevity. Also, fungi can spread to other parts of the tree and neighboring trees, which can lead to more widespread infections and increased management challenges for growers. Additionally, the cost of managing and treating leaf spot diseases can add to production expenses. In conclusion, leaf spot diseases on olive trees are important because they can negatively affect the tree's health, fruit quality, and overall productivity. Olive growers need to monitor for leaf spot diseases and implement effective management strategies to minimize their impact and ensure a healthy and productive orchard. It is also necessary to conduct further research

that will include monitoring these fungal diseases and studying the effectiveness of various substances or treatments in inhibiting the growth and reproduction of these fungi.

To our knowledge, this paper is the first report of *Nigrospora* species causing diseases on olives and the first report of *Nigrospora philosophiae-doctoris* causing plant disease.

Author Contributions: Conceptualization, E.P. and S.G.; methodology, E.P. and S.G.; investigation, E.P. and S.G., writing—original draft preparation, E.P.; writing—review and editing, S.G., K.V., J.Ć. and E.Đ. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Croatian Science Foundation Installation Research Project "Natural bioactive compounds as a source of potential antimicrobial agents in the control of bacterial and other fungal pathogens of olives", Anti-Mikrobi-OL (AMO), UIP-2020-02-7413, and "Young Researchers' Career Development Project" DOK-2021-02-2882.

Data Availability Statement: All sequence data are available in NCBI GenBank in accordance with the accession numbers in the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. FAO. Food and Agriculture Organization of the United Nations. Crop and Livestock Products. Available online: https://www.fao.org/faostat/en/#data/QCL (accessed on 19 January 2023).
2. FAO. Food and Agriculture Organization of the United Nations. Land & Water. Available online: https://www.fao.org/land-water/databases-and-software/crop-information/olive/en/ (accessed on 19 January 2023).
3. Varanda, C.M.R.; Materatski, P.; Landum, M.; Campos, M.D.; Rosário Félix, M.D. Fungal communities associated with peacock and cercospora leaf spots in olive. *Plants* **2019**, *8*, 169. [CrossRef] [PubMed]
4. Figueres, G. *Repilos del Olivo: Ataque en Fruto*; Phytoma España: Valencia, Spain, 1991; pp. 31–36.
5. Civantes, M. *Olive Pest and Disease Management*; IOCC: Towson, MD, USA, 1999.
6. Benitez, Y.; Botella, M.A.; Trapero, A.; Alsalimiya, M.; Caballero, J.L.; Dorado, G.; Muñoz-Blanco, J. Molecular analysis of the interaction between *Olea europaea* and the biographic fungus *Spilocaea oleagina*. *Mol. Plant Pathol.* **2005**, *6*, 425–438. [CrossRef] [PubMed]
7. Obanor, F.; Jaspers, M.; Jones, E.E.; Walter, M. Greenhouse and field evaluation of fungicides for control of olive leaf spot in New Zealand. *Crop Prot.* **2008**, *27*, 1335–1342. [CrossRef]
8. Sanei, S.; Razav, S. Survey of *Spilocaea oleagina*, causal agent of olive leaf spot, in North of Iran. *J. Yeast Fungal Res.* **2011**, *2*, 33–38. [CrossRef]
9. Basim, E.; Basim, H.; Abdulai, M.; Baki, D.; Ozturk, N. Identification and characterization of *Alternaria alternata* causing leaf spot of olive tree (*Olea europaea*) in Turkey. *Crop Prot.* **2017**, *92*, 79–88. [CrossRef]
10. Sergeeva, V.; Spooner-Hart, R.; Nair, N.G. First report of *Colletotrichum acutatum* and *C. gloeosporioides* causing leaf spots of olives (*Olea europaea*) in Australia. *Australas. Plant Dis. Notes* **2008**, *3*, 143–144.
11. Trouillas, F.P.; Nouri, M.T.; Lawrence, D.P.; Moral, J.; Travadon, R.; Aegerter, B.J.; Lightle, D. Identification and Characterization of *Neofabraea kienholzii* and *Phlyctema vagabunda* Causing Leaf and Shoot Lesions of Olive in California. *Plant Dis.* **2019**, *103*, 3018–3030. [CrossRef]
12. Zimmerman, A. Ueber einige an tropischen Kulturpfianzen beobachtete Pilze III. *Zentralblatt Bakteriol. Parasitenkd.* **1992**, *8*, 216–221.
13. Barnett, H.L.; Hunter, B.B. *Illustrated Genera of Imperfect Fungi*, 4th ed.; APS Press: St. Paul, MN, USA, 1998.
14. Kirk, P.M.; Cannon, P.F.; Minter, D.W.; Stalpers, J.A. Dictionary of the Fungi. *Mycol. Res.* **2008**, *113*, 908–910.
15. Wang, M.; Liu, F.; Crous, P.W.; Cai, L. Phylogenetic reassessment of *Nigrospora*: Ubiquitous endophytes, plant and human pathogens. *Persoonia* **2017**, *39*, 118–142. [CrossRef]
16. Hyde, K.D.; Norphanphoun, C.; Maharachchikumbura, S.S.N.; Bhat, D.J.; Jones, E.B.G.; Bundhun, D.; Chen, Y.J.; Bao, D.F.; Boonmee, S.; Calabon, M.S.; et al. Refiend families of *Sordariomycetes*. *Mycosphere* **2020**, *11*, 305–1059. [CrossRef]
17. MycoBank Database. Available online: https://www.mycobank.org/Basic%20names%20search (accessed on 18 September 2023).
18. Fisher, P.J.; Petrini, O.; Petrini, L.E.; Descals, E. A preliminary study of fungi inhabiting xylem and whole stems of Olea europaea. *Sydowia* **1992**, *44*, 117–121.
19. Landum, M.C.; Félix, M.d.R.; Alho, J.; Garcia, R.; Cabrita, M.J.; Rei, F.; Varanda, C.M. Antagonistic activity of fungi of *Olea europaea* L. against *Colletotrichum acutatum*. *Microbiol. Res.* **2016**, *183*, 100–108. [CrossRef] [PubMed]
20. Su, Y.-Y.; Qi, Y.-L.; Cai, L. Induction of sporulation in plant pathogenic fungi. *Mycology* **2012**, *3*, 195–200.
21. Kindo, A.J.; Tupaki-Sreepurna, A.; Yuvaraj, M. Banana peel culture as an indigenous medium for easy identification of late-sporulation human fungal pathogens. *Indian J. Med. Microbiol.* **2016**, *34*, 457–461. [CrossRef] [PubMed]

22. White, T.J.; Bruns, T.D.; Lee, S.B.; Taylor, J.W. 38—Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics. In *PCR—Protocols and Applications—A Laboratory Manual*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press, Inc.: Cambridge, MA, USA, 1990; pp. 315–322.
23. EPPO. European and Mediterranean Plant Protection Organization. PM 7/129 (1) DNA barcoding as an identification tool for a number of regulated pests. *Bull. OEPP* **2016**, *46*, 501–537. [CrossRef]
24. Woudenberg, J.H.C.; Aveskamp, M.M.; de Grutyer, J.; Spiers, A.G.; Crous, P.W. Multiple *Didymella* teleomorphs are linked to the *Phoma clematidina* morphotype. *Persoonia* **2009**, *22*, 56–62. [CrossRef]
25. Carbone, I.; Kohn, L.M. A Method for Designing Primer Sets for Speciation Studies in Filamentous Ascomycetes. *Mycologia* **1995**, *91*, 553–556. [CrossRef]
26. Hao, Y.; Aluthmuhandiram, J.V.S.; Chethana, K.W.T.; Manawasinghe, I.S.; Li, X.; Liu, M.; Hyde, D.K.; Phillips, A.J.L.; Zhang, W. *Nigrospora* Species Associated with Various Hosts from Shandong Peninsula, China. *Mycobiology* **2020**, *48*, 169–183. [CrossRef]
27. Saitou, N.; Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **1987**, *4*, 406–425.
28. Chen, Q.; Bakhshi, M.; Balci, Y.; Broders, K.D.; Cheewangkoon, R.; Chen, S.F.; Fan, X.L.; Gramaje, D.; Halleen, F.; Jung, M.H.; et al. Genera of phytopathogenic fungi: GOPHY 4. *Stud. Mycol.* **2022**, *101*, 417–564. [CrossRef] [PubMed]
29. Slippers, B.; Crous, P.W.; Denman, S.; Coutinho, T.A.; Wingfield, B.D.; Wingfield, M. Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. *Mycologia* **2004**, *96*, 83–101. [CrossRef] [PubMed]
30. Raza, M.; Zhang, Z.-F.; Hyde, K.D.; Diao, Y.-Z.; Cai, L. Culturable plant pathogenic fungi associated with sugarcane in southern China. *Fungal Diver* **2019**, *99*, 1–104. [CrossRef]
31. Crous, P.W.; Carnegie, A.J.; Wingfield, M.J.; Sharma, R.; Mughini, G.; Noordeloos, M.E.; Santini, A.; Shouche, Y.S.; Bezerra, J.D.P.; Dima, B.; et al. Fungal Planet description sheets: 868–950. *Persoonia* **2019**, *42*, 291–473. [CrossRef] [PubMed]
32. Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **1985**, *39*, 783–791. [CrossRef]
33. Tamura, K.; Nei, M.; Kumar, S. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11030–11035. [CrossRef] [PubMed]
34. Tamura, K.; Stecher, G.; Kumar, S. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [CrossRef]
35. Rashmi, M.; Kushveerm, J.; Sarma, V. A worldwide list of endophytic fungi with notes on ecology and diversity. *Mycosphere* **2019**, *10*, 798–1079. [CrossRef]
36. Sun, X.D.; Cai, X.L.; Pang, Q.Q.; Zhou, M.; Zhang, W.; Chen, Y.S.; Bian, Q. First record of leaf spot disease on *Costus speciosus* caused by *Nigrospora oryzae* in Hainan, China. *Plant Dis.* **2021**, *105*, 506. [CrossRef]
37. Ngoko, Z.; Marasas, W.F.O.; Rheeder, J.P.; Shephard, G.S.; Wingfield, M.J.; Cardwell, K.F. Fungal infection and mycotoxin contamination of maize in the humid forest and the western highlands of Cameroon. *Phytoparasitica* **2001**, *29*, 352–360. [CrossRef]
38. Barkat, E.H.; Hardy, G.E.S.J.; Ren, Y.; Calver, M.; Bayliss, K.L. Fungal contaminants of stored wheat vary between Australian states. Australas. *Plant Pathol.* **2016**, *45*, 621–628.
39. Moreira, S.I.; Dutra, D.; Rodrigues, A.; de Oliveira, J.; Dhingra, O.; Pereira, O. Fungi and bacteria associated with post-harvest rot of ginger rhizomes in Espírito Santo, Brazil. *Trop. Plant Pathol.* **2013**, *38*, 218–226.
40. Kwon, Y.; Kim, M.; Kwack, Y.-B.; Kwak, Y.-S. First report of *Nigrospora* sp. causing kiwifruit postharvest black rot. *N. Z. J. Crop Hortic. Sci.* **2016**, *45*, 75–79. [CrossRef]
41. Li, L.; Pan, H.; Chen, M.Y.; Zhang, S.J.; Zhong, C.H. First report of *Nigrospora oryzae* causing brown/black spot disease of kiwifruit in China. *Plant Dis.* **2018**, *102*, 243. [CrossRef]
42. Shahzad, S.; Ghaffar, A. New records of soilborne root infecting fungi in Pakistan. *Pak. J. Bot.* **1995**, *27*, 209–216.
43. Lucca, A.J.D.; Mich, M.; Boue, S.; Cleveland, T.E.; Sien, T.; Walsh, T.J. Fungicidal activity of plant saponin CAY-i for fungi isolated from diseased vitis fruit and stems. *Am. J. Enol. Vitic.* **2008**, *59*, 67–72. [CrossRef]
44. Dahlberg, K.R.; Etten, J. Physiology and biochemistry of fungal sporulation. *Annu. Rev. Phytopathol.* **1982**, *20*, 281–301. [CrossRef]
45. Riddell, R.W. Permanent stained mycological preparations obtained by slide culture. *Mycologia* **1950**, *42*, 265–270. [CrossRef]
46. Masangkay, R.F.; Paulitz, T.C.; Hallett, S.G.; Watson, A.K. Characterization of sporulation of *Alternaria alternata* f. sp. *sphenocleae*. *Biocontrol Sci. Technol.* **2000**, *10*, 385–397. [CrossRef]
47. Wu, P.-C.; Tsai, J.-C.; Li, F.-C.; Lung, S.-C.; Su, H.-J. Increased levels of ambient fungal spore in Taiwan are associated with dust events from China. *Atmos. Environ.* **2004**, *38*, 4879–4886. [CrossRef]
48. Webster, J. Spore projection in hyphomycete *Nigrospora sphaerica*. *New Phytol.* **1952**, *51*, 229–235. [CrossRef]
49. Savulescu, T.; Rayss, T. Contribution a la connaissance de la hiologie de *Nigrospora oryzae* (B. et Br.) Petch Parasite du mats. *Rec. Trav. Cryptogam. dédiés à Louis Mangin. Muséum Nat. d'Hist. Nat. Paris* **1931**, 233–240.
50. Alfaro, A. El Ácara *Pediculopsis graminum* Reut. y el hongo *Nigrospora oryzae* (Berk, et Br.). Petch en asociación parasitaria sobre Tigos arogeneses. *Bol. Pathol. Veg. Entomol. Agric.* **1946**, *16*, 321.
51. Njokuocha, R.C.; Agwu, C.O.C.; Okezie, C.E.A. Effects of weather conditions on selected airborne fungal spores in the southern part of the state of Enugu, Nigeria. *Grana* **2017**, *56*, 263–272. [CrossRef]
52. Sarkar, S.; Girisham, S.; Reddym, S.M. Influence of temperature and relative humidity on the development of post-harvest rot of banana. *Proc. Natl. Acad. Sci. India Sect.* **2011**, *81*, 285–287.

53. Li, W.J.; Hu, M.; Xue, Y.; Li, Z.; Zhang, Y.; Zheng, D.; Lu, G.; Wang, J.; Zhou, J. Five fungal pathogens are responsible for bayberry twig blight and fungicides were screened for disease control. *Microorganisms* **2020**, *8*, 689. [CrossRef] [PubMed]
54. Rahman, A.U.; Ashraf, M.; Choudary, M.I.; Rehman, H.U.; Kazmi, M.H. Antifungal aryltetralin lignans from leaves of *Podophyllum-hexandrum*. *Phytochemistry* **1995**, *40*, 427–431. [CrossRef]
55. Reyes-Ramirez, A.; Escudero-Abarca, B.I.; Aguilar-Uscanga, G.; Hayward-Jones, P.M.; Barboza-Corona, J.E. Antifungal activity of *Bacillus thuringiensis* chitinase and its potential for the biocontrol of phytopathogenic fungi in soybean seeds. *J. Food Sci.* **2020**, *69*, M131–M134. [CrossRef]
56. Mohd-Baseri, N.; Anuar, M.S.K.; Shamsuhazli, N.A.S.; Zulkifli, M.A.F.; Wasoh, H.; Yusof, M.T. Antagonistic activity of wild growing mushrooms against various fungal rice pathogen. *Int. Microbiol.* **2023**, *26*, 91–98. [CrossRef]
57. Sornakili, A.; Thankappan, S.; Sridharan, A.P.; Nithya, P.; Uthandi, S. Antagonistic fungal endophytes and their metabolite-mediated interactions against phytopathogens in rice. *Physiol. Mol. Plant Pathol.* **2020**, *112*, 101525. [CrossRef]
58. Lu, L.; Karunarathna, S.C.; Hyde, K.D.; Suwannarach, N.; Elgorban, A.M.; Stephenson, S.L.; Al-Rejaie, S.; Jayawardena, R.S.; Tibpromma, S. Endophytic fungi associated with coffee leaves in China exhibited in vitro antagonism against fungal and bacterial pathogens. *J. Fungi* **2022**, *8*, 698. [CrossRef]
59. Ramesha, K.P.; Mohana, N.C.; Nuthan, B.R.; Rakshith, D.; Satish, S. Antimicrobial metabolite profiling of *Nigrospora sphaerica* from *Adiantum philippense* L. *J. Genet. Eng. Biotechnol.* **2020**, *18*, 66. [CrossRef] [PubMed]
60. Kim, J.-C.; Choi, G.J.; Park, J.-H.; Kim, H.T.; Cho, K.Y. Activity against plant pathogenic fungi of phomalactone isolated from *Nigrospora sphaerica*. *Pest Manag. Sci.* **2001**, *57*, 554–559. [CrossRef] [PubMed]
61. Ibrahim, D.; Lee, C.C.; Yenn, T.W.; Zakaria, L.; Sheh-Hong, L. Effect of the extract of endophytic fungus, *Nigrospora sphaerica* CL-OP 30, against the growth of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Klebsiella pneumonia* cells. *Trop. J. Pharm. Res.* **2015**, *14*, 2091–2097. [CrossRef]
62. Luo, F.; Li, W.; Zhu, T.; Han, S.; Qiao, T.; Li, S. First report of *Nigrospora aurantiaca* causing leaf spot disease of *Castanea mollissima* in China. *Plant Dis.* **2020**, *104*, 2730. [CrossRef]
63. Khoo, Y.W.; Tan, H.T.; Khaw, Y.S.; Li, S.-F.; Chong, K.P. First report of *Nigrospora aurantiaca* causing leaf spot on *Pandanus amaryllifolius* in Malaysia. *J. Plant Pathol.* **2022**, *104*, 1205–1206. [CrossRef]
64. Huang, Y.; Li, Z.; Wang, H.-C.; Chen, Q.; Li, W.H. First report of leaf spot caused by *Nigrospora aurantiaca* in tobacco in China. *Plant Dis.* **2021**, *105*, 1569. [CrossRef]
65. Manawasinghe, I.S.; Jayawardena, R.S.; Li, H.L.; Zhou, Y.Y.; Zhang, W.; Phillips, A.J.L.; Wanasinghe, D.N.; Dissanayake, A.J.; Li, X.H.; Li, Y.H.; et al. Microfungi associated with *Camellia sinensis*: A case study of leaf and shoot necrosis on Tea in Fuijan, China. *Mycosphere* **2021**, *12*, 430–518. [CrossRef]
66. Qin, S.; Chen, X.; Zhou, X.; Zhao, J.; Baccelli, I.; Cernava, T. First report of *Camellia oleifera* leaf blight caused by *Nigrospora chiensis*. *J. Plant Pathol.* **2021**, *103*, 711–712. [CrossRef]
67. Zeng, Y.; Li, L.; Zhu, T.; Han, S.; Li, S. First report of brown leaf spot disease caused by *Nigrospora guiliensis* on *Phellodendron chinense* in China. *Plant Disease* **2020**, *104*, 2518. [CrossRef]
68. Conforto, C.; Lima, N.B.; Silva, F.J.A.; Câmara, M.P.S.; Macharachchikumbura, S.; Michereeff, S.J. Characterization of fungal species associated with cladode brown spot on *Nopalea cochenillifera* in Brazil. *Eur. J. Plant Pathol.* **2019**, *155*, 1179–1194. [CrossRef]
69. Zheng, T.; Zhao, L.; Huang, M.-G.; Deng, J.-X.; Wang, Y.-H. First report of leaf spot caused by *Nigrospora hainanensis* on *Oxalis corymbose*—China. *Plant Dis.* **2022**, *106*, 1986. [CrossRef]
70. Al-Nadabi, H.; Maharachchikumbura, S.S.N.; Al-Gahaffi, Z.S.; Al-Hasani, A.S.; Velazhahan, R.; Al-Sadi, A.M. Molecular identification of fungal pathogens associated with leaf spot disease of date palms (*Phoenix dactylifera*). *All Life* **2020**, *13*, 587–597. [CrossRef]
71. Kee, Y.J.; Hafifi, A.B.M.; Huda-Shakirah, A.R.; Wong, K.L.; Jin, X.L.; Nordahliawate, M.S.S.; Zakaria, L.; Mohd, M.H. First report of reddish brown spot disease of red-fleshed dragon fruit (*Hypocereus polyrhizus*) caused by *Nigrospora lacticolonia* and *Nigrospora sphaerica* in Malaysia. *Crop Prot.* **2019**, *122*, 165–170. [CrossRef]
72. Li, M.; Gao, Z.; Wang, Y.; Zhang, W.; Yang, J.; Gong, D.; Ma, Y.; Li, Y.; Hu, M. First report of *Nigrospora lacticolonia* causing leaf spot of *Bougainvillea spectabilis* in China. *Can. J. Plant Pathol.* **2022**, *44*, 695–701. [CrossRef]
73. Zhai, L.F.; Liu, J.; Zhang, M.X.; Hong, N.; Wang, G.P.; Wang, L.P. The first report of leaf spot in *Aloe vera* caused by *Nigrospora oryzae* in China. *Plant Dis.* **2013**, *97*, 1256. [CrossRef]
74. Alam, M.W.; Rehman, A.; Saira, M.; Khan, N.A.; Aslam, S.; Fiaz, M.; Muhammad, S. First report of leaf spots in *Aloe vera* caused by *Nigrospora oryzae* in Pakistan. *Plant Dis.* **2017**, *101*, 841. [CrossRef]
75. Begum, M.; Hamza, A.; Tanny, T.; Das, K.C.; Mahmud, M.T.; Salimullah, M.; Alam, I. First report of leaf spot disease in *Aloe vera* caused by *Nigrospora oryzae* in Bangladesh. *Plant Dis.* **2018**, *102*, 1461. [CrossRef]
76. Qiu, C.; Zhu, W.; Niu, T.; Liu, Z. *Nigrospora oryzae* causing leaf spot on asiatic dayflower on Chongqing, China. *Plant Dis.* **2022**, *106*, 763. [CrossRef]
77. Zhang, L.Q.; Jiang, S.; Meng, J.J.; An, H.S.; Zhang, X.Y. First report of leaf spot caused by *Nigrospora oryzae* on blueberry in Shangai, China. *Plant Dis.* **2019**, *103*, 2473. [CrossRef]
78. He, B.Y.; Cernava, T.; He, H.D.; Li, H.X.; Chen, X.Y.L.; Yang, H. First report of leaf spon on *Photinia serrulata* caused by *Nigrospora oryzae* in China. *Plant Dis.* **2019**, *103*, 2480. [CrossRef]

79. Zhang, L.X.; Li, S.S.; Tan, G.J.; Shen, J.T.; He, T. First report of *Nigrospora oryzae* causing leaf spot on cotton in China. *Plant Dis.* **2012**, *96*, 1379. [CrossRef] [PubMed]
80. Han, S.; Yu, S.T.; Zhu, T.; Li, S.; Qiao, T.; Liu, Y.; Lin, T.; Yang, C. *Nigrospora oryzae* causing black leaf spot disease of *Hibiscus mutabilis* in China. *Plant Dis.* **2021**, *105*, 2255. [CrossRef]
81. Wu, J.B.; Zhang, C.L.; Mao, P.P.; Qian, Y.S.; Wang, H.Z. First report of leaf spot caused by *Nigrospora oryzae* on *Dendrobium candidum* in China. *Plant Dis.* **2014**, *98*, 996. [CrossRef] [PubMed]
82. Yang, H.L.; Du, C.M.; Liang, L.Y.; Qin, Q.Y.; Wang, C.; Cui, D.X.; Wu, Q.G.; Zou, L. First report of *Davida involucrata* leaf blight caused by *Nigrospora oryzae* in Sichuan, China. *Plant Dis.* **2022**, *106*, 2520. [CrossRef] [PubMed]
83. Özer, G.; Paulitz, T.C.; Imren, M.; Alkan, M.; Muminjanov, H.; Dababat, A.A. Identity and pathogenicity of fungi associated with crown and root rot of dryland winter wheat in Azerbaijan. *Plant Dis.* **2020**, *104*, 2149–2157. [CrossRef]
84. Widmer, T.; Kirk, A.; Kirk, G.; Guermache, F. Foliar and cane rot of *Arundo donax* caused by *Nigrospora oryzae* in Europe. *Plant Dis.* **2006**, *90*, 1107. [CrossRef]
85. Liu, Z.; Zhou, S.; Qi, L.; Wang, X.; Song, J.; Li, D.; Chen, T.; Wang, Q. First report of *Nigrospora oryzae* causing leaf spot on ginger in China. *Plant Dis.* **2022**, *106*, 316. [CrossRef]
86. Zhang, Q.H.; Huang, L.L.; Liu, Y.J.; Ai, Y.; Pend, D.H. First report of leaf spot of lotus (*Nelumbo nucifera*) caused by *Nigrospora oryzae* in China. *Plant Dis.* **2018**, *102*, 1038. [CrossRef]
87. Sharma, P.; Meena, P.D.; Chauhan, J.S. First report of *Nigrospora oryzae* (Berk. & Broome) petch causing stem blight on *Brassica juncea* in India. *J. Plant Pathol.* **2013**, *161*, 439–441.
88. Zheng, L.; Shi, F.; Kelly, D.; Hsiang, T. First report of leaf spot of Kentucky bluegrass (*Poa pratensis*) caused by *Nigrospora oryzae* in Ontario. *Plant Dis.* **2012**, *96*, 909. [CrossRef] [PubMed]
89. Luo, M.-Y.; Jiang, Y.-L. First report of leaf spot on kidney bean caused by *Nigrospora oryzae* in China. *Plant Dis.* **2022**, *106*, 1064. [CrossRef] [PubMed]
90. Li, L.; Pan, H.; Liu, Y.F.; Li, D.W.; Zhang, Q.; Deng, L.; Chen, M.Y.; Zhong, C.H. First report of *Nigrospora sphaerica* causing kiwifruit postharvest rot disease in China. *Plant Dis.* **2018**, *102*, 1666. [CrossRef]
91. Borrelli, N.P.; Stancanelli, S.; Papone, M.L.; Moreno, M.V.; Stenglein, S.; Wright, E.R.; Hagiwara, J.C.; Rivera, M.C. Leaf spots on calibrachoa caused by *Nigrospora oryzae*. *Ornam. Hortic.* **2020**, *26*, 591–597. [CrossRef]
92. Kalati, T.H.; Jahani, M.; Zare, R.; Mirzaee, M. First report of Nigrospora leaf spot on *Pennisetum americanum* in Iran. *J. Plant Pathol.* **2014**, *96*, 606.
93. Farid, K.; Zafari, D.; Soleimani, M.J.; Bagherabadi, S. First report of *Nigrospora oryzae* causing brown leaf spot on *Mentha spicata*. *J. Plant Pathol.* **2020**, *102*, 1281. [CrossRef]
94. Zhang, H.; Kong, N.; Ji, S.; Liu, B.; Tian, Z.; Qi, J.; Liu, Z. First report of leaf blight caused by *Nigrospora oryzae* on Poplar in China. *Plant Dis.* **2022**, *106*, 1063. [CrossRef]
95. Shamsi, S.; Khan, A.; Shahjahan, A.K.M.; Miah, S.A. Fungal species associated with sheaths and grains of sheath rot affected rice varietes from Bangladesh. *Bangladesh J. Bot.* **2003**, *32*, 17–22.
96. Wang, D.; Li, Y.; Sun, G.; Ai, Y.F.; Wang, F.; Wang, X. First report of leaf spot disease caused by *Nigrospora oryzae* on *Nicotiana tabacum* in China. *Plant Dis.* **2022**, *106*, 2526. [CrossRef]
97. Chen, X.; Wang, N.; Yang, M.F.; Li, H.-X. First report of *Nigrospora* leaf spot caused by *Nigrospora oryzae* on watermelon in China. *Plant Dis.* **2019**, *103*, 1019. [CrossRef]
98. Eken, C.; Spanbayev, A.; Tulegenova, Z.; Yechshzhanov, T. First report of *Nigrospora oryzae* on wheat iz Kazahstan. *Plant Dis.* **2016**, *100*, 861. [CrossRef]
99. Bozoğlu, T.; Dervis, S.; Imren, M.; Amer, M.; Özdemir, F.; Paulitz, T.C.; Morgounov, A.; Dababat, A.A.; Özer, G. Fungal pathogens associated with crown and root rot of wheat in Central, Eastern, and Southeastern Kazahstan. *J. Fungi* **2022**, *8*, 417. [CrossRef] [PubMed]
100. Liu, Y.L.; Tang, J.R.; Li, Y.; Zhou, H.K. First report of leaf spot caused by *Nigrospora oryzae* in wild rice in China. *Plant Dis.* **2021**, *105*, 3293. [CrossRef] [PubMed]
101. Kido, L.R.; Uzabakirihno, J.D.; Chimwamourombe, P.M. Isolation and identification of pathogenic fungi associated with *Aloe zebrina* flower malformation—First report. *J. Pure Appl. Microbiol.* **2012**, *6*, 125–129.
102. Liu, J.; Yang, L.; Miao, P.; Wu, D.; Cai, G.; Li, X.; Lu, J. First report of leaf blight on *Ficus pinduara* caused by *Nigrospora osmanthi* in China. *Plant Dis.* **2019**, *103*, 2685. [CrossRef]
103. Ismail, S.I.; Norzaki, N.A.M.; Ya'acob, M.E.; Jamian, S. First report of *Nigrospora osmanthi* causing leaf blight on *Orthosiphon stamineus* in Malaysia. *Plant Dis.* **2022**, *106*, 770. [CrossRef] [PubMed]
104. Mei, S.S.; Wang, Z.Y.; Zhang, J.; Rong, W. First report of leaf blight on *Stenotaphrum secundatum* caused by *Nigrospora osmanthi* in China. *Plant Dis.* **2019**, *103*, 1783. [CrossRef]
105. Shen, Q.; Peng, X.; He, F.; Li, S.; Xiao, Z.; Wang, H.; Tang, X.; Zhou, M. First report of *Nigrospora osmanthi* causing leaf spot on *Tartary* buckwheat in China. *Plant Dis.* **2021**, *105*, 1227. [CrossRef]
106. Aktar, M.; Shamsi, S. Mycloflora associated with infected plant parts of *Tagetes erecta* L. and *Tagetes patula* L. *Bangladesh J. Bot.* **2021**, *50*, 131–140. [CrossRef]
107. Chen, X.-Y.-L.; Zhang, C.; Yang, H.; Yang, M.-F.; Cernava, T. First report of leaf spot on *Chenopodium album* caused by *Nigrospora pyriformis* in China. *Plant Dis.* **2020**, *104*, 1872. [CrossRef]

108. Barman, A.; Nath, A.; Thakur, D. Identification and characterization of fungi associated with blister blight lesion of tea (*Camellia sinensis* L. Kuntze) isolated from Meghalaya, India. *Microbiol. Res.* **2020**, *240*, 126561. [CrossRef] [PubMed]
109. Szilagyi-Zecchin, V.J.; Adamoski, D.; Rodrigues Gomes, R.; Hungria, M.; Ikeda, A.C.; Kava-Cordeiro, V.; Glienke, C.; Galli-Terasawa, L.V. Composition of endophytics fungal community associated with leaves of maize cultivated in south Brazilian field. *Acta Microbiol. Immunol. Hung.* **2016**, *63*, 449–466. [CrossRef] [PubMed]
110. Dutta, J.; Gupta, S.; Thakur, D.; Handique, P.J. First report of *Nigrospora* leaf blight on tea caused by *Nigrospora sphaerica* in India. *Plant Dis.* **2015**, *99*, 417. [CrossRef]
111. Liu, Y.J.; Tang, Q.; Fang, L. First report of *Nigrospora sphaerica* causing leaf blight on *Camellia sinensis* in China. *Plant Dis.* **2016**, *100*, 221. [CrossRef]
112. Wright, E.R.; Folgado, M.; Rivera, M.C.; Crelier, A.; Vasquez, P.; Lopez, S.E. *Nigrospora sphaerica* causing leaf spot and twig and shoot blight on blueberry: A new host of the pathogen. *Plant Dis.* **2008**, *92*, 171. [CrossRef]
113. Li, Y.G.; Huang, M.H.; Sun, L.P.; Ji, P. Occurence of leaf spot on calabash caused by *Nigrospora sphaerica* in Georgia. *Plant Dis.* **2016**, *100*, 1506.
114. Xu, Y.M.; Liu, Y.J. First report of *Nigrospora sphaerica* causing leaf bligh on *Cunninghamia lanceolata*—China. *Plant Dis.* **2017**, *101*, 389. [CrossRef]
115. Soylu, S.; Dervis, S.; Soylu, E.M. First report of *Nigrospora sphaerica* causing leaf spots on Chinese Wisteria: A new host of the pathogen. *Plant Dis.* **2011**, *95*, 219. [CrossRef]
116. Sun, X.D.; Cai, X.L.; Pang, Q.Q.; Zhou, M.; Zhang, W.; Chen, Y.S.; Bian, Q. First report of leaf blight on *Mentha canadensis* caused by *Nigrospora sphaerica* in China. *Plant Dis.* **2020**, *104*, 3059. [CrossRef]
117. Deepika, Y.S.; Mahadevakumar, S.; Amruthesh, K.N.; Lakshmidevi, N. First report of *Nigrospora sphaerica* associated with leaf spot disease of Cowpea (*Vigna unguiculata*) from India. *Plant Dis.* **2020**, *105*, 506. [CrossRef]
118. Zhang, L.X.; Song, J.H.; Tan, G.J.; Li, S.S. First report of leaf blight caused by *Nigrospora sphaerica* on Curcuma in China. *Plant Dis.* **2011**, *95*, 1190. [CrossRef]
119. Abass, M.H.; Hameed, M.A.; Ahmed, A.N. First report of *Nigrospora sphaerica* (Sacc.) Mason as potential pathogen on date palm (*Phoenix dactylifera* L.). *Can. J. Plant Pathol.* **2012**, *35*, 75–80. [CrossRef]
120. Al-Sadi, A.M.; Al-Jabri, A.H.; Al-Mazroui, S.S.; Al-Mahmooli, I.H. Characterization and pathogenicity of fungi and oomycetes associated with root disease of date palm in Oman. *Crop Prot.* **2012**, *37*, 1–6. [CrossRef]
121. Alam, M.W.; Rehman, A.; Ahmad, S.; Sarwar, M.; Nawaz, A.; Khan, S.M.; Ali, S.; Aslam, S.; Mannan, A. First report of *Nigrospora sphaerica* causing leaf spot of date palm in Pakistan. *J. Plant Pathol.* **2020**, *102*, 223. [CrossRef]
122. Yasmin, Z.; Shamsi, S. Mycoflora associated with symptomatic leaves of *Rauvolfia serpentina* (L.) Benth. Ex. Kurz. in Bangladesh. *Bangladesh J. Plant Taxon.* **2020**, *27*, 129–136. [CrossRef]
123. Taguiam, J.D.; Evallo, E.; Bengoa, J.; Maghirang, R.; Balendres, M.A. Detection of *Nigrospora sphaerica* in the Philippines and the suscpetibility of three *Hylocereus* species to reddish-brown spot disease. *J. Prof. Assoc. Cactus Dev.* **2020**, *22*, 49–61. [CrossRef]
124. Liu, F.; Wu, J.B.; Zhan, R.L.; Ou, X.C. First report of reddish brown spot disease on pitaya caused by *Nigrospora sphaerica* in China. *Plant Dis.* **2016**, *100*, 1792. [CrossRef]
125. Han, Y.Z.; Fan, Z.W.; Wu, C.F.; Li, M.Y.; Zhou, D.D. First report of *Nigrospora* leaf blight on elephant grass caused by *Nigrospora sphaerica* in China. *Plant Dis.* **2019**, *103*, 2681. [CrossRef]
126. Gautam, A.K. First report of *Nigrospora sphaerica* causing leaf spot on *Celtis australis* from Himachal Pradesh, India. *Int. Lett. Nat. Sci.* **2015**, *40*, 16–18. [CrossRef]
127. Qiu, C.; Liu, C.; Niu, T.; Zhu, W.; Liu, Z. *Nigrospora sphaerica* causing leaf spot in a new host, *Eclipta prostrata* (False Daisy), in China. *J. Phytopathol.* **2022**, *170*, 242–246. [CrossRef]
128. Alam, M.W.; Rehman, A.; Gleason, M.L.; Riaz, K.; Saira, M.; Aslam, S.; Rosli, H.; Muhammad, S. First report of *Nigrospora sphaerica* causing leaf spot on Kinnow mandarin in Pakistan. *J. Plant Pathol.* **2017**, *99*, 295.
129. Chen, Y.; Yang, X.; Zhang, A.F.; Zhang, H.Y.; Gu, C.Y.; Hameed, U.; Qi, Y.J.; Xu, Y.L. First report of leaf spot caused by *Nigrospora sphaerica* on kiwifruit in China. *Plant Dis.* **2016**, *100*, 2326. [CrossRef]
130. Verma, O.P.; Gupta, R.B.L. A new host for *Nigrospora sphaerica* causing leaf spots on *Glycyrrhiza glabra*. *Plant Pathol.* **2008**, *57*, 782. [CrossRef]
131. Pandey, A.; Pandey, S.; Awasthi, A.K. A new host record of *Nigrospora sphaerica* on *Mangifera indica* from Jabalpur, India. *J. Mycol. Plant Pathol.* **2013**, *43*, 255–256.
132. Youssef, K.; Mosa, M.A.; Kamhawy, M.A. First report of *Nigrospora sphaerica* causing twig dieback and leaf spot of mango in Egypt. *J. Plant Pathol.* **2022**, *104*, 1571. [CrossRef]
133. Chen, J.; Xiang, T.T.; Liu, X.Y.; Wang, W.H.; Zhang, B.L.; Liu, J.; Zhou, W.; Wan, Y.J.; Chen, G.; Zhu, H.S. First report of *Nigrospora sphaerica* causing shot hole disease on mulberry in China. *Plant Dis.* **2018**, *102*, 245. [CrossRef]
134. Arunakumar, G.S.; Gnanesh, B.N.; Supriya, M.; Sivaprasad, V. First report of *Nigrospora sphaerica* causing shot hole disease on mulberry in India. *Plant Dis.* **2019**, *103*, 1783. [CrossRef]
135. Wang, Y.; Cernava, T.; Zhou, X.; Yang, L.; Baccelli, I.; Wang, J.; Gou, Y.; Sang, W.; Chen, X. First report of passion fruit leaf blight caused by *Nigrospora sphaerica* in China. *Plant Dis.* **2022**, *106*, 323. [CrossRef]
136. Liu, X.; Yu, F.; Fu, D.; Yang, W. First report of leaf blight on peanut caused by *Nigrospora sphaerica* in China. *J. Plant Pathol.* **2020**, *102*, 1269. [CrossRef]

137. Hernández-Cubero, L.C.; Ampofo, P.; Montes, J.M.; Voegele, R.T. Identification of pathogenic fungi and preliminary screening for resistance in *Jatropha curcas* L. germplasm. *Eur. J. Plant Pathol.* **2017**, *149*, 325–336. [CrossRef]
138. Zheng, X.; Liu, C.; Zhang, M.; Shang, X.; Fang, S.; Chen, F. First report of leaf blight of *Cyclocarya paliurus* caused by *Nigrospora sphaerica* in China. *Crop Prot.* **2021**, *140*, 105453. [CrossRef]
139. Zhao, H.; Liu, H.Y.; Yang, X.S.; Liu, Y.X.; Ni, Y.X.; Wang, F.; Tang, L. First report of *Nigrospora* leaf blight on sesame caused by *Nigrospora sphaerica* in China. *Plant Dis.* **2014**, *98*, 842. [CrossRef]
140. Rehman, A.; Alam, M.W.; Saira, M.; Naz, S.; Mushtaq, R.; Chohan, T.A.; Din, S.U.; Noureen, A.; Gilani, K.; Hussain, D. *Nigrospora sphaerica* causing leaf blight disease on sesame in Palkistan. *Plant Dis.* **2022**, *106*, 317. [CrossRef]
141. Cui, Y.P.; Wu, B.; Peng, A.T.; Li, Z.L.; Lin, J.F.; Song, X.B. First report of *Nigrospora* leaf blight on sugarcane caused by *Nigrospora sphaerica* in China. *Plant Dis.* **2018**, *102*, 824. [CrossRef]
142. Hong, X.; Chen, S.; Wang, L.; Liu, B.; Yang, Y.; Tang, X.; Liu, Y.S.; Huang, S. First report of *Nigrospora sphaerica* causing fruit dried-shrink disease in *Akebia trifolitata* from China. *Plant Dis.* **2021**, *105*, 2244. [CrossRef]
143. Liu, Y.J.; Hu, F.; Chen, L.S.; Xu, S.W. First report of *Nigrospora sphaerica* causing leaf blight on oil tea (*Camellia oleifera*) in China. *Plant Dis.* **2020**, *104*, 3252. [CrossRef]
144. Ismail, S.I.; Razak, N.F.A. First report of *Nigrospora sphaerica* causing leaf spot on watermelon (*Citrullus lanatus* L.) in Malaysia. *Plant Dis.* **2020**, *105*, 488. [CrossRef]
145. Bernardi, C.; Busso, C.; Borin, R.C.; Mazaro, S.M.; Saburo, R.S.S. The first report of *Nigrospora sphaerica* associated with *Helicarpus americanus* seeds in Brazil. *Floresta Ambiente* **2021**, *28*, e20190103. [CrossRef]

Naslov izvornog znanstvenog rada broj 3: First Report of *Nigrospora* Species Causing Leaf Spot on Olive (*Olea europaea* L.)

Proširenи sažetak:

U 2021. godini u maslinicima u Istri, Hrvatska, primijećeni su simptomi pjegavosti lišća na stablima masline (*Olea europaea* L.). Simptomi su se manifestirali kao tamne nekrotične pjege različite veličine, često okružene žućkastom bojom. Kako bi se identificirao uzročnik, provedena je izolacija patogena iz simptomatskih listova. Izolirane gljive su preliminarno, na temelju morfoloških karakteristika, klasificirane kao pripadnici roda *Nigrospora*. Daljnja molekularna analiza (sekvenciranje ITS, TUB i TEF1-α regija genoma) i filogenetska analiza potvrdile su prisutnost triju vrsta: *Nigrospora gorlenkoana* Novobr., *Nigrospora osmanthi* Mei Wang & L. Cai i *Nigrospora philosophiae-doctoris* M. Raza, Qian Chen & L. Cai. Patogenost izoliranih vrsta ispitana je inokulacijom zdravih listova masline u *in vitro* i *in vivo* uvjetima. Inficirani listovi razvili su simptome slične onima viđenim na terenu, što je dokazalo uzročnu povezanost između izoliranih *Nigrospora* spp. i pojave bolesti. Ovo je prvi izvještaj u Hrvatskoj koji dokumentira *Nigrospora* spp. kao patogene na maslini. Štoviše, ovo je ujedno i prvi izvještaj u svijetu za navedene tri vrste kao uzročnike bolesti na maslini te prvi izvještaj o vrsti *N. philosophiae-doctoris* kao uzročniku bolesti biljaka.

Ključne riječi: gljivična bolest; izolat; maslina; patogenost

Izvorni znanstveni rad broj 4 u obliku i izvornom jeziku na kojem je objavljen u znanstvenom časopisu

Naslova rada: Identification and Pathogenicity of *Biscogniauxia* and *Sordaria* Species Isolated from Olive Trees

Autori: Elena Petrović, Sara Godena, Jasenka Ćosić, Karolina Vrandečić

Tip rada: Izvorni znanstveni članak

Časopis: Horticulturae

Kategorija: A1

Impakt faktor: 3,1 (2024.)

Kvartil: Q1

Primljen na recenziju: 21. prosinac 2023.

Prihvaćen za objavljivanje: 29. veljače 2024.

Status: Objavljen

Volumen: 10

Broj: 3

Broj rada: 243

WOS broj: 001193349800001

horticulturae

MDPI

Article

Identification and Pathogenicity of *Biscogniauxia* and *Sordaria* Species Isolated from Olive Trees

Elena Petrović [1], Sara Godena [1,*], Jasenka Ćosić [2] and Karolina Vrandečić [2]

[1] Institute of Agriculture and Tourism, Karla Huguesa 8, 52440 Poreč, Croatia; elena@iptpo.hr
[2] Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Vladimira Preloga 1, 31000 Osijek, Croatia; jcosic@fazos.hr (J.Ć.); kvrandecic@fazos.hr (K.V.)
* Correspondence: sara@iptpo.hr

Abstract: A field investigation of olive trees in Istria, Croatia, revealed branch dieback and cracked bark. Samples of diseased branches were collected from eight different locations and analysed. Additionally, meteorological data from two locations were analysed to determine if there was a connection between climatic changes and the appearance of pathogens in the region. Pathogenicity tests were conducted on olive seedlings. This study provides a description of *Biscogniauxia* and *Sordaria* species' morphology and elucidates their phylogeny based on the internal transcribed spacer (ITS), beta-tubulin (*TUB2*) and translation elongation factor 1-alpha (*TEF1-α*) regions. This research represents the first documented occurrence of *Biscogniauxia mediterranea* causing charcoal disease in olive trees in Croatia. Additionally, it is the first report of *Biscogniauxia nummularia* (Bull.) Kuntze and *Sordaria fimicola* causing diseases in olive trees anywhere in the world. Furthermore, this study marks one of the initial forays into molecular investigations of these species isolated from olive trees. Considering the potential threat posed by the inherent aggressiveness of *Biscogniauxia* species, further research is deemed necessary to curb the development of charcoal disease.

Keywords: charcoal disease; *Biscogniauxia mediterranea*; *Biscogniauxia nummularia*; first report; *Sordaria fimicola*

Citation: Petrović, E.; Godena, S.; Ćosić, J.; Vrandečić, K. Identification and Pathogenicity of *Biscogniauxia* and *Sordaria* Species Isolated from Olive Trees. *Horticulturae* **2024**, *10*, 243. https://doi.org/10.3390/horticulturae10030243

Academic Editors: Miguel de Cara-García and Harald Scherm

Received: 21 December 2023
Revised: 26 February 2024
Accepted: 29 February 2024
Published: 2 March 2024

1. Introduction

Olive cultivation serves not only as a vital agricultural industry but also as a cherished cultural tradition in numerous regions, playing a pivotal role in the production of olive oil and table olives worldwide. While olive cultivation boasts a rich history, particularly in Mediterranean countries, it has also expanded to other regions with favorable climates such as Australia and California [1], while Italy, Spain and Tunisia rank as some of the world's foremost producers of olives. While Croatia's olive production may be relatively modest compared to that of Italy, Spain and Tunisia, it remains a key agricultural sector in numerous coastal regions. According to FAO, the Croatian national production of olives in 2022 was 40,130 tons, while the olive cultivation area covered 19,900 hectares. The production of olive oil in 2021 amounted to 3500 tons [2]. The region's warm, sunny summers and mild winters create a favourable environment for the cultivation of olive trees. Due to these unique climatic conditions, Mediterranean climate ecosystems host remarkable levels of biodiversity that hold global significance [3]. The Mediterranean littoral vegetation belt encompasses the majority of the Adriatic islands, Central and South Dalmatia and the narrow coastal region of the Croatian littoral. It is divided into three horizontal vegetation zones: the Steno-Mediterranean, Eu-Mediterranean and Sub-Mediterranean. Istria falls within the Eu-Mediterranean region. The absolute minimum temperatures occur here during more months of the year than in the Steno-Mediterranean. In the areas of Rovinj and Poreč, Istria, absolute minimum temperatures begin in October. The rainy period is in the autumn, with the main precipitation minimum occurring in winter, accompanied by a

shorter dry period in summer [4]. However, variability in climate can sometimes impact olive production.

Trunk diseases in olive trees target the woody tissues of the tree, encompassing the branches, trunk and occasionally even the roots. These ailments have the potential to result in substantial economic losses within the olive production industry. Trunk diseases affecting olive trees are instigated by a range of fungal pathogens. Among the most prevalent fungal species implicated in these conditions are *Cytospora* spp., *Diplodia* spp., *Dothiorella* spp., *Neofusicoccum* spp., *Phaeoacremonium* spp. and *Pseudophaeomoniella* spp. *Cytospora* spp. has been identified as an olive pathogen in California [5], Croatia [6], Greece [7], Spain [8] and South Africa [9,10]. Similarly, species from the *Botryosphaeriacea* family, namely *Diplodia* spp., *Dothiorella* spp. and *Neofusicoccum* spp., have been recognised as pathogens in regions including California [11], Croatia [12,13], Italy [14–16], Spain [8,17], South Africa [18] and Uruguay [19]. *Phaeoacremonium* spp. have been confirmed as a pathogen in Croatia [20], California [11], Italy [15,21] and South Africa [10,18], while *Pseudophaeomoniella* spp. have been identified as a pathogen in Greece [22], South Africa [18] and Spain [23].

These fungi enter the tree through wounds, naturally occurring openings or pruning incisions [8]. The manifestations of trunk diseases in olive trees can exhibit variability but frequently encompass branch dieback, diminished fruit production and wilting [6,20]. Pycnidia and/or perithecia of certain fungi linked to olive dieback can be discovered embedded in the bark of the affected areas [11]. Additionally, hallmark indications involve the emergence of dark lesions or cankers on the trunk [11,20]. These cankers have the potential to encircle the tree, disrupting the flow of water and nutrients. Trunk diseases can spread among trees through the use of pruning tools, contaminated soil or infected plant material. The dispersal of spores of trunk pathogens can take place through various mechanisms, including rain, wind and both internal or external dissemination facilitated by insects [24–27].

One of the causative agents of trunk disease in olives is a species from the *Biscogniauxia* genus [28]. *Biscogniauxia* is a fungal genus belonging to the family *Graphostromataceae*, the order *Xylariales* and the *class Sordariomycetes*. These fungi are renowned as cosmopolitan and omnipresent wood decomposers, as well as prevalent endophytes [29]. The *Biscogniauxia* genus (basionym *Nummularia* Tul. and C. Tul.) was introduced in 1891 for seven species by Kuntze (Table 1), while *Nummularia* Tul. and C. Tul. was introduced in 1863 by Tulasne and Tulasne.

Table 1. *Biscogniauxia* genus—seven species described by Kuntze [30].

Species	Basionym
Biscogniauxia baileyi (Berk. and Broome ex Cooke) Kuntze	*Nummularia baileyi* Berk. and Broome ex Cooke
Biscogniauxia bulliardii (Tul. and C. Tul.) Kuntze	*Nummularia bulliardii* Tul. and C. Tul.
Sphaeria discreta Schwein.	*Sphaeria discreta* Schwein.
Obolarina dryophila (Tul. and C. Tul.)	*Nummularia dryophila* Tul. and C. Tul.
Camillea obularia (Fr.) Læssøe	*Hypoxylon obularium* Fr.
Biscogniauxia repanda (Fr.) Kuntze	*Sphaeria repanda* Fr.
Biscogniauxia mediterranea (De Not.) Kuntze	*Sphaeria mediterranea* De Not.

B. mediterranea and *B. nummularia* (Bull.) Kuntze are members of this fungus group. Granata and Sidoti [31] state that *B. nummularia* is naturally found in beech stands and has not been traditionally regarded as a primary pathogenic agent. Considering that pathogenicity is a variable trait, some species can acquire pathogenicity under certain conditions. According to some authors, *B. mediterranea* and *B. nummularia* exist in a latent form within symptomless host tissues during a portion of their life cycle. According to Petrini [32], this would qualify them as endophytes during this latent period. Some studies

have demonstrated that these species can rapidly transform from a benign endophyte to a primary pathogen [33,34]. Under stress conditions, they can swiftly infiltrate woody tissues, sometimes becoming noticeable as fungal stroma on branches and trunks [35]. Van Dyk et al. [10] note that drought conditions in the Mediterranean region can influence the aggressiveness of the species and lead to the onset of the disease. The high temperatures and extended periods of summer drought influence the rapid spread of *B. mediterranea* and *B. nummularia* [36,37]. *B. mediterranea* is typically found in association with trees affected by drought, fires and mechanical injuries [38]. Nugent et al. [34] mention water stress as a key factor predisposing trees to susceptibility to attacks by this fungus. *Biscogniauxia* species are recognised for inducing cankers on the bark of trees. *B. mediterranea* can affect both the trunk and leaves of olive trees, causing the development of longitudinal bark cracks, detached bark and the withering of the crown [28]. In more severe instances, *Biscogniauxia* infections can lead to stem girdling, where the cankers encircle the tree trunk, as observed in wild almond [39]. This girdling disrupts the flow of nutrients and water, contributing to branch dieback and, eventually, tree mortality. The pathogen can fatally affect the host within a single growing season [39].

B. mediterranea is among the most common fungal pathogens in the Mediterranean region, leading to charcoal canker (syn. charcoal disease) in different plant species, including oak (*Quercus castaneifolia* C.A.Mey., *Q. brantii* Lindl. and *Q. suber* L.), *Prunus scoparia* (Spach) C.K.Schneid (syn. *Amygdalus scoparia*) and *Zelkova carpinifolia* [38–41].

On the other hand, *B. nummularia* is known to cause a disease called strip cankering (syn. beech tarcrust, charcoal canker, hypoxylon canker) on European beech (*Fagus sylvatica* L.) and sedge (*Carex brevicollis* DC.) [36,41,42].

Some authors highlight species from the *Biscogniauxia* genus for its increasing economic impact, which has been attributed to climate change [36,41,43].

The genus *Sordaria* is classified within the family *Sordariaceae*, belonging to the fungal order *Sordariales* and the class *Sordariomycetes*. These fungi typically inhabit herbivore dung, decaying plant material and, infrequently, coniferous needles. They exhibit a brief life cycle, typically spanning 7 to 12 days [44]. Due to its ease of cultivation in laboratory settings, the fungus is frequently used in microbiology laboratories, for research purposes, as a model organism.

S. fimicola is a homothallic species. Any haploid nucleus within the mycelium can pair with any other nucleus, leading to the abundant production of selfed perithecia alongside hybrid ones. Notably, individual perithecia may give rise to clusters of intermixed hybrid and homozygous asci [45]. An important aspect of this species' life cycle is that it does not involve the formation of macroconidia. Instead, microconidia are produced, serving as male gametes in sexual reproduction. While microconidia germination is possible, it tends to be very poor [46]. *S. fimicola* has been recorded as a pathogen on the species *Acer palmatum* 'Atropurpureum' [46]. In a study conducted by Newcombe et al. [47], *S. fimicola* reduced the growth and fecundity of two out of three populations of *Bromus tectorum* L., the host from which they had been isolated. The same isolate of *S. fimicola* reproduced sexually on inoculated host plant tissues as well as in dung after passage through sheep, thus demonstrating a facultative rather than an obligate life cycle.

Currently, 116 *Biscogniauxia* species and 262 *Sordaria* species are listed in the MycoBank database. In contrast, the NCBI database includes 37 identified *Biscogniauxia* species along with 55 unidentified *Biscogniauxia* isolates, and 20 identified *Sordaria* species as well as 23 unidentified *Sordaria* isolates.

The objectives of this research were to identify the causal agent of symptoms observed on olive trees in Croatia; to morphologically characterise and molecularly identify the isolates of phytopathogenic fungal species using PCR and DNA sequence analysis of ITS, *TUB2* and *TEF1-α* gene regions; to assess the isolates' pathogenicity in pathogenicity tests; and to examine the impact of weather conditions on the appearance of pathogens.

2. Materials and Methods

2.1. Fieldwork and Isolation of Fungi

The symptoms were spotted on olive trees between August and October of 2021 in eight olive orchards in Istria, Croatia while searching for the causative agents of Botriosphaeria dieback of olive trees. The trees exhibited symptoms such as branch and twig dieback, the dieback and shedding of leaves and the premature dropping of fruits. In addition, substantial drying and intensive cracking of the bark on olive trees were observed. After removing the upper layer of the tree bark, dried dark tissue was noticed. The presence of stroma on olive trees was not observed. Samples were gathered from a total of twelve trees, i.e., one in Kaštelir (45°17′30″ N, 13°40′51″ E), one in Fažana (44°56′21″ N, 13°50′18″ E), one in Novigrad (45°20′08.8″ N, 13°33′33.6″ E), three in Španidiga (45°03′02.2″ N, 13°42′43.9″ E), two in Rovinj (45°03′46″ N, 13°42′71″ E), one in Sveti Lovreč (45°09′26″ N, 13°45′02″ E) and three in Poreč (45°13′18.5″ N, 13°36′11.9″ E and 45°13′14.7″ N, 13°36′09.2″ E). Affected trees were aged between 12 and 48 years old. Among these locations, six practised conventional management methods for olive production, while one—located in Vodnjan—followed organic farming practices, and another—situated in Novigrad—employed integrated farming methods. Ten branches from each symptomatic tree were collected and placed in individual sterile black plastic bags. The branches were obtained from areas of the trees exhibiting decline, specifically focusing on sections where the transition from the healthy to the dry part was visible. Each bag was properly labelled and then stored in a portable refrigerator at a temperature of +4 °C. The samples were then promptly transported to the Laboratory for Plant Protection at the Institute of Agriculture and Tourism in Poreč, Croatia, for analysis. The branch samples underwent thorough washing with tap water. Using a sterile surgical scalpel, the bark was carefully removed from the branches, after which the samples were cut using fruit shears. The branch pieces (5 × 5 cm) were immersed in a 10% bleach solution for 30 s, followed by three rinses in sterile distilled water for 30 s. Afterwards, they were arranged on a sterile paper sheet within a laminar flow cabinet to allow for surface drying. Once dried, the pieces were placed onto potato dextrose agar (PDA) supplemented with 25 mg/L of streptomycin. They were then incubated in a dark environment at 25 °C within an incubator. Following the establishment of pure cultures, small pieces of mycelium were transferred using a sterile laboratory needle onto PDA, malt extract agar (MEA) and water agar (WA) for further examination. Pure cultures were preserved in 2 mL cryovial screw cap tubes containing a 50% glycerol solution at temperatures of −20 °C and −80 °C.

2.2. Morphological Characterisation

After incubating for two weeks at 25 °C in the absence of light, pure fungal cultures were examined. From all 10 collected samples per tree (total of 12 trees), the same fungus was consistently isolated. Morphological characterisation was performed on all isolates and, subsequently, one representative isolate per tree was selected for further detailed morphological study. Fungal species from 12 isolates (1 isolate per tree) were characterised by evaluating colony traits (such as colour, shape, elevation, margin, surface and opacity) as well as the features of spores (including colour, shape, presence or absence of septum and dimensions). The morphological characteristics were compared with those from the relevant literature [39,41,44,48].

Macroscopic characteristics were observed using a BOECO zoom stereo microscope BSZ-405 and photographed with a fitted B-CAM16 industrial digital camera and B-View software (Boeckel, Hamburg, Germany). Microscopic characteristics were observed using a BOECO BM-2000 microscope, BOECO BCAM10 camera and B-View software (Boeckel) at the Institute of Agriculture and Tourism Poreč and with an Olympus BX41 microscope (Olympus, Tokyo, Japan) at the Faculty of Agrobiotechnical Sciences Osijek.

2.3. DNA Extraction and Amplification

Fresh fungal mycelia from fungal isolates, that had been cultured on PDA for one week at 25 °C in the dark, were gently scraped from the colony margins using a sterile laboratory needle. This mycelium was then used for the extraction of genomic DNA. The total genomic DNA from the isolates was extracted using a Maxwell® RSC Instrument (Promega, Madison, WI, USA) along with the Maxwell® RSC Plant DNA Kit (Promega). A Maxwell Promega Quantus™ fluorometer (Promega) was used to determine the amount of genomic DNA in samples after DNA isolation. The regions of the internal transcribed spacer (ITS) were amplified using primers ITS4 (5′ TCCTCCGCTTATTGATATGC 3′) and ITS1 (5′ TCCGTAGGTGAACCTGCGG 3′) [49]. Partial beta-tubulin (TUB2) was amplified using oligonucleotide primers Bt2a (5′ GGTAACCAAATCGGTGCTGCTTTC 3′) and Bt2b (5′ ACCCTCAGTGTAGTGACCCTTGGC 3′) [50]. For amplifying portions of the translation elongation factor 1-alpha (*TEF1- α*), the PCR primer pair of EF-728F (5′ CATCGAGAAGTTCGAGAAGG 3′) and EF-986R (5′ TACTTGAAGGAACCCTTACC 3′) [51] was used. The components of a 25 µL PCR mixture were as follows: 12.5 µL of EmeraldAmp® GT PCR Master Mix (Takara Bio Inc., San Jose, CA), 0.5 µL of each primer (10 µM), 6.5 µL of nuclease-free water and 5 µL of genomic DNA (at a concentration of 5 ng/µL). The PCR was carried out using a MiniAmpPlus Thermal Cycler (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). The PCR conditions for *Biscogniauxia* species were as follows: initial denaturation step at 95 °C for 2 min and 30 s, 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, elongation at 72 °C for 32 s and final extension at 72 °C for 5 min [52]. The PCR conditions for *Sordaria* species were as follows: initial denaturation step at 94 °C for 2 min, 40 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, elongation at 72 °C for 1 min and 30 s and final extension at 72 °C for 5 min [53]. Electrophoresis was conducted using a 1% agarose gel at 110 V for 25 min in 1× TAE buffer, using an omniPAC Midi CS-300V electrophoresis power supply (Cleaver Scientific, Rugby, Warwickshire, UK). Following electrophoresis, the PCR products were visualised using an iBright CL1000 Imaging System (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA).

2.4. DNA Sequence Assembly and Phylogenetic Analysis

Sequencing of the PCR products was carried out by Macrogen Europe (Amsterdam, The Netherlands). The obtained sequences were subsequently edited using Sequencher® 5.4.6. software (Gene Codes, Ann Arbor, MI, USA). These edited sequences were then compared with sequences available in GenBank. For phylogenetic analyses, relevant sequences from the GenBank database were used. The details of these employed sequences are presented in Table 2. The ITS, *TUB2* and *TEF1- α* sequence data were assembled individually using ClustalX2 (UCD Dublin) software. Subsequently, multiple sequence alignments of the ITS, *TUB2* and *TEF1- α* gene regions were combined and adjusted, also using ClustalX2 (UCD Dublin, Dublin, Ireland) software. A phylogenetic tree was made using MEGA11 software (Pennsylvania State University, State College, PA, USA), and the evolutionary history was inferred using the Neighbour Joining method [54].

Table 2. List of species and isolates used in the phylogenetic analysis and their isolation source, country of origin, GenBank accession number and references.

Species	Isolate	Isolation Source	Country	GenBank Accession Number			References
				ITS	*TUB2*	*TEF1- α*	
Biscogniauxia anceps	YMJ 123	*Corylus avellana* L.	France	EF026132	AY951671	/	[55,56]
B. arima F. San Martín, Y.M. Ju and J.D. Rogers	YMJ 122	Wood	Mexico	EF026150	AY951672	/	[56,57]
B. atropunctata	YMJ 128	Wood	United States of America	JX507799	AY951673	/	[57,58]
	ATCC 38987	Unknown	United States of America	AF201705	/	/	[59]

Table 2. *Cont.*

Species	Isolate	Isolation Source	Country	GenBank Accession Number			References
				ITS	*TUB2*	*TEF1-α*	
B. atropunctata var. intermedia	B70M	*Quercus* sp.	Costa Rica	AJ390412	/	/	[60]
B. bartholomaei	ATCC 38992	Unknown	United States of America	AF201719	/	/	[59]
B. capnodes	YMJ 142	*Nothofagus solandri* (Hook.f.) Oerst.	New Zealand	/	AY951674	/	[56]
	YMJ 138	Corticated wood	Taiwan	EF026131	AY951675	/	[57,58]
B. citriformis	YMJ 88113012	Wood	Taiwan	JX507800	AY951677	/	[56]
	YMJ 129	*Casuarina equisetifolia* L.	Hawaii, USA	JX507801	AY951678	/	[57,58]
B. cylindrispora Y.M. Ju and J.D. Rogers	YMJ 89092701	Bark of *Cinnamomum* Schaeff.	Taiwan	EF026133	AY951679	/	[56,61]
B. formosana Y.M. Ju and J.D. Rogers	YMJ 89032201	Bark	Taiwan	JX507802	AY951680	/	[56,58,61]
B. grammoi	YMJ 135	Bark of *Prunus padus* L.	Austria	JX507803	AY951681	/	[56–58]
B. latirima Y.M. Ju and J.D. Rogers	YMJ 90080703	Bark	Taiwan	EF026135	AY951683	/	[56,61]
B. magna Samarak. and K.D. Hyde	MFLU 18-0850	Dead branch from unidentified host	Thailand	MW240616	MW775577	MW759498	[62]
B. marginata	MFLUCC 12-0740	Unknown	France	KJ958407	/	/	[63]
	B74A	*Quercus* sp.	Pennsylvania, USA	AJ390417	/	/	[60]
B. mediterranea	YMJ 147	Corticated wood	France	EF026134	AY951684	/	[56–58]
	Bx63	*Quercus pubescens* Willd.	Italy	KT253501	KT253535	/	[64]
	Bx70	*Q. pubescens*	Italy	KT253502	KT253536	/	[64]
	Bx85	*Q. pubescens*	Italy	KT253503	KT253537	/	[64]
	Bm8L-19Aa	*Abies alba* Mill.	Poland	MN538267	MZ221959	MZ221965	[42]
	Bm04.001	*Quercus suber* L.	Portugal	KM216752	KM267202	KM216788	[65]
	Bm07.003	*Q. suber*	Portugal	KM216754	KM267203	KM216790	[65]
	Bm10.019	*Q. suber*	Portugal	KM216761	KM267210	KM216797	[65]
	Bm10.001	*Q. suber*	Portugal	KM216756	KM267205	KM216792	[65]
	Bm10.006	*Q. suber*	Portugal	KM216757	KM267206	KM216793	[65]
	Bm10.012	*Q. rotundifolia* Lam.	Portugal	KM216758	KM267207	KM216794	[65]
	Bm11.003	*Q. suber*	Portugal	KM216764	KM267212	KM216800	[65]
	Bm12.027	*Q. robur* L.	Portugal	KM216775	KM267223	KM216811	[65]
	Bm12.032	*Q. suber*	Portugal	KM216777	KM267225	KM216813	[65]
	Pc08.002	Insects *Platypus cylindrus* Fabricius which were collected directly from their galleries on *Q. suber*	Portugal	KM216785	KM267234	KM216822	[65]
	Pc96.009	Insects *Platypus cylindrus* Fabricius which were collected directly from their galleries on *Q. suber*	Portugal	KM216786	KM267233	KM216821	[65]
	Bm10.016	*Q. suber*	Italy	KM216759	KM267208	KM216795	[65]
	CBS101016	*Q. robur*	Netherlands	KM216787	KM267235	KM216823	[65]
	Bm13.013	*Q. suber*	Spain	KM216784	KM267232	KM216820	[65]
	BM03 (BM01-BM03)	*Erica multiflora* L.	Tunisia	MH356285	MK210238	MK189173	[38]
	CPC:18215	*Quercus castaneifolia* C.A.Mey.	Iran	JF295127	/	/	[66]
	CPC:18216	*Q. castaneifolia*	Iran	JF295128	/	/	[66]
	CPC:18217	*Q. castaneifolia*	Iran	JF295129	/	/	[66]
	ARIZ:AZ0703	*Pseudevernia intensa*	Arizona, USA	HM123416	KU684122	/	[67]
	Oe.Bm 1	*Olea europaea* L.	Tunisia	KY275264	KY275263	/	[28]

Table 2. *Cont.*

Species	Isolate	Isolation Source	Country	GenBank Accession Number			References
				ITS	*TUB2*	*TEF1-α*	
B. nummularia	B72C	*Fagus sylvatica* L.	England	AJ390415	/	/	[60]
	BNUMM3	Unknown	Italy	AJ246231	/	/	[52]
	H86	*Salix alba* L.	Slovakia	GQ428318	GQ428324	/	[68]
	Bn3W-19Pu	*Pinus mugo* subsp. *uncinata* (Raymond ex A.DC.) Domin	Poland	MN595068	MZ221954	MZ221960	[42]
	Bn5L-19Pu	*P. mugo* subsp. *uncinata*	Poland	MN588203	MZ221955	MZ221961	[42]
	Bn6L-19Pu	*P. mugo* subsp. *uncinata*	Poland	MN588202	MZ221956	MZ221962	[42]
	Bn31M-20Aa	*A. alba*	Poland	MT936553	MZ221957	MZ221963	[42]
	Bn56C-20Aa	*A. alba*	Poland	MT937244	MZ221958	MZ221964	[42]
	GLMC 829	*Prunus avium* (L.) L.	Germany	MT153623	/	/	[69]
	NWFVA4756	*Pinus sylvestris* (L.) L.	Germany	MT790313	/	/	[70]
B. petrensis Z.F. Zhang, F. Liu and L. Cai	LC5698	Rock	China	KU746670	KU746763	KX855216	[70]
	LC5751	Rock	China	KU746671	KU746761	KX855215	[71]
B. philippinensis var. *microspora*	YMJ 89041101	Bark	Taiwan	EF026136	AY951685	/	[56,57]
B. repanda	B75A	Unknown	Unknown	AJ390418	/	/	[60]
B. rosacearum A. Carlucci and M. L. Raimondo	Bx3	*Cydonia oblonga* Mill.	Italy	KT253487	KT253521	/	[64]
	Bx14	*C. oblonga*	Italy	KT253488	KT253522	/	[64]
	Bx19	*C. oblonga*	Italy	KT253489	KT253523	/	[64]
	Bx25	*Q. pubescens*	Italy	KT253499	KT253533	/	[64]
	CSN1052	*O. europaea*	South Africa	MT813910	/	/	[18]
	CSN1055	Wild olive	New Zealand	MT813912	/	/	[18]
	CSN1056	Wild olive	South Africa	MT813913	/	/	[18]
	PMM2071	*O. europaea*	South Africa	MT813997	/	/	[18]
B. simplicior Pouzar	YMJ 136	*Rhamnus cathartica* L.	France	EF026130	AY951686	/	[56]
B. uniapiculata	YMJ 90080608	Bark	Taiwan	JX507805	AY951687	/	[56]
Phaeoacremonium iranianum L. Mostert, Grafenhan, W. Gams and Crous	R18B4	*O. europaea*	Croatia	OP627795	OP684932	OP684933	[20]
Sordaria alcina N. Lundqvist	CBS 109460	Unknown	Unknown	AY681198	AY681232	/	[72]
S. equicola Crous	CBS 1146992	Zebra dung	Namibia	NR173047	MZ078267	MZ078226	Unpublished
S. fimicola	DAFE_SP16-17	*Lupinus* sp.	Italy	MK560171	MK567929	/	[73]
	FGSC 2918	Unknown	New York, USA	/	FR774339	FR774388	[74]
	CBS 398.63	Unknown	Argentina	MH858315	/	/	[75]
	CBS 485.64	Unknown	Netherlands	MH858489	/	/	[75]
	CBS 508.50	Unknown	Unknown	AY681188	AY681228	/	[72]
S. lappae Potebnia	CBS 154.97	Unknown	Unknown	AY681171	AY681205	/	[72]
S. macrospora Auerswald	SORDMGRF46	*Grevillea robusta* A.Cunn. ex R.Br.	Kenya	/	/	FJ904898	Unpublished
S. tomentoalba Cailleux	CBS 260.78	Unknown	Unknown	AY681195	AY681229	/	[72]

2.5. Pathogenicity Tests on Olive Seedlings

For a whole plant assay, 3-year-old olive seedlings were inoculated in a greenhouse at the Institute of Agriculture and Tourism in Poreč. In total, two seedlings of the 'Buža' variety, two of the 'Istarska bjelica' variety, two of the 'Leccino' variety, two of the 'Porečka rosulja' variety and two of the 'Rosinjola' variety were each inoculated with the respective fungal isolate of *B. mediterranea*, *B. nummularia* and *S. fimicola* species. To initiate the inoculation process, wounds measuring 5 mm in diameter were created in the bark (which was wiped with a cotton ball soaked in 70% ethanol) using a sterile cork borer. These wounds were designed to remove the outer bark while preserving the inner bark. A 5 mm diameter mycelium plug from a 2-week-old PDA culture of the fungal isolates was then

carefully inserted into each wound. Subsequently, the inoculated wounds were sealed with Vaseline and covered with Parafilm. PDA plugs without mycelium served as a control. The inoculated plants were cultivated in the greenhouse at approximately 23 °C and a relative air humidity of 83% for a duration of 11 months, spanning from January to November 2023, for *Biscogniauxia* species. To induce more pronounced disease symptoms, the plants underwent water stress and were watered every 10 days throughout the summer months (June to August). For *S. fimicola*, the inoculated plants were cultivated in the greenhouse at approximately 18 °C and a relative air humidity of 93% for a duration of two months, spanning from October to November 2023, during which they were closely monitored for the presence of symptoms. After the incubation period, observations of changes in the olive trees were recorded and samples were collected in black plastic bags, labelled and analysed. In an attempt to adhere to Koch's postulate, small fragments of necrotic tissue from the edges of the developed lesions were placed on PDA medium to re-isolate the initially inoculated fungus.

2.6. Analysis of Meteorological Data

Meteorological and hydrological data were retrieved from the database maintained by the Croatian Meteorological and Hydrological Service, sourced from measurement stations close to the study sites. Specifically, information was gathered from two meteorological stations located in the cities of Poreč and Rovinj. Precise details regarding the geographic locations of these measurement stations are accessible through the official website of the Croatian Meteorological and Hydrological Service [76]. Data retrieval for Poreč spanned the years 1982 to 2023, while, in the Rovinj region, data from 1970 to 2023 were collected. The collected dataset comprised key variables, including the average monthly temperature (°C) measured by a dry thermometer, monthly and annual precipitation levels (mm) and average monthly relative air humidity (%). Subsequently, the acquired dataset underwent analysis in Microsoft Office Excel 2021, and linear trends of the observed variables were made.

3. Results

3.1. Field Symptoms

The observed symptoms on the olive trees included branch and twig dieback, canker formations, extensive drying and cracking of the bark, leaf dieback, necrosis and wilting. Upon removing the bark, the wood underneath exhibited a reddish-brown to black-brown discoloration. Additionally, the change in bark colour to reddish occurred as a result of branch decline. Trees infected with *Biscogniauxia* species displayed all the mentioned symptoms (Figure 1a–d), while those infected with the *Sordaria* species only showed symptoms of branch and leaf dieback and bark discolouration (Figure 1e).

3.2. Morphological Characterisation

The culture isolates were deposited in the collection of the Laboratory for Plant Protection, Department of Agriculture and Nutrition, at the Institute of Agriculture and Tourism in Poreč, Croatia.

3.2.1. *Biscogniauxia mediterranea*

The colonies expanded to a diameter of 9 cm within 3 days at 25 °C on PDA, and after 4 days on MEA. In contrast, the diameter of the colony on WA reached 7.1 cm after 40 days. The colonies on PDA and MEA exhibited rapid growth, as observed for *B. nummularia*, with a thick texture and a similar colour. On WA, the colonies appeared whitish to light grey and displayed poor growth (Figure 2). The colony surface colour on PDA differed among isolates, ranging from olivaceous to dark grey with beige spots, or a surface that was lightish grey with white spots, to cinnamon-brown and beige. Typically, it appeared as light grey initially and became darker with age, developing beige spots. The mycelium had a woolly, cottony and fluffy appearance. Reverse colonies were grey to beige with dark spots. The hyphae were aseptate, branched and hyaline. The perithecia exhibited

an ovoid-to-tubular shape, measuring 712.3–825.2 µm in length and 111.4–175.7 µm in width (x = 802.2 × 172.9 µm, n = 30). The asci were stipitate and had dimensions ranging from 118.1 to 174.9 µm in length and from 8.7 to 10.8 µm in width (x = 129.2 × 9.2 µm, n = 30). The ascospores were brown and ellipsoidal, measuring 14.7–21.2 µm in length and 6.9–8.8 µm in width (x = 19.9 × 8.2 µm, n = 30).

Figure 1. Symptoms on olive trees: (**a**–**d**) tree bark cracking caused by species of *Biscogniauxia*; (**e**) dieback of branches and leaves caused by *Sordaria fimicola*.

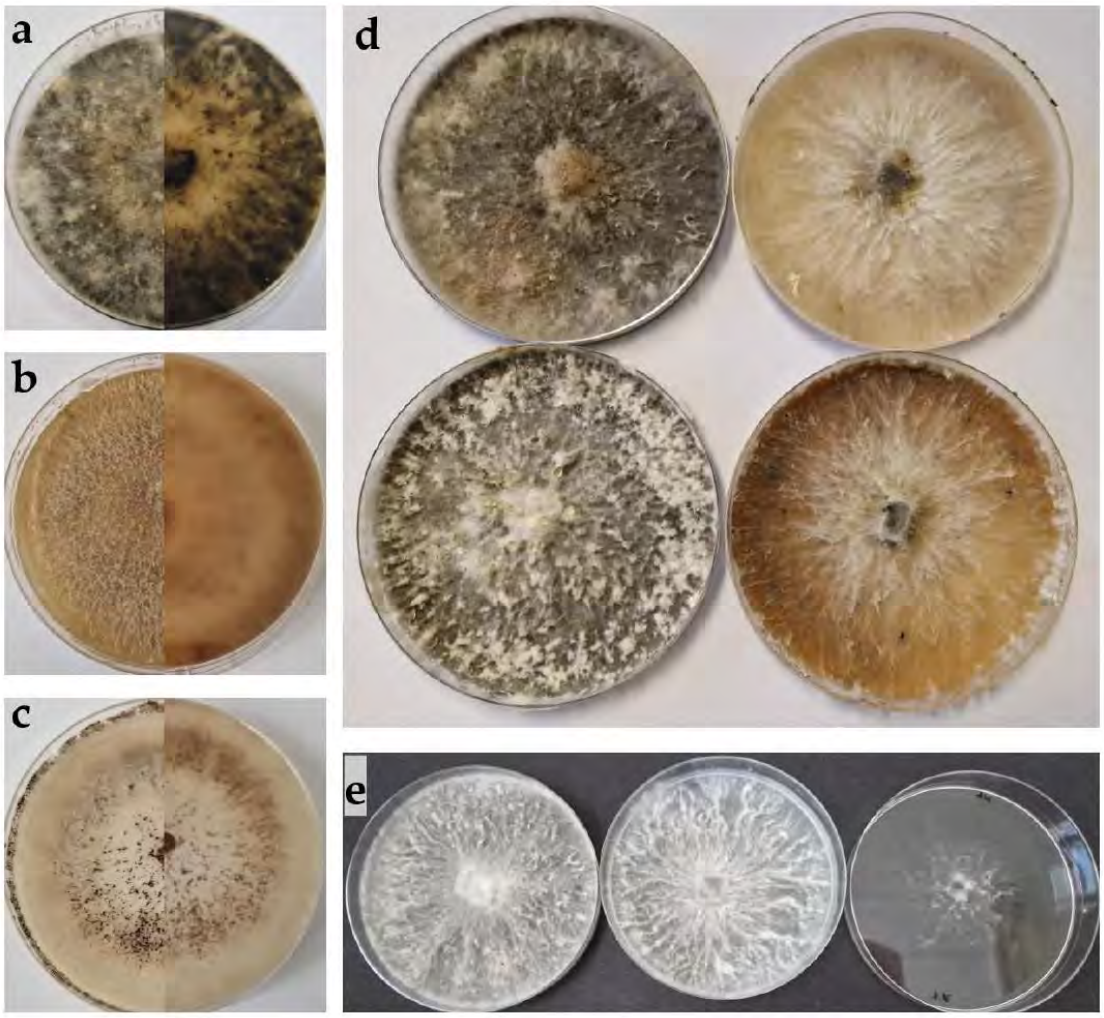


Figure 2. (**a**–**c**) Upper and reverse view of cultures two weeks after incubation at 25 °C on potato dextrose agar (PDA) medium: (**a**) *Biscogniauxia mediterranea*, (**b**) *B. nummularia*, (**c**) *Sordaria fimicola*. (**d**) Differences in colony colour between isolates of *B. mediterranea* on PDA after one month. (**e**) Difference in the growth of *B. mediterranea* isolates on PDA (**left**), malt extract agar (MEA) (**middle**) and water agar (WA) (**right**).

3.2.2. *Biscogniauxia nummularia*

The colonies expanded to a diameter of 9 cm within 5 days at 25 °C on PDA, and after 11 days on MEA. On WA, the colony diameter reached 8.7 cm after 40 days. The colonies on PDA and MEA exhibited rapid growth, with a thick texture and a similar colour. In contrast, the colonies on WA appeared whitish to light beige and displayed poor growth. The colony surface colour on PDA ranged from beige to light orange, occasionally featuring olivaceous spots (Figure 2). The mycelium had a cottony appearance. Reverse colonies were beige with grey discolouration and black to grey spots. The hyphae were septate, branched and hyaline to yellowish. The perithecia exhibited an ovoid-to-tubular shape, measuring 423.1–733.3 µm in length and 289.3–455.3 µm in width (x = 652.5 × 301.2 mm,

n = 30). The asci had dimensions ranging from 87.3 to 125.7 µm in length and from 8.1 to 11.1 µm in width (x = 95.2 × 9.1 mm, n = 30). The ascospores were dark brown and ovoid in shape, with tapered ends, measuring 10.2–13.6 µm in width and 6.9–8.7 µm in length (x = 12.9 × 7.8 µm, n = 30).

3.2.3. *Sordaria fimicola*

The colonies expanded to a diameter of 9 cm within 5 days at 25 °C on PDA, after 6 days on MEA and after 14 days on WA. Colonies developed rapidly on PDA and MEA, while growth on WA was somewhat slower, and the mycelium on the latter was less developed, white and with perithecia. The colony colour on PDA ranged from light in the initial stages of development to dark (Figure 2). The mycelium was aerial, with visible black perithecia formed after 4–5 days on PDA, 9 days on MEA and 19 days on WA. Reverse colonies were dark grey with visible perithecia. The hyphae were septate, branched and hyaline. The perithecium was dark-brown to black, solitary, superficial and pear-shaped, with colourless hairs, measuring 440.2–608.5 µm in length and 227.9–367.9 µm in width (x = 506.3 × 299.4 µm, n = 30). Asci were cylindrical with an apical ring (Figure 3), and contained eight uniseriate ascospores arranged obliquely in rosettes, growing from the base of the perithecium. The asci had dimensions ranging from 125.2 to 167.9 µm in length and from 15.6 to 17.9 µm in width (x = 152.3 × 15.7 µm, n = 30). The ascospores were brown to dark-brown, aseptate, fusiform to ovoid and surrounded by a hyaline, gelatinous sheath, measuring 13.9–18.3 µm in width and 8.1–11.9 µm in length (x = 16.1 × 9.9 µm, n = 30).

3.3. Molecular Phylogenetic Identification

The sequences obtained in this study were deposited in the GenBank database and are available under the accession numbers presented in Table 3.

Table 3. Data on the olive variety from which fungi were isolated, the date of sample collection, location and GenBank accession number.

				GenBank Accession Number		
Isolate	**Olive Variety**	**Collection Date**	**Location**	**ITS**	***TUB2***	***TEF1- α***
SL2 PRI	Porečka rosulja	25 August 2021	Kaštelir-Labinci	OQ734646	OQ744688	OQ754165
N17 BJA1	Istarska bjelica	14 October 2021	Novigrad	OQ733307	OQ744682	OQ744689
R18 B3I	Buža	14 October 2021	Rovinj	OQ733361	OQ744684	OQ744691
R18 LECII	Leccino	14 October 2021	Rovinj	OQ746452	OQ744687	OQ744694
R18 LEC1	Leccino	14 October 2021	Rovinj	OQ733486	OQ744686	OQ744693
R19 B1	Buža	14 October 2021	Rovinj	OQ733324	OQ744683	OQ744690
ISN9 LDC3I	Leccio del Corno	13 September 2021	Poreč	OQ729816	OQ942633	OQ744681
IMK9 36II	Buža puntoža	13 September 2021	Poreč	OQ646787	OQ725012	OQ725013
R18 BII	Buža	14 October 2021	Rovinj	OQ933231	OQ744685	OQ744682
V16 B3	Buža	14 October 2021	Vodnjan	OQ743781	OQ754166	OQ754167
SL1 NP2	Unknown	25 August 2021	Sveti Lovreč	OQ828656	OQ835632	OQ835629
ISN9PEN	Pendolino	13 September 2021	Poreč	OQ828658	OQ835630	OQ835631

The BLAST analysis of sequences derived from *Biscogniauxia* and *Sordaria* isolates in this study revealed a 99–100% similarity for the ITS, *TUB2* and *TEF1- α* gene regions, aligning closely with corresponding species documented in the GenBank database.

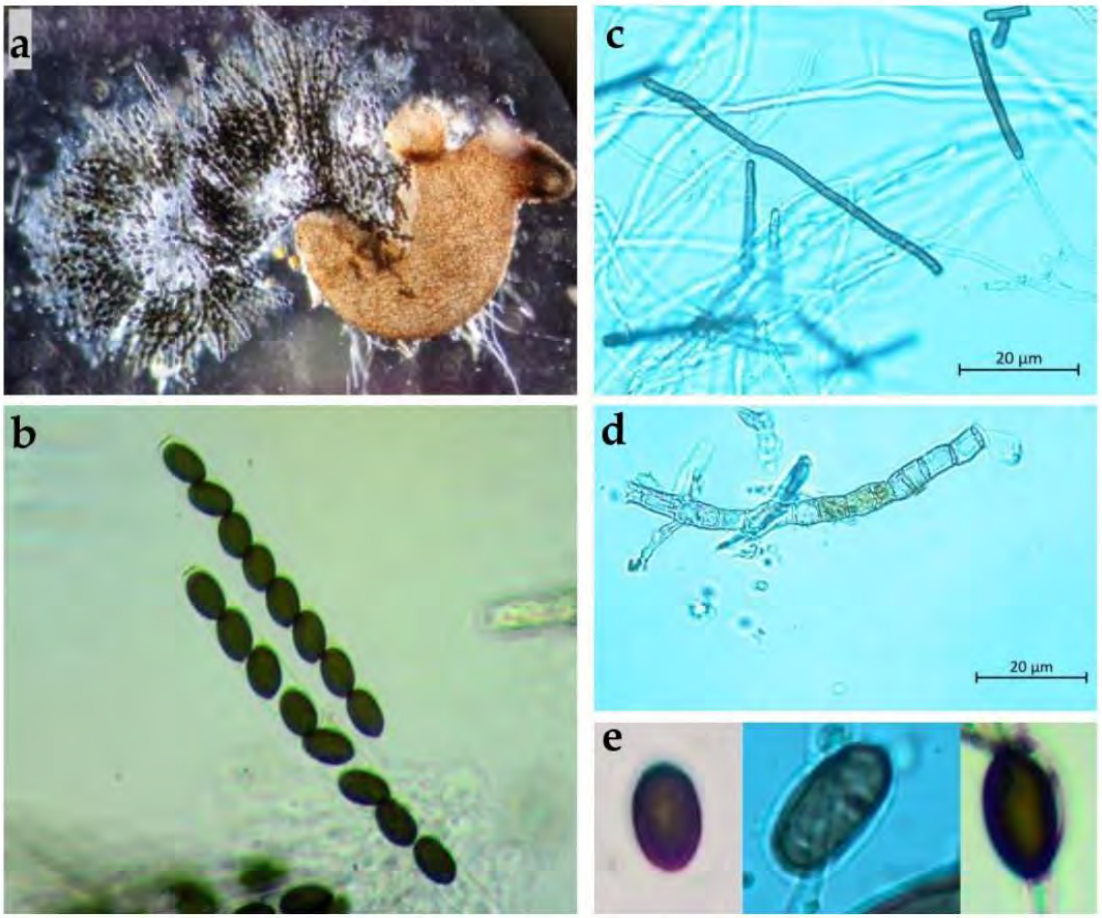


Figure 3. (**a**,**b**) Perithecia, asci and ascospores of the species *Sordaria fimicola* with a visible apical ring. (**c**,**d**) Mycelium and hyphae: (**c**) *Biscogniauxia mediterranea*, (**d**) *B. nummularia*. (**e**) Differences in spore appearance among species: *B. nummularia* (**left**), *B. mediterranea* (**middle**), *S. fimicola* (**right**).

To depict the evolutionary relationships among the isolates, phylogenetic trees were constructed by aligning ITS, *TUB2* and *TEF1-* α sequences, employing the Neighbour Joining method [54]. A comprehensive tree, combining ITS, *TUB2* and *TEF1-* α sequence alignments, was generated, and the optimal trees are depicted in Figures 4–7. The percentage values beneath branches represent the replicates in which related taxa clustered together in the bootstrap test, conducted with 1000 replicates [77]. Evolutionary distances, measured in base substitutions per site, were calculated using the Maximum Composite Likelihood method [78]. The *Phaeoacremonium iranianum* L. Mostert, Grafenhan, W. Gams and Crous isolate R18 BI served as the outgroup in the analysis. Ambiguous positions were excluded through pairwise detection using MEGA11 software [79].

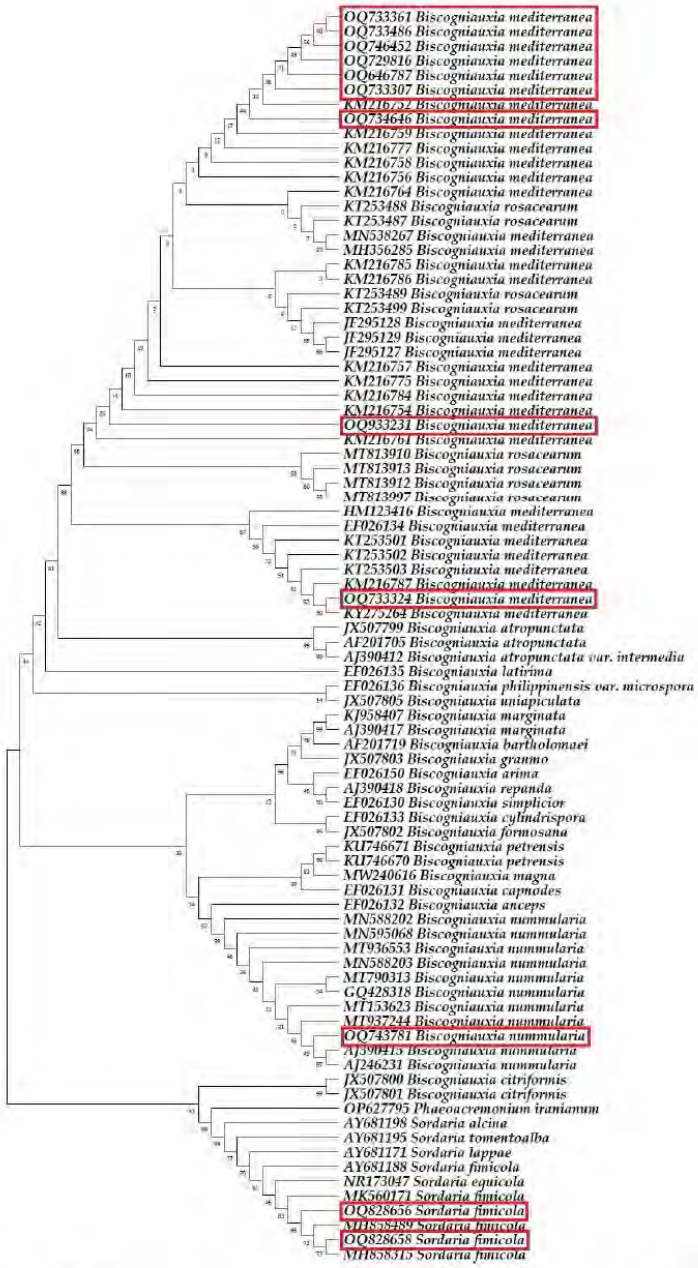


Figure 4. Phylogenetic tree based on internal transcribed spacer sequence alignment. Sequences identified from olive isolates are highlighted with red rectangles.

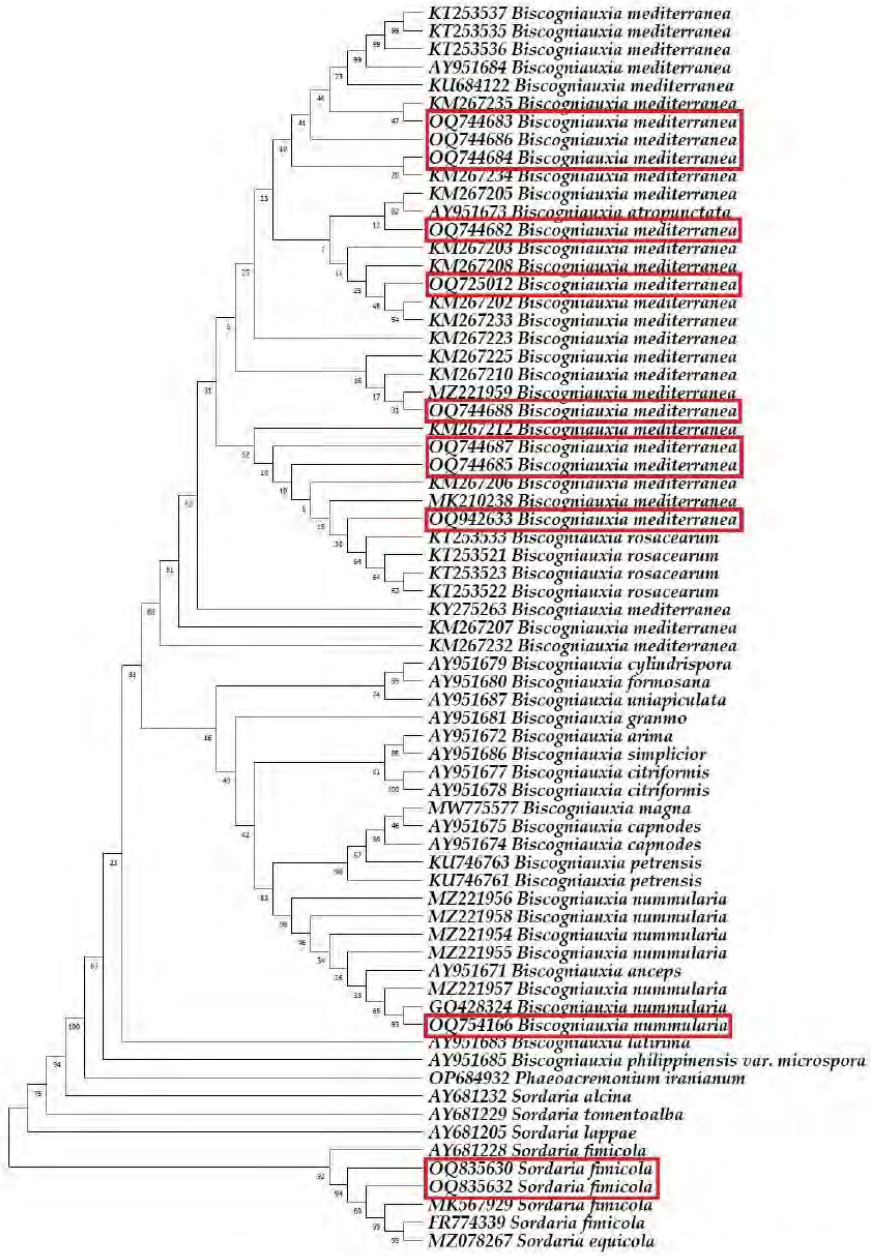


Figure 5. Phylogenetic tree based on beta-tubulin sequence alignment. Sequences identified from olive isolates are highlighted with red rectangles.

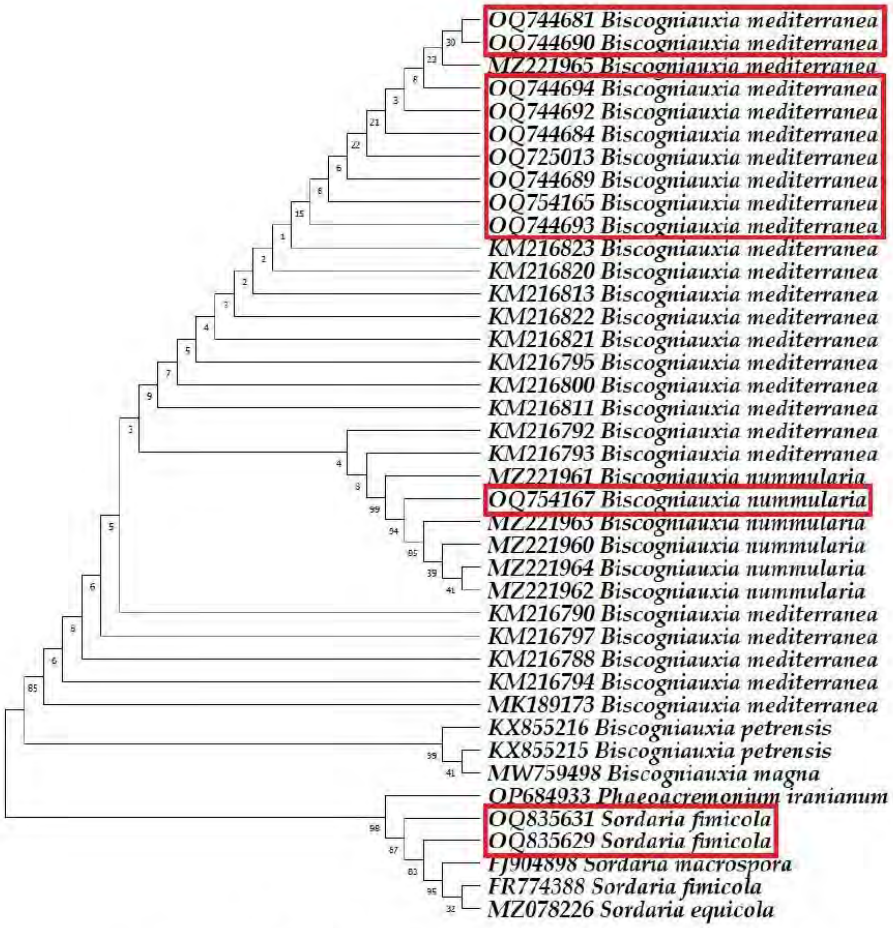


Figure 6. Phylogenetic tree based on translation elongation factor 1-alpha sequence alignment. Sequences identified from olive isolates are highlighted with red rectangles.

The majority of isolates from the *Biscogniauxia* genus included in the phylogenetic analysis originated from *Quercus* spp., while six isolates originated from olive trees. The isolation source of a total of five isolates of *Biscogniauxia* spp. and seven isolates of *Sordaria* spp. remains unknown. Notably, isolates of the *B. petrensis* species were sourced from rock, while an isolate of *S. equicola* originated from zebra dung. Additional data regarding the isolation source and location of isolates used in the phylogenetic analysis can be found in Table 1. Phylogenetic trees, based on the ITS, *TUB2* and *TEF1-* α genomic regions, and a multilocus phylogenetic tree, collectively affirmed the identification of nine isolates belonging to *B. mediterranea*, one isolate belonging to *B. nummularia* and two isolates belonging to *S. fimicola*. The analysis of the phylogenetic tree revealed that the clustering of isolates was not influenced by host species or geographical location. This observation underscores the robustness of the genetic relationships identified within the studied fungal isolates, indicating that their phylogenetic grouping remains consistent across different host species and locations.

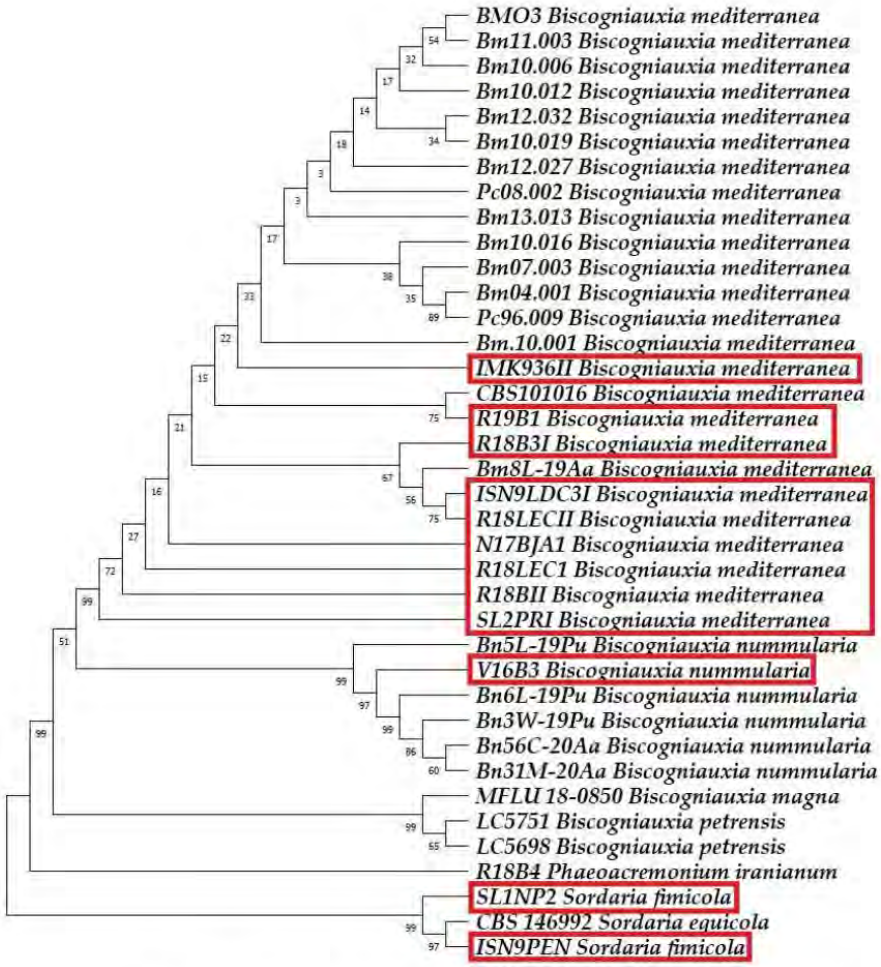


Figure 7. Multilocus tree based on internal transcribed spacer, beta-tubulin and translation elongation factor 1-alpha sequence alignment. Sequences identified from olive isolates are highlighted with red rectangles.

In the specific analyses of individual gene regions, the ITS sequence analysis featured a dataset of 86 nucleotide sequences, culminating in a final dataset of 1536 positions. The *TUB2* sequence analysis involved 69 nucleotide sequences, resulting in a final dataset of 1831 positions. Simultaneously, the *TEF1-* α sequence analysis incorporated 40 nucleotide sequences, with a final dataset of 973 positions. Additionally, the multilocus analysis integrated 38 nucleotide sequences, resulting in a final dataset of 2696 positions. This extensive dataset enabled a comprehensive evaluation of genetic relationships across multiple genomic regions.

The availability of reference material in GenBank for the *TUB2* and *TEF1-* α gene regions, particularly for *S. fimicola*, was limited. Specifically, only one isolate from the *Sordaria* genus in GenBank possesses sequences for ITS, *TUB2* and *TEF1-* α gene regions. The isolate is *S. equicola*, which was previously mentioned as having been isolated from zebra dung. This scarcity of reference material posed a challenge in constructing a multilocus phylogenetic tree, given the necessity for comprehensive data across multiple genomic regions.

3.4. Pathogenicity Test

The first symptoms began to appear approximately between 90 and 100 days after inoculating olive seedlings with *Biscogniauxia* species. Around the site of inoculation, the colour of the bark started to become reddish. The occurrence of necrosis and branch and twig dieback was also observed (Figure 8). In olive seedlings inoculated with *Sordaria* species, the first symptoms appeared after only 3 weeks following inoculation. A black mass of perithecia formed at the entry wound, and the plant tissue began to necrotise (Figure 9). Additionally, a change in the colour of the bark was noticed. Similar symptoms were observed in the field. The largest lesion diameter for species *B. mediterranea* and *B. nummularia* was observed on the variety 'Istarska bjelica', and for *S. fimicola* on the variety 'Leccino'. The average lesion lengths for all tested olive varieties were 26.4 mm for *B. mediterranea*, 15.8 mm for *B. nummularia* and 10.3 mm for *S. fimicola*. Regarding olives inoculated with the pure PDA (control plants), no changes were recorded. The fungus re-isolated from the diseased seedlings was identical to the inoculated species, thereby confirming Koch's postulate.

Figure 8. Results of pathogenicity tests. (**a**) Defoliation and branch dieback symptoms on olive seedlings inoculated with *Biscogniauxia mediterranea*, (**b**) collected samples from all inoculated olive seedlings, (**c**) difference between the branch of the control seedling inoculated with potato dextrose agar (PDA) (**upper**) and the olive branch inoculated with *B. nummularia* (**lower**). (**d**,**f**) Necrosis on branches caused by *Biscogniauxia* species: (**d**,**f**) branches inoculated with *B. mediterranea* (**left**), *B. nummularia* (**middle**) and control branches inoculated with PDA (**right**). (**e**) Necrosis visible on the cross-section of the branches caused by *B. mediterranea* (**left**) and *B. nummularia* (**right**). (**g**) Branch dieback caused by *B. mediterranea*. (**h**) Branch dieback and cracking caused by *B. nummularia*.

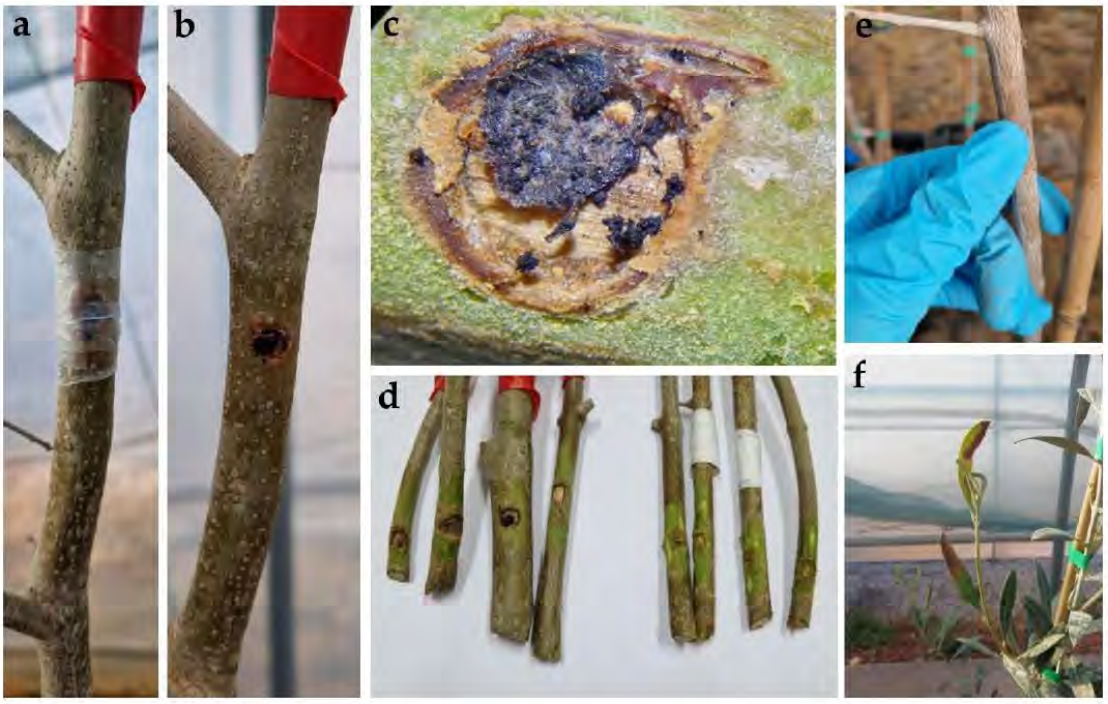


Figure 9. Symptoms of infection on olive seedlings caused by *Sordaria fimicola*: (**a**–**c**) presence of perithecia during inoculation with *S. fimicola*; (**d**) differences between infected (**left**) and control branches (**right**); (**e**) change in the colour of the bark to reddish; (**f**) leaf wilting.

3.5. Meteorological Data

The graphical representations in Figure 10 show the average annual values of temperature, precipitation and relative humidity for the cities of Poreč and Rovinj. The linear trend, represented by the red line, serves as a visual guide, highlighting the discernible patterns of increase or decrease in meteorological variables over the depicted period. Above each graph in the upper right corner, a linear trend equation is displayed, based on which decreases or increases during the observed time period were calculated.

Upon closer examination of the data, it becomes evident that both locations, Poreč and Rovinj, have experienced notable shifts in their climatic conditions. Poreč is situated at an elevation of 15 m above sea level, while Rovinj is at an elevation of 20 m above sea level. The average annual temperature during the observed period was 13.9 °C at both locations. Over the observed period, the temperature increased, on average, by 2.3 °C in Poreč and by 2.2 °C in Rovinj.

Regarding the average annual precipitation, the highest amount was recorded in 2010 at both locations (1403 mm in Poreč and 1422 mm in Rovinj), while the lowest was in 1983 (567 mm in Poreč and 510 mm in Rovinj). During the observed period, there was an increase in precipitation of 193.3 mm in Poreč and 59.1 mm in Rovinj.

Conversely, the relative humidity trend reveals a contrasting pattern, with a noticeable decrease at both locations. In Poreč, the relative humidity decreased by 2.6%, while in Rovinj it decreased by 4.3%.

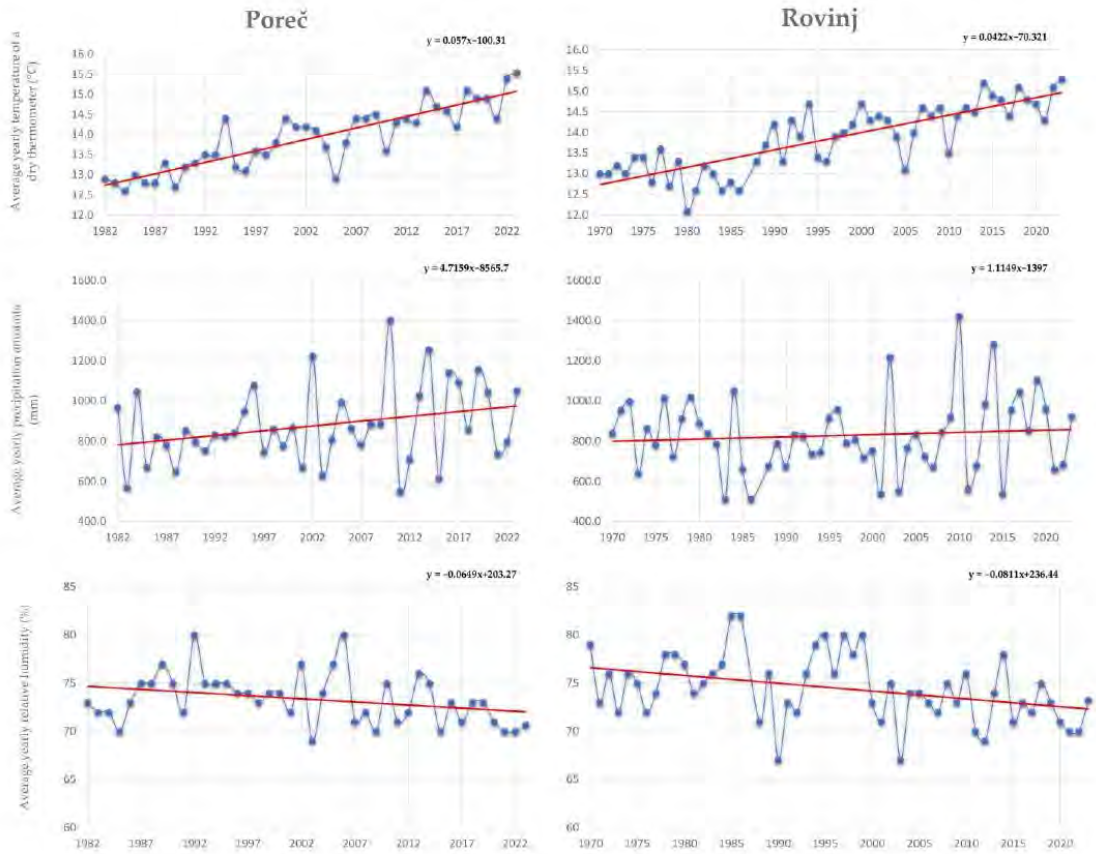


Figure 10. Graphical representation of meteorological data for the locations Poreč and Rovinj. The average annual values of temperature, precipitation, and relative humidity for each year are marked with blue dots. The red line represents the linear trend, highlighting discernible patterns of increase or decrease in meteorological variables over the depicted period.

4. Discussion

Patejuk et al. [42] state that many authors have emphasised that *B. nummularia* behaves mainly as a saprophyte in the northern Mediterranean basin, where the climate is more temperate. In Europe, it is most commonly described as a pathogen of the plant species *F. sylvatica* [37]. Petrini and Petrini [33] identified the endophytic nature of the fungus, facilitating its rapid transition from a benign endophyte to a primary pathogen, a phenomenon well-acknowledged within the *Xylariaceae* group of fungi [80]. In a field study conducted in a Portuguese forest in 1974, it was observed that 41.5% of cork oak trees were weakened, and the primary factor contributing to the decline of cork oaks was identified as *B. mediterranea* [81]. *Biscogniauxia* species have also been described as pathogens on olive trees in Sfax, Tunisia, which is characterised by a Mediterranean climate [28], but also as an endophyte on olives in Portugal [82]. Another *Biscogniauxia* species, *B. rosaceum*, was assessed as having an intermediate level of virulence on olive trees [10]. *B. mediterranea* has been isolated from olives in Croatia; however, it has not been described as a pathogen on olive trees [83]. The occurrence of *B. mediterranea* has also been recorded on oak trees (*Quercus cerris* L., *Q. pubescens* Willd. and *Q. ilex* L.) in Croatia, on the island of Cres and in Istria. Considering that oaks make up 82–100% of the trees in these stands [84], a question

arises regarding its transmission to olive trees and vice versa. In this study, the species *B. mediterranea* and *B. nummularia* were identified as the causative agents of charcoal disease in the olive trees from which samples were taken.

The native habitat of *Sordaria* species is primarily associated with herbivorous animal dung [85]. Ecologically, these fungi fulfil an important role in the decomposition of organic matter. As mentioned earlier, *S. fimicola* has been identified as the etiological agent responsible for leaf and twig blight of *A. palmatum* [46]. On the other hand, while *Sordaria* species have been isolated from *Pinus coulteri* D.Don, their pathogenicity towards this plant species has not been established [44]. In this study, the species *S. fimicola* was identified as the causative agent of olive branch dieback in the olive trees from which samples were taken.

Regarding the phylogenetic analysis, the constrained availability of sequences in GenBank for some loci emphasises the need for continued efforts in genomic research and data deposition to enhance the depth and breadth of genetic information accessible for diverse fungal species. This scarcity of genetic information is particularly noted for *S. fimicola*, but also for *Biscogniauxia* species. Despite these challenges, the study proceeded with the available data, acknowledging the limitations and contributing to the understanding of genetic diversity within the constraints of the existing reference material. A genetic analysis of the isolates revealed no significant genetic distinctions compared to the majority of other isolates. As indicated by Patejuk et al. [42] in their research, this implies a potential natural drift of the fungus into Europe.

B. mediterranea exhibits resilience in diverse environmental conditions owing to its notable adaptability. It demonstrates tolerance to a broad spectrum of temperatures and pH levels, exhibits rapid growth and possesses the capacity for expeditiously causing host mortality [38,86]. Saharan and Mehta [87] posit that the observed variability in morphological characteristics among conspecific isolates may be ascribed to a combination of environmental factors, underlying genetic determinants and the influence of mycoviral presence. In a study conducted by Yangui et al. [38], notable correlations were identified between ecological variables, including temperature and rainfall, and certain morphological traits such as the surface colour of the colony and the presence of exudates. Temperature stands out as a key abiotic factor exerting a profound influence on the developmental processes of fungi [88,89]. Henriques et al. [90] found that isolates of *B. mediterranea* exhibited considerable variability, particularly in culture characteristics such as pigmentation and the presence of aerial mycelium. In this study, a difference in the colour of the colony surface of *B. mediterranea* was also observed among isolates. The colour ranged from olivaceous-white-grey to brown-beige, and even to a cinnamon hue. The morphological characteristics of all isolates matched previously published data for the mentioned species.

The pronounced genetic variability of *B. mediterranea* has been associated with its heterothallic mating system, and sexual reproduction contributes to the generation of a substantial number of diverse ascospores [91,92]. Ascospores serve as dispersal and inoculum units capable of being disseminated over extensive distances—facilitated by airborne vectors such as insects—thereby significantly contributing to fungal spread [92,93].

Patejuk et al. [42] emphasise the importance of conducting pathogenicity tests to demonstrate the pathogenic nature of the species. As part of this study, pathogenicity tests were conducted on seedlings of five different olive varieties: 'Buža', 'Istarska bjelica', 'Leccino', 'Porečka rosulja' and 'Rosinjola'. The pathogenicity of isolates on all tested varieties was confirmed. The chosen three-year-old olive seedlings exhibited excellent development. Younger seedlings generally manifest disease or infection symptoms more quickly, facilitating faster results and simpler symptom identification. Moreover, younger seedlings offer improved control over experimental conditions, as they can be equally treated and maintained in controlled environments. This helps eliminate variables and ensures a more reliable assessment of pathogenicity. Those are some of the main reasons why predominantly two-, three-, or four-year-old olive seedlings are used in research studies where pathogenicity tests are conducted.

Henriques et al. [94] note recent observations highlighting an elevated occurrence of *B. mediterranea* and the manifestation of atypical symptoms on cork, particularly in young trees. These symptoms include cork cracking, the presence of a brown powdery mass in the liber, as well as discoloured and desiccated leaves. These observations raise questions as to whether alterations in the epidemiology of the disease may have occurred, as previously suggested by Sousa et al. [95]. Patejuk et al. [42] assert that the increasing distribution of *B. mediterranea* and *B. nummularia* can be ascribed to climate change, specifically the elevation in annual temperatures and the regular incidence of droughts. The reported symptoms in infected olive trees in Tunisia [28] include the development of lengthwise bark cracks, detached bark, the withering of the crown and the extensive chlorosis of the leaves. Intensive bark cracking was also observed in olives from which samples were taken as part of this study. Additionally, as mentioned earlier, a considerable change in climatic conditions was recorded, including an increase in temperature and precipitation, as well as a decrease in relative humidity values at the observed locations. Dminić et al. [84] also underscore the influence of changing climatic conditions in this area and their impact on the emergence of *B. mediterranea*. High temperatures and low air humidity can cause the faster evaporation of water from the soil and plants. This leads to the dehydration of plants, which can affect their growth, development and survival, consequently impacting their resistance to pathogens and making it easier for pathogens to invade and colonise more rapidly. Changes in temperature and humidity can influence the diversity and balance of ecosystems [96]. Some microorganisms may be sensitive to such changes, leading to disruptions in the ecosystem. Increased temperature can stimulate faster growth and the reproduction of fungi, while decreased air humidity can enhance the dispersal of their spores. Pathogenic fungi may exhibit greater aggressiveness when subjected to stress conditions. Stressful conditions can contribute to the selection and adaptation of pathogenic fungi, leading to changes in the genetic composition of their populations. Consequently, this could potentially result in the emergence of new strains with increased aggressiveness or resistance to specific conditions [97]. Interestingly, the initial detection of *B. mediterranea* on olive trees in Croatia took place in 2016 in the northern part of Istria during the execution of the VIP project [83]. This project involved the inspection of olive orchards and the collection and analysis of samples from olive trees displaying symptoms of fungal disease. Back then, one isolate of the species *B. mediterranea* was documented. Presently, the presence of *Biscogniauxia* spp. has been documented at a total of eight locations in Istria.

Stress caused by elevated temperatures and reduced air humidity is often linked to global climate change. Increased greenhouse gas emissions contribute to climate change, resulting in more extreme weather conditions. According to Kim et al. [98], the Mediterranean region has been recognised as one of the world's most vulnerable regions to the impacts of climate change.

The spectrum of pathogens identified on distinct hosts shows substantial diversity, potentially linked to the evolutionary history of their hosts or ecological factors, such as the architectural complexity of the host or the extent of the pathogen's natural range [99]. Co-evolutionary processes between hosts and pathogens, coupled with the ecological criteria of host organisms, contribute to the observed variations in pathogen–host interactions. Host architectural features, including anatomical structures, can influence the susceptibility and resistance to specific pathogens, thereby shaping infection patterns and disease outcomes. Furthermore, the natural range of pathogens, encompassing factors such as geographic distribution and environmental conditions, adds complexity to the myriad host–pathogen relationships evident in diverse ecosystems [100–102]. A comprehensive understanding of these factors is essential for unravelling the intricate dynamics of disease ecology and formulating effective strategies for disease management across varied ecological settings.

Patejuk et al. [42] state that findings of *B. mediterranea* and *B. nummularia* species are very rare in Europe. They note that, in the last five years, *B. mediterranea* has been reported in Europe for the first time in history, indicating a growth of *Biscogniauxia* spp. in central Europe. Furthermore, they emphasise the importance of monitoring these pathogenic

species given their aggressive nature and the significant threat they pose. Given all the aforementioned information, it is crucial to monitor the development and movement of this pathogen, particularly in olive production. Pathogens in olive production can pose significant risks, affecting both the quantity and quality of olive yields. They may affect tree health, resulting in a diminished harvest. Pathogens can compromise the quality of olives, affecting attributes such as size, colour and taste. This can impact the marketability and economic value of the olives and their derived products, such as olive oil. Some pathogens can spread easily, posing a risk of transmission to neighbouring olive trees. This can lead to the rapid spread of diseases within an orchard or across different groves. In addition to compromising tree health, the impact of *Biscogniauxia* infections extends to the quality of timber. The structural integrity of the wood can be compromised, influencing its strength and suitability for various applications. This aspect is particularly relevant for olive producers engaged in the cultivation of trees for furniture and other applications. Moreover, *Biscogniauxia* infections create potential entry points for secondary pathogens, heightening the susceptibility of trees to additional diseases. This scenario further compounds the overall impact on tree health, emphasising the importance of proactive measures for disease management to mitigate the multifaceted threats posed by *Biscogniauxia* species.

Given that *Sordaria* is mostly described in the literature as a saprophytic fungus, control methods against this fungus have not been extensively researched. Effectively managing *Biscogniauxia* infections presents formidable challenges. Once these fungi establish themselves in affected trees, their persistence becomes a notable concern. Successful management typically requires a multifaceted approach, encompassing cultural practices, strategic pruning and, in certain cases, the necessary removal of severely infected trees. The fungicides carbendazim and propiconazole are reported to be effective in controlling *Biscogniauxia* sp. [103]. Among the plant-derived antifungal agents, *B. mediterranea* has demonstrated strong resistance to certain essential oils, including those from *Eucalyptus lehmannii* Benth. and *E. sideroxylon* A.Cunn. ex Woolls. However, it exhibited high sensitivity to *E. camaldulensis* Dehnh oil (IC50 = 3.83 mg/mL) and *Malus communis* Desf. oil from Zaghouan (IC50 = 1 mg/mL). This sensitivity was observed to be associated with specific compounds present in the essential oils, such as carvacrol, cuminaldehyde, linalool and p-cymene [104]. In terms of biological control, the antagonistic fungus *Trichoderma* sp. also proved to be effective in controlling *Biscogniauxia* species [103].

5. Conclusions

This study provides a comprehensive exploration of the morphological characteristics, phylogenetic analysis and symptoms of *B. mediterranea*, *B. nummularia* and *S. fimicola* in olive trees. The findings highlight the versatility and adaptability of *Biscogniauxia* species in diverse environmental conditions, showcasing their resilience and ability to cause significant damage to olive trees. Pathogenicity tests conducted on various olive varieties confirm the ability of *B. mediterranea*, *B. nummularia* and *S. fimicola* to cause disease, emphasising the importance of proactive measures in disease management. The observed atypical symptoms on cork and olive trees, coupled with changes in climatic conditions, prompt further investigation into alterations in disease epidemiology. It is necessary to monitor the development and movement of these pathogenic species, especially in olive production, where the impact extends beyond tree health to affect olive quantity, quality and economic value. Management strategies, including cultural practices, pruning and the use of fungicides and biological control agents, are essential tools in mitigating the multifaceted threats posed by *Biscogniauxia* species [103,104]. It is also important to emphasise that the limited availability of sequences in GenBank for specific gene regions underscores the need to continue research in the genomic field and deposit data to enhance the depth and breadth of genetic information accessible for various fungal species.

To our knowledge, this research represents the first documented occurrence of *B. mediterranea* causing charcoal disease on olive trees in Croatia. Additionally, it is the first report of *B. nummularia* and *S. fimicola* causing diseases on olive trees on a global scale.

Furthermore, this study marks one of the initial forays into molecular investigations of these species isolated from olive trees.

Author Contributions: Conceptualisation, E.P. and S.G.; methodology, E.P. and S.G.; investigation, E.P., S.G. and K.V., writing—original draft preparation, E.P.; writing—review and editing, S.G., K.V. and J.Ć. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Croatian Science Foundation Installation Research Project, 'Natural bioactive compounds as a source of potential antimicrobial agents in the control of bacterial and other fungal pathogens of olives', Anti-Mikrobi-OL (AMO), UIP-2020-02-7413 and the 'Young Researchers' Career Development Project', DOK-2021-02-2882.

Data Availability Statement: All sequence data are available in NCBI GenBank in accordance with the accession numbers in the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Torres, M.; Pierantozzi, P.; Searles, P.; Rousseaux, M.C.; García-Inza, G.; Miserere, A.; Bodoira, R.; Contreras, C.; Maestri, D. Olive cultivation in the southern hemisphere: Flowering, water requirements and oil quality responses to new crop environments. *Front. Plant Sci.* **2017**, *8*, 1830. [CrossRef]
2. FAO. Food and Agriculture Organization of the United Nations. Crop and Livestock Products. Available online: https://www.fao.org/faostat/en/#data/QCL (accessed on 25 January 2024).
3. Cowling, R.M.; Rundel, P.W.; Lamont, B.B.; Arroyo, M.K.; Arianoutsou, M. Plant diversity in mediterranean climate regions. *Trends Ecol. Evol.* **1996**, *11*, 362–366. [CrossRef]
4. Seletković, Z.; Tikvić, I.; Vučetič, M.; Ugarović, D. Climatic features and the vegetation of Mediterranean Croatia. In *Šume Hrvatskog Sredozemlja*; Akademija Šumarskih Znanosti: Zagreb, Croatia, 2011.
5. Úrbez-Torres, J.R.; Lawrence, D.P.; Hand, F.P.; Trouillas, F.P. Olive twig and branch dieback in California caused by *Cytospora oleicola* and the newly described species *Cytospora olivarum* sp. nov. *Plant Dis.* **2020**, *104*, 1908–1917. [CrossRef] [PubMed]
6. Petrović, E.; Vrandečić, K.; Ivić, D.; Ćosić, J.; Godena, S. First Report of Olive Branch Dieback in Croatia Caused by *Cytospora pruinosa* Defago. *Microorganisms* **2023**, *11*, 1679. [CrossRef]
7. Rumbos, I.C. *Cytospora oleina* causing canker and dieback of olive in Greece. *Plant Pathol.* **1988**, *37*, 441–444. [CrossRef]
8. Moral, J.; Agusti-Brisach, C.; Pérez-Rodríguez, M.; Xaviér, C.; Carmen-Raya, M.; Rhouma, A.; Trapero, A. Identification of fungal species associated with branch dieback of olive and resistance of table cultivars to *Neofusicoccum mediterraneum* and *Botryosphaeria Dothidea*. *Plant Dis.* **2017**, *101*, 306–316. [CrossRef] [PubMed]
9. Adams, G.C.; Roux, J.; Wingfield, M.J. *Cytospora* species (*Ascomycota*, *Diaporthales*, *Valsaceae*): Introduced and native pathogens of trees in South Africa. *Australas. Plant Pathol.* **2006**, *35*, 521–548. [CrossRef]
10. van Dyk, M.; Spies, C.F.J.; Mostert, L.; van der Rijst, M.; du Plessis, I.L.; Moyo, P.; van Jaarsveld, W.J.; Haleen, F. Pathogenicity testing of fungal isolates associated with olive trunk diseases in South Africa. *Plant Dis.* **2021**, *105*, 4060–4073. [CrossRef] [PubMed]
11. Úrbez-Torres, J.R.; Peduto, F.; Vossen, P.M.; Krueger, W.H.; Gubler, W.D. Olive twig and branch dieback: Etiology, incidence, and distribution in California. *Plant Dis.* **2013**, *97*, 231–244. [CrossRef]
12. Kaliterna, J.; Miličević, T.; Ivić, D.; Benčić, D.; Mesić, A. First report of *Diplodia seriata* as causal agent of olive dieback in Croatia. *Plant Dis.* **2012**, *96*, 290. [CrossRef]
13. Ivić, D.; Petrović, E.; Godena, S. Fungi associated with canker diseases on olive in Istria (Croatia). *J. Cent. Eur. Agric.* **2023**, *24*, 470–475. [CrossRef]
14. Lazzizera, C.; Frisullo, S.; Alves, A.; Phillips, A.J.L. Phylogeny and morphology of *Diplodia* species on olives in southern Italy and description of *Diplodia olivarum* sp nov. *Fungal Divers.* **2008**, *21*, 63–71.
15. Carlucci, A.; Raimondo, M.l.; Cibelli, F.; Phillips, A.J.I.; Lops, F. *Pleurostomophora richardsiae*, *Neofusicoccum parvum* and *Phaeoacremonium aleophilum* associated with a decline of olives in southern Italy. *Phytopathol. Mediterr.* **2013**, *52*, 517–527.
16. Linaldeddu, B.T.; Rossetto, G.; Maddau, L.; Vatrano, T.; Bregant, C. Diversity and Pathogenicity of *Botryosphaeriaceae* and *Phytophthora* Species Associated with Emerging Olive Diseases in Italy. *Agriculture* **2023**, *13*, 1575. [CrossRef]
17. Palou, L.; Taberner, V.; Montesinos-Herrero, C. First Report of *Diplodia seriata* Causing Loquat Fruit Rot in Spain. *Plant Dis.* **2013**, *97*, 421–422. [CrossRef] [PubMed]
18. Spies, C.F.J.; Mostert, L.; Carlucci, A.; Moyo, P.; van Jaarsveld, W.J.; du Plessis, I.L.; van Dyk, M.; Halleen, F. Dieback and decline pathogens of olive trees in South Africa. *Persoonia* **2020**, *45*, 196–220. [CrossRef] [PubMed]
19. Hernández-Rodríguez, L.; Mondino-Hintz, P.; Alaniz-Ferro, S. Diversity of Botryosphaeriaceae species causing stem canker and fruit rot in olive trees in Uruguay. *J. Phytopathol.* **2022**, *170*, 264–277. [CrossRef]
20. Petrović, E.; Vrandečić, K.; Ćosić, J.; Kanižai Šarić, G.; Godena, S. First Report of *Phaeoacremonium iranianum* Causing Olive Twig and Branch Dieback. *Plants* **2022**, *11*, 3578. [CrossRef]

21. Raimondo, M.L.; Lops, F.; Carlucci, A. First Report of *Phaeoacremonium oleae* and *P. viticola* Associated with Olive Trunk Diseases in Italy. *Plant Dis.* **2022**, *106*, 331. [CrossRef]
22. Markakis, E.A.; Soultatos, S.K.; Koubouris, G.C.; Psarras, G.; Kanetis, L.; Papadaki, A.A.; Goumas, D.E. First Report of *Pseudophaeomoniella oleae* Causing Wood Streaking and Decay on Olive Trees in Greece. *Plant Dis.* **2022**, *106*, 2263. [CrossRef] [PubMed]
23. Agustí-Brisach, C.; Jiménez-Urbano, J.P.; Raya, M.D.; López-Moral, A.; Trapero, A. Vascular Fungi Associated with Branch Dieback of Olive in Super-High-Density Systems in Southern Spain. *Plant Dis.* **2021**, *105*, 797–818. [CrossRef]
24. Ahimera, N.; Gisler, S.; Morgan, D.P.; Michailides, T.J. Effects of single-drop impactions and natural and simulated rains on the dispersal of *Botryosphaeria dothidea* conidia. *Phytopathology* **2004**, *94*, 1189–1197. [CrossRef]
25. van Niekerk, J.M.; Calitz, F.J.; Halleen, F.; Fourie, P.H. Temporal spore dispersal patterns of grapevine trunk pathogens in South Africa. *Eur. J. Plant Pathol.* **2010**, *127*, 375–390. [CrossRef]
26. Moyo, P.; Allsopp, E.; Roets, F.; Mostert, L.; Halleen, F. Arthropods Vector Grapevine Trunk Disease Pathogens. *Phytopathology* **2014**, *104*, 1063–1069. [CrossRef] [PubMed]
27. Bertrant, P.F.; English, H. Release and dispersal of conidia of *Valsa leucostoma*. *Phytopathology* **1976**, *66*, 987–991. [CrossRef]
28. Gharbi, Y.; Ennouri, K.; Bouazizi, E.; Cheffi, M.; Ali Triki, M. First report of charcoal disease caused by *Biscogniauxia mediterranea* on *Olea europaea* in Tunisia. *J. Plant Pathol.* **2020**, *102*, 961. [CrossRef]
29. Stadler, M. Importance of secondary metabolites in the *Xylariaceae* as parameters for assessment of their taxonomy, phylogeny, and functional biodiversity. *Curr. Res. Environ. Appl. Mycol.* **2011**, *1*, 75–133. [CrossRef]
30. Kuntze, O. *Revisio Generum Plantarum*; Arthur Felix: Leipzig, Germany, 1891; pp. 375–1001.
31. Granata, G.; Sidoti, A. *Biscogniauxia nummularia*: Pathogenic agent of a beech decline. *For. Pathol.* **2004**, *34*, 363–367. [CrossRef]
32. Petrini, O. Fungal endophytes of trees leaves. In *Microbial Ecology of Leaves*; Abdews, J.H., Hirano, S.S., Eds.; Springer: New York, NY, USA, 1991; pp. 179–197.
33. Petrini, L.; Petrini, O. Xylariaceous fungi as endophytes. *Sydowia* **1985**, *38*, 216–234.
34. Nugent, L.K.; Sihanonth, P.; Thienhirun, S.; Whalley, A.J.S. *Biscogniauxia*: A genus of latent invaders. *Mycologist* **2005**, *19*, 40–43. [CrossRef]
35. Collado, J.; Platas, G.; Pelaez, F. Identification of an endophytic *Nodulisporium* sp. from *Quercus ilex* in central Spain as the anamorph of *Biscogniauxia mediterranea* by rDNA sequences analysis and effect of different ecological factors on distribution of the fungus. *Mycologia* **2001**, *93*, 875–886. [CrossRef]
36. Luchi, N.; Capretti, P.; Feducci, M.; Vannini, A.; Ceccarelli, B.; Vettraino, A.M. Latent infection of Biscogniauxia nummularia in Fagus sylvatica: A possible bioindicator of beech health conditions. *iForest* **2015**, *9*, 49–54. [CrossRef]
37. Hendry, S.J.; Boddy, L.; Lonsdale, D. Abiotic variables effect differential expression of latent infections in beech (*Fagus sylvatica*). *New Phytol.* **2002**, *155*, 449–460. [CrossRef] [PubMed]
38. Yangui, I.; Boutiti, M.Z.; Messaoud, C.; Lahbib Ben Jamma, M.; Vannini, A.; Vettraino, A.M. First report of *Biscogniauxia mediterranea* causing canker on *Erica multiflora* L. in Tunisia. *J. Plant Pathol.* **2019**, *1001*, 1273. [CrossRef]
39. Rostamian, M.; Kavosi, M.R.; Bazgir, E.; Babanezhad, M. First report of *Biscogniauxia mediterranea* causing canker on wild almond (*Amygdalus scoparia*). *Australas. Plant Dis. Notes* **2016**, *11*, 30. [CrossRef]
40. Mirabolpathi, M. Outbreak of charcoral disease on *Quercus* spp. and *Zelkova carpinifolia* trees in forest of Zagros and Albroz Mountains in Iran. *Iran. J. Plant Pathol.* **2013**, *49*, 257–263.
41. Zabalgogeazcoa, I.; Pedro, J.; Canalis, R.M. *Biscogniauxia nummularia* infecting beech (*Fagus sylvatica*) trees and sympatric plants of the sedge *Carex brevicollis*. *For. Pathol.* **2015**, *45*, 346–348. [CrossRef]
42. Patejuk, K.; Baturo-Cieśniewska, A.; Pusz, W.; Kaczmarek-Pieńczewska, A. Biscogniauxia Charcoal Canker—A New Potential Threat for Mid-European Forests as an Effect of Climate Change. *Forests* **2022**, *13*, 89. [CrossRef]
43. La Porta, N.; Capretti, P.; Thomsen, I.M.; Kasanen, R.; Hietala, A.M.; Von Weissenberg, K. Forest pathogens with higher damage potential due to climate change in Europe. *Can. J. Plant Pathol.* **2008**, *30*, 177–195. [CrossRef]
44. Ivanová, H.; Onderková, A.; Pristaš, P. *Sordaria fimicola*-like ascomycete isolated from *Pinus coulteri* needles in Slovakia. *Biologia* **2018**, *73*, 553–559. [CrossRef]
45. Olive, L.S. Sordaria. In *Bacteria, Bacteriophages, and Fungi*; King, R.C., Ed.; Springer: Boston, MA, USA, 1974; pp. 553–562.
46. Ivanová, H. Sordaria fimicola (Ascomycota, Sordariales) on Acer palmatum. *Folia Oecologica* **2015**, *42*, 67–71.
47. Newcombe, G.; Campbell, J.; Griffith, D.; Baynes, M.; Launchbaugh, K.; Pendleton, R. Revisiting the life cycle of dung fungi, including *Sordaria fimicola*. *PLoS ONE* **2016**, *11*, e0147425. [CrossRef] [PubMed]
48. Hanlin, R.T. *Illustrated Genera of Ascomycetes*; APS Press: St. Paul, MN, USA, 1990.
49. White, T.J.; Bruns, T.D.; Lee, S.B.; Taylor, J.W. 38—Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR—Protocols and Applications—A Laboratory Manual*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press, Inc.: Cambridge, MA, USA, 1990; pp. 315–322.
50. Glass, N.L.; Donaldson, G.C. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl. Environ. Microbiol.* **1995**, *61*, 1323–1330. [CrossRef] [PubMed]
51. Carbone, I.; Kohn, L.M. A Method for Designing Primer Sets for Speciation Studies in Filamentous Ascomycetes. *Mycologia* **1995**, *91*, 553–556. [CrossRef]

52. Mazzaglia, A.; Anselmi, N.; Gasbarri, A.; Vannini, A. Development of a Polymerase Chain Reaction (PCR) assay for the specific detection of *Biscogniauxia mediterranea* living as an endophyte in oak tissues. *Mycol. Res.* **2001**, *105*, 952–956. [CrossRef]
53. Slippers, B.; Crous, P.W.; Denman, S.; Coutinho, T.A.; Wingfield, B.D.; Wingfield, M.J. Combined Multiple Gene Genealogies and Phenotypic Characters Differentiate Several Species Previously Identified as *Botryosphaeria dothidea*. *Mycologia* **2004**, *96*, 83. [CrossRef] [PubMed]
54. Saitou, N.; Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **1987**, *4*, 406–425.
55. Rogers, J.D.; Ju, Y.-M.; Candoussau, F. *Biscogniauxia anceps* comb. nov. and *Vivantia guadalupensis* gen. et sp. nov. *Mycol. Res.* **1996**, *100*, 669–674. [CrossRef]
56. Hsieh, H.M.; Ju, Y.M.; Rogers, J.D. Molecular phylogeny of *Hypoxylon* and closely related genera. *Mycologia* **2005**, *97*, 844–865. [CrossRef]
57. Ju, Y.-M.; Rogers, J.D.; San Martín, F.; Granmo, A. The genus *Biscogniauxia*. *Mycotaxon* **1998**, *66*, 1–98.
58. Mirabolfathy, M.; Ju, Y.-M.; Hsieh, H.-M.; Rogers, J.D. *Obolarina persica* sp. nov., associated with dying *Quercus* in Iran. *Mycoscience* **2013**, *54*, 315–320. [CrossRef]
59. Pinto-Sherer, T.J. *The Phyloecology of Hypaxylan Sensu Lata*; B.A. Florida International University: Miami, FL, USA, 1996.
60. Sánchez-Ballesteros, J.; González, V.; Salazar, O.; Acero, J.; Portal, M.A.; Julián, M.; Rubio, V.; Bills, G.F.; Polishook, J.D.; Platas, G.; et al. Phylogenetic study of *Hypoxylon* and related genera based on ribosomal ITS sequences. *Mycologia* **2000**, *92*, 964–977. [CrossRef]
61. Ju, Y.-M.; Rogers, J.D. New and interesting *Biscogniauxia* taxa, with a key to the world species. *Mycol. Res.* **2001**, *105*, 1123–1133. [CrossRef]
62. Samarakoon, M.C.; Hyde, K.D.; Maharachchikumbura, S.S.N.; Stadler, M.; Jones, E.B.G.; Promputtha, I.; Suwannarach, N.; Camporesi, E.; Bulgakov, T.S.; Liu, J.-K. Taxonomy, phylogeny, molecular dating and ancestral state reconstruction of *Xylariomycetidae* (*Sordariomycetes*). *Fungal Divers.* **2022**, *112*, 1–88.
63. Daranagama, D.A.; Camporesi, E.; Tian, Q.; Liu, X.; Chamyuang, S.; Stadler, M.; Hyde, K.D. *Anthostomella* is polyphyletic comprising several genera in *Xylariaceae*. *Fungal Divers.* **2015**, *73*, 203–238. [CrossRef]
64. Raimondo, M.L.; Lops, F.; Carlucci, A. Charcoal canker of pear, plum, and quince trees caused by *Biscogniauxia rosaceum* sp. nov. in southern Italy. *Plant Dis.* **2016**, *100*, 1813–1822. [CrossRef] [PubMed]
65. Henriques, J.; Nóbrega, F.; Sousa, E.; Lima, A. Analysis of the genetic diversity and phylogenetic relationships of *Biscogniauxia mediterranea* isolates associated with cork oak. *Phytoparasitica* **2016**, *44*, 19–34. [CrossRef]
66. Mirabolfathy, M.; Groenewald, J.Z.; Crous, P.W. The Occurrence of Charcoal Disease Caused by *Biscogniauxia mediterranea* on Chestnut-Leaved Oak (*Quercus castaneifolia*) in the Golestan Forests of Iran. *Plant Dis.* **2011**, *95*, 876. [CrossRef] [PubMed]
67. U'Ren, J.M.; Miadlikowska, J.; Zimmerman, N.B.; Lutzoni, F.; Stajich, J.E.; Arnold, A.E. Contributions of North American endophytes to the phylogeny, ecology, and taxonomy of *Xylariaceae* (*Sordariomycetes*, Ascomycota). *Mol. Phylogenetics Evol.* **2016**, *98*, 210–232. [CrossRef] [PubMed]
68. Pažoutová, S.; Šrůtka, P.; Holuša, J.; Chudíčková, M.; Kolařík, M. The phylogenetic position of *Obolarina dryophila* (Xylariales). *Mycol. Prog.* **2010**, *9*, 501–507. [CrossRef]
69. Bien, S.; Damm, U. *Prunus* trees in Germany—A hideout of unknown fungi? *Mycol. Prog.* **2020**, *19*, 667–690. [CrossRef]
70. Blumenstein, K.; Bußkamp, J.; Langer, G.J.; Langer, E.J.; Terhonen, E. The Diplodia tip blight pathogen *Sphaeropsis sapinea* is the most common fungus in scots pines' mycobiome, irrespective of health status-a case study from Germany. *J. Fungi* **2021**, *7*, 607. [CrossRef]
71. Zhang, Z.F.; Liu, F.; Zhou, X.; Liu, X.Z.; Liu, S.J.; Cai, L. Culturable mycobiota from Karst caves in China, with descriptions of 20 new species. *Persoonia* **2017**, *39*, 1–31. [CrossRef]
72. Cai, L.; Jeewon, R.; Hyde, K.D. Phylogenetic investigations of *Sordariaceae* based on multiple gene sequences and morphology. *Mycol. Res.* **2006**, *110*, 137–150. [CrossRef]
73. Pecchia, S.; Caggiano, B.; Da Lio, D.; Cafà, G.; Le Floch, G.; Baroncelli, R. Molecular Detection of the Seed-Borne Pathogen *Colletotrichum lupini* Targeting the Hyper-Variable IGS Region of the Ribosomal Cluster. *Plants* **2019**, *8*, 222. [CrossRef]
74. Nygren, K.; Strandberg, R.; Wallberg, A.; Nabholz, B.; Gustafsson, T.; García, D.; Cano, J.; Guarro, J.; Johannesson, H. A comprehensive phylogeny of *Neurospora* reveals a link between reproductive mode and molecular evolution in fungi. *Mol. Phylogenet. Evol.* **2011**, *59*, 649–663. [CrossRef]
75. Vu, D.; Groenewald, M.; de Vries, M.; Gehrmann, T.; Stielow, B.; Eberhardt, U.; Al-Hatmi, A.; Groenewald, J.Z.; Cardinali, G.; Houbraken, J.; et al. Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. *Stud. Mycol.* **2019**, *92*, 135–154. [CrossRef]
76. The Croatian Meteorological and Hydrological Service. Available online: https://meteo.hr/infrastruktura.php?section=mreze_postaja&param=pmm&el=klimatoloske (accessed on 4 December 2023).
77. Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **1985**, *39*, 783–791. [CrossRef]
78. Tamura, K.; Nei, M.; Kumar, S. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11030–11035. [CrossRef]
79. Tamura, K.; Stecher, G.; Kumar, S. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [CrossRef]

80. Osono, T.; Tateno, O.; Masuya, H. Diversity and ubiquity of xylariaceous endophytes in live and dead leaves of temperate forest trees. *Mycoscience* **2013**, *54*, 54–61. [CrossRef]
81. Macara, A.M. *Estimativa em 1975 dos Prejuízos Causados Pelas Principais Doenças do Sobreiro Num Montado da Região Ribatejana*; Boletim do Instituto dos Podutos Florestais; Cortiça N°444: Lisabon, Portugal, 1975.
82. Materatski, P.; Varanda, C.; Carvalho, T.; Dias, A.B.; Campos, M.D.; Rei, F.; Félix, M.R. Spatial and temporal variation of fungal enophytic richness and diversity associated to the phyllosphere of olive cultivars. *Fungal Biol.* **2019**, *123*, 66–76. [CrossRef]
83. Godena, S.; Ivić, D.; Goreta Ban, S. *Uzročnici Djelomičnog ili Potpunog Sušenja Stabala Maslina*; Priručnik o Rezultatima VIP Projekta; Institut za Poljoprivredu i Turizam: Poreč, Croatia, 2019; pp. 1–49.
84. Dminić, D.; Orlović, J.K.; Lukić, I.; Ježić, M.; Ćurković Perica, M.; Pernek, M. First Report of Charcoal Disease of Oak (*Biscogniauxia mediterranea*) on *Quercus* spp. in Croatia. *Plant Dis.* **2019**, *103*, 2687. [CrossRef]
85. Fields, W.G. An introduction to the genus *Sordaria*. *Neurospora Newsl.* **1970**, *16*, 14–17. [CrossRef]
86. Vaninni, A.; Paganini, R.; Anselmi, N. Factors affecting discharge and germination of ascospores of *Hypoxylon mediterraneum* (De Not.) Mill. *Eur. J. For. Pathol.* **1996**, *26*, 12–24. [CrossRef]
87. Saharan, G.S.; Mehta, N. *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*; Springer: New York, NY, USA, 2008.
88. Ouedraogo, A.; Fargues, J.; Goettel, M.S.; Lomer, C.J. Effect of temperature on vegetative growth among isolates of *Metarhizium anisopliae* and *M. flavoviride*. *Mycopathologia* **1997**, *137*, 37–43. [CrossRef]
89. Gauthier, G.M. Dimorphism in fungal pathogens of mammals, plants, and insects. *PLoS Pathog.* **2015**, *11*, e1004608. [CrossRef]
90. Henriques, J.; Nóbrega, F.; Sousa, E.; Lima, A. Diversity of *Biscogniauxia mediterranea* within single stromata on cork oak. *J. Mycol.* **2014**, *2014*, 324349. [CrossRef]
91. Vannini, A.; Mazzaglia, A.; Anselmi, N. Use of random amplified polymorphic DNA (RAPD) for detection of genetic variation and proof of the heterothallic mating system in Hypoxylom mediterraneum. *Eur. J. Forrest Pathol.* **1999**, *29*, 209–218. [CrossRef]
92. Jiménez, J.J.; Sánchez, M.E.; Trapero, A. El chancro carbonoso de Quercus III: Dispersión de ascosporas del agente causal. *Boletín Sanid. Veg. Plagas* **2005**, *31*, 577–585.
93. Henriques, J.; Barrento, M.J.; Bonifácio, L.; Gomes, A.A.; Lima, A.; Sousa, E. Factors affecting the dispersion of *Biscogniauxia mediterranea* in Portuguese cork oak stands. *Silva Lusit.* **2014**, *22*, 83–97.
94. Henriques, J.; Inácio, M.L.; Lima, A.; Sousa, E. New outbreaks of charcoal canker on young cork oak trees in Portugal. *Integr. Prot. Oak For. IOBC/Wprs Bull.* **2012**, *76*, 85–88.
95. Sousa, E.M.R.d.; Santos, M.N.S.; Varela, M.C.; Henriques, J. *Perda do Vigor dos Montados de Sobro e Azinho: Análise da Situação e Perspetivas*; Instituto Nacional de Investigação Agrária e Veterinária, I.P.: Oeiras, Portugese, 2007.
96. García, F.; Bestiona, E.; Warfielda, R.; Yvon-Durochera, G. Changes in temperature alter the relationship between biodiversity and ecosystem functioning. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 201805518. [CrossRef]
97. Brown, A.J.P.; Budge, S.; Kaloriti, D.; Tillmann, A.; Jacobsen, M.D.; Yin, Z.; Ene, I.V.; Bohovych, I.; Sandai, D.; Stavroula, K.; et al. Stress adaptation in a pathogenic fungus. *J. Exp. Biol.* **2014**, *217*, 144–155. [CrossRef]
98. Kim, G.-U.; Chen, D. Climate change over the Mediterranean and current destruction of marine ecosystem. *Sci. Rep.* **2019**, *9*, 18813. [CrossRef]
99. Burdon, J.J.; Silk, J. Sources and Patterns of Diversity in Plant-Pathogenic Fungi. *Phytopathology* **1997**, *87*, 664–669. [CrossRef]
100. Ostfeld, R.S.; Keesing, F.; Eviner, V.T. The Ecology of Infectious Diseases: Progress, Challenges, and Frontiers. In *Infectious Disease Ecology: Effects of Ecosystems on Disease and of Disease on Ecosystems*; Ostfeld, R.S., Keesing, F., Eviner, V.T., Eds.; Princeton University Press: Princeton, NJ, USA, 2008; pp. 469–482.
101. Rupp, S.; Sohn, K. Host-Pathogen Interactions: Methods and protocols. In *Methods in Molecular Biology*; Walker, J.M., Ed.; Humana Press: Totowa, NJ, USA, 2009; Volume 470.
102. Lugtenberg, B. *Principles of Plant-Microbe Interactions: Microbe for Sustainable Agriculture*; Springer: New York, NY, USA, 2015.
103. Karami, J.; Kavosi, M.R.; Babanezhad, M.; Kiapasha, K. Integrated management of the charcoal disease by silviculture, chemical and biological methods in forest parks. *J. Sustain. For.* **2018**, *37*, 429–444. [CrossRef]
104. Yangui, I.; Boutiti, M.Z.; Boussaid, M.; Messaoud, C. Essential Oils of Myrtaceae Species Growing Wild in Tunisia: Chemical Variability and Antifungal Activity Against *Biscogniauxia mediterranea*, the Causative Agent of Charcoal Canker. *Chem. Biodivers.* **2017**, *14*, e1700058. [CrossRef]

Naslov izvornog znanstvenog rada broj 4: Identification and Pathogenicity of *Biscogniauxia* and *Sordaria* Species Isolated from Olive Trees

Prošireni sažetak:

U 2021. godini u maslinicima u Istri, Hrvatska, primijećeni su simptomi gljivičnog oboljenja na stablima masline (*Olea europaea* L.). Simptomi su uključivali sušenje i pucanje kore stabla, često uz prisutnost nekrotičnih tkiva i sušenja grana maslina. Kako bi se utvrdili uzročnici, provedena je izolacija gljiva iz simptomatskog tkiva masline. Izolirane gljive su preliminarno, na temelju morfoloških karakteristika, klasificirane kao pripadnici rodova *Biscogniauxia* i *Sordaria*. Daljnja molekularna analiza (sekvenciranje ITS, TUB2 i TEF1-α regija genoma) i filogenetska analiza potvrdile su prisutnost triju vrsta: *Biscogniauxia mediterranea* (De Not.) Kuntze, *Biscogniauxia nummularia* (Bull.) Kuntze i *Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not. Patogenost izoliranih vrsta ispitana je inokulacijom zdravih biljaka maslina u plasteničkim uvjetima. Zaražene biljke razvile su simptome slične onima viđenima na terenu, što je dokazalo uzročnu povezanost između izoliranih gljiva i pojave bolesti. Analiza meteoroloških podataka za razdoblje prije pojave simptoma sugerira da su produženi sušni periodi i visoke temperature mogli pogodovati zarazi i razvoju bolesti, što je u skladu s ranijim nalazima o utjecaju klimatskih promjena na učestalost bolesti drvenastih biljaka i pojave vrsta roda *Biscogniauxia*. Ovo je prvi izvještaj u Hrvatskoj koji dokumentira *B. mediterranea* kao uzročnika bolesti na maslini, dok su *B. nummularia* i *S. fimicola* prvi put u svijetu zabilježene kao patogeni na maslini.

Ključne riječi: bolest ugljene truleži; *Biscogniauxia mediterranea*; *Biscogniauxia nummularia*; prvi izvještaj; *Sordaria fimicola*

Izvorni znanstveni rad broj 5 u obliku i izvornom jeziku na kojem je objavljen u znanstvenom časopisu

Naslova rada: Diversity and Pathogenicity of *Botryosphaeriaceae* Species Isolated from Olives in Istria, Croatia, and Evaluation of Varietal Resistance

Autori: Elena Petrović, Karolina Vrandečić, Andreina Belušić Vozila, Jasenka Ćosić, Sara Godena

Tip rada: Izvorni znanstveni članak

Časopis: Plants

Kategorija: A1

Impakt faktor: 4,0 (2024.)

Kvartil: Q1

Primljen na recenziju: 13. lipanj 2024.

Prihvaćen za objavljivanje: 28. lipanj 2024.

Status: Objavljen

Volumen: 13

Broj: 13

Broj rada: 1813

WOS broj: 001271264900001

plants

MDPI

Article

Diversity and Pathogenicity of *Botryosphaeriaceae* Species Isolated from Olives in Istria, Croatia, and Evaluation of Varietal Resistance

Elena Petrović [1,*], Karolina Vrandečić [2], Andreina Belušić Vozila [1], Jasenka Ćosić [2] and Sara Godena [1]

[1] Institute of Agriculture and Tourism, Karla Huguesa 8, 52440 Poreč, Croatia; andreina@iptpo.hr (A.B.V.); sara@iptpo.hr (S.G.)
[2] Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Vladimira Preloga 1, 31000 Osijek, Croatia; kvrandecic@fazos.hr (K.V.); jcosic@fazos.hr (J.Ć.)
* Correspondence: elena@iptpo.hr

Abstract: During 2021 and 2022, a field investigation was conducted in Istria, Croatia, searching for trees exhibiting signs of Botryosphaeria dieback. Samples of symptomatic trees were collected from 26 different locations and analysed. Isolates that morphologically corresponded to species from the *Botryosphaeriaceae* family were selected, and detailed morphological characterisation and molecular identification of the isolates were conducted. Based on morphological characteristics and phylogenetic analysis using the internal transcribed spacer (ITS), beta-tubulin (*TUB2*), and translation elongation factor 1-alpha (*TEF1-α*) regions, six species of fungi from the *Botryosphaeriaceae* family were identified: *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & De Not.; *Diplodia mutila* (Fr.) Fr.; *Diplodia seriata* De Not.; *Dothiorella iberica* A.J.L. Phillips, J. Luque & A. Alves; *Dothiorella sarmentorum* (Fr.) A.J.L. Phillips, Alves & Luque; and *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips. This is the first report of *D. mutila*, *Do. sarmentorum*, and *Do. iberica* causing Botryosphaeria dieback on olive trees in Croatia, and the first study investigating the resistance of Croatian olive varieties to species from the *Botryosphaeriaceae* family. Pathogenicity testing of selected isolates and assessment of variety resistance were conducted on four different olive varieties, namely Buža, Istarska bjelica, Leccino, and Rosinjola, using representative isolates of the mentioned species. The most aggressive species was found to be *N. parvum*. Olive varieties exhibited differences in susceptibility depending on the fungus they were infected with.

Keywords: Botryosphaeria dieback; *Botryosphaeria dothidea*; *Diplodia* spp.; *Dothiorella* spp.; first report; *Neofusicoccum* sp.

Citation: Petrović, E.; Vrandečić, K.; Belušić Vozila, A.; Ćosić, J.; Godena, S. Diversity and Pathogenicity of *Botryosphaeriaceae* Species Isolated from Olives in Istria, Croatia, and Evaluation of Varietal Resistance. *Plants* **2024**, *13*, 1813. https://doi.org/10.3390/plants13131813

Academic Editors: Muhammad Shahid, Bin Li, Temoor Ahmed and Muhammad Noman

Received: 13 June 2024
Revised: 27 June 2024
Accepted: 28 June 2024
Published: 1 July 2024

1. Introduction

The olive (*Olea europea* L.) is one of the oldest cultivated plants, spread across the Mediterranean from 45° north to 35° south [1]. Material evidence from ancient times indicates the significance of olive growing and olive oil, mostly used as food, fuel for oil lamps, cosmetic purposes, etc. [1]. Numerous tales suggest how olive production expanded. The prevalence of olives in Istria, as well as in Sicily and southern Spain, is attributed by historians to Aristeus, the ancient pastoral god of the Arcadians, Boeotians, and Thessalians, who was considered the inventor of olives and olive oil [2,3]. Pribetić [1] suggests the assumption that the olive's homeland is Palestine or Asia Minor, from where it first spread to Egypt. Mythology tells of the goddess Minerva, challenged to a contest by Neptune, who plucked the first olive plant from the ground, already in bloom and bearing fruit, thus making it a symbol of peace [4]. It is interesting to note that a golden olive branch was left on the moon's surface by Apollo 11 crew members as a symbol of peace. Olive cultivation in Istria dates back to the 1st century BC, mainly along the western and southern coastal areas [5]. During the period between 1500 and 1700, there was a significant decline in

production, but in the 19th and 20th centuries, its production once again spread throughout the Istrian Peninsula [3]. As stated by Godena et al. [6], the Istrian Peninsula stands as the northernmost olive-growing region in Croatia and is also one of the northernmost olive-growing regions globally. Croatia's olive strategy lies in producing extra-high-quality olive oil, precisely due to suitable climatic conditions [7]. According to the FAO [8], there are no data available for the annual olive production in the world for the year 2023, but in 2022, the production amounted to 21.4 million t. Olive production in Croatia amounted to approximately 40,100 t in 2022 [8], and 29,800 t in 2023 [9]. The production of a high-quality olive oil strongly depends on the quality of the fruit from which the oil is extracted. However, the quality of the fruit depends on numerous factors, such as agroecological conditions, disease and pest attacks, varieties, etc.

Olive varieties exhibit a vast range of diversity. A key question is whether this differentiation occurred post-domestication or whether olives have multiple origins [10]. Typically, olives are propagated through cuttings or grafts, resulting in varieties that are essentially clones [10]. It is assumed that over 1000 varieties and types of olives are cultivated in the Mediterranean region [1]. According to Rugini [11], the number of cultivated olive varieties is estimated to be around 2500. In studies investigating the pathogenicity of fungi and the susceptibility of olive varieties, two terms frequently appear: olive variety and olive cultivar. When discussing olives and olive trees, there is no difference between these terms, as the term "cultivar" is short for "cultivated variety" and is used frequently in olive growing to refer to the different varieties of olives produced. Therefore, the olive cultivar is a synonym for olive variety [12,13]. A significant advantage of Croatian olive cultivation is the indigenous assortment that distinguishes certain areas with the uniqueness of olive oil aroma and flavour, especially since nowadays, oil and olives with a geographical origin achieve twice the price of oil without an origin [7]. In Istrian olive groves, slightly more than a third of the trees belong to indigenous varieties: Buža (50.69%), Istarska bjelica (30.22%), Rosinjola (5.72%), Crnica (syn. Karbonera, 5.60%), and other less represented varieties (7.77%). New plantations primarily consist of foreign varieties (Leccino, Frantoio, Pendolino) [5].

The Buža variety (syn. Buga, Burgaca, Domaća, Gura, Morgaca) [14] is widespread in Istria. Pribetić [1] lists it as the most widespread variety in that area. It is highly valued inland for its excellent oil. It is sensitive to early autumn cold, which reduces oil yields [3]. It is known that in Istria entire plantations suffered from low temperatures in certain years [1]. The name "Buža" comes from the ancient term "bugio," meaning pitted, hollow. Hugues [3] suggests that the name of this variety could originate from the frequent cavities or holes in its trunk near the stump. In the Vodnjan area (Istria), the Buža variety is often grown. This variety is susceptible to peacock spot disease caused by the species *Venturia oleaginea* (Castagne) Rossman & Crous and the appearance of sooty mould, and it is susceptible to olive fruit fly (*Bactrocera oleae* Rossi) and olive moth (*Prays oleae* Bernard) [1].

The Rosinjola variety (syn. Rošinjola, Rosulja, Rovinječka, Rušinjola) [14] is mostly grown in the southern part of Istria, around Vrsar, Rovinj, and Vodnjan [1]. It produces good-quality oil, with an intense aroma and a pronounced bitter taste [1]. Due to its dense canopy, it is often attacked by insects, which causes the appearance of sooty mould, but it shows good resistance to other pests and diseases [1].

The Istarska bjelica variety (syn. Bjelica, Bjankera, Bianchera) [14] is also widespread in Istria and on the Kvarner islands. Unlike Buža, it is somewhat more resistant to low temperatures and the wind [1]. It has a high oil yield, abundant productivity, and excellent oil quality, but it is susceptible to *B. oleae* [1].

The Leccino variety (syn. Leccio) [14] originates from Italy, and it has been cultivated in Istria since 1940 [1]. It is one of the most widespread varieties globally due to its significant adaptability to different agroecological conditions [1]. The Leccino variety is resistant to low temperatures, suitable for intensive plantations, shows good and constant productivity and, finally, produces outstanding-quality oil [1]. Moreover, it is noted to be more resistant to

peacock spot disease [1,7], olive knot disease caused by the bacteria *Pseudomonas savastanoi* pv. *savastanoi*, and the pest *P. oleae*, but it is susceptible to *B. oleae* [1].

A large number of fungi and pests have been reported to cause damage to olive trees. Among the most significant pest of olive trees is *B. oleae*, which is the most economically significant pest in Croatian olive cultivation. On the other hand, in terms of diseases, there is peacock spot disease, as well as patula, caused by *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & De Not., and anthracnose of fruits caused by the species *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (syn. *Gloeosporium olivarum*) [7]. Fungi from the *Botryosphaeriaceae* family identified as pathogens of olive trees in Croatia include the species *B. dothidea*, *Diplodia seriata* De Not. and *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips [15–18]. In addition to the fungi from the *Botryosphaeriaceae* family, other pathogens of olive trees in Croatia include the species *Armillaria mellea* (Vahl) P. Kumm. [17]; *Biscogniauxia mediterranea* and *Biscogniauxia nummularia* (Bull.) Kuntze [19]; *Colletotrichum* spp. [15]; *Comoclathris incompta* (Sacc. & Martelli) Ariyaw. & K.D. Hyde (syn. *Phoma incompta* Sacc. & Mart.) [20]; *Cytospora pruinosa* Défago [21]; *Diaporthe* sp. [18]; *Nigrospora gorlenkoana* Novobr., *Nigrospora osmanthi* Mei Wang & L. Cai, and *Nigrospora philosophiae-doctoris* M. Raza, Qian Chen & L. Cai [22]; *Phaeoacremonium iranianum* L. Mostert, Gräfenhan, W. Gams & Crous [23]; *Pleurostoma richardsiae* (Nannfeldt) Réblová & Jaklitsch [24]; *Pseudocercospora cladosporioides* (Sacc.) U. Braun [15]; *Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not [19]; *Venturia oleaginea* (Castagne) Rossman & Crous (syn. *Spilocaea oleaginea*) [25,26]; and *Verticillium dahliae* Klebahn [27].

Peacock spot disease and patula are among the first described fungal diseases of olives in Croatia. Peacock spot disease was first described in 1901 in Croatia [28]. Patula (syn. Dalmatian disease, Botryosphaeria dieback, escudete, maricume delle drupe, lepre des olives, etc.) was first described in 1883 in Dalmatia by the German–Australian botanist Thumen, and now it is present in almost all olive-growing countries in the Mediterranean region [7]. It compromises the quality of oil and diminishes the value of table olives. The vector of *B. dothidea* is the olive fruit fly and its predator *Lasioptera berlesiana* Paoli (syn. *Prolasioptera berlesiana*). *L. berlesiana* carries *B. dothidea* spores in a mycangium. While the mosquito deposits its egg adjacent to the fly egg, it also inoculates the puncture made by *B. olea* with the fungus [29]. The symptoms of the disease resemble an attack by *C. gloeosporioides*. Signs of the disease in commercial groves encompass necrotic, sunken, and distinctly demarcated lesions on fruits [30]. Besides *B. dothidea*, fungi from the *Botryosphaeriaceae* family are known as some of the most common pathogens of olive trees [31,32]. For instance, causal agents of Botryosphaeria dieback and fruit rot include species such as *D. olivarum* A.J.L. Phillips & Lazzizera; *N. vitifusiforme* (Van Niekerk & Crous) Crous, Slippers & A.J.L. Phillips; *N. parvum*; and *N. mediterraneum* Crous, M.J. Wingf. & A.J.L. Phillips [33,34]. Disease symptoms can be observed on olive fruits, leaves, branches, and trunks. They cause fruit rot, leaf wilting and defoliation, bark discolouration, branch dieback, canker formations, and the appearance of necrosis [35,36]. Fruit contamination with fungi is also a concern due to mycotoxins, which can develop through fruit decay and prolonged storage of damaged fruit [7].

The *Botryosphaeriaceae* family belongs to the class *Dothideomycetes* and the order *Botryosphaeriales*. It encompasses a range of morphologically diverse fungi that can be pathogens, endophytes, or saprobes, primarily on woody hosts [37]. This family was introduced by Theissen & Sydow in 1918 as a sub-family of *Pseudosphaeriaceae*. These species spread through conidia [38], with conidiomata (pycnidia), the fungi's fruiting bodies, releasing conidia during rain and high humidity [39]. Fungi from the *Botryosphaeriaceae* family are also found in healthy tissue parts of the plant and usually cause diseases after the plant is exposed to stressful conditions (post-harvest and heavy rain). These species can remain in a latent stage until favourable conditions for development arise (biotic and/or abiotic stress) [40]. Species from this family are considered aggressive pathogens. According to Schoch et al. [41], 33 genera with over 1200 species of fungi in this family have been identified. The MycoBank database currently encompass 85 genera of fungi in this family [42].

The overall objectives of this study were to identify the causative agent responsible for symptoms observed in olive trees in Istria, Croatia; to conduct morphological characterisation and molecular identification of the fungal isolates through PCR and DNA sequencing of the ITS, *TUB2*, and *TEF1-α* gene regions; to evaluate the pathogenicity of fungal isolates via pathogenicity tests; and to investigate the resistance of various olive varieties to the identified fungal species.

2. Results

2.1. Field Symptoms

Symptoms observed during field research included branch and twig dieback, leaf wilting and defoliation, fruit rot, bark cracking, reddish-brown discolouration of bark, and the appearance of dark-brown necrotic lesions. Necrotic lesions were particularly evident in cross-sectional branch cuts (Figure 1).

Figure 1. Symptoms of infection observed during field research caused by fungi from the *Botryosphaeriaceae* family: (**a**) branch and twig dieback and defoliation caused by the species *Botriosphaeria dothidea*; (**b**) reddish-yellow discolouration of bark caused by the species *B. dothidea*; (**c**) cracking of twig bark, branch dieback, and leaf wilting caused by the species *Diplodia mutila*; (**d**,**e**) necrotic changes in branch cross-sections: (**d**) *Neofusicoccum parvum*, (**e**) *Diplodia seriata*; (**f**,**g**) cracking of tree bark and the appearance of necrotic lesions: (**f**) *Dothiorella sarmentorum*, (**g**) *N. parvum*; (**h**) branch drying and bark discolouration caused by the species *Dothiorella iberica*; (**i**) bark cracking and the appearance of necrosis caused by the species *B. dothidea*.

Out of the 26 locations visited in total, species from the *Botryosphaeriaceae* family were identified in 10, representing 38.46% of the total. Among the 112 sampled trees, species from the *Botryosphaeriaceae* family were identified in 13, making up 11.6% of the total count. The species *B. dothidea* and *N. parvum* were found at four locations in Istria, while the species *Do. sarmentorum* was found at two locations. The species *D. mutila*, *D. seriata*, and *Do. iberica* were each found at one location. The highest number of isolates was collected in Vodnjan (5), followed by Rovinj (4), Poreč (3), and Novigrad (1).

None of the olive groves were equipped with irrigation systems. Pruning was carried out manually at every location, except for grove R18, where a combination of manual and mechanical methods was utilised. Moreover, pruning and burning of plant residues were standard practices across these olive groves. More information about the agricultural practices implemented in olive groves is presented in Table 1.

Table 1. Compilation of production methods, tree density, fertilisation practices, cultivation techniques, pre-culture, surrounding vegetation, and harvesting methods in olive groves.

Location *	Production Method **	Tree Density	Fertilisation ***	Cultivation ****	Pre-Culture	Surrounding Vegetation	Harvesting Method
R8	O	2 × 3	NA	UC	Low shrubbery	Olive grove	Manual
IKB9	C	6 × 6	CAN	C	Grains, vineyards, forage crops	Olive grove	Handheld shakers
IMK9	C	6 × 6	CAN	C	Grains, vineyards, forage crops	Vineyards, forest	Handheld shakers
V12	C	5 × 5	NA	UC	Forest, meadow	Olive grove	Tractor-mounted shakers
V16	O	6 × 6 and 8 × 8	NA	C	Fruit trees	Olive grove, forest	Handheld shakers
N17	C	6 × 7 and 5 × 6	CAN	UC	Low shrubbery, meadow	Vineyards, forest	Manual, handheld shakers
R18	C	6 × 6	CAN	C	Vineyards	Meadow, field, forest	Handheld shakers
R19	C	6 × 6	Stallatico	C	Vineyards	Olive grove, vineyards, meadow, forest	Manual, handheld shakers
PL1	C	10	NA	UC	Low shrubbery, meadow	Olive trees located by the sea, surrounded by a forest	Manual
V21	C/I	6 × 6, 5 × 7 and 7 × 7	Sheep manure, compost	C/UC	Olive	Olive grove	Handheld shakers

* Location: The location tags are identical to the first half of the isolate names. ** C—conventional, I—integrated, O—organic. *** NA—not applicable, CAN—calcium ammonium nitrate. **** C—cultivated, UC—uncultivated.

2.2. Morphological Characterisation

2.2.1. Botryosphaeria Dothidea

The colonies expanded to a diameter of nine cm within five days at 25 °C on potato dextrose agar (PDA) and after seven days on water agar (WA). On WA, the mycelium was poorly developed, ranging in colour from white to greyish. On PDA, the colony colour in the initial stages of growth was white-grey, gradually turning olive green to grey with white tips. As the colony aged, it developed a black-grey colour. The mycelium was dense and matte, with a woolly, cottony, and fluffy appearance (Figure 2). The reverse colonies were white to olivaceous green with dark grey spots. The hyphae were septate, branched, and hyaline. Pycnidia formed on the nutrient medium WA + *Pinus*. The pycnidia were fluffy, olivaceous green with white tips, appearing individually or in clusters. Conidia were fusiform, thicker in the middle and thinner at the ends, aseptate, and hyaline to brownish. The dimensions of the conidia are shown in Table 2.

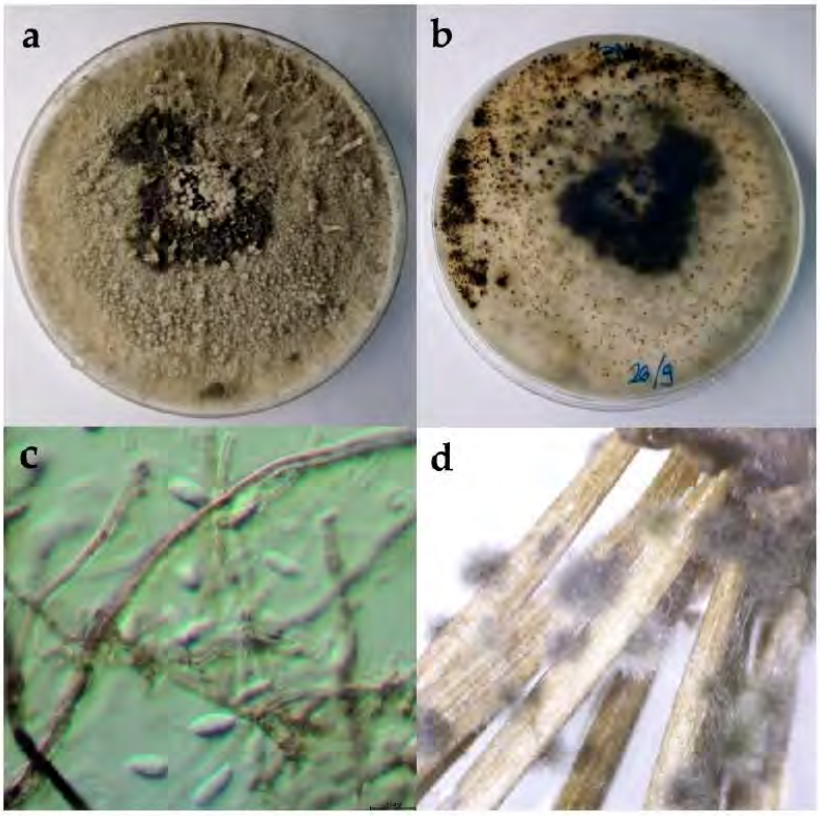


Figure 2. (**a**,**b**) Upper and reverse view of *Botryospheria dothidea* isolate 15 days after incubation at 25 °C on potato dextrose agar (PDA) medium; (**c**) hyphae and conidia of *B. dothidea* isolate observed under the microscope, scale bar = 20 µm; (**d**) pycnidia developed on WA + *Pinus*.

Table 2. Conidial dimensions of fungal isolates.

Species	Conidial Size (µm)		
	Length × Width *	**Average ± SD ****	**95% Conf. *****
Botryosphaeria dothidea	(20.0) 22.9–24.2 (27.3) ± (5.1) 6.0–6.6 (7.8)	23.5 ± 1.9 × 6.3 ± 0.8	0.7–0.3
Diplodia mutila	(20.4) 23.5–24.8 (27.4) ± (9.1) 10.8–11.6 (14.2)	24.1 ± 1.8 ×11.2 ± 1.1	0.6–0.4
Diplodia seriata	(16.2) 22.1–23.7 (27.6) ± (8.3) 10.7–11.7 (13.9)	22.9 ± 2.1 × 11.2 ± 1.3	0.8–0.5
Dothiorella iberica	(15.7) 20.4–21.7 (23.9) ± (7.1) 8.4–9.1 (10.7)	21.0 ± 1.8 × 8.7 ± 0.9	0.7–0.4
Dothiorella sarmentorum	(15.5) 18.9–20.1 (22.9) ± (7.3) 8.3–8.9 (10.9)	19.5 ± 1.7 × 8.6 ± 0.9	0.6–0.3
Neofusicoccum parvum	(9.2) 11.7–12.6 (15.5) ± (3.6) 5.4–6.0 (7.1)	12.2 ± 1.3 × 5.7 ± 0.9	0.5–0.3

* Dimensions are expressed as a range from the lower to the upper limit of the 95% confidence interval, with the minimum and maximum values of the measured dimensions shown in parentheses. ** Mean dimension values (length × width) and standard deviation (SD) are presented. *** The length-to-width ratio of conidia is depicted as a range from the lower to the upper limit of the 95% confidence interval.

2.2.2. Diplodia Mutila

The colonies expanded to a diameter of nine cm within four days at 25 °C on PDA and after seven days on WA. On WA, the mycelium was poorly developed, ranging in colour from white to greyish. On PDA, the colony colour was olivaceous green. As the colony aged, it developed a black-grey colour. The mycelium was dense and matte, with a woolly, cottony, and fluffy appearance (Figure 3). The reverse colonies were white with black spots. The hyphae were septate, branched, and hyaline. Pycnidia formed on the nutrient medium WA + *Pinus*. The pycnidia were fluffy and white, appearing individually or in clusters. Conidia were capsule shaped, hyaline, and aseptate, and over time changed from beige to dark brown, with one septum. The dimensions of the conidia are shown in Table 2.

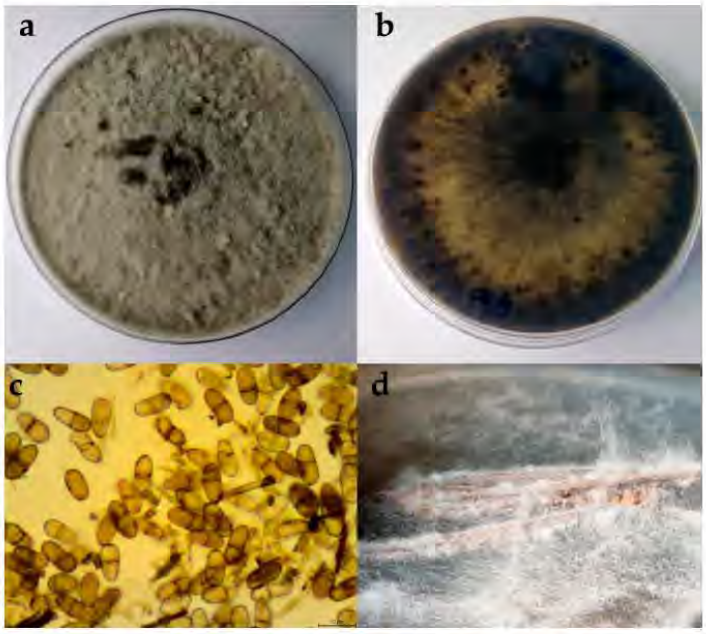


Figure 3. (**a**,**b**) Upper and reverse view of *Diplodia mutila* isolate 15 days after incubation at 25 °C on potato dextrose agar (PDA) medium; (**c**) conidia of *D. mutila* isolate observed under the microscope, scale bar = 20 µm; (**d**) pycnidia developed on WA + *Pinus*.

2.2.3. Diplodia Seriata

The colonies expanded to a diameter of nine cm within four days at 25 °C on PDA and after seven days on WA. On WA, the mycelium was poorly developed, ranging in colour from white to greyish. On PDA, the colony colour was white-grey. As the colony aged, it developed a dark grey colour. The mycelium was dense and matte, with a woolly, cottony, and fluffy appearance (Figure 4). In certain areas, fluffy, whitish clumps of mycelium formed. The reverse colonies were dark grey. The hyphae were septate, branched, and hyaline. Pycnidia formed on the nutrient medium WA + *Pinus*. The pycnidia were fluffy and brown with white tips, appearing in clusters. The conidia were ovoid, rounded or slightly pointed at the edges, aseptate, and hyaline, and over time turned dark brown. The dimensions of the conidia are shown in Table 2.

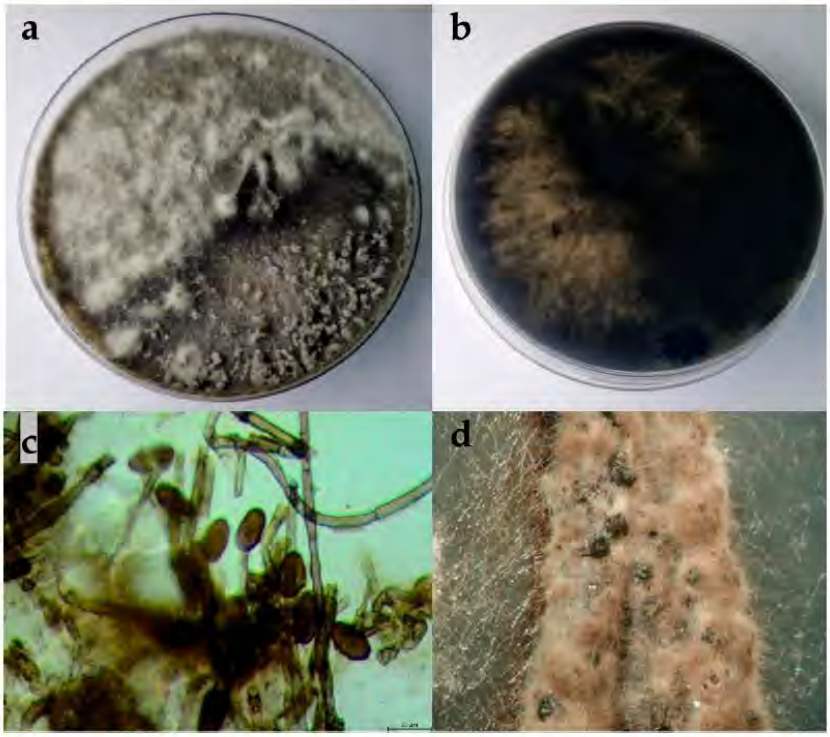


Figure 4. (**a**,**b**) Upper and reverse view of *Diplodia seriata* isolate 15 days after incubation at 25 °C on potato dextrose agar (PDA) medium; (**c**) hyphae and conidia of *D. seriata* isolate observed under the microscope, scale bar = 20 µm; (**d**) pycnidia developed on WA + *Pinus*.

2.2.4. Dothiorella Iberica

The colonies expanded to a diameter of nine cm within four days at 25 °C on PDA and after eight days on WA. On WA, the mycelium was poorly developed, ranging in colour from white to greyish. On PDA, the colony colour was dark grey. The mycelium was dense and matte, with a cottony appearance (Figure 5). The aerial mycelium was more adherent to the substrate, forming rounded cushions, and was not as fluffy as in the previously mentioned species. The reverse colonies were dark grey. The hyphae were septate, branched, and hyaline. Pycnidia formed on the nutrient medium WA + *Pinus*. The pycnidia were fluffy and grey, appearing in clusters. Conidia were capsule shaped, rounded at the edges, and hyaline, and over time turned light brown, with one septum. The dimensions of the conidia are shown in Table 2.

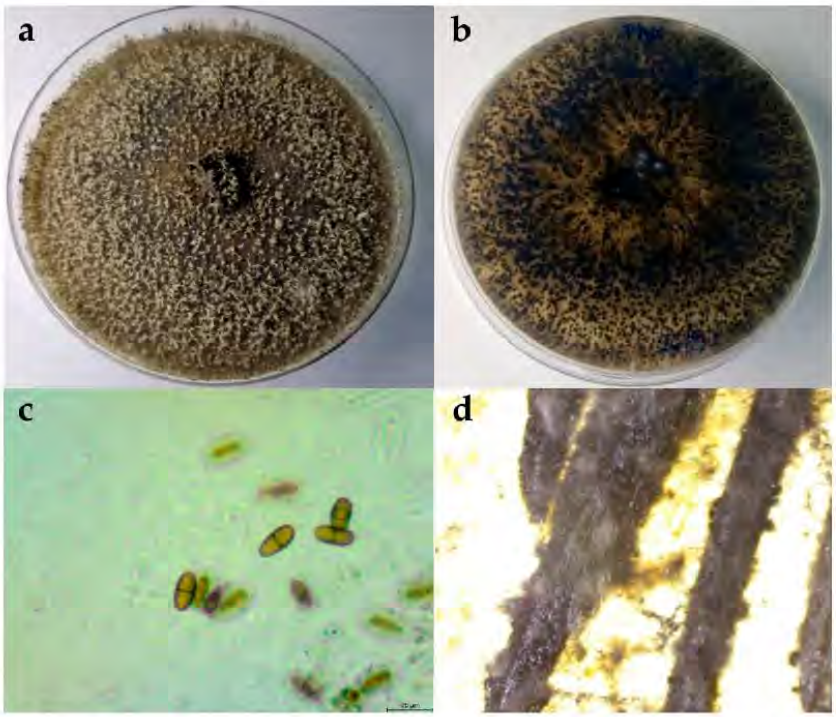


Figure 5. (**a**,**b**) Upper and reverse view of *Dothiorella iberica* isolate 15 days after incubation at 25 °C on potato dextrose agar (PDA) medium; (**c**) conidia of *D. iberica* isolate observed under the microscope, scale bar = 20 µm; (**d**) pycnidia developed on WA + *Pinus*.

2.2.5. Dothiorella Sarmentorum

The colonies expanded to a diameter of nine cm within three days at 25 °C on PDA and after eight days on WA. On WA, the mycelium was poorly developed, ranging in colour from white to greyish. On PDA, the colony colour was dark grey with a white coating and brown edges. The mycelium was dense and matte, with a cottony appearance (Figure 6). The mycelium was adherent to the substrate with little cushion-like protrusions. The reverse colonies were black with brown edges. The hyphae were septate, branched, and hyaline. Pycnidia formed on the nutrient medium WA + *Pinus*. The pycnidia were fluffy and brown with white tips, appearing in clusters. The conidia were capsule-shaped, rounded at the edges, hyaline, and aseptate, and over time turned light brown, with one septum. The dimensions of the conidia are shown in Table 2.

2.2.6. Neofusicoccum Parvum

The colonies expanded to a diameter of nine cm within four days at 25 °C on PDA and after six days on WA. On WA, the mycelium was poorly developed, ranging in colour from white to greyish. On PDA, the colony colour varied among isolates, ranging from light grey to brownish grey, and up to dark grey. The mycelium was dense and matte, with a cottony appearance (Figure 7). The aerial mycelium was adherent to the substrate. The reverse colonies were dark grey with beige-brown edges. The hyphae were septate, branched, and hyaline. Pycnidia formed on the nutrient medium WA + *Pinus*. The pycnidia were fluffy and grey with white tips, appearing in clusters. The conidia were ellipsoidal, with a round apex but sometimes slightly pointed at the ends, aseptate, and hyaline. The dimensions of the conidia are shown in Table 2.

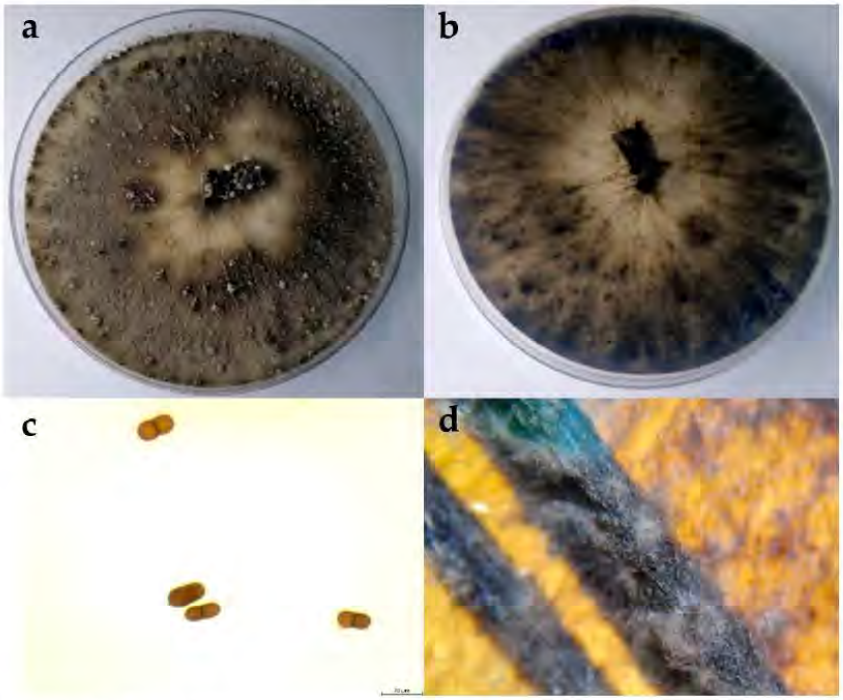


Figure 6. (**a**,**b**) Upper and reverse view of *Dothiorella sarmentorum* isolate 15 days after incubation at 25 °C on potato dextrose agar (PDA) medium; (**c**) conidia of *D. sarmentorum* isolate observed under the microscope, scale bar = 20 µm; (**d**) pycnidia developed on WA + *Pinus*.

2.2.7. Rate of Mycelial Growth

In tests of mycelial growth rate conducted at eight different temperatures, after 48 h, none of the isolates showed growth at 5 °C or 40 °C. At 10 °C, 15 °C, 20 °C, and 25 °C, the species *Do. sarmentorum*, *Do. iberica*, and *D. seriata* exhibited the fastest growth rates. At 25 °C, the highest growth was recorded for all isolates except for the species *N. parvum*, which had the highest growth at 30 °C (Table 3). According to empirically derived data, a temperature of 25 °C proved optimal for the growth of *B. dothidea*, *D. seriata*, *D. mutila*, *Do. iberica*, and *Do. sarmentorum*, while for *N. parvum*, the optimal temperature was 30 °C. At 30 °C, the growth rate of *Do. iberica* and *Do. sarmentorum* drastically decreased. At 35 °C, *B. dothidea* exhibited the fastest growth, followed by *N. parvum*, *D. seriata*, and *D. mutila*, whereas *Do. iberica* and *Do. sarmentorum* did not show any mycelial growth.

According to the empirical mathematical modelling (Table 4), optimal temperatures for species growth ranged between 21.3 °C and 28.1 °C, minimum temperatures between 5.1 °C and 5.4 °C, and maximum temperatures between 31.8 °C and 39.9 °C. As evident from the obtained data, the highest optimal growth temperature was recorded for the species *N. parvum*, while the lowest optimal growth temperature was recorded for the species *Do. sarmentorum*.

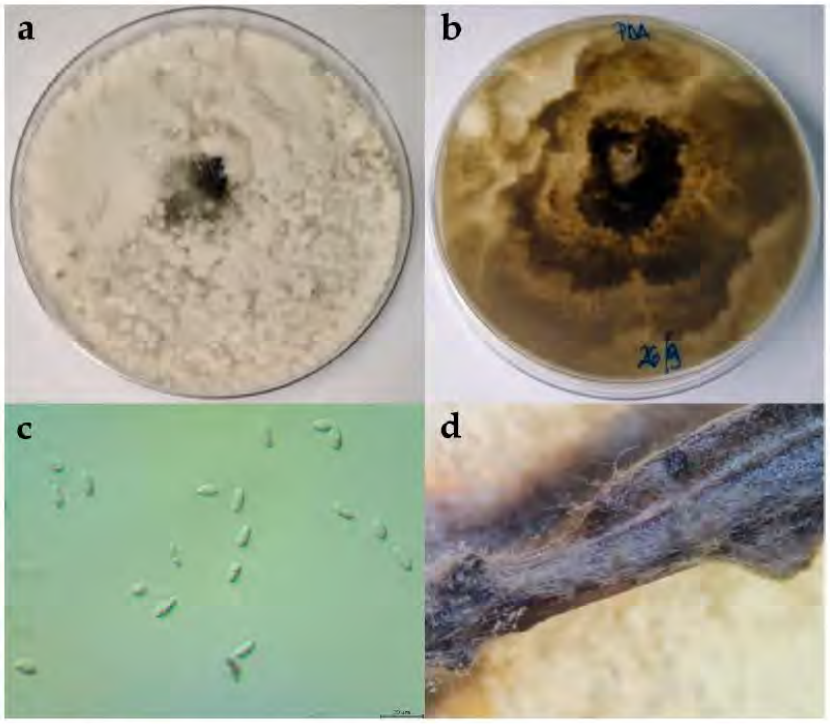


Figure 7. (**a**,**b**) Upper and reverse view of *Neofusicoccum parvum* isolate 15 days after incubation at 25 °C on potato dextrose agar (PDA) medium; (**c**) conidia of *N. parvum* isolate observed under the microscope, scale bar = 20 µm; (**d**) pycnidia developed on WA + *Pinus*.

Table 3. The average mycelial growth rate (mm) after 48 h, incubated at eight different temperatures.

Temperature (°C)	*Botryosphaeria dothidea*	*Diplodia mutila*	*Diplodia seriata*	*Dothiorella iberica*	*Dothiorella sarmentorum*	*Neofusicoccum parvum*
	Growth of Mycelium (mm)					
5	0	0	0	0	0	0
10	2.58	5.16	8.5	11.0	18.33	7.03
15	8.87	12.0	19.16	23.5	38.0	10.21
20	24.29	33.3	45.33	45.66	57.0	28.16
25	39.04	48.33	55.0	50.33	57.41	40.95
30	30.17	31.16	44.66	7.16	16.83	51.95
35	16.47	1.83	4.16	0	0	5.04
40	0	0	0	0	0	0

Table 4. The cardinal temperature for mycelium isolate growth derived from empirically determined growth rate values and mathematical modelling outcomes.

Species	Cardinal Temperatures (°C) for Mycelial Growth Based on Empirically Determined Growth Rate Values			Cardinal Temperatures (°C) for Mycelial Growth Estimated through Mathematical Modelling		
	Minimum	Optimal	Maximum	Minimum	Optimal	Maximum
Botryosphaeria dothidea	5–10	25	35–40	5.1	25.7	39.9
Diplodia mutila	5–10	25	35–40	5.1	24.9	35.3
Diplodia seriata	5–10	25	35–40	5.1	25.4	35.6
Dothiorella iberica	5–10	25	30–35	5.1	26.9	31.8
Dothiorella sarmentorum	5–10	25	30–35	5.2	21.3	34.3
Neofusicoccum parvum	5–10	30	35–40	5.4	28.1	35.5

2.3. Molecular Phylogenetic Identification

Six fungal species were identified as belonging to the *Botryosphaeriaceae* family. All 39 nucleotide sequences obtained from 13 isolates, analysed via BLAST in this study, exhibited a 100% match for the ITS, *TUB2*, and *TEF1-α* gene regions, closely aligning with the species listed in the GenBank database. The sequences generated from this research were added to the GenBank database and are available under the accession numbers indicated in Table 5.

Table 5. List of accession numbers of isolates deposited in GenBank.

Isolate	Species	GenBank Accession Number		
		ITS	*TUB2*	*TEF1-α*
R8 NP	*Botryosphaeria dothidea*	OQ338370	OQ348378	OQ348385
PL1 NP	*Botryosphaeria dothidea*	OQ352832	OQ361692	OQ553927
N17 BJA3	*Botryosphaeria dothidea*	OQ353073	OQ361697	OQ553926
R19 F	*Botryosphaeria dothidea*	OQ354201	OQ361700	OQ361701
IKB9 B2II	*Diplodia mutila*	OQ338569	OQ348379	OQ348386
V16 K2II	*Diplodia seriata*	OQ352870	OQ361695	OQ361696
V16 BI	*Dothiorella iberica*	OQ339205	OQ348381	OQ348388
V12 PEN	*Dothiorella sarmentorum*	OQ339150	OQ348380	OQ348387
R18 PEN1	*Dothiorella sarmentorum*	OQ341230	OQ348383	OQ348390
IMK9 IBVI	*Neofusicoccum parvum*	OQ352837	OQ361693	OQ361694
V16 K1	*Neofusicoccum parvum*	OQ341191	OQ348382	OQ348389
R18 B1	*Neofusicoccum parvum*	OQ353087	OQ361698	OQ361699
V21 B5I	*Neofusicoccum parvum*	OQ341428	OQ348384	OQ553928

Phylogenetic analysis incorporated 100 nucleotide sequences per tree, with all ambiguous positions removed via the pairwise deletion option. The *Biscogniauxia mediterranea* isolates Bm04.001 and Bm10.019 were used as outgroups. The final dataset comprised 1189 positions for ITS sequence analysis, 1450 positions for *TUB2* sequence analysis, 783 positions for *TEF1-α* sequence analysis, and 2838 positions for multilocus analysis. The optimal trees are shown in Figures 8–11.

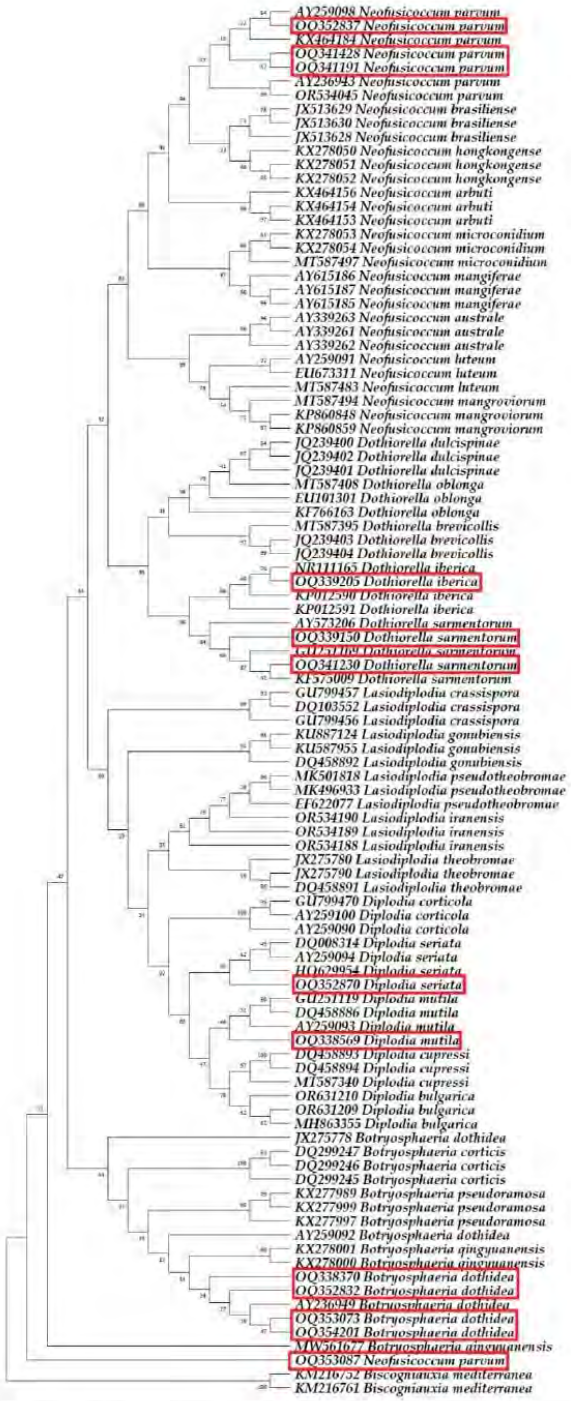


Figure 8. The phylogenetic tree, based on the alignment of internal transcribed spacer sequences, highlights the sequences identified in this research with red rectangles.

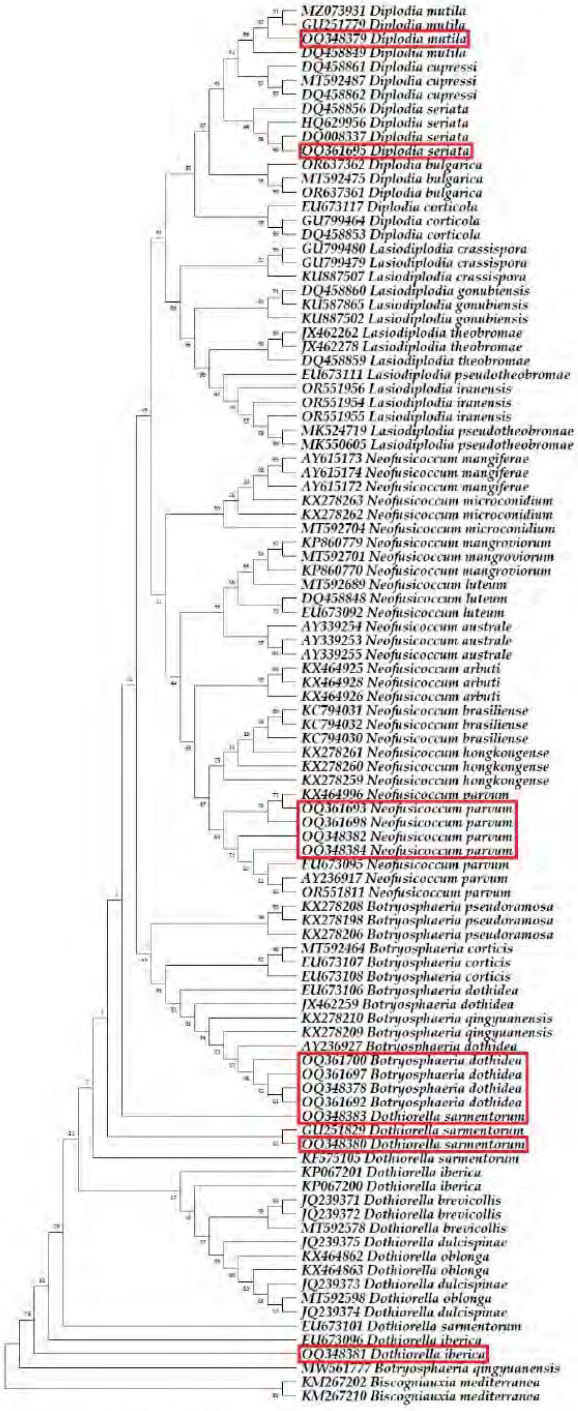


Figure 9. The phylogenetic tree, based on the alignment of beta-tubulin sequences, highlights the sequences identified in this research with red rectangles.

Figure 10. The phylogenetic tree, based on the alignment of translation elongation factor 1-alpha sequences, highlights the sequences identified in this research with red rectangles.

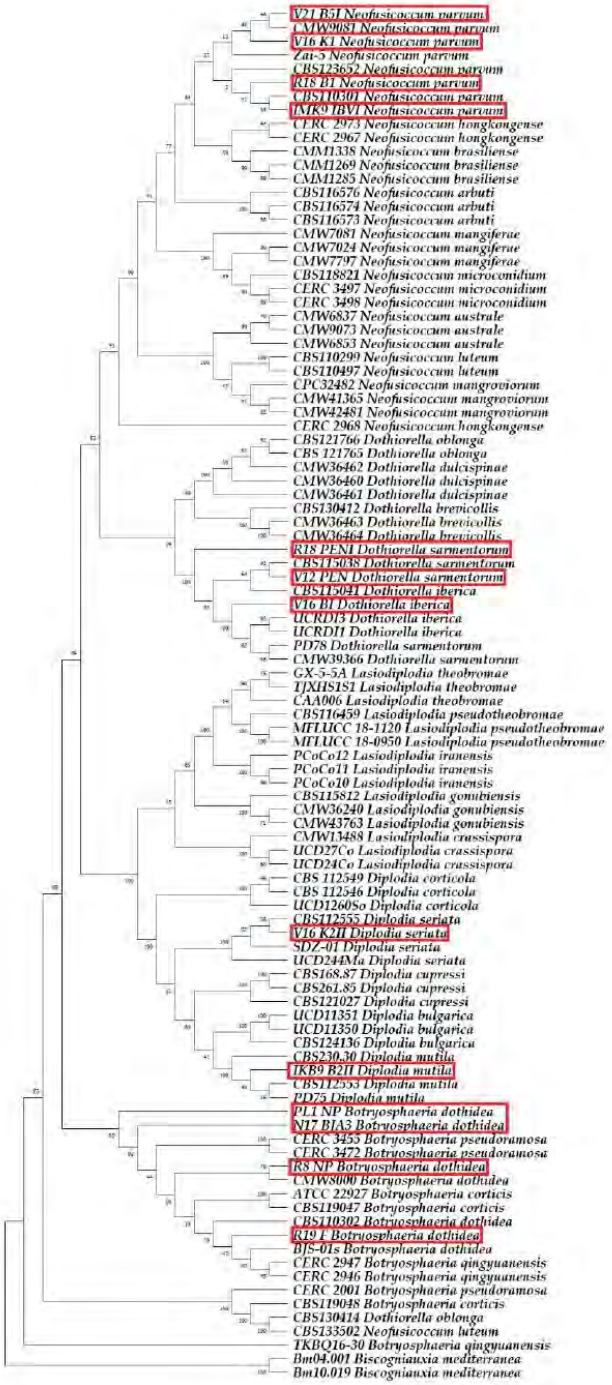


Figure 11. The multilocus phylogenetic tree, based on the alignment of the internal transcribed spacer, beta-tubulin, and translation elongation factor 1-alpha sequences, highlights the sequences identified in this research with red rectangles.

Based on the combination of morphological characteristics, sequence analysis using the BLAST system, and phylogenetic analysis, the isolates R8 NP, PL1 NP, N17 BJA3, and R19 F were identified as *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & De Not.; isolate IKB9 B2II as *Diplodia mutila*; isolate V16 K2II as *Diplodia seriata* De Notaris; isolate V16 BI as *Dothiorella iberica* A.J.L. Phillips, J. Luque & A. Alves; isolates V12 PEN and R18 PEN1 as *Dothiorella sarmentorum* (Fr.) A.J.L. Phillips, Alves & Luque; and isolates IMK9 IBVI, V16 K1, R18 B1, and V21 B5I as *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips.

2.4. Pathogenicity Test and Evaluation of Variety Resistance

2.4.1. Pathogenicity Test

The first symptom observed on olive seedlings inoculated with species from the *Botryosphaeriaceae* family was leaf wilting, defoliation, and twig and branch dieback (Figure 12), which appeared after three months. This was most pronounced in seedlings inoculated with *B. dothidea* and *D. mutila*, and least pronounced in seedlings inoculated with *D. seriata*.

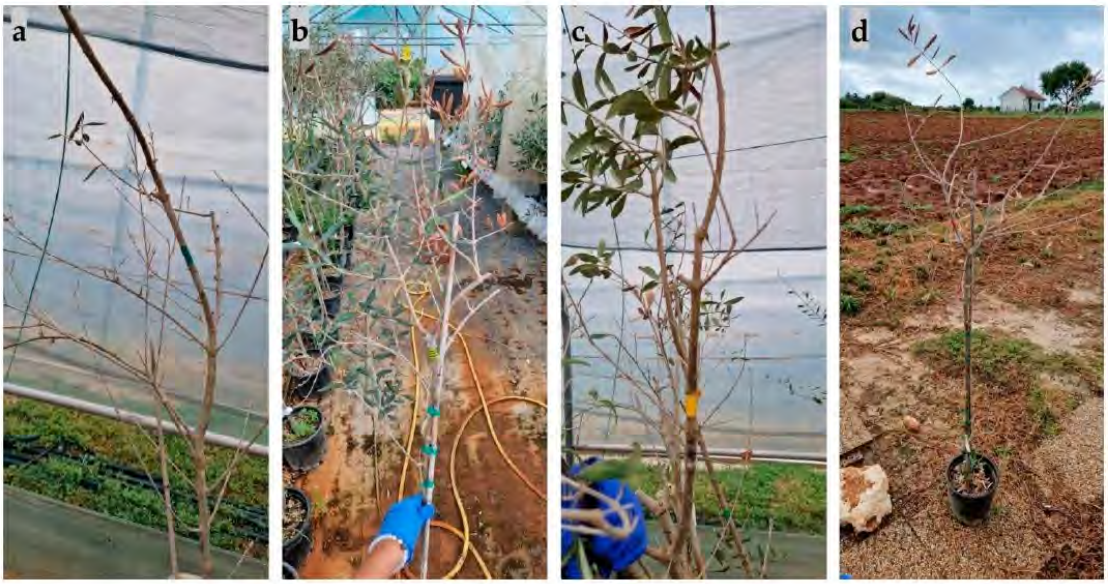


Figure 12. Defoliation and branch dieback symptoms on olive seedlings inoculated with (**a**) *Diplodia mutila*, (**b**) *Neofusicoccum parvum*, (**c**) *Diplodia seriata*, and (**d**) *Botryosphaeria dothidea*.

Other symptoms that appeared included a change in bark colour to reddish due to branch dieback, followed by the appearance of canker formations, bark cracking (Figure 13), and necrosis (Figure 14). The most aggressive species was *N. parvum*, with a total average necrotic lesion diameter of 93.45 mm. This was followed by *D. mutila*, with 33.2 mm; *B. dothidea*, with 17.8 mm; and *Do. sarmentorum*, with 11.3 mm. The least aggressive species were *Do. iberica,* with a total necrotic lesion diameter of 4.46 mm, and *D. seriata*, with 7.45 mm. For the olives inoculated with pure PDA (control group), there were no observed changes. The fungus that was re-isolated from the infected seedlings matched the originally inoculated species, thus validating Koch's postulate.

Figure 13. Canker formations, bark splitting and colour change on branches inoculated with the species (**a**) *Botryosphaeria dothidea*, (**b**) *Diplodia mutila*, (**c**,**d**) *Neofusicoccum parvum*, and (**e**) *Diplodia mutila*.

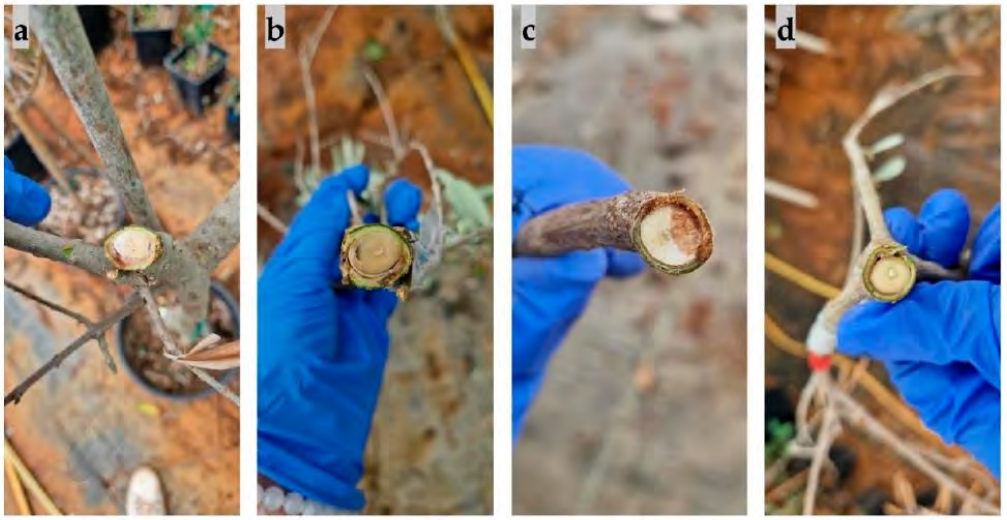


Figure 14. Necrosis observed in the cross-sections of branches caused by (**a**) *Diplodia mutila*, (**b**) *Dothiorella iberica*, (**c**) *Neofusicoccum parvum*, and (**d**) *Botryosphaeria dothidea*.

2.4.2. Variety Resistance

The olive variety Buža exhibited the highest resistance to *Do. sarmentorum*, with a statistically significant difference compared to other species (Table 6). This was followed in descending order by resistance to *Do. iberica*, *D. seriata*, and *B. dothidea*. Conversely, Buža showed the greatest susceptibility to *D. mutila* and *N. parvum*. The Istarska bjelica variety demonstrated the greatest resistance to *Do. iberica* and *Do. sarmentorum*, followed by *D. seriata* and *B. dothidea*. Its highest susceptibility was observed for *N. parvum*, with a statistically significant difference compared to other species. Istarska bjelica also showed notable susceptibility to *D. mutila*. The Leccino variety had the highest resistance to *Do. iberica* and *D. seriata*. It was most susceptible to *B. dothidea*, followed by *Do. sarmentorum* and *N. parvum*, with significant susceptibility also noted for *D. mutila*. The Rosinjola variety exhibited the greatest resistance to *Do. sarmentorum* and *Do. iberica*, followed by *D. seriata* and *B. dothidea*. It displayed significant susceptibility to *N. parvum* and *D. mutila*.

Table 6. The results of the pathogenicity test/variety resistance test with average values of the length of necrotic changes (mean ± standard deviation, in mm) of six isolates of fungi from the *Botryosphaeriaceae* family on olive seedlings, with sterile PDA as a negative control.

Species	Variety *			
	Buža	**Istarska Bjelica**	**Leccino**	**Rosinjola**
Botryosphaeria dothidea	9.30 ± 3.69 b	31.75 ± 11.88 c	65.41 ± 17.82 a	12.87 ± 6.26 b
Diplodia mutila	19.16 ± 6.27 a	84.33 ± 33.28 b	44.95 ± 20.75 b	22.25 ± 6.36 a
Diplodia seriata	5.50 ± 1.85 b	11.15 ± 5.25 c	5.10 ± 2.32 c	10.05 ± 5.41 bc
Dothiorella iberica	5.50 ± 1.03 b	3.65 ± 1.93 c	3.75 ± 2.02 c	4.95 ± 2.85 cd
Dothiorella sarmentorum	4.70 ± 1.92 bc	4.55 ± 1.42 c	53.99 ± 20.04 ab	4.85 ± 0.75 cd
Neofusicoccum parvum	16.15 ± 5.32 a	320.75 ± 87.39 a	48.45 ± 18.69 ab	24.11 ± 7.87 a
Control	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 d
Minimum significant difference	4.87	48.62	19.99	6.90

* Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$).

When evaluating resistance by fungus, for *B. dothidea*, Buža demonstrated the highest resistance (9.30 ± 3.69), followed by Rosinjola (12.87 ± 6.26) and Istarska bjelica (31.75 ± 11.88), while Leccino was the most susceptible (65.41 ± 17.82). Similarly, for *D. mutila*, Buža demonstrated the highest resistance (19.16 ± 6.27), followed by Rosinjola (22.25 ± 6.36) and Leccino (44.95 ± 20.75), with Istarska bjelica being the most susceptible (84.33 ± 33.28). For *D. seriata*, Leccino demonstrated the highest resistance (5.10 ± 2.32), followed by Buža (5.50 ± 1.85) and Rosinjola (10.05 ± 5.41), with Istarska bjelica showing moderate susceptibility (11.15 ± 5.25). In the case of *Do. iberica*, Istarska bjelica was the most resistant (3.65 ± 1.93), followed by Leccino (3.75 ± 2.02) and Rosinjola (4.95 ± 2.85), while Buža was the most susceptible (5.50 ± 1.03). For *Do. sarmentorum*, Istarska bjelica also exhibited the highest resistance (4.55 ± 1.42), followed by Buža (4.70 ± 1.92) and Rosinjola (4.85 ± 0.75), with Leccino being significantly more susceptible (53.99 ± 20.04). For *N. parvum*, Buža again showed the greatest resistance (16.15 ± 5.32), followed by Rosinjola (24.11 ± 7.87) and Leccino (48.45 ± 18.69), whereas Istarska bjelica was highly susceptible (320.75 ± 87.39). This range of susceptibility highlights the varying resistance levels among different olive varieties to specific pathogenic fungi (Figures 15 and 16).

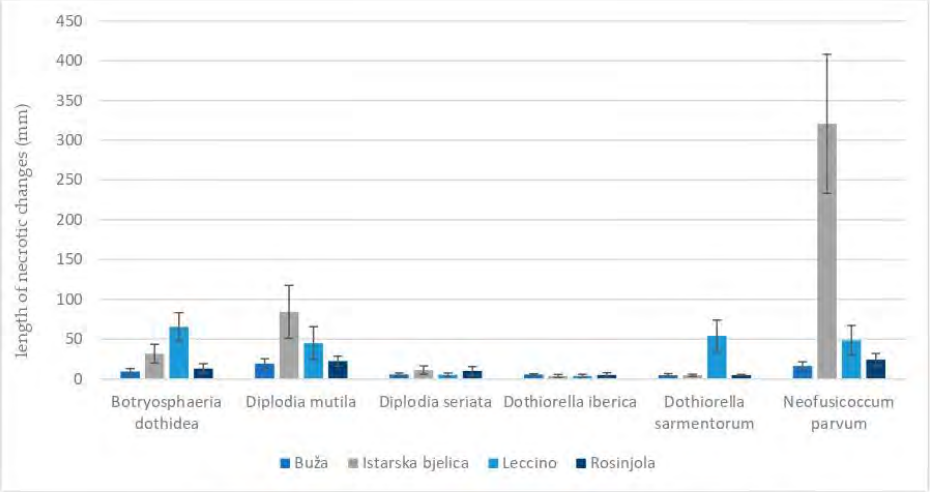


Figure 15. Results of pathogenicity test/variety resistance test. Average values of the length of necrotic changes (mm) per variety are shown in columns of different colours, ranging from light to dark blue. Each column represents the mean value of 10 measurements corresponding to Table 6. The vertical error bars marked in black indicate the standard deviation.

Figure 16. Results of pathogenicity test/variety resistance test: (**a**) samples collected from all inoculated olive seedlings; (**b**) differences between control plants and plants inoculated with species from the *Botryosphaeriaceae* family observed as follows: the white mark indicates control, the red mark indicates *Botryosphaeria dothidea*, the green mark indicates *Diplodia mutila*, the yellow mark indicates *Diplodia seriata*, the blue mark indicates *Dothiorella iberica*, the black mark indicates *Dothiorella sarmentorum*, and the yellow–green mark indicates *Neofusicoccum parvum*; (**c**–**h**) symptoms on branches per variety (from left to right: three branches from Buža, three from Istarska bjelica, three from Leccino, and three from Rosinjola): (**c**) *B. dothidea*, (**d**) *D. mutila*, (**e**) *D. seriata*, (**f**) *Do. iberica*, (**g**) *Do. sarmentorum*, (**h**) *N. parvum*.

3. Discussion

In the past, species within the *Botryosphaeriaceae* family were identified mainly by their ascospores. However, relying solely on the sexual state for classification is inadequate, particularly because some species are known only in their asexual state, and in others, the sexual state is exceedingly rare. On the other hand, conidia of the *Botryosphaeriaceae* display great variation between genera and species [37]. Two types of conidia appear: thin-walled, narrow or spindle-shaped (fusicoccum-like), and thick-walled, broader (diplodia-like) conidia [37]. *D. mutila*, *D. seriata*, *Do. iberica*, and *Do. sarmentorum* belong to the first group, as they have diplodia-like conidia, while *B. dothidea* and *N. parvum* have fusicoccum-like conidia. In this study, isolates were morphologically characterised based on the appearance of mycelium and rate of mycelial growth, as well as the appearance and dimensions of spores, and the appearance of pycnidia. These morphological characteristics aligned with those reported for species in the relevant literature [37]. According to empirical mathematical modelling, the optimal temperatures for species growth varied depending on the species and ranged between 21.3 °C and 28.1 °C. The minimum temperatures ranged between 5.1 °C and 5.4 °C, and the maximum temperatures between 31.8 °C and 39.9 °C. *N. parvum* exhibited the highest values of optimal growth temperature, while *Do. sarmentorum* exhibited the lowest. Hernandez-Rodriguez et al. [32] examined the growth rates of various *Botryosphaeriaceae* species at six temperatures (10, 15, 20, 25, 30, and 35 °C). The optimal temperature was found to be 25 °C. All isolates grew at all temperatures evaluated between 10 and 30 °C. In this study, the isolates also grew at 35 °C, but none of the isolates grew at 5 °C or 40 °C. Kaliterna [43] tested the growth rates of *B. dothidea*, *D. seriata*, *Do. sarmentorum*, and *N. parvum*. Small deviations in temperature values estimated by the empirical mathematical modelling were recorded between the mentioned study and this study. Additionally, in the mentioned study, no mycelial growth was recorded at 5 °C or 40 °C, except for *B. dothidea* at 40 °C, which ranged between 1 and 4 mm. As the author notes, the exact reason for the difference in cardinal temperatures for mycelial growth, as well as mycelial growth rates, is not fully understood, likely due to intraspecific variability among isolates.

Phillips et al. [37] consider morphological characteristics alone to be inadequate for defining genera or identifying species, given the confusion it has caused in the past, their variation during development, and inevitable overlap as representation grows. The most accurate identification of fungi is achieved through a combination of morphological characteristics of fungi and molecular methods. As a standard for the molecular identification of fungi, the ITS region of the genome is commonly used [44]. However, in recent research, additional genes such as *TUB2*, *TEF1-α*, *CALM*, *ACT*, and *COI* are also utilised [44]. As highlighted by Kaliterna [43], a drawback of such research is its high cost. In studies of species from the *Botryosphaeriaceae* family, identification is most commonly performed based on the ITS, *TUB2*, and *TEF1-α* regions of the genome [32,36,37,43]. In this study, species were molecularly identified based on the ITS, *TUB2*, and *TEF1-α* regions of the genome, as well as utilising four phylogenetic trees derived from these gene regions. A total of six species were identified on olive trees in Croatia, namely, *B. dothidea*, *D. mutila*, *D. seriata*, *Do. iberica*, *Do. sarmentorum*, and *N. parvum*.

As pathogens on olives, the following species from the *Botryosphaeriaceae* family have been identified globally: *B. dothidea*; *B. wangensis* G.Q. Li & S.F. Chen; *D. africana* Damm & Crous; *D. fraxini* Fries; *D. mutila*; *D. olivarum*; *D. seriata*; *D. subglobosa* A.J.L. Phillips, Deidda & Linald; *L. theobromae* (Pat.) Griffon & Maubl.; *Do. iberica*; *Do. omnivora* Linaldeddu, Deidda & Scanu; *Do. sarmentorum*; *Do. sempervirentis* Abdollahz., Zare & A.J.L. Phillips; *N. australe*; *N. cryptoaustrale* Pavlic, Maleme, Slippers & M.J. Wingf.; *N. luteum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips; *N. mediterraneum*; *N. occulatum* Sakalidis & T. Burgess; *N. parvum*; *N. ribis*; *N. vitifusiforme*; and *Sardiniella urbana* Linaldeddu, A. Alves & A.J.L. Phillips [16,32,35,36,45–48]. In Croatia, three species from the *Botryosphaeriaceae* family have been identified as pathogens on olive trees: *B. dothidea*, *D. seriata*, and *N. parvum* [15–18]. It is known that species from this family attack numerous plant species,

predominantly woody ones, such as grapevine (*V. vinifera*) [49], European beech (*Fagus sylvatica* L.) [50], plum (*Prunus salicina* Lindl.) [51], etc. In Croatia, besides on olive trees, they have also been found on grapevine (*V. vinifera*) [43], walnut (*Juglans* sp.) [52], and giant sequoia (*Sequoiadendron giganteum* (Lindl.) J. Buchholz) [53]. On grapevines, the species *B. dothidea*, *D. coryli*, *D. seriata*, *Do. sarmentorum*, and *N. parvum* have been identified [43]. On walnut trees, *B. dothidea* and *N. parvum* have also been identified, while in giant sequoia, *B. dothidea* and *N. yunnanense* G.Q. Li & S.F. Chen have been recorded [52,53].

Various other fungi described in our previous studies were also identified at some locations. In grove R18 (Table 1), the fungal species *B. mediterranea* [19], *N. philosophiae-doctoris* [22], and *P. iranianum* [23] were identified, along with *Do. sarmentorum* and *N. parvum* detected in this study. In grove V16, fungi including *B. nummularia* (Bull.) Kuntze [19] and *C. pruinosa* [21] were identified, alongside *D. seriata*, *Do. iberica*, and *N. parvum*. In grove IMK9, the fungi *B. mediterranea* [19] and *N. parvum* were identified, while in grove N17 and R19, the species *B. mediterranea* [19] and *B. dothidea* were found. Mutual infection was not observed on all sampled trees. In this research, the species *B. dothidea* and *N. parvum* were the most frequently found *Botryosphaeriaceae* species on olive trees. In the study by Linaldeddu et al. [48], fungi from the *Botryosphaeriaceae* family were the main species isolated from olive trees with branch cankers and fruit rots. Similarly, Hernandez-Rodriguez et al. [32] predominantly isolated species from the genera *Botryosphaeria* (41%) and *Neofusicoccum* (51%) from olives, with *Diplodia* being less common (8%). In the study by Lazzizera et al. [33], *Botryosphaeria* and *Neofusicoccum* species were isolated from over 60% of the affected drupes, indicating they are the primary contributors to the disease. The most frequently isolated species was *B. dothidea*, found in 34% of the drupes. *B. dothidea* ranks among the most globally prevalent species [32]. The prevalence and distribution of these species may be influenced by climatic and geographical factors, which could be particularly pronounced in Croatia, as noted by Kaliterna [43]. When the climate conditions are optimal, phytopathogenic fungus can grow exponentially and devastate crops, which can cause high economic losses [54]. Humans and animals can also suffer the consequences of fungi attacks because of the mycotoxins produced by some fungi.

Most fungal species responsible for causing fruit rot in olives are typically common saprophytes or secondary invaders that usually enter through wounds inflicted by biotic or abiotic factors. Fungi of the *Botryosphaeriaceae* family have been recognised as some of the most aggressive and prevalent pathogens impacting olives in olive cultivation areas like California, Italy, South Africa, and others [16,32,35,36]. Different fungi can attack different plant organs, so fungal infections cause an enormous range of disease symptoms, such as colour and shape changes, rotting, wilting, and wounds. Cell death causes parts of the plant to decompose and turns plant tissues into a dark colour; this can appear as spots on leaves or rotten spots on fruits [54]. According to the literature, species from the *Botryosphaeriaceae* family cause symptoms that include fruit rot, leaf wilting and defoliation, branch and twig dieback, canker formation, and necrosis of internal tissue, as well as discolouration of the bark, among others [16,32,35,36]. These symptoms were also observed in this research, both in the field and on olive seedlings following pathogenicity tests.

In research conducted by Moral et al. [31], pathogenicity tests using unripe olive fruit and olive branches showed that *D. seriata* isolates were the least aggressive on both the fruit and branches, while *N. mediterraneum* isolates were the most aggressive in both tissues. Isolates of *B. dothidea* were not pathogenic on branches and only weakly aggressive on fruit. The most aggressive species in our research were *N. parvum*, *D. mutila*, and *B. dothidea*, whereas the least aggressive species was *Do. iberica*. As stated by Hernandez-Rodriguez et al. [32] in their studies, the *Neofusicoccum* isolates were also considerably more aggressive than the *Botryosphaeria* and *Diplodia* isolates. The same case was recorded in the pathogenicity tests in the study by Linaldeddu et al. [48], where *N. parvum* caused much larger lesions than species *B. dothidea*, *D. fraxini*, *D. mutila*, *D. olivarum*, and *D. subglobulosa*. Contrary to that, in the study by Godena et al. [17], *D. seriata* proved to be more aggressive than *N. parvum*.

The disease resistance of olive varieties offers an economically feasible alternative to chemical control, with minimal environmental impact, and can be integrated into pest management strategies [46]. Theophrastus [2] observed that the Greeks preferred to propagate olives through cuttings, knowing from experience that olives grown from seeds were inferior, thus requiring improvement through grafting and thereby creating more resistant trees. As a measure against *B. dothidea*, the use of varieties resistant to olive fruit fly attack may serve (since the parasite of the fly, *L. berlesiana*, cannot penetrate the fruit on its own), along with monitoring the fly occurrence using traps with attractants [46]. Varieties identified as more susceptible to olive fruit fly infestation include St. Catarina and Ascolara Tenera, while varieties such as Dužica [7], Nocellara etnea, Oliva di Cerignola, Orbetana, and Capolga have shown resistance [4]. As a preventive measure, varieties more resistant to disease-causing agents can be planted. Latinović et al. [30] tested the resistance of 17 olive varieties to *B. dothidea*. The most resistant were Crnjaka and Gloginja, along with Pendolino and Cassanesse. Moderately resistant varieties included Picholine, Grossa di Spagna, and Conserviola, while Rogganiella, Lumbardeška, Sant Agostino, Manzanilla, and Noccelara del Belice were rated as intermediate. Leccino, Coratina, and Žutica showed moderate susceptibility, while Giarraffa and Ascolana tenera were highly susceptible. Moral et al. [46] tested the resistance of the 11 most important table varieties to *N. mediterraneum* and *B. dothidea*. Testing results on branches showed that Gordal Sevillana, followed by Santa Caterina and San Agostino, were the most susceptible to *N. parvum*. Manzanilla Cacereña was the most resistant, followed by Verdial de Huévar and Morona. Concerning potted plant inoculation with *N. mediterraneum*, Manzanilla Cacereña and Gordal Sevillana were the most susceptible, while the most resistant were Verdial de Huévar, Hojiblanca, and Aloreña de Atarfe. The results of testing resistance on olive fruit showed that the most resistant varieties to *B. dothidea* were San Agostino and Hojiblanca, while Aloreña de Atarfe was the most susceptible. According to the authors, detached branches may experience significant stress and may not behave physiologically in the same manner as branches attached to a tree. Therefore, results obtained from detached branches may not be entirely indicative of varieties' resistance. In this study, the susceptibility of varieties varied depending on the fungal species with which the olive seedlings were inoculated. When evaluating resistance by fungus, for *B. dothidea*, Buža demonstrated the highest resistance, while Leccino was the most susceptible. For *D. mutila*, Buža showed the highest resistance, whereas Istarska bjelica was the most susceptible. In the case of *D. seriata*, Leccino exhibited the highest resistance, with Istarska bjelica being most susceptible. For *Do. iberica* and *Do. sarmentorum*, Istarska bjelica showed the highest resistance, while Buža and Leccino were the most susceptible, respectively. For *N. parvum*, Buža had the highest resistance, while Istarska bjelica was highly susceptible. An important factor is not only the resistance of varieties but also the timing of fruit ripening, i.e., the occurrence of apparent resistance. The fruits of most varieties ripen between November and February, and the moment they must be harvested depends on the olive tree's location, exposure, and meteorological conditions [4]. Early-ripening varieties harvested in late September and October avoid the most intense periods of olive fruit fly attacks [7]. Therefore, when establishing an olive grove, it is advisable to arrange varieties in separate rows to facilitate harvest segregation based on genotype and relative ripening period [4]. As noted by Latinović et al. [30], the identification of resistant varieties could represent the fundamental element of cost-effective disease management, especially for numerous small-scale growers unable to afford pesticide spraying for large olive trees.

Besides resistant varieties, disease control strategies include the removal of infected plant parts [55]. Pruning should be conducted during dry weather with clean and disinfected tools, and coating wounds after pruning is also crucial. Pitt et al. [56] and Díaz and Latorre [57] report that treating wounds with fungicides and horticultural wax helps reduce the incidence of Botryosphaeria dieback. However, it is important to carefully select the fungicide to be applied, as in vitro studies have shown that not all fungicides are equally effective against all species of this family [56,58]. Among the most effective fungicides are those containing the active ingredients benomyl, carbendazim, fluazinam, flusila-

zole, fludioxonil, iprodione, myclobutanil, penconazole, procymidone, pyraclostrobin, thiophanate–methyl, and tebuconazole [55,56]. When establishing an olive grove, it is also essential to consider the choice of location.

4. Materials and Methods

4.1. Fieldwork and Isolation of Fungi

As part of the olive disease research conducted in Istria County in 2021 and 2022, a total of 26 locations were surveyed, and samples were collected from a total of 112 olive trees. The Botryosphaeria dieback of olive was confirmed at 10 of these locations (Figure 17), i.e., the disease was observed on a total of 13 trees.

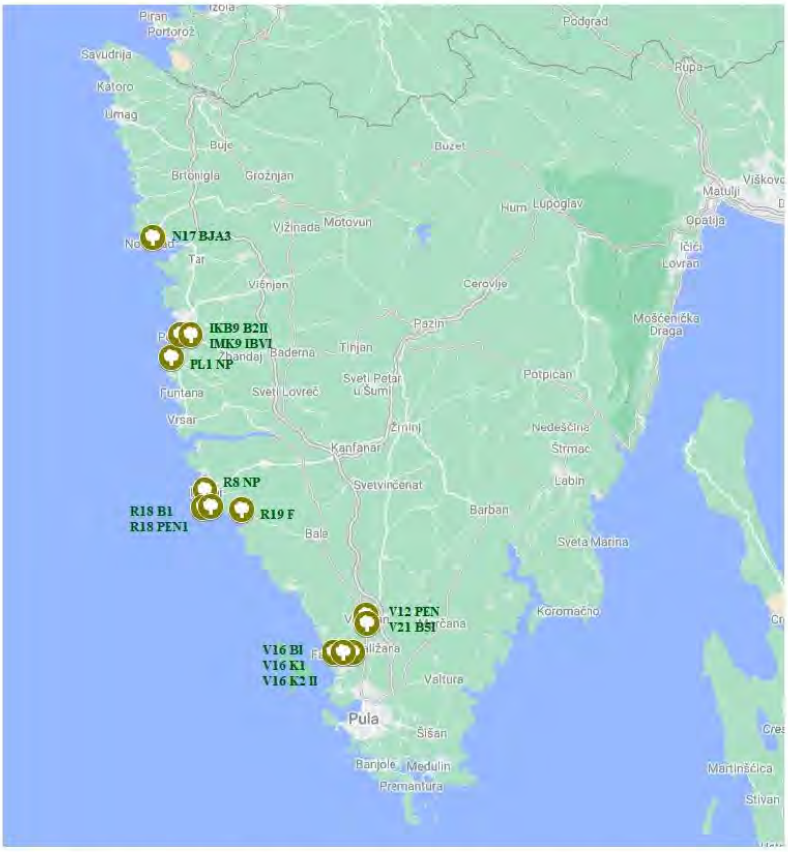


Figure 17. Locations of samples of species from the *Botryosphaeriaceae* family collected from olive trees in Istria, Croatia, are marked with green–white labels, with the isolate names next to the labels. The figure was made using Google Maps.

Information regarding the precise location and coordinates of the site where species from the family *Botryosphaeriaceae* were found, including the variety from which the sample was taken, collection date, olive grove area, and tree age, are presented in Table 7.

Table 7. Isolate label, location, and coordinates of *Botryosphaeriaceae* species site, olive variety from which the sample was taken, collection date, olive grove area, and tree age.

Isolate	Location	Coordinates	Variety from Which Sample Was Taken	Collection Date	Olive Grove Area (Ha)	Tree Age (Years)
R8 NP	Rovinj	45°05′20″ N, 13°38′51″ E	Unknown	6 September 2021	0.01	15
IKB9 B2II	Poreč	45°13′19.9″ N, 13°36′07.7″ E	Buža	13 September 2021	1.49	>30
IMK9 IBVI	Poreč	45°13′13″ N 13°36′09.8″ E	Istarska bjelica	13 September 2021	1.49	>30
V12 PEN	Vodnjan	44°57′65″ N, 13°50′19″ E	Pendolino	24 September 2021	0.1	20–100
V16 BI	Fažana near Vodnjan	44°56′21″ N; 13°50′18″ E	Buža	14 October 2021	1	13
V16 K1			Karbonaca			
V16 K2II			Karbonaca			
N17 BJA3	Novigrad	45°20′08.8″ N, 13°33′33.6″ E	Istarska bjelica	14 October 2021	3	20–25
R18 B1	Rovinj	45°03′02.2″ N 13°42′43.9″ E	Buža	14 October 2021	0.43	39
R18 PEN1			Pendolino			
R19 F	Rovinj	45°03′46″ N, 13°42′71″ E	Frantoio	14 October 2021	0.38	25–30
PL1 NP	Poreč	45°12′26″ N 13°35′29″ E	Unknown	23 March 2022	0.02	10
V21 B5I	Vodnjan	44°57′34″ N, 13°50′37″ E	Buža	24 March 2022	8.2	12–300

The trees exhibited symptoms such as leaf wilting and defoliation, twig and branch dieback, and the appearance of necrosis and cankers. A total of 10 branch samples per tree were collected. Samples were taken from the parts of the branches where the transition between healthy and infected parts were visibly apparent. The samples were placed in sterile black plastic bags, labelled, and stored in a portable refrigerator at a temperature of +4 °C. The collected samples were promptly transported to the Laboratory for Plant Protection at the Institute of Agriculture and Tourism in Poreč, Croatia, for analysis. Branch samples from affected trees were photographed and documented and then underwent a washing under tap water. With the use of a sterile surgical scalpel, the bark was removed from the branches, and subsequently, the samples were cut using fruit shears. The branch pieces (5 × 5 cm) were immersed in 70% ethanol for two minutes, followed by rinses in sterile distilled water for two minutes. After this process, they were carefully arranged on a sterile paper sheet within a laminar flow cabinet to facilitate surface drying. Once adequately dried, the pieces were placed on PDA supplemented with 35 mg/L of penicillin and incubated in a dark environment at 25 °C within an incubator. Upon the development of the culture on PDA, isolates were transferred to a medium containing WA and pine needles (*Pinus* L.) (WA + *Pinus*). The medium preparation involved cutting fresh green pine needles, washing them under tap water, and autoclaving them twice in a glass jar at 121 °C for 15 min. Two to three pieces of pine needles were placed on the surface of WA after the agar had solidified by 50%, submerging half of the needle in the medium while leaving the rest exposed. Fungal isolates were then inoculated into the nutrient medium WA + *Pinus*. After an incubation period of 20 days and the subsequent development of pycnidia, spores were extracted to create single-spore isolates. Pure cultures were preserved in 2 mL cryovial screw cap tubes containing a 50% glycerol solution at temperatures of −20 °C and −80 °C, as well as in sterilised water in plastic tubes at 4 °C. The preserved cultures are kept in

the Laboratory for Plant Protection collection at the Institute of Agriculture and Tourism in Poreč.

4.2. Morphological Characterisation

Following incubation at 25 °C in the absence of light for 2, 15, and 30 days, pure fungal cultures were subjected to examination. The preliminary determination involved an inspection of colonies, considering their overall appearance and colour. Additionally, the observation of conidia included an assessment of colour, appearance, septation, and shape. Out of the 10 samples collected per tree (totalling 13 trees), the identical fungus was consistently isolated. For a detailed analysis of morphological characteristics, one representative isolate per tree was selected, and a total of 13 fungal isolates were analysed. This involved a detailed evaluation of colony traits, encompassing parameters such as colour, shape, elevation, margin, surface, and opacity. Macroscopic characteristics were observed using a BOECO zoom stereo microscope BSZ-405 and photographed with a fitted B-CAM16 industrial digital camera and B-View software (Boeckel, Hamburg, Germany) at the Institute of Agriculture and Tourism Poreč, as well as with an Olympus SZX10 microscope and Olympus N547 camera (Olympus, Tokyo, Japan) at the Faculty of Agrobiotechnical Sciences Osijek. Additionally, the determination of growth rate and cardinal temperatures for mycelial growth was performed. To determine the cardinal temperatures for growth, five-day-old isolates were inoculated in triplicate on PDA in Petri dishes with a diameter of 90 mm. A circular section of mycelium with a diameter of five mm was placed at the centre of the PDA. The isolates were incubated in darkness at eight different temperatures (5, 10, 15, 20, 25, 30, 35, and 40 °C), and measurements were taken after 48 h. Colony diameter was measured at two positions perpendicular to each other, and the obtained values were reduced by the diameter of the circular section of the mycelium. The average values were then calculated. Empirical mathematical modelling, employing the least squares method, as described by Sanchez et al. [59], was conducted within the suitable frameworks of third, fourth, fifth, and sixth-degree polynomial regressions using Microsoft Office Excel. This approach was used to generate graphs along with corresponding equations, serving to determine cardinal temperatures. Since the sixth-degree polynomial equation fits the examined data best and closely follows the points of determined growth rates, it was utilised for further determination of cardinal temperatures. In order to obtain the minimum, maximum, and optimal cardinal temperatures from the equation, the described empirical mathematical modelling using the least squares method was performed with the Wolfram Alpha (WolframAlpha LLC) mathematical program.

Furthermore, the features of spores, including characteristics such as colour, shape, the presence or absence of septum, and dimensional measurements, were examined. To determine the morphological characteristics of the conidia, they were extracted from pycnidia cut with a laboratory needle. Microscopic analysis was conducted using an LABOSGEN camera and LABOSGEN software at the Institute of Agriculture and Tourism Poreč, as well as with an Olympus BX41 microscope (Olympus, Tokyo, Japan) at the Faculty of Agrobiotechnical Sciences Osijek. Additionally, measurements of 30 conidia per isolate were performed. Subsequently, mean values, standard deviations of measured conidia and their minimum and maximum values, and the length-to-width ratio of conidia were calculated. Furthermore, 95% confidence intervals for the determined mean dimensions and length-to-width ratio were established using Microsoft Office Excel. The morphological profile derived from this analysis was systematically compared with relevant scientific literature sources [37].

4.3. DNA Extraction and Amplification

Molecular analysis was used to confirm the identification of all 13 isolates at the species level. Fungal isolates were cultured on PDA for seven days at 25 °C in the dark. Subsequently, a small portion of mycelium from the colony margins was aseptically sampled using a sterile laboratory needle for genomic DNA extraction. Maxwell® RSC Instrument

(Promega, Madison, WI, USA) and Maxwell® RSC Plant DNA Kit (Promega, Madison, WI, USA) were used to extract total genomic DNA. The amount of genomic DNA in samples post-isolation was quantified using a Maxwell Promega Quantus fluorometer (Promega). The internal transcribed spacer (ITS) regions were subjected to amplification and subsequent sequencing using the primer pairs ITS1 (5′ TCCGTAGGTGAACCTGCGG 3′) and ITS4 (5′ TCCTCCGCTTATTGATATGC 3′) [60]. Amplification of a segment of the beta-tubulin (*TUB2*) gene was carried out utilising the oligonucleotide primers Bt2a (5′ GGTAACCAAATCGGTGCTGCTTTC 3′) and Bt2b (5′ ACCCTCAGTGTAGTGACCCTTGGC 3′) [61]. Furthermore, a part of the translation elongation factor 1-alpha gene (*TEF1-α*) was amplified and sequenced using the primer pairs EF-728F (5′ CATCGAGAAGTTCGAGAAGG 3′) and EF1-986R (5′ TACTTGAAGGAACCCTTACC 3′) [62]. Each PCR mixture, with a final volume of 25 µL, was composed of 12.5 µL of EmeraldAmp® GT PCR Master Mix, 0.5 µL of each primer (10 µM), 6.5 µL of nuclease-free water, and 5 µL of template DNA at a concentration of 5 ng/µL. PCR amplification was performed using a SureCycler 8800 Thermal Cycler (Agilent Technologies, Santa Clara, CA, USA). The amplification program comprised an initial denaturation step at 94 °C for two minutes, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, elongation at 72 °C for one minute and 30 s, and a final extension step at 72 °C for five minutes [63]. Gel electrophoresis was performed utilising a 1% agarose gel at 110 V for 25 min in 1× TAE buffer, employing an omniPAC Midi CS-300V electropshoresis power supply (Cleaver Scientific, Rugby, Warwickshire, UK). Following electrophoresis, visualisation of PCR products was accomplished using an iBright CL1000 Imaging System (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). After visualisation, purification of the PCR products was carried out using the GenElute™ PCR Clean-Up Kit (Sigma-Aldrich, Burlington, MA, USA).

4.4. DNA Sequence Assembly and Phylogenetic Analysis

Sequencing of the PCR products was carried out by the Macrogen Europe sequencing service (Amsterdam, the Netherlands). Sequencing was performed bidirectionally using the same primers that were used for amplification. Subsequently, nucleotide sequences were read and edited using Sequencher® software (Gene Codes Corporation, Ann Arbor, MI, USA). Comparative analysis was conducted against existing sequences from the *Botryosphaeriaceae* family available in the National Center for Biotechnology Information GenBank database. Consensus sequences resulting from this study were submitted to NCBI GenBank. Phylogenetic analyses were conducted for individual gene regions as well as for combined regions. Sequence data from isolates used in this study and relevant isolates from GenBank were utilised for phylogenetic analysis. The list of species/isolates included in the phylogenetic analysis is represented in Table 8. Sequence alignment was performed using ClustalX2 software (UCD, Dublin, Ireland) using the following multiple alignment parameters: gap opening = 15, gap extension = 6.66, delay divergent sequences = 30%, DNA transition weight = 0.5. The evolutionary history was deduced utilising the neighbour–joining method [64], with the optimal tree depicted. Bootstrap analysis (1000 replicates) indicated the percentage of replicate trees in which the associated taxa clustered together, shown next to the branches [65]. The evolutionary distances were calculated using the maximum composite likelihood method and are presented as the number of base substitutions per site [66]. MEGA11 (Pennsylvania State University, State College, PA, USA) was employed for the evolutionary analyses [67].

Table 8. List of species/isolates included in the phylogenetic analysis, detailing their host, country of origin, GenBank accession numbers, and references.

Species	Isolate	Host	Country	GenBank Accession Number			References
				ITS	*TUB2*	*TEF1-α*	
Biscogniauxia mediterranea	Bm04.001	*Quercus suber* L.	Portugal	KM216752	KM267202	KM216788	[68]
B. mediterranea	Bm10.019	*Q. suber*	Portugal	KM216761	KM267210	KM216797	[68]
Botryosphaeria corticis (Demaree & Wilcox) Arx & E. Mull.	ATCC 22927	*Vaccinium corymbosum* L.	USA	DQ299247	EU673108	EU673291	[33,69]
Bo. corticis	CBS119047	*V. corymbosum*	USA	DQ299245	EU673107	EU017539	[33,69]
Bo. corticis	CBS119048	*V. corymbosum*	USA	DQ299246	MT592464	EU017540	[33,69]
Bo. dothidea (Moug. ex Fr.) Ces. & De Not.	CBS110302	*Vitis vinifera* L.	Portugal	AY259092	EU673106	AY573218	[70–72]
Bo. dothidea	CMW8000	*Prunus* sp.	Switzerland	AY236949	AY236927	AY236898	[63]
Bo. dothidea	BJS-01s	*V. vinifera*	China	JX275778	JX462259	JX462285	[49]
Bo. pseudoramosa G.Q. Li & S.F. Chen	CERC 2001	*Eucalyptus urophylla* × *Eucalyptus grandis*	China	KX277989	KX278198	KX278094	[73]
Bo. pseudoramosa	CERC 3455	*E. urophylla* × *E. grandis*	China	KX277997	KX278206	KX278102	[73]
Bo. pseudoramosa	CERC 3472	*E. urophylla* × *E. grandis*	China	KX277999	KX278208	KX278104	[73]
Bo. qingyuanensis G.Q. Li & S.F. Chen	CERC 2947	*E. urophylla* × *E. grandis*	China	KX278001	KX278210	KX278106	[73]
Bo. qingyuanensis	CERC 2946	*E. urophylla* × *E. grandis*	China	KX278000	KX278209	KX278105	[73]
Bo. qingyuanensis	TKBQ16-30	*Carya cathayensis* Sarg.	China	MW561677	MW561777	MW561687	[74]
Diplodia bulgarica A.J.L. Phillips, J. Lopes & S.G. Bobev	UCD11351	*Malus domestica* (Suckow) Borkh.	USA	OR631210	OR637362	OR637364	[75]
D. bulgarica	UCD11350	*M. domestica*	USA	OR631209	OR637361	OR637363	[75]
D. bulgarica	CBS124136	*M. sylvestris* (L.) Mill.	Bulgaria	MH863355	MT592475	GQ923822	[76–78]
D. corticola A.J.L. Phillips, A. Alves & J. Luque	CBS 112546	*Q. ilex* L.	Spain	AY259090	EU673117	EU673310	[70,72]
D. corticola	CBS 112549	*Q. suber*	Portugal	AY259100	DQ458853	AY573227	[70,71]
D. corticola	UCD1260So	*V. vinifera*	California	GU799470	GU799464	GU799467	[79]
D. cupressi A.J.L. Phillips & A. Alves	CBS168.87	*Cupressus sempervirens* L.	Israel	DQ458893	DQ458861	DQ458878	[80]
D. cupressi	CBS261.85	*C. sempervirens*	Israel	DQ458894	DQ458862	DQ458879	[80]
D. cupressi	CBS121027	*C. sempervirens*	Cyprus	MT587340	MT592487	MT592045	[78]
D. mutila	CBS112553	*V. vinifera*	Portugal	AY259093	MZ073931	AY573219	[70,71,81]
D. mutila	CBS230.30	*Phoenix dactylifera* L.	USA	DQ458886	DQ458849	DQ458869	[80]

Table 8. *Cont.*

Species	Isolate	Host	Country	GenBank Accession Number			References
				ITS	*TUB2*	*TEF1-α*	
D. mutila	PD75	Holly	USA	GU251119	GU251779	GU251251	[82]
D. seriata De Notaris	UCD244Ma	*V. vinifera*	California	DQ008314	DQ008337	EU012406	[79]
D. seriata	CBS 112555	*V. vinifera*	Portugal	AY259094	DQ458856	AY573220	[70]
D. seriata	SDZ-01	*V. vinifera*	China	HQ629954	HQ629956	HQ629958	[49]
Dothiorella brevicollis Jami, Gryzenh., Slippers & M.J. Wingf.	CMW36463	*Vachellia karroo* (Hayne) Banfi & Galasso	South Africa	JQ239403	JQ239371	JQ239390	[83]
Do. brevicollis	CMW36464	*V. karroo*	South Africa	JQ239404	JQ239372	JQ239391	[83]
Do. brevicollis	CBS130412	*V. karroo*	South Africa	MT587395	MT592578	MT592107	[78]
Do. dulcispinae Jami, Gryzenh., Slippers & M.J. Wingf.	CMW36460	*V. karroo*	South Africa	JQ239400	JQ239373	JQ239387	[83]
Do. dulcispinae	CMW36461	*V. karroo*	South Africa	JQ239401	JQ239374	JQ239388	[83]
Do. dulcispinae	CMW36462	*V. karroo*	South Africa	JQ239402	JQ239375	JQ239389	[83]
Do. iberica A.J.L. Phillips, J. Luque & A. Alves	CBS115041	*Q. ilex*	Spain	NR111165	EU673096	AY573222	[72,84]
Do. iberica	UCRDI3	*Prunus dulcis* (Mill.) D.A.Webb	California	KP012591	KP067201	KP828802	[85]
Do. iberica	UCRDI1	*P. dulcis*	California	KP012590	KP067200	KP828801	[85]
Do. oblonga F.J.J. van der Walt, Slippers & G.J. Marais	CBS130414	*V. karroo*	South Africa	MT587408	MT592598	MT592120	[78]
Do. oblonga	CBS121766	*Senegalia mellifera* (Vahl) L.A. Silva & J. Freitas	South Africa	EU101301	KX464863	EU101346	[86,87]
Do. oblonga	CBS121765	*S. mellifera*	South Africa	KF766163	KX464862	EU101345	[86–88]
Do. sarmentorum (Fr.) A.J.L. Phillips, Alves & Luque	CMW39366	*Aesculus hippocastanum* L.	Serbia	KF575009	KF575105	KF575047	[89]
Do. sarmentorum	PD78	*P. dulcis*	California	GU251169	GU251829	GU251301	[82]
Do. sarmentorum	CBS115038	*Malus pumila* Mill.	Netherlands	AY573206	EU673101	AY573223	[71]
Lasiodiplodia crassispora T.I. Burgess & P.A. Barber	UCD27Co	*V. vinifera*	California	GU799457	GU799480	GU799488	[90]
L. crassispora	CMW13488	*Eucalyptus urophylla* S.T. Blake	Venezuela	DQ103552	KU887507	DQ103559	[91,92]

Table 8. *Cont.*

Species	Isolate	Host	Country	GenBank Accession Number			References
				ITS	*TUB2*	*TEF1-α*	
L. crassispora	UCD24Co	*V. vinifera*	California	GU799456	GU799479	GU799487	[90]
L. gonubiensis Pavlic, Slippers & M.J. Wingf	CBS115812	*Syzygium cordatum* Hochst.	South Africa	DQ458892	DQ458860	DQ458877	[80]
L. gonubiensis	CMW36240	*Adansonia* sp.	Africa	KU887124	KU887502	KU887001	[92]
L. gonubiensis	CMW43763	*Bruguiera gymnorhiza* (L.) *Savigny*	South Africa	KU587955	KU587865	KU587944	[93]
L. iranensis Abdollahz., Zare & A.J.L. Phillips	PCoCo10	*Theobroma cacao* L.	Taiwan	OR534188	OR551954	OR552312	[94]
L. iranensis	PCoCo11	*T. cacao*	Taiwan	OR534189	OR551955	OR552313	[94]
L. iranensis	PCoCo12	*T. cacao*	Taiwan	OR534190	OR551956	OR552314	[94]
L. pseudotheobromae A.J.L. Phillips, A. Alves & Crous	CBS116459	*Gmelina arborea* Roxb. ex Sm.	Costa Rica	EF622077	EU673111	EF622057	[72,95]
L. pseudotheobromae	MFLUCC 18-1120	*Magnolia liliifera* (L.) L.	China	MK496933	MK524719	MK521585	[96]
L. pseudotheobromae	MFLUCC 18-0950	*M. liliifera*	China	MK501818	MK550605	MK521586	[96]
L. theobromae (Pat.) Griffon & Maubl.	CAA006	*V. vinifera*	USA	DQ458891	DQ458859	DQ458876	[80]
L. theobromae	GX-5-5A	*V. vinifera*	China	JX275780	JX462262	JX462288	[49]
L. theobromae	TJXHS1S1	*V. vinifera*	China	JX275790	JX462278	JX462304	[49]
N. arbuti (D.F. Farr & M. Elliott) Crous, Slippers & A.J.L. Phillips	CBS116576	*Arbutus menziesii* Pursh	USA	KX464156	KX464928	KX464651	[87]
N. arbuti	CBS116574	*A. menziesii*	USA	KX464154	KX464926	KX464649	[87]
N. arbuti	CBS116573	*A. menziesii*	USA	KX464153	KX464925	KX464648	[87]
N. australe	CMW6837	*Acacia* sp.	Australia	AY339262	AY339254	AY339270	[97]
N. australe	CMW9073	*Acacia* sp.	Australia	AY339261	AY339253	AY339269	[97]
N. australe	CMW6853	*Sequoiadendron giganteum* (Lindl.) J. Buchholz	Australia	AY339263	AY339255	AY339271	[97]
N. brasiliense M.W. Marques, A.J.L. Phillips & M.P.S. Camara	CMM1285	*Mangifera indica* L.	Brazil	JX513628	KC794030	JX513608	[98]
N. brasiliense	CMM1338	*M. indica*	Brazil	JX513630	KC794031	JX513610	[98]
N. brasiliense	CMM1269	*M. indica*	Brazil	JX513629	KC794032	JX513609	[98]
N. hongkongense G.Q. Li & S.F. Chen	CERC 2967	*Araucaria cunninghamii* Mudie	China	KX278050	KX278259	KX278155	[73]
N. hongkongense	CERC 2968	*A. cunninghamii*	China	KX278051	KX278260	KX278156	[73]
N. hongkongense	CERC 2973	*A. cunninghamii*	China	KX278052	KX278261	KX278157	[73]

Table 8. *Cont.*

Species	Isolate	Host	Country	GenBank Accession Number			References
				ITS	*TUB2*	*TEF1-α*	
N. mangroviorum J.A. Osorio, Jol. Roux & Z.W. de Beer	CPC32482	*Diospyros dichrophylla* (Gand.) De Winter	South Africa	MT587494	MT592701	MT592209	[78]
N. mangroviorum	CMW41365	*Avicennia marina* (Forssk.) Vierh.	South Africa	KP860859	KP860779	KP860702	[93]
N. mangroviorum	CMW42481	*Bruguiera gymnorhiza* (L.) Savigny,	South Africa	KP860848	KP860770	KP860692	[93]
N. mangiferae (Syd. & P.Syd.) Crous et al.	CMW7024	*Magnifera indica* L.	Australia	AY615185	AY615172	DQ093221	[99]
N. mangiferae	CMW7797	*M. indica*	Australia	AY615186	AY615173	DQ093220	[99]
N. mangiferae	CMW7081	*M. indica*	Australia	AY615187	AY615174	KF766425	[99]
N. microconidium	CERC 3497	*E. urophylla* × *E. grandis*	China	KX278053	KX278262	KX278158	[73]
N. microconidium	CERC 3498	*E. urophylla* × *E. grandis*	China	KX278054	KX278263	KX278159	[73]
N. microconidium	CBS118821	*Syzygium cordatum* Hochst.	South Africa	MT587497	MT592704	MT592212	[78]
N. luteum (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips	CBS110299	*V. vinifera*	Portugal	AY259091	DQ458848	AY573217	[70,71]
N. luteum	CBS110497	*V. vinifera*	Portugal	EU673311	EU673092	EU673277	[72]
N. luteum	CBS133502	*Persea americana* Mill.	USA	MT587483	MT592689	MT592197	[78]
N. parvum (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips	CBS110301	*V. vinifera*	Portugal	AY259098	EU673095	AY573221	[70–72]
N. parvum	CMW9081	*Populus nigra* L.	New Zealand	AY236943	AY236917	AY236888	[97]
N. parvum	CBS123652	*S. cordatum*	South Africa	KX464184	KX464996	KX464710	[87]
N. parvum	Zai-5	*Syzygium samarangense* Merr. & L.M. Perry	Taiwan	OR534045	OR551811	OR552360	[94]

4.5. Pathogenicity Test and the Evaluation of Variety Resistance

For confirmation of species pathogenicity and evaluation of variety resistance to identified species, a greenhouse experiment was set up using olive seedlings. One representative isolate of each *Botryosphaeriaceae* species (a total of six isolates) identified in this study was selected for pathogenicity testing. Since multiple isolates were collected for certain species while only one for others, isolates with similar growth rates on PDA were chosen. The experiment utilised five-year-old seedlings of three Croatian indigenous olive varieties: Buža, Istarska bjelica, and Rosinjola, as well as one introduced variety: Leccino. The bark

at the intended inoculation site was wiped with cotton soaked in 70% ethanol. Wounds measuring five mm in diameter were then created using a sterile cork borer. The outer bark was removed while preserving the inner bark. A 5 mm-diameter mycelium plug from a 10-day-old colony on PDA was inserted into the wound using a sterile cork borer. Inoculated wounds were coated with Vaseline and covered with Parafilm. Pure PDA plugs served as controls. Ten seedlings were inoculated per isolate. The inoculated plants were grown in the greenhouse for nine months, from December 2022 to October 2023. The seedlings were irrigated using a drip irrigation system. The average temperature during the experimental period in the greenhouse ranged between 24 and 25 °C, with a relative humidity of 85%. Changes were recorded over time. After the incubation period, samples were collected in black plastic bags, and the total length of surface necrotic changes above and below the inoculation site was measured. In accordance with Koch's postulates, small necrotic tissue fragments from the periphery of lesions that had developed on each seedling were inoculated onto PDA medium to isolate the originally introduced fungus. The data obtained from the pathogenicity assay underwent analysis of variance (ANOVA), followed by Tukey's test to identify significant differences between mean values at a significance level of 5% [43]. Statistical analysis was conducted using the SAS Enterprise Guide 8.4 statistical software.

5. Conclusions

In conclusion, six different species have been identified as causative agents of Botryosphaeria dieback of olive in Istria: *Botryosphaeria dothidea*, *Diplodia mutila*, *D. seriata*, *Dothiorella iberica*, *Do. sarmentorum*, and *Neofusicoccum parvum*. To our knowledge, *D. mutila*, *Do. iberica*, and *Do. sarmentorum* have not been previously identified in olive trees in Croatia, making this the first report of their presence. Species from the *Botryosphaeriaceae* family are economically significant pathogens due to their detrimental impact on olive trees, causing fruit rot, leaf wilting, necrosis, and other symptoms. They rank among the most aggressive pathogens attacking olive trees. Therefore, it is necessary to monitor olive groves and track the further movement of these pathogens to minimise the damage they cause. Preventive measures are of utmost importance in controlling the further spread of these pathogens. These measures include disinfection of tools, pruning of olive trees and burning of residues, selection of planting locations, selection of resistant varieties, etc. The varieties tested in this study showed differences in resistance depending on the fungus with which they were infected. In addition to preventive measures, it is important to protect olive groves by using preparations that have proven effectiveness against these pathogens.

Author Contributions: Conceptualisation, E.P. and S.G.; methodology, E.P., K.V. and A.B.V.; investigation, E.P., S.G., K.V. and J.Ć.; writing—original draft preparation, E.P.; writing—review and editing, S.G., K.V., A.B.V. and J.Ć. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Croatian Science Foundation Installation Research Project "Natural bioactive compounds as a source of potential antimicrobial agents in the control of bacterial and other fungal pathogens of olives", Anti-Mikrobi-OL (AMO), UIP-2020-02-7413, and the "Young Researchers' Career Development Project" DOK-2021-02-2882.

Data Availability Statement: All sequence data are available in NCBI GenBank in accordance with the accession numbers in the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Pribetić, D. *Sorte Maslina u Istri*; MIH d.o.o.: Poreč, Croatia, 2006.
2. Rosa, G. *Storia dell' Agricoltura Nella Civilitá*; Forni: Bologna, Italy, 1883; p. 83.
3. Hugues, C. *Maslinarstvo Istre. Elaiografia Istriana*; Ceres: Zagreb, Croatia, 1999.
4. Del Fabro, A. *Maslina—Uzgoj, Berba, korištenje*; Leo Commerce d.o.o.: Rijeka, Croatia, 2015.
5. Bertoša, M. *Istarska Enciklopedija*; Leksikografski Zavod Miroslav Krleža: Zagreb, Croatia, 2005.

6. Godena, S.; Ivić, D.; Ban, D.; Gorena Ban, S. Characterization of *Verticillium dahliae* isolates from olive and susceptibility of local olive cultivars to Verticillium wilt in Istria, Croatia. *Sci. Hortic.* **2022**, *292*, 110630. [CrossRef]
7. Bjeliš, M. *Zaštita Masline u Ekološkoj Proizvodnji*; Graf Form: Solin, Croatia, 2005.
8. FAO—Food and Agriculture Organization of United Nations. Crop and Livestock Products. FAOSTAT. Available online: https://www.fao.org/faostat/en/#data (accessed on 29 May 2024).
9. DZS—Državni Zavod za Statistiku. Proizvodnja Povrća, Voća i Grožđa u 2023—Privremeni Podaci. Available online: https://podaci.dzs.hr/2023/hr/58459 (accessed on 29 May 2024).
10. Breton, C.; Terral, F.; Pinatel, C.; Médail, F.; Bonhomme, F.; Bervillé, A. The origins of the domestication of the olive tree. *Comptes Rendus Biol.* **2009**, *332*, 1059–1064. [CrossRef] [PubMed]
11. Rugini, E.; Mencuccini, M.; Biasi, R.; Altamura, M.M. Olive (*Olea europea* L.). In *Protocol for Somatic Embryogenesis in Woody Plants*; Jain, S.M., Gupta, P.K., Eds.; Foresty Sciences; Springer: Dordrecht, The Netherlands, 2005; Volume 77, pp. 345–360.
12. Schiestel, A. Olive Variety versus Cultivar. Available online: https://www.documentingolives.com/knowledge-centre/olive-variety-versus-cultivar/ (accessed on 29 May 2024).
13. Monini. Olive Varieties. Available online: https://www.monini.com/en-gl/olive-varieties (accessed on 29 May 2024).
14. HAPIH—Hrvatska Agencija za Poljoprivredu i Hranu. *Popis Sorti Voćnih Vrsta*. Available online: https://www.hapih.hr/csr/sortne-liste/ (accessed on 13 May 2024).
15. Cvjetković, B. *Mikoze i Pseudomikoze Voćnjaka i Vinoze Loze*; Zrinski d.d.: Čakovec, Croatia, 2010.
16. Kaliterna, J.; Miličević, T.; Ivić, D.; Benčić, D.; Mešić, A. First Report of *Diplodia seriata* as Causal Agent of Olive Dieback in Croatia. *Plant Dis.* **2012**, *96*, 290. [CrossRef]
17. Godena, S.; Ivić, D.; Goreta Ban, S. *Uzročnici Djelomičnog ili Potpunog Sušenja Stabala Maslina. Priručnik o Rezultatima VIP Projekta*; Institute of Agriculture and Tourism: Poreč, Croatia, 2019.
18. Ivić, D.; Petrović, E.; Godena, S. Fungi associated with canker diseases on olive in Istria (Croatia). *J. Cent. Eur. Agric.* **2023**, *24*, 470–475. [CrossRef]
19. Petrović, E.; Godena, S.; Ćosić, J.; Vrandečić, K. Identification and Pathogenicity of *Biscogniauxia* and *Sordaria* Species Isolated from Olive Trees. *Horticulturae* **2024**, *10*, 243. [CrossRef]
20. Ivić, D.; Ivanović, A.; Miličević, T.; Cvjetković, B. Shoot necrosis of olive caused by *Phoma incompta*, a new disease of olive in Croatia. *Phytopathol. Mediterr.* **2010**, *49*, 414–416.
21. Petrović, E.; Vrandečić, K.; Ivić, D.; Ćosić, J.; Godena, S. First report of olive branch and fruit dieback in Croatia caused by *Cytospora pruinosa* Défago. *Microorganisms* **2023**, *11*, 1679. [CrossRef] [PubMed]
22. Petrović, E.; Vrandečić, K.; Ćosić, J.; Godena, S. First report of *Nigrospora* species causing leaf spot on olive (*Olea europaea* L.). *Horticulturae* **2023**, *9*, 1067. [CrossRef]
23. Petrović, E.; Vrandečić, K.; Ćosić, J.; Kanižai Šarić, G.; Godena, S. First report of *Phaeoacremonium iranianum* causing olive twig and branch dieback. *Plants* **2022**, *11*, 3578. [CrossRef]
24. Ivić, D.; Tomić, Z.; Godena, S. First Report of *Pleurostomophora richardsiae* Causing Branch Dieback and Collar Rot of Olive in Istria, Croatia. *Plant Dis.* **2018**, *102*, 2648. [CrossRef]
25. Buljubašić, I.; Bjeliš, M.; Marušić, I. Ocjena intenziteta napada paunovog oka [*Spilocaea oleagina* (Castagne) Hughes] na uzgojnim područjima masline. *Glas. Biljn. Zaštite* **2012**, *12*, 341–347.
26. Cvjetković, B.; Vončina, D. Paunovo oko [*Spilocaea oleaginea* (Castagne) Hughes] najučestalija je bolest masline. *Glas. Biljn. Zaštite* **2012**, *102*, 336–340.
27. Kaliterna, J.; Miličević, D.; Benčić, D.; Mešić, A. First Report of Verticillium Wilt Caused by *Verticillium dahliae* on Olive Trees in Croatia. *Plant Dis.* **2016**, *100*, 2526. [CrossRef]
28. Vrsalović, M. *Maslinarstvo i Uljarstvo za Puk*; Vtalijani: Zadar, Croatia, 1901.
29. Eldesouki-Arafat, I. Interacciones de *Batrocera oleae* Gmel. (Mosca del Olivo) con *Botryosphaeria dothidea* Moug. (Escudete de la Aceituna) y de *Phloeotribus scarabaeoides* Bern. (Barrenillo del Olivo) con *Verticillium dahliae* Kleb. Causante de la Verticilosis del Olivo. Ph.D. Thesis, University of Cordoba, Cordoba, Spain, 2013.
30. Latinović, J.; Mazzaglia, A.; Latinović, N.; Ivanović, M.; Gleason, M.L. Resistance of olive cultivars to *Botryosphaeria dothidea*, causal agent of olive fruit rot in Montenegro. *Crop Prot.* **2013**, *48*, 35–40. [CrossRef]
31. Moral, J.; Muñoz-Diez, C.; Gonzalez, N.; Trapero, A.; Michailides, T.J. Characterization and Pathogenicity of *Botryosphaeriaceae* Species Collected from Olive and Other Hosts in Spain and California. *Phytopathology* **2010**, *100*, 1340–1351. [CrossRef]
32. Hernández-Rodríguez, L.; Mondino-Hintz, P.; Alaniz-Ferro, S. Diversity of *Botryosphaeriaceae* species causing stem canker and fruit rot in olive trees in Uruguay. *J. Phytopathol.* **2022**, *170*, 264–277. [CrossRef]
33. Lazzizera, C.; Frisullo, S.; Alves, A.; Phillips, A.J.L. Morphology, phylogeny and pathogenicity of *Botryosphaeria* and *Neofusicoccum* species associated with drupe rot of olives in southern Italy. *Plant Pathol.* **2008**, *57*, 948–956. [CrossRef]
34. Lazzizera, C.; Frisullo, S.; Alves, A.; Lopes, J.; Phillips, A.J.L. Phylogeny and morphology of *Diplodia* species on olives in southern Italy and description of *Diplodia olivarum* sp. nov. *Fungal Divers.* **2008**, *31*, 63–71.
35. Carlucci, A.; Raimondo, M.L.; Cibelli, F.; Phillips, A.J.L.; Lops, F. *Pleurostomophora richardsiae*, *Neofusicoccum parvum* and *Phaeoacremonium aleophilum* associated with a decline of olives in southern Italy. *Phytopathol. Mediterr.* **2013**, *52*, 517–527.
36. Úrbez-Torres, J.R.; Peduto, F.; Vossen, P.M.; Krueger, W.H.; Gubler, W.D. Olive Twig and Branch Dieback: Etiology, Incidence, and Distribution in California. *Plant Dis.* **2013**, *97*, 231–244. [CrossRef]

37. Phillips, A.J.L.; Alves, A.; Abdollahzadeh, J.; Slippers, B.; Wingfield, M.J.; Groenewald, J.Z.; Crous, P.W. The *Botryosphaeriaceae*: Genera and species known from culture. *Stud. Mycol.* **2013**, *76*, 51–167. [CrossRef] [PubMed]
38. Amponsah, N.T.; Jones, E.E.; Ridgway, H.J.; Jaspers, M.V. Rainwater dispersal of *Botryosphaeria* conidia from infected grapevines. *N. Z. Plant Prot.* **2009**, *62*, 228–233. [CrossRef]
39. Lehoczky, J. Black dead-arm disease of grapevine caused by *Botryosphaeria stevensii* infection. *Acta Phytopathol. Acad. Sci. Hung.* **1974**, *9*, 319–327.
40. Van Nieker, J.M.; Fourie, P.H.; Hallen, F.; Crous, P.W. *Botryosphaeria* spp. as grapevine trunk disease pathogens. *Phytopathol. Mediterr.* **2006**, *45*, S43–S54.
41. Schoch, C.L.; Ciufo, S.; Domrachev, M.; Hotton, C.L.; Kannan, S.; Khovanskaya, R.; Leipe, D.; Mcveigh, R.; O'Neill, K.; Robbertse, B.; et al. NCBI Taxonomy: A comprehensive update on curation, resources and tools. *Database* **2020**, *2020*, baaa062. [CrossRef] [PubMed]
42. Mycobank. *Botryosphaeriaceae*. Available online: https://www.mycobank.org/page/Name%20details%20page/name/Botryosphaeriaceae (accessed on 3 June 2024).
43. Kaliterna, J. Identifikacija, Patogenost i Rasprostranjenost Vrsta Gljiva iz Porodice *Botryosphaeriaceae* i *Diaporthaceae* na Vinovoj Lozi u Hrvatskoj. Ph.D. Thesis, University in Zagreb, Faculty of Agriculture, Zagreb, Croatia, 2013.
44. EPPO. European and Mediterranean Plant Protection Organization. *Bull. OEPP/EPPO Bull.* **2016**, *46*, 501–537.
45. Romero, M.A.; Sánchez, M.E.; Trapero, A. First report of *Botryosphaeria ribis* as a branch dieback pathogen of olive trees in Spain. *Plant Dis.* **2005**, *89*, 208. [CrossRef] [PubMed]
46. Moral, J.; Agustí-Brisach, C.; Pérez-Rodríguez, M.; Xavier, C.; Raya, M.C.; Rhouma, A.; Trapero, A. Identification of Fungal Species Associated with Branch Dieback of Olive and Resistance of Table Cultivars to *Neofusicoccum mediterraneum* and *Botryosphaeria dothidea*. *Plant Dis.* **2017**, *107*, 306–316. [CrossRef] [PubMed]
47. Spies, C.F.J.; Mostert, L.; Carlucci, A.; Moyo, P.; van Jaarsveld, W.J.; du Plessis, I.L.; van Dyk, M.; Halleen, F. Dieback and decline pathogens of olive trees in South Africa. *Persoonia* **2020**, *45*, 196–220. [CrossRef]
48. Linaldeddu, B.T.; Rossetto, G.; Maddau, L.; Vatrano, T.; Bregant, C. Diversity and Pathogenicity of *Botryosphaeriaceae* and *Phytophthora* Species Associated with Emerging Olive Diseases in Italy. *Agriculture* **2023**, *13*, 1575. [CrossRef]
49. Yan, J.-Y.; Xie, Y.; Zhang, W.; Wang, Y.; Liu, J.K.; Hyde, K.D.; Seem, R.C.; Zhang, G.-Z.; Wang, Z.-Y.; Yao, S.-W.; et al. Species of *Botryosphaeriaceae* involved in grapevine dieback in China. *Fungal Divers.* **2013**, *61*, 221–236. [CrossRef]
50. Langer, G.J.; Bußkamp, J. Fungi Associated with Woody Tissues of European Beech and Their Impact on Tree Health. *Front. Microbiol.* **2021**, *12*, 702467. [CrossRef] [PubMed]
51. Endes, A.; Kayim, M. Morphological and Molecular Characterization of *Botryosphaeriaceae* Species Associated with Dieback And Gummosis On Plum Trees In Turkey. *Comptes Rendus L Acad. Bulg. Sci.* **2022**, *75*, 295–302. [CrossRef]
52. Novak, A.; Ivić, D.; Sever, Z.; Fazinić, T.; Šimunac, K. Gljivični rak oraha u Hrvatskoj. *Glas. Biljn. Zaštite* **2018**, *3*, 316–321.
53. Kovač, M.; Diminić, D.; Orlović, S.; Zlatković, M. *Botryosphaeria Dothidea* and *Neofusicoccum Yunnanense* Causing Canker and Die-Back of Sequoiadendron Giganteum in Croatia. *Forests* **2021**, *12*, 695. [CrossRef]
54. Marcianó, D.; Mizzotti, C.; Maddalena, G.; Toffolatti, S. The dark side of fungi: How they cause diseases in plants. *Front. Young Minds* **2021**, *9*, 560315. [CrossRef]
55. Gramaje, D.; Úrbez-Torres, J.R.; Sosnowski, M.R. Managing Grapevine Trunk Diseases with Respect to Etiology and Epidemiology: Current Strategies and Future Prospects. *Plant Dis.* **2018**, *102*, 12–39. [CrossRef] [PubMed]
56. Pitt, W.M.; Sosnowski, M.R.; Huang, R.; Qiu, Y.; Steel, C.C.; Savocchia, S. Evaluation of Fungicides for the Management of Botryosphaeria Canker of Grapevines. *Plant Dis.* **2012**, *96*, 1303–1308. [CrossRef] [PubMed]
57. Díaz, G.; Latorre, B. Efficacy of paste and liquid fungicide formulations to protect pruning wounds against pathogens associated with grapevine trunk diseases in Chile. *Crop Prot.* **2013**, *46*, 106–112. [CrossRef]
58. Amponsah, N.T.; Jones, E.; Ridgway, H.J.; Jaspers, M.V. Evaluation of fungicides for the management of *Botryosphaeria* dieback diseases of grapevines. *Pest Manag. Sci.* **2012**, *68*, 676–683. [CrossRef]
59. Sánchez, M.E.; Venegas, J.; Romero, M.A.; Phillips, A.J.L.; Trapero, A. *Botryosphaeria* and related taxa causing oak canker in southwestern Spain. *Plant Dis.* **2003**, *87*, 1515–1521. [CrossRef]
60. White, T.J.; Bruns, T.D.; Lee, S.B.; Taylor, J.W. 38—Amplification and direct sequencing of fungal ribosomal RNA genes forphylogenetics. In *PCR—Protocols and Applications—A Laboratory Manual*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press Inc.: Cambridge, MA, USA, 1990; pp. 315–322.
61. Glass, N.L.; Donaldson, G.C. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl. Environ. Microbiol.* **1995**, *61*, 1323–1330. [CrossRef]
62. Carbone, I.; Kohn, L.M. A Method for Designing Primer Sets for Speciation Studies in Filamentous Ascomycetes. *Mycologia* **1995**, *91*, 553–556. [CrossRef]
63. Slippers, B.; Crous, P.W.; Denman, S.; Coutinho, T.A.; Wingfield, B.D.; Wingfield, M.J. Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. *Mycologia* **2004**, *96*, 83–101. [CrossRef] [PubMed]
64. Saitou, N.; Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **1987**, *4*, 406–425. [PubMed]
65. Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **1985**, *39*, 783–791. [CrossRef]

66. Tamura, K.; Nei, M.; Kumar, S. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11030–11035. [CrossRef]
67. Tamura, K.; Stecher, G.; Kumar, S. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [CrossRef] [PubMed]
68. Henriques, J.; Nóbrega, F.; Sousa, E.; Lima, A. Analysis of the genetic diversity and phylogenetic relationships of *Biscogniauxia mediterranea* isolates associated with cork oak. *Phytoparasitica* **2016**, *44*, 19–34. [CrossRef]
69. Phillips, A.J.L.; Oudemans, P.V.; Correia, A.; Alves, A. Characterisation and epitypification of *Botryosphaeria corticis*, the cause of blueberry cane canker. *Fungal Divers.* **2006**, *2*, 141–155.
70. Alves, A.; Correia, A.; Luque, J.; Phillips, A. *Botryosphaeria corticola*, sp. nov. on *Quercus* species, with notes and description of *Botryosphaeria stevensii* and its anamorph, Diplodia mutila. *Mycologia* **2004**, *96*, 598–613. [PubMed]
71. Phillips, A.; Alves, A.; Correia, A.; Luque, J. Two new species of *Botryosphaeria* with brown, 1-septate ascospores and *Dothiorella* anamorphs. *Mycologia* **2005**, *97*, 513–529. [CrossRef] [PubMed]
72. Phillips, A.J.L.; Alves, A.; Pennycook, S.R.; Johnston, P.R.; Ramaley, A.; Akulov, A.; Crous, P.W. Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the *Botryosphaeriaceae*. *Persoonia* **2008**, *21*, 29–55. [CrossRef] [PubMed]
73. Li, G.Q.; Liu, F.F.; Li, J.Q.; Liu, Q.L.; Chen, S.F. *Botryosphaeriaceae* from *Eucalyptus* plantations and adjacent plants in China. *Persoonia* **2018**, *40*, 63–95. [CrossRef] [PubMed]
74. Zhuang, C.J.; Wang, Q.W.; Wu, Q.Q.; Qiu, Z.L.; Xu, B.C.; Zhang, C.Q. Diversity of *Botryosphaeriaceae* Species Associated with Chinese Hickory Tree (*Carya cathayensis*) Trunk Cankers. *Plant Dis.* **2021**, *105*, 3869–3879. [CrossRef] [PubMed]
75. Elfar, K.; Carachure, C.; Bustamante, M.I.; Andrews, E.; Eskalen, A. First report of *Diplodia bulgarica* causing black canker on apple in California. *Plant Dis.* **2024**, *108*, 531. [CrossRef]
76. Phillips, A.J.L.; Lopes, J.; Abollahzadeh, J.; Bobev, S.; Alves, A. Resolving the *Diplodia* complex on apple and other *Rosaceae* hosts. *Persoonia* **2012**, *29*, 29–38. [CrossRef]
77. Vu, D.; Groenewald, M.; de Vries, M.; Gehrmann, T.; Stielow, B.; Eberhardt, U.; Al-Hatmi, A.; Groenewald, J.Z.; Cardinali, G.; Houbraken, J.; et al. Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. *Stud. Mycol.* **2019**, *92*, 135–154. [CrossRef]
78. Zhang, W.; Groenewald, J.Z.; Lombard, L.; Schumacher, R.K.; Phillips, A.J.L.; Crous, P.W. Evaluating species in *Botryosphaeriales*. *Persoonia* **2021**, *46*, 63–115. [CrossRef]
79. Úrbez-Torres, J.R.; Peduto, F.; Rooney-Latham, S.; Gubler, W.D. First Report of *Diplodia corticola* Causing Grapevine (*Vitis vinifera*) Cankers and Trunk Cankers and Dieback of Canyon Live Oak (*Quercus chrysolepis*) in California. *Plant Dis.* **2010**, *94*, 785. [CrossRef]
80. Alves, A.; Correia, A.; Phillips, A.J.L. Multi-gene genealogies and morphological data support *Diplodia cupressi* sp. nov., previously recognized as *D. pinea* f. sp. *cupressi*, as a distinct species. *Fungal Divers.* **2006**, *23*, 1–15.
81. Zhao, P.; Crous, P.W.; Hou, L.W.; Duan, W.J.; Cai, L.; Ma, Z.Y.; Liu, F. Fungi of quarantine concern for China I: *Dothideomycetes*. *Persoonia* **2021**, *47*, 45–105. [CrossRef] [PubMed]
82. Inderbitzin, P.; Bostock, R.M.; Trouillas, F.P.; Michailides, T.J. A six locus phylogeny reveals high species diversity in *Botryosphaeriaceae* from California almond. *Mycologia* **2010**, *102*, 1350–1368. [CrossRef] [PubMed]
83. Jami, F.; Slippers, B.; Wingfield, M.; Gryzenhout, M. Five new species of *Botryosphaeriaceae* from Acacia Karroo in South Africa. *Cryptogam. Mycol.* **2012**, *33*, 245–266. [CrossRef]
84. Schoch, C.L.; Robbertse, B.; Robert, V.; Vu, D.; Cardinali, G.; Irinyi, L.; Meyer, W.; Nilsson, R.H.; Hughes, K.; Miller, A.N.; et al. Finding needles in haystacks: Linking scientific names, reference specimens and molecular data for Fungi. *Database (Oxford)* **2014**, *2014*, bau061. [CrossRef] [PubMed]
85. Doll, D.A.; Rolshausen, P.E.; Pouzoulet, J.; Michailides, T.J. First Report of *Dothiorella iberica* Causing Trunk and Scaffold Cankers of Almond in California. *Plant Dis.* **2015**, *99*, 1185. [CrossRef]
86. der Walt, F.J.J. Botryosphaeriaceae Associated with Acacia Species in Southern Africa with Special Reference to *A. mellifera*. Magister Scientae, University of Pretoria, Faculty of Natural and Agricultural Sciences, Pretoria, South Africa, 2008.
87. Yang, T.; Groenewald, J.Z.; Cheewangkoon, R.; Jami, F.; Abdollahzadeh, J.; Lombard, L.; Crous, P.W. Families, genera, and species of *Botryosphaeriales*. *Fungal Biol.* **2017**, *121*, 322–346. [CrossRef] [PubMed]
88. Slippers, B.; Boissin, E.; Phillips, A.J.L.; Groenewald, J.Z.; Lombard, L.; Wingfield, M.J.; Postma, A.; Burgess, T.; Crous, P.W. Phylogenetic lineages in the *Botryosphaeriales*: A systematic and evolutionary framework. *Stud. Mycol.* **2013**, *76*, 31–49. [CrossRef] [PubMed]
89. Zlatković, M.; Keča, N.; Wingfield, M.J.; Jami, F.; Slippers, B. *Botryosphaeriaceae* associated with the die-back of ornamental trees in the Western Balkans. *Antonie Leeuwenhoek* **2016**, *109*, 543–564.
90. Úrbez-Torres, J.R.; Peduto, F.; Gubler, W. First Report of Grapevine Cankers Caused by *Lasiodiplodia crassispora* and *Neofusicoccum mediterraneum* in California. *Plant Dis.* **2010**, *94*, 785. [CrossRef]
91. Burgess, T.I.; Barber, P.A.; Mohali, S.; Pegg, G.; de Beer, W.; Wingfield, M.J. Three new *Lasiodiplodia* spp. from the tropics, recognized based on DNA sequence comparisons and morphology. *Mycologia* **2006**, *98*, 423–435. [CrossRef] [PubMed]
92. Cruywagen, E.M.; Slippers, B.; Roux, J.; Wingfield, M.J. Phylogenetic species recognition and hybridisation in *Lasiodiplodia*: A case study on species from baobabs. *Fungal Biol.* **2017**, *121*, 420–436. [CrossRef] [PubMed]

93. Osorio, J.A.; Crous, C.J.; de Beer, Z.W.; Wingfield, M.J.; Roux, J. Endophytic *Botryosphaeriaceae*, including five new species, associated with mangrove trees in South Africa. *Fungal Biol.* **2017**, *121*, 361–393. [CrossRef] [PubMed]
94. Ko, Y.Z.; Liyanage, W.K.; Shih, H.C.; Tseng, M.N.; Shiao, M.S.; Chiang, Y.C. Unveiling Cryptic Species Diversity and Genetic Variation of *Lasiodiplodia* (Botryosphaeriaceae, Botryosphaeriales) Infecting Fruit Crops in Taiwan. *J. Fungi* **2023**, *9*, 950. [CrossRef] [PubMed]
95. Alves, A.; Crous, P.W.; Correia, A.C.M.; Phillips, A.J.L. Morphological and molecular data reveal cryptic species in *Lasiodiplodia theobromae*. *Fungal Divers.* **2008**, *28*, 1–13.
96. de Silva, N.I.; Phillips, A.J.L.; Liu, J.K.; Lumyong, S.; Hyde, K.D. Phylogeny and morphology of *Lasiodiplodia* species associated with Magnolia forest plants. *Sci. Rep.* **2019**, *9*, 14355. [CrossRef] [PubMed]
97. Slippers, B.; Fourie, G.; Crous, P.W.; Coutinho, T.A.; Wingfield, B.D.; Wingfield, M.J. Multiple gene sequences delimit *Botryosphaeria australis* sp. nov. from *B. lutea*. *Mycologia* **2004**, *96*, 1030–1041. [CrossRef] [PubMed]
98. Marques, M.W.; Lima, N.B.; de Morais, M.A., Jr.; Michereff, S.J.; Phillips, A.J.L.; Câmara, M.-P.S. *Botryosphaeria*, *Neofusicoccum*, *Neoscytalidium* and *Pseudofusicoccum* species associated with mango in Brazil. *Fungal Divers.* **2013**, *61*, 195–208. [CrossRef]
99. Slippers, B.; Johnson, G.I.; Crous, P.W.; Coutinho, T.A.; Wingfield, B.D.; Wingfield, M.J. Phylogenetic and morphological re-evaluation of the *Botryosphaeria* species causing diseases of *Mangifera indica*. *Mycologia* **2005**, *97*, 99–110. [CrossRef]

Naslov izvornog znanstvenog rada broj 5: Diversity and Pathogenicity of Botryosphaeriaceae Species Isolated from Olives in Istria, Croatia, and Evaluation of Varietal Resistance

Prošireni sažetak:

U 2021. i 2022. godini u maslinicima u Istri, Hrvatska, primijećeni su simptomi odumiranja grana, venuća i otpadanja lišća, truleži plodova te pucanja kore na stablima masline (*Olea europaea* L.). Kako bi se identificirali uzročnici ovih simptoma, provedena je izolacija patogena iz simptomatskih dijelova biljaka. Izolirane gljive preliminarno su, na temelju morfoloških karakteristika, klasificirane kao pripadnici porodice *Botryosphaeriaceae*. Daljnja molekularna analiza (sekvenciranje ITS, TUB2 i TEF1-α regija genoma) potvrdila je prisutnost šest vrsta: *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & De Not, *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, *Diplodia mutila* (Fr.) Fr., *Diplodia seriata* De Notaris, *Dothiorella iberica* A.J.L. Phillips, J. Luque & A. Alves i *Dothiorella sarmentorum* (Fr.) A.J.L. Phillips, Alves & Luque. Filogenetska analiza dodatno je poduprla ovu identifikaciju. Među izoliranim vrstama, *N. parvum* i *D. seriata* pokazale su najveću učestalost na zaraženim biljkama. Patogenost izoliranih vrsta ispitana je inokulacijom zdravih biljaka masline u plasteničkim uvjetima. Zaražene biljke razvile su simptome slične onima viđenima na terenu, uključujući nekroze tkiva, odumiranje izbojaka i pucanje kore. Rezultati su pokazali da su *N. parvum* i *D. mutila* izazvale najizraženije simptome s prosječnom dužinom lezija većom od 30 mm, dok su vrste iz roda *Dothiorella* pokazale slabiju patogenost s manjim lezijama.

Osim toga, ispitana je otpornost različitih sorti masline na izolirane patogene. Sorte su pokazale različite stupnjeve otpornosti na gljive. Ovi rezultati ukazuju na mogućnost selekcije otpornijih sorti u budućim uzgojnim programima i sugeriraju potrebu za sortno specifičnim strategijama zaštite.

Prema saznanjima, vrste *D. mutila*, *Do. iberica* i *Do. sarmentorum* do sada nisu bile zabilježene na stablima maslina u Hrvatskoj, što ovo čini prvim izvješćem o njihovoj prisutnosti. Otkriće naglašava važnost kontinuiranog praćenja novih patogena u maslinarstvu te potrebu za razvojem učinkovitih mjera kontrole kako bi se spriječile potencijalne štete u proizvodnji. Također, rezultati ukazuju na ključnu ulogu izbora sorti u smanjenju pojave gljivičnih bolesti maslina.

Ključne riječi: Botriosferijsko sušenje; *Botryosphaeria dothidea*; *Diplodia* spp.; *Dothiorella* spp.; prvi izvještaj; *Neofusicoccum* sp.

Izvorni znanstveni rad broj 6 u obliku i izvornom jeziku na kojem je objavljen u znanstvenom časopisu

Naslova rada: Antifungal Efficacy of Essential Oils and Their Predominant Components Against Olive Fungal Pathogens

Autori: Elena Petrović, Karolina Vrandečić, Jasenka Ćosić, Tamara Siber, Sara Godena

Tip rada: Izvorni znanstveni članak

Časopis: Agriculture

Kategorija: A1

Impakt faktor: 3.3 (2025.)

Kvartil: Q1

Primljen na recenziju: 17. listopad 2024.

Prihvaćen za objavljivanje: 18. studeni 2024.

Status: Objavljen

Volumen: 15

Broj: 3

Broj rada: 340

WOS broj: 001418418200001

agriculture

MDPI

Article

Antifungal Efficacy of Essential Oils and Their Predominant Components Against Olive Fungal Pathogens

Elena Petrović [1], Karolina Vrandečić [2], Jasenka Ćosić [2], Tamara Siber [2] and Sara Godena [1,*]

[1] Institute of Agriculture and Tourism, Karla Huguesa 8, 52440 Poreč, Croatia; elena@iptpo.hr
[2] Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Vladimira Preloga 1, 31000 Osijek, Croatia; kvrandecic@fazos.hr (K.V.); jcosic@fazos.hr (J.Ć.); tsiber@fazos.hr (T.S.)
* Correspondence: sara@iptpo.hr

Abstract: The antifungal effectiveness of essential oils (EOs) and their predominant components were tested on 14 phytopathogenic fungi isolated from olive trees. Commercial EOs from holy basil (*Ocimum tenuiflorum* L.), Chinese cinnamon (*Cinnamomum aromaticum* Ness), lemon (*Citrus* × *limon*), peppermint (*Mentha* × *piperita* L.), oregano (*Origanum compactum* Benth), and thyme (*Thymus vulgaris* L.) and components eugenol, e-cinnamaldehyde, limonene, menthol, carvacrol, and thymol were used. Antifungal efficacy was tested on six species from the *Botryosphaeriaceae* family: *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & De Not.; *Diplodia mutila* (Fr.) Fr.; *D. seriata* De Not.; *Dothiorella iberica* A.J.L. Phillips, J. Luque & A. Alves; *Do. sarmentorum* (Fr.) A.J.L. Phillips, Alves & Luque; and *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips. Other tested species included *Biscogniauxia mediterranea* (De Not.) Kuntze, *B. nummularia* (Bull.) Kuntze; *Cytospora pruinosa* Défago; *Nigrospora gorlenkoana* Novobr.; *N. osmanthi* Mei Wang & L. Cai; *N. philosophiae-doctoris* M. Raza, Qian Chen & L. Cai; *Phaeoacremonium iranianum* L. Mostert, Grafenhan, W. Gams & Crous; and *Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not. The results show that Chinese cinnamon and oregano EOs, along with their components, completely inhibited the growth of all tested fungi, indicating their potential as biological control agents in sustainable agriculture. In contrast, the least effective treatments were the EOs derived from lemon and peppermint, as well as the components limonene, menthol, and thymol. Notably, the fungi *Do. iberica* and *N. gorlenkoana* were among the most sensitive to all the treatments applied.

Keywords: carvacrol; Chinese cinnamon; e-cinnamaldehyde; metiram + pyraclostrobin; oregano; trifloxystrobin + tebuconazole

Academic Editor: Francesca Laudani

Received: 17 October 2024
Revised: 15 November 2024
Accepted: 18 November 2024
Published: 4 February 2025

Citation: Petrović, E.; Vrandečić, K.; Ćosić, J.; Siber, T.; Godena, S. Antifungal Efficacy of Essential Oils and Their Predominant Components Against Olive Fungal Pathogens. *Agriculture* **2025**, *15*, 340. https://doi.org/10.3390/agriculture15030340

1. Introduction

Olive cultivation likely dates back further than that of any other tree species [1]. Traditionally, olive groves in the Mediterranean have been associated with high biodiversity, serving as an example of a high-natural-value agricultural system with an important environmental role [1]. According to the latest data, in 2022, global olive production amounted to approximately 21.4 million t grown over 10.9 million ha [2]. In the European Union (EU), olive farming is transitioning from traditional low-density methods toward modern high-density systems, including super-intensive plantations. This shift aims to enhance yields while reducing harvesting costs. However, these changes have significantly impacted the prevalence and severity of plant diseases [1]. Moreover, as outlined in our previous research [3], the increasing incidence of plant diseases is also directly linked to climate change [4]. In the Mediterranean, due to its unique climate and high biodiversity, a

growing number of new pathogenic species have been reported. Kim et al. [5] identified the Mediterranean as one of the most vulnerable regions in the world in the context of climate change, suggesting that the number of reports of plant pathogens will continue to rise in the future. According to the literature, common causes of olive diseases include species from the family *Botryosphaeriaceae* [6,7], *Colletotrichum* spp. [8,9], *Diatrype* spp. [6], *Venturia oleaginea* (Castagne) Rossman & Crous [10], *Verticillium dahliae* Klebahn [11], and others. Additionally, reports of new olive pathogens continue to grow [3,12–16].

Fungicides are commonly employed to protect olive crops from fungal diseases. However, concerns have been raised about their environmental impact, risks to non-target organisms, potential human health hazards, and effects on product quality [1]. The overuse and uncontrolled application of fungicides have led to the emergence of more virulent strains, which are resistant to previously effective treatments [17]. Fungicides contain various harmful substances that can negatively affect human and animal health as well as biodiversity [18]. The EU aims to reduce dependence on pesticides in olive farming by promoting agricultural strategies with a low environmental impact and by developing new management systems [1]. In response to the issues related to pesticide use, the EU has adopted two key strategic documents: the "European Green Deal", which includes strategies for achieving sustainability in the EU economy, and the "Biodiversity Strategy", which aims to reduce pesticide use by 50% by 2030.

In light of the above, there is a growing demand for alternative plant protection methods, including the use of biological agents. As an alternative to chemical plant protection agents, various plant compounds and extracts with antifungal properties against various phytopathogenic fungi can be used [19]. Plants produce phytochemicals with antimicrobial properties, serving as defense mechanisms against harmful microorganisms. These substances, known as botanical pesticides or botanicals, can be toxic to fungal pathogens when applied to infected crops [20].

Essential oils (EOs) have become increasingly studied due to their proven efficacy against specific fungal species. These natural compounds, which are secondary metabolites of plants, possess strong antimicrobial properties and have applications in various industries, such as textiles, cosmetics, and food [21–24]. EOs have been recognized and valued since ancient times [25]. They serve as natural defenses for plants against predators and appear as volatile, liquid mixtures of various aromatic compounds [26]. The International Organization for Standardization (ISO) defines an EO as a product obtained through water or steam distillation, mechanical processing, or dry distillation of natural materials [21]. The extraction of EOs from herbs dates back to ancient Persian and Egyptian alchemists, who boiled aromatic herbs in a sealed flask, allowed them to cool, and collected the thin layer of EO floating on the surface of the water [27]. Modern extraction processes achieve similar results through distillation in which steam is passed over the herbs rather than boiling them directly in water [27]. For example, citrus EOs, typically extracted by crushing the peel between rollers in a process known as expression or cold pressing, can also be obtained through steam distillation, as in the case of lemon EO, which is derived from the peel and does not contain vitamin C or citric acid; peppermint EO, extracted from the aerial parts, primarily the leaves, of the hybrid *Mentha aquatica* L. and *Mentha spicata* L., is harvested before flowering to avoid the incorporation of odor-producing molecules, and thyme EO is obtained by steam distillation of the plant's leaves [27]. EOs differ from vegetable oils in their chemical structure, which gives them distinct aromas. They are highly flammable and evaporate quickly at temperatures between 40 and 80 °C [27]. Approximately 3000 different EOs are known [21].

EOs consist of a flexible combination of components, with the percentage varying depending on the year, batch, and other factors. For instance, EOs derived from the same

botanical species, even when cloned from identical plants, produce different chemical compositions when grown in varying climatic conditions or geographic regions [27]. EOs can contain over 60 components, with two or three major components present in high concentrations [22]. The primary components of EOs are often terpenes and terpenoids, with nitrogen and sulfur components, coumarins, and phenylpropanoid homologs appearing less frequently [28,29]. Generally, up to three main components account for 90% of the EO, while others are present in less than 1% [30]. Burt [31] noted that trace components play a key role in the antimicrobial properties of EOs, potentially due to their synergistic interactions with other components. The chemical composition of EOs is typically analyzed using gas chromatography and a combination of gas chromatography and mass spectrometry [32].

Ćosić et al. [33] and Karimi et al. [34] reported that the inhibitory potential of EOs against microorganisms depends on the type of EO, chemical composition, pathogen type, host plant species, applied concentrations or volumes, and agricultural practices. Some hypotheses suggest that the antimicrobial activity of EOs is attributed to their lipophilicity, free hydroxyl groups, and unsaturated cyclic structures [22].

The aim of this study was to explore the antifungal potential of commercial EOs and their predominant components against 14 pathogenic fungal species affecting olive trees, previously identified in our research, in pursuit of a sustainable and environmentally friendly alternative to conventional chemical fungicides. This research further pursued to evaluate their fungistatic and fungicidal properties, and to determine their minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs).

2. Materials and Methods

2.1. Phytopathogenic Fungi

To assess the impact of EOs and their predominant components, representative isolates of phytopathogenic fungi obtained from olive trees were utilized. A list of the utilized species is provided in Table 1. Isolates of pure cultures were inoculated onto potato dextrose agar (PDA) and incubated at 25 °C for 7 days, in the dark, for species from the *Botryosphaeriaceae* family, *Biscogniauxia* spp., *Cytospora* sp., and *Sordaria* sp., and for 10 days for *Phaeoacremonium* sp. Additionally, *Nigrospora* spp. was incubated at 28 °C for 7 days in the dark.

Table 1. List of fungal species used, isolated from olives in Croatia, and their isolate names, with references.

Class *Dothideomycetes*		
Species	**Isolate**	**References**
Botryosphaeria dothidea (Moug. ex Fr.) Ces. & De Not.	R19 F	[7]
Diplodia mutila (Fr.) Fr.	IKB9 B2II	[7]
Diplodia seriata De Not.	V16 K2II	[7]
Dothiorella iberica A.J.L. Phillips, J. Luque & A. Alves	V16 BI	[7]
Dothiorella sarmentorum (Fr.) A.J.L. Phillips, Alves & Luque	V12 PEN	[7]
Neofusicoccum parvum (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips	V21 B5I	[7]
Class *Sordariomycetes*		
Biscogniauxia mediterranea (De Not.) Kuntze	R18 LEC1	[3]
Biscogniauxia nummularia (Bull.) Kuntze	V16 B3	[3]
Cytospora pruinosa Défago	SL2 PRIV	[16]

Table 1. *Cont.*

Class *Sordariomycetes*		
Species	**Isolate**	**References**
Nigrospora gorlenkoana Novobr.	P13 LECIII	[35]
Nigrospora osmanthi Mei Wang & L. Cai	JA20 NP	[35]
Nigrospora philosophiae-doctoris M. Raza, Qian Chen & L. Cai	R18 BI	[35]
Phaeoacremonium iranianum L. Mostert, Grafenhan, W. Gams & Crous	R18 B4	[14]
Sordaria fimicola (Roberge ex Desm.) Ces. & De Not.	ISN9 PEN	[3]

2.2. Essential Oils and Components

To test antifungal activity, six commercial EOs were used, along with the predominant component of each EO. The EOs were derived from *Cinnamomum aromaticum* Nees, *Citrus* × *limon*, *Mentha* × *piperita* L., *Ocimum tenuiflorum* L., *Origanum compactum* Benth, and *Thymus vulgaris* L. The full names of the plants from which the EOs were derived, their Latin names and synonyms, as well as their predominant components present in concentrations greater than 0.1% are listed in Table S1, along with the names of the fungicides used and their active substances. EOs, except *C.* × *limon* EO, were sourced from Pranarōm International Ltd. (Ath, Belgium), while *C.* × *limon* EO was obtained from Fagron Hrvatska d.o.o. (Donja Zelina, Croatia). All EOs were analyzed using gas chromatography–mass spectrometry (GC-MS) in the manufacturer's laboratory. The components carvacrol, eugenol, and menthol were sourced from Thermo Fisher Scientific Inc. (Fair Lawn, NJ, USA), e-cinnamaldehyde and limonene from Thermo Fisher GmbH (Kandel, Germany), and thymol from VWR International (Leuven, Belgium).

The EOs were tested at five concentrations, namely 10 µL/10 mL, 20 µL/10 mL, 50 µL/10 mL, 75 µL/10 mL, and 100 µL/10 mL, corresponding to volume ratios of 0.1%, 0.2%, 0.5%, 0.75%, and 1.0%, respectively, in the substrate. The tested concentration of the predominant component was calculated based on its percentage within the EO for each tested concentration of the EO [36,37]. Pure PDA was used as a positive control. As a negative control, two commercial fungicides commonly applied for controlling phytopathogenic fungi in olive cultivation were used: Nativo 75WG (Bayer d.o.o., Zagreb, Croatia) and Cabrio TOP (BASF Croatia, Zagreb, Croatia). The fungicides were diluted to the working concentration recommended for treating olive trees according to the manufacturer's instructions. The concentration for Nativo 75WG was 20 g/100 L, and for Cabrio TOP, it was 200 g/100 L. Instead of water, the same amount of potato dextrose agar (PDA) was used according to Palfi [37].

2.3. Procedure

EOs and their components were used to evaluate the contact phase effect on the mycelial growth of fungal isolates. PDA was prepared following the manufacturer's instructions, and the temperature of the entire substrate was monitored until it cooled to approximately 50 °C. Once cooled, 10 mL of the PDA was poured into a sterile Falcon tube. The appropriate amount of EO/component/fungicide was then pipetted into the tube, and the mixture was stirred with a glass rod and gently vortexed until a homogeneous solution was obtained. The solution was poured into sterile 90 mm Petri dishes. After the substrate solidified, a 4 mm diameter plug of the actively growing fungal culture was cut using a cork borer and inoculated onto the center of the substrate with a sterile laboratory needle, ensuring that the top side of the plug was facing down on the medium. The Petri dishes were sealed with parafilm and incubated under the appro-

priate conditions. The entire procedure was performed in a laminar flow cabinet (Nüve LN 090, Ankara, Turkey). For *Botryosphaeriaceae* spp., *Biscogniauxia* spp., *Cytospora* sp., *Phaeoacremonium* sp., and *Sordaria* sp., the incubation temperature was set to 25 °C, while for *Nigrospora* spp., it was set to 28 °C. The experiment was conducted in triplicate for each treatment (EOs/components/fungicides) and concentration. Fungal mycelial growth was measured after 2, 4, and 10 days of inoculation. The diameter of the mycelium plug was subtracted from the displayed values, so the maximum growth for all species except *P. iranianum* is 86 mm, and for *P. iranianum*, it is 26.7 mm.

For isolates where no fungal growth was observed at the final measurement, half of the mycelium plug was transferred with a sterile laboratory needle onto a new sterile Petri dish containing pure PDA. The samples were incubated under the same conditions used during inoculation with treatments. If fungal growth resumed after incubation, the treatment was classified as fungistatic, while if no mycelial growth occurred, the treatment was considered fungicidal. The lowest concentration of EO or component that resulted in the complete inhibition of mycelial growth was recorded as the MIC, and the lowest concentration that exhibited fungicidal activity was recorded as the MFC.

2.4. Statistical Data Analysis

To determine whether there were statistically significant differences in the treatments applied and to assess interactions between all groups, a two-way analysis of variance (ANOVA) was first conducted, taking into account all measurement days, treatments, and concentrations using Python 3.8.10 (Python Software Foundation, Wilmington, DE, USA). Subsequently, a one-way ANOVA and Tukey's multiple comparison tests were performed using the SAS Enterprise Guide 8.4 (SAS Institute, Cary, NC, USA) to assess the antifungal efficacy of treatments. Data were expressed as arithmetic means, standard deviations, and 95% confidence intervals for the mean. In specific cases where the one-way ANOVA results showed no significant differences between treatments, but visual differences were observed, additional *T*-tests were conducted using the SAS Enterprise Guide 8.4 (SAS Institute, Cary, NC, USA) to compare specific pairs. The inhibitory effect of EOs and components on the mycelial growth of phytopathogenic fungi was calculated using Microsoft Office Excel based on the formula by Wu et al. [38]:

$$I\,(\%) = \frac{\mathrm{C} - \mathrm{T}}{\mathrm{C} - 0.4} * 100$$

where *I* (%) is the percentage of mycelial growth inhibition by the tested compounds, C is the diameter of fungal growth on control, and T is the diameter of fungal growth on treated PDA.

3. Results

The results of the two-way ANOVA, conducted for each fungus while considering all factors—namely applied treatments, measurement days, and concentrations—revealed that the combination of all three factors significantly affected mycelial growth. When examining the interaction between the treatments (EO/component/fungicide) and concentration, a highly significant interaction was found, indicating that the effect of concentration on mycelial growth depends on the type of treatments. Similarly, when analyzing the interaction between treatments and measurement days, a highly significant interaction was observed, suggesting that the effects of the treatments change over time. Furthermore, when assessing the interaction between applied concentrations and measurement days, a significant interaction was detected, indicating that the effect of concentration depends on the day of measurement.

Additionally, when considering all three factors—treatments, applied concentrations, and measurement days—a highly significant three-way interaction was identified. This implies that the optimal treatment efficacy depends on the specific combination of EO/component/fungicide type and concentration, and the effectiveness of individual treatments decreases over time.

The following section presents the results of the statistical analysis sorted by fungal species. The results of the one-way ANOVA and the inhibition percentages of mycelial growth are shown in tables in the Supplementary File (Tables S2–S29). The average mycelial growth values on the 10th day are displayed graphically using Microsoft Office Excel. The EC50 value could not be calculated due to a lack of variability in the data.

3.1. Dothideomycetes

3.1.1. *Botryosphaeria dothidea*

Based on the results of the one-way ANOVA (Table S2), Chinese cinnamon, oregano, and thyme EOs, as well as components carvacrol and e-cinnamaldehyde, were the most effective in inhibiting mycelial growth. At a concentration of 0.1%, they completely inhibited mycelial growth (Table S3). These treatments demonstrated greater efficacy than the commercial fungicides tested (Figure 1). The fungicide Cabrio TOP inhibited growth by 71.7% after two days, by 22.9% after four days, and by 9.7% after 10 days. In comparison, Nativo 75WG was more effective in suppressing mycelial growth, with 100% inhibition on the second and fourth days of measurement and 95.7% inhibition on the 10th day.

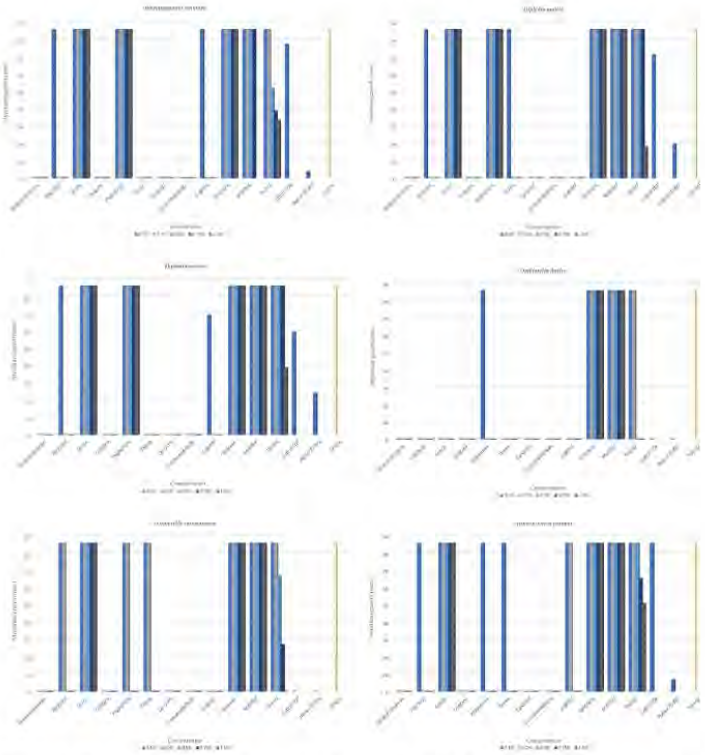


Figure 1. The average mycelial growth values on the 10th day of measurement are shown by concentration for all essential oils/components/fungicides used and the control for the species *Botryosphaeria dothidea*, *Diplodia mutila*, *D. seriata*, *Dothiorella iberica*, *Do. sarmentorum*, and *Neofusicoccum parvum*.

Thymol demonstrated a slightly weaker effect on mycelial growth inhibition compared to the previously mentioned EOs and components. At a concentration of 0.1%, thymol achieved 68.6% inhibition on the second day; however, by the fourth day, the mycelium had completely colonized the Petri dish. At a concentration of 0.2%, the mycelium fully covered the dish by the 10th day. At concentrations of 0.75% and 1.0%, no mycelial growth was observed by the fourth day, but by the 10th day, growth had resumed with inhibition rates of 54.7% and 60.9%, respectively.

Holy basil EO showed limited effectiveness at 0.1%, but at 0.2%, it completely inhibited growth. When treated with the main component of holy basil EO, eugenol, the mycelium exhibited slower growth compared to the control at a concentration of 0.1%. However, at 0.2%, mycelial growth was entirely inhibited.

Peppermint EO began inhibiting mycelial growth at a concentration of 0.2%. As the concentration increased, inhibition also increased. Although this EO significantly slowed fungal growth compared to the control, the mycelium still completely filled the Petri dish by the 10th day at a concentration of 1.0%. Lemon EO, along with components menthol and limonene, proved to be ineffective. Even at a concentration of 1.0%, these treatments had no impact on mycelial growth throughout the entire measurement period.

3.1.2. *Diplodia mutila*

Based on the results of the one-way ANOVA (Table S4), the EOs of Chinese cinnamon and oregano and the components carvacrol, e-cinnamaldehyde, and eugenol proved to be highly effective in inhibiting mycelial growth, outperforming the fungicides (Figure 1). At a concentration of 0.1%, they inhibited mycelial growth by 100% across all measurement days, whereas the fungicides Cabrio TOP and Nativo 75 WG inhibited growth on the second day by 81.2% and 100%, on the fourth day by 57.8% and 90.3%, and on the 10th day by 17.1% and 76.7% (Table S5).

The EOs of holy basil and thyme showed slightly lower efficacy. Holy basil inhibited mycelial growth by 73.6% on the second day at a concentration of 0.1%, but by the fourth day, the mycelium had filled the Petri dish. Thyme was somewhat more effective, inhibiting growth by 100% on the second day and 73.3% on the fourth day, but by the 10th day, the mycelium had filled the Petri dish. Both EOs inhibited 100% mycelial growth at a concentration of 0.2% across all measurement days. The component thymol showed an inhibitory effect on mycelial growth at the lowest concentration compared to the control. At the highest concentration, it inhibited mycelial growth by 100% on the second and fourth days and by 78.7% on the 10th day.

The EOs of peppermint and lemon had a weaker effect on mycelial growth inhibition. Peppermint showed efficacy at a concentration of 0.1%, while lemon showed efficacy at a concentration of 0.2% and higher. For both EOs, the inhibitory effect decreased over time, with the most pronounced effect on the second day. As the concentration increased, the inhibition percentage increased, but this effect was not lasting and declined by the 10th day. Comparing these two EOs showed that peppermint was more effective, inhibiting growth at a concentration of 1.0% by 89.5% and 48.8% on the second and fourth days, respectively, while by the 10th day, the mycelium had filled the Petri dish. Their main components, menthol and limonene, were less effective than the EOs. Menthol suppressed growth at concentrations of 0.75% and 1.0% only on the second day, while limonene was ineffective even at the highest concentration.

3.1.3. *Diplodia seriata*

Based on the results of the one-way ANOVA (Table S6), the EOs of Chinese cinnamon, oregano, and thyme, as well as the components carvacrol and e-cinnamaldehyde, proved to

be the most effective in inhibiting mycelial growth, outperforming the fungicides (Figure 1). At a concentration of 0.1%, they inhibited mycelial growth by 100% across all measurement days. Holy basil EO and its component eugenol also showed high efficacy, inhibiting mycelial growth by 100% at a concentration of 0.2% (Table S7). Cabrio TOP and Nativo 75 WG did not completely suppress fungal mycelial growth at the applied concentrations. Comparing these two fungicides, Nativo 75WG demonstrated greater effectiveness, inhibiting mycelial growth by 71.7% on the 10th day, while Cabrio TOP achieved an inhibition of 30.6%. The component thymol also exhibited a strong inhibitory effect on mycelial growth with an increasing concentration. At a concentration of 1.0%, the component was more effective than the fungicide Cabrio TOP, although on the 10th day of measurement, it showed a weaker effect than the fungicide Nativo 75WG at the same concentration.

Peppermint EO showed a very weak effect on growth inhibition at a concentration of 0.2%, inhibiting growth by only 3.1% on the second day. At the highest concentration, no mycelial growth was observed on the second day of measurement, but by the fourth day, significant growth reduction occurred with a 41.1% inhibition rate, and by the 10th day, the mycelium had filled the Petri dish. A similar pattern was observed with lemon EO, which proved to be the least effective. At a concentration of 1.0%, growth inhibition was recorded only on the second day of measurement, at 26.4%. The components limonene and menthol had no effect on mycelial growth inhibition at any of the applied concentrations.

3.1.4. *Dothiorella iberica*

Based on the one-way ANOVA results (Table S8), Chinese cinnamon, holy basil, lemon, oregano, and thyme EOs, as well as the components carvacrol, e-cinnamaldehyde, and eugenol, demonstrated the highest efficacy in inhibiting mycelial growth. At a concentration of 0.1%, they inhibited mycelial growth by 100% (Table S9). Both fungicides completely inhibited mycelial growth at the applied concentrations. In contrast to thyme EO, which was effective at all concentrations, its component thymol exhibited a slightly weaker effect on mycelial growth inhibition. After the second and fourth days, mycelial growth inhibition was 100% at concentrations of 0.1% and 0.2%. However, by the 10th day, the mycelium had fully colonized the Petri dish, while at a concentration 0.5%, mycelial growth was completely inhibited for all measurement days.

Peppermint EO demonstrated significant mycelial growth inhibition, even at a concentration of 0.1%, on the second day. However, by the 10th day, the mycelium had overgrown the Petri dish (Figure 1). At a concentration of 0.2%, peppermint EO completely inhibited mycelial growth.

The components menthol and limonene had the least inhibitory effect on mycelial growth compared to the other treatments. Despite this, at all concentrations on the second day, they showed mycelial growth inhibition. However, by the fourth day, the mycelium had fully colonized the Petri dish. When comparing these two components, menthol proved to be slightly more effective than limonene at concentrations ranging from 0.1% to 0.75%, based on the second day of measurement, where an inhibition effect on mycelial growth was observed. However, at a concentration of 1.0%, limonene demonstrated a marginally higher inhibition percentage, surpassing menthol by 1.6%.

3.1.5. *Dothiorella sarmentorum*

Based on the results of the one-way ANOVA (Table S10), the EOs of Chinese cinnamon and oregano and the components carvacrol, e-cinnamaldehyde, and eugenol proved to be the most effective in inhibiting mycelial growth (Figure 1). At a concentration of 0.1%, they inhibited mycelial growth by 100% (Table S11). Also, both fungicides inhibited mycelial growth at the applied concentrations. Holy basil EO also showed high efficacy compared

to the control, inhibiting mycelial growth by 100% at a concentration of 0.2% on second and fourth day. At a lower concentration of 0.1%, inhibition of 51.9% was recorded on the second day of measurement, but by the fourth day, the mycelium had filled the Petri dish.

Thyme EO, at concentrations of 0.1% and 0.2%, significantly inhibited mycelial growth on the second and fourth days, but by the 10th day, the mycelium had filled the Petri dish. However, a concentration of 0.5% inhibited growth by 100%. Its component, thymol, began to show inhibitory effects at a concentration of 0.2%, while a concentration of 1.0% completely inhibited mycelial growth throughout all the days of measurement. Peppermint EO, at concentrations of 0.1% and 0.2%, inhibited mycelial growth by 100% on the second day, but by the fourth day, the mycelium had filled the Petri dish. A concentration of 0.5% completely suppressed mycelial growth. For the menthol component, concentrations ranging from 0.1% to 0.5% had no effect on the inhibition of mycelial growth. Slower growth was noted on the second day compared to the control, with concentrations of 0.75% and 1.0% showing growth inhibition of 10.5% and 17.1%, respectively, but by the fourth day of measurement, the mycelium had filled the Petri dish in both cases. Lemon EO and its component limonene showed no effect on mycelial growth inhibition.

3.1.6. *Neofusicoccum parvum*

Based on the results of the one-way ANOVA (Table S12), the EOs of Chinese cinnamon and oregano, along with their major components e-cinnamaldehyde and carvacrol, were the most effective in inhibiting mycelial growth and outperformed the fungicides (Figure 1). At a concentration of 0.1%, they inhibited mycelial growth by 100% across all days (Table S13). Holy basil, peppermint, and thyme EOs were also highly effective, inhibiting mycelial growth by 100% at a concentration of 0.2%. Of the three EOs mentioned, holy basil was the most effective at lower concentrations. Eugenol completely inhibited growth at a concentration of 0.5%.

The fungicide Nativo 75WG demonstrated high efficacy, reducing mycelial growth by 92.2% on the 10th day. In contrast, Cabrio TOP was significantly less effective, reducing growth by only 56.6% on the second day, with 1.9% on the fourth day, and by the 10th day, the mycelium had filled the Petri dish. Thymol impacted mycelial growth even at the lowest concentration, and at the highest concentration, it also inhibited mycelial growth on the 10th day by 40.7%. Lemon, limonene, and menthol had no effect on inhibiting fungal mycelial growth.

3.2. *Sordariomycetes*

3.2.1. *Biscogniauxia mediterranea*

Based on the results of the one-way ANOVA (Table S14), the EOs of holy basil, Chinese cinnamon, oregano, and thyme, along with the components carvacrol, e-cinnamaldehyde, and eugenol, were shown to be the most effective in inhibiting mycelial growth (Figure 2), with a minimum concentration of 0.1% inhibiting growth by 100% across all days (Table S15). Peppermint and thymol inhibited mycelial growth by 100% at a concentration of 0.5%. At lower concentrations, peppermint was more effective, inhibiting mycelial growth by 18.6% and 54.3% on the fourth day at concentrations of 0.1% and 0.2%, respectively. The fungicide Nativo 75 WG was more effective compared to Cabrio TOP, achieving an inhibition of 86.8% on the 10th day, while Cabrio TOP showed an inhibition of 75.9%.

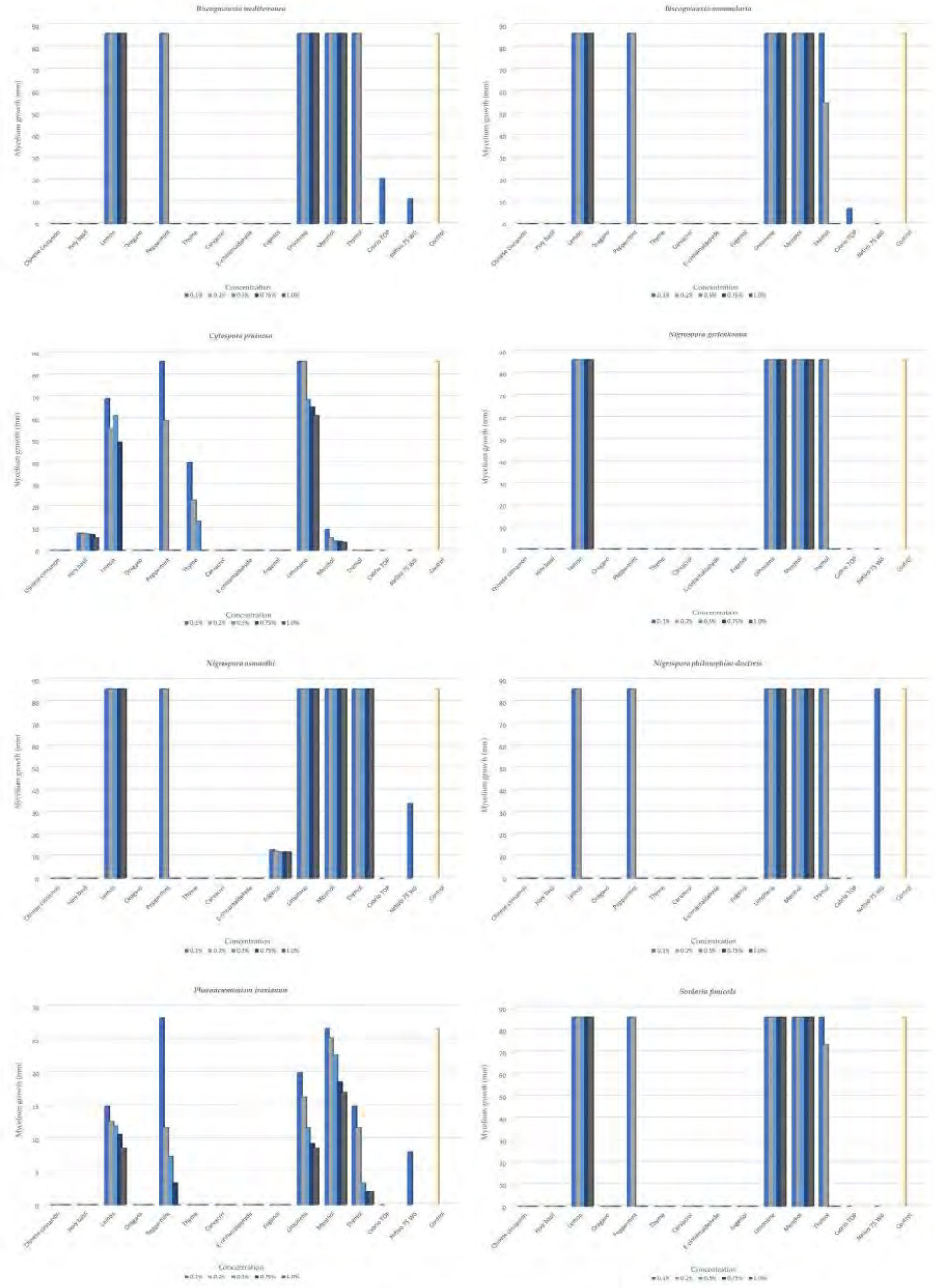


Figure 2. The average mycelial growth values on the 10th day of measurement are shown by the concentrations of all essential oils/components/fungicides used and the control for the species *Biscogniauxia mediterranea*, *B. nummularia*, *Cytospora pruinosa*, *Nigrospora gorlenkoana*, *N. osmanthi*, *N. philosophiae-doctoris*, *Phaeoacremonium iranianum*, and *Sordaria fimicola*.

Lemon EO and the components limonene and menthol had an impact on mycelial growth inhibition at the lowest concentration, but only on the second day of measurement. At the highest concentration, inhibition was also only observed on the second day. Among these treatments, lemon EO was the most effective, with an inhibition percentage of 77.3% on the second day of measurement. Limonene was the least effective treatment, with an inhibition percentage of 29.9% on the second day at the highest concentration.

3.2.2. *Biscogniauxia nummularia*

Based on the results of the one-way ANOVA (Table S16), the EOs of holy basil, Chinese cinnamon, oregano, and thyme, as well as the components carvacrol, e-cinnamaldehyde, and eugenol, along with fungicide Nativo 75WG, were found to be the most effective in inhibiting mycelial growth. At a minimum concentration of 0.1%, EOs and components inhibited mycelial growth by 100% across all measurement days (Table S17). Peppermint inhibited mycelial growth by 100% at a concentration of 0.5%. At lower concentrations, it also had an effect on the inhibition of mycelial growth. However, at the minimal concentration, the inhibition percentage was only 2.7% on the second day, and 28.7 on the fourth day, and by the 10day, the mycelium had filled the Petri dish. The fungicide Cabrio TOP showed slightly lower efficacy compared to Nativo 75WG but was still significant compared to the control across all days, inhibiting growth by 100% on the second day, 97.3% on fourth day, and 92.2% on the 10th day.

The EO of lemon and the components limonene and menthol showed noticeable effects on inhibiting mycelial growth at the lowest concentration on the second and fourth days. However, even at the highest concentration, the mycelium completely filled the Petri dish by the 10th day in all three treatments (Figure 2). Among these treatments, menthol was the least effective at the highest applied concentration, inhibiting mycelial growth by 50% on the fourth day, while limonene was the most effective, inhibiting growth by 90.3% on the same day.

3.2.3. *Cytospora pruinosa*

Based on the results of the one-way ANOVA (Table S18), the EOs of Chinese cinnamon and oregano, as well as the components carvacrol, e-cinnamaldehyde, eugenol, and thymol, were highly effective in inhibiting mycelial growth. At a minimum concentration of 0.1%, they inhibited mycelial growth by 100% across all days. Both fungicides also inhibited mycelial growth at the applied concentration. Peppermint EO inhibited mycelial growth by 100% at a concentration of 0.5%, thyme EO at 0.75%, and lemon EO at 1.0% (Table S19). All three treatments had an effect on the inhibition of mycelial growth at lower concentrations.

Holy basil EO and the menthol component showed no mycelial growth at any concentration after two and four days, but growth was observed on the 10th day. Between these two treatments, menthol was more effective with an inhibition of 95.3% at a concentration of 1.0% on the 10th day. The least effective component for inhibiting mycelial growth was limonene (Figure 2). The inhibition percentage at a concentration of 1.0% on the 10th day of measurement was 28.3%.

3.2.4. *Nigrospora gorlenkoana*

Based on the results of the one-way ANOVA (Table S20), the EOs of Chinese cinnamon, holy basil, oregano, peppermint, and thyme, as well as the components carvacrol, e-cinnamaldehyde, and eugenol, proved to be highly effective in inhibiting mycelial growth. At a minimum concentration of 0.1%, EOs and components inhibited mycelial growth by 100% across all measurement days. Additionally, both fungicides inhibited mycelial growth by 100% at the applied concentrations. The component thymol inhibited mycelial growth by 100% at a concentration of 0.5% (Table S21), but it was also effective at lower concentra-

tions, suppressing growth by 24.0% at a concentration of 0.1% on the second day, and at a concentration of 0.2%, it suppressed growth by 89.5% and 64.7% on the second and fourth days, respectively.

Among the tested EOs, only lemon exhibited a weak effect on inhibiting mycelial growth (Figure 2). Even at the minimum concentration, inhibition was observed, but by the fourth measurement day, the mycelium had overgrown the Petri dish at all concentrations. At a concentration of 1.0%, the inhibition percentage on the second day was a high of 86.4%. The component limonene showed an effect on inhibition at a concentration of 0.5% only on the second day. At lower concentrations, it had no effect on the inhibition of mycelial growth. At the highest concentration, the inhibition percentage on the second measurement day was also a high of 84.5%. The component menthol, like limonene, inhibited growth at higher concentrations, in this case from 0.75% and above, with inhibition at the highest concentration of 56.6% on the second day.

3.2.5. *Nigrospora osmanthi*

Based on the results of the one-way ANOVA (Table S22), the EOs of holy basil, Chinese cinnamon, oregano, and thyme, along with the components e-cinnamaldehyde and carvacrol, were found to be the most effective in inhibiting mycelial growth. At a minimum concentration of 0.1%, they inhibited mycelial growth by 100% across all measurement days (Table S23). The fungicide Cabrio TOP also inhibited mycelial growth at the applied concentration (Figure 2). Peppermint EO inhibited mycelial growth by 100% at a concentration of 0.5%. At lower concentrations, it also had a visible effect on mycelial growth. The fungicide Nativo 75WG also showed an effect on mycelial inhibition with 100% inhibition on the second day, 79.1% on the fourth day, and 60.1% on the 10th day. Lemon EO, even at the highest concentration, did not completely inhibit mycelial growth, though an increase in concentration led to a visible rise in inhibition, similar to the component limonene. At a concentration of 1.0%, lemon EO inhibited growth by 80.5% on the second day and by 57.8% on the fourth day, while limonene showed 77.4% and 57.8% inhibition on the same days.

Eugenol had a particularly strong impact on inhibiting mycelial growth, with consistent values being observed across all concentrations. Due to the similarity in the results, a two-way ANOVA was conducted, confirming that different concentrations of eugenol had no statistically significant impact on the inhibition results, and there was no significant interaction between eugenol concentration and measurement days. Menthol displayed similar results, where changes in concentration had no significant effect on inhibition outcomes. Its impact was only noticeable on the second day.

The component thymol exhibited a very strong inhibitory effect on mycelial growth at higher concentrations, particularly at a concentration of 0.5%, although, by the 10th day, mycelial growth had completely filled the Petri dish (Figure 2).

3.2.6. *Nigrospora philosophiae-doctoris*

Based on the results of the one-way ANOVA (Table S24), the EOs of Chinese cinnamon, holy basil, oregano, and thyme, as well as the components carvacrol, e-cinnamaldehyde, and eugenol, were shown to be the most effective in inhibiting mycelial growth. At a minimum concentration of 0.1%, they inhibited mycelial growth by 100% throughout all measurement days (Table S25). The fungicide Cabrio TOP also completely inhibited mycelial growth (Figure 2). Peppermint, lemon, and thymol inhibited mycelial growth by 100% at a concentration of 0.5%. Of these three treatments, at lower concentrations, thymol was the most effective. The fungicide Nativo 75WG showed significant inhibition of mycelial growth on the second day, but by the fourth day, the inhibition percentage was only 2.6%, and by the 10th day, the mycelium had completely filled the Petri dish.

Limonene and menthol exhibited similar effects on mycelial growth inhibition. Growth inhibition was noted on the second and fourth days, although by the 10th day, the mycelium had completely filled the Petri dish. Inhibition was similar across all tested concentrations, though a slight increase in inhibition was observed with higher concentrations of these two components. Limonene showed slightly better results, inhibiting growth by 18.2% and 12.2% on the second and fourth days at a concentration of 1.0%, while menthol showed inhibition rates of 14.1% and 7.9%, respectively.

3.2.7. *Phaeoacremonium iranianum*

Based on the results of the one-way ANOVA (Table S26), the EOs of Chinese cinnamon, holy basil, oregano, and thyme, along with the components carvacrol, e-cinnamaldehyde, and eugenol and the fungicide Cabrio TOP, were shown to be the most effective in inhibiting mycelial growth. At a minimum concentration of 0.1%, EOs and components inhibited mycelial growth by 100% throughout all measurement days, while peppermint EO inhibited growth by 100% at a concentration of 1.0% (Table S27). Nativo 75WG was less effective compared to the fungicide Cabrio TOP (Figure 2), with inhibition percentages of 22.2% on the second day, 61.9% on the fourth day, and 70% on the 10th day.

Lemon EO had the weakest performance among the tested EOs. On the second day, no growth occurred, but by the 10th day, the inhibition percentage was 67.5% at the highest concentration.

Limonene, thymol, and menthol were components that, even at the highest concentrations, did not fully suppress mycelial growth. Limonene slowed mycelial growth, and no growth occurred on the second day, but by the fourth day, growth resumed even at the highest concentration. Although limonene was more effective in the initial stages of growth compared to thymol, it had a weaker effect than thymol by the final measurement day. Among the three components, menthol was the least effective, inhibiting growth by only 36.3% on the 10th day at the highest concentration.

3.2.8. *Sordaria fimicola*

Based on the results of the one-way ANOVA (Table S28), the EOs of Chinese cinnamon, holy basil, oregano, and thyme, along with the components carvacrol, e-cinnamaldehyde, and eugenol and both fungicides, were the most effective in inhibiting mycelial growth. At a minimal concentration of 0.1%, EOs and components inhibited mycelial growth by 100% across all measurement days. Additionally, both fungicides were 100% effective at the applied concentrations. Peppermint and thymol inhibited mycelial growth by 100% at a concentration $\geq$0.5% (Table S29 and Figure 2). Comparing these two treatments, thymol was more effective at lower concentrations.

Lemon, limonene, and menthol, as in some previous cases, showed the lowest effectiveness in inhibiting phytopathogenic fungi growth. Among them, lemon was the most effective, with an inhibition percentage of 85.5% on the second day of measurement at a concentration of 1.0%. For all three treatments, growth inhibition was observed only on the second day.

3.3. Determined MIC and MFC Values

Based on the MIC and MFC values determined in this study, it was found that Chinese cinnamon and oregano EOs produced the best results among the EOs tested (Figure 3), with MFC values of 0.1% for all fungal species (Table S30). Holy basil EO also demonstrated high efficacy with MFC values ranging between 0.1% and 0.2%, depending on the fungal species, except for *Do. sarmentorum*, for which it was 0.5%. For *C. pruinosa*, the MFC value was not determined. Thyme EO showed an MFC range of 0.1% to 0.75%. The MFC for thyme EO was not established for *B. nummularia*, but the MIC value was determined to

be 0.1%. For lemon EO, the MIC value was 0.1% for eight out of 14 species. The treatment had no effect on *B. dothidea*, *N. parvum* or *Do. sarmentorum*. The MFC value was only determined for *Do. iberica* (0.1%), *C. pruinosa* (1.0%) and *N. philosophiae-doctoris* (0.5%). The MIC values for peppermint EO ranged from 0.1% to 0.2% depending on the fungal species. The lowest MFC value was found for *N. gorlenkoana* (0.1%), and the highest was found for *P. iranianum* (1.0%), while no MFC value was established for *B. dothidea*, *D. mutila*, or *D. seriata*.

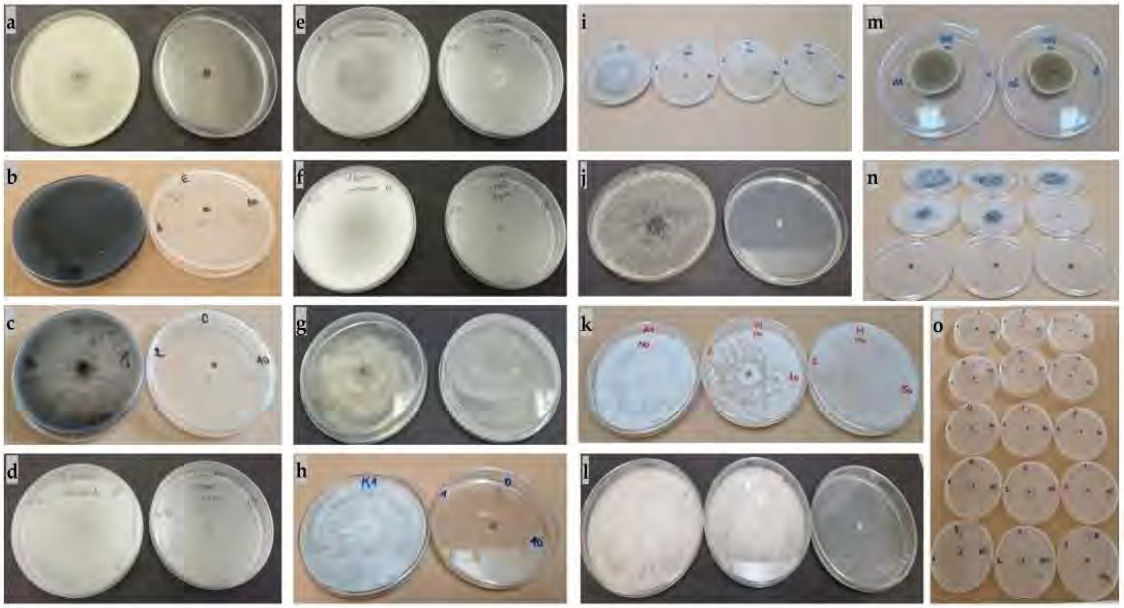


Figure 3. Testing the antifungal potential of essential oils (EOs) on isolates of phytopathogenic fungi from olive trees: (**a**) *Botryosphaeria dothidea*—the control on the left, holy basil EO at a concentration of 0.2% on the right, day 10; (**b**) *Dothiorella iberica*—the control on the left, the eugenol component at a concentration of 0.1% on the right, day 10; (**c**) *Do. sarmentorum*—the control on the left, Chinese cinnamon EO at a concentration of 0.1% on the right, day 10; (**d**) *Diplodia seriata*—the control on the left, thyme EO at a concentration of 0.1% on the right, day 10; (**e**) *D. mutila*—the control on the left, holy basil EO at a concentration of 0.1% on the right, day 2; (**f**) *Neofusicoccum parvum*—the control on the left, Chinese cinnamon EO at a concentration of 0.1% on the right, day 10; (**g**) *Cytospora pruinosa*—treatment with lemon EO: a 0.1% concentration on the left and a 1.0% concentration on the right, day 10; (**h**) *Biscogniauxia mediterranea*—the control on the left, oregano EO at a concentration of 0.1% on the right, measured on day 10; (**i**) *B. nummularia*—the one control on the far left, with three replicates of treatment with thyme EO at a concentration of 0.1% on the right, day 10; (**j**) *Nigrospora gorlenkoana*—the control on the left, treatment with the thymol component at a concentration of 0.5% on the right, day 10; (**k**) *N. osmanthi*—the control on the left, treatment with peppermint EO at a concentration of 0.2% in the middle and 0.5% on the right, with a fogged Petri dish from EO vapors, day 10; (**l**) *N. philosophiae-doctoris*—treatment with lemon EO: a 0.1% concentration on the left, 0.2% in the middle, and 0.5% on the right, day 10; (**m**) *Phaeoacremonium iranianum*—treatment with the menthol component: a 0.1% concentration on the left and 0.2% on the right, day 10; (**n**) *Sordaria fimicola*—three replicates of the treatment with the thymol component: a top concentration of 0.1%, a middle concentration of 0.2%, and a bottom concentration of 0.5%, day 10; (**o**) *Botryosphaeria dothidea*—treatment with oregano EO, with three replicates for each concentration, on day 10, from top to bottom as follows: 0.1%, 0.2%, 0.5%, 0.75%, and 1.0%.

Regarding EO components, the best results based on MFC values were achieved by carvacrol and e-cinnamaldehyde. For the component eugenol, the MFC value was 0.1% for most species, except for *B. dothidea* and *D. seriata*, where it was 0.2%, and *N. parvum*, where it was 0.5%. For *B. nummularia* and *N. osmanthi*, the MFC value was not determined, but the MIC value was 0.1% for both species. The MFC value for the component limonene was not established for any species, and the MIC value was not determined for *B. dothidea*, *D. mutila*, *D. seriata*, *Do. Sarmentorum*, and *N. parvum*. For other species, the MIC value was 0.1%, except for *N. gorlenkoana*, where it was 0.5%. Similarly, no MFC value was determined for the component menthol for any species. Menthol was ineffective against *B. dothidea*, *D. seriata*, and *N. parvum*, while the MIC value was 0.75% for *D. mutila*, *Do. sarmentorum*, and *N. gorlenkoana*, and 0.1% for the remaining species. These findings indicate that limonene and menthol exhibited the weakest antifungal activity against phytopathogenic fungi. For the component thymol, the MIC value was 0.1% for most fungi, except for *Do. sarmentorum*, where it was 0.2%. The MFC values ranged between 0.1% and 0.5% and were not determined for *B. dothidea*, *D. mutila*, *D. seriata*, *Do. sarmentorum*, *N. parvum*, *N. osmanthi*, and *P. iranianum*.

4. Discussion

The expanded cultivation of olive trees has greatly heightened the demand for the use of plant protection products. Additionally, the development of resistance in phytopathogenic fungi presents a substantial challenge for producers [18]. Currently, the management of olive tree diseases largely relies on the application of chemical pesticides, typically applied according to predetermined schedules rather than being based on monitoring or predictive systems. However, growing public awareness of environmental pollution in this vital Mediterranean agroecosystem, issues stemming from the side effects of these chemicals, and EU policies have led to a decrease in the number of approved active ingredients for olive disease management. Consequently, there is a growing emphasis on the sustainable use of pesticides and the adoption of non-chemical alternatives or Integrated Pest Management (IPM) strategies [1].

In recent times, natural compounds have been investigated for inclusion in disease control programs due to their faster degradation rates and distinct ecotoxicological profiles [1]. Considering that this study included certain pathogens whose presence on olive trees has only recently been detected, there is limited research on their control, particularly in olives.

Species from the family *Botryosphaeriaceae* are among the most aggressive pathogens of olive trees, with 22 species being identified on olives to date [7]. *B. dothidea* has been identified as a causative agent of olive diseases in California [6], Croatia [7], Greece [39], Italy [40], Montenegro [41], Spain [42], Tunisia [43], Turkey [44], and Uruguay [45]. *D. mutila* has been detected on olive trees in California [6], Croatia [7], Italy [40], and Uruguay [45]. *D. seriata* has been detected on olive trees in California [6], Croatia [7], Italy [40], Spain [46], and Uruguay [45]. *Do. iberica* has been detected on olive trees in California [6] and Croatia [7]. *Do. sarmentorum* has been detected on olive trees in Croatia [7] and Italy [47], while *N. parvum* has been detected on olive trees in Croatia [7], Italy [48], South Africa [49], and Uruguay [45].

Ammad et al. [50] conducted in vitro tests on the antifungal efficacy of lemon EO (*C. limon*) against three pathogenic species, including *B. dothidea*. Lemon EO inhibited the growth of *B. dothidea* at a concentration of 0.25%. All tested concentrations were found to be lethal under the test conditions. In our study, lemon EO and the component limonene (which was also the predominant component of their EO) did not inhibit the growth of *B. dothidea* mycelium, even at a concentration of 1.0%. Li et al. [51] examined the

antifungal effect of 33 EO components on *B. dothidea* isolated from kiwi, including carvacrol, cinnamaldehyde, eugenol, limonene, and thymol. The components carvacrol and thymol had the highest antifungal effect on mycelial growth. In our research, the most effective components were carvacrol and e-cinnamaldehyde. Zhang et al. [52] tested the antifungal activity of 20 pure compounds against *B. dothidea* at a concentration of 400 μg/mL, where menthol inhibited growth by approximately 40.2%. In our study, menthol had no effect on mycelial growth, even at the highest concentration. Wang et al. [53] examined the antifungal effect of cinnamon and clove EOs on *B. dothidea*, where cinnamon EO exhibited a stronger inhibition effect compared with clove EO. The most abundant components in their cinnamon EO were trans-cinnamaldehyde and benzaldehyde, whereas in our study, they were e-cinnamaldehyde and trans-o-methoxy-cinnamaldehyde.

Dai et al. [54] tested 17 fungicides for their in vitro activity against *B. dothidea*. Difenoconazole, tebuconazole, and prochloraz were the most effective in inhibiting mycelial growth, while trifloxystrobin had the most significant inhibitory activity against spore germination. Fan et al. [55] found that resistance levels in *B. dothidea* to tebuconazole significantly increased over time. In the study by Latorre et al. [56], in the initial screening of protectant compounds against *N. parvum* in blueberry stems, 0.5% of tebuconazole was the most effective fungicide treatment. Twizeyimana et al. [57] examined the effect of 12 fungicides on fungi from the *Botryosphaeriaceae* family, including *N. parvum* and *Do. iberica*. Based on their in vitro fungicide screening results, five fungicide treatments were the most effective, including azoxystrobin, fludioxonil, metconazole, propiconazole, and pyraclostrobin. In our research, the fungicide Nativo 75WG (trifloxystrobin + tebuconazole) was more effective for all species from the *Botryosphaeriaceae* family compared to Cabrio TOP, except for *Do. iberica* and *Do. sarmentorum*, where both fungicides were 100% effective.

No research has been conducted so far on the effects of EOs on *Do. iberica* and *Do. sarmentorum*. In our study, Chinese cinnamon and oregano EOs, as well as the components e-cinnamaldehyde, eugenol, and carvacrol, completely suppressed growth at the lowest concentration, while for *Do. iberica* basil, lemon and thyme EOs were effective.

No research is available on the effects of EOs on *D. seriata* and *D. mutila*. However, Štůsková et al. [58] examined the effects of phenolic components on the growth of grapevine pathogens, including *D. seriata* and *N. parvum*. Eugenol and thymol had an inhibitory effect on fungal mycelial growth, with eugenol showing a better effect in both cases, achieving 100% growth inhibition at a concentration of 2.5 μL/mL. In our study, for *D. mutila*, the most effective EOs at the lowest concentration were Chinese cinnamon and oregano and the components e-cinnamaldehyde, eugenol, and carvacrol, while for *D. seriata*, the most effective were Chinese cinnamon, oregano, thyme, and the components e-cinnamaldehyde and carvacrol. Sarkhosh et al. [19] tested eight different EOs against the growth of phytopathogenic fungi, including the species *Botryosphaeria* sp. At 250 μL/L, thyme and savory EO completely inhibited the mycelial growth of this fungus. At an application rate of 1500 μL/L, peppermint EO completely inhibited the mycelial growth of *Botryosphaeria* sp. None of the other EOs completely inhibited the mycelial growth of *Botryosphaeria* sp. at any of the concentrations tested. The major components of their peppermint EO were menthol, menthone, and 1,8-cineole, as in the EO used in our study. Some other EO components also have an inhibitory effect on fungal growth, such as monoterpenes [52], limonene, thymol, and 31 other essential oil components [51]. According to the literature, the most abundant components of Chinese cinnamon EO are benzaldehyde, phenylpropyl aldehyde, e-cinnamaldehyde, 2-methoxy-cinnamaldehyde, and (E)-cinnamyl acetate, which make up 90% of the EO [59,60]. In our study, as we mentioned earlier, they were e-cinnamaldehyde, trans-o-methoxy-cinnamaldehyde, and cinnamyl acetate. The major components of lemon

EO are limonene, neral, and trans-verbenol [61]. In our study, they were limonene, β-pinene, and γ-terpinene. The major components of peppermint EO are menthol, menthyl acetate, menthone, menthofuran, β-phellandrene, and isomenthone [62,63], as were menthol, menthone, and 1,8-cineole in the EO used in our study. The major components of basil EO are eugenol, (E)-caryophyllene, and β-elemen, which make up about 98% of the EO [64]. In our study, they were eugenol, β-caryophyllene, and estragole. The major components of thyme EO are thymol, p-cymene, carvacrol, α-pinene, durenol, and camphor [62,65]. In our study, they were thymol, p-cymene, and γ-terpinene. The major components of oregano (*O. compactum*) EO are carvacrol (2.18–63.65%), p-cymene (6.69–42.64%), thymol (0.16–34.29%), and γ-terpinene [66,67]. In our study, they were carvacrol, γ-terpinene, and thymol.

B. mediterranea has been reported as a pathogen in both Croatia and Tunisia [3,12], while *B. nummularia* has been identified as a pathogen on olive trees in Croatia [3]. There is currently no available data on the control measures for *B. nummularia*, whereas studies on *B. mediterranea* have investigated the use of antagonistic microorganisms, such as *Trichoderma* spp., *Paecilomyces variotii* (syn. Bainier *Byssochlamys spectabilis* (Udagawa & Shoji Suzuki) Houbraken & Samson), etc., [68–71], as well as fungicides including carbendazim, copper oxychloride 35% WP, mancozeb, and propiconazole, along with silvicultural methods. Yangui et al. [72] tested EOs from five *Eucalyptus* species and five EOs derived from *Myrtus communis* L. from five different locations in Tunisia. The most effective were the EOs of *E. camaldulensis* Dehnh. and *Myrtus* sp. collected from Zaghouan, which completely inhibited fungus growth at a concentration of 45 mg/mL. Based on our results, the EOs of holy basil, Chinese cinnamon, oregano, and thyme, as well as the components e-cinnamaldehyde, eugenol, and carvacrol, completely inhibited the mycelial growth of both fungi at a concentration of 0.1%.

C. pruinosa has been identified as a pathogen in Croatia, South Africa, and Spain [16,42,73]. To date, no studies have been conducted on the control of this pathogen in any plant species. In our study, the EOs of Chinese cinnamon and oregano, as well as the components e-cinnamaldehyde, eugenol, carvacrol, and thymol and both tested fungicides, were found to be the most effective in inhibiting the mycelial growth of this pathogen.

N. gorlenkoana, *N. osmanthi*, and *N. philosophiae-doctoris* are newly described pathogens on olive trees globally [35], although some species of *Nigrospora* are known to cause leaf spot disease on various crops such as fiddle-leaf fig, java tea, sugarcane, and others [74–76]. There are no available data on control measures specifically for these three pathogens, although studies have been conducted on fungicides and other antifungal substances for other *Nigrospora* species [77,78]. We determined that the EOs of holy basil, Chinese cinnamon, oregano, and thyme, as well as the components e-cinnamaldehyde and carvacrol, along with the fungicide Cabrio TOP, were found to be the most effective against all three species. Additionally, for *N. gorlenkoana*, peppermint EO and the fungicide Nativo 75WG were also the most effective, completely inhibiting mycelial growth at minimal concentrations. The component eugenol was effective for both *N. gorlenkoana* and *N. philosophiae-doctoris*.

P. iranianum is a pathogen responsible for olive twig and branch dieback, and so far, it has only been detected in Croatia [14]. A single study has been conducted on the potential control of this pathogen. Idbella et al. [79] examined the potential application of biochar in controlling this pathogen in grapevines, and the results indicate that the pathogen was exclusively present in untreated soils. In our research, EOs of holy basil, Chinese cinnamon, oregano, and thyme, as well as the components e-cinnamaldehyde, eugenol, and carvacrol and the fungicide Cabrio TOP, have proven to be the most effective in inhibiting the growth of this pathogen's mycelium.

S. fimicola is a pathogen that causes olive twig and branch dieback and has been identified as the cause of olive disease in Croatia [3], and it also affects other plant species [80]. Only older data, from 1979 [81], are available regarding fungicide testing on this species. The author found that triadimefon, triadimenol, fenarimol, nuarimol, and imazalil had toxic effects on *S. fimicola*. No studies have been conducted with EOs. In our research, the EOs of holy basil, Chinese cinnamon, oregano, and thyme, along with the components e-cinnamaldehyde, carvacrol, and eugenol and both fungicides, proved to be the most effective in inhibiting mycelial growth.

Overall, the best effect on inhibiting the mycelial growth of fungi used in this study was achieved by cinnamon and oregano EOs, along with their predominant components, e-cinnamaldehyde and carvacrol, which completely inhibited the mycelial growth of all tested fungi. In contrast, the least effective results were observed with lemon and peppermint EOs, as well as their components limonene, menthol, and thymol. Notably, among the *Dothideomycetes*, the species *Do. iberica* demonstrated the highest sensitivity to all the treatments applied, while within the *Sordariomycetes*, this was observed for the species *N. gorlenkoana*.

EOs reduce the growth of mycelium of certain phytopathogenic fungi [50]; disrupt cell metabolism and normal cell function [22]; lead to morphological degeneration including cytoplasmic coagulation, hyphal degeneration, and leakage of protoplasm from fungal hyphae [82]; induce mitochondrial dysfunction caused by reactive oxygen species [83]; reduce mycotoxin production [84,85]; and decrease spore germination [86]. Due to their hydrophobic nature, EOs pass through membranes, causing disruption in the lipid bilayer of the cell membrane, leading to the leakage of cellular contents and slowing down ergosterol biosynthesis (a specific sterol of fungi) [87]. EOs cause cell death by destroying the structure of the cell membrane [88]. Li et al. [51] examined the effect of carvacrol on the fungus *B. dothidea*. They concluded that carvacrol affects the morphology and structure of the fungal mycelium. After treatment with carvacrol, the fungal hyphae appeared shrunken and deformed, and wrinkles, dryness, and other irreversible changes were observed, along with more branching and fractures in the mycelia. Furthermore, they concluded that carvacrol causes cell death in the mycelium, disrupts the ultrastructure of the pathogen's hyphae, and significantly enlarges the periplasmic space between the cell membrane and the cell wall. Plasmolysis was also observed in the cells, with the cellular contents either being degraded or unevenly distributed. Compared to the mycelia in the control group, carvacrol significantly reduces the total lipid and ergosterol contents in the hyphae of *B. dothidea* and significantly increases the MDA content in the pathogen compared to the control group. The extracellular conductivity of the mycelium also increased with carvacrol treatment, as did the extracellular content of soluble proteins and sugars. This indicates that carvacrol can target the permeability of the cell membrane of *B. dothidea* and may have a significant effect on the mitochondrial activity of *B. dothidea*.

In conclusion, considering the increasing prevalence of pathogens affecting olive trees and the dangers and consequences associated with pesticide use, the development and application of alternative biological plant protection methods are crucial for sustainable agriculture. Climate change is expected to influence the incidence and severity of infectious diseases in olives. However, predicting these consequences is challenging due to the complex interactions between specific pathogens, olive genotypes, and environmental factors (such as climate and agronomic practices). Excessive moisture and recurrent rainfall favor the prevalence and epidemic spread of fungal diseases. Milder winters and higher nocturnal or overall temperatures may enhance the overwintering of certain pathogens on olive trees or promote sporulation. Heavy rains can lead to soil saturation, increasing long-term problems like root rot caused by various soil-borne pathogens. Additionally,

adjustments in the timing and dosage of chemical or biological products may be necessary, as rainfall intensity, frequency, and temperature fluctuations can influence the persistence and effectiveness of plant protection products, potentially leading to their degradation [1]. Moreover, the development of new plant protection strategies is essential in light of the EU's efforts to transition from conventional production systems to environmentally friendly agriculture [89].

EOs offer a promising alternative to chemical plant protection products. However, when testing their efficacy, it is essential to conduct measurements over an extended period, as most studies have only evaluated their effects after 48 h. This research indicates that the impact of EOs weakens over time. Further studies are needed to explore their effects on pathogens and plants, as well as to determine the optimal dosage that is effective against pathogens without causing harm to the plant or the environment.

5. Conclusions

The increasing prevalence of olive tree pathogens, coupled with the risks and adverse effects associated with pesticide use, underscores the urgent need for the development and implementation of alternative biological plant protection strategies to promote sustainable agricultural practices. EOs have emerged as a promising alternative to synthetic chemicals due to their potent antimicrobial properties. Research has shown that the synergistic effect of EO components is primarily responsible for their antifungal activity. The chemical profiles of EOs vary depending on plant species and are influenced by numerous factors. In this study, Chinese cinnamon and oregano EOs, along with their key components, e-cinnamaldehyde and carvacrol, demonstrated the most effective inhibition of fungal mycelial growth, completely suppressing the growth of all fungi tested. In contrast, lemon and peppermint EOs, along with the components limonene, menthol, and thymol, showed the weakest antifungal effects. However, for the practical application of EOs, further investigation is needed to assess their long-term efficacy, their impact on pathogens, and their potential effects on the treated plants themselves. Given the observed variations in the effects of EOs on pathogens, it is crucial to conduct studies that elucidate the precise mechanisms of action against pathogens. Additionally, understanding the interaction between EOs and the host plants is essential for optimizing their practical use. A detailed examination of these interactions could help tailor treatments to specific pathogens and host plants. Determining appropriate dosages that are both effective and environmentally safe is also critical for broader application.

In conclusion, continued research and innovation in plant protection strategies are essential to address future agricultural challenges while meeting the EU's sustainability goals. By advancing the understanding and application of EOs, we can move closer to achieving effective, sustainable, and eco-friendly solutions for plant health management.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agriculture15030340/s1, Table S1. List of used essential oils (EOs), Latin names of plant species, and EO components present in concentrations greater than 0.1%, as well as the commercial names of used fungicides, type of fungicide, and active ingredients; Table S2. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Botryosphaeria dothidea* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$); Table S3. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Botryosphaeria dothidea*; Table S4. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Diplodia mutila* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$); Table S5. Inhibitory effect (%) of essential oils, components, and

fungicides on the mycelial growth of *Diplodia mutila*; Table S6. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Diplodia seriata* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$); Table S7. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Diplodia seriata*; Table S8. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Dothiorella iberica* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$); Table S9. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Dothiorella iberica*; Table S10. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Dothiorella sarmentorum* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$); Table S11. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Dothiorella sarmentorum*; Table S12. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Neofusicoccum parvum* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$); Table S13. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Neofusicoccum parvum*, Table S14. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Biscogniauxia mediterranea* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$); Table S15. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Biscogniauxia mediterranea*, Table S16. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Biscogniauxia nummularia* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$); Table S17. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Biscogniauxia nummularia*; Table S18. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Cytospora pruinosa* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$); Table S19. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Cytospora pruinosa;* Table S20. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Nigrospora gorlenkoana* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$); Table S21. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Nigrospora gorlenkoana*; Table S22. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Nigrospora osmanthi* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$); Table S23. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Nigrospora osmanthi*; Table S24. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Nigrospora philosophiae-doctoris* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$); Table S25. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Nigrospora philosophiae-doctoris*; Table S26. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Phaeoacremonium iranianum* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$); Table S27. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Phaeoacremonium iranianum;* Table S28. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Sordaria fimicola* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test

($p < 0.05$); Table S29. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Sordaria fimicola;* Table S30. The determined MIC (Minimum Inhibitory Concentration) and MFC (Minimum Fungicidal Concentration) values for the treatments.

Author Contributions: Conceptualization, E.P., K.V., and J.Ć.; methodology, E.P., K.V., and J.Ć.; investigation, E.P.; resources, S.G.; data curation, E.P.; writing—original draft preparation, E.P.; writing—review and editing, E.P., K.V., T.S., J.Ć., and S.G.; visualization, E.P. and T.S.; project administration, S.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Croatian Science Foundation Installation Research Project titled "Natural bioactive compounds as a source of potential antimicrobial agents in the control of bacterial and other fungal pathogens of olives", Anti-Mikrobi-OL (AMO), UIP-2020-02-7413, and the "Young Researchers' Career Development Project" DOK-2021-02-2882.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: All data are available in the manuscript and Supplementary Materials.

Acknowledgments: We would like to extend our sincere gratitude to Monika Lisjak, a technician at the Faculty of Agrobiotechnical Sciences Osijek, for her assistance in setting up and conducting the experiments.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Landa, B.B. *Pests and Diseases of the Olive Tree*; EIP-AGRI Focus Group; European Commission: Brussels, Belgium, 2019; pp. 1–20. Available online: https://ec.europa.eu/eip/agriculture/sites/default/files/eip-agri_fg33_pests_and_diseases_olive_tree_starting_paper_2019_en.pdf (accessed on 15 October 2024).
2. FAO (Food and Agriculture Organization of United Nations). Crop and Livestock Products. Available online: https://www.fao.org/faostat/en/#data/QCL (accessed on 7 October 2024).
3. Petrović, E.; Godena, S.; Ćosić, J.; Vrandečić, K. Identification and Pathogenicity of *Biscogniauxia* and *Sordaria* Species Isolated from Olive Trees. *Horticulturae* **2024**, *10*, 243. [CrossRef]
4. Patejuk, K.; Baturo-Cieśniewska, A.; Pusz, W.; Kaczmarek-Pieńczewska, A. Biscogniauxia Charcoal Canker—A New Potential Threat for Mid-European Forests as an Effect of Climate Change. *Forests* **2022**, *13*, 89. [CrossRef]
5. Kim, G.-U.; Seo, K.-H.; Chen, D. Climate change over the Mediterranean and current destruction of marine ecosystem. *Sci. Rep.* **2019**, *9*, 18813. [CrossRef] [PubMed]
6. Úrbez-Torres, J.R.; Peduto, F.; Vossen, P.M.; Krueger, W.H.; Gubler, W.D. Olive Twig and Branch Dieback: Etiology, Incidence, and Distribution in California. *Plant Dis.* **2013**, *97*, 231–244. [CrossRef]
7. Petrović, E.; Vrandečić, K.; Belušić Vozila, A.; Ćosić, J.; Godena, S. Diversity and Pathogenicity of *Botryosphaeriaceae* Species Isolated from Olives in Istria, Croatia, and Evaluation of Varietal Resistance. *Plants* **2024**, *13*, 1813. [CrossRef]
8. Cvjetković, B. *Mikoze i Pseudomikoze Voćnjaka Ivinove Loze*; Zrinski d.d: Čakovec, Croatia, 2010.
9. Schena, L.; Mosca, S.; Cacciola, S.O.; Faedda, R.; Sanzani, S.M.; Agosteo, G.E.; Sergeeva, V.; Magnano di San Lio, G. Species of the *Colletotrichum gloeosporioides* and *C. boninense* complexes associated with olive anthracnose. *Plant Pathol.* **2013**, *63*, 437–446. [CrossRef]
10. Cvjetković, B.; Vončina, D. Paunovo oko [Spilocaea oleaginea (Castagne) Hughes] najučestalijaje bolest masline. *Glas. Biljn. Zašt.* **2012**, *12*, 336–340.
11. Kaliterna, J.; Miličević, T.; Benčić, D.; Mešić, A. First report of Verticillium wilt caused by *Verticilium dahlie* on olive trees in Croatia. *Plant Dis.* **2016**, *100*, 2526. [CrossRef]
12. Gharbi, Y.; Ennouri, K.; Bouazizi, E.; Cheffi, M.; Ali Triki, M. First report of charcoal disease caused by *Biscogniauxia mediterranea* on *Olea europaea* in Tunisia. *J. Plant Pathol.* **2020**, *102*, 961. [CrossRef]
13. Santilli, E.; Riolo, M.; La Spada, F.; Pane, A.; Cacciola, S.O. First Report of Root Rot Caused by *Phytophthora bilorbang* on *Olea europaea* in Italy. *Plants* **2020**, *9*, 826. [CrossRef]
14. Petrović, E.; Vrandečić, K.; Ćosić, J.; Kanižai Šarić, G.; Godena, S. First Report of *Phaeoacremonium iranianum* Causing Olive Twig and Branch Dieback. *Plants* **2022**, *11*, 3578. [CrossRef] [PubMed]

15. Brugneti, F.; Turco, S.; Drais, M.I.; Giubilei, I.; Mazzaglia, A. First report of the *Fusarium arthrosporioides/avenaceum* complex causing olive fruit rot in Italy. *New Dis. Rep.* **2023**, *48*, e12198. [CrossRef]
16. Petrović, E.; Vrandečić, K.; Ivić, D.; Ćosić, J.; Godena, S. First Report of Olive Branch Dieback in Croatia Caused by *Cytospora pruinosa* Défago. *Microorganisms* **2023**, *11*, 1679. [CrossRef] [PubMed]
17. Alzohairy, S.A.; Gillett, J.; Saito, S.; Naegele, R.N.; Xiao, C.L.; Miles, T.D. Fungicide Resistance Profiles of *Botrytis cinerea* Isolates from Michigan Vineyards and Development of a TaqMan Assay for Detection of Fenhexamid Resistance. *Plant Dis.* **2020**, *105*, 258–294. [CrossRef] [PubMed]
18. Petrović, E.; Vrandečić, K.; Ćosić, J.; Godena, S. Chemical Control of Olive Fungal Diseases: Strategies and Risks. *Poljoprivreda* **2024**, *30*, 44–53. [CrossRef]
19. Sarkhosh, A.; Schaffer, B.; Vargas, A.I.; Palmateer, A.J.; Lopez, P.; Soleymani, A. *In Vitro* Evaluation of Eight Plant Essential Oils for Controlling Colletotrichum, Botryosphaeria, Fusarium and Phytophthora Fruit Rots of Avocado, Mango and Papaya. *Plant Prot. Sci.* **2018**, *54*, 153–162. [CrossRef]
20. Rahman, S.M.A.; Yusef, H.; Halawi, J. Biological and chemical control of some tomato fungal diseases. *Egypt. J. Bot.* **2022**, *62*, 45–58. [CrossRef]
21. Burt, S.A.; Reinders, R.D. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. *Lett. Appl. Microbiol.* **2003**, *36*, 162–167. [CrossRef]
22. Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological effects of essential oils—A review. *Food Chem. Toxicol.* **2008**, *46*, 446–475. [CrossRef]
23. Tongnuanchan, P.; Benjakul, S. Essential oils: Extraction, bioactivities, and their uses for food preservation. *J. Food Sci.* **2014**, *79*, R1231–R1249. [CrossRef]
24. Božović, M.; Garzoli, S.; Sabatino, M.; Pepi, F.; Baldisserotto, A.; Andreotti, E.; Romagnoli, C.; Mai, A.; Manfredini, S.; Ragno, R. Essential oil extraction, chemical analysis and anti-*Candida* activity of *Calamintha nepeta* (L.) Savi subsp. *glandulosa* (Req.) Ball-New Approaches. *Molecules* **2017**, *22*, 203. [CrossRef] [PubMed]
25. Del Principe, S.; Mondo, L. *Eterična Ulja*; Trsat: Zagreb, Croatia, 2001; pp. 1–126.
26. Nazzaro, F.; Fratianni, F.; Coppola, R.; De Feo, V. Essential oils and antifungal activity. *Pharmaceuticals* **2017**, *10*, 86. [CrossRef] [PubMed]
27. Bowles, E.J. *Eterična Ulja*; Veble Commerce: Zagreb, Croatia, 2012.
28. Niu, C.; Gilbert, E.S. Colorimetric method for identifying plant essential oil components that affect biofilm formation and structure. *Appl. Environ. Microbiol.* **2004**, *70*, 6951–6956. [CrossRef] [PubMed]
29. Hyldgaard, M.; Mygind, T.; Meyer, R.L. Essential oils in food preservation: Mode of action, synergies and interactions with food matrix components. *Front. Microbiol.* **2012**, *3*, 12. [CrossRef]
30. Dorman, H.J.D.; Deans, S.G. Antimicrobial agents from plants: Antimicrobial activity of plant volatile oils. *J. Appl. Microbiol.* **2000**, *88*, 308–316. [CrossRef]
31. Burt, S. Essential oils: Their antibacterial properties and potential applications in foods—A review. *Int. J. Food Microbiol.* **2004**, *94*, 223–253. [CrossRef]
32. Iacobelis, N.S.; Lo Cantore, P.; Capasso, F.; Senatore, F. Antibacterial Activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. *J. Agric. Food Chem.* **2005**, *53*, 57–61. [CrossRef]
33. Ćosić, J.; Vrandečić, K.; Jurković, D. The effect of essential oils on the development of phytopathogenic fungi. In *Biological Controls for Preventing Food Deterioration—Strategies for Pre- and Postharvest Management*; Sharma, N., Ed.; John Wiley & Sons: Hoboken, NJ, USA, 2014; pp. 273–292.
34. Karimi, K.; Arzanlou, M.; Pertot, I. Antifungal activity of the dill (*Anethum graveolens* L.) seed essential oil against strawberry anthracnose under *in vitro* and *in vivo* conditions. *Arch. Phytopathol. Plant Prot.* **2016**, *49*, 554–566. [CrossRef]
35. Petrović, E.; Vrandečić, K.; Ćosić, J.; Đermić, E.; Godena, S. First Report of *Nigrospora* Species Causing Leaf Spot on Olive (*Olea europaea* L.). *Horticulturae* **2023**, *9*, 1067. [CrossRef]
36. Edris, A.E.; Farrag, E.S. Antifungal activity of peppermint and sweet basil essential oils and their major aroma constituents on some plant pathogenic fungi from the vapor phase. *Food/Nahrung* **2003**, *47*, 117–121. [CrossRef]
37. Palfi, M. Antifungalno Djelovanje Eteričnih Ulja i Njihovih Komponenti na Fitopatogene Gljivice u In Vitro Uvjetima. Ph.D. Thesis, Josip Juraj Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences, Osijek, Croatia, 2017.
38. Wu, J.; Kang, S.; Luo, L.; Shi, Q.; Ma, J.; Yin, J.; Song, B.; Hu, D.; Yang, S. Synthesis and antifungal activities of novel nicotinamide derivatives containing 1, 3, 4-oxadiazole. *Chem. Cen. J.* **2013**, *7*, 64. [CrossRef] [PubMed]
39. Markakis, E.A.; Roditakis, E.N.; Kalantzakis, G.S.; Chatzaki, A.; Soultatos, S.K.; Stavrakaki, M.; Soultatos, S.K.; Stavrakaki, M.; Tavlaki, G.I.; Koubouris, G.C.; et al. Characterization of Fungi Associated with Olive Fruit Rot and Olive Oil Degradation in Crete, Southern Greece. *Plant Dis.* **2021**, *105*, 3623–3635. [CrossRef] [PubMed]
40. Lazzizera, C.; Frisullo, S.; Alves, A.; Phillips, A.J.L. Morphology, phylogeny and pathogenicity of *Botryosphaeria* and *Neofusicoccum* species associated with drupe rot of olives in southern Italy. *Plant Pathol.* **2008**, *57*, 948–956. [CrossRef]

41. Latinović, J.; Mazzaglia, A.; Latinović, N.; Ivanović, M.; Gleason, M.L. Resistance of olive cultivars to *Botryosphaeria dothidea*, causal agent of olive fruit rot in Montenegro. *Crop Prot.* **2013**, *48*, 35–40. [CrossRef]
42. Moral, J.; Agustí-Brisach, C.; Pérez-Rodríguez, M.; Xaviér, C.; Carmen-Raya, M.; Rhouma, A.; Trapero, A. Identification of fungal species associated with branch dieback of olive and resistance of table cultivars to *Neofusicoccum mediterraneum* and *Botryosphaeria dothidea*. *Plant Dis.* **2017**, *101*, 306–316. [CrossRef]
43. Chattaoui, M.; Rhouma, A.; Krid, S.; Ali Triki, M.; Moral, J.; Msallem, M.; Trapero, A. First Report of Fruit Rot of Olives Caused by *Botryosphaeria dothidea* in Tunisia. *Plant Dis.* **2011**, *95*, 770. [CrossRef]
44. Avan, M. Identification and Characterization of Leaf Blight Caused by *Botryosphaeria dothidea* (Moug. Ex Fr.) Ces. & De Not. on Olive Tree (*Olea europaea*) in Adıyaman, Turkiye. *Appl. Fruit Sci.* **2024**, *66*, 1487–1492. [CrossRef]
45. Hernández-Rodríguez, L.; Mondino-Hintz, P.; Alaniz-Ferro, S. Diversity of *Botryosphaeriaceae* species causing stem canker and fruit rot in olive trees in Uruguay. *J. Phytopathol.* **2022**, *170*, 264–277. [CrossRef]
46. Moral, J.; Luque, F.; Trapero, A. First report of *Diplodia seriata*, the anamorph of "*Botryosphaeria*" *obtusa*, causing fruit rot of olive in Spain. *Plant Dis.* **2008**, *92*, 311. [CrossRef]
47. Linaldeddu, B.T.; Rossetto, G.; Maddau, L.; Vatrano, T.; Bregant, C. Diversity and Pathogenicity of *Botryosphaeriaceae* and *Phytophthora* Species Associated with Emerging Olive Diseases in Italy. *Agriculture* **2023**, *13*, 1575. [CrossRef]
48. Carlucci, A.; Raimondo, M.L.; Cibelli, F.; Phillips, A.J.L.; Lops, F. *Pleurostomophora richardsiae*, *Neofusicoccum parvum* and *Phaeoacremonium aleophilum* associated with a decline of olives in southern Italy. *Phytopathol. Mediterr.* **2013**, *52*, 517–527. [CrossRef]
49. van Dyk, M.; Spies, C.F.J.; Mostert, L.; van der Rijst, M.; du Plessis, I.L.; Moyo, P.; van Jaarsveld, W.J.; Halleen, F. Pathogenicity Testing of Fungal Isolates Associated with Olive Trunk Diseases in South Africa. *Plant Dis.* **2021**, *105*, 4060–4073. [CrossRef] [PubMed]
50. Ammad, F.; Moumen, O.; Gasem, A.; Othmane, S.; Hisashi, K.-N.; Zebib, B.; Merah, O. The potency of lemon (*Citrus limon* L.) essential oil to control some fungal diseases of grapevine wood. *Comptes Rendus Biol.* **2018**, *341*, 97–101. [CrossRef] [PubMed]
51. Li, J.; Fu, S.; Fan, G.; Li, D.; Yang, S.; Peng, L.; Pan, S. Active compound identification by screening 33 essential oil monomers against *Botryosphaeria dothidea* from postharvest kiwifruit and its potential action mode. *Pestic. Biochem. Physiol.* **2021**, *179*, 104957. [CrossRef] [PubMed]
52. Zhang, Z.; Xie, Y.; Hu, X.; Shi, H.; Wei, M.; Lin, Z. Antifungal Activity of Monoterpenes against *Botryosphaeria dothidea*. *Nat. Prod. Commun.* **2018**, *13*, 1721–1724. [CrossRef]
53. Wang, D.; Wang, G.; Wang, J.; Zhai, H.; Xue, X. Inhibitory effect and underlying mechanism of cinnamon and clove essential oils on *Botryosphaeria dothidea* and *Colletotrichum gloeosporioides* causing rots in postharvest bagging-free apple fruits. *Front. Microbiol.* **2023**, *14*, 1109028. [CrossRef]
54. Dai, D.J.; Wang, H.D.; Wang, Y.P.; Zhang, C.Q. Management of Chinese hickory (*Carya cathayensis*) trunk canker through effective fungicide application programs and baseline sensitivity of *Botryosphaeria dothidea* to trifloxystrobin. *Australas. Plant Pathol.* **2017**, *46*, 75–82. [CrossRef]
55. Fan, K.; Fu, L.; Liu, H.; Qu, J.; Zhang, G.; Zhang, S.; Qiao, K. Reduced Sensitivity to Tebuconazole in *Botryosphaeria dothidea* Isolates Collected from Major Apple Production Areas of China. *Plant Dis.* **2022**, *106*, 2817–2822. [CrossRef]
56. Latorre, B.A.; Torres, R.; Silva, T.; Elfar, K. Evaluation of the use of wound-protectant fungicides and biological control agents against stem canker (*Neofusicoccum parvum*) of blueberry. *Cienc. Investig. Agrar.* **2013**, *40*, 547–557. [CrossRef]
57. Twizeyimana, M.; McDonald, V.; Mayorquin, J.S.; Wang, D.H.; Na, F.; Akgül, D.S.; Eskalen, A. Effect of Fungicide Application on the Management of Avocado Branch Canker (Formerly Dothiorella Canker) in California. *Plant Dis.* **2013**, *97*, 897–902. [CrossRef]
58. Štůsková, K.; Mondello, V.; Hakalová, E.; Tekielska, D.; Fontaine, F.; Eichmeier, A. Phenolic compounds inhibit viability and infectivity of the grapevine pathogens *Diplodia seriata*, *Eutypa lata*, *Fomitiporia mediterranea*, and *Neofusicoccum parvum*. *Phytopathol. Mediterr.* **2023**, *62*, 307–319. [CrossRef]
59. Tao, D.; Li, Y.; Lu, D.; Luo, Y.; Yu, S.; Ye, S. The essential oil components of *Cinnamomum cassia*: An analysis under different thinning models of plantation *Pinus massoniana*. *J. For. Res.* **2015**, *27*, 707–717. [CrossRef]
60. Le, V.D.; Tran, V.T.; Dang, V.S.; Nguyen, D.T.; Dang, C.H.; Nguyen, T.D. Physicochemical characterizations, antimicrobial activity and non-isothermal decomposition kinetics of *Cinnamomum cassia* essential oils. *J. Essent. Oil Res.* **2019**, *32*, 158–168. [CrossRef]
61. Paw, M.; Begm, T.; Gogoi, R.; Pandey, S.K.; Lal, M. Chemical composition of *Citrus limon* L. burmf peel essential oil from north east India. *J. Essent. Oil Bear. Plants* **2020**, *23*, 337–344. [CrossRef]
62. Soković, M.D.; Vukojević, J.; Marin, P.D.; Brkić, D.D.; Vajs, V.; Van Griensven, L.J.L.D. Chemical composition of essential oils of *Thymus* and *Mentha* species and their antifungal activities. *Molecules* **2009**, *14*, 238–249. [CrossRef]
63. Moghaddam, M.; Pourbaige, M.; Tabar, H.K.; Farhadi, N.; Hosseini, S.M.A. Composition and antifungal activity of peppermint (*Mentha piperita*) essential oil from Iran. *J. Essent. Oil Bear. Plants* **2013**, *16*, 506–512. [CrossRef]
64. Awasthi, P.K.; Dixit, S.C. Chemical compositions of *Ocimum sanctum* Shyama and *Ocimum sanctum* Rama oils from the plains of northern India. *J. Essent. Oil Bear. Plants* **2007**, *10*, 292–296. [CrossRef]

65. Kazem, M. Phytochemical composition of *Thymus vulgaris* L. essential oil. *J. Essent. Oil Bear. Plants* **2015**, *18*, 751–753. [CrossRef]
66. Laghmouchi, Y.; Belmehdi, O.; Senhaji, N.S.; Abrini, J. Chemical composition and antibacterial activity of *Origanum compactum* Benth. essential oils from different areas at northern Morocco. *S. Afr. J. Bot.* **2018**, *115*, 120–125. [CrossRef]
67. Jeldi, L.; Taarabt, K.O.; Mazri, M.A.; Ouahmane, L.; Alfeddy, M.N. Chemical composition, antifungal and antioxidant activities of wild and cultivated *Origanum compactum* essential oils from the municipality of Chaoun, Morocco. *S. Afr. J. Bot.* **2022**, *147*, 852–858. [CrossRef]
68. Karami, J.; Kavosi, M.R.; Babanezhad, M.; Kiapasha, K. Integrated management of the charcoal disease by silviculture, chemical and biological methods in forest parks. *J. Sustain. For.* **2017**, *37*, 429–444. [CrossRef]
69. Rodrigo, S.; Santamaria, O.; Halecker, S.; Lledó, S.; Stadler, M. Antagonism between *Byssochlamys spectabilis* (anamorph *Paecilomyces variotii*) and plant pathogens: Involvement of the bioactive compounds produced by the endophyte. *Ann. Appl. Biol.* **2017**, *171*, 464–476. [CrossRef]
70. Coelho, V.; Nunes, L.; Gouveia, E. Short and long term efficacy and prevalence of *Cryphonectria parasitica* hypovirulent strains released as biocontrol agents of chestnut blight. *Eur. J. Plant Pathol.* **2021**, *159*, 769–781. [CrossRef]
71. Costa, D.; Tavares, R.M.; Baptista, P.; Lino-Neto, T. Cork Oak Endophytic Fungi as Potential Biocontrol agents Against *Biscogniauxia mediterranea* and *Diplodia corticola*. *J. Fungi* **2020**, *6*, 287. [CrossRef]
72. Yangui, I.; Boutiti, M.Z.; Boussaid, M.; Messaoud, C. Essential Oils of Myrtaceae Species Growing Wild in Tunisia: Chemical Variability and Antifungal Activity Against *Biscogniauxia mediterranea*, the Causative Agent of Charcoal Canker. *Chem. Biodivers.* **2017**, *14*, e1700058. [CrossRef]
73. Adams, G.C.; Roux, J.; Wingfield, M.J. *Cytospora* species (*Ascomycota*, *Diaporthales*, *Valsaceae*): Introduced and native pathogens of trees in South Africa. *Australas. Plant Pathol.* **2006**, *35*, 521–548. [CrossRef]
74. Liu, Y.J.; Hu, F.; Chen, L.S.; Xu, S.W. First report of *Nigrospora sphaerica* causing leaf blight on oil tea (*Camellia oleifera*) in China. *Plant Dis.* **2020**, *104*, 3252. [CrossRef]
75. Ismail, S.I.; Razak, N.F.A. First report of *Nigrospora sphaerica* causing leaf spot on watermelon (*Citrullus lanatus* L.) in Malaysia. *Plant Dis.* **2020**, *105*, 488. [CrossRef]
76. Raza, M.; Zhang, Z.-F.; Hyde, K.D.; Diao, Y.-Z.; Cai, L. Culturable plant pathogenic fungi associated with sugarcane in southern China. *Fungal Divers.* **2019**, *99*, 1–104. [CrossRef]
77. Li, W.J.; Hu, M.; Xue, Y.; Li, Z.; Zhang, Y.; Zheng, D.; Lu, G.; Wang, J.; Zhou, J. Five fungal pathogens are responsible for bayberry twig blight and fungicides were screened for disease control. *Microorganisms* **2020**, *8*, 689. [CrossRef]
78. Rahman, A.U.; Ashraf, M.; Choudary, M.I.; Rehman, H.U.; Kazmi, M.H. Antifungal aryltetralin lignans from leaves of *Podophyllum hexandrum*. *Phytochemistry* **1995**, *40*, 427–431. [CrossRef]
79. Idbella, M.; Baronti, S.; Vaccari, F.P.; Abd-ElGawad, A.M.; Bonanomi, G. Long-Term Application of Biochar Mitigates Negative Plant–Soil Feedback by Shaping Arbuscular Mycorrhizal Fungi and Fungal Pathogens. *Microorganisms* **2024**, *12*, 810. [CrossRef] [PubMed]
80. Ivanová, H. *Sordaria fimicola* (Ascomycota, Sordariales) on *Acer palmatum*. *Folia Oecol.* **2015**, *42*, 67–71.
81. Buchenauer, H. Comparative studies on the antifungal activity of triadimefon, triadimenol, fenarimol, nuarimol, imazalil and fluotrimazole in vitro. *J. Plant Dis. Prot.* **1979**, *86*, 341–354.
82. Adebayo, O.; Dang, T.; Bèlanger, A.; Khanizadeh, S. Antifungal studies of sellected essential oils and commercial formulation against *Botrytis cinerea*. *J. Food Res.* **2013**, *2*, 217–226. [CrossRef]
83. Tian, J.; Ban, X.; Zeng, H.; He, J.; Chen, Y.; Wang, Y. The mechanism of antifungal action of essential oil from dill (*Anethum graveolens* L.) on *Aspergillus flavus*. *PLoS ONE* **2012**, *7*, e30147. [CrossRef]
84. El Khoury, R.; Atoui, A.; Verheecke, C.; Maroun, R.; El Khoury, A.; Mathieu, F. Essential oils modulate gene expression and ochratoxin A production in *Aspergillus carbonarius*. *Toxins* **2016**, *8*, 242. [CrossRef]
85. Rodrigues, M.P.; Astoreca, A.L.; de Oliviera, A.A.; Salvato, L.A.; Biscoto, G.L.; Keller, L.A.M.; Rosa, C.A.d.R.; Cavaglieri, L.R.; de Azevedo, M.I.; Keller, K.M. In vitro activity of Neem (*Azadirachta indica*) oil on growth and Ochratoxin A production by *Aspergillus carbonarius* isolates. *Toxins* **2019**, *11*, 579. [CrossRef]
86. Vitoratos, A.; Bilalis, D.; Karkanis, A.; Efthimiadou, A. Antifungal activity of plant essential oils against *Botrytis cinerea*, *Penicillium italicum* and *Penicillium digitatum*. *Not. Bot. Hortic. Agrobot. Cluj-Napoca* **2013**, *41*, 86–92. [CrossRef]
87. Kedia, A.; Jha, D.K.; Dubey, N.K. Plant essential oils as natural fungicides against stored product fungi. the battle against microbial pathogens: Basic science. *Technol. Adv. Educ. Programs* **2015**, *2*, 208–214.
88. Harris, R. Progress with superficial mycoses using essential oils. *Int. J. Aromather.* **2002**, *12*, 83–91. [CrossRef]
89. Bažok, R. Je li održiva uporaba pesticida doista održiva? *Glas. Biljn. Zaštite* **2020**, *20*, 384–389.

Table S1. List of used essential oils (EOs), Latin names of plant species, and EO components present in concentrations greater than 0.1%, as well as the commercial names of used fungicides, type of fungicide, and active ingredients.

Essential oil source plant	Latin name	Components	Content (%)
Chinese cinnamon, cassia-bark	*Cinnamomum aromaticum* Nees (syn. *Cinnamomum cassia* (L.) J.Presl)	E-cinnamaldehyde	78.32
		Trans-o-methoxy-cinnamaldehyde	4.15
		Cinnamyl acetate	2.54
		Aromatic compound	1.87
		Coumarin	1.77
		Benzaldehyde	1.73
		Benzene propanal	0.96
		Phenylethyl alcohol	0.77
		Salicylic aldehyde	0.56
		α-copaene	0.54
		Z-cinnamaldehyde	0.43
		Delta-cadinene + sesquiterpene	0.38
		2-methoxy-benzaldehyde	0.35
		2-methyl-benzofuran	0.27
		Styrene	0.26
		Cinnamic alcohol	0.25
		β-caryophyllene	0.24
		2-methoxyphenyl acetone	0.22
		β-bisabolene	0.21
		γ-muurolene	0.21
		Allo-aromadendrene + acetophenone	0.19
		Nerolidol	0.18
		α-muurolene	0.14
		2-phenyl ethyl acetate	0.12
		Ledene	0.11
		Cinnamic acid	0.11
		α-pinene	0.11
		Borneol	0.10
Holy basil	*Ocimum tenuiflorum* L. (syn. *Ocimum sanctum* L.)	Eugenol	56.60
		β -caryophyllene	29.82
		Estragole	4.31
		Geraniol	1.03
		Caryophyllene oxide	0.78
		Citronellol	0.77
		α-copaene	0.71
		1,8-cineole	0.58
		Delta-cadinene + sesquiterpene	0.39
		Sesquiterpene epoxide	0.38
		Sesquiterpenol	0.24
		α-humulene	0.21
		Caryophylla-3.7-dien-6-ol	0.21
		Sesquiterpene epoxide	0.17
		α-pinene	0.15
		Limonene	0.15

		α-cubebene	0.14
		Linalool	0.14
		6,7-humulene epoxide	0.14
		Calamenene	0.13
		α-farnesene	0.12
		Compound mw=164	0.11
		Sesquiterpene epoxide	0.11
		Caryophyllenol	0.10
		γ-eudesmol	0.10
Lemon	*Citrus × limon* (syn. *Citrus limonum* Risso)	Limonene	68.90
		β-pinene	11.84
		γ-terpinene	7.94
		Sabinene	1.86
		α-pinene	1.75
		β-myrcene	1.52
		Geranial	1.01
		Neral	0.61
		α-thujene	0.46
		β-bisabolene	0.43
		p-cymene	0.39
		1,8-cineole	0.36
		β-phellandrene	0.36
		Terpinolene	0.36
		Neryl acetate	0.31
		β-caryophyllene	0.30
		Terpinene-4-ol	0.30
		α-trans-bergamotene	0.27
		α-terpineol	0.24
		α-terpinene	0.20
		Geranyl acetate	0.16
		Linalool	0.11
		Nonanal	0.10
Oregano	*Origanum compactum* Benth	Carvacrol	57.58
		γ-terpinene	14.12
		Thymol	8.21
		p-cymene	7.84
		β-caryophyllene	1.72
		α-terpinene	1.64
		β-myrcene	1.60
		Linalool	1.38
		α-thujene	0.90
		α-pinene	0.56
		Terpinene-4-ol	0.42
		1-octen-3-ol	0.26
		α-phellandrene + psi-limonene	0.25
		Limonene	0.22
		α-terpineol	0.20
		β-phellandrene	0.19
		Trans-thujanol	0.19
		Borneol	0.17
		Methyl carvacrol ether	0.13

		Caryophyllene oxide	0.13
		β-pinene	0.12
		Trans- β-ocimene	0.11
		3-octanone	0.10
Peppermint	*Mentha × piperita* L.	Menthol	36.37
		Menthone	24.08
		1,8-cineole	5.51
		Menthyl acetate	5.48
		Isomenthone	3.64
		β-caryophyllene	3.05
		Neomenthol	2.91
		Limonene	2.47
		Menthofuran	2.07
		Pulegone	1.21
		β-pinene	1.08
		Germacrene D	1.08
		Piperitone	1.04
		α-pinene	0.97
		Terpinene-4-ol	0.64
		Neoisomenthol	0.61
		α-terpineol	0.55
		Sabinene	0.46
		γ-terpinene	0.35
		β-bourbonene	0.33
		Lavandulol	0.25
		Trans-thujanol	0.24
		β-myrcene	0.23
		Bicyclogermacrene	0.22
		Isomenthol	0.21
		Trans-isopiperitenol	0.21
		3-octanol	0.19
		Isomenthyl acetate	0.19
		Epsilon-cadinene	0.19
		α-terpinene	0.18
		p-cymene	0.18
		E-β-farnesene	0.18
		Cis-β-ocimene	0.17
		Linalool	0.17
		Isopulegol	0.17
		Neomenthyl acetate	0.16
		1-nonanol	0.13
		α-humulene	0.13
		β-elemene	0.12
		Germacrene A	0.11
		γ-muurolene	0.11
		Terpinolene	0.10
		Sesquiterpene	0.10
Thyme, garden thyme	*Thymus vulgaris* L.	Thymol	45.73
		p-cymene	17.03
		γ-terpinene	10.05
		Linalool	4,52

		Carvacrol	4.10
		β-caryophyllene	2.09
		Borneol	2.07
		Terpinene-4-ol	1.55
		β-myrcene	1.52
		α-terpinene	1.23
		α-thujene	1.22
		α-pinene	1.16
		Camphene	1.15
		Camphor	0.66
		Limonene	0.37
		α-terpineol	0.31
		β-pinene	0.25
		Methyl thymol ether	0.24
		β-phellandrene	0.23
		Trans-thujanol	0.20
		Caryophyllene oxide	0.20
		α-phellandrene	0.18
		Isocarvacrol	0.14
		Terpinolene	0.12
		Cis-linalool oxide	0.12
		Isothymol	0.12
		Trans-linalool oxide	0.10
		Cis-thujanol	0.10
		Aromadendrene	0.10
		Delta-cadinene + sesquiterpene	0.10
Commercial name of the fungicide	**Type of fungicide**	**Active ingredient**	**g/kg**
Cabrio TOP	Dithiocarbamaten	Metiram	550
	Strobilurin	Pyraclostrobin	50
Nativo 75WG	Strobilurin	Trifloxystrobin	250
	Triazole	Tebuconazole	500

Table S2. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Botryosphaeria dothidea* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$).

Treatment	Day 2	Day 4	Day 10
	Concentration: 0.1%		
Chinese cinnamon	**0.00 ± 0.00 d**	0.00 ± 0.00 d	0.00 ± 0.00 c
Holy basil	6.75 ± 0.00 c	75.00 ± 11.00 b	86.00 ± 0.00 a
Lemon	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 c
Peppermint	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 c
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 c
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 c
Eugenol	0.00 ± 0.00 d	10.00 ± 4.00 c	86.00 ± 0.00 a
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	27.00 ± 0.00 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Cabrio TOP	24.33 ± 4.51 b	66.33 ± 2.88 b	77.66 ± 7.37 b
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 d	3.66 ± 0.58 c
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	3.99	9.37	5.74
	Concentration: 0.2%		
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Lemon	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Peppermint	72.50 ± 4.50 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 d	71.33 ± 8.74 b	86.00 ± 0.00 a
Cabrio TOP	24.33 ± 4.51 c	66.33 ± 2.88 b	77.66 ± 7.37 b
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 c	3.67 ± 0.58 c
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	4.95	7.15	5.74
	Concentration: 0.5%		
Chinese cinnamon	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
Lemon	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
Peppermint	0.67 ± 1.15 c	73.66 ± 13.65 b	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a

Thymol	0.00 ± 0.00 c	2.33 ± 1.53 c	52.33 ± 5.13 c
Cabrio TOP	24.33 ± 4.51 b	66.33 ± 2.88 b	77.66 ± 7.37 b
Nativo 75WG	0.00 ± 0.00 c	0.00 ± 0.00 c	3.67 ± 0.58 d
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	3.62	10.9	6.99
		Concentration: 0.75%	
Chinese cinnamon	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 d
Lemon	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 d
Peppermint	0.67 ± 1.15 c	3.00 ± 1.00 c	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 d
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 c	0.00 ± 0.00 d	39.00 ± 3.00 c
Cabrio TOP	24.33 ± 4.51 b	66.33 ± 2.88 b	77.66 ± 7.37 b
Nativo 75WG	0.00 ± 0.00 c	0.00 ± 0.00 d	3.67 ± 0.58 d
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	3.62	2.37	6.19
		Concentration: 1.0%	
Chinese cinnamon	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 d
Lemon	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 d
Peppermint	0.67 ± 1.15 c	3.33 ± 1.53 c	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 d
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 c	0.00 ± 0.00 d	33.67 ± 7.51 c
Cabrio TOP	24.33 ± 4.51 b	66.33 ± 2.88 b	77.66 ± 7.37 b
Nativo 75WG	0.00 ± 0.00 c	0.00 ± 0.00 d	3.67 ± 0.58 d
Control	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 d
LSD	3.62	2.54	8.18

Table S3. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Botryosphaeria dothidea*.

Essential oil/component/fungicide	Concentration (%)	Inhibition (%)		
		Day 2	Day 4	Day 10
Chinese cinnamon	0.1	100	100	100
	0.2	100	100	100
	0.5	100	100	100
	0.75	100	100	100
	1.0	100	100	100
Holy basil	0.1	92.2	12.8	0

	0.2	100	100	100
	0.5	100	100	100
	0.75	100	100	100
	1.0	100	100	100
Lemon	0.1	0	0	0
	0.2	0	0	0
	0.5	0	0	0
	0.75	0	0	0
	1.0	0	0	0
Oregano	0.1	100	100	100
	0.2	100	100	100
	0.5	100	100	100
	0.75	100	100	100
	1.0	100	100	100
Peppermint	0.1	0	0	0
	0.2	15.7	0	0
	0.5	99.2	14.3	0
	0.75	99.2	96.5	0
	1.0	99.2	96.1	0
Thyme	0.1	100	100	100
	0.2	100	100	100
	0.5	100	100	100
	0.75	100	100	100
	1.0	100	100	100
Carvacrol	0.1	100	100	100
	0.2	100	100	100
	0.5	100	100	100
	0.75	100	100	100
	1.0	100	100	100
E-cinnamaldehyde	0.1	100	100	100
	0.2	100	100	100
	0.5	100	100	100
	0.75	100	100	100
	1.0	100	100	100
Eugenol	0.1	100	88.4	0
	0.2	100	100	100
	0.5	100	100	100
	0.75	100	100	100
	1.0	100	100	100
Limonene	0.1	0	0	0
	0.2	0	0	0
	0.5	0	0	0
	0.75	0	0	0
	1.0	0	0	0
Menthol	0.1	0	0	0
	0.2	0	0	0
	0.5	0	0	0
	0.75	0	0	0
	1.0	0	0	0
Thymol	0.1	68.6	0	0
	0.2	100	17.1	0

	0.5	100	97.3	39.1
	0.75	100	100	54.7
	1.0	100	100	60.9
Cabrio TOP	0.2	71.7	22.9	9.7
Nativo 75WG	0.02	100	100	95.7

Table S4. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Diplodia mutila* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$).

Treatment	Day 2	Day 4	Day 10
	Concentration: 0.1%		
Chinese cinnamon	**0.00 ± 0.00 e**	0.00 ± 0.00 e	0.00 ± 0.00 d
Holy basil	21.00 ± 0.00 d	86.00 ± 0.00 a	86.00 ± 0.00 a
Lemon	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 e	0.00 ± 0.00 e	0.00 ± 0.00 d
Peppermint	76.33 ± 6.43 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 e	23.00 ± 1.00 c	86.00 ± 0.00 a
Carvacrol	0.00 ± 0.00 e	0.00 ± 0.00 e	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 e	0.00 ± 0.00 e	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 e	0.00 ± 0.00 e	0.00 ± 0.00 d
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	42.67 ± 1.15 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Cabrio TOP	15.00 ± 2.65 d	36.33 ± 0.58 b	71.33 ± 1.15 b
Nativo 75WG	0.00 ± 0.00 e	8.30 ± 1.15 d	20.00 ± 2.00 c
Control	79.67 ± 6.11 ab	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	7.25	1.27	1.79
	Concentration: 0.2%		
Chinese cinnamon	0.00 ± 0.00 e	0.00 ± 0.00 e	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 e	0.00 ± 0.00 e	0.00 ± 0.00 d
Lemon	64.67 ± 8.32 b	78.67 ± 6.66 b	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 e	0.00 ± 0.00 e	0.00 ± 0.00 d
Peppermint	64.67 ± 7.77 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 e	0.00 ± 0.00 e	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 e	0.00 ± 0.00 e	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 e	0.00 ± 0.00 e	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 e	0.00 ± 0.00 e	0.00 ± 0.00 d
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	35.33 ± 2.08 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Cabrio TOP	15.00 ± 2.65 d	36.33 ± 0.58 c	71.33 ± 1.15 b
Nativo 75WG	0.00 ± 0.00 e	8.33 ± 1.15 d	20.00 ± 2.00 c
Control	79.67 ± 6.11 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	10.37	5.27	1.79
	Concentration: 0.5%		
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 f	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 f	0.00 ± 0.00 d
Lemon	60.33 ± 13.50 b	77.67 ± 7.64 b	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 f	0.00 ± 0.00 d
Peppermint	24.00 ± 4.00 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 f	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 f	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 f	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 f	0.00 ± 0.00 d
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a

Thymol	0.00 ± 0.00 d	19.33 ± 3.21 d	86.00 ± 0.00 a
Cabrio TOP	15.00 ± 2.65 c	36.33 ± 0.58 c	71.33 ± 1.15 b
Nativo 75WG	0.00 ± 0.00 d	8.33 ± 1.15 e	20.00 ± 2.00 c
Control	79.67 ± 6.11 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	12.1	6.52	1.79
		Concentration: 0.75%	
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Lemon	41.33 ± 6.43 b	75.67 ± 14.43 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Peppermint	16.67 ± 2.52 c	62.33 ± 8.14 b	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	77.00 ± 7.94 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 d	9.00 ± 2.65 d	86.00 ± 0.00 a
Cabrio TOP	15.00 ± 2.65 c	36.33 ± 0.58 c	71.33 ± 1.15 b
Nativo 75WG	0.00 ± 0.00 d	8.33 ± 1.15 d	20.00 ± 2.00 c
Control	79.67 ± 6.11 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	9.67	13.08	1.79
		Concentration: 1.0%	
Chinese cinnamon	0.00 ± 0.00 e	0.00 ± 0.00 f	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 e	0.00 ± 0.00 f	0.00 ± 0.00 d
Lemon	40.00 ± 1.00 c	68.67 ± 9.07 b	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 e	0.00 ± 0.00 f	0.00 ± 0.00 d
Peppermint	8.33 ± 2.31 de	44.00 ± 3.46 c	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 e	0.00 ± 0.00 f	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 e	0.00 ± 0.00 f	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 e	0.00 ± 0.00 f	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 e	0.00 ± 0.00 f	0.00 ± 0.00 d
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	71.33 ± 8.33 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 e	0.00 ± 0.00 f	18.33 ± 0.58 c
Cabrio TOP	15.00 ± 2.65 d	36.33 ± 0.58 d	71.33 ± 1.15 b
Nativo 75WG	0.00 ± 0.00 e	8.33 ± 1.15 e	20.00 ± 2.00 c
Control	79.67 ± 6.11 ab	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	8.51	7.61	1.85

Table S5. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Diplodia mutila*.

Essential oil/component/fungicide	Concentration (%)	Concentration	Inhibition (%)		
			Day 2	Day 4	Day 10
Chinese cinnamon	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Holy basil	0.1	10	73.6	0	0
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Lemon	0.1	10	0	0	0
	0.2	20	18.8	8.5	0
	0.5	50	24.3	9.7	0
	0.75	75	48.1	12.0	0
	1.0	100	49.8	20.2	0
Oregano	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Peppermint	0.1	10	4.7	0	0
	0.2	20	18.8	0	0
	0.5	50	69.9	0	0
	0.75	75	79.1	27.5	0
	1.0	100	89.5	48.8	0
Thyme	0.1	10	100	73.3	0
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Carvacrol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
E-cinnamaldehyde	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Eugenol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100

Limonene	0.1	10	0	0	0
	0.2	20	0	0	0
	0.5	50	0	0	0
	0.75	75	0	0	0
	1.0	100	0	0	0
Menthol	0.1	10	0	0	0
	0.2	20	0	0	0
	0.5	50	0	0	0
	0.75	75	5.9	0	0
	1.0	100	10.5	0	0
Thymol	0.1	10	46.4	0	0
	0.2	20	55.6	0	0
	0.5	50	100	77.5	0
	0.75	75	100	89.5	0
	1.0	100	100	100	78.7
Cabrio TOP	0.2	20	81.2	57.8	17.1
Nativo 75WG	0.02	2	100	90.3	76.7

Table S6. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Diplodia seriata* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$).

Treatment	Day 2	Day 4	Day 10
	Concentration: 0.1%		
Chinese cinnamon	**0.00 ± 0.00 d**	0.00 ± 0.00 e	0.00 ± 0.00 e
Holy basil	14.33 ± 6.11 c	76.67 ± 8.14 b	86.00 ± 0.00 a
Lemon	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 e
Peppermint	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 e
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 e
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 e
Eugenol	0.00 ± 0.00 d	18.00 ± 4.36 d	69.33 ± 7.02 b
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	62.67 ± 1.53 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Cabrio TOP	15.00 ± 3.00 c	32.67 ± 0.58 c	59.67 ± 1.53 c
Nativo 75WG	1.33 ± 1.15 d	14.00 ± 3.00 d	24.33 ± 4.04 d
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	5.49	7.56	6.41
	Concentration: 0.2%		
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Lemon	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Peppermint	83.33 ± 3.06 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	8.67 ± 1.15 c	70.67 ± 7.57 b	86.00 ± 0.00 a
Cabrio TOP	15.00 ± 3.00 b	32.67 ± 0.58 c	59.67 ± 1.53 b
Nativo 75WG	1.33 ± 1.15 d	14.00 ± 3.00 d	24.33 ± 4.04 c
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	3.56	6.34	3.36
	Concentration: 0.5%		
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Lemon	85.67 ± 0.58 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Peppermint	72.00 ± 6.56 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a

Thymol	5.33 ± 2.08 d	67.33 ± 7.57 b	86.00 ± 0.00 a
Cabrio TOP	15.00 ± 3.00 c	32.67 ± 0.58 c	59.67 ± 1.53 b
Nativo 75WG	1.33 ± 1.15 d	14.00 ± 3.00 d	24.33 ± 4.04 c
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	5.92	6.34	3.36
		Concentration: 0.75%	
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Lemon	81.33 ± 5.03 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Peppermint	36.67 ± 5.86 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	4.00 ± 0.00 d	4.67 ± 1.15 d	86.00 ± 0.00 a
Cabrio TOP	15.00 ± 3.00 c	32.67 ± 0.58 b	59.67 ± 1.53 b
Nativo 75WG	1.33 ± 1.15 d	14.00 ± 3.00 c	24.33 ± 4.04 c
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	6.49	2.54	3.36
		Concentration: 1.0%	
Chinese cinnamon	0.00 ± 0.00 c	0.00 ± 0.00 f	0.00 ± 0.00 e
Holy basil	0.00 ± 0.00 c	0.00 ± 0.00 f	0.00 ± 0.00 e
Lemon	63.33 ± 21.19 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 c	0.00 ± 0.00 f	0.00 ± 0.00 e
Peppermint	0.00 ± 0.00 c	50.67 ± 3.06 b	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 c	0.00 ± 0.00 f	0.00 ± 0.00 e
Carvacrol	0.00 ± 0.00 c	0.00 ± 0.00 f	0.00 ± 0.00 e
E-cinnamaldehyde	0.00 ± 0.00 c	0.00 ± 0.00 f	0.00 ± 0.00 e
Eugenol	0.00 ± 0.00 c	0.00 ± 0.00 f	0.00 ± 0.00 e
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	4.00 ± 0.00 c	4.33 ± 0.58 e	39.00 ± 1.00 c
Cabrio TOP	15.00 ± 3.00 c	32.67 ± 0.58 c	59.67 ± 1.53 b
Nativo 75WG	1.33 ± 1.15 c	14.00 ± 3.00 d	24.33 ± 4.04 d
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	16.66	3.39	3.45

Table S7. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Diplodia seriata*.

Essential oil/component/fungicide	Concentration (%)	Concentration	Inhibition (%)		
			Day 2	Day 4	Day 10
Chinese cinnamon	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Holy basil	0.1	10	83.3	10.9	0
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Lemon	0.1	10	0	0	0
	0.2	20	0	0	0
	0.5	50	0.4	0	0
	0.75	75	5.4	0	0
	1.0	100	26.4	0	0
Oregano	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Peppermint	0.1	10	0	0	0
	0.2	20	3.1	0	0
	0.5	50	16.3	0	0
	0.75	75	57.4	0	0
	1.0	100	100	41.1	0
Thyme	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Carvacrol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
E-cinnamaldehyde	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Eugenol	0.1	10	100	79.1	19.4
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100

	1.0	100	100	100	100
Limonene	0.1	10	0	0	0
	0.2	20	0	0	0
	0.5	50	0	0	0
	0.75	75	0	0	0
	1.0	100	0	0	0
Menthol	0.1	10	0	0	0
	0.2	20	0	0	0
	0.5	50	0	0	0
	0.75	75	0	0	0
	1.0	100	0	0	0
Thymol	0.1	10	27.1	0	0
	0.2	20	89.9	17.8	0
	0.5	50	93.8	21.7	0
	0.75	75	95.3	94.6	0
	1.0	100	95.3	94.9	54.7
Cabrio TOP	0.2	20	82.6	62.0	30.6
Nativo 75WG	0.02	2	98.4	83.7	71.7

Table S8. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Dothiorella iberica* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$).

Treatment	Day 2	Day 4	Day 10
		Concentration: 0.1%	
Chinese cinnamon	**0.00 ± 0.00 d**	0.00 ± 0.00 c	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Lemon	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Peppermint	0.00 ± 0.00 d	74.00 ± 10.39 b	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Limonene	53.33 ± 6.03 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	43.67 ± 3.06 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 d	0.00 ± 0.00 c	86.00 ± 0.00 a
Cabrio TOP	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	5.25	8.07	0
		Concentration: 0.2%	
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Lemon	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Peppermint	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Limonene	53.33 ± 2.65 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	37.33 ± 4.04 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 d	0.00 ± 0.00 b	86.00 ± 0.00 a
Cabrio TOP	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	3.75	0	0
		Concentration: 0.5%	
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Lemon	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Peppermint	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Limonene	47.67 ± 7.64 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	36.33 ± 4.04 c	86.00 ± 0.00 a	86.00 ± 0.00 a

Thymol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Cabrio TOP	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	6.71	0	0
		Concentration: 0.75%	
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Lemon	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Peppermint	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Limonene	47.33 ± 3.21 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	35.00 ± 5.29 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Cabrio TOP	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	4.81	0	0
		Concentration: 1.0%	
Chinese cinnamon	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Lemon	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Oregano	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Peppermint	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Thyme	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Carvacrol	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Limonene	28.67 ± 2.52 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	30.00 ± 3.61 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Cabrio TOP	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Nativo 75WG	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	3.42	0	0

Table S9. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Dothiorella iberica*.

Essential oil/component/fungicide	Concentration (%)	Concentration	Inhibition (%)		
			Day 2	Day 4	Day 10
Chinese cinnamon	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Holy basil	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Lemon	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Oregano	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Peppermint	0.1	10	100	13.9	0
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Thyme	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Carvacrol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
E-cinnamaldehyde	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Eugenol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Limonene	0.1	10	38.0	0	0

	0.2	20	38.4	0	0
	0.5	50	44.6	0	0
	0.75	75	45.0	0	0
	1.0	100	66.7	0	0
Menthol	0.1	10	49.2	0	0
	0.2	20	56.6	0	0
	0.5	50	57.8	0	0
	0.75	75	59.3	0	0
	1.0	100	65.1	0	0
Thymol	0.1	10	100	100	0
	0.2	20	100	100	0
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Cabrio TOP	0.2	20	100	100	100
Nativo 75WG	0.02	2	100	100	100

Table S10. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Dothiorella sarmentorum* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$).

Treatment	Day 2	Day 4	Day 10
	Concentration: 0.1%		
Chinese cinnamon	**0.00 ± 0.00 c**	0.00 ± 0.00 c	0.00 ± 0.00 b
Holy basil	41.33 ± 6.11 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Lemon	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b
Peppermint	0.00 ± 0.00 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Thyme	3.33 ± 1.53 c	24.33 ± 12.66 b	86.00 ± 0.00 a
Carvacrol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Cabrio TOP	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b
Nativo 75WG	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	4.89	9.84	0
	Concentration: 0.2%		
Chinese cinnamon	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 c	0.00 ± 0.00 b	86.00 ± 0.00 a
Lemon	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Peppermint	0.00 ± 0.00 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 c	0.00 ± 0.00 b	86.00 ± 0.00 a
Carvacrol	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	62.00 ± 8.66 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Cabrio TOP	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Nativo 75WG	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	6.73	0	0
	Concentration: 0.5%		
Chinese cinnamon	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Holy basil	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Lemon	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Peppermint	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Thyme	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Carvacrol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
E-cinnamaldehyde	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Eugenol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a

Thymol	15.33 ± 4.04 b	67.00 ± 9.00 b	67.00 ± 9.00 b
Cabrio TOP	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Nativo 75WG	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	3.14	6.99	6.99
		Concentration: 0.75%	
Chinese cinnamon	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Holy basil	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Lemon	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Peppermint	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Thyme	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Carvacrol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
E-cinnamaldehyde	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Eugenol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	77.00 ± 7.94 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 c	2.00 ± 0.00 b	27.33 ± 3.51 b
Cabrio TOP	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Nativo 75WG	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	6.17	0	2.73
		Concentration: 1.0%	
Chinese cinnamon	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Lemon	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Peppermint	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Thyme	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Carvacrol	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	71.33 ± 8.34 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Cabrio TOP	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Nativo 75WG	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	6.47	0	0

Table S11. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Dothiorella sarmentorum*.

Essential oil/component/fungicide	Concentration (%)	Concentration	Inhibition (%)		
			Day 2	Day 4	Day 10
Chinese cinnamon	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Holy basil	0.1	10	51.9	0	0
	0.2	20	100	100	0
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Lemon	0.1	10	0	0	0
	0.2	20	0	0	0
	0.5	50	0	0	0
	0.75	75	0	0	0
	1.0	100	0	0	0
Oregano	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Peppermint	0.1	10	100	0	0
	0.2	20	100	0	0
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Thyme	0.1	10	96.1	71.7	0
	0.2	20	100	100	0
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Carvacrol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
E-cinnamaldehyde	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Eugenol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Limonene	0.1	10	0	0	0

	0.2	20	0	0	0
	0.5	50	0	0	0
	0.75	75	0	0	0
	1.0	100	0	0	0
Menthol	0.1	10	0	0	0
	0.2	20	0	0	0
	0.5	50	0	0	0
	0.75	75	10.5	0	0
	1.0	100	17.1	0	0
Thymol	0.1	10	0	0	0
	0.2	20	27.9	0	0
	0.5	50	82.2	22.1	22.1
	0.75	75	100	97.7	68.2
	1.0	100	100	100	100
Cabrio TOP	0.2	20	100	100	100
Nativo 75WG	0.02	2	100	100	100

Table S12. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Neofusicoccum parvum* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$).

Treatment	Day 2	Day 4	Day 10
	Concentration: 0.1%		
Chinese cinnamon	**0.00 ± 0.00 f**	0.00 ± 0.00 d	0.00 ± 0.00 c
Holy basil	42.50 ± 2.50 d	50.33 ± 6.11 b	86.00 ± 0.00 a
Lemon	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 f	0.00 ± 0.00 d	0.00 ± 0.00 c
Peppermint	27.33 ± 4.51 e	86.00 ± 0.00 a	86.00 ± 0.00 a
Thyme	70.33 ± 5.13 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Carvacrol	0.00 ± 0.00 f	0.00 ± 0.00 d	0.00 ± 0.00 c
E-cinnamaldehyde	0.00 ± 0.00 f	0.00 ± 0.00 d	0.00 ± 0.00 c
Eugenol	67.67 ± 5.51 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	58.67 ± 4.04 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Cabrio TOP	37.33 ± 4.04 d	84.33 ± 2.89 a	86.00 ± 0.00 a
Nativo 75WG	0.00 ± 0.00 f	5.33 ± 1.15 c	6.67 ± 2.52 b
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	8.36	5.33	1.96
	Concentration: 0.2%		
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Lemon	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Peppermint	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Eugenol	0.00 ± 0.00 d	40.67 ± 7.64 b	86.00 ± 0.00 a
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	57.67 ± 3.79 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Cabrio TOP	37.33 ± 4.04 c	84.33 ± 2.89 a	86.00 ± 0.00 a
Nativo 75WG	0.00 ± 0.00 d	5.33 ± 1.15 c	6.67 ± 2.52 b
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	4.3	6.41	1.96
	Concentration: 0.5%		
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Lemon	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Peppermint	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a

Thymol	42.67 ± 1.53 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Cabrio TOP	37.33 ± 4.04 c	84.33 ± 2.59 a	86.00 ± 0.00 a
Nativo 75WG	0.00 ± 0.00 d	5.33 ± 1.15 b	6.67 ± 2.52 b
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	3.36	2.42	1.96
		Concentration: 0.75%	
Chinese cinnamon	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
Lemon	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
Peppermint	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
Thyme	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 c	0.00 ± 0.00 c	65.67 ± 5.69 b
Cabrio TOP	37.33 ± 4.04 b	84.33 ± 2.89 a	86.00 ± 0.00 a
Nativo 75WG	0.00 ± 0.00 c	5.33 ± 1.15 b	6.67 ± 2.52 c
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	3.14	2.41	4.83
		Concentration: 1.0%	
Chinese cinnamon	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
Lemon	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
Peppermint	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
Thyme	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 c	0.00 ± 0.00 c	51.00 ± 2.00 b
Cabrio TOP	37.33 ± 4.04 b	84.33 ± 2.89 a	86.00 ± 0.00 a
Nativo 75WG	0.00 ± 0.00 c	5.33 ± 1.15 b	6.67 ± 2.52 c
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	3.14	2.42	2.49

Table S13. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Neofusicoccum parvum*.

Essential oil/component/fungicide	Concentration (%)	Concentration	Inhibition (%)		
			Day 2	Day 4	Day 10
Chinese cinnamon	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Holy basil	0.1	10	50.6	41.5	0
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Lemon	0.1	10	0	0	0
	0.2	20	0	0	0
	0.5	50	0	0	0
	0.75	75	0	0	0
	1.0	100	0	0	0
Oregano	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Peppermint	0.1	10	68.2	0	0
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Thyme	0.1	10	18.2	0	0
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Carvacrol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
E-cinnamaldehyde	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Eugenol	0.1	10	21.3	0	0
	0.2	20	100	52.7	0
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Limonene	0.1	10	0	0	0

	0.2	20	0	0	0
	0.5	50	0	0	0
	0.75	75	0	0	0
	1.0	100	0	0	0
Menthol	0.1	10	0	0	0
	0.2	20	0	0	0
	0.5	50	0	0	0
	0.75	75	0	0	0
	1.0	100	0	0	0
Thymol	0.1	10	31.8	0	0
	0.2	20	32.9	0	0
	0.5	50	50.4	0	0
	0.75	75	100	100	23.6
	1.0	100	100	100	40.7
Cabrio TOP	0.2	20	56.6	1.9	0
Nativo 75WG	0.02	2	100	93.8	92.2

Table S14. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Biscogniauxia mediterranea* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$).

Treatment	Day 2	Day 4	Day 10
	Concentration: 0.1%		
Chinese cinnamon	**0.00 ± 0.00 d**	0.00 ± 0.00 d	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Lemon	32.33 ± 2.89 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Peppermint	8.50 ± 5.50 c	70.00 ± 9.17 b	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Limonene	51.67 ± 4.04 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	47.67 ± 1.53 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	31.67 ± 4.04 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Cabrio TOP	2.00 ± 1.00 cd	9.33 ± 1.53 c	20.67 ± 1.53 b
Nativo 75WG	0.00 ± 0.00 d	3.33 ± 2.08 cd	11.33 ± 3.51 c
Control	51.33 ± 0.58 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	6.72	7.39	2.98
	Concentration: 0.2%		
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Lemon	28.33 ± 1.53 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Peppermint	29.00 ± 2.00 c	39.33 ± 3.06 b	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Limonene	43.33 ± 3.79 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	45.67 ± 1.53 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	29.00 ± 2.65 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Cabrio TOP	2.00 ± 1.00 d	9.33 ± 1.53 c	20.67 ± 1.53 b
Nativo 75WG	0.00 ± 0.00 d	3.33 ± 2.08 d	11.33 ± 3.51 c
Control	51.33 ± 0.58 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	4.35	3.11	2.98
	Concentration: 0.5%		
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Lemon	17.33 ± 4.16 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Peppermint	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Limonene	43.00 ± 8.67 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	39.33 ± 2.89 b	86.00 ± 0.00 a	86.00 ± 0.00 a

Thymol	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Cabrio TOP	2.00 ± 1.00 d	9.33 ± 1.53 b	20.67 ± 1.53 b
Nativo 75WG	0.00 ± 0.00 d	3.33 ± 2.08 c	11.33 ± 3.51 c
Control	51.33 ± 0.58 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	7.85	2.01	2.98
		Concentration: 0.75%	
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Lemon	17.00 ± 3.61 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Peppermint	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Limonene	37.00 ± 0.00 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	38.00 ± 1.73 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 d
Cabrio TOP	2.00 ± 1.00 d	9.33 ± 1.53 b	20.67 ± 1.53 b
Nativo 75WG	0.00 ± 0.00 d	3.33 ± 2.08 c	11.33 ± 3.51 c
Control	51.33 ± 0.58 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	3.23	2.01	2.98
		Concentration: 1.0%	
Chinese cinnamon	0.00 ± 0.00 e	0.00 ± 0.00 d	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 e	0.00 ± 0.00 d	0.00 ± 0.00 d
Lemon	11.67 ± 2.89 d	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 e	0.00 ± 0.00 d	0.00 ± 0.00 d
Peppermint	0.00 ± 0.00 e	0.00 ± 0.00 d	0.00 ± 0.00 d
Thyme	0.00 ± 0.00 e	0.00 ± 0.00 d	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 e	0.00 ± 0.00 d	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 e	0.00 ± 0.00 d	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 e	0.00 ± 0.00 d	0.00 ± 0.00 d
Limonene	36.00 ± 0.00 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	25.00 ± 2.00 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 e	0.00 ± 0.00 d	0.00 ± 0.00 d
Cabrio TOP	2.00 ± 1.00 e	9.33 ± 1.53 b	20.67 ± 1.53 b
Nativo 75WG	0.00 ± 0.00 e	3.33 ± 2.08 c	11.33 ± 3.51 c
Control	51.33 ± 0.58 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	2.87	2.01	2.98

Table S15. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Biscogniauxia mediterranea*.

Essential oil/component/fungicide	Concentration (%)	Concentration	Inhibition (%)		
			Day 2	Day 4	Day 10
Chinese cinnamon	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Holy basil	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Lemon	0.1	10	37.0	0	0
	0.2	20	42.9	0	0
	0.5	50	66.2	0	0
	0.75	75	66.9	0	0
	1.0	100	77.3	0	0
Oregano	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Peppermint	0.1	10	83.4	18.6	0
	0.2	20	43.5	54.3	0
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Thyme	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Carvacrol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
E-cinnamaldehyde	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Eugenol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100

Limonene	0.1	10	2.4	0	0
	0.2	20	15.6	0	0
	0.5	50	16.2	0	0
	0.75	75	27.9	0	0
	1.0	100	29.9	0	0
Menthol	0.1	10	7.1	0	0
	0.2	20	11.0	0	0
	0.5	50	23.4	0	0
	0.75	75	25.9	0	0
	1.0	100	51.3	0	0
Thymol	0.1	10	38.3	0	0
	0.2	20	43.5	0	0
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Cabrio TOP	0.2	20	96.1	89.1	75.9
Nativo 75WG	0.02	2	100	96.1	86.8

Table S16. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Biscogniauxia nummularia* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$).

Treatment	Day 2	Day 4	Day 10
		Concentration: 0.1%	
Chinese cinnamon	**0.00 ± 0.00 d**	0.00 ± 0.00 c	0.00 ± 0.00 c
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Lemon	30.00 ± 0.00 ab	60.67 ± 1.53 b	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Peppermint	32.33 ± 2.08 a	61.33 ± 4.62 b	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Limonene	30.00 ± 1.73 ab	65.00 ± 5.19 b	86.00 ± 0.00 a
Menthol	28.00 ± 2.00 b	63.67 ± 1.53 b	86.00 ± 0.00 a
Thymol	20.00 ± 3.61 c	60.00 ± 1.00 b	86.00 ± 0.00 a
Cabrio TOP	0.00 ± 0.00 d	2.33 ± 0.58 c	6.67 ± 1.53 b
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Control	32.67 ± 1.53 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	4.01	5.73	1.19
		Concentration: 0.2%	
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 f	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 f	0.00 ± 0.00 d
Lemon	25.00 ± 2.66 b	56.00 ± 2.65 c	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 f	0.00 ± 0.00 d
Peppermint	2.67 ± 1.15 d	9.33 ± 3.06 e	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 f	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 f	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 f	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 f	0.00 ± 0.00 d
Limonene	28.33 ± 2.52 b	63.33 ± 5.13 b	86.00 ± 0.00 a
Menthol	26.00 ± 2.00 b	65.00 ± 2.65 b	86.00 ± 0.00 a
Thymol	7.67 ± 2.52 c	34.33 ± 0.58 d	54.67 ± 5.86 b
Cabrio TOP	0.00 ± 0.00 d	2.33 ± 0.58 f	6.67 ± 1.53 c
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 f	0.00 ± 0.00 d
Control	32.67 ± 1.53 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	4.06	5.51	4.7
		Concentration: 0.5%	
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 c
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 c
Lemon	23.67 ± 2.52 b	52.33 ± 0.58 c	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 c
Peppermint	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 c
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 c
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 c
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 c
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 c
Limonene	26.00 ± 0.00 b	56.00 ± 0.00 b	86.00 ± 0.00 a
Menthol	14.00 ± 2.65 c	54.33 ± 4.51 bc	86.00 ± 0.00 a

Thymol	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 c
Cabrio TOP	0.00 ± 0.00 d	2.33 ± 0.58 d	6.67 ± 1.53 b
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 d	0.00 ± 0.00 c
Control	32.67 ± 1.53 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	3.07	3.56	1.19
	Concentration: 0.75%		
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 c
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 c
Lemon	22.00 ± 1.73 b	34.67 ± 2.31 d	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 c
Peppermint	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 c
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 c
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 c
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 c
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 c
Limonene	15.00 ± 4.36 c	44.67 ± 2.08 c	86.00 ± 0.00 a
Menthol	13.33 ± 0.58 c	47.67 ± 2.08 b	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 c
Cabrio TOP	0.00 ± 0.00 d	2.33 ± 0.58 e	6.67 ± 1.53 b
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 c
Control	32.67 ± 1.53 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	3.86	2.94	1.19
	Concentration: 1.0%		
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 c
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 c
Lemon	1.67 ± 0.58 d	32.00 ± 4.58 c	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 c
Peppermint	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 c
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 c
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 c
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 c
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 c
Limonene	4.67 ± 2.08 c	8.33 ± 4.51 d	86.00 ± 0.00 a
Menthol	13.00 ± 0.00 b	43.00 ± 3.61 b	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 c
Cabrio TOP	0.00 ± 0.00 d	2.33 ± 0.58 e	6.67 ± 1.53 b
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 c
Control	32.67 ± 1.53 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	2.06	5.74	1.19

Table S17. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Biscogniauxia nummularia*.

Essential oil/component/fungicide	Concentration (%)	Concentration	Inhibition (%)		
			Day 2	Day 4	Day 10
Chinese cinnamon	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Holy basil	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Lemon	0.1	10	8.2	29.5	0
	0.2	20	23.5	34.9	0
	0.5	50	27.6	39.1	0
	0.75	75	32.7	59.7	0
	1.0	100	94.9	62.8	0
Oregano	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Peppermint	0.1	10	2.7	28.7	0
	0.2	20	91.8	89.1	0
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Thyme	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Carvacrol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
E-cinnamaldehyde	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Eugenol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100

Limonene	0.1	10	8.2	24.4	0
	0.2	20	13.3	26.4	0
	0.5	50	20.4	34.9	0
	0.75	75	54.1	48.1	0
	1.0	100	85.7	90.3	0
Menthol	0.1	10	14.3	25.9	0
	0.2	20	20.4	24.4	0
	0.5	50	57.1	36.8	0
	0.75	75	59.2	44.6	0
	1.0	100	60.2	50	0
Thymol	0.1	10	38.8	30.2	0
	0.2	20	76.5	60.1	36.4
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Cabrio TOP	0.2	20	100	97.3	92.2
Nativo 75WG	0.02	2	100	100	100

Table S18. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Cytospora prunosa* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$).

Treatment	Day 2	Day 4	Day 10
	Concentration: 0.1%		
Chinese cinnamon	**0.00 ± 0.00 c**	0.00 ± 0.00 c	0.00 ± 0.00 e
Holy basil	0.00 ± 0.00 c	0.00 ± 0.00 c	8.00 ± 0.00 d
Lemon	11.00 ± 1.00 b	29.67 ± 3.06 a	69.00 ± 4.64 b
Oregano	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 e
Peppermint	0.00 ± 0.00 c	31.33 ± 3.06 a	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 c	11.67 ± 3.21 b	40.33 ± 2.08 c
Carvacrol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 e
E-cinnamaldehyde	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 e
Eugenol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 e
Limonene	15.00 ± 2.66 a	30.00 ± 2.65 a	86.00 ± 0.00 a
Menthol	0.00 ± 0.00 c	0.00 ± 0.00 c	9.67 ± 0.58 d
Thymol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 e
Cabrio TOP	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 e
Nativo 75WG	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 e
Control	16.33 ± 0.58 a	30.00 ± 2.65 a	86.00 ± 0.00 a
LSD	2.24	5.09	3.78
	Concentration: 0.2%		
Chinese cinnamon	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 c	0.00 ± 0.00 b	8.00 ± 0.00 d
Lemon	8.33 ± 2.31 b	24.67 ± 1.15 a	55.67 ± 9.61 b
Oregano	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 d
Peppermint	0.00 ± 0.00 c	0.00 ± 0.00 b	59.00 ± 1.00 b
Thyme	0.00 ± 0.00 c	2.33 ± 0.58 b	23.33 ± 5.13 c
Carvacrol	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 d
Limonene	14.00 ± 2.00 a	27.67 ± 6.66 a	86.00 ± 0.00 a
Menthol	0.00 ± 0.00 c	0.00 ± 0.00 b	6.00 ± 0.00 d
Thymol	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 d
Cabrio TOP	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 d
Nativo 75WG	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 d
Control	16.33 ± 0.58 a	30.00 ± 2.65 a	86.00 ± 0.00 a
LSD	2.42	5.66	8.49
	Concentration: 0.5%		
Chinese cinnamon	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 f
Holy basil	0.00 ± 0.00 c	0.00 ± 0.00 c	7.67 ± 0.58 e
Lemon	0.00 ± 0.00 c	22.33 ± 2.08 b	61.67 ± 1.15 c
Oregano	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 f
Peppermint	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 f
Thyme	0.00 ± 0.00 c	0.00 ± 0.00 c	13.67 ± 3.51 d
Carvacrol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 f
E-cinnamaldehyde	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 f
Eugenol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 f
Limonene	10.00 ± 0.00 b	28.00 ± 1.00 a	68.67 ± 3.51 b
Menthol	0.00 ± 0.00 c	0.00 ± 0.00 c	4.67 ± 0.58 e

Thymol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 f
Cabrio TOP	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 f
Nativo 75WG	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 f
Control	16.33 ± 0.58 a	30.00 ± 2.65 a	86.00 ± 0.00 a
LSD	0.45	2.73	4.01
		Concentration: 0.75%	
Chinese cinnamon	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 e
Holy basil	0.00 ± 0.00 c	0.00 ± 0.00 c	7.33 ± 0.58 d
Lemon	0.00 ± 0.00 c	19.33 ± 1.53 b	49.33 ± 5.03 c
Oregano	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 e
Peppermint	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 e
Thyme	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 e
Carvacrol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 e
E-cinnamaldehyde	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 e
Eugenol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 e
Limonene	10.67 ± 1.53 b	29.33 ± 2.52 a	65.33 ± 1.15 b
Menthol	0.00 ± 0.00 c	0.00 ± 0.00 c	4.33 ± 0.58 d
Thymol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 e
Cabrio TOP	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 e
Nativo 75WG	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 e
Control	16.33 ± 0.58 a	30.00 ± 2.65 a	86.00 ± 0.00 a
LSD	1.27	3.07	4.06
		Concentration: 1.0%	
Chinese cinnamon	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 c	0.00 ± 0.00 b	6.00 ± 0.00 c
Lemon	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 d
Oregano	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 d
Peppermint	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 d
Thyme	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 d
Limonene	10.33 ± 3.79 b	26.67 ± 4.04 a	61.67 ± 7.02 b
Menthol	0.00 ± 0.00 c	0.00 ± 0.00 b	4.00 ± 0.00 cd
Thymol	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 d
Cabrio TOP	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 d
Nativo 75WG	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 d
Control	16.33 ± 0.58 a	30.00 ± 2.65 a	86.00 ± 0.00 a
LSD	2.98	3.75	5.46

Table S19. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Cytospora pruinosa*.

Essential oil/component/fungicide	Concentration (%)	Concentration	Inhibition (%)		
			Day 2	Day 4	Day 10
Chinese cinnamon	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Holy basil	0.1	10	100	100	90.7
	0.2	20	100	100	90.7
	0.5	50	100	100	91.1
	0.75	75	100	100	91.5
	1.0	100	100	100	93.0
Lemon	0.1	10	32.7	1.1	19.8
	0.2	20	48.9	17.8	35.3
	0.5	50	100	25.6	28.3
	0.75	75	100	35.6	42.6
	1.0	100	100	100	100
Oregano	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Peppermint	0.1	10	100	0	0
	0.2	20	100	100	31.4
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Thyme	0.1	10	100	61.1	53.1
	0.2	20	100	92.2	72.9
	0.5	50	100	100	84.1
	0.75	75	100	100	100
	1.0	100	100	100	100
Carvacrol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
E-cinnamaldehyde	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Eugenol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Limonene	0.1	10	9.5	3.3	0

	0.2	20	14.3	11.1	0
	0.5	50	38.8	6.7	20.2
	0.75	75	34.7	4.4	24.0
	1.0	100	36.7	12.2	28.3
Menthol	0.1	10	100	100	88.8
	0.2	20	100	100	93.0
	0.5	50	100	100	94.5
	0.75	75	100	100	94.9
	1.0	100	100	100	95.3
Thymol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Cabrio TOP	0.2	20	100	100	100
Nativo 75WG	0.02	2	100	100	100

Table S20. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Nigrospora gorlenkoana* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$).

Treatment	Day 2	Day 4	Day 10
	Concentration: 0.1%		
Chinese cinnamon	**0.00 ± 0.00 d**	0.00 ± 0.00 b	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Lemon	62.67 ± 1.15 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Peppermint	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	65.33 ± 3.06 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Cabrio TOP	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	2.54	0	0
	Concentration: 0.2%		
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Lemon	56.00 ± 4.58 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Peppermint	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Limonene	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	9.00 ± 2.65 c	30.33 ± 2.08 b	86.00 ± 0.00 a
Cabrio TOP	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	4.11	1.62	0
	Concentration: 0.5%		
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Lemon	27.33 ± 1.53 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Peppermint	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Limonene	57.00 ± 1.73 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a

Thymol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Cabrio TOP	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	1.79	0	0
		Concentration: 0.75%	
Chinese cinnamon	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Lemon	13.67 ± 3.21 d	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Peppermint	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Thyme	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Carvacrol	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Limonene	48.67 ± 5.69 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	75.67 ± 4.16 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Cabrio TOP	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Nativo 75WG	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	6.02	0	0
		Concentration: 1.0%	
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Lemon	11.67 ± 0.58 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Peppermint	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Limonene	13.33 ± 2.89 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	37.33 ± 3.21 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Cabrio TOP	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Control	86.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	3.39	0	0

Table S21. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Nigrospora gorlenkoana*.

Essential oil/component/fungicide	Concentration (%)	Concentration	Inhibition (%)		
			Day 2	Day 4	Day 10
Chinese cinnamon	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Holy basil	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Lemon	0.1	10	27.1	0	0
	0.2	20	34.9	0	0
	0.5	50	68.2	0	0
	0.75	75	84.1	0	0
	1.0	100	86.4	0	0
Oregano	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Peppermint	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Thyme	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Carvacrol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
E-cinnamaldehyde	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Eugenol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Limonene	0.1	10	0	0	0

	0.2	20	0	0	0
	0.5	50	33.7	0	0
	0.75	75	43.4	0	0
	1.0	100	84.5	0	0
Menthol	0.1	10	0	0	0
	0.2	20	0	0	0
	0.5	50	0	0	0
	0.75	75	12.0	0	0
	1.0	100	56.6	0	0
Thymol	0.1	10	24.0	0	0
	0.2	20	89.5	64.7	0
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Cabrio TOP	0.2	20	100	100	100
Nativo 75WG	0.02	2	100	100	100

Table S22. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Nigrospora osmanthi* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$).

Treatment	Day 2	Day 4	Day 10
		Concentration: 0.1%	
Chinese cinnamon	**0.00 ± 0.00 f**	0.00 ± 0.00 d	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 f	0.00 ± 0.00 d	0.00 ± 0.00 d
Lemon	51.33 ± 2.52 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 f	0.00 ± 0.00 d	0.00 ± 0.00 d
Peppermint	35.00 ± 2.00 d	86.00 ± 0.00 a	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 f	0.00 ± 0.00 d	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 f	0.00 ± 0.00 d	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 f	0.00 ± 0.00 d	0.00 ± 0.00 d
Eugenol	6.00 ± 0.00 e	7.00 ± 1.00 c	13.00 ± 1.00 c
Limonene	59.67 ± 2.31 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	73.33 ± 2.52 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	72.67 ± 1.53 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Cabrio TOP	0.00 ± 0.00 f	0.00 ± 0.00 d	0.00 ± 0.00 d
Nativo 75WG	0.00 ± 0.00 f	18.00 ± 2.65 b	34.33 ± 4.16 b
Control	73.67 ± 3.06 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	4.51	2.19	3.33
		Concentration: 0.2%	
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Lemon	39.33 ± 2.89 b	57.33 ± 4.16 b	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Peppermint	0.00 ± 0.00 d	0.00 ± 0.00 e	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Eugenol	6.00 ± 0.00 d	7.00 ± 1.00 d	12.33 ± 0.58 c
Limonene	32.00 ± 6.56 bc	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	72.33 ± 2.52 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	30.33 ± 6.66 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Cabrio TOP	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Nativo 75WG	0.00 ± 0.00 d	18.00 ± 2.65 c	34.33 ± 4.16 b
Control	73.67 ± 3.06 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	8.19	3.91	3.27
		Concentration: 0.5%	
Chinese cinnamon	0.00 ± 0.00 e	0.00 ± 0.00 f	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 e	0.00 ± 0.00 f	0.00 ± 0.00 d
Lemon	27.00 ± 2.65 b	47.33 ± 5.51 b	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 e	0.00 ± 0.00 f	0.00 ± 0.00 d
Peppermint	0.00 ± 0.00 e	0.00 ± 0.00 f	0.00 ± 0.00 d
Thyme	0.00 ± 0.00 e	0.00 ± 0.00 f	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 e	0.00 ± 0.00 f	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 e	0.00 ± 0.00 f	0.00 ± 0.00 d
Eugenol	6.00 ± 0.00 d	7.33 ± 0.58 e	12.00 ± 0.00 c
Limonene	22.67 ± 3.79 bc	43.67 ± 4.62 bc	86.00 ± 0.00 a
Menthol	72.00 ± 1.73 a	86.00 ± 0.00 a	86.00 ± 0.00 a

Thymol	21.00 ± 2.65 c	38.00 ± 5.29 c	86.00 ± 0.00 a
Cabrio TOP	0.00 ± 0.00 e	0.00 ± 0.00 f	0.00 ± 0.00 d
Nativo 75WG	0.00 ± 0.00 e	18.00 ± 2.65 d	34.33 ± 4.16 b
Control	73.67 ± 3.06 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	4.95	7.25	3.23
		Concentration: 0.75%	
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Lemon	19.67 ± 2.31 b	38.00 ± 3.00 b	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Peppermint	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Eugenol	6.00 ± 0.00 c	7.00 ± 1.00 d	12.00 ± 0.00 c
Limonene	21.33 ± 0.58 b	41.33 ± 0.58 b	86.00 ± 0.00 a
Menthol	71.33 ± 0.58 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	2.33 ± 0.58 d	6.67 ± 2.31 d	86.00 ± 0.00 a
Cabrio TOP	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Nativo 75WG	0.00 ± 0.00 d	18.00 ± 2.65 c	34.33 ± 4.16 b
Control	73.67 ± 3.06 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	3.07	3.69	3.23
		Concentration: 1.0%	
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Lemon	14.33 ± 4.51 b	36.33 ± 0.58 b	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Peppermint	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Eugenol	6.00 ± 00.00 c	6.67 ± 1.15 d	12.00 ± 0.00 c
Limonene	16.67 ± 3.06 b	36.33 ± 5.51 b	86.00 ± 0.00 a
Menthol	70.00 ± 1.73 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	2.00 ± 0.00 cd	4.00 ± 0.00 de	86.00 ± 0.00 a
Cabrio TOP	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d
Nativo 75WG	0.00 ± 0.00 d	18.00 ± 2.65 c	34.33 ± 4.16 b
Control	73.67 ± 3.06 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	5.03	4.85	3.23

Table S23. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Nigrospora osmanthi*.

Essential oil/component/fungicide	Concentration (%)	Concentration	Inhibition (%)		
			Day 2	Day 4	Day 10
Chinese cinnamon	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Holy basil	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Lemon	0.1	10	30.3	0	0
	0.2	20	46.6	33.3	0
	0.5	50	63.3	44.9	0
	0.75	75	73.3	55.8	0
	1.0	100	80.5	57.8	0
Oregano	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Peppermint	0.1	10	52.5	0	0
	0.2	20	100	100	0
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Thyme	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Carvacrol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
E-cinnamaldehyde	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Eugenol	0.1	10	91.9	91.9	84.9
	0.2	20	91.9	91.9	85.7
	0.5	50	91.9	91.5	86.0
	0.75	75	91.9	91.9	86.0
	1.0	100	91.9	92.2	86.0
Limonene	0.1	10	19.0	0	0

	0.2	20	56.6	0	0
	0.5	50	69.2	49.2	0
	0.75	75	71.0	51.9	0
	1.0	100	77.4	57.8	0
Menthol	0.1	10	1.5	0	0
	0.2	20	1.8	0	0
	0.5	50	2.4	0	0
	0.75	75	3.2	0	0
	1.0	100	3.8	0	0
Thymol	0.1	10	1.5	0	0
	0.2	20	58.8	0	0
	0.5	50	71.5	55.8	0
	0.75	75	96.8	92.2	0
	1.0	100	97.3	95.3	0
Cabrio TOP	0.2	20	100	100	100
Nativo 75WG	0.02	2	100	79.1	60.1

Table S24. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Nigrospora philosophiae-doctoris* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$).

Treatment	Day 2	Day 4	Day 10
	Concentration: 0.1%		
Chinese cinnamon	**0.00 ± 0.00 c**	0.00 ± 0.00 d	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 b
Lemon	0.00 ± 0.00 c	39.00 ± 7.81 b	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 b
Peppermint	31.33 ± 2.52 a	63.00 ± 3.61 a	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 b
Carvacrol	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 b
Limonene	32.67 ± 0.58 a	62.33 ± 1.15 a	86.00 ± 0.00 a
Menthol	32.67 ± 0.58 a	62.00 ± 1.00 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 c	25.00 ± 3.61 c	86.00 ± 0.00 a
Cabrio TOP	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 b
Nativo 75WG	4.00 ± 2.65 b	62.67 ± 4.16 a	86.00 ± 0.00 a
Control	33.00 ± 1.00 a	63.00 ± 2.65 a	86.00 ± 0.00 a
LSD	3.01	8.28	0
	Concentration: 0.2%		
Chinese cinnamon	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b
Lemon	0.00 ± 0.00 c	6.33 ± 0.58 b	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b
Peppermint	0.00 ± 0.00 c	0.00 ± 0.00 c	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b
Carvacrol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b
Limonene	32.33 ± 1.15 a	61.67 ± 0.58 a	86.00 ± 0.00 a
Menthol	32.67 ± 0.58 a	61.33 ± 1.53 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 c	0.00 ± 0.00 c	86.00 ± 0.00 a
Cabrio TOP	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b
Nativo 75WG	4.00 ± 2.65 b	62.67 ± 4.16 a	86.00 ± 0.00 a
Control	33.00 ± 1.00 a	63.00 ± 2.65 a	86.00 ± 0.00 a
LSD	2.42	4.06	0
	Concentration: 0.5%		
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Lemon	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Peppermint	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Limonene	30.33 ± 0.58 b	61.33 ± 0.58 a	86.00 ± 0.00 a
Menthol	31.00 ± 1.00 ab	61.00 ± 1.00 a	86.00 ± 0.00 a

Thymol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Cabrio TOP	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Nativo 75WG	4.00 ± 2.65 c	62.67 ± 4.16 a	86.00 ± 0.00 a
Control	33.00 ± 1.00 a	63.00 ± 2.65 a	86.00 ± 0.00 a
LSD	2.37	3.94	0
		Concentration: 0.75%	
Chinese cinnamon	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Lemon	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Oregano	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Peppermint	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Thyme	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Carvacrol	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Limonene	29.33 ± 1.53 a	60.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	30.33 ± 3.51 a	58.67 ± 4.16 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Cabrio TOP	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
Nativo 75WG	4.00 ± 2.65 b	62.67 ± 4.16 a	86.00 ± 0.00 a
Control	33.00 ± 1.00 a	63.00 ± 2.65 a	86.00 ± 0.00 a
LSD	3.69	5.01	0
		Concentration: 1.0%	
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Lemon	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Peppermint	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Limonene	27.00 ± 1.00 b	55.33 ± 1.15 b	86.00 ± 0.00 a
Menthol	28.33 ± 1.53 b	58.00 ± 1.00 b	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Cabrio TOP	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 b
Nativo 75WG	4.00 ± 2.65 c	62.67 ± 4.16 a	86.00 ± 0.00 a
Control	33.00 ± 1.00 a	63.00 ± 2.65 a	86.00 ± 0.00 a
LSD	2.62	4.01	0

Table S25. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Nigrospora philosophiae-doctoris*.

Essential oil/component/fungicide	Concentration (%)	Concentration	Inhibition (%)		
			Day 2	Day 4	Day 10
Chinese cinnamon	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Holy basil	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Lemon	0.1	10	100	38.1	0
	0.2	20	100	89.9	0
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Oregano	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Peppermint	0.1	10	5.1	0	0
	0.2	20	100	100	0
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Thyme	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Carvacrol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
E-cinnamaldehyde	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Eugenol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Limonene	0.1	10	1.0	1.1	0

	0.2	20	2.0	2.1	0
	0.5	50	8.1	2.7	0
	0.75	75	11.1	4.8	0
	1.0	100	18.2	12.2	0
Menthol	0.1	10	1.0	1.6	0
	0.2	20	1.0	2.7	0
	0.5	50	6.1	3.2	0
	0.75	75	8.1	6.9	0
	1.0	100	14.1	7.9	0
Thymol	0.1	10	100	60.3	0
	0.2	20	100	100	0
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Cabrio TOP	0.2	20	100	100	100
Nativo 75WG	0.02	2	87.9	2.6	0

Table S26. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Phaeoacremonium iranianum* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$).

Treatment	DAY 2	DAY 4	DAY 10
	Mean	Mean	Mean
Chinese cinnamon	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 d
Lemon	0.00 ± 0.00 b	4.00 ± 0.00 bc	15.00 ± 2.65 b
Oregano	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 d
Peppermint	0.00 ± 0.00 b	4.00 ± 1.00 bc	28.33 ± 2.08 a
Thyme	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 d
Limonene	0.00 ± 0.00 b	7.67 ± 3.06 a	20.00 ± 4.36 b
Menthol	2.67 ± 1.15 a	7.00 ± 1.73 ab	26.67 ± 2.08 a
Thymol	2.67 ± 1.15 a	4.67 ± 0.58 abc	15.00 ± 1.00 b
Cabrio TOP	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 d
Nativo 75WG	2.67 ± 1.15 a	2.67 ± 1.15 cd	8.00 ± 2.00 c
Control	3.00 ± 1.00 a	7.00 ± 1.73 ab	26.67 ± 4.16 a
LSD	1.55	3.29	5.87
		Concentration: 0.2%	
Chinese cinnamon	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 d
Lemon	0.00 ± 0.00 b	4.00 ± 0.00 b	12.67 ± 2.89 bc
Oregano	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 d
Peppermint	0.00 ± 0.00 b	0.00 ± 0.00 c	11.67 ± 1.53 bc
Thyme	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 d
Limonene	0.00 ± 0.00 b	7.00 ± 1.73 a	16.33 ± 2.52 b
Menthol	2.67 ± 1.15 a	6.67 ± 1.53 a	25.33 ± 1.15 a
Thymol	2.33 ± 0.58 a	4.00 ± 0.00 b	11.67 ± 1.16 bc
Cabrio TOP	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 d
Nativo 75WG	2.67 ± 1.15 a	2.67 ± 1.15 b	8.00 ± 2.00 c
Control	3.00 ± 1.00 a	7.00 ± 1.73 a	26.67 ± 4.16 a
LSD	1.55	2.42	4.97
		Concentration: 0.5%	
Chinese cinnamon	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 e
Holy basil	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 e
Lemon	0.00 ± 0.00 b	3.67 ± 0.58 b	12.00 ± 1.00 b
Oregano	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 e
Peppermint	0.00 ± 0.00 b	0.00 ± 0.00 c	7.33 ± 3.21 cd
Thyme	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 e
Carvacrol	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 e
E-cinnamaldehyde	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 e
Eugenol	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 e
Limonene	0.00 ± 0.00 b	3.67 ± 1.15 b	11.67 ± 1.15 bc
Menthol	3.00 ± 1.00 a	6.00 ± 0.00 a	22.67 ± 0.58 a

Thymol	2.33 ± 0.58 a	2.33 ± 0.58 b	3.33 ± 0.58 de
Cabrio TOP	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 e
Nativo 75WG	2.67 ± 1.15 a	2.67 ± 1.15 b	8.00 ± 2.00 bc
Control	3.00 ± 1.00 a	7.00 ± 1.73 a	26.67 ± 4.16 a
LSD	1.49	1.96	4.57
		Concentration: 0.75%	
Chinese cinnamon	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 d
Lemon	0.00 ± 0.00 b	2.00 ± 0.00 b	10.67 ± 0.58 c
Oregano	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 d
Peppermint	0.00 ± 0.00 b	0.00 ± 0.00 c	3.33 ± 1.53 d
Thyme	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 d
Limonene	0.00 ± 0.00 b	3.00 ± 0.00 b	9.33 ± 1.53 c
Menthol	2.00 ± 0.00 a	6.00 ± 0.00 a	18.67 ± 1.53 b
Thymol	2.00 ± 0.00 a	2.00 ± 0.00 b	2.00 ± 0.00 d
Cabrio TOP	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 d
Nativo 75WG	2.67 ± 1.15 a	2.67 ± 1.15 b	8.00 ± 2.00 c
Control	3.00 ± 1.00 a	7.00 ± 1.73 a	26.67 ± 4.16 a
LSD	1.19	1.16	4.16
		Concentration: 1.0%	
Chinese cinnamon	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 d
Holy basil	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 d
Lemon	0.00 ± 0.00 b	2.00 ± 0.00 c	8.67 ± 0.58 c
Oregano	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 d
Peppermint	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 d
Thyme	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 d
Carvacrol	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 d
E-cinnamaldehyde	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 d
Eugenol	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 d
Limonene	0.00 ± 0.00 b	2.33 ± 0.58 c	8.67 ± 1.53 c
Menthol	2.00 ± 0.00 a	5.00 ± 1.00 b	17.00 ± 2.65 b
Thymol	2.00 ± 0.00 a	2.00 ± 0.00 c	2.00 ± 0.00 d
Cabrio TOP	0.00 ± 0.00 b	0.00 ± 0.00 d	0.00 ± 0.00 d
Nativo 75WG	2.67 ± 1.15 a	2.67 ± 1.15 c	8.00 ± 2.00 c
Control	3.00 ± 1.00 a	7.00 ± 1.73 a	26.67 ± 4.16 a
LSD	1.19	1.85	4.33

Table S27. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Phaeoacremonium iranianum*.

Essential oil/component/fungicide	Concentration (%)	Concentration	Inhibition (%)		
			Day 2	Day 4	Day 10
Chinese cinnamon	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Holy basil	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Lemon	0.1	10	100	42.9	43.8
	0.2	20	100	42.9	52.5
	0.5	50	100	47.6	55
	0.75	75	100	71.4	60
	1.0	100	100	71.4	67.5
Oregano	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Peppermint	0.1	10	100	42.9	0.8
	0.2	20	100	100	56.3
	0.5	50	100	100	72.5
	0.75	75	100	100	87.5
	1.0	100	100	100	100
Thyme	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Carvacrol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
E-cinnamaldehyde	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Eugenol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Limonene	0.1	10	100	9.5	25.0

	0.2	20	100	9.5	38.8
	0.5	50	100	47.6	56.2
	0.75	75	100	57.1	65.0
	1.0	100	100	66.7	67.5
Menthol	0.1	10	11.1	9.5	2.9
	0.2	20	11.1	9.5	5.0
	0.5	50	11.1	14.3	15.0
	0.75	75	33.3	14.3	30.0
	1.0	100	33.3	28.6	36.3
Thymol	0.1	10	11.1	33.3	43.8
	0.2	20	22.2	42.9	56.3
	0.5	50	22.2	66.7	87.5
	0.75	75	33.3	71.4	92.5
	1.0	100	33.3	71.4	92.5
Cabrio TOP	0.2	20	100	100	100
Nativo 75WG	0.02	2	22.2	61.9	70.0

Table S28. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Sordaria fimicola* under the effects of various treatments. Mean values with the same letters in a row are not significantly different, according to Tukey's honestly significant difference test ($p < 0.05$).

Treatment	DAY 2	DAY 4	DAY 10
	Mean	Mean	Mean
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Lemon	51.00 ± 0.00 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Peppermint	44.00 ± 9.00 bc	86.00 ± 0.00 a	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Limonene	74.67 ± 1.15 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	71.33 ± 0.58 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	36.67 ± 7.14 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Cabrio TOP	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 b	0.00 ± 0.00 b
Control	75.67 ± 1.53 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	9.56	0	0
		Concentration: 0.2%	
Chinese cinnamon	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Holy basil	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Lemon	32.00 ± 4.36 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Peppermint	13.00 ± 1.00 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Thyme	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Carvacrol	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
E-cinnamaldehyde	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Eugenol	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Limonene	70.33 ± 10.69 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	71.00 ± 0.00 a	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	15.33 ± 1.53 c	61.33 ± 2.51 b	73.33 ± 4.16 b
Cabrio TOP	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 c
Control	75.67 ± 1.53 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	9.16	1.96	3.23
		Concentration: 0.5%	
Chinese cinnamon	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Lemon	21.33 ± 2.89 d	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Peppermint	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Thyme	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Carvacrol	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Limonene	57.00 ± 1.73 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	71.00 ± 0.00 b	86.00 ± 0.00 a	86.00 ± 0.00 a

Thymol	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Cabrio TOP	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Nativo 75WG	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Control	75.67 ± 1.53 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	2.87	0	
		Concentration: 0.75%	
Chinese cinnamon	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Lemon	17.67 ± 1.53 d	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Peppermint	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Thyme	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Carvacrol	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Limonene	41.67 ± 2.08 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	60.67 ± 2.52 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Cabrio TOP	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Nativo 75WG	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Control	75.67 ± 1.53 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	3.04	0	0
		Concentration: 1.0%	
Chinese cinnamon	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Holy basil	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Lemon	11.00 ± 2.65 d	86.00 ± 0.00 a	86.00 ± 0.00 a
Oregano	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Peppermint	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Thyme	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Carvacrol	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
E-cinnamaldehyde	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Eugenol	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Limonene	40.67 ± 0.58 c	86.00 ± 0.00 a	86.00 ± 0.00 a
Menthol	46.33 ± 5.51 b	86.00 ± 0.00 a	86.00 ± 0.00 a
Thymol	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Cabrio TOP	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Nativo 75WG	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 b
Control	75.67 ± 1.53 a	86.00 ± 0.00 a	86.00 ± 0.00 a
LSD	4.91	0	0

Table S29. Inhibitory effect (%) of essential oils, components, and fungicides on the mycelial growth of *Sordaria fimicola*.

Essential oil/component/fungicide	Concentration (%)	Concentration	Inhibition (%)		
			Day 2	Day 4	Day 10
Chinese cinnamon	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Holy basil	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Lemon	0.1	10	32.6	0	0
	0.2	20	57.7	0	0
	0.5	50	71.8	0	0
	0.75	75	76.7	0	0
	1.0	100	85.5	0	0
Oregano	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Peppermint	0.1	10	41.9	0	0
	0.2	20	82.8	0	0
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Thyme	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Carvacrol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
E-cinnamaldehyde	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Eugenol	0.1	10	100	100	100
	0.2	20	100	100	100
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Limonene	0.1	10	1.5	0	0

	0.2	20	7.8	0	0
	0.5	50	24.7	0	0
	0.75	75	44.9	0	0
	1.0	100	46.3	0	0
Menthol	0.1	10	5.8	0	0
	0.2	20	6.2	0	0
	0.5	50	6.2	0	0
	0.75	75	19.8	0	0
	1.0	100	38.8	0	0
Thymol	0.1	10	51.5	0	0
	0.2	20	79.7	28.7	14.7
	0.5	50	100	100	100
	0.75	75	100	100	100
	1.0	100	100	100	100
Cabrio TOP	0.2	20	100	100	100
Nativo 75WG	0.02	2	100	100	100

Table S30. The determined MIC (Minimum Inhibitory Concentration) and MFC (Minimum Fungicidal Concentration) values for the treatments.

Essential oil/component	Species	MIC	MFC
		%	
Chinese cinnamon	***Botryosphaeria dothidea***	/	0.1
	Diplodia mutila	/	0.1
	D. seriata	/	0.1
	Dothiorella iberica	/	0.1
	Do. sarmentorum	/	0.1
	Neofusicoccum parvum	/	0.1
	Biscogniauxia mediterranea	/	0.1
	B. nummularia	/	0.1
	Cytospora pruinosa	/	0.1
	Nigrospora gorlenkoana	/	0.1
	N. osmanthi	/	0.1
	N. philosophiae-doctoris	/	0.1
	Phaeoacremonium iranianum	/	0.1
	Sordaria fimicola	/	0.1
Holy basil	*Botryosphaeria dothidea*	0.1	0.2
	Diplodia mutila	0.1	0.2
	D. seriata	0.1	0.2
	Dothiorella iberica	/	0.1
	Do. sarmentorum	0.1	0.5
	Neofusicoccum parvum	0.1	0.2
	Biscogniauxia mediterranea	/	0.1
	B. nummularia	/	0.1
	Cytospora pruinosa	0.1	/
	Nigrospora gorlenkoana	/	0.1
	N. osmanthi	/	0.1
	N. philosophiae-doctoris	/	0.1
	Phaeoacremonium iranianum	/	0.1
	Sordaria fimicola	/	0.1
Lemon	*Botryosphaeria dothidea*	/	/
	Diplodia mutila	0.2	/
	D. seriata	0.5	/
	Dothiorella iberica	/	0.1
	Do. sarmentorum	/	/
	Neofusicoccum parvum	/	/
	Biscogniauxia mediterranea	0.1	/
	B. nummularia	0.1	/
	Cytospora pruinosa	0.1	1.0
	Nigrospora gorlenkoana	0.1	/
	N. osmanthi	0.1	/
	N. philosophiae-doctoris	0.1	0.5
	Phaeoacremonium iranianum	0.1	/
	Sordaria fimicola	0.1	/
Oregano	*Botryosphaeria dothidea*	/	0.1
	Diplodia mutila	/	0.1
	D. seriata	/	0.1
	Dothiorella iberica	/	0.1
	Do. sarmentorum	/	0.1

	Neofusicoccum parvum	/	0.1
	Biscogniauxia mediterranea	/	0.1
	B. nummularia	/	0.1
	Cytospora pruinosa	/	0.1
	Nigrospora gorlenkoana	/	0.1
	N. osmanthi	/	0.1
	N. philosophiae-doctoris	/	0.1
	Phaeoacremonium iranianum	/	0.1
	Sordaria fimicola	/	0.1
Peppermint	*Botryosphaeria dothidea*	0.2	/
	Diplodia mutila	0.1	/
	D. seriata	0.2	/
	Dothiorella iberica	0.1	0.2
	Do. sarmentorum	0.1	0.5
	Neofusicoccum parvum	0.1	0.2
	Biscogniauxia mediterranea	0.1	0.5
	B. nummularia	0.1	0.5
	Cytospora pruinosa	0.1	0.5
	Nigrospora gorlenkoana	/	0.1
	N. osmanthi	0.1	0.5
	N. philosophiae-doctoris	0.1	0.5
	Phaeoacremonium iranianum	0.1	1.0
	Sordaria fimicola	0.1	0.5
Thyme	*Botryosphaeria dothidea*	/	0.1
	Diplodia mutila	0.1	0.2
	D. seriata	/	0.1
	Dothiorella iberica	/	0.1
	Do. sarmentorum	0.1	0.5
	Neofusicoccum parvum	0.1	0.2
	Biscogniauxia mediterranea	/	0.1
	B. nummularia	0.1	/
	Cytospora pruinosa	0.1	0.75
	Nigrospora gorlenkoana	/	0.1
	N. osmanthi	/	0.1
	N. philosophiae-doctoris	/	0.1
	Phaeoacremonium iranianum	/	0.1
	Sordaria fimicola	/	0.1
Carvacrol	*Botryosphaeria dothidea*	/	0.1
	Diplodia mutila	/	0.1
	D. seriata	/	0.1
	Dothiorella iberica	/	0.1
	Do. sarmentorum	/	0.1
	Neofusicoccum parvum	/	0.1
	Biscogniauxia mediterranea	/	0.1
	B. nummularia	/	0.1
	Cytospora pruinosa	/	0.1
	Nigrospora gorlenkoana	/	0.1
	N. osmanthi	/	0.1
	N. philosophiae-doctoris	/	0.1
	Phaeoacremonium iranianum	/	0.1
	Sordaria fimicola	/	0.1

E-cinnamaldehyde	*Botryosphaeria dothidea*	/	0.1
	Diplodia mutila	/	0.1
	D. seriata	/	0.1
	Dothiorella iberica	/	0.1
	Do. sarmentorum	/	0.1
	Neofusicoccum parvum	/	0.1
	Biscogniauxia mediterranea	/	0.1
	B. nummularia	/	0.1
	Cytospora pruinosa	/	0.1
	Nigrospora gorlenkoana	/	0.1
	N. osmanthi	/	0.1
	N. philosophiae-doctoris	/	0.1
	Phaeoacremonium iranianum	/	0.1
	Sordaria fimicola	/	0.1
Eugenol	*Botryosphaeria dothidea*	0.1	0.2
	Diplodia mutila	/	0.1
	D. seriata	0.1	0.2
	Dothiorella iberica	/	0.1
	Do. sarmentorum	/	0.1
	Neofusicoccum parvum	0.1	0.5
	Biscogniauxia mediterranea	/	0.1
	B. nummularia	0.1	/
	Cytospora pruinosa	/	0.1
	Nigrospora gorlenkoana	/	0.1
	N. osmanthi	0.1	/
	N. philosophiae-doctoris	/	0.1
	Phaeoacremonium iranianum	/	0.1
	Sordaria fimicola	/	0.1
Limonene	*Botryosphaeria dothidea*	/	/
	Diplodia mutila	/	/
	D. seriata	/	/
	Dothiorella iberica	0.1	/
	Do. sarmentorum	/	/
	Neofusicoccum parvum	0.1	/
	Biscogniauxia mediterranea	0.1	/
	B. nummularia	0.1	/
	Cytospora pruinosa	0.1	/
	Nigrospora gorlenkoana	0.5	/
	N. osmanthi	0.1	/
	N. philosophiae-doctoris	0.1	/
	Phaeoacremonium iranianum	0.1	/
	Sordaria fimicola	0.1	/
Menthol	*Botryosphaeria dothidea*	/	/
	Diplodia mutila	0.75	/
	D. seriata	/	/
	Dothiorella iberica	0.1	/
	Do. sarmentorum	0.75	/
	Neofusicoccum parvum	0.1	/
	Biscogniauxia mediterranea	0.1	/
	B. nummularia	0.1	/
	Cytospora pruinosa	0.1	/

	Nigrospora gorlenkoana	0.75	/
	N. osmanthi	0.1	/
	N. philosophiae-doctoris	0.1	/
	Phaeoacremonium iranianum	0.1	/
	Sordaria fimicola	0.1	/
Thymol	*Botryosphaeria dothidea*	0.1	/
	Diplodia mutila	0.1	/
	D. seriata	0.1	/
	Dothiorella iberica	0.1	0.5
	Do. sarmentorum	0.2	/
	Neofusicoccum parvum	0.1	/
	Biscogniauxia mediterranea	0.1	0.5
	B. nummularia	0.1	0.5
	Cytospora pruinosa	/	0.1
	Nigrospora gorlenkoana	0.1	0.5
	N. osmanthi	0.1	/
	N. philosophiae-doctoris	0.1	0.5
	Phaeoacremonium iranianum	0.1	/
	Sordaria fimicola	0.1	0.5

Naslov izvornog znanstvenog rada broj 6: Antifungal Efficacy of Essential Oils and Their Predominant Components Against Olive Fungal Pathogens

Prošireni sažetak:

Provedeno je istraživanje s ciljem procjene antifungalne učinkovitosti eteričnih ulja (EtU) i njihovih glavnih komponenti protiv fitopatogenih gljiva izoliranih iz masline (*Olea europaea* L.). Uočivši rastuću potrebu za održivim alternativama kemijskim fungicidima, istraživanje je obuhvatilo ispitivanje prirodnih tvari kao potencijalnih sredstava u zaštiti bilja.

Testirana su komercijalna EtU svetog bosiljka (*Ocimum tenuiflorum* L.), kineskog cimeta (*Cinnamomum aromaticum* Nees), limuna (*Citrus × limon* (L.) Osbeck), paprene metvice (*Mentha × piperita* L.), origana (*Origanum compactum* Benth) i timijana (*Thymus vulgaris* L.), kao i njihove najzastupljenije komponente: eugenol, e-cinamaldehid, limonen, mentol, karvakrol i timol. EtU i komponente testirani su u različitim koncentracijama. Antifungalna aktivnost ispitana je protiv 14 gljiva, među kojima su vrste iz porodice *Botryosphaeriaceae* (*Botryosphaeria dothidea*, *Diplodia mutila*, *Diplodia seriata*, *Dothiorella iberica*, *Do. sarmentorum*, *Neofusicoccum parvum*), kao i druge patogene iz razreda Sordariomycetes poput *Biscogniauxia mediterranea*, *B. nummularia*, *Cytospora pruinosa*, *Nigrospora gorlenkoana*, *N. osmanthi*, *N. philosophiae-doctoris*, *Phaeoacremonium iranianum* i *Sordaria fimicola*.

Rezultati su pokazali da su EtU kineskog cimeta i origana, te njihove glavne sastavnice e-cinamaldehid i karvakrol, pokazali najvišu antifungalnu aktivnost, potpuno inhibirajući rast svih testiranih gljiva već pri najnižoj koncentraciji. Nasuprot tome, EtU limuna i paprene metvice te sastojci limonen, mentol i timol pokazali su značajno slabiju antifungalnu učinkovitost, s potrebom za znatno višim koncentracijama za postizanje inhibicije rasta.

Gljive *Do. iberica* i *N. gorlenkoana* bile su među najosjetljivijima na primijenjene tretmane, dok su *S. fimicola* i *P. iranianum* pokazale relativno višu otpornost. Uočena je varijabilnost osjetljivosti među gljivama, što upućuje na potrebu za preciznim prilagođavanjem tretmana ovisno o specifičnom patogenu prisutnom u nasadu.

Ovi nalazi sugeriraju da EtU, osobito ona bogata e-cinamaldehidom i karvakrolom, mogu predstavljati učinkovitu, prirodnu alternativu sintetskim fungicidima u zaštiti maslina. Njihova primjena mogla bi značajno pridonijeti održivom maslinarstvu, smanjujući ekološki otisak, sprječavajući razvoj rezistentnih sojeva patogena i smanjujući potencijalne rizike za zdravlje ljudi.

Ključne riječi: karvakrol; kineski cimet; e-cinamaldehid; metiram + piraklostrobin; origano;

trifloksistrobin + tebukonazol

Izvorni znanstveni rad broj 7 u obliku i izvornom jeziku na kojem je objavljen u znanstvenom časopisu

Naslova rada: Integrated Analysis of Olive Mill Wastewaters: Physicochemical Profiling, Antifungal Activity, and Biocontrol Potential Against Botryosphaeriaceae

Autori: Elena Petrović, Karolina Vrandečić, Alen Albreht, Igor Gruntar, Nikola Major, Jasenka Ćosić, Zoran Užila, Smiljana Goreta Ban, Sara Godena

Tip rada: Izvorni znanstveni članak

Časopis: Horticulturae

Kategorija: A1

Impakt faktor: 3.1 (2025.)

Kvartil: Q1

Primljen na recenziju: 02. lipanj 2025.

Prihvaćen za objavljivanje: 03. srpanj 2025.

Status: Objavljen

Volumen: 11

Broj: 7

Broj rada: 819

WOS broj: 001535381400001

horticulturae

MDPI

Article

Integrated Analysis of Olive Mill Wastewaters: Physicochemical Profiling, Antifungal Activity, and Biocontrol Potential Against *Botryosphaeriaceae*

Elena Petrović [1], Karolina Vrandečić [2], Alen Albreht [3], Igor Gruntar [4], Nikola Major [1], Jasenka Ćosić [2], Zoran Užila [1], Smiljana Goreta Ban [1] and Sara Godena [1,*]

[1] Institute of Agriculture and Tourism, Karla Huguesa 8, 52440 Poreč, Croatia; elena@iptpo.hr (E.P.); nikola@iptpo.hr (N.M.); zoran@iptpo.hr (Z.U.); smilja@iptpo.hr (S.G.B.)
[2] Department of Phytopathology, Faculty of Agrobiotechnical Sciences Osijek, Vladimira Preloga 1, 31000 Osijek, Croatia; kvrandecic@fazos.hr (K.V.); jcosic@fazos.hr (J.Ć.)
[3] Laboratory for Food Chemistry, Department of Analytical Chemistry, National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia; alen.albreht@ki.si
[4] Veterinary Faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana, Slovenia; igor.gruntar@vf.uni-lj.si
* Correspondence: sara@iptpo.hr

Abstract

The disposal of olive mill wastewater (OMWW) poses significant environmental challenges due to its high content of phytotoxic and pollutant compounds. This study aims to explore the chemical composition of OMWW derived from various olive varieties (Buža, Buža puntoža, Istarska bjelica, Leccino, and Rosinjola) and assess its antifungal potential against phytopathogenic fungi from the *Botryosphaeriaceae* family. OMWW samples were analyzed for their physicochemical properties, phenolic composition via LC-MS/MS, and antifungal activity against *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & De Not., *Diplodia mutila* (Fr.) Fr., *D. seriata* De Not., *Dothiorella iberica* A.J.L. Phillips, J. Luque & A. Alves, *Do. sarmentorum* (Fr.) A.J.L. Phillips, Alves & Luque, and *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips. Antifungal efficacy was tested at varying concentrations, alongside the phenolic compounds hydroxytyrosol and vanillic acid. Antifungal activity varied across fungal species and OMWW concentrations. Lower OMWW concentrations inhibited mycelial growth in some pathogens, while higher concentrations often had a stimulatory effect. Among the OMWW treatments, Leccino and Buža showed the most significant antifungal activity against species from the *Botryosphaeriaceae* family. The results demonstrated significant variability in OMWW composition, with Istarska bjelica exhibiting the highest concentrations of phenolic compounds, sugars, dry matter, and carbon and nitrogen content. The results also highlight the impact of acidification on the phenolic profile of OMWW. Treatment with HCl significantly altered the concentration of individual phenolic compounds, either enhancing their release or contributing to their degradation. Among the two compounds, vanillic acid showed greater efficacy than hydroxytyrosol. In addition, microorganisms isolated from OMWW, including *Bacillus velezensis* Ruiz-Garcia et al., *Rhodotorula mucilaginosa* (A. Jörg.) F.C. Harrison, *Nakazawaea molendiniolei* (N. Cadez, B. Turchetti & G. Peter) C. P. Kurtzman & C. J. Robnett, and *Penicillium crustosum* Thom, demonstrated antagonistic potential against fungal pathogens, with *B. velezensis* showing the strongest inhibitory effect. The greatest antagonistic effect against fungi was observed with the species *Do. Iberica*. The findings highlight the potential of OMWW as a sustainable alternative to chemical fungicides, simultaneously contributing to the management of waste and protection of plants through circular economy principles.

check for updates

Academic Editor: Francesco Lops

Received: 2 June 2025
Revised: 23 June 2025
Accepted: 3 July 2025
Published: 10 July 2025

Citation: Petrovič, E.; Vrandečič, K.; Albreht, A.; Gruntar, I.; Major, N.; Ćosić, J.; Užila, Z.; Goreta Ban, S.; Godena, S. Integrated Analysis of Olive Mill Wastewaters: Physicochemical Profiling, Antifungal Activity, and Biocontrol Potential Against *Botryosphaeriaceae*. *Horticulturae* **2025**, *11*, 819. https://doi.org/10.3390/horticulturae11070819

Keywords: antagonism; *Bacillus velezensis*; biological control; phenols; yeast

1. Introduction

The olive tree has been cultivated in all Mediterranean regions for over 2500 years [1]. It is of great economic value in this area. According to the latest data from 2023, olives are grown on approximately 11.1 million ha, yielding an average of 20.2 million t of olives annually [2]. The most important product of olive processing is olive oil. According to the latest data from 2022, global olive oil production amounted to 2.7 million t [2].

Plant pathogenic fungi are among the leading causes of yield loss in agricultural production, and the primary challenge in their management lies in the continued reliance on chemical agents for plant protection. Most of these pesticides are classified as toxic substances, posing a risk to ecosystems and contributing to environmental degradation. Although the number of studies focusing on alternative protection methods, such as plant-based formulations, composts, and similar approaches, is increasing, they remain insufficiently researched to enable widespread practical application. Utilizing agricultural waste as a natural solution for plant protection could facilitate a circular economy and potentially reduce the release of harmful chemicals into the environment. Olive mill wastewater (OMWW) is a byproduct generated during olive oil extraction using two-phase or three-phase systems. OMWW contains high concentrations of salts, organic matter, and chemicals (mainly phenols), which are phytotoxic and can adversely affect the physical, chemical, and biological properties of the soil [3]. A significant environmental concern with OMWW lies in its disposal, as it is considered a major environmental pollutant [4]. Typically, OMWW is discarded directly in landfills without any prior treatment [5].

However, due to its high content of mineral and organic matter, OMWW can also have positive effects on plants if properly treated (e.g., filtration, centrifugation, thermal treatment, etc.) and applied correctly [3]. Studies have also shown that OMWW exhibits antimicrobial effects against phytopathogenic fungi and bacteria [6–8]. The antimicrobial activity of OMWW has been found to depend on the olive variety from which it is derived, as phenolic concentrations and chemical composition vary between varieties. Differences in the chemical composition of OMWW are attributed to geographic, agronomic, seasonal, and other factors [8].

OMWW components also have potential applications in other industries, such as the food industry [9] and cosmetics [10]. Among the most prominent components of OMWW is hydroxytyrosol, a phenolic compound predominantly found in olive leaves and pulp, with smaller amounts present in olive oil, that has been shown to be effective in combating bacterial and fungal pathogens [11–13].

Given the growing awareness of the dangers associated with chemical pesticides, such as their impact on human and animal health, and the emergence of resistant microorganisms, significant efforts are being made to replace chemical pesticides with alternative and less toxic agents. The development of new plant protection strategies is particularly important in the context of the European Union's (EU) efforts to transition from conventional agriculture to environmentally friendly practices with minimal environmental impact [14]. To this end, the EU has introduced two strategic documents: the "European Green Deal", which outlines strategies for achieving sustainable economic growth, and the "Biodiversity Strategy", aimed at promoting the sustainable use of plant protection agents and achieving a 50% reduction in their use by 2030. The antimicrobial properties of olive mill waste could be utilized to combat plant pathogens, opening up new possibilities for recycling these distinctive bioactive byproducts [15]. The amount of research regarding the effects

of OMWW on pathogens remains limited, with experiments being carried out on only a narrow range of fungal species.

The aim of this study was to determine the chemical composition of OMWW derived from various olive varieties, analyze the microbial population present in OMWW, and assess the antifungal potential of these OMWW along with two phenolic compounds—one identified as the most studied in previous research and the other as the most abundant in the analyzed OMWW. Specifically, this study focused on their application in controlling phytopathogenic fungi from the *Botryosphaeriaceae* family and evaluating the antagonistic interactions between these pathogens and microorganisms isolated from OMWW, with the aim of exploring potential utilization strategies for OMWW as an industrial byproduct. Species from the *Botryosphaeriaceae* family are among the most aggressive olive pathogens, causing branch and twig dieback, fruit and leaf drop, and, consequently, yield reduction and economic losses for producers [16,17]. Given their high aggressiveness, limited treatment options, and significant impact on olive productivity, effective and sustainable control strategies against *Botryosphaeriaceae* are urgently needed.

2. Materials and Methods

2.1. OMWW Collection

The OMWW was collected directly from olive processors in Istria County, Croatia, in 2021. The interval between harvest and oil processing was four hours, with the paste processing temperature maintained at 24 °C. Malaxation was performed with continuous cooling, using water at 12 °C. The olive oil was extracted through centrifugation, employing a two-phase Pieralisi system with a hammer mill (Pieralisi Group, Ancona, Italy). The OMWW was derived from the Croatian olive cultivars Buža, Buža Puntoža, Istarska Bjelica, and Rosinjola, as well as the Italian olive cultivar Leccino.

2.2. Pretreatment of the Sample

After collecting the OMWW samples from the olive mill, they were stored under refrigeration at 4 °C for nine days [18]. The samples' color was initially determined, followed by filtration through a vacuum filtration system using high-flow-rate 21/N-grade filter paper, 80 g/m^2, a thickness of 0.28 mm, a filtration speed of 10 s/10 mL, and a pore size of 20–25 µm (Munktell, Fisher Scientific, Göteborg, Sweden). After filtration, the samples were subjected to centrifugation at 4000 rpm for 10 min at +4 °C, utilizing a Hettich 320 R centrifuge (Merck, Darmstadt, Germany). The pH of the OMWW samples was determined at room temperature using a pH meter (MP220 Basic pH/mV/°C Meter, Mettler-Toledo GmbH, Giessen, Germany) that had been calibrated with certified pH buffers (Mettler-Toledo GmbH, Greifensee, Switzerland) as reference materials.

Subsequently, the samples were divided into two fractions. Hydrochloric acid (HCl) was added to one fraction to lower the final pH to 2, aiming to prevent oxidation and thus preserve phenolic compounds [8,19,20], while the other fraction was left in its natural form without any additives. Each fraction was then further divided, with one aliquot stored at −20 °C and the other at room temperature.

2.3. Determination of Physical and Chemical Parameters

2.3.1. Sample Preparation and LC-MS/MS Analysis of Phenolic Compounds

OMWW (300 µL) was combined with 1200 µL of methanol to achieve a 1:5 dilution ratio. The mixture was thoroughly vortexed and centrifuged at 16,000× *g* for five minutes at 25 °C using a Domel Centric 350 centrifuge (Železniki, Slovenia). After centrifugation, 300 µL of the supernatant was freeze-dried using a Labogene Coolsafe 95-15 Pro system (Allerød, Denmark) and reconstituted in 600 µL of the initial mobile phase, consisting of

water with 2% methanol and 0.1% acetic acid. The reconstituted solution was vortexed for 30 s and transferred to HPLC vials for analysis. The phenolic profile was determined using LC-MS/MS, comprising an autosampler (Shimadzu Nexera SIL-40CX3, Kyoto, Japan), two solvent delivery units (Shimadzu Nexera LC-40DX3, Kyoto, Japan), a thermostatic column compartment (Shimadzu Nexera CTO-40C, Kyoto, Japan), and a triple quadrupole mass spectrometer (Shimadzu LCMS8045, Kyoto, Japan). Separation was carried out on a C18 core-shell column (2.1 mm × 150 mm, 2.7 µm, Advanced Materials Technology, Wilmington, DE, USA) maintained at 37 °C. A 1 µL sample was injected, and separation was achieved using a linear gradient elution of mobile phase A (water/0.1% acetic acid) and mobile phase B (methanol/0.1% acetic acid) at a flow rate of 0.35 mL/min. The gradient conditions were as follows: 0–0.75 min, 98% A; 0.75–15 min, 98% A to 50% A; 15–15.1 min, 50% A to 0% A; 15.1–20 min, 0% A; 20–20.1 min, 0% A to 98% A; and 20.1–25 min, 98% A. Polyphenolic compounds were identified and quantified using analytical standards. Each sample was analyzed in quadruplicate, and the phenolic content was expressed as the mean of these four measurements ± standard error.

2.3.2. Spectrophotometric Determination of Sugar

The determination of sugar concentration was performed according to the method of DuBois et al. [21], using a spectrophotometer (PerkinElmer, Lambda 45, Waltham, MA, USA) at wavelengths of 480 nm and 490 nm. The absorbance of the samples was measured in 1 cm quartz cuvettes after the addition of the DuBois reagent. The measurements were performed at room temperature, and the concentration of sugar in each sample was calculated from a calibration curve generated using glucose standards in the range of 0 to 100 mg/L. Three replicates were performed for each OMWW sample. The concentration of sugar is expressed as the mean value of these replicates ± standard error.

2.3.3. Determination of Dry Matter and Water Content

To determine the dry matter content and water volume fraction, 3 mL of each sample was pipetted into a glass beaker. The samples were placed in a drying chamber (Binder, FD 56, Tuttlingen, Germany) at 103 °C for 24 h. The mass of the empty glass beaker, as well as the mass of the sample before and after drying, was measured using an analytical balance (Mettler Toledo, XP205, Switzerland). Each measurement was conducted in three replicates. The dry matter mass was calculated using the following formula: $m_{dm} = m_3 - m_1$, where m_{dm} is the mass of the dry matter (in mg), m_1 is the mass of the empty glass beaker, m_2 is the mass of the sample and glass beaker before drying, and m_3 is the mass of the sample and glass beaker after 24 h of drying. The initial (wet) mass of the sample was determined as: $m_{wet} = m_2 - m_1$, where m_{wet} represents the mass of the sample before drying (excluding the beaker). The water content (WH_2O) was then calculated using the following formula: $W_{H2O} = 100\% - W_{dm}$, where W_{dm} represents the percentage of dry matter, calculated as: $W_{dm} = (m_{dm}/m_{wet}) \times 100\%$. The amount of dry matter and the percentage of water content were expressed as the mean value of three replicates ± standard error.

2.3.4. Determination of Carbon and Nitrogen Content

The total carbon and nitrogen content was simultaneously analyzed in the sample using a TOC-L-CPH/TNM-L-ROHS analyzer (Shimadzu Corporation, Kyoto, Japan). A 3 mL sample was mixed with 27 mL of a 0.2% HCl solution. The sample was homogenized for one min and subsequently analyzed using the instrument. The concentration of carbon and nitrogen is expressed as the mean value of three replicates.

2.4. Antifungal Efficacy of OMWW and Components

2.4.1. Utilized Isolates

To evaluate the effects of OMWW and its components, representative isolates of phytopathogenic fungi sourced from olive trees were employed. The list of species used is presented in Table 1. Fungal isolates were cultured on potato dextrose agar (PDA) and maintained at 25 °C for five days in darkness.

Table 1. List of used fungal species, isolate names, and GenBank IDs, with references.

Species	Isolate	GenBank ID			Reference
		ITS	TUB2	TEF1-Alpha	
Botryosphaeria dothidea (Moug. ex Fr.) Ces. & De Not.	R19 F	OQ354201	OQ361700	OQ361701	[17]
Diplodia mutila (Fr.) Fr.	IKB9 B2II	OQ338569	OQ348379	OQ348386	
Diplodia seriata De Notaris	V16 K2II	OQ352870	OQ361695	OQ361696	
Dothiorella iberica A.J.L. Phillips, J. Luque & A. Alves	V16 BI	OQ339205	OQ348381	OQ348388	
Dothiorella sarmentorum (Fr.) A.J.L. Phillips, Alves & Luque	V12 PEN	OQ339150	OQ348380	OQ348387	
Neofusicoccum parvum (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips	V21 B5I	OQ341428	OQ348384	OQ553928	

2.4.2. Experimental Setup

To evaluate antifungal efficacy, five untreated OMWWs were used, along with the compound vanillic acid, which was either the most abundant or among the most abundant components of the OMWWs examined in this study. Additionally, hydroxytyrosol, identified in previous studies as a dominant OMWW component with potent antimicrobial activity [8,12], was included. Vanillic acid and hydroxytyrosol were sourced from Sigma Aldrich (Merck KGaA, Darmstadt, Germany).

Antifungal testing was conducted using OMWW at five concentrations, corresponding to volume ratios of 0.2%, 0.5%, 2%, 6%, and 10% in the substrate, while hydroxytyrosol and vanillic acid were tested at two concentrations, corresponding to volume ratios of 0.1% and 0.5% in the substrate [6,12]. Pure PDA served as the negative control, while Nativo 75WG (Bayer d.o.o., Zagreb, Croatia), commonly used against phytopathogenic fungi in olive cultivation, was the positive control. Nativo 75WG is a fungicide formulated as a water-dispersible granule. It contains two active ingredients: trifloxystrobin (250 g/kg) and tebuconazole (500 g/kg). The fungicide was diluted to the recommended concentration for olive tree treatment, according to the manufacturer's guidelines, of 20 g/100 l. PDA was used instead of water [22].

The temperature of PDA was monitored until it cooled to approximately 45 °C [6]. Once cooled, 10 mL of PDA was transferred into a sterile Falcon tube. The appropriate quantity of the OMWW, compound, or fungicide was pipetted into the tube, stirred with a glass rod, and gently vortexed to achieve a homogeneous mixture. The solution was then poured into Petri dishes, and a 4 mm diameter plug of an actively growing fungal culture was inoculated onto the center of the substrate, with the plug's upper surface facing the medium. The Petri dishes were sealed with parafilm and incubated in darkness at 25 °C. Each treatment and concentration were conducted in triplicate.

Fungal mycelial growth was assessed at two and seven days post-inoculation. For isolates showing no fungal growth at the final measurement, half of the mycelial plug

was transferred with a sterile needle onto fresh PDA in sterile Petri dishes. The samples were incubated under the same conditions. Treatments were classified as fungistatic if fungal growth resumed or fungicidal if no growth was observed. The minimum inhibitory concentration (MIC) was recorded as the lowest concentration that completely inhibited mycelial growth, while the minimum fungicidal concentration (MFC) was the lowest concentration resulting in fungicidal activity.

2.5. Antagonistic Activity of Microorganisms Isolated from OMWW

2.5.1. Isolation of Microorganisms from OMWW

The OMWW samples were shaken and vortexed. Subsequently, 100 µL of untreated OMWW was pipetted onto three different media: PDA, malt dextrose agar (MEA), and nutrient agar (NA). Three replicates were prepared for each medium. After seven days of incubation at room temperature (23 °C), microorganisms that developed on the media were subcultured onto pure media. For fungi, a PDA medium supplemented with the antibiotic streptomycin was used. For bacterial purification, the samples were placed in sterile PBS (phosphate-buffered saline), vortexed, and centrifuged at 1000× *g* for 5 min to separate bacteria and yeast, after which they were inoculated onto pure NA. These procedures were repeated until pure cultures were obtained. In total, three yeast isolates, one bacterial isolate, and three fungal isolates were obtained.

2.5.2. Identification of Microorganisms from OMWW

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used for the identification of yeast and bacterial isolates. Briefly, the yeast strains were grown on PDA + streptomycin agar, and bacterial strains were grown aerobically on NA agar plates for four days at room temperature (23 °C). A small proportion of yeast/bacterial colonies was subsequently analyzed using a Bruker Microflex LT MS machine (Bruker Daltonics, Manning Park Billerica, MA, USA). Rapid on-plate formic acid (FA) treatment, followed by the application of α-cyano-4-hydroxycinnamic acid (HCCA), was used to extract protein from bacterial/yeast cells, according to the manufacturer's instructions. For each spot, 40 sub-spectra for each of the 40 randomized positions within the spot were collected and assembled into one main spectrum. The mass spectra profiles were assessed, visualized, and analyzed/compared to reference spectra from the Bruker Biotyper version 3.1 (Build 65) database using Bruker flexControl 3.4 software. A mass range of 2000 to 20,000 Da was used for the analysis.

For the identification of filamentous fungi, the PCR method was used. The fungal isolates were cultured on PDA for five days at 25 °C in darkness. A small portion of mycelium from the colony margins was aseptically collected using a sterile laboratory needle for genomic DNA extraction. Total genomic DNA was extracted using the Maxwell® RSC Instrument (Promega, Madison, WI, USA) and the Maxwell® RSC Plant DNA Kit (Promega). The genomic DNA concentration was quantified post-isolation using a Maxwell Promega Quantus fluorometer (Promega). The internal transcribed spacer (ITS) regions were amplified and sequenced using the primer pairs ITS1 (5′ TCCGTAGGTGAACCT-GCGG 3′) and ITS4 (5′ TCCTCCGCTTATTGATATGC 3′) [23]. Each PCR mixture had a final volume of 25 µL, containing 12.5 µL of EmeraldAmp® GT PCR Master Mix, 0.5 µL of each primer (10 µM), 6.5 µL of nuclease-free water, and 5 µL of template DNA at a concentration of 5 ng/µL. PCR amplification was conducted using a SureCycler 8800 Thermal Cycler (Agilent Technologies, Santa Clara, CA, USA) according to a slightly modified program described by [24]. In the case of the yeast isolate R_BB, MALDI-TOF did not yield results; therefore, PCR analysis was also performed using the same equipment and chemicals. The difference lay in the PCR amplification conditions. The program included an initial

denaturation step at 95 °C for 2 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, elongation at 72 °C for 1 min, and a final extension step at 72 °C for 5 min [23]. Gel electrophoresis was performed using a 1% agarose gel at 110 V for 20 min in 1x TAE buffer, powered by an omniPAC Midi CS-300V electrophoresis power supply (Cleaver Scientific, Rugby, Warwickshire, UK). The PCR products were visualized with an iBright CL1000 Imaging System (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), purified using the GenElute™ PCR Clean-Up Kit (Sigma-Aldrich, Burlington, MA, USA), and subsequently sent to Macrogen Europe for sequencing. The nucleotide sequences were examined and refined using Sequencher 5.0 software (Gene Codes Corporation, Ann Arbor, MI, USA). A comparative analysis was performed using relevant sequences from species in the GenBank database, National Center for Biotechnology Information (NCBI). The consensus sequences obtained in this study were submitted to GenBank. Both the sequence data from the isolates analyzed in this study and additional relevant sequences from GenBank were incorporated into the phylogenetic analysis. The evolutionary relationships were inferred using the neighbor-joining method [25], with the resulting optimal phylogenetic tree illustrated. Bootstrap analysis, based on 1000 replicates, was conducted to assess the reliability of clustering, with the bootstrap values displayed adjacent to the branches [26]. Evolutionary distances were computed using the maximum composite likelihood approach, expressed as the number of nucleotide substitutions per site [27]. The phylogenetic analyses were carried out using MEGA11 software (Pennsylvania State University, State College, PA, USA) [28].

2.5.3. Antagonistic Assay

The antagonistic assay was conducted following the dual-culture method [29], as described in Živković et al. [30]. Isolates were pre-incubated in darkness at 25 °C for seven days. Sterile Petri dishes were filled with 10 mL of PDA. Using a 4 mm diameter cork borer, a mycelial disk of the pathogen was cut and placed on one side of the Petri dish, ensuring that the top side of the plug faced the medium. On the opposite side of the Petri dish, a loopful of bacteria or yeast (1 µL) was placed 3 cm away from the mycelial plug using a sterile inoculation loop. A pure PDA plate with a pathogen mycelial plug served as the control. The assay was performed in triplicate. For the antagonistic assay with Penicillium sp., the same procedure was followed, except that a 4 mm mycelial disk of the antagonist was used instead of the loopful.

The percentage of growth inhibition (PGI) was determined using the formula described in Živković et al. [30]: PGI (%) = (KR − R1)/KR × 100, where KR represents the distance (in mm) from the point of inoculation to the colony margin on the control plates, and R1 denotes the distance from the point of inoculation to the colony margin on the treated plates, specifically in the direction of the antagonist [31]. The PGI values were categorized using a growth inhibition category (GIC) scale ranging from 0 to 4 as follows: 0 = no growth inhibition, 1 = 1–25% growth inhibition, 2 = 26–50% growth inhibition, 3 = 51–75% growth inhibition, and 4 = 76–100% growth inhibition. The zone of inhibition was measured as the distance between the fungal pathogen and the area of antagonist growth after seven days of incubation [30].

2.6. Statistical Analysis

Statistical analysis of the chemical profile of the OMWW, calculations related to the antagonistic effect, percentage inhibition calculations, MIC and MFC values, and bar chart creation for antagonism were performed using Microsoft Office Excel. Graphical representations (HeatMaps) were generated using Python 3.10.12. The antifungal efficacy of OMWW, its components, and the fungicide were assessed using SAS Enterprise Guide

8.4. Data were expressed as arithmetic means, standard deviations, and 95% confidence intervals for the mean.

3. Results

3.1. Physicochemical Properties of the OMWW

The color of the OMWW varied among olive varieties, ranging from yellow to brown (Table 2). The darkest color was observed in OMWW from Istarska bjelica, while the lightest was found in OMWW from Buža puntoža. Regarding pH values, they tended to lean towards acidic, except for OMWW from Buža puntoža, which had a neutral pH of 7.17. The highest levels of dry matter and sugars were recorded in OMWW from Istarska Bjelica (35.72 mg/mL and 4.05 mg/mL, respectively), while the lowest levels were observed in OMWW from Buža Puntoža (1.54 mg/mL and 0.17 mg/mL, respectively).

Table 2. Results of the physicochemical analysis of OMWW.

Parameter	Type of OMWW	OMWW				
		Buža	**Buža Puntoža**	**Istarska Bjelica**	**Leccino**	**Rosinjola**
Color	O	yellow-brown	light orange-brown	dark orange-brown	light grey-white	Brown
pH	O	6	7.17	5.26	6.06	6.66
Dry matter (mg)	O	5.61 ± 0.19	1.54 ± 0.44	35.72 ± 0.74	19.42 ± 0.29	10.04 ± 0.25
	H-O	5.42 ± 0.15	3.07 ± 0.34	4.1 ± 0.72	10.85 ± 0.24	10.13 ± 0.02
Water volume fraction (%)	O	99.81 ± 0.01	99.94 ± 0.01	98.79 ± 0.02	99.34 ± 0.01	99.65 ± 0.01
	H-O	99.81 ± 0.01	99.89 ± 0.01	99.86 ± 0.02	99.59 ± 0.07	99.65 ± 0.00
Sugar content (mg/mL)	O	0.65 ± 0.02	0.17 ± 0.002	4.05 ± 0.20	2.95 ± 0.10	1.66 ± 0.01
	H-O	0.73 ± 0.02	0.25 ± 0.006	5.0 ± 0.1	1.99 ± 0.07	1.82 ± 0.01
Carbon content (mg C/L)	O	618.55 ± 0.29	297.30 ± 1.69	4453.50 ± 9.97	2251.50 ± 2.89	1245.50 ± 0.49
	H-O	557.00 ± 2.48	245.40 ± 0.16	4448.50 ± 4.31	1705.50 ± 1.06	1383.50 ± 0.49
Nitrogen content (mg N/L)	O	8.37 ± 0.03	7.05 ± 0.01	37.09 ± 0.08	16.83 ± 0.04	12.19 ± 0.02
	H-O	9.15 ± 0.01	6.60 ± 0.01	44.44 ± 0.06	15.42 ± 0.05	14.34 ± 0.01

O: OMWW without HCl; H-O: OMWW with HCl. The concentration of sugars is expressed as the average value measured at the wavelengths of 480 nm and 490 nm.

Following HCl treatment, OMWW from Buža puntoža also exhibited the lowest concentration of dry matter and sugars (3.07 mg/mL and 0.25 mg/mL, respectively), although these levels were higher compared to the untreated sample. Surprisingly, OMWW from Istarska bjelica, which had the highest dry matter and sugar content without HCl, demonstrated a sharp reduction in dry matter to 4.1 mg post-HCl treatment, while retaining the highest sugar concentration among all varieties and treatments (5.0 mg/mL). The highest concentration of dry matter after HCl treatment was recorded in OMWW from Leccino.

For all OMWW samples, except those from Leccino, the sugar concentrations were higher in HCl-treated samples compared to untreated samples.

Regarding the concentrations of carbon and nitrogen in the OMWW samples, the highest concentrations were observed in OMWW from Istarska bjelica, while the lowest were found in OMWW from Buža puntoža. Concerning the HCl treatments and carbon content, lower carbon concentrations were recorded in OMWW treated with HCl from Buža, Buža puntoža, Istarska bjelica, and Leccino. However, the opposite trend was observed for Rosinjola, where higher carbon concentrations were found in HCl-treated OMWW compared to untreated samples. Regarding nitrogen content, higher nitrogen concentrations were observed in HCl-treated OMWW from Buža, Istarska bjelica, and Rosinjola compared to untreated samples, while lower nitrogen concentrations were found in HCl-treated OMWW from Buža puntoža and Leccino.

3.2. HPLC Analysis of Phenolic Compounds

The data presented indicate a significant impact of acidification with HCl on the phenolic profiles of OMWW. The effects can be categorized into three main trends: an increase in phenolic concentrations, a decrease in certain compounds, and the complete absence of specific phenolics following acidification (Figure 1). For instance, HCl treatment increased the concentrations of the most abundant phenolics in OMWW from Buža, Buža puntoža, and Istarska bjelica, while a decrease was observed in OMWW from Leccino and Rosinjola. In some cases, such as in OMWW from Buža puntoža, caffeic acid was not detected in the sample without HCl treatment but was present following HCl treatment. Conversely, quercetin-3,4′-diglucoside was detected without HCl treatment but was absent after HCl treatment.

Significant variations in isorhamnetin concentrations were observed across all four measurements for each OMWW, as indicated by the standard deviation. Furthermore, larger deviations in concentration between measurements were recorded for other phenolics, such as luteolin and quercetin, particularly in OMWW from Buža and Buža puntoža.

The most abundant components in OMWW from Buža without HCl treatment were vanillic acid, luteolin-7-rutinoside, isorhamnetin, dihydroquercetin (taxifolin), and 3,4,5-trihydroxy benzoic acid (gallic acid). In contrast, with HCl treatment, the most abundant phenolics were luteolin-7-rutinoside, vanillic acid, vanillic-4-glucoside, p-coumaric acid, and 3,4,5-trihydroxybenzoic acid (gallic acid).

For OMWW from Buža puntoža, the most abundant components without HCl treatment were vanillic acid, isorhamnetin, vanillic-4-glucoside, 3,4,5-trihydroxybenzoic acid (gallic acid), and p-coumaric acid. Following HCl treatment, the most abundant phenolics were 3,4-dihydroxybenzoic acid (protocatechuic acid), vanillic acid, vanillic-4-glucoside, p-coumaric acid, and luteolin-7-rutinoside.

In OMWW from Istarska bjelica, the most abundant phenolics without HCl treatment were luteolin-7-rutinoside, vanillic-4-glucoside, vanillic acid, quercetin-3-rutinoside (rutin), and luteolin-7-glucoside. After HCl treatment, the most abundant phenolics were luteolin-7-rutinoside, verbascoside, vanillic-4-glucoside, quercetin-3-rutinoside (rutin), and luteolin-7-glucoside.

For OMWW from Leccino, the most abundant phenolics without HCl treatment were vanillic-4-glucoside, vanillic acid, luteolin-7-rutinoside, apigenin-7-glucoside, and luteolin. After HCl treatment, the most abundant components were luteolin-7-rutinoside, vanillic-4-glucoside, vanillic acid, luteolin-7-glucoside, and luteolin.

In OMWW from Rosinjola, the most abundant phenolics without HCl treatment were vanillic-4-glucoside, luteolin-7-rutinoside, vanillic acid, p-coumaric acid, and apigenin-7-glucoside. With HCl treatment, the most abundant were luteolin-7-rutinoside, vanillic-4-glucoside, vanillic acid, 3,4-dihydroxybenzoic acid (protocatechuic acid), and p-coumaric acid.

Overall, vanillic acid, vanillic-4-glucoside, and luteolin-7-rutinoside were the most abundant phenolics in OMWW samples collected in this study. The highest phenolic concentration was recorded in OMWW from Istarska bjelica, while the lowest was observed in OMWW from Buža puntoža. This is consistent with the findings for total dry matter content, which was also highest in Istarska bjelica and lowest in Buža puntoža. Acidification with HCl generally resulted in higher total phenolic concentrations across all OMWW samples, except for Buža puntoža.

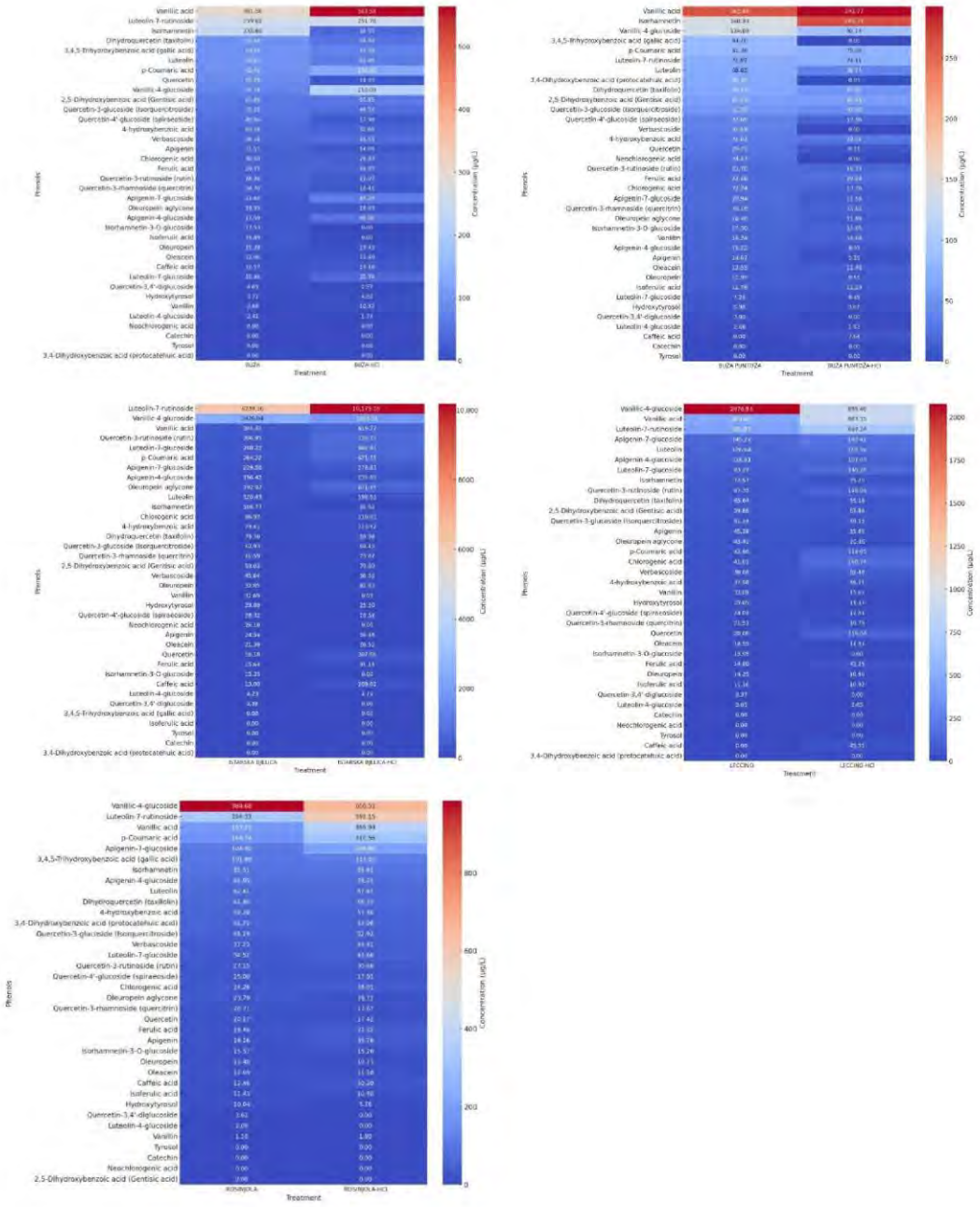


Figure 1. The heatmap presents the relative concentrations of individual phenolic compounds identified in OMWW from five olive varieties: Buža, Buža puntoža, Istarska bjelica, Leccino, and Rosinjola. For each variety, untreated samples (left in each pair) are compared to acidified samples treated with HCl (right in each pair). Rows represent specific phenolic compounds, while columns correspond to individual samples. Within each OMWW sample, phenolic compounds are ordered from the most to the least abundant based on the untreated condition. The color gradient indicates relative concentration levels, with red representing higher values and blue indicating lower concentrations.

3.3. Antifungal Activity of OMWW

The results of the ANOVA analysis showed significant differences between the applied treatments and their effects on the growth of phytopathogenic fungi mycelia. The results of the ANOVA analysis and the inhibition percentage calculations are presented in the Supplementary File in Tables S1–S12. For clarity, the text discusses the results of the inhibition percentage calculations.

Overall, among the tested fungi, *N. parvum* proved to be the most resistant species, where only the vanillic acid component and fungicide had an inhibitory effect on fungal mycelial growth (Figure 2). Conversely, *Do. iberica* was the most susceptible species to all treatments. Inhibitory effects of OMWW on fungal mycelial growth on the seventh day of measurement were observed exclusively for *Do. sarmentorum*. For *D. seriata*, all OMWW treatments stimulated mycelial growth. In *D. mutila*, only the lowest concentration of all OMWWs had an inhibitory effect, whereas higher concentrations exhibited a stimulatory effect across all treatments. A similar pattern was observed for *B. dothidea*, where lower OMWW concentrations inhibited mycelial growth, while higher concentrations stimulated growth. Vanillic acid was more effective compared to the component hydroxytyrosol, and the fungicide Nativo 75WG also demonstrated high efficacy in inhibiting fungal mycelial growth. Among the OMWW treatments, the most significant effects were observed with OMWW from Leccino, followed by Buža. Considering that the treatments' effects were more pronounced on the second day of measurement and that most treatments acted fungistatically, the percentage inhibition of fungal mycelial growth by fungi and treatments on the second day was graphically represented.

When analyzing each treatment individually and its effects on fungi, OMWW from Buža at lower concentrations (0.2%, 0.5%, and 2%) inhibited the mycelial growth of *B. dothidea*; however, at higher concentrations (6% and 10%), greater growth than the control was recorded. For *D. mutila*, the inhibitory effects of this OMWW from Buža were observed at a concentration of 0.2%, whereas at concentrations $\geq$0.5%, significantly higher mycelial growth compared to the control was recorded, indicating a stimulatory effect. For *Do. iberica*, 100% inhibition was observed at all concentrations of this OMWW on the second day of measurement. For *Do. sarmentorum*, no inhibition was observed at the lowest concentration. At higher concentrations, mycelial growth was inhibited by 100% on both the second and seventh days of measurement.

OMWW from Buža puntoža inhibited the growth of *B. dothidea* at lower concentrations (0.2% and 0.5%) by 31.95% and 22.19%, respectively. At a concentration of 2%, it had no effect, while at 6% and 10%, it stimulated mycelial growth. For *D. mutila*, only a minimal inhibitory effect (3.11%) was observed at the lowest concentration, with higher concentrations resulting in a stimulatory effect. For *Do. iberica*, the greatest inhibition (62.65%) was recorded at the lowest concentration, with inhibitory effects diminishing at higher concentrations. For *Do. sarmentorum*, inhibitory effects were recorded at concentrations of 0.2% and 0.5% (22.71% and 21.12%), but higher concentrations had no significant effect on mycelial growth.

OMWW from Istarska bjelica showed negligible effects on mycelial inhibition at a concentration of 0.2%, while higher concentrations exhibited a stimulatory effect. For *D. mutila* and *B. dothidea*, inhibitory effects were recorded only at 0.2% (2.7% and 1.87%). For *Do. iberica*, the greatest inhibitory effects were observed at the lowest concentrations (48.19% and 46.18%), diminishing with higher concentrations. For *Do. sarmentorum*, 100% inhibition was recorded at the lowest concentration; at 0.5%, inhibition was mild (8.76%), and at higher concentrations, the effects ranged from neutral to slightly stimulatory.

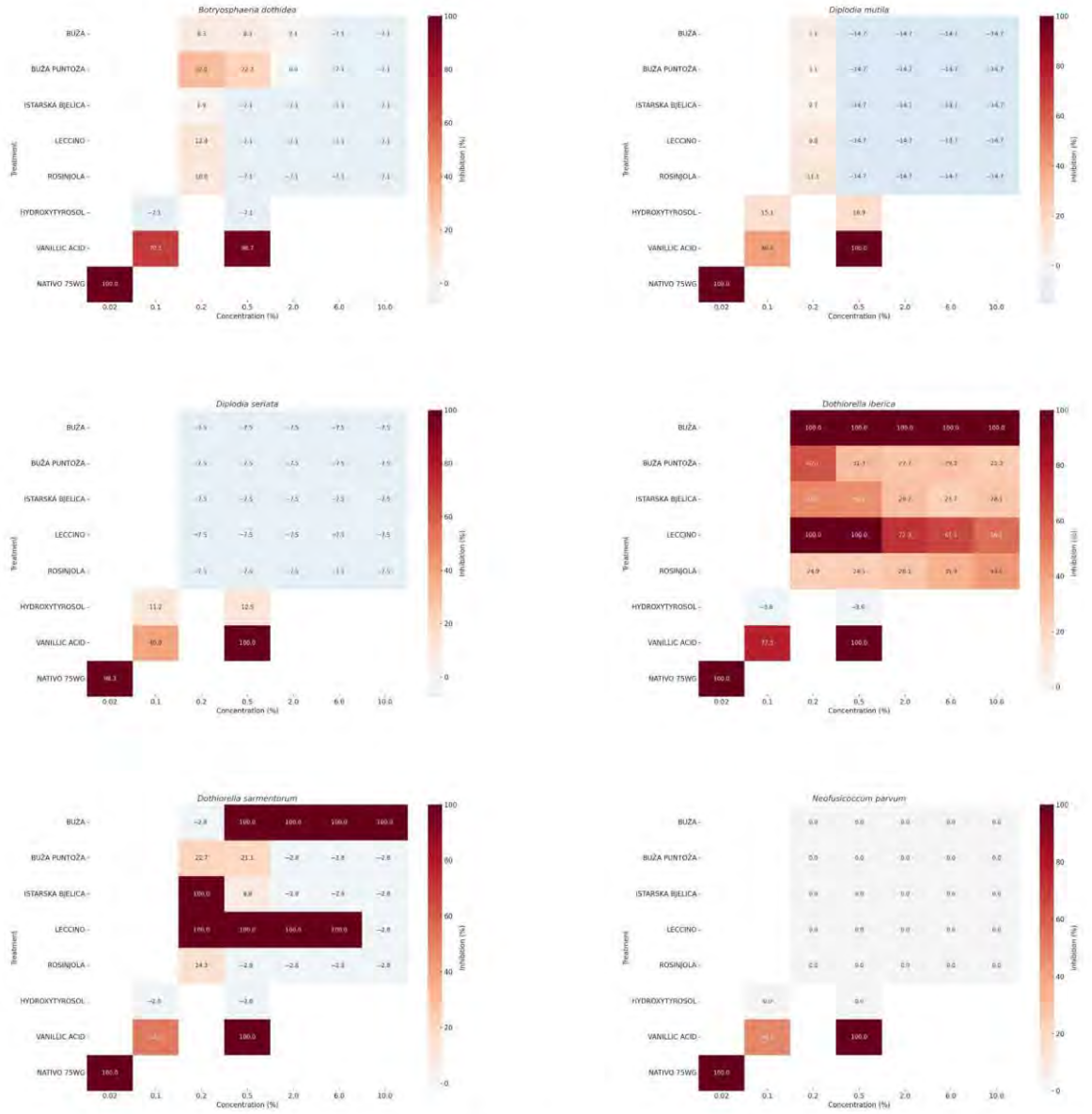


Figure 2. The heatmap presents the percentage of fungal mycelial growth inhibition measured on day two for six species of the *Botryosphaeriaceae* family: *Botryosphaeria dothidea*, *Diplodia mutila*, *Diplodia seriata*, *Dothiorella iberica*, *Dothiorella sarmentorum*, and *Neofusicoccum parvum*. Each panel represents one fungal species. Treatments include OMWW from five olive varieties, two phenolic compounds (hydroxytyrosol and vanillic acid), and a commercial fungicide (Nativo 75WG). The x-axis displays treatment concentrations, while the y-axis lists the applied treatments with inhibition percentages. The color gradient reflects the intensity of fungal inhibition, from blue (lowest or negative/stimulatory effect) to red (highest inhibition, up to 100%). Negative values indicate a stimulatory effect on fungal growth.

OMWW from Leccino inhibited the growth of *B. dothidea* at a concentration of 0.2% by 12.45%, with higher concentrations showing stimulatory effects. For *D. mutila*, a similar pattern was observed, with a 9.78% inhibition at 0.2%. For *Do. iberica*, 100% inhibition was recorded at concentrations of 0.2% and 0.5%, while inhibition diminished with higher concentrations. For *Do. sarmentorum*, 100% inhibition was observed at concentrations of 0.2–6% on both the second and seventh days of measurement. Only this species showed mycelial inhibition on the seventh day (with OMWW from Leccino and Buža showing 100% inhibition).

OMWW from Rosinjola inhibited the growth of *B. dothidea* and *D. mutila* only at the lowest concentration (10.79% and 11.11%, respectively), while higher concentrations showed a stimulatory effect. For *Do. iberica*, higher concentrations resulted in greater inhibition, with the highest inhibition (42.97%) at 10%. For *Do. sarmentorum*, growth inhibition was observed only at the lowest concentration (14.34%).

Regarding hydroxytyrosol, this component stimulated the growth of *B. dothidea*. For *D. mutila* and *D. seriata*, inhibitory effects on mycelial growth were observed only on the second day of measurement (15.11% and 16.89%; and 11.25 and 12.50). No inhibitory effects were observed on *Do. iberica*, *Do. sarmentorum*, or *N. parvum*. In contrast, vanillic acid exhibited inhibitory effects on all fungi, with greater inhibition at higher concentrations. For *B. dothidea*, inhibition at 0.1% was 70.12% and 12.02% on the second and seventh days, respectively, and 96.68% and 77.13% at 0.5%. For *D. mutila*, inhibition at 0.1% was observed only on the second day (40.44%), while at 0.5%, inhibition was 100% on both days. For *D. seriata*, inhibition was observed on both days at both concentrations, while higher concentrations resulted in 100% inhibition. For *Do. iberica* and *Do. sarmentorum*, inhibition at lower concentrations occurred on both days, while at higher concentrations, inhibition reached 100% for both species on both days. For *N. parvum*, inhibition at the lower concentration occurred only on the second day (48.45%), while at the higher concentration, inhibition was 100% on both days.

The fungicide Nativo 75WG was more effective than all other treatments for *B. dothidea*, *D. mutila*, and *D. seriata*. For *Do. Iberica*, the fungicide outperformed all other treatments, except vanillic acid at 0.5%. For *Do. sarmentorum*, the fungicide was effective (100%) on both days, as were OMWW from *Buža* at concentrations of 0.5–10% and OMWW from *Leccino* at 0.2–6%. For *N. parvum*, Nativo 75WG was also highly effective (100% and 93.41%), but vanillic acid at a concentration of 0.5% was more effective, achieving 100% inhibition on both days.

Regarding the MIC and MFC values (Table S13), the fungicide, as expected, showed the best results, exhibiting a fungicidal effect on all tested fungi except *Diplodia seriata*, where only a fungistatic effect was observed. Among the other treatments, MFC values were recorded only for certain species in the Buža, Leccino, and vanillic acid treatments.

3.4. Antagonistic Assay

3.4.1. Identification of Microorganisms

The list of all species isolated from the OMWW is presented in Table 3. A total of one bacterium, three yeasts, and three filamentous fungi were isolated. The bacterium was found only in OMWW from Buža. From the OMWW of Istarska bjelica and Leccino, only *Penicillium crustosum* Thom was isolated. The ITS region sequences of the isolates were deposited in GenBank under accession numbers PV092539 for isolate R_BB, PQ826427 for isolate BJ_P, PQ826435 for isolate L_P, and PQ826436 for isolate BP_P. The phylogenetic analysis confirms the identification of this species (Figure S1).

Table 3. The list of species isolated from each OMWW.

OMWW	Isolate	Isolated Organism
Buža	B_RB	*Rhodotorula mucilaginosa* (A. Jörg.) F.C. Harrison
	B_BB	*Bacillus velezensis* Ruiz-Garcia et al.
Buža puntoža	BP_P	*Penicillium crustosum* Thom
Istarska bjelica	BJ_P	*P. crustosum*
Leccino	L_P	*P. crustosum*
Rosinjola	R_RB	*R. mucilaginosa*
	R_BB	*Nakazawaea molendiniolei* (N. Cadez, B. Turchetti & G. Peter) C. P. Kurtzman & C. J. Robnett

3.4.2. Antagonistic Assay Results

The greatest antagonistic effect against fungi was observed with the species *Do. iberica*, where four out of seven tested isolates exhibited a strong antagonistic impact, with a GIC value of 4 (Table S14). The highest antagonistic effect on *B. dothidea* was shown by *P. crustosum* from OMWW Istarska bjelica (BJ_P) (70.54%), while *P. crustosum* from OMWW Buža puntoža (BP_P) (67.86%) was also effective (Figure 3). In contrast, *N. molendiniolei* (R_BB) demonstrated the weakest effect (11.76%). For *D. mutila*, the highest inhibition percentage was observed with *P. crustosum* from OMWW Leccino (L_P) (96.67%), and *P. crustosum* from OMWW Istarska bjelica (BJ_P) also showed significant effectiveness (85%). Against *D. seriata*, *P. crustosum* from OMWW Leccino (L_P) (82.22%) and *P. crustosum* from OMWW Buža puntoža (BP_P) (81.67%) exhibited the highest inhibition. In comparison, *B. velezensis* (B_BB) was substantially less effective.

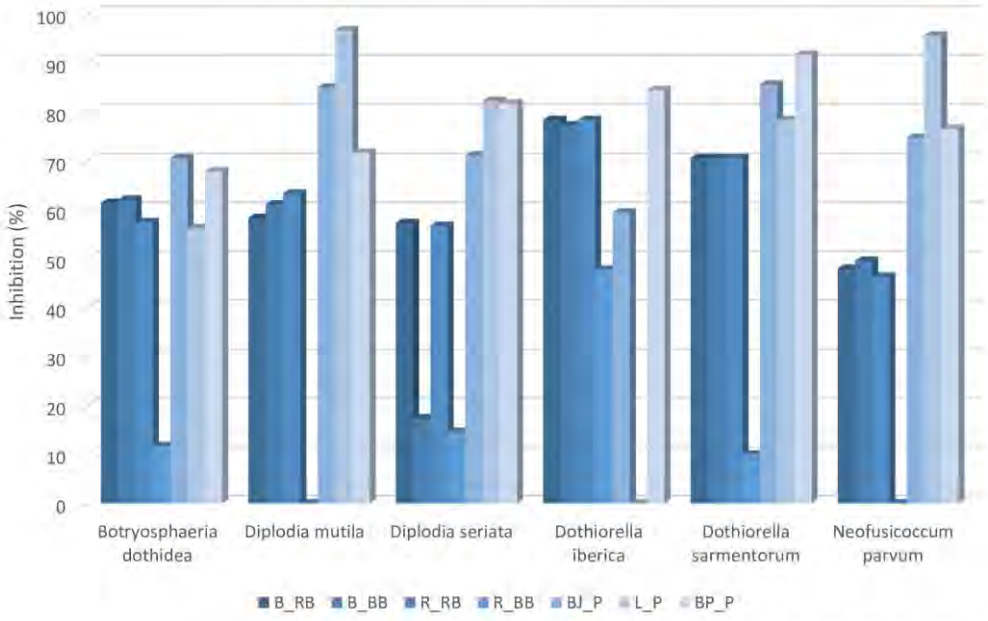


Figure 3. Results of the statistical analysis of the antagonistic activity of microorganisms isolated from OMWWs against phytopathogenic fungi. The graph represents the calculated percentage of inhibition of fungal mycelial growth by microorganisms isolated from OMWWs. Different isolates were tested against six phytopathogenic fungi: *Botryosphaeria dothidea*, *Diplodia mutila*, *Diplodia seriata*, *Dothiorella iberica*, *Dothiorella sarmentorum*, and *Neofusicoccum parvum*. Higher inhibition percentages indicate stronger antagonistic effects.

The best effect on *Do. iberica* was observed with *P. crustosum* from OMWW Buža puntoža (BP_P) (84.44%), while *P. crustosum* from OMWW Istarska bjelica (BJ_P) (59.44%) showed moderate effectiveness. For *Do. sarmentorum*, *P. crustosum* from OMWW Buža puntoža (BP_P) (91.67%) and Istarska bjelica (BJ_P) (85.56%) demonstrated notably high inhibition, while *N. molendiniolei* (R_BB) exhibited a very weak effect (10%). For *N. parvum*, *P. crustosum* from OMWW Leccino (L/P) almost completely inhibited pathogen growth (95.57%), while those from Istarska bjelica (BJ_P) (74.69%) and Buža puntoža (BP_P) (76.54%) were also effective.

Among the tested *Penicillium* isolates, L_P emerged as the most efficient antagonistic organism, particularly against *D. mutila* and *N. parvum* (Figure S2). The weakest antagonistic effect among the treatments was observed with the R_BB isolate, *N. molendiniolei*, as no antagonistic interaction was recorded between this isolate and *D. mutila* or *N. parvum*. Additionally, no antagonistic effect was observed between the L_P isolate and *Do. iberica*.

4. Discussion

As previously mentioned, OMWW and its management pose a significant challenge in agriculture. However, some OMWWs have demonstrated effectiveness in inhibiting the growth of phytopathogenic fungi, as well as the potential to stimulate plant growth due to their organic content. Moreover, OMWWs can serve as a resource for extracting phenols or microorganisms that have proven effective in suppressing the growth of phytopathogenic fungi.

Among the OMWW treatments in this study, the most significant effects were observed with OMWWs from the Leccino variety, followed by Buža. While these olive varieties are highly praised and considered among the favorites of olive oil enthusiasts, the *Botryosphaeriaceae* species seem to "lose the will to live", indicating that the OMWW from these olive varieties inhibits the growth of fungi as effectively as their oils delight gourmets. A varied effect of olive mill wastes on phytopathogenic fungi was reported in the study by Cayuela et al. [15]. The authors noted that the diverse effects observed in certain cases, such as with *Globisporangium ultimum* (Trow) Uzuhashi, Tojo & Kakish (syn. *Pythium ultimum*) and *Botrytis cinerea* Pers., showed no correlation with the measured chemical properties of the residues but were likely linked to specific compounds present in varying concentrations within the residues. Cayuela et al. [15] emphasize that the limited research examining the antifungal potential of olive mill wastes often yield conflicting results. This variability arises from the numerous factors that can influence the effectiveness of these wastes in suppressing pathogens. For instance, Bonanomi et al. [32] observed that dry olive mill residue exhibited phytotoxic effects on various crop species, which, in certain cases, increased the number of fungal diseases. Similarly, in our research, it was confirmed that treatments had a stimulative effect on certain fungi, while in some cases, the effect depended on the concentration. At lower concentrations, the impact was inhibitory, whereas at higher concentrations, it became stimulative, and vice versa.

Istrian olive oils are known to contain a relatively high amount of polyphenols compared to values reported in the literature [33]. Among these, olive oils from the Istarska bjelica variety stand out for their exceptionally high polyphenol content [33]. Similarly, in our research, the highest phenolic concentration was recorded in OMWWs derived from Istarska bjelica. This can also be linked to the fact that this OMWW had the highest dry matter content, which corresponded with elevated levels of sugars, nitrogen, and carbon. Although the phenolic concentration was the highest in this OMWW, and the antimicrobial activity of OMWW is often associated with the presence of phenols, it did not demonstrate the strongest inhibition of fungal mycelium growth. The antifungal properties of phenolic compounds against pathogenic fungi have been documented in several studies. These

studies describe the effects of phenolics on fungi such as *Verticillium dahliae* Klebahn [34], *Phytophthora* sp., *Alternaria* sp., *Fusarium* sp., and others [35,36]. Additionally, Krid et al. [12] identified hydroxytyrosol as the main antimicrobial compound in OMWW. In our research, vanillic acid, vanillic-4-glucoside, and luteolin-7-rutinoside were identified as the most abundant phenolic compounds in OMWW. Regarding hydroxytyrosol, which is widely cited as the phenol with the strongest antimicrobial activity, its effectiveness was limited to *D. mutila* and *D. seriata*. It did not affect *N. parvum*, and its impact on *B. dothidea*, *Do. iberica*, and *Do. sarmentorum* was mildly stimulatory. However, this stimulatory effect should be interpreted with caution, given the sensitivity and variability of biological assays. In the case of vanillic acid, an increase in its concentration was associated with greater inhibitory effects. At a concentration of 0.5%, it completely inhibited the growth of tested fungal species, demonstrating its significant antifungal potential.

Regarding the acidification of OMWW with HCl and its impact on phenols, it significantly influences the phenolic composition of OMWW, with both positive and negative effects. In many cases, acidification with HCl resulted in either an increase or decrease in specific phenols. This suggests that acidification may enhance the stability or release of these phenols, possibly by breaking down glycosylated forms into their free phenolic counterparts. Conversely, reductions in phenolic content could be attributed to the degradation of sensitive phenols under acidic conditions or their transformation into other phenolic derivatives that were not quantified in this dataset. Certain phenols are particularly susceptible to degradation under specific conditions [37,38].

In addition to phenols, other agents responsible for the antimicrobial activity of OMWW are mentioned in the literature. Yangui et al. [6] suggested the potential application of OMWW and bacteria isolated from OMWW (*Bacillus subtilis* (Ehrenberg 1835) Cohn, *Trinickia caryophylli* (Burkholder 1942) Estrada-de los Santos (syn. *Burkholderia caryophylli*), and *Pseudomonas fluorescens* Migula for controlling the pathogenic fungus *Armillaria mellea* (Vahl) P. Kumm). In the work of Alfano et al. [35], the antifungal potential of olive waste compost was examined. The compost was found to contain significant populations of active microbes capable of degrading chitin and cellulose. Plate inhibition assays demonstrated that extracts from compost water strongly inhibited the growth of several pathogens, including *Fusarium oxysporum* f.sp. *lycopersici*, *Globisporangium ultimum* (Trow) Uzuhashi, Tojo & Kakish (syn. *Pythium ultimum*), *Phytophthora infestans* (Mont.) de Bary, *Sclerotinia sclerotiorum* (Lib.) de Baryand, and *V. dahliae*. The inhibitory effects were attributed to the antagonistic activities of microorganisms present in the compost, including large populations of aerobic spore-forming bacteria and actinomycetes. Muzzalupo et al. (2020) [36] reported the great effectiveness of olive leaf extracts in controlling fungal pathogens, either in their free form or encapsulated in chitosan-tripolyphosphate nanoparticles. Their study documented high inhibition rates for the germination and growth of *Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg.

Several microorganisms were isolated from the OMWW used in this study, including *P. crustosum*, *B. velezensis*, *R. mucilaginosa*, and *N. molendiniolei*.

P. crustosum is a common fungal species frequently associated with food contamination, leading to the spoilage of various foods. This species has been previously reported on olives and their byproducts in Spain [39]. It has shown significant potential for future industrial applications due to its pronounced enzymatic activities [39]. Gharsallah et al. [40] also identified *P. crustosum* from insects collected in olive orchards. The authors demonstrated the pathogenicity of *P. crustosum* through assays performed on excised shoots, where the isolate *P. crustosum* F14 caused browning in the cortex. Additionally, the study documented antagonistic interactions between this isolate and fungal species such as *Aspergillus calidoustus* Varga, Houbraken & Samson, *Penicillium chrysogenum* Thom, and *Alternaria consortialis*

(Thm.) J.W. Groves & S. Hughes. In contrast, no antagonistic effects were observed with the isolate *P. crustosum* F33, which was also collected from insects in olive orchards. This study also confirmed differences in the antagonistic potential among *Penicillium* sp. isolates, as well as variations in the antagonistic effects of the same isolate on different fungal species. The strongest antagonistic effect was observed between the L/P isolate and the species *N. parvum* and *D. mutila*, while the weakest interaction was noted between L/P and *Do. iberica*, where no antagonistic effect of the isolate on the pathogen was recorded.

B. velezensis Ruiz-Garcia et al. is an aerobic, Gram-positive bacterium capable of forming endospores and enhancing plant growth. Various strains of this species have been documented for their ability to inhibit the growth of microbial pathogens, including fungi, bacteria, and nematodes [41]. *B. velezensis* OEE1, isolated from the endogenous root tissue of olive trees, exhibited antifungal activity under in vitro conditions against *V. dahliae*, with an inhibition rate exceeding 92%. Under in vivo conditions, *B. velezensis* OEE1 significantly reduced the final mean disease severity index, the percentage of dead plants, the area under the disease progress curve, and the microsclerotia density in naturally infested soil [42]. In the study by Castro et al. [43], under in vitro conditions, strain XT1 demonstrated the ability to reduce fungal mycelium by 34–100%. When applied directly to young olive trees, it decreased the incidence rate of Verticillium wilt and the severity of symptoms. Additionally, it increased polyphenol oxidase (PPO) activity by 395%, indicating the enhanced resistance of plant tissues to the disease, and reduced the number of fungal microsclerotia in the soil. The *B. velezensis* isolate in this study exhibited a strong antagonistic effect on pathogens, with the greatest impact observed on *Do. iberica* and the least on *D. seriata*.

R. mucilaginosa is a biotechnologically significant yeast that has garnered considerable attention as a potential platform strain due to its ability to utilize a wide range of substrates, exceptional stress tolerance, and other advantageous traits. *R. mucilaginosa* is considered a highly suitable candidate for producing carotenoids, lipids, enzymes, and other valuable bioproducts, particularly through the biorefining of low-cost agricultural waste materials [44]. Ghilardi et al. [45] demonstrated that substrates derived from olive mill waste can be effectively utilized for carotenoid production by *R. mucilaginosa*. Interestingly, Jarboui et al. [46,47] identified that *R. mucilaginosa* CH4 can play a significant role in the purification of OMWW by removing polyphenolic compounds, including catechol, gallic acid, p-coumaric acid, protocatechuic acid, tyrosol, vanillic acid, etc. In this study, the yeast mentioned was isolated from the OMWW of Buža and Rosinjola, with the R_RB isolated from the OMWW of Rosinjola showing a stronger impact on pathogens.

N. molendiniolei (syn. *Nakazawaea molendini-olei* or *Candida molendinolei*) has been recognized for its resistance to phenolic compounds and its ability to convert oleuropein into hydroxytyrosol [48]. It has also been utilized as a starter culture for controlled olive fermentation, as demonstrated in the study by Ciafardini and Zullo [49]. Furthermore, *N. molendiniolei* exhibits significant enzymatic activities, such as β-glucosidase and peroxidase. These activities contribute to limiting the increase in the acidity of olive oil during storage; however, they are also associated with an increase in oxidative parameters, which ultimately result in a decline in olive oil quality over time [50].

5. Conclusions

This study confirms the potential of OMWW as a sustainable alternative to chemical fungicides. Rich in bioactive compounds such as phenols, OMWW represents an interesting and environmentally friendly solution for crop protection in the Mediterranean region while simultaneously reducing environmental burdens. Among the OMWW treatments, Leccino and Buža showed the most significant antifungal activity against aggressive pathogens

from the *Botryosphaeriaceae* family, which are known for causing substantial yield losses in woody crops.

The results also highlight the impact of acidification on the phenolic profile of OMWW. Treatment with HCl significantly altered the concentration of individual phenolic compounds, either enhancing their release or contributing to their degradation, suggesting that pH manipulation could be a tool for optimizing OMWW bioactivity. Furthermore, among the two phenols tested, vanillic acid demonstrated exceptional antifungal activity, while microorganisms isolated from OMWW, such as *B. velezensis*, further emphasized the biological potential of this waste. These findings open up possibilities for integrating OMWW into sustainable crop protection systems.

Future studies should focus on the standardization of production processes, the optimization of concentrations, and the combination of OMWW with other bioactive compounds or microorganisms. This could make OMWW a key component in integrated management systems, offering the dual benefit of crop protection and the promotion of sustainable agricultural practices. In addition, OMWW serves as a valuable source for the isolation of microorganisms that can be used for various purposes, including pigment production, in the food industry, and others.

In conclusion, OMWW exemplifies the principles of the circular economy by transforming agricultural waste into a valuable product. While the results are promising, additional research is essential to address challenges, such as the stimulatory effects of higher OMWW concentrations on certain pathogens. This study lays the foundation for the further development of eco-friendly crop protection methods aligned with the goals of the European Green Deal and the target of reducing pesticide use by 50% by 2030.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae11070819/s1, Table S1. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Botryosphaeria dothidea* under different treatments. Table S2. Inhibitory effect (%) of treatment on the mycelial growth of *Botryosphaeria dothidea*. Table S3. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Diplodia mutila* under different treatments. Table S4. Inhibitory effect (%) of treatment on the mycelial growth of *Diplodia mutila*. Table S5. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Diplodia seriata* under different treatments. Table S6. Inhibitory effect (%) of treatment on the mycelial growth of *Diplodia seriata*. Table S7. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Dothiorella iberica* under different treatments. Table S8. Inhibitory effect (%) of treatment on the mycelial growth of *Dothiorella iberica*. Table S9. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Dothiorella sarmentorum* under different treatments. Table S10. Inhibitory effect (%) of treatment on the mycelial growth of *Dothiorella sarmentorum*. Table S11. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Neofusicoccum parvum* under different treatments. Table S12. Inhibitory effect (%) of treatment on the mycelial growth of *Neofusicoccum parvum*. Table S13. MIC and MFC values of treatments. Table S14. Growth Inhibition Category (GIC) scale results from the antagonism test, where a higher number indicates a greater level of inhibition. Figure S1. The evolutionary relationships were determined using the Neighbor-Joining method, and the optimal phylogenetic tree is presented. Figure S2. Results of the conducted tests.

Author Contributions: Conceptualization—E.P., K.V., J.Ć., and S.G.; Data curation—E.P., A.A., and Z.U., Formal analysis—E.P., Funding acquisition—S.G., A.A., I.G., K.V., S.G.B., N.M., and Z.U. Methodology—E.P., K.V., A.A., I.G., N.M., J.Ć., Z.U., and S.G.; Investigation—E.P., A.A., I.G., N.M., and Z.U., Project administration—S.G. and A.A., Resources—S.G., N.M., S.G.B., Z.U., A.A., and I.G., Supervision—K.V. and S.G., Visualization—E.P., Writing—original draft preparation, E.P.; Writing—review and editing—E.P., K.V., A.A., I.G., N.M., J.Ć., Z.U., S.G.B., and S.G. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Croatian Science Foundation under the project numbers HRZZ-UIP-2020-02-7413 and HRZZ-DOK-2021-02-2882, and the Slovenian Research and Innovation Agency (research program P1-0005 and P4-0092).

Data Availability Statement: Data is contained within the article and Supplementary Materials.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Žužić, I. *Buđenje Istarskog Maslinarstva 1960-ih Godina*; Propaganda d.o.o.: Poreč, Croatia, 2023.
2. FAO, Food and Agriculture Organization of the United Nations. FAOSTAT: Crops and Livestock Products. Available online: https://www.fao.org/faostat/en/#data/QCL (accessed on 19 February 2025).
3. Shabir, S.; Ilyas, N.; Saeed, M.; Bibi, F.; Sayyed, R.Z.; Almaki, W.H. Treatment technologies for olive mill wastewater with impacts on plants. *Environ. Res.* **2023**, *216*, 114399. [CrossRef]
4. Ghilardi, C.; Negrete, P.S.; Gutierrez, G.R.; Monetta, P.; Arroyo-Lopez, F.N.; Hornero-Mendez, D.; Carelli, A.A.; Borroni, V. Influence of olive mill waste phenolic compounds levels on carotenoid production by *Rhodotorula* spp. *Process Biochem.* **2022**, *120*, 275–286. [CrossRef]
5. Bouhia, Y.; Hafidi, M.; Ouhdouch, Y.; El Boukhari, M.E.; El Fels, L.; Zeroual, Y.; Lyamlouli, K. Microbial community succession and organic pollutants removal during olive mill waste sludge and green waste co-composting. *Front. Microbiol.* **2022**, *12*, 814553. [CrossRef] [PubMed]
6. Yangui, T.; Rhouma, A.; Ali Triki, M.; Gargouri, K.; Bouzid, J. Control of damping-off caused by *Rhizoctonia solani* and *Fusarium solani* using olive mill waste water and some of its indigenous bacterial strains. *Crop Prot.* **2008**, *27*, 189–197. [CrossRef]
7. Cibelli, F.; Bevilacqua, A.; Raimondo, M.L.; Campaniello, D.; Carlucci, A.; Ciccarone, C.; Sinigaglia, M.; Corbo, M.R. Evaluation of fungal growth on olive-mill wastewaters treated at high temperature and by high-pressure homogenization. *Front. Microbiol.* **2017**, *8*, 2515. [CrossRef]
8. Yakhlef, W.; Arhab, R.; Romero, C.; Brenes, M.; de Castro, A.; Medina, E. Phenolic composition and antimicrobial activity of Algerian olive products and by-products. *LWT* **2018**, *93*, 323–328. [CrossRef]
9. Galanakis, C.M. Phenols recovered from olive mill wastewater as additives in meat products. *Trends Food Sci. Technol.* **2018**, *79*, 98–105. [CrossRef]
10. Galanakis, C.M.; Tsatalas, P.; Galanakis, I.M. Implementation of phenols recovered from olive mill wastewater as UV booster in cosmetics. *Ind. Crops Prod.* **2018**, *111*, 30–37. [CrossRef]
11. Medina, E.; Romero, C.; de los Santos, B.; de Castro, A.; García, A.; Romero, F.; Brenes, M. Antimicrobial activity of olive solutions from stored alpeorujo against plant pathogenic microorganisms. *J. Agric. Food Chem.* **2011**, *59*, 6921–6929. [CrossRef]
12. Krid, S.; Bouaziz, M.; Ali Triki, M.; Gargouri, A.; Rhouma, A. Inhibition of olive knot disease by polyphenols extracted from olive mill waste water. *J. Plant Pathol.* **2011**, *93*, 561–568.
13. Bleve, G.; Gallo, A.; Altomare, C.; Vurro, M.; Maiorano, G.; Cardinali, A.; D'Antuono, I.; Marchi, G.; Mita, G. In vitro activity of antimicrobial compounds against *Xylella fastidiosa*, the causal agent of the olive quick decline syndrome in Apulia (Italy). *FEMS Microbiol. Lett.* **2018**, *365*, fnx281. [CrossRef] [PubMed]
14. Bažok, R. Je li održiva uporaba pesticida doista održiva? *Glas. Biljn. Zaštite* **2020**, *20*, 384–389.
15. Cayuela, M.L.; Millner, P.D.; Meyer, S.L.; Roig, A. Potential of olive mill waste and compost as biobased pesticides against weeds, fungi, and nematodes. *Sci. Total Environ.* **2008**, *399*, 11–18. [CrossRef]
16. Úrbez-Torres, J.R.; Peduto, F.; Vossen, P.M.; Krueger, W.H.; Gubler, W.D. Olive twig and branch dieback: Etiology, incidence, and distribution in California. *Plant Dis.* **2013**, *97*, 231–244. [CrossRef] [PubMed]
17. Petrović, E.; Vrandečić, K.; Belušić Vozila, A.; Ćosić, J.; Godena, S. Diversity and pathogenicity of *Botryosphaeriaceae* species isolated from olives in Istria, Croatia, and evaluation of varietal resistance. *Plants* **2024**, *13*, 1813. [CrossRef]
18. Russo, E.; Spallarossa, A.; Comite, A.; Pagliero, M.; Guida, V.; Belotti, V.; Caviglia, D.; Schito, A.M. Valorization and potential antimicrobial use of olive mill wastewater (OMW) from Italian olive oil production. *Antioxidants* **2022**, *11*, 903. [CrossRef]

19. Klen, T.J.; Vodopivec, B.M. Ultrasonic extraction of phenols from olive mill wastewater: Comparison with conventional methods. *J. Agric. Food Chem.* **2011**, *59*, 12725–12731. [CrossRef]
20. Klen, T.J.; Wondra, A.G.; Vrhovšek, U.; Vodopivec, B.M. Phenolic profiling of olives and olive oil process-derived matrices using UPLC-DAD-ESI-QTOF-HRMS analysis. *J. Agric. Food Chem.* **2015**, *63*, 3859–3872. [CrossRef] [PubMed]
21. DuBois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350–356. [CrossRef]
22. Palfi, M. Antifungal Activity of Essential Oils and Their Components Against Phytopathogenic Fungi Under In Vitro Conditions. Ph.D. Thesis, Josip Juraj Strossmayer University of Osijek, Ruđer Bošković Institute, Zagreb, Croatia, 2017.
23. White, T.J.; Bruns, T.D.; Lee, S.B.; Taylor, J.W. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*, 1st ed.; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press Inc.: Cambridge, MA, USA, 1990; pp. 315–322.
24. Demjanová, S.; Jevinová, P.; Pipová, M.; Regecová, I. Identification of *Penicillium verrucosum*, *Penicillium commune*, and *Penicillium crustosum* isolated from chicken eggs. *Processes* **2021**, *9*, 53. [CrossRef]
25. Saitou, N.; Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **1987**, *4*, 406–425. [PubMed]
26. Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **1985**, *39*, 783–791. [CrossRef] [PubMed]
27. Tamura, K.; Nei, M.; Kumar, S. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11030–11035. [CrossRef]
28. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [CrossRef] [PubMed]
29. Fokkema, N.J. Fungal antagonism in the phyllosphere. *Ann. Appl. Biol.* **1978**, *89*, 115–117. [CrossRef]
30. Živković, S.; Stojanović, S.; Ivanović, Ž.; Gavrilović, V.; Popović, T.; Balaž, J. Screening of antagonistic activity of microorganisms against *Colletotrichum acutatum* and *Colletotrichum gloeosporioides*. *Arch. Biol. Sci. Belgrade* **2010**, *62*, 611–623. [CrossRef]
31. Korsten, L.; De Jager, E.S. Mode of action of *Bacillus subtilis* for control of avocado postharvest pathogens. *S. Afr. Avocado Grow. Assoc. Yearb.* **1995**, *18*, 124–130.
32. Bonanomi, G.; Giorgi, V.; Del Sorbo, G.; Neri, D.; Scala, F. Olive mill residues affect saprophytic growth and disease incidence of foliar and soilborne plant fungal pathogens. *Agric. Ecosyst. Environ.* **2006**, *115*, 194–200. [CrossRef]
33. Bertoša, M.; Matijašić, R. (Eds.) *Istarska Enciklopedija*; Leksikografski zavod Miroslav Krleža: Zagreb, Croatia, 2005.
34. Markakis, E.A.; Tjamos, S.E.; Antoniou, P.P.; Roussos, P.A.; Paplomatas, E.J.; Tjamos, E.C. Phenolic responses of resistant and susceptible olive cultivars induced by defoliating and nondefoliating *Verticillium dahliae* pathotypes. *Plant Dis.* **2010**, *94*, 1156–1162. [CrossRef]
35. Alfano, G.; Lustrato, G.; Lima, G.; Vitullo, D.; Ranalli, G. Characterization of composted olive mill wastes to predict potential plant disease suppressiveness. *Biol. Control* **2011**, *58*, 199–207. [CrossRef]
36. Muzzalupo, I.; Badolati, G.; Chiappetta, A.; Picci, N.; Muzzalupo, R. In vitro antifungal activity of olive (*Olea europaea*) leaf extracts loaded in chitosan nanoparticles. *Front. Bioeng. Biotechnol.* **2020**, *8*, 151. [CrossRef] [PubMed]
37. Brenes, M.; García, A.; García, P.; Garrido, A. Acid hydrolysis of secoiridoid aglycons during storage of virgin olive oil. *J. Agric. Food Chem.* **2001**, *49*, 5609–5614. [CrossRef]
38. Romero, C.; Brenes, M.; García, P.; García, A.; Garrido, A. Polyphenol changes during fermentation of naturally black olives. *J. Agric. Food Chem.* **2004**, *52*, 1973–1979. [CrossRef]
39. Baffi, M.A.; Romo-Sánchez, S.; Úbeda-Iranzo, J.; Briones-Pérez, A.I. Fungi isolated from olive ecosystems and screening of their potential biotechnological use. *New Biotechnol.* **2012**, *29*, 451–456. [CrossRef] [PubMed]
40. Gharsallah, H.; Ksentini, I.; Naayma, S.; Taieb, K.H.; Abdelhedi, N.; Schuster, C.; Triki, M.A.; Ksantini, M.; Leclerque, A. Identification of fungi in Tunisian olive orchards: Characterization and biological control potential. *BMC Microbiol.* **2020**, *20*, 307. [CrossRef]
41. Rabbee, M.F.; Ali, M.S.; Choi, J.; Hwang, B.S.; Jeong, S.C.; Baek, K.H. *Bacillus velezensis*: A valuable member of bioactive molecules within plant microbiomes. *Molecules* **2019**, *24*, 1046. [CrossRef] [PubMed]
42. Cheffi Azabou, M.; Gharbi, Y.; Medhioub, I.; Ennouri, K.; Barham, H.; Tounsi, S.; Triki, M.A. The endophytic strain *Bacillus velezensis* OEE1: An efficient biocontrol agent against Verticillium wilt of olive and a potential plant growth-promoting bacteria. *Biol. Control* **2020**, *142*, 104168. [CrossRef]
43. Castro, D.; Torres, M.; Sampedro, I.; Martínez-Checa, F.; Torres, B.; Béjar, V. Biological control of Verticillium wilt on olive trees by the salt-tolerant strain *Bacillus velezensis* XT1. *Microorganisms* **2020**, *8*, 1080. [CrossRef]
44. Li, Z.; Li, C.; Cheng, P.; Yu, G. *Rhodotorula mucilaginosa*—Alternative sources of natural carotenoids, lipids, and enzymes for industrial use. *Heliyon* **2022**, *8*, e11505. [CrossRef]

45. Ghilardi, C.; Sanmartin Negrete, P.; Carelli, A.A.; Borroni, V. Evaluation of olive mill waste as substrate for carotenoid production by *Rhodotorula mucilaginosa*. *Bioresour. Bioprocess.* **2020**, *7*, 52. [CrossRef]
46. Jarboui, R.; Baati, H.; Fetoui, F.; Gargouri, A.; Gharsallah, N.; Ammar, E. Yeast performance in wastewater treatment: Case study of *Rhodotorula mucilaginosa*. *Environ. Technol.* **2012**, *33*, 951–960. [CrossRef] [PubMed]
47. Jarboui, R.; Magdich, S.; Ayadi, R.J.; Gargouri, A.; Gharsallah, N.; Ammar, E. *Aspergillus niger* P6 and *Rhodotorula mucilaginosa* CH4 used for olive mill wastewater (OMW) biological treatment in single pure and successive cultures. *Environ. Technol.* **2013**, *34*, 629–636. [CrossRef] [PubMed]
48. Ghomari, O.; Merzouki, M.; Benlemlih, M. Optimization of bioconversion of oleuropein, of olive leaf extract, to hydroxytyrosol by *Nakazawaea molendini-olei* using HPLC-UV and a method of experimental design. *J. Microbiol. Methods* **2020**, *176*, 106010. [CrossRef] [PubMed]
49. Ciafardini, G.; Zullo, B.A. Use of selected yeast starter cultures in industrial-scale processing of brined Taggiasca black table olives. *Food Microbiol.* **2019**, *84*, 103250. [CrossRef]
50. Giavalisco, M.; Zotta, T.; Parente, E.; Siesto, G.; Capece, A.; Ricciardi, A. Effect of oil-borne yeasts on the quality of extra-virgin olive oils of Basilicata region. *Int. J. Food Microbiol.* **2023**, *386*, 110041. [CrossRef]

Table S1. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Botryosphaeria dothidea* under different treatments. Means sharing the same letters within a row indicate no significant differences, as determined by Tukey's test for honestly significant differences ($p < 0.05$).

TREATMENT	DAY 2	DAY 7
Concentration = 0.2		
Buža	73.67 ± 1.53 a	86.00 ± 0.00 a
Buža puntoža	54.67 ± 14.01 b	86.00 ± 0.00 a
Istarska bjelica	78.83 ± 2.56 a	86.00 ± 0.00 a
Leccino	70.33 ± 9.81 ab	86.00 ± 0.00 a
Rosinjola	71.67 ± 9.50 ab	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	86.00 ± 0.00 a	86.00 ± 0.00 a
Vanilic acid (0.1)	24.00 ± 3.61 c	75.67 ± 3.79 b
Vanilic acid (0.5)	2.67 ± 1.53 d	19.67 ± 5.51 c
Nativo 75WG	0.00 ± 0.00 d	3.00 ± 1.00 d
Control	80.33 ± 0.58 a	86.00 ± 0.00 a
MSD	17.77	5.95
Concentration = 0.5		
Buža	73.67 ± 2.31 c	86.00 ± 0.00 a
Buža puntoža	62.50 ± 6.50 d	86.00 ± 0.00 a
Istarska bjelica	86.00 ± 0.00 a	86.00 ± 0.00 a
Leccino	86.00 ± 0.00 a	86.00 ± 0.00 a
Rosinjola	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	86.00 ± 0.00 a	86.00 ± 0.00 a
Vanilic acid (0.1)	24.00 ± 3.61 e	75.67 ± 3.79 b
Vanilic acid (0.5)	2.67 ± 1.53 f	19.67 ± 5.51 c
Nativo 75WG	0.00 ± 0.00 f	3.00 ± 1.00 d
Control	80.33 ± 0.58 b	86.00 ± 0.00 a
MSD	6.99	5.94
Concentration = 2		
Buža	78.67 ± 6.43 a	86.00 ± 0.00 a
Buža puntoža	80.33 ± 9.82 a	86.00 ± 0.00 a
Istarska bjelica	86.00 ± 0.00 a	86.00 ± 0.00 a
Leccino	86.00 ± 0.00 a	86.00 ± 0.00 a
Rosinjola	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	86.00 ± 0.00 a	86.00 ± 0.00 a
Vanilic acid (0.1)	24.00 ± 3.61 b	75.67 ± 3.79 b
Vanilic acid (0.5)	2.67 ± 1.53 c	19.67 ± 5.51 c
Nativo 75WG	0.00 ± 0.00 c	3.00 ± 1.00 d
Control	80.33 ± 0.58 a	86.00 ± 0.00 a
MSD	10.89	5.94
Concentration = 6 and 10		
Buža	86.00 ± 0.00 a	86.00 ± 0.00 a

Buža puntoža	86.00 ± 0.00 a	86.00 ± 0.00 a
Istarska bjelica	86.00 ± 0.00 a	86.00 ± 0.00 a
Leccino	86.00 ± 0.00 a	86.00 ± 0.00 a
Rosinjola	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	86.00 ± 0.00 a	86.00 ± 0.00 a
Vanilic acid (0.1)	24.00 ± 3.61 c	75.67 ± 3.79 b
Vanilic acid (0.5)	2.67 ± 1.53 d	19.67 ± 5.51 c
Nativo 75WG	0.00 ± 0.00 d	3.00 ± 1.00 d
Control	80.33 ± 0.58 b	86.00 ± 0.00 a
MSD	3.48	5.94

Table S2. Inhibitory effect (%) of treatment on the mycelial growth of *Botryosphaeria dothidea*.

TREATMENT	CONCENTRATION	DAY 2	DAY 7
Buža	0.2 and 0.5	8.29	0
	2	2.07	0
	6 and 10	-7.05	0
Buža puntoža	0.2	31.95	0
	0.5	22.19	0
	2	0	0
	6 and 10	-7.05	0
Istarska bjelica	0.2	1.87	0
	0.5, 2, 6 and 10	-7.05	0
Leccino	0.2	12.45	0
	0.5, 2, 6 and 10	-7.05	0
Rosinjola	0.2	10.79	0
	0.5, 2, 6 and 10	-7.05	0
Hydroxytyrosol	0.1 and 0.5	-7.05	0
Vanillic acid	0.1	70.12	12.02
	0.5	96.68	77.13
Nativo 75WG	0.02	100	96.51

Table S3. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Diplodia mutila* under different treatments. Means sharing the same letters within a row indicate no significant differences, as determined by Tukey's test for honestly significant differences ($p < 0.05$).

TREATMENT	DAY 2	DAY 7
Concentration = 0.2		
Buža	69.67 ± 2.52 abc	86.00 ± 0.00 a
Buža puntoža	72.67 ± 2.08 ab	86.00 ± 0.00 a
Istarska bjelica	73.00 ± 1.73 ab	86.00 ± 0.00 a
Leccino	67.67 ± 2.89 abc	86.00 ± 0.00 a
Rosinjola	66.67 ± 0.58 bc	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	63.66 ± 4.62 c	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	62.33 ± 1.53 c	86.00 ± 0.00 a
Vanilic acid (0.1)	44.67 ± 3.06 d	86.00 ± 0.00 a

Vanilic acid (0.5)	0.00 ± 0.00 e	0.00 ± 0.00 c
Nativo 75WG	0.00 ± 0.00 e	14.00 ± 1.00 b
Control	75.00 ± 4.35 a	86.00 ± 0.00 a
MSD	7.59	0.88
Concentration = 0.5, 2, 6 and 10		
Buža	86.00 ± 0.00 a	86.00 ± 0.00 a
Buža puntoža	86.00 ± 0.00 a	86.00 ± 0.00 a
Istarska bjelica	86.00 ± 0.00 a	86.00 ± 0.00 a
Leccino	86.00 ± 0.00 a	86.00 ± 0.00 a
Rosinjola	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	63.67 ± 4.62 c	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	62.33 ± 1.53 c	86.00 ± 0.00 a
Vanilic acid (0.1)	44.67 ± 3.06 d	86.00 ± 0.00 a
Vanilic acid (0.5)	0.00 ± 0.00 e	0.00 ± 0.00 c
Nativo 75WG	0.00 ± 0.00 e	14.00 ± 1.00 b
Control	75.00 ± 4.35 b	86.00 ± 0.00 a
MSD	6.35	0.88

Table S4. Inhibitory effect (%) of treatment on the mycelial growth of *Diplodia mutila*.

TREATMENT	CONCENTRATION	DAY 2	DAY 7
Buža	0.2	7.11	0
	0.5, 2, 6 and 10	-14.67	0
Buža puntoža	0.2	3.11	0
	0.5, 2, 6 and 10	-14.67	0
Istarska bjelica	0.2	2.67	0
	0.5, 2, 6 and 10	-14.67	0
Leccino	0.2	9.78	0
	0.5, 2, 6 and 10	-14.67	0
Rosinjola	0.2	11.11	0
	0.5, 2, 6 and 10	-14.67	0
Hydroxytyrosol	0.1	15.11	0
	0.5	16.89	0
Vanillic acid	0.1	40.44	0
	0.5	100	100
Nativo 75WG	0.02	100	83.72

Table S5. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Diplodia seriata* under different treatments. Means sharing the same letters within a row indicate no significant differences, as determined by Tukey's test for honestly significant differences ($p < 0.05$).

TREATMENT	DAY 2	DAY 7
Concentration = 0.2, 0.5, 2, 6 and 10		
Buža	86.00 ± 0.00 a	86.00 ± 0.00 a
Buža puntoža	86.00 ± 0.00 a	86.00 ± 0.00 a
Istarska bjelica	86.00 ± 0.00 a	86.00 ± 0.00 a
Leccino	86.00 ± 0.00 a	86.00 ± 0.00 a

Rosinjola	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	71.00 ± 7.81 bc	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	70.00 ± 1.00 c	86.00 ± 0.00 a
Vanilic acid (0.1)	48.00 ± 6.08 d	80.33 ± 6.02 b
Vanilic acid (0.5)	00.00 ± 0.00 e	0.00 ± 0.00 d
Nativo 75WG	1.33 ± 0.58 e	18.00 ± 1.00 c
Control	80.00 ± 2.65 ab	86.00 ± 0.00 a
MSD	9.08	5.38

Table S6. Inhibitory effect (%) of treatment on the mycelial growth of *Diplodia seriata*.

TREATMENT	CONCENTRATION	DAY 2	DAY 7
Buža	0.2, 0.5, 2, 6 and 10	-7.50	0
Buža puntoža	0.2, 0.5, 2, 6 and 10	-7.50	0
Istarska bjelica	0.2, 0.5, 2, 6 and 10	-7.50	0
Leccino	0.2, 0.5, 2, 6 and 10	-7.50	0
Rosinjola	0.2, 0.5, 2, 6 and 10	-7.50	0
Hydroxytyrosol	0.1	11.25	0
	0.5	12.50	0
Vanillic acid	0.1	40.00	6.59
	0.5	100	100
Nativo 75WG	0.02	98.33	79.07

Table S7. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Dothiorella iberica* under different treatments. Means sharing the same letters within a row indicate no significant differences, as determined by Tukey's test for honestly significant differences ($p < 0.05$).

TREATMENT	DAY 2	DAY 7
Concentration = 0.2		
Buža	0.00 ± 0.00 f	86.00 ± 0.00 a
Buža puntoža	31.00 ± 1.00 d	86.00 ± 0.00 a
Istarska bjelica	43.00 ± 5.00 c	86.00 ± 0.00 a
Leccino	0.00 ± 0.00 f	86.00 ± 0.00 a
Rosinjola	62.33 ± 1.15 b	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	86.00 ± 0.00 a	86.00 ± 0.00 a
Vanilic acid (0.1)	18.67 ± 2.08 e	59.00 ± 1.73 b
Vanilic acid (0.5)	0.00 ± 0.00 f	0.00 ± 0.00 c
Nativo 75WG	0.00 ± 0.00 f	0.00 ± 0.00 c
Control	83.00 ± 0.00 a	86.00 ± 0.00 a
MSD	4.95	1.52
Concentration = 0.5		
Buža	0.00 ± 0.00 e	86.00 ± 0.00 a
Buža puntoža	57.00 ± 1.00 b	86.00 ± 0.00 a

Istarska bjelica	44.67 ± 1.53 c	86.00 ± 0.00 a
Leccino	0.00 ± 0.00 e	86.00 ± 0.00 a
Rosinjola	62.67 ± 7.64 b	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	86.00 ± 0.00 a	86.00 ± 0.00 a
Vanilic acid (0.1)	18.67 ± 2.08 d	59.00 ± 1.73 b
Vanilic acid (0.5)	0.00 ± 0.00 e	0.00 ± 0.00 c
Nativo 75WG	0.00 ± 0.00 e	0.00 ± 0.00 c
Control	83.00 ± 0.00 a	86.00 ± 0.00 a
MSD	7.15	1.52
Concentration = 2		
Buža	0.00 ± 0.00 d	86.00 ± 0.00 a
Buža puntoža	60.00 ± 1.00 b	86.00 ± 0.00 a
Istarska bjelica	58.33 ± 1.53 b	86.00 ± 0.00 a
Leccino	23.00 ± 3.46 c	86.00 ± 0.00 a
Rosinjola	59.67 ± 2.31 b	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	86.00 ± 0.00 a	86.00 ± 0.00 a
Vanilic acid (0.1)	18.67 ± 2.08 c	59.00 ± 1.73 b
Vanilic acid (0.5)	0.00 ± 0.00 d	0.00 ± 0.00 c
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 c
Control	83.00 ± 0.00 a	86.00 ± 0.00 a
MSD	4.4	1.52
Concentration = 6		
Buža	0.00 ± 0.00 f	86.00 ± 0.00 a
Buža puntoža	58.67 ± 0.57 bc	86.00 ± 0.00 a
Istarska bjelica	63.33 ± 5.86 b	86.00 ± 0.00 a
Leccino	27.33 ± 2.08 d	86.00 ± 0.00 a
Rosinjola	53.67 ± 6.81 c	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	86.00 ± 0.00 a	86.00 ± 0.00 a
Vanilic acid (0.1)	18.67 ± 2.08 e	59.00 ± 1.73 b
Vanilic acid (0.5)	0.00 ± 0.00 f	0.00 ± 0.00 c
Nativo 75WG	0.00 ± 0.00 f	0.00 ± 0.00 c
Control	83.00 ± 0.00 a	86.00 ± 0.00 a
MSD	8.33	1.52
Concentration = 10		
Buža	0.00 ± 0.00 e	86.00 ± 0.00 a
Buža puntoža	62.00 ± 1.00 b	86.00 ± 0.00 a
Istarska bjelica	59.67 ± 6.35 b	86.00 ± 0.00 a
Leccino	36.33 ± 2.52 c	86.00 ± 0.00 a
Rosinjola	47.33 ± 11.02 c	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	86.00 ± 0.00 a	86.00 ± 0.00 a

Hydroxytyrosol (0.5)	86.00 ± 0.00 a	86.00 ± 0.00 a
Vanilic acid (0.1)	18.67 ± 2.08 d	59.00 ± 1.73 b
Vanilic acid (0.5)	0.00 ± 0.00 e	0.00 ± 0.00 c
Nativo 75WG	0.00 ± 0.00 e	0.00 ± 0.00 c
Control	83.00 ± 0.00 a	86.00 ± 0.00 a
MSD	11.59	1.52

Table S8. Inhibitory effect (%) of treatment on the mycelial growth of *Dothiorella iberica*.

TREATMENT	CONCENTRATION	DAY 2	DAY 7
Buža	0.2, 0.5, 2, 6 and 10	100	0
Buža puntoža	0.2	62.65	0
	0.5	31.33	0
	2	27.71	0
	6	29.32	0
	10	25.30	0
Istarska bjelica	0.2	48.19	0
	0.5	46.18	0
	2	29.72	0
	6	23.69	0
	10	28.11	0
Leccino	0.2 and 0.5	100	0
	2	72.29	0
	6	67.07	0
	10	56.22	0
Rosinjola	0.2	24.89	0
	0.5	24.49	0
	2	28.11	0
	6	35.34	0
	10	42.97	0
Hydroxytyrosol	0.1 and 0.5	-3.61	0
Vanillic acid	0.1	77.51	31.39
	0.5	100	100
Nativo 75WG	0.02	100	100

Table S9. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Dothiorella sarmentorum* under different treatments. Means sharing the same letters within a row indicate no significant differences, as determined by Tukey's test for honestly significant differences ($p < 0.05$).

TREATMENT	DAY 2	DAY 7
Concentration = 0.2		
Buža	86.00 ± 0.00 a	86.00 ± 0.00 a
Buža puntoža	64.67 ± 3.06 b	86.00 ± 0.00 a
Istarska bjelica	0.00 ± 0.00 d	86.00 ± 0.00 a
Leccino	0.00 ± 0.00 d	0.00 ± 0.00 c

Rosinjola	71.67 ± 9.50 b	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	86.00 ± 0.00 a	86.00 ± 0.00 a
Vanilic acid (0.1)	39.67 ± 1.53 c	70.67 ± 0.57 b
Vanilic acid (0.5)	0.00 ± 0.00 d	0.00 ± 0.00 c
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 c
Control	83.67 ± 0.58 a	86.00 ± 0.00 a
MSD	8.9	0.51
Concentration = 0.5		
Buža	0.00 ± 0.00 e	0.00 ± 0.00 c
Buža puntoža	66.00 ± 2.65 c	86.00 ± 0.00 a
Istarska bjelica	76.33 ± 1.15 b	86.00 ± 0.00 a
Leccino	0.00 ± 0.00 e	0.00 ± 0.00 c
Rosinjola	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	86.00 ± 0.00 a	86.00 ± 0.00 a
Vanilic acid (0.1)	39.67 ± 1.53 d	70.67 ± 0.57 b
Vanilic acid (0.5)	0.00 ± 0.00 e	0.00 ± 0.00 c
Nativo 75WG	0.00 ± 0.00 e	0.00 ± 0.00 c
Control	83.67 ± 0.58 a	86.00 ± 0.00 a
MSD	2.92	0.51
Concentration = 2, 6 and 10		
Buža	0.00 ± 0.00 d	0.00 ± 0.00 c
Buža puntoža	86.00 ± 0.00 a	86.00 ± 0.00 a
Istarska bjelica	86.00 ± 0.00 a	86.00 ± 0.00 a
Leccino	0.00 ± 0.00 d	0.00 ± 0.00 c
Rosinjola	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	86.00 ± 0.00 a	86.00 ± 0.00 a
Vanilic acid (0.1)	39.67 ± 1.53 c	70.67 ± 0.57 b
Vanilic acid (0.5)	0.00 ± 0.00 d	0.00 ± 0.00 c
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 c
Control	83.67 ± 0.58 b	86.00 ± 0.00 a
MSD	1.44	0.51

Table S10. Inhibitory effect (%) of treatment on the mycelial growth of *Dothiorella sarmentorum*.

TREATMENT	CONCENTRATION	DAY 2	DAY 7
Buža	0.2	-2.79	0
	0.5, 2, 6 and 10	100	100
Buža puntoža	0.2	22.71	0
	0.5	21.12	0
	2, 6 and 10	-2.79	0

Istarska bjelica	0.2	100	0
	0.5	8.76	0
	2, 6 and 10	-2.79	0
Leccino	0.2, 0.5, 2 and 6	100	100
	10	-2.78	0
Rosinjola	0.2	14.34	0
	0.5, 2, 6 and 10	-2.79	0
Hydroxytyrosol	0.1 and 0.5	-2.79	0
Vanillic acid	0.1	52.59	17.83
	0.5	100	100
Nativo 75WG	0.02	100	100

Table S11. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Neofusicoccum parvum* under different treatments. Means sharing the same letters within a row indicate no significant differences, as determined by Tukey's test for honestly significant differences ($p < 0.05$).

TREATMENT	DAY 2	DAY 7
Concentration = 0.2, 0.5, 2, 6 and 10		
Buža	86.00 ± 0.00 a	86.00 ± 0.00 a
Buža puntoža	86.00 ± 0.00 a	86.00 ± 0.00 a
Istarska bjelica	86.00 ± 0.00 a	86.00 ± 0.00 a
Leccino	86.00 ± 0.00 a	86.00 ± 0.00 a
Rosinjola	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	86.00 ± 0.00 a	86.00 ± 0.00 a
Vanilic acid (0.1)	44.33 ± 2.52 b	86.00 ± 0.00 a
Vanilic acid (0.5)	0.00 ± 0.00 c	0.00 ± 0.00 c
Nativo 75WG	0.00 ± 0.00 c	5.67 ± 1.53 b
Control	86.00 ± 0.00 a	86.00 ± 0.00 a
MSD	2.21	2.03

Table S12. Inhibitory effect (%) of treatment on the mycelial growth of *Neofusicoccum parvum*.

TREATMENT	CONCENTRATION	DAY 2	DAY 7
Buža	0.2, 0.5, 2, 6 and 10	0	0
Buža puntoža	0.2, 0.5, 2, 6 and 10	0	0
Istarska bjelica	0.2, 0.5, 2, 6 and 10	0	0
Leccino	0.2, 0.5, 2, 6 and 10	0	0
Rosinjola	0.2, 0.5, 2, 6 and 10	0	0
Hydroxytyrosol	0.1 and 0.5	0	0
Vanillic acid	0.1	48.45	0
Vanillic acid	0.5	100	100
Nativo 75WG	0.02	100	93.41

Table S13. MIC and MFC values of treatments.

Treatment	Species	MIC	MFC
		%	
Buža	*Botryosphaeria dothidea*	0.2	/
	Diplodia mutila	0.2	/
	Diplodia seriata	/	/
	Dothiorella iberica	/	0.2
	Dothiorella sarmentorum	/	0.5
	Neofusicoccum parvum	/	/
Buža puntoža	*Botryosphaeria dothidea*	0.2	/
	Diplodia mutila	0.2	/
	Diplodia seriata	/	/
	Dothiorella iberica	0.2	/
	Dothiorella sarmentorum	0.2	/
	Neofusicoccum parvum	/	/
Istarska bjelica	*Botryosphaeria dothidea*	0.2	/
	Diplodia mutila	0.2	/
	Diplodia seriata	/	/
	Dothiorella iberica	0.2	/
	Dothiorella sarmentorum	0.2	/
	Neofusicoccum parvum	/	/
Leccino	*Botryosphaeria dothidea*	0.2	/
	Diplodia mutila	0.2	/
	Diplodia seriata	/	/
	Dothiorella iberica	0.2	/
	Dothiorella sarmentorum	/	0.2
	Neofusicoccum parvum	/	/
Rosinjola	*Botryosphaeria dothidea*	0.2	/
	Diplodia mutila	0.2	/
	Diplodia seriata	/	/
	Dothiorella iberica	0.2	/
	Dothiorella sarmentorum	0.2	/
	Neofusicoccum parvum	/	/
Hydroxytyrosol	*Botryosphaeria dothidea*	/	/
	Diplodia mutila	0.1	/
	Diplodia seriata	0.1	/
	Dothiorella iberica	/	/
	Dothiorella sarmentorum	/	/
	Neofusicoccum parvum	/	/
Vanillic acid	*Botryosphaeria dothidea*	0.1	/
	Diplodia mutila	0.1	/
	Diplodia seriata	0.1	/
	Dothiorella iberica	0.1	0.5
	Dothiorella sarmentorum	0.1	/
	Neofusicoccum parvum	0.1	/
Nativo 75WG	*Botryosphaeria dothidea*	/	0.02
	Diplodia mutila	0.02	/
	Diplodia seriata	0.02	/
	Dothiorella iberica	/	0.02

	Dothiorella sarmentorum	/	0.02
	Neofusicoccum parvum	0.02	/

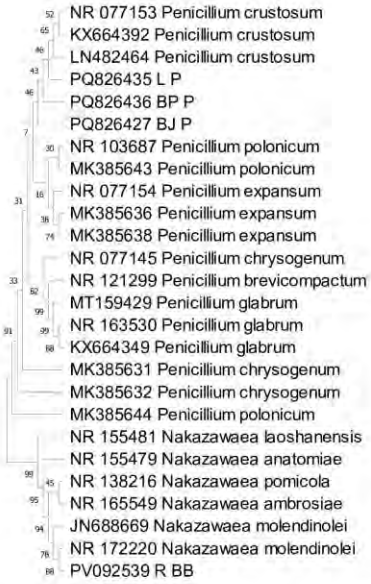


Figure S1. The evolutionary relationships were determined using the Neighbor-Joining method, and the optimal phylogenetic tree is presented. The bootstrap test (1,000 replicates) was used to assess clustering reliability, with the percentage of replicate trees in which taxa grouped together displayed next to the branches. This analysis included 26 nucleotide sequences, with all ambiguous positions removed for each sequence pair using the pairwise deletion method. The final dataset comprised a total of 1,213 positions.

Table S14. Growth Inhibition Category (GIC) scale results from the antagonism test, where a higher number indicates a greater level of inhibition.

Isolates	***Botryosphaeria dothidea***	***Diplodia mutila***	***Diplodia seriata***	***Dothiorella iberica***	***Dothiorella sarmentorum***	***Neofusicoccum parvum***
B_RB	3	3	3	4	3	2
B_BB	3	3	1	4	3	2
R_RB	3	3	3	4	3	2
R_BB	1	0	1	2	1	0
BJ_P	3	4	3	3	4	3
L_P	3	4	4	0	4	4
BP_P	3	3	4	4	4	4

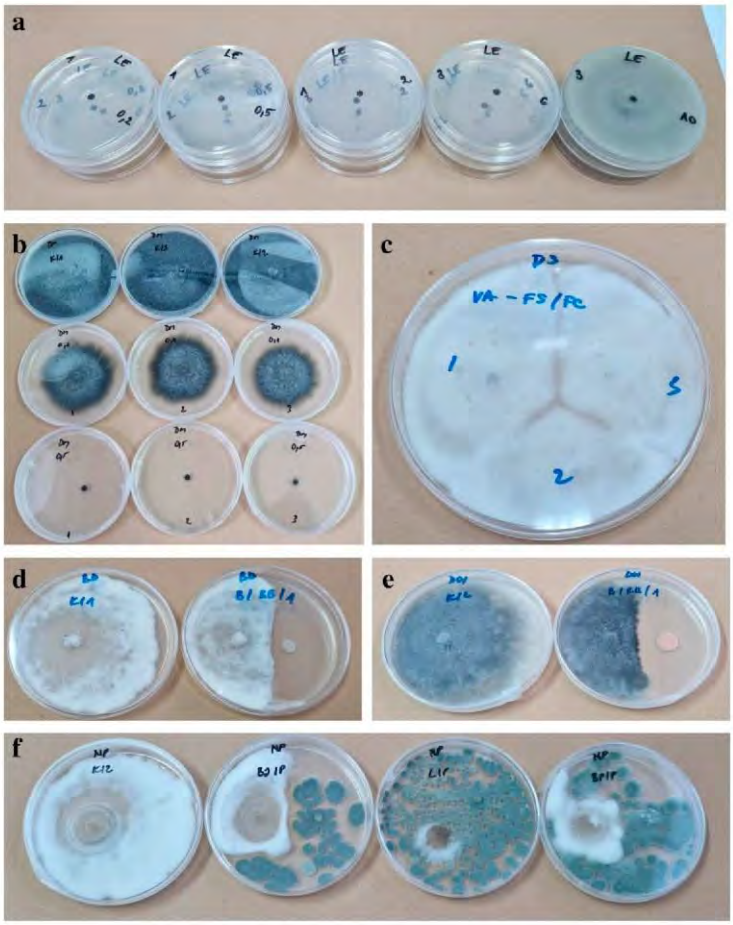


Figure S2. Results of the conducted tests: (a) Evaluation of the antifungal effect of OMWW from Leccino on *Do. sarmentorum* on day 10 at different concentrations (from right to left – from the lowest to the highest concentration, three replicates for each treatment). (b) Evaluation of the antifungal effect of vanillic acid on *D. mutila* on day 10 (top – control, middle – 0.1% concentration, bottom – 0.5% concentration, three replicates for each treatment). (c) Evaluation of the fungistatic/fungicidal effect of vanillic acid on *D. seriata*. (d) Evaluation of the antagonistic effect of *B. velezensis* isolate on *B. dothidea* (left – control, right – treatment). (e) Evaluation of the antagonistic effect of *R. mucilaginosa* on *Do. iberica* (left – control, right – treatment). (f) Evaluation of the antagonistic effect of *Penicillium* sp. isolates on *N. parvum* (from left to right: control, isolates BJ_P, L_P, BP_P).

Naslov izvornog znanstvenog rada broj 7: Integrated Analysis of Olive Mill Wastewaters: Physicochemical Profiling, Antifungal Activity, and Biocontrol Potential Against Botryosphaeriaceae

Prošireni sažetak:

Provedeno je sveobuhvatno istraživanje s ciljem procjene kemijskog sastava i antifungalne aktivnosti otpadnih biljnih voda maslina (OBVM) dobivenih iz različitih sorata maslina (Buža, Buža puntoža, Istarska bjelica, Leccino i Rosinjola) protiv fitopatogenih gljiva iz porodice Botryosphaeriaceae. S obzirom na sve veću potrebu za održivim alternativama kemijskim fungicidima, ovo istraživanje je fokusiralo se na evaluaciju potencijala OBVM kao sredstva za zaštitu bilja i valorizaciju otpada prehrambene industrije.

Provedena je detaljna kemijska analiza OBVM, uključujući određivanje fizikalno-kemijskih svojstava, sadržaja fenolnih spojeva pomoću LC-MS/MS metode, koncentracije šećera, suhe tvari te ukupnog sadržaja ugljika i dušika. Rezultati su pokazali značajne razlike između sorata, pri čemu je OBVM iz Istarske bjelice imala najviše koncentracije fenola, suhe tvari, šećera, ugljika i dušika.

Antifungalna aktivnost ispitana je protiv šest gljiva: *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & De Not., *Diplodia mutila* (Fr.) Fr., *Diplodia seriata* De Notaris, *Dothiorella iberica* A.J.L. Phillips, J. Luque & A. Alves, *Dothiorella sarmentorum* (Fr.) A.J.L. Phillips, Alves & Luque i *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips. Uočeno je da niske koncentracije OBVM inhibiraju rast nekih patogena, dok su više koncentracije često imale stimulativni učinak na rast micelija. Među fenolima, vanilinska kiselina pokazala je veću antifungalnu učinkovitost u odnosu na hidroksitirosol, s potpunom inhibicijom rasta pojedinih gljiva već pri koncentraciji od 0,5%.

Uz antifungalna svojstva OBVM, istraženo je i antagonističko djelovanje mikroorganizama izoliranih iz OBVM. Izolirane vrste uključivale su *Bacillus velezensis* Ruiz-Garcia et al., *Rhodotorula mucilaginosa* (A. Jörg.) F.C. Harrison, *Nakazawaea molendiniolei* (N. Cadez, B. Turchetti & G. Peter) C. P. Kurtzman & C. J. Robnett i *Penicillium crustosum* Thom. Među njima, *B. velezensis* pokazala je najsnažniju inhibiciju rasta patogena, posebno protiv *Do. iberica*.

Rezultati su potvrdili da OBVM sorti Leccino i Buža imaju izraženo antifungalno djelovanje, posebno pri nižem koncentracijama. S druge strane, OBVM sorte Istarska bjelica, iako bogata fenolima, nije pokazala najočekivaniju antifungalnu učinkovitost, što ukazuje da sama koncentracija fenola nije jedini čimbenik koji utječe na antifungalnu aktivnost OBVM.

Zaključno, ovo istraživanje naglašava potencijal OBVM kao prirodnog sredstva za suzbijanje

bolesti maslina, što predstavlja značajan doprinos održivom razvoju poljoprivrede i kružnom gospodarstvu. Istraživanje također otvara mogućnosti daljnje upotrebe OBVM za izolaciju korisnih mikroorganizama i prostor za daljnje istraživanje optimalnih koncentracija, sinergijskih efekata i standardizacije primjene kako bi se osigurala maksimalna antifungalna učinkovitost uz minimalne negativne učinke na okoliš.

Ključne riječi: antagonizam, *Bacillus velezensis*, biološka kontrola, fenoli, kvasci

Izvorni znanstveni rad broj 8 u obliku i izvornom jeziku na kojem je objavljen u znanstvenom časopisu

Naslova rada: The Antifungal Efficacy of Olive Mill Wastewater on Phytopathogenic Fungi from the Class Sordariomycetes

Autori: Elena Petrović, Karolina Vrandečić, Tamara Siber, Jasenka Ćosić, Sara Godena

Tip rada: Izvorni znanstveni članak

Časopis: Microorganisms

Kategorija: A1

Impakt faktor: 4,1 (2023.)

Kvartil: Q1

Primljen na recenziju: 03. rujan 2025.

Prihvaćen za objavljivanje:

Status:

Volumen:

Broj:

Broj rada:

WOS broj:

microorganisms

MDPI

Article

The antifungal efficacy of olive mill wastewater on phytopathogenic fungi from the class *Sordariomycetes*

Elena Petrović [1], Karolina Vrandečić [2], Tamara Siber [2], Jasenka Ćosić [2] and Sara Godena [1]*

1 Laboratory for Plant Protection, Department of Agriculture and Nutrition, Institute of Agriculture and Tourism, Karla Huguesa 8, Croatia, elena@iptpo.hr
2 Laboratory for Plant Protection, Department of Phytopathology, Faculty of Agrobiotechnical Sciences Osijek, Vladimira Preloga 1, Croatia, kvrandecic@fazos.hr , tsiber@fazos.hr , jcosic@fazos.hr
*Corresponding author: sara@iptpo.hr

Abstract: Olive mill wastewater (OMWW) is a byproduct of olive oil production, characterised by a complex composition and has gained attention as a potential natural alternative for controlling plant pathogens. This study investigates the antifungal potential of OMWW derived from various olive varieties (Buža, Buža puntoža, Istarska bjelica, Leccino and Rosinjola) against phytopathogenic fungi from the *Sordariomycetes* class, including *Biscogniauxia mediterranea* (De Not.) Kuntze, *B. nummularia* (Bull.) Kuntze, *Cytospora pruinosa* Défago, *Nigrospora gorlenkoana* Novobr., *N. osmanthi* Mei Wang & L. Cai, *N. philosophiae-doctoris* M. Raza, Qian Chen & L. Cai, *Phaeoacremonium iranianum* L. Mostert, Grafenhan, W. Gams & Crous, and *Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not. In addition, microorganisms isolated from OMWW, *Bacillus velezensis* Ruiz-Garcia et al., *Rhodotorula mucilaginosa* (A. Jörg.) F.C. Harrison, *Nakazawaea molendiniolei* (N. Cadez, B. Turchetti & G. Peter) C. P. Kurtzman & C. J. Robnett, and *Penicillium crustosum* Thom, were tested for their antagonistic potential against these pathogens. The antifungal efficacy of OMWW was evaluated at different concentrations, alongside phenols hydroxytyrosol and vanillic acid. Among the tested pathogens, *N. philosophiae-doctoris* was the most susceptible, whereas, in the case of *N. gorlenkoana*, all OMWW treatments exhibited a stimulatory effect. Although the results varied significantly between species, OMWW from Rosinjola demonstrated the highest efficacy in inhibiting mycelial growth, while OMWW from Leccino was the least effective. Regarding the antagonistic activity of microorganisms, substantial differences were observed in their effects on different fungal species. Neither bacteria nor yeasts successfully inhibited the growth of *B. mediterranea*. Additionally, variations in antagonistic activity were detected among isolates of the same species. In some cases, microbial isolates from OMWW even had a stimulatory effect on pathogen growth. Given the increasing demand for eco-friendly disease management strategies, OMWW-derived treatments could represent an innovative approach to mitigating fungal infections in agriculture.

Keywords: antagonism; bacteria; ecology; plant protection; yeast

Academic Editor: Firstname Lastname

Received: date
Revised: date
Accepted: date
Published: date

Citation: To be added by editorial staff during production.

1. Introduction

The olive tree is widely cultivated in Mediterranean regions of the world, with Spain, Italy, Portugal, Tunisia, Morocco, and Egypt being the leading producers. In 2023, global olive production reached 20.2 million t, covering an area of 11.1 million ha [1].

Oil extraction from olive paste can be performed using different methods, such as the traditional batch press, the three-phase centrifugal system, and the two-phase centrifugal extraction system, resulting in three products—olive oil, solid pomace, and olive mill wastewater (OMWW)—in the first two methods, while the two-phase system yields only olive oil and wet pomace, eliminating liquid wastewater [2,3]. However, the production

of olive oil results in substantial amounts of waste, which can pose a significant environmental threat due to its strong phytotoxic properties, harmful effects on aquatic life, and inhibitory impact on soil microbial communities [4]. OMWW consists of vegetation water naturally present in the olive fruit, water used for processing and cleaning, as well as remnants of olive pulp and residual oil [4]. The presence of phytotoxic compounds in OMWW makes it unsuitable for direct use in agricultural irrigation or uncontrolled discharge into surface waters [5]. Addressing the challenge of OMWW reuse in agriculture is essential for safeguarding environmental and public health [5]. Unfortunately, the absence of cost-effective and technically feasible solutions has resulted in the uncontrolled disposal of OMWW, becoming a serious ecological concern across Mediterranean regions [4].

Due to the increasing challenges in controlling fungal diseases in agriculture, primarily driven by the rising resistance of microorganisms and the potential risks associated with chemically synthesized plant protection agents for human health, animals, and the environment, there is a growing demand for environmentally sustainable protection methods. Additionally, the need for the valorization of industrial waste has become increasingly relevant, offering a dual benefit, both reducing waste accumulation and repurposing it for plant protection applications. According to the literature, OMWW exhibits antimicrobial properties against phytopathogenic fungi and bacteria, which have been attributed to its rich content of phenolic compounds and the presence of naturally occurring antagonistic microorganisms [6,7].

This study aimed to assess the antifungal potential of OMWW derived from different olive varieties and bioactive phenolic compounds, hydroxytyrosol and vanillic acid, against phytopathogenic fungi belonging to the *Sordariomycetes* class. The fungal species investigated, previously identified in our research, included *Biscogniauxia mediterranea* (De Not.) Kuntze, *B. nummularia* (Bull.) Kuntze, *Cytospora pruinosa* Défago, *Nigrospora gorlenkoana* Novobr., *N. osmanthi* Mei Wang & L. Cai, *N. philosophiae-doctoris* M. Raza, Qian Chen & L. Cai, *Phaeoacremonium iranianum* L. Mostert, Grafenhan, W. Gams & Crous, and *Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not. Additionally, microorganisms isolated from OMWW, including *Bacillus velezensis* Ruiz-Garcia et al., *Rhodotorula mucilaginosa* (A. Jörg.) F.C. Harrison, *Nakazawaea molendiniolei* (N. Cadez, B. Turchetti & G. Peter) C. P. Kurtzman & C. J. Robnett, and *Penicillium crustosum* Thom, were examined for their antagonistic activity against these fungal pathogens.

The fungi tested in this study represent emerging pathogens affecting olive trees. The increasing occurrence of new pathogens is attributed to changes in agricultural practices, climate change, the importation of seedlings, and other factors. *Biscogniauxia* sp., *C. pruinosa*, *P.iranianum*, and *S. fimicola* are responsible for olive tree decline, causing symptoms such as branch and twig dieback, necrosis, bark discolouration, etc. [8,9,10]. Additionally, *Nigrospora* sp. induces leaf spot disease, which subsequently leads to leaf desiccation and premature defoliation [11].

2. Materials and Methods

2.1. OMWW collection

The OMWW samples were directly obtained from olive processing facilities in Istria County, Croatia, in 2021. The interval between olive harvest and oil extraction was maintained at four hours, ensuring minimal degradation. During processing, the paste temperature was controlled at 24 °C, with malaxation performed under continuous cooling using water at 12 °C. Olive oil extraction was carried out through centrifugation, utilizing a two-phase Pieralisi system equipped with a hammer mill. The OMWW originated from Croatian olive cultivars (Buža, Buža Puntoža, Istarska Bjelica, and Rosinjola) and the Italian cultivar Leccino.

2.2. Pretreatment of the sample

Following collection, OMWW samples were stored under refrigerated conditions at 4 °C for nine days, as described by Russo et al. [12]. Samples were then subjected to vacuum filtration using high-flow rate filter paper to remove particulate matter. After filtration, the samples underwent centrifugation at 4000 rpm for 10 minutes at +4 °C, utilizing a Hettich 320 R centrifuge (Merck, Darmstadt, Germany) to further clarify the liquid phase. The pH of the OMWW samples was measured at room temperature using a calibrated pH meter (MP220 Basic pH/mV/°C Meter, Mettler-Toledo GmbH, Giessen, Germany). Calibration was performed with certified pH buffer solutions (Mettler-Toledo GmbH, Greifensee, Switzerland) as reference materials. Finally, the processed samples were stored at -20 °C to preserve their chemical stability.

2.3. Determination of physical and chemical parameters

The OMWW samples underwent comprehensive chemical and physical analysis, during which the following parameters were determined: colour, phenolic compound content, nitrogen and carbon content, dry matter, and sugar content. The detailed methodology and results are described in our previous work [13].

2.4. Antifungal efficacy of OMWW and components.

2.4.1. Utilized isolates

To assess the impact of OMWW and its constituents, representative phytopathogenic fungal isolates originating from olive trees were utilized. The list of tested species is provided in Table 1. Fungal isolates were cultivated on potato dextrose agar (PDA) and incubated at 25 °C for seven days for *Biscogniauxia* spp., *C. pruinosa*, *P. iranianum*, and *S. fimicola*, and at 28 °C for *Nigrospora* spp., in complete darkness.

Table 1. List of used fungal species and isolate names, and GenBank ID, with references.

Species	Isolate	ITS	TUB2	TEF1-alpha	References
Biscogniauxia mediterranea (De Not.) Kuntze	R18 LEC1	OQ733486	OQ744686	OQ744693	[8]
Biscogniauxia nummularia (Bull.) Kuntze	V16 B3	OQ743781	OQ754166	OQ754167	[8]
Cytospora pruinosa Défago	SL2 PRIV	OQ642321	OQ652101	/	[9]
Nigrospora gorlenkoana Novobr.	P13 LECIII	OP999642	OQ286068	OQ286069	[11]
Nigrospora osmanthi Mei Wang & L. Cai	JA20 NP	OP999639	OQ275027	OQ275028	[11]
Nigrospora philosophiae-doctoris M. Raza, Qian Chen & L. Cai	R18 BI	OP999644	OQ286067	OQ286066	[11]
Phaeoacremonium iranianum L. Mostert, Grafenhan, W. Gams & Crous	R18 B4	OP627795	OP684932	OP684933	[10]
Sordaria fimicola (Roberge ex Desm.) Ces. & De Not.	ISN9 PEN	OQ828658	OQ835630	OQ835631	[8]

2.4.2. Experimental setup

To assess antifungal activity, five untreated OMWW samples were tested alongside vanillic acid, a compound identified as either the predominant or among the most abundant components within the analysed OMWWs. Additionally, hydroxytyrosol, previously described for its strong antimicrobial properties as a key constituent of OMWW [3,14], was included in the study. Both vanillic acid and hydroxytyrosol were obtained from

Sigma Aldrich (Merck KGaA, Darmstadt, Germany). The antifungal evaluation was performed using OMWW at five concentration levels (0.2%, 0.5%, 2%, 6%, and 10% volume ratios within the substrate), while hydroxytyrosol and vanillic acid were tested at two concentrations (0.1% and 0.5% volume ratios) [7,14]. Pure PDA served as the positive control, whereas Nativo 75WG (Bayer d.o.o., Zagreb, Croatia), a fungicide commonly applied in olive disease management, was used as the negative control. The fungicide was diluted following the manufacturer's recommendations for olive tree treatments, achieving a final concentration of 20 g/100 L. Instead of water, PDA was used as a suspension medium [15]. PDA was prepared as per the manufacturer's instructions, and the substrate temperature was monitored until it cooled to approximately 45 °C [7]. After cooling, 10 mL of PDA was transferred into a sterile Falcon tube, followed by the addition of the appropriate OMWW, chemical compound, or fungicide. The mixture was stirred with a glass rod and gently vortexed to ensure uniform distribution. The final solution was poured into sterile 90-mm Petri dishes. Once the medium solidified, a 4-mm plug of actively growing fungal culture was excised with a sterile cork borer and placed at the centre of the plate using a sterile laboratory needle, ensuring that the plug's upper surface remained in contact with the medium. The Petri dishes were sealed with parafilm and incubated in complete darkness at 25 °C for *Biscogniauxia* spp., *C. pruinosa*, *P. iranianum*, and *S. fimicola*, and at 28 °C for *Nigrospora* spp. Each treatment, including all concentrations, was tested in triplicate. Fungal mycelial growth was measured at two and seven days post-inoculation. In cases where no fungal growth was observed by the final measurement, half of the mycelial plug was transferred onto a fresh PDA plate under sterile conditions and incubated under the same conditions. The treatments were categorized as fungistatic if mycelial growth resumed and fungicidal if no regrowth occurred. The minimum inhibitory concentration (MIC) was determined as the lowest concentration that completely inhibited fungal growth, whereas the minimum fungicidal concentration (MFC) was the lowest concentration at which no fungal regrowth was detected, confirming fungicidal activity.

2.5.1. Isolation of microorganisms from OMWW

The procedure for the isolation and identification of microorganisms from OMWW is described in our previous work [13]. A list of utilized species are listed in Table 2.

Table 2. The list of species isolated from each OMWW.

OMWW	Isolate	Isolated organism
Buža	B_RB	*Rhodotorula mucilaginosa* (A. Jörg.) F.C. Harrison
	B_BB	*Bacillus velezensis* Ruiz-Garcia et al
Buža puntoža	BP_P	*Penicillium crustosum* Thom
Istarska bjelica	BJ_P	*P. crustosum*
Leccino	L_P	*P. crustosum*
Rosinjola	R_RB	*R. mucilaginosa*
	R_BB	*Nakazawaea molendiniolei* (N. Cadez, B. Turchetti & G. Peter) C. P. Kurtzman & C. J. Robnett

2.5.3. Antagonistic assay

The antagonistic assessment was carried out using the dual culture technique outlined by Fokkema [16] and further detailed by Živković et al. [17]. Before the assay, isolates were incubated in darkness at 25 °C for seven days. Sterile Petri dishes were prepared by pouring 10 mL of PDA medium. A 4-mm mycelial disc of the target pathogen was excised using a sterile cork borer and positioned on one side of the plate, ensuring that the upper surface of the plug remained in direct contact with the medium. On the opposite

side, using a sterile inoculation loop, a 1-µl loopful of bacterial or yeast suspension was introduced at a distance of three cm from the pathogen disc. Control plates consisted of PDA containing only the pathogen mycelial plug without any antagonist. Each experiment was conducted in triplicate. For assays involving *Penicillium* species, a similar approach was followed; however, instead of a loopful of microbial culture, a 4-mm mycelial disc of the antagonistic isolate was used. The percentage of growth inhibition (PGI) was calculated according to the formula established by Živković et al. [17]: PGI(%) = KR(KR−R1)×100, where KR represents the distance (in mm) from the inoculation site to the colony margin on control plates, and R1 denotes the corresponding distance on plates treated with the antagonist [18].

To classify inhibition levels, the Growth Inhibition Category (GIC) scale was applied as follows:

0 = No inhibition
1 = 1–25% inhibition
2 = 26–50% inhibition
3 = 51–75% inhibition
4 = 76–100% inhibition

The inhibition zone was quantified by measuring the distance between the fungal pathogen and the antagonist growth zone following a seven-day incubation period [17].

2.6. Statistical analysis

All statistical analyses related to antagonistic effects, percentage inhibition, MIC and MFC determinations, and the generation of bar charts illustrating antagonism were conducted using Microsoft Office Excel. Heat maps for graphical visualization were created with Python 3.10.12. The antifungal effectiveness of OMWW, phenols, and fungicide was evaluated through SAS Enterprise Guide 8.4. Results were presented as arithmetic means with standard deviations at 95% confidence intervals.

3. Results

3.1. Antifungal Efficacy of OMWW and Phenolic Compounds

Although the antifungal efficacy of the applied treatments varied significantly depending on the concentration (Table S1-S16) and fungal species, it can be concluded that OMWW from Rosinjola exhibited the highest effectiveness in inhibiting the mycelial growth of phytopathogenic fungi, whereas OMWW from Leccino demonstrated the weakest results. Among the tested fungal species, *N. philosophiae-doctoris* was the most susceptible to all treatments, while *N. gorlenkoana* exhibited a stimulatory response to all OMWW treatments. To facilitate result interpretation, the following sections present the mycelial growth inhibition percentages.

Regarding *B. mediterranea*, an inhibitory effect of OMWW from Buža at the lowest concentration was observed only on the second day of measurement. Increasing the concentration led to a stronger inhibitory effect, reaching 81% inhibition at the highest concentration by day seven (Figure 1). A similar trend was observed with OMWW from Buža puntoža. In contrast, treatments with OMWW from Istarska bjelica showed stronger effects at lower concentrations, but only on day two, whereas by day seven, the mycelium had fully colonized the Petri dish. At the highest concentration, no inhibitory effect was recorded. OMWW from Leccino at 0.2–2% concentrations exhibited a stimulatory effect, with greater mycelial growth observed at higher concentrations. However, 6% and 10% concentrations showed inhibitory effects only on day two, with inhibition percentages of 2.15% and 23.02%, respectively. In contrast, OMWW from Rosinjola demonstrated inhibitory effects on both days, with inhibition increasing in a concentration-dependent manner. Among the two tested phenolic compounds, vanillic acid exhibited the highest efficacy, completely inhibiting mycelial growth (100%) on both measurement days at a

concentration of 0.5%. Hydroxytyrosol displayed a similar effect at both concentrations (53.95% and 51.79%), but only on day two. The fungicide Nativo 75WG was the second most effective treatment after vanillic acid, achieving 92.64% inhibition on day seven.

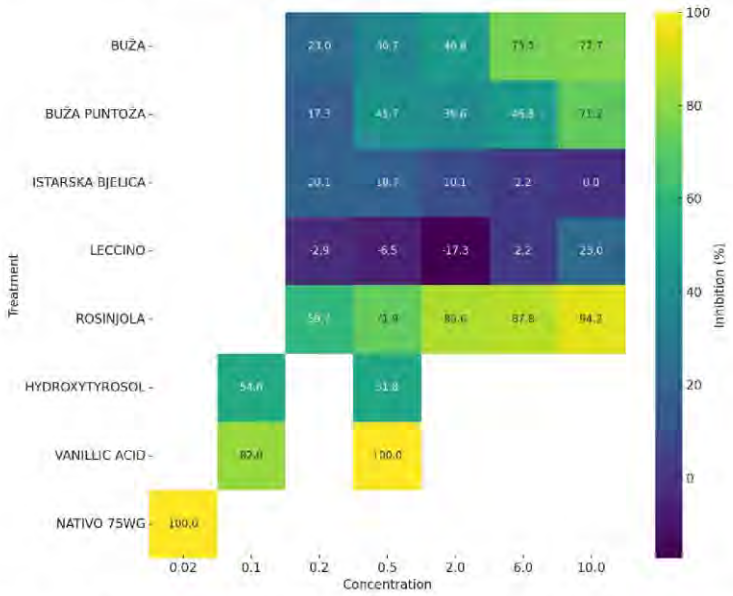


Figure 1. The inhibitory effect of different treatments (OMWW, phenols, and fungicide) at various concentrations on the *B. mediterranea*. The dataset covers treatments using OMWW derived from five olive cultivars, two individual phenolics (hydroxytyrosol and vanillic acid), and a reference fungicide (Nativo 75WG). Along the x-axis are the treatment doses; the y-axis lists each treatment with its corresponding percent growth inhibition. Color shading represents inhibition strength—blues mark minimal or even growth-promoting effects, while yellows indicate strong inhibition approaching 100%. Values below zero denote stimulation of fungal growth.

As for *B. nummularia*, OMWW from Buža had a stimulatory effect (Figure 2) at a concentrations ≥ 0.5%, which increased with concentration, although at 10%, inhibition was slightly lower than at 6% (by 3.57%). OMWW from Buža puntoža at 0.2% and 0.5% concentrations exhibited a stimulatory effect on day two, whereas by day seven, an inhibitory effect of 26.74% and 23.25% was recorded. Concentrations ranging from 2% to 10% showed inhibition on both days, with inhibition increasing linearly with concentration. OMWW from Istarska bjelica and Leccino exhibited a stimulatory effect that increased with concentration, except for OMWW from Leccino at 6%, which had a lower inhibition percentage than lower concentrations, but higher than the subsequent highest concentration. OMWW from Rosinjola exhibited a remarkable inhibitory effect, reaching 100% inhibition at 2–10% concentrations on both days, with significant inhibition even at lower concentrations. Similar to *B. mediterranea*, vanillic acid was more effective than hydroxytyrosol, inhibiting mycelial growth at 0.5% concentration by 98.80% and 93.02% on days two and seven, respectively. Nativo 75WG was the most effective treatment, completely inhibiting growth (100%) on both days.

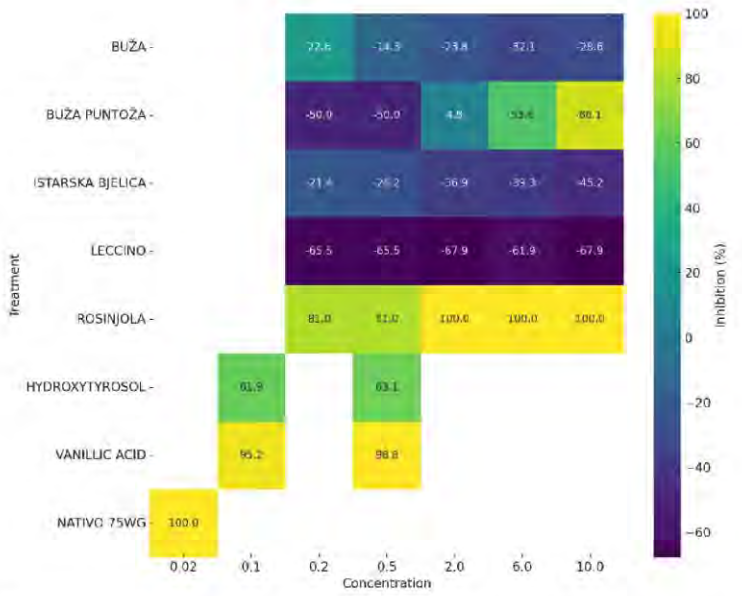


Figure 2. The inhibitory effect of different treatments (OMWW, phenols, and fungicide) at various concentrations on the *B. nummularia*. The dataset covers treatments using OMWW derived from five olive cultivars, two individual phenolics (hydroxytyrosol and vanillic acid), and a reference fungicide (Nativo 75WG). Along the x-axis are the treatment doses; the y-axis lists each treatment with its corresponding percent growth inhibition. Color shading represents inhibition strength—blues mark minimal or even growth-promoting effects, while yellows indicate strong inhibition approaching 100%. Values below zero denote stimulation of fungal growth.

Regarding *C. pruinosa*, OMWW from Buža exhibited a weak inhibitory effect, observed only on day two at 0.2% and 0.5% concentrations (Figure 3), while on day seven, a stimulatory effect was recorded. Concentrations above 2% showed stimulatory effect on both days, with stimulatory effect increasing with concentration. OMWW from Buža puntoža demonstrated a strong inhibitory effect, with 100% inhibition at ≥ 0.5% concentration on day two, and at 6% and 10% on both days. OMWW from Istarska bjelica at 0.2% and 0.5% exhibited weak inhibition (6.45%) on day two, but a stimulatory effect on day seven. Concentrations from 2% to 10% had a stimulatory effect on both days. OMWW from Leccino at 0.2–2% concentrations exhibited an inhibitory effect (77.41%, 51.61%, and 3.22%) on day two, but a stimulatory effect on day seven. At 6% and 10%, a stimulatory effect was observed on both days. OMWW from Rosinjola at 0.2–6% concentrations exhibited a decreasing inhibitory effect with increasing concentration on day two, whereas at >10%, a stimulatory effect was recorded on both days. Hydroxytyrosol had a stronger effect at higher concentrations, while vanillic acid was again more potent, achieving 100% inhibition on both days at a concentration of 0.5%., similar to Nativo 75WG.

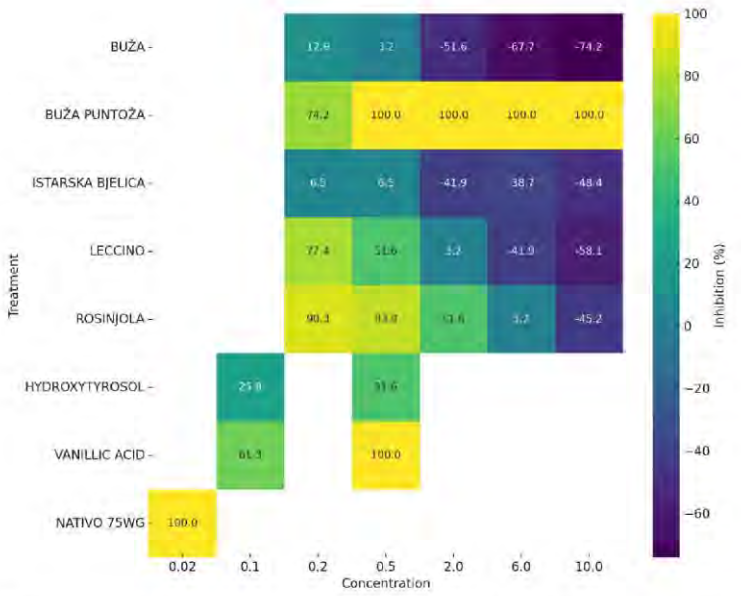


Figure 3. The inhibitory effect of different treatments (OMWW, phenols, and fungicide) at various concentrations on *C. pruinosa*. The dataset covers treatments using OMWW derived from five olive cultivars, two individual phenolics (hydroxytyrosol and vanillic acid), and a reference fungicide (Nativo 75WG). Along the x-axis are the treatment doses; the y-axis lists each treatment with its corresponding percent growth inhibition. Color shading represents inhibition strength—blues mark minimal or even growth-promoting effects, while yellows indicate strong inhibition approaching 100%. Values below zero denote stimulation of fungal growth.

When it comes to *N. gorlenkoana*, all OMWW treatments exhibited a uniform stimulatory effect (Figure 4). Hydroxytyrosol suppressed mycelial growth (100%) at both concentrations but only on day two, while by day seven, the mycelium had completely colonized the Petri dish. Vanillic acid at a higher concentration exhibited better inhibition (91.18% and 83.33%), whereas Nativo 75WG was the most effective, achieving 100% inhibition on both days.

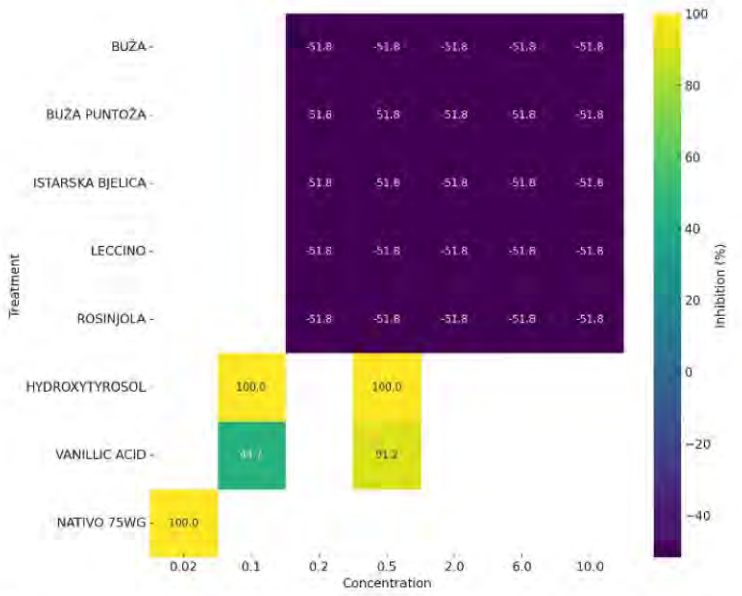


Figure 4. The inhibitory effect of different treatments (OMWW, phenols, and fungicide) at various concentrations on the *Nigrospora gorlenkoana*. The dataset covers treatments using OMWW derived from five olive cultivars, two individual phenolics (hydroxytyrosol and vanillic acid), and a reference fungicide (Nativo 75WG). Along the x-axis are the treatment doses; the y-axis lists each treatment with its corresponding percent growth inhibition. Color shading represents inhibition strength—blues mark minimal or even growth-promoting effects, while yellows indicate strong inhibition approaching 100%. Values below zero denote stimulation of fungal growth.

As for *N. osmanthi*, OMWW from Buža showed an inhibitory effect that increased with concentration, reaching 82.32% and 79.84% inhibition at 10%. A similar trend was observed for OMWW from Buža puntoža (Figure 5), although at 0.2% and 0.5%, inhibition was only observed on day two, with inhibition at 10% reaching 56.74% and 56.20%. OMWW from Istarska bjelica at 0.2% had a mild stimulatory effect, while higher concentrations showed inhibition only on day two, with inhibition increasing with concentration. OMWW from Leccino exhibited a stimulatory effect that increased with concentration. OMWW from Rosinjola demonstrated inhibition on both days, with inhibition increasing with concentration, reaching 85.11% and 84.49% at 10%. Hydroxytyrosol had a stronger inhibitory effect at a higher concentration but was only effective on day two, while vanillic acid completely inhibited growth (100%) at 0.5% on both days. Nativo 75WG suppressed growth by 100% on day two and by 75.19% on day seven.

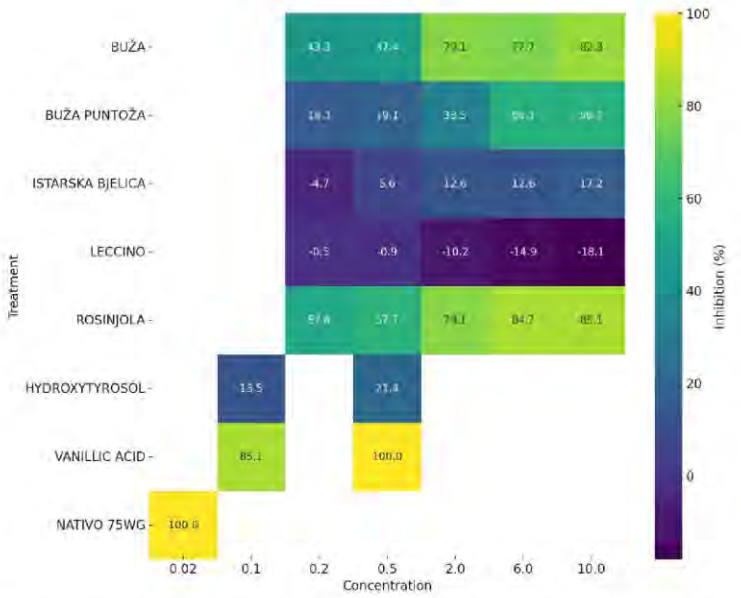


Figure 5. The inhibitory effect of different treatments (OMWW, phenols, and fungicide) at various concentrations on the *Nigrospora osmanthi*. The dataset covers treatments using OMWW derived from five olive cultivars, two individual phenolics (hydroxytyrosol and vanillic acid), and a reference fungicide (Nativo 75WG). Along the x-axis are the treatment doses; the y-axis lists each treatment with its corresponding percent growth inhibition. Color shading represents inhibition strength—blues mark minimal or even growth-promoting effects, while yellows indicate strong inhibition approaching 100%. Values below zero denote stimulation of fungal growth.

Regarding *N. philosophiae-doctoris*, OMWW from Buža and Buža puntoža completely inhibited growth at all concentrations (Figure 6) (except OMWW from Buža puntoža at 0.2%, where inhibition was 96%), although by day seven, the mycelium fully colonized the Petri dish. OMWW from Istarska bjelica exhibited increasing inhibition with concentration, reaching 54.66% at 10%, but by day seven, the mycelium had fully grown. OMWW from Leccino showed decreasing inhibition with increasing concentration, with inhibition ranging from 88% at 0.2% to 46.66% at 10% on second day. OMWW from Rosinjola exhibited a stronger inhibition with increasing concentration, reaching 100% inhibition at 10%, but by day seven, the mycelium had fully colonized the plate. Hydroxytyrosol showed similar inhibition (62.66% and 66.66%) at both concentrations on day two, but no effect on day seven. Vanillic acid at 0.1% had a stimulatory effect, whereas at 0.5%, it exhibited strong inhibition on both days (96% and 96.51%). Nativo 75WG inhibited mycelial growth by 82.66% only on day two.

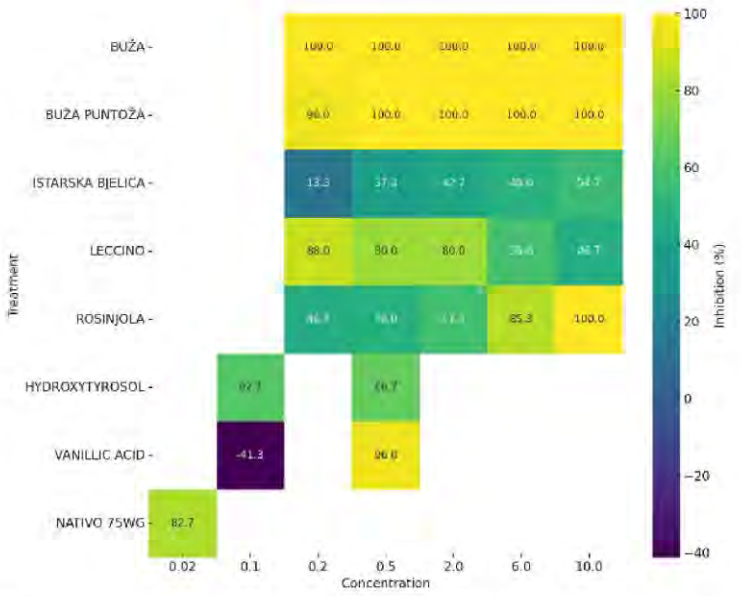


Figure 6. The inhibitory effect of different treatments (OMWW, phenols, and fungicide) at various concentrations on the *Nigrospora philosophiae-doctoris*. The dataset covers treatments using OMWW derived from five olive cultivars, two individual phenolics (hydroxytyrosol and vanillic acid), and a reference fungicide (Nativo 75WG). Along the x-axis are the treatment doses; the y-axis lists each treatment with its corresponding percent growth inhibition. Color shading represents inhibition strength—blues mark minimal or even growth-promoting effects, while yellows indicate strong inhibition approaching 100%. Values below zero denote stimulation of fungal growth.

In relation to *P. iranianum*, OMWW from Buža exhibited an inhibitory effect on mycelial growth (Figure 7), on both days of measurement, reaching 65.21% and 65.67% at the highest concentration on day two and day seven, respectively. A similar pattern was observed with OMWW from Buža puntoža, although with a slightly lower inhibitory effect recorded at the 0.5% concentration compared to lower and higher concentrations. OMWW from Istarska bjelica showed a strong inhibitory effect, particularly at concentrations of 6% and 10%, where 100% inhibition was observed on day two. However, on day seven, inhibition at the 10% concentration was slightly lower than at 6%. OMWW from Leccino also demonstrated an inhibitory effect, which remained consistent across most concentrations (0.5% to 10%) on day two. OMWW from Rosinjola completely inhibited the growth of this pathogen at all tested concentrations and on both days of measurement. The phenolic compound vanillic acid inhibited growth by 100% at a concentration of 0.5% on both days. Hydroxytyrosol showed the same inhibitory effect at both tested concentrations on day two, while on day seven, the lower concentration was more effective. The fungicide Nativo 75WG was effective, inhibiting mycelial growth by 82.60% and 77.61% on day two and day seven, respectively.

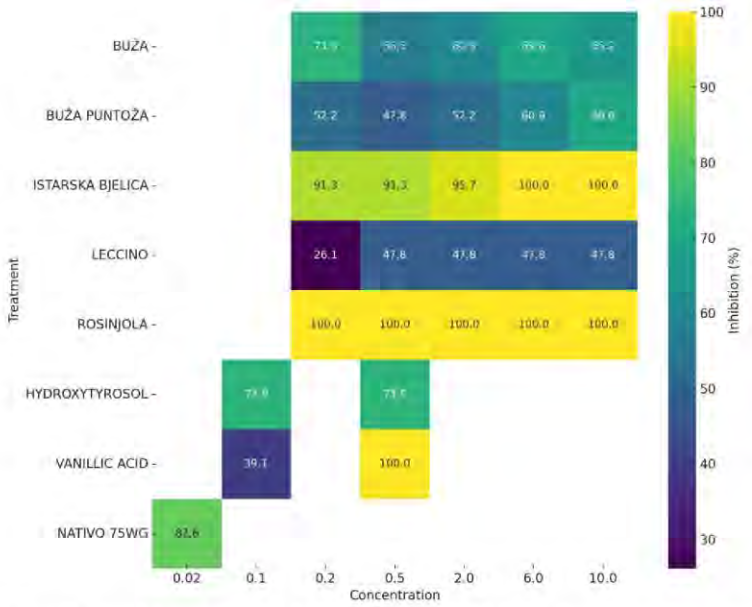


Figure 7. The inhibitory effect of different treatments (OMWW, phenols, and fungicide) at various concentrations on the *Phaeoacremonium iranianum*. The dataset covers treatments using OMWW derived from five olive cultivars, two individual phenolics (hydroxytyrosol and vanillic acid), and a reference fungicide (Nativo 75WG). Along the x-axis are the treatment doses; the y-axis lists each treatment with its corresponding percent growth inhibition. Color shading represents inhibition strength—blues mark minimal or even growth-promoting effects, while yellows indicate strong inhibition approaching 100%.

Regarding *S. fimicola*, OMWW from Buža, Buža puntoža, and Istarska bjelica showed no inhibitory effect on the pathogen on day seven. On day two, Buža exhibited a very weak inhibitory effect at concentrations ranging from 0.2% to 2%, while at 6% and 10%, a slight stimulatory effect was observed. OMWW from Buža puntoža demonstrated the highest inhibitory effect (15.45%) at the lowest concentration, which decreased with increasing concentration. In contrast, OMWW from Istarska bjelica showed a stronger inhibitory effect with increasing concentration, reaching 41.63% at 10% concentration on day two. OMWW from Leccino exhibited a concentration-dependent increase in inhibition on both days, reaching 87.55% and 86.04% at 10% concentration on day two and day seven, respectively. A similar trend was observed with OMWW from Rosinjola, where inhibition at 10% concentration was 96.56% on day two and 69.76% on day seven. Hydroxytyrosol showed a weaker effect on growth inhibition, and no inhibitory activity was recorded on day seven. Both vanillic acid at 0.5% concentration and Nativo 75WG achieved 100% growth inhibition on both days of measurement.

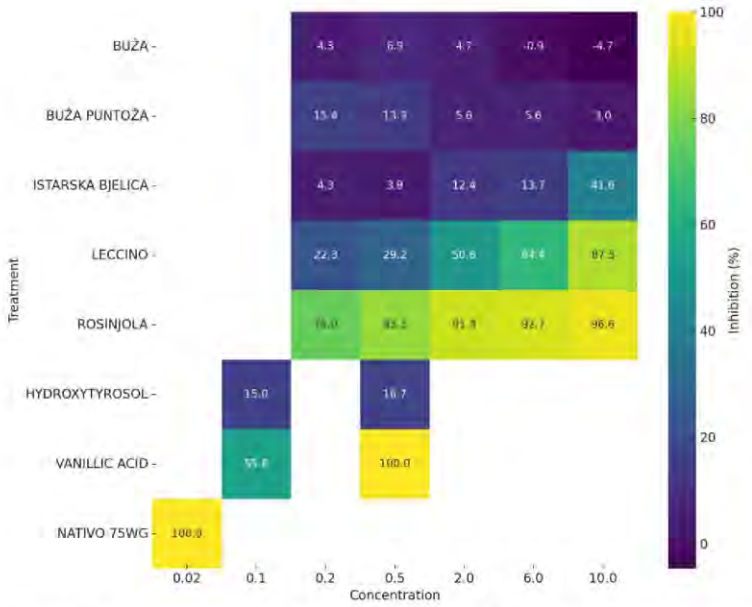


Figure 8. The inhibitory effect of different treatments (OMWW, phenols, and fungicide) at various concentrations on the *Sordaria fimicola*. The dataset covers treatments using OMWW derived from five olive cultivars, two individual phenolics (hydroxytyrosol and vanillic acid), and a reference fungicide (Nativo 75WG). Along the x-axis are the treatment doses; the y-axis lists each treatment with its corresponding percent growth inhibition. Color shading represents inhibition strength—blues mark minimal or even growth-promoting effects, while yellows indicate strong inhibition approaching 100%. Values below zero denote stimulation of fungal growth.

Regarding MIC and MFC values, the MFC was determined only for treatments with OMWW from Buža puntoža on the species *C. pruinosa*, OMWW from Rosinjola on *B. nummularia* and *P. iranianum*, and treatments with vanillic acid on *B. mediterranea* and *S. fimicola*. For fungicide, MFC values were established for effects on *B. nummularia*, *C. pruinosa*, *N. gorlenkoana*, and *S. fimicola*. The MIC value was not determined for OMWW from Buža on *N. gorlenkoana*, OMWW from Buža puntoža on *N. gorlenkoana*, Istarska bjelica on *B. nummularia* and *N. gorlenkoana*, Leccino on *B. nummularia*, *N. gorlenkoana*, and *N. osmanthi*, as well as OMWW from Rosinjola on *N. gorlenkoana* and *P. iranianum*. The most common MIC values ranged between 0.1% and 0.2% (Table S17).

3.2. Antagonistic Effects of Tested Isolates on Phytopathogenic Fungi

Significant variations were observed in the antagonistic effects of the tested isolates on phytopathogenic fungi. However, regarding *R. mucilaginosa*, the isolate from OMWW Buža (B_RB) exhibited a stronger antagonistic effect compared to the one isolated from OMWW Rosinjola. In the case of *P. crustosum*, substantial differences in antagonistic activity were detected among isolates of this species. For *P. crustosum* isolated from OMWW Istarska bjelica (BJ_P), a GIC of 4 was recorded for three fungal species, GIC 3 for four species, while on *B. nummularia* showed no inhibition (GIC 0), indicating a stimulatory effect (Table S18). The following section presents the results of antagonistic tests, categorized by fungal species.

Neither bacterial nor yeast isolates exhibited any inhibitory effects on the growth of *B. mediterranea*. However, *P. crustosum* demonstrated a high percentage of mycelial growth inhibition, with the most effective isolate being *P. crustosum* from OMWW Istarska bjelica (BJ_P) (Figure 9), which achieved an 82.78% inhibition rate.

The *P. crustosum* isolate from OMWW Istarska bjelica (BJ_P) had a stimulatory effect on *B. nummularia* growth. The highest inhibition rate was observed with *P. crustosum* isolated from OMWW Buža puntoža (BP_P) at 81.94%, followed by BB_B at 80.56%, while all other antagonists exhibited inhibition rates above 70%.

The *P. crustosum* isolate from OMWW Leccino had a stimulatory effect on fungal growth of *C. pruinosa*. The strongest antagonist was *P. crustosum* from OMWW Istarska bjelica, which achieved an 77.64% inhibition rate, whereas the weakest antagonist was *R. mucilaginosa* from OMWW Rosinjola (R_RB), with an inhibition rate of only 7.78%.

The *P. crustosum* isolate from OMWW Buža puntoža (BP_P) exhibited no inhibitory effect on *N. gorlenkoana* mycelial growth. The strongest antagonistic activity was observed in the isolate from OMWW Istarska bjelica (BJ_P), with an inhibition rate of 82.22%.

Antagonistic effects on *N. osmanthi* were relatively similar, ranging between 40% and 60% inhibition. The most effective isolate was *P. crustosum* from OMWW Istarska bjelica (BJ_P), achieving 68.24% inhibition, while the least effective was *R. mucilaginosa* from OMWW Rosinjola (R_RB) with an inhibition rate of 40.86%.

Neither *R. mucilaginosa* from OMWW Rosinjola (R_RB) nor *N. molendiniolei* (R_BB) had any inhibitory effect on *N. philosophiae doctoris*. The most effective antagonist was *P. crustosum* from OMWW Leccino (L_P), which exhibited an inhibition rate of 84.44%, followed by *P. crustosum* from OMWW Buža puntoža with 73.33% inhibition.

The *P. crustosum* isolates from OMWW Buža puntoža (BP_P) and Leccino (L_P) stimulated the mycelial growth of *P. iranianum*. In contrast, *P. crustosum* from OMWW Istarska bjelica (BJ_P) exhibited the strongest antagonistic effect among all tested isolates, with an inhibition rate of 55%, whereas the weakest antagonist was *R. mucilaginosa* from OMWW Rosinjola (R_RB), which achieved only 11.76% inhibition.

The *R. mucilaginosa* isolate from OMWW Rosinjola (R_RB) did not influence the inhibition of *S. fimicola* mycelial growth. Conversely, the most effective isolate was *R. mucilaginosa* from OMWW Buža (B_RB), with an inhibition rate of 74.44%, followed by *N. molendiniolei* (R_BB) with 71.11% inhibition.

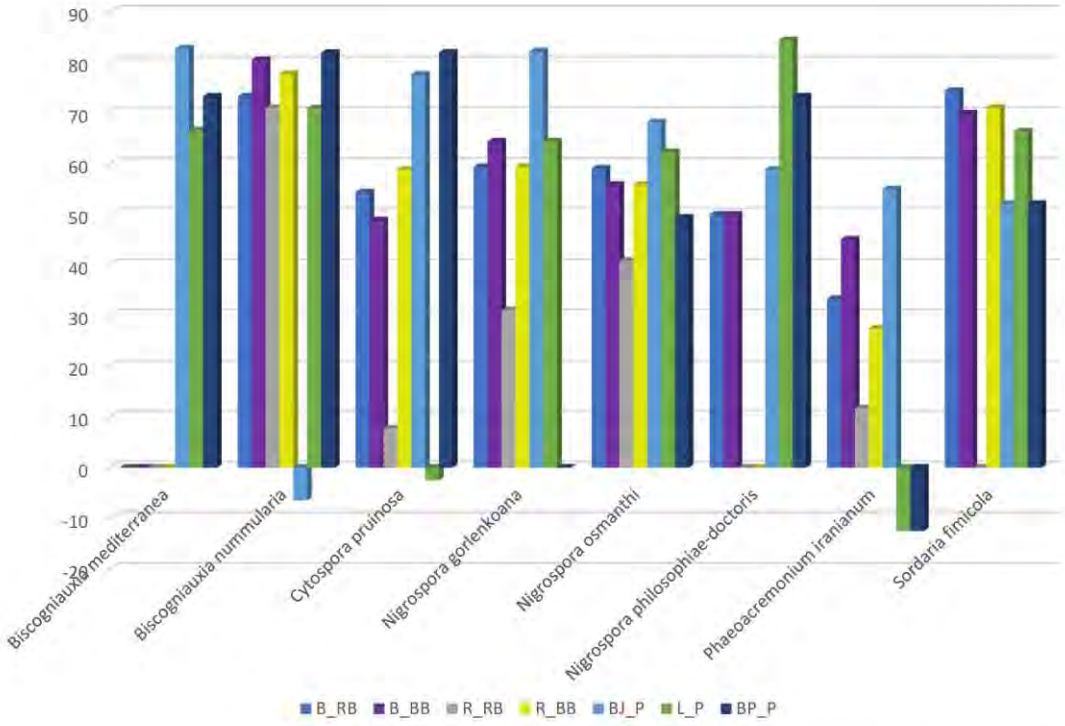


Figure 9. The inhibitory effect of antagonistic microorganisms on the fungi Biscogniauxia mediterranea, B. nummularia, Cytospora pruinosa, Nigrospora gorlenkoana, N. osmanthi, N. philosophiae-doctoris, P. iranianum, and S. fimicola. Negative values indicate a stimulatory effect of the antagonistic microorganisms on the growth of the pathogens. B_RB (from OMWW Buža) and R_RB (from OMWW Rosinjola) are isolates of Rhodotorula mucilaginosa, R_BB (from OMWW Rosinjola) is an isolate of Nakazawaea molendiniolei, B_BB (from OMWW Buža) is an isolate of Bacillus velezensis, and BP_P (from OMWW Buža puntoža), BJ_P (from OMWW Istarska bjelica), and L_P (from OMWW Leccino) are isolates of Penicillium crustosum.

4. Discussion

Although tested fungi represent relatively new and/or emerging pathogens affecting olive trees, it is crucial to identify effective disease control measures. For most of the listed fungal species, no fungicide testing or evaluations of natural plant protection products have been conducted, despite some species being previously described as pathogens on other plant hosts. Patejuk et al. [19], for example, report that the occurrence of *B. mediterranea* and *B. nummularia* is exceptionally rare in Europe, underscoring the need for continuous monitoring of these pathogens due to their aggressive nature and the potential threat they pose. To date, antifungal efficacy assessments have not been performed for *C. pruinosa, N. gorlenkoana, N. philosophiae-doctoris, N. osmanthi,* and *P. iranianum*, except for our prior research involving essential oils and two commonly used fungicides (Nativo 75WG and Cabrio TOP) in the management of olive fungal diseases [20]. These evaluations were also extended to other fungal species presented in this study, as well as those from the *Botryosphaeriaceae* family. Among the tested treatments, oregano and cinnamon essential oils, along with their major active components (carvacrol and e-cinnamaldehyde), exhibited the highest antifungal activity. Additionally, both fungicides demonstrated strong efficacy against most tested fungi.

For *Biscogniauxia* species, previous studies have indicated that the fungicides carbendazim and propiconazole are effective in controlling *Biscogniauxia* spp. [21]. Furthermore, *B. mediterranea* has demonstrated high susceptibility to certain essential oils, including *Eucalyptus camaldulensis Dehnh* and *Malus communis Desf.* Since *Sordaria* is predominantly classified in the literature as a saprophytic fungus, control strategies for this species remain largely unexplored. However, one study investigated the antifungal activity of triadimefon, triadimenol, fenarimol, nuarimol, imazalil, and fluotrimazole against *S. fimicola* [18], although it dates back several decades.

Our findings indicate that certain OMWW and phenolic compounds have an inhibitory effect on the mycelial growth of these fungi. Among the tested treatments, OMWW derived from the Rosinjola cultivar exhibited the highest antifungal activity, whereas OMWW from Leccino showed the weakest inhibition, although the results varied significantly. Among the examined fungal species, *N. philosophiae-doctoris* demonstrated the highest susceptibility to all treatments, while *N. gorlenkoana* displayed a stimulatory response to all OMWW applications. *B. mediterranea* emerged as the most resistant species to antagonist treatments. This species is known as an aggressive pathogen on specific plant hosts and has been widely recognized as a causal agent of canker disease in forest trees [19].

In other studies, the antimicrobial properties of OMWW have been demonstrated as a potential means to combat plant pathogens, opening new possibilities for recycling these distinctive bioactive by-products [23]. In the study by Cibelli et al. [5], non-thermal high-pressure homogenization of OMWW significantly slowed the growth of eight out of 12 tested fungal species. Similarly, Vagelas et al. [24] reported that filter-sterilized OMWW inhibited the growth of *Botrytis cinerea* Pers. *in vitro*, primarily due to the activity of phenolic compounds present in OMWW, while sterile (autoclaved) water exhibited an effect similar to the control. Furthermore, the results of Vagelas et al. [24] showed that filter-sterilized OMWW inhibited the mycelial growth of various phytopathogenic fungi *in vitro*.

In the study by Yangui et al. [7], OMWW and its indigenous bacterial strains were tested both *in vitro* and *in vivo* to evaluate their efficacy in suppressing damping-off disease caused by soil-borne fungi *Rhizoctonia solani* J.G. Kühn and *Fusarium solani* (Mart.) Sacc. The results demonstrated that OMWW and its polyphenols had a strong antifungal effect against *R. solani*, whereas *F. solani* exhibited greater resistance. Only the highest applied dose (2%) of OMWW successfully inhibited *F. solani* mycelial growth. Additionally, nine indigenous bacterial strains isolated from OMWW (*Bacillus* spp., *Burkholderia caryophylli* Burkholder, and *Pseudomonas fluorescens* Migula) were examined for their potential antagonistic activity against pathogenic fungi.

However, OMWW can serve as a valuable resource for various applications, including fertilizers, antioxidants, and other bio-based products [25]. Furthermore, Yakhlef et al. [3] highlighted that the high concentrations of antimicrobial compounds in by-products of olive oil extraction—including olive oil, pomace, and OMWW—represent a promising source of natural antimicrobials with potential applications in healthcare, animal nutrition, the food industry, and as potential pesticides in agriculture. Some studies have suggested that the organic matter in OMWW can significantly improve soil quality by increasing organic matter content, which in turn may enhance populations of saprophytic microorganisms in the soil, including potential antagonists of soil-borne pathogens such as *Trichoderma* spp. [26].

Based on our research, OMWW is a valuable source of microorganisms and phenolic compounds that can be utilized in plant protection. Nevertheless, further investigations are required to optimize protocols, determine optimal concentrations and application rates, and further explore antagonistic interactions.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: title; Table S1: title; Video S1: title.

Author Contributions: Conceptualization, E.P.; methodology, E.P.; investigation, E.P.; resources, S.G.; data curation, E.P., T.S.; writing—original draft preparation, E.P.; writing—review and editing, E.P., K.V., T.S., J.Ć., S.G.; visualization, E.P.; supervision, K.V., S.G.; project administration, S.G. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Croatian Science Foundation under the project numbers HRZZ-UIP-2020-02-7413 and HRZZ-DOK-2021-02-2882.

Data Availability Statement: All data are represented in the Manuscript and Supplementary file.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. FAO, Food and Agriculture Organization of the United Nations. FAOSTAT: Crops and livestock products. Available online: https://www.fao.org/faostat/en/#data/QCL (accessed on 10 May 2025).
2. Klen, T.J.; Vodopivec, B.M. Ultrasonic extraction of phenols from olive mill wastewater: comparison with conventional methods. J. Agric. *Food Chem.* **2011**, *59*, 12725–12731. https://doi.org/10.1021/jf202800n.
3. Yakhlef, W.; Arhab, R.; Romero, C.; Brenes, M.; de Castro, A.; Medina, E. Phenolic composition and antimicrobial activity of Algerian olive products and by-products. *LWT* **2018**, *93*, 323–328. https://doi.org/10.1016/j.lwt.2018.03.044.
4. European Commission. Recovery, recycling, resource. Valorisation of olive mill effluents by recovering high added value bio-products. Life Public Database. Available online: LIFE 3.0 - LIFE07 ENV/IT/000421 (accessed on 21 March 2025).
5. Cibelli, F.; Bevilacqua, A.; Raimondo, M.L.; Campaniello, D.; Carlucci, A.; Ciccarone, C.; Sinigaglia, M.; Corbo, M.R. Evaluation of Fungal Growth on Olive-Mill Wastewaters Treated at High Temperature and by High-Pressure Homogenization. *Front Microbiol.* **2017**, *8*, 2515. https://doi.org/10.3389/fmicb.2017.02515.
6. D'Annibale, A.; Casa, R.; Pieruccetti, F.; Ricci, M.; Marabottini, R. Lentinula edodes removes phenols from olive-mill wastewater: impact on durum wheat (*Triticum durum* Desf.) germinability. *Chemosphere* **2004**, *54*, 887–894. https://doi.org/10.1016/j.chemosphere.2003.10.
7. Yangui, T.; Rhouma, A.; Triki, M.A.; Gargouri, K.; Bouzid, J. Control of damping-off caused by *Rhizoctonia solani* and *Fusarium solani* using olive mill wastewater and some of its indigenous bacterial strains. *Crop Prot.* **2008**, *27*, 189–197. https://doi.org/10.1016/j.cropro.2007.05.005.
8. Petrović, E.; Godena, S.; Ćosić, J.; Vrandečić, K. Identification and Pathogenicity of *Biscogniauxia* and *Sordaria* Species Isolated from Olive Trees. *Horticulturae* **2024**, *10*, 243. https://doi.org/10.3390/horticulturae10030243.
9. Petrović, E.; Vrandečić, K.; Ivić, D.; Ćosić, J.; Godena, S. First Report of Olive Branch Dieback in Croatia Caused by *Cytospora pruinosa* Défago. *Microorganisms* **2023**, *11*, 1679. https://doi.org/10.3390/microorganisms11071679.
10. Petrović, E.; Vrandečić, K.; Ćosić, J.; Kanižai Šarić, G.; Godena, S. First Report of *Phaeoacremonium iranianum* Causing Olive Twig and Branch Dieback. *Plants* **2022**, *11*, 3578. https://doi.org/10.3390/plants11243578.
11. Petrović, E.; Vrandečić, K.; Ćosić, J.; Đermić, E.; Godena, S. First Report of *Nigrospora* Species Causing Leaf Spot on Olive (*Olea europaea* L.). *Horticulturae* **2023**, *9*, 1067. https://doi.org/10.3390/horticulturae9101067.
12. Russo, E.; Spallarossa, A.; Comite, A.; Pagliero, M.; Guida, V.; Belotti, V.; Caviglia, D.; Schito, A.M. Valorization and potential antimicrobial use of olive mill wastewater (OMW) from Italian olive oil production. *Antioxidants* **2022**, *11*, 903. https://doi.org/10.3390/antiox11050903.
13. Petrović, E.; Vrandečić, K.; Albreht, A.; Gruntar, I.; Major, N.; Ćosić, J.; Užila, Z.; Goreta Ban, S.; Godena, S. Integrated Analysis of Olive Mill Wastewaters: Physicochemical Profiling, Antifungal Activity, and Biocontrol Potential Against *Botryosphaeriaceae*. *Horticulturae* **2025**, *11*, 819. https://doi.org/10.3390/horticulturae11070819.
14. Krid, S.; Bouaziz, M.; Ali Triki, M.; Gargouri, A.; Rhouma, A. Inhibition of olive knot disease by polyphenols extracted from olive mill waste water. *J. Plant Pathol.* **2011**, *93*, 561–568.
15. Palfi, M. *Antifungal activity of essential oils and their components against phytopathogenic fungi under in vitro conditions*. PhD Thesis, Josip Juraj Strossmayer University of Osijek, Ruđer Bošković Institute, Zagreb, Croatia, 2017.
16. Fokkema, N.J. Fungal antagonism in the phyllosphere. *Ann. Appl. Biol.* **1978**, *89*, 115–117.
17. Živković, S.; Stojanović, S.; Ivanović, Ž.; Gavrilović, V.; Popović, T.; Balaž, J. Screening of antagonistic activity of microorganisms against *Colletotrichum acutatum* and *Colletotrichum gloeosporioides*. *Arch. Biol. Sci. Belgrade* **2010**, *62*, 611–623. https://doi.org/10.2298/ABS1003611Z.
18. Korsten, L.; De Jager, E.S. Mode of action of *Bacillus subtilis* for control of avocado postharvest pathogens. *S. Afr. Avocado Growers Assoc. Yearb.* **1995**, *18*, 124–130.
19. Patejuk, K.; Baturo-Cieśniewska, A.; Pusz, W.; Kaczmarek-Pieńczewska, A. Biscogniauxia Charcoal Canker—A New Potential Threat for Mid-European Forests as an Effect of Climate Change. *Forests* **2022**, *13*, 89. https://doi.org/10.3390/f13010089.
20. Petrović, E.; Vrandečić, K.; Ćosić, J.; Siber, T.; Godena, S. Antifungal Efficacy of Essential Oils and Their Predominant Components Against Olive Fungal Pathogens. *Agriculture* **2025**, *15*, 340. https://doi.org/10.3390/agriculture15030340.
21. Karami, J.; Kavosi, M.R.; Babanezhad, M.; Kiapasha, K. Integrated management of the charcoal disease by silviculture, chemical and biological methods in forest parks. *J. Sustain. For.* **2018**, *37*, 429–444. http://dx.doi.org/10.1080/10549811.2017.1416642.

22. Buchenauer, H. Comparative studies on the antifungal activity of triadimefon, triadimenol, fenarimol, nuarimol, imazalil and fluotrimazole in vitro. *J. Plant Dis. Prot*. **1979**, *86*, 341–354.
23. Cayuela, M.L.; Millner, P.D.; Meyer, S.L.; Roig, A. Potential of olive mill waste and compost as biobased pesticides against weeds, fungi, and nematodes. *Sci. Total Environ.* **2008**, *399*, 11–18. https://doi.org/10.1016/j.scitotenv.2008.03.031.
24. Vagelas, I.; Papachatzis, A.; Kalorizou, H.; Wogiatzi, E. Biological control of Botrytis fruit rot (gray mold) on strawberry and red pepper fruits by olive oil mill wastewater. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2014**, *42*, 1489–1491. https://doi.org/10.2478/V10133-009-0017-3.
25. Morillo, J.A.; Antizar-Ladislao, B.; Monteoliva-Sánchez, M.; Ramos-Cormenzana, A.; Russell, N.J. Bioremediation and biovalorisation of olive-mill wastes. *Appl. Microbiol. Biotechnol*. **2009**, *82*, 25–39. https://doi.org/10.1007/s00253-008-1801-y.
26. Manici, L.M.; Caputo, F.; Bambini, V. Effect of green manure on *Pythium* spp. Population and microbial communities in intensive cropping systems. *Plant Soil* **2004**, *263*, 133–142. https://doi.org/10.1023/B:PLSO.0000047720.40918.29.

Table S1. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Biscogniauxia mediterranea* under different treatments. Values within the same row marked with identical letters are not significantly different, based on Tukey's Honest Significant Difference (HSD) test at a significance level of $p < 0.05$. The concentrations of the OMWWs are shown above the values, while the concentrations of hydroxytyrosol and vanillic acid are indicated in parentheses next to their names.

Treatment	Day 2	Day 7
Concentration of OMWW = 0.2		
Buža	35.67 ± 6.11 b	86.00 ± 0.00 a
Buža puntoža	38.33 ± 0.58 b	86.00 ± 0.00 a
Istarska bjelica	40.33 ± 5.13 b	86.00 ± 0.00 a
Leccino	47.67 ± 1.53 a	86.00 ± 0.00 a
Rosinjola	18.67 ± 2.31 c	32.00 ± 6.93 b
Hydroxytyrosol (0.1)	21.33 ± 0.58 c	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	22.33 ± 2.89 c	86.00 ± 0.00 a
Vanilic acid (0.1)	8.33 ± 0.58 d	86.00 ± 0.00 a
Vanilic acid (0.5)	0.00 ± 0.00 e	0.00 ± 0.00 d
Nativo 75WG	0.00 ± 0.00 e	6.33 ± 0.58 c
Control	46.33 ± 2.08 a	86.00 ± 0.00 a
MSD	6.91	6.12
Concentration of OMWW = 0.5		
Buža	29.33 ± 2.08 c	46.67 ± 8.02 b
Buža puntoža	27.00 ± 5.29 cd	51.00 ± 0.00 b
Istarska bjelica	37.67 ± 1.53 b	86.00 ± 0.00 a
Leccino	49.33 ± 1.53 a	86.00 ± 0.00 a
Rosinjola	13.00 ± 2.65 e	31.67 ± 1.53 c
Hydroxytyrosol (0.1)	21.33 ± 0.58 d	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	22.33 ± 2.89 d	86.00 ± 0.00 a
Vanilic acid (0.1)	8.33 ± 0.58 e	86.00 ± 0.00 a
Vanilic acid (0.5)	0.00 ± 0.00 f	0.00 ± 0.00 d
Nativo 75WG	0.00 ± 0.00 f	6.33 ± 0.58 d
Control	46.33 ± 2.08 a	86.00 ± 0.00 a
MSD	6.67	7.2
Concentration of OMWW = 2		
Buža	24.67 ± 4.04 c	29.00 ± 3.00 c
Buža puntoža	28.00 ± 3.61 c	40.00 ± 1.00 b
Istarska bjelica	41.67 ± 4.04 b	86.00 ± 0.00 a
Leccino	54.33 ± 3.06 a	86.00 ± 0.00 a
Rosinjola	6.67 ± 2.31 de	28.33 ± 4.04 c
Hydroxytyrosol (0.1)	21.33 ± 0.58 c	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	22.33 ± 2.89 c	86.00 ± 0.00 a
Vanilic acid (0.1)	8.33 ± 0.58 d	86.00 ± 0.00 a

Vanilic acid (0.5)	0.00 ± 0.00 e	0.00 ± 0.00 d
Nativo 75WG	0.00 ± 0.00 e	6.33 ± 0.58 d
Control	46.33 ± 2.08 b	86.00 ± 0.00 a
MSD	7.55	4.54
Concentration of OMWW = 6		
Buža	11.33 ± 4.51 cd	17.33 ± 2.31 d
Buža puntoža	24.67 ± 5.77 b	35.67 ± 5.86 b
Istarska bjelica	45.33 ± 1.15 a	86.00 ± 0.00 a
Leccino	45.33 ± 10.07 a	86.00 ± 0.00 a
Rosinjola	5.67 ± 0.58 d	24.00 ± 2.65 c
Hydroxytyrosol (0.1)	21.33 ± 0.58 bc	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	22.33 ± 2.89 bc	86.00 ± 0.00 a
Vanilic acid (0.1)	8.33 ± 0.58 d	86.00 ± 0.00 a
Vanilic acid (0.5)	0.00 ± 0.00 d	0.00 ± 0.00 f
Nativo 75WG	0.00 ± 0.00 d	6.33 ± 0.58 e
Control	46.33 ± 2.08 a	86.00 ± 0.00 a
MSD	11.48	6.03
Concentration of OMWW = 10		
Buža	10.33 ± 1.15 d	16.33 ± 1.15 d
Buža puntoža	13.33 ± 2.52 d	21.33 ± 4.51 c
Istarska bjelica	46.33 ± 6.43 a	86.00 ± 0.00 a
Leccino	35.67 ± 3.51 b	86.00 ± 0.00 a
Rosinjola	2.67 ± 0.58 ef	26.00 ± 0.00 b
Hydroxytyrosol (0.1)	21.33 ± 0.58 c	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	22.33 ± 2.89 c	86.00 ± 0.00 a
Vanilic acid (0.1)	8.33 ± 0.58 de	86.00 ± 0.00 a
Vanilic acid (0.5)	0.00 ± 0.00 f	0.00 ± 0.00 f
Nativo 75WG	0.00 ± 0.00 f	6.33 ± 0.58 e
Control	46.33 ± 2.08 a	86.00 ± 0.00 a
MSD	7.62	4.13

Table S2. Inhibition percentage (%) of mycelial growth of *Biscogniauxia mediterranea* under different treatment conditions.

Treatment	Concentration	Day 2	Day 7
Buža	0.2	23.02	0.00
	0.5	36.69	45.73
	2	46.76	66.27
	6	75.53	79.84
	10	77.69	81.00
Buža puntoža	0.2	17.26	0.00
	0.5	41.72	40.69
	2	39.56	53.48
	6	46.76	58.52
	10	71.22	75.19

Istarska bjelica	0.2	20.14	0.00
	0.5	18.70	0.00
	2	10.07	0.00
	6	2.15	0.00
	10	0.00	0.00
Leccino	0.2	-2.87	0.00
	0.5	-6.47	0.00
	2	-17.26	0.00
	6	2.15	0.00
	10	23.02	0.00
Rosinjola	0.2	59.71	62.79
	0.5	71.94	60.46
	2	85.61	67.05
	6	87.76	72.09
	10	94.24	69.76
Hydroxytyrosol	0.1	53.95	0.00
	0.5	51.79	0.00
Vanillic acid	0.1	82.01	0.00
	0.5	100	100
Nativo 75WG	0.02	100	92.63

Table S3. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Biscogniauxia nummularia* under different treatments. Values within the same row marked with identical letters are not significantly different, based on Tukey's Honest Significant Difference (HSD) test at a significance level of $p < 0.05$. The concentrations of the OMWWs are shown above the values, while the concentrations of hydroxytyrosol and vanillic acid are indicated in parentheses next to their names.

Treatment	Day 2	Day 7
Concentration of OMWW = 0.2		
Buža	21.67 ± 6.43 c	86.00 ± 0.00 a
Buža puntoža	42.00 ± 3.46 a	63.00 ± 3.61 b
Istarska bjelica	34.00 ± 2.00 b	86.00 ± 0.00 a
Leccino	46.33 ± 0.58 a	86.00 ± 0.00 a
Rosinjola	5.33 ± 2.08 de	6.00 ± 1.00 d
Hydroxytyrosol (0.1)	10.67 ± 3.21 d	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	10.33 ± 2.89 d	86.00 ± 0.00 a
Vanilic acid (0.1)	1.33 ± 0.58 e	42.67 ± 4.51 c
Vanilic acid (0.5)	0.33 ± 0.58 e	6.00 ± 2.00 d
Nativo 75WG	0.00 ± 0.00 e	0.00 ± 0.00 e
Control	28.00 ± 1.00 bc	86.00 ± 0.00 a
MSD	7.99	5.45
Concentration of OMWW = 0.5		
Buža	32.00 ± 4.00 cd	86.00 ± 0.00 a
Buža puntoža	42.00 ± 3.00 ab	66.00 ± 5.29 b
Istarska bjelica	35.33 ± 2.08 bc	86.00 ± 0.00 a
Leccino	46.33 ± 0.57 a	86.00 ± 0.00 a

Rosinjola	5.33 ± 0.58 ef	5.67 ± 0.58 d
Hydroxytyrosol (0.1)	10.67 ± 3.21 e	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	10.33 ± 2.89 e	86.00 ± 0.00 a
Vanilic acid (0.1)	1.33 ± 0.58 f	42.67 ± 4.51 c
Vanilic acid (0.5)	0.33 ± 0.58 f	6.00 ± 2.00 d
Nativo 75WG	0.00 ± 0.00 f	0.00 ± 0.00 d
Control	28.00 ± 1.00 d	86.00 ± 0.00 a
MSD	6.24	6.39
Concentration of OMWW = 2		
Buža	34.67 ± 4.16 bc	86.00 ± 0.00 a
Buža puntoža	26.67 ± 8.39 c	36.33 ± 12.67 b
Istarska bjelica	38.33 ± 1.53 ab	86.00 ± 0.00 a
Leccino	47.00 ± 1.73 a	86.00 ± 0.00 a
Rosinjola	0.00 ± 0.00 e	0.00 ± 0.00 c
Hydroxytyrosol (0.1)	10.67 ± 3.21 d	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	10.33 ± 2.89 d	86.00 ± 0.00 a
Vanilic acid (0.1)	1.33 ± 0.58 de	42.67 ± 4.51 b
Vanilic acid (0.5)	0.33 ± 0.58 e	6.00 ± 2.00 c
Nativo 75WG	0.00 ± 0.00 e	0.00 ± 0.00 c
Control	28.00 ± 1.00 c	86.00 ± 0.00 a
MSD	9.37	11.96
Concentration of OMWW = 6		
Buža	37.00 ± 1.00 b	86.00 ± 0.00 a
Buža puntoža	13.00 ± 1.73 d	14.33 ± 2.08 c
Istarska bjelica	39.00 ± 1.00 b	86.00 ± 0.00 a
Leccino	45.33 ± 1.15 a	86.00 ± 0.00 a
Rosinjola	0.00 ± 0.00 e	0.00 ± 0.00 e
Hydroxytyrosol (0.1)	10.67 ± 3.21 d	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	10.33 ± 2.89 d	86.00 ± 0.00 a
Vanilic acid (0.1)	1.33 ± 0.58 e	42.67 ± 4.51 b
Vanilic acid (0.5)	0.33 ± 0.58 e	6.00 ± 2.00 d
Nativo 75WG	0.00 ± 0.00 e	0.00 ± 0.00 e
Control	28.00 ± 1.00 c	86.00 ± 0.00 a
MSD	4.54	4.71
Concentration of OMWW = 10		
Buža	36.00 ± 0.00 c	86.00 ± 0.00 a
Buža puntoža	3.33 ± 0.58 f	6.00 ± 2.65 c
Istarska bjelica	40.67 ± 0.58 b	86.00 ± 0.00 a
Leccino	47.00 ± 1.73 a	86.00 ± 0.00 a
Rosinjola	0.00 ± 0.00 f	0.00 ± 0.00 d
Hydroxytyrosol (0.1)	10.67 ± 3.21 e	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	10.33 ± 2.89 e	86.00 ± 0.00 a
Vanilic acid (0.1)	1.33 ± 0.58 f	42.67 ± 4.51 b
Vanilic acid (0.5)	0.33 ± 0.58 f	6.00 ± 2.00 c
Nativo 75WG	0.00 ± 0.00 f	0.00 ± 0.00 d
Control	28.00 ± 1.00 d	86.00 ± 0.00 a
MSD	4.31	4.93

Table S4. Inhibition percentage (%) of mycelial growth of *Biscogniauxia nummularia* under different treatment conditions.

Treatment	Concentration	Day 2	Day 7
Buža	0.2	22.61	0.00
	0.5	-14.28	0.00
	2	-23.80	0.00
	6	-32.14	0.00
	10	-28.57	0.00
Buža puntoža	0.2	-50	26.74
	0.5	-50	23.25
	2	4.76	57.75
	6	53.57	83.33
	10	88.09	93.02
Istarska bjelica	0.2	-21.42	0.00
	0.5	-26.19	0.00
	2	-36.90	0.00
	6	-39.28	0.00
	10	-45.23	0.00
Leccino	0.2	-65.47	0.00
	0.5	-65.47	0.00
	2	-67.85	0.00
	6	-61.90	0.00
	10	-67.85	0.00
Rosinjola	0.2	80.95	93.02
	0.5	80.95	93.02
	2	100	100
	6	100	100
	10	100	100
Hydroxytyrosol	0.1	61.90	0.00
	0.5	63.09	0.00
Vanillic acid	0.1	95.23	50.38
	0.5	98.80	93.02
Nativo 75WG	0.02	100	100

Table S5. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Cytospora pruinosa* under different treatments. Values within the same row marked with identical letters are not significantly different, based on Tukey's Honest Significant Difference (HSD) test at a significance level of $p < 0.05$. The concentrations of the OMWWs are shown above the values, while the concentrations of hydroxytyrosol and vanillic acid are indicated in parentheses next to their names.

Treatment	Day 2	Day 7
Concentration of OMWW = 0.2		
Buža	9.00 ± 3.00 ab	86.00 ± 0.00 a
Buža puntoža	2.67 ± 0.58 de	4.67 ± 1.15 d

Istarska bjelica	9.67 ± 1.53 a	86.00 ± 0.00 a
Leccino	2.33 ± 0.58 de	86.00 ± 0.00 a
Rosinjola	1.00 ± 0.00 de	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	7.67 ± 0.58 abc	60.67 ± 7.78 b
Hydroxytyrosol (0.5)	5.00 ± 2.65 bcd	53.67 ± 8.39 b
Vanilic acid (0.1)	4.00 ± 2.00 cde	28.33 ± 1.53 c
Vanilic acid (0.5)	0.00 ± 0.00 e	0.00 ± 0.00 d
Nativo 75WG	0.00 ± 0.00 e	0.00 ± 0.00 d
Control	10.33 ± 0.58 a	58.67 ± 1.53 b
MSD	4.28	10.29
Concentration of OMWW = 0.5		
Buža	10.00 ± 1.73 a	86.00 ± 0.00 a
Buža puntoža	0.00 ± 0.00 e	1.00 ± 0.00 d
Istarska bjelica	9.67 ± 2.89 ab	86.00 ± 0.00 a
Leccino	5.00 ± 2.65 bcd	86.00 ± 0.00 a
Rosinjola	1.67 ± 0.58 de	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	7.67 ± 0.58 abc	60.67 ± 7.78 b
Hydroxytyrosol (0.5)	5.00 ± 2.65 bcd	53.67 ± 8.39 b
Vanilic acid (0.1)	4.00 ± 2.00 cde	28.33 ± 1.53 c
Vanilic acid (0.5)	0.00 ± 0.00 e	0.00 ± 0.00 d
Nativo 75WG	0.00 ± 0.00 e	0.00 ± 0.00 d
Control	10.33 ± 0.58 a	58.67 ± 1.53 b
MSD	4.84	10.24
Concentration of OMWW = 2		
Buža	15.67 ± 1.53 a	86.00 ± 0.00 a
Buža puntoža	0.00 ± 0.00 f	1.00 ± 0.00 d
Istarska bjelica	14.67 ± 0.58 ab	86.00 ± 0.00 a
Leccino	10.00 ± 4.00 bcd	86.00 ± 0.00 a
Rosinjola	5.00 ± 2.00 def	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	7.67 ± 0.58 cde	60.67 ± 7.78 b
Hydroxytyrosol (0.5)	5.00 ± 2.65 def	53.67 ± 8.39 b
Vanilic acid (0.1)	4.00 ± 2.00 ef	28.33 ± 1.53 c
Vanilic acid (0.5)	0.00 ± 0.00 f	0.00 ± 0.00 d
Nativo 75WG	0.00 ± 0.00 f	0.00 ± 0.00 d
Control	10.33 ± 0.58 bc	58.67 ± 1.53 b
MSD	5.16	10.24
Concentration of OMWW = 6		
Buža	17.33 ± 0.58 a	86.00 ± 0.00 a
Buža puntoža	0.00 ± 0.00 e	0.00 ± 0.00 d
Istarska bjelica	14.33 ± 0.58 a	86.00 ± 0.00 a
Leccino	14.67 ± 0.58 a	86.00 ± 0.00 a
Rosinjola	10.00 ± 2.00 b	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	7.67 ± 0.58 bc	60.67 ± 7.78 b
Hydroxytyrosol (0.5)	5.00 ± 2.65 cd	53.67 ± 8.39 b
Vanilic acid (0.1)	4.00 ± 2.00 d	28.33 ± 1.53 c
Vanilic acid (0.5)	0.00 ± 0.00 e	0.00 ± 0.00 d
Nativo 75WG	0.00 ± 0.00 e	0.00 ± 0.00 d

Control	10.33 ± 0.58 b	58.67 ± 1.53 b
MSD	3.59	10.24
Concentration of OMWW = 10		
Buža	18.00 ± 0.00 a	86.00 ± 0.00 a
Buža puntoža	0.00 ± 0.00 e	0.00 ± 0.00 d
Istarska bjelica	15.33 ± 0.58 a	86.00 ± 0.00 a
Leccino	16.33 ± 1.15 a	86.00 ± 0.00 a
Rosinjola	15.00 ± 1.73 a	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	7.67 ± 0.58 bc	60.67 ± 7.78 b
Hydroxytyrosol (0.5)	5.00 ± 2.65 cd	53.67 ± 8.39 b
Vanilic acid (0.1)	4.00 ± 2.00 d	28.33 ± 1.53 c
Vanilic acid (0.5)	0.00 ± 0.00 e	0.00 ± 0.00 d
Nativo 75WG	0.00 ± 0.00 e	0.00 ± 0.00 d
Control	10.33 ± 0.58 b	58.67 ± 1.53 b
MSD	3.56	10.24

Table S6. Inhibition percentage (%) of mycelial growth of *Cytospora pruinosa* under different treatment conditions.

Treatment	Concentration	Day 2	Day 7
Buža	0.2	12.90	-46.59
	0.5	3.22	-46.59
	2	-51.61	-46.59
	6	-67.74	-46.59
	10	-74.19	-46.59
Buža puntoža	0.2	74.19	92.04
	0.5	100	98.29
	2	100	98.29
	6	100	100
	10	100	100
Istarska bjelica	0.2	6.45	-46.59
	0.5	6.45	-46.59
	2	-41.93	-46.59
	6	-38.70	-46.59
	10	-48.38	-46.59
Leccino	0.2	77.41	-46.59
	0.5	51.61	-46.59
	2	3.22	-46.59
	6	-41.93	-46.59
	10	-58.06	-46.59
Rosinjola	0.2	90.32	-46.59
	0.5	83.87	-46.59
	2	51.61	-46.59
	6	3.22	-46.59
	10	-45.16	-46.59
Hydroxytyrosol	0.1	25.80	-3.40
	0.5	51.61	8.52

Vanillic acid	0.1	61.29	51.70
	0.5	100	100
Nativo 75WG	0.02	100	100

Table S7. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Nigrospora gorlenkoana* under different treatments. Values within the same row marked with identical letters are not significantly different, based on Tukey's Honest Significant Difference (HSD) test at a significance level of $p < 0.05$. The concentrations of the OMWWs are shown above the values, while the concentrations of hydroxytyrosol and vanillic acid are indicated in parentheses next to their names.

Treatment	Day 2	Day 7
Concentration of OMWW = 0.2		
Buža	86.00 ± 0.00 a	86.00 ± 0.00 a
Buža puntoža	86.00 ± 0.00 a	86.00 ± 0.00 a
Istarska bjelica	86.00 ± 0.00 a	86.00 ± 0.00 a
Leccino	86.00 ± 0.00 a	86.00 ± 0.00 a
Rosinjola	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	0.00 ± 0.00 d	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	0.00 ± 0.00 d	86.00 ± 0.00 a
Vanilic acid (0.1)	31.33 ± 4.51 c	71.67 ± 7.09 b
Vanilic acid (0.5)	5.00 ± 2.00 d	14.33 ± 2.31 c
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 d
Control	56.67 ± 8.32 b	86.00 ± 0.00 a
MSD	8.52	6.57
Concentration of OMWW = 0.5		
Buža	86.00 ± 0.00 a	86.00 ± 0.00 a
Buža puntoža	86.00 ± 0.00 a	86.00 ± 0.00 a
Istarska bjelica	86.00 ± 0.00 a	86.00 ± 0.00 a
Leccino	86.00 ± 0.00 a	86.00 ± 0.00 a
Rosinjola	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	0.00 ± 0.00 d	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	0.00 ± 0.00 d	86.00 ± 0.00 a
Vanilic acid (0.1)	31.33 ± 4.51 c	71.67 ± 7.09 b
Vanilic acid (0.5)	5.00 ± 2.00 d	14.33 ± 2.31 c
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 d
Control	56.67 ± 8.32 b	86.00 ± 0.00 a
MSD	8.52	6.57
Concentration of OMWW = 2		
Buža	86.00 ± 0.00 a	86.00 ± 0.00 a
Buža puntoža	86.00 ± 0.00 a	86.00 ± 0.00 a
Istarska bjelica	86.00 ± 0.00 a	86.00 ± 0.00 a
Leccino	86.00 ± 0.00 a	86.00 ± 0.00 a
Rosinjola	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	0.00 ± 0.00 d	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	0.00 ± 0.00 d	86.00 ± 0.00 a
Vanilic acid (0.1)	31.33 ± 4.51 c	71.67 ± 7.09 b

Vanilic acid (0.5)	5.00 ± 2.00 d	14.33 ± 2.31 c
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 d
Control	56.67 ± 8.32 b	86.00 ± 0.00 a
MSD	8.52	6.57
Concentration of OMWW = 6		
Buža	86.00 ± 0.00 a	86.00 ± 0.00 a
Buža puntoža	86.00 ± 0.00 a	86.00 ± 0.00 a
Istarska bjelica	86.00 ± 0.00 a	86.00 ± 0.00 a
Leccino	86.00 ± 0.00 a	86.00 ± 0.00 a
Rosinjola	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	0.00 ± 0.00 d	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	0.00 ± 0.00 d	86.00 ± 0.00 a
Vanilic acid (0.1)	31.33 ± 4.51 c	71.67 ± 7.09 b
Vanilic acid (0.5)	5.00 ± 2.00 d	14.33 ± 2.31 c
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 d
Control	56.67 ± 8.32 b	86.00 ± 0.00 a
MSD	8.52	6.57
Concentration of OMWW = 10		
Buža	86.00 ± 0.00 a	86.00 ± 0.00 a
Buža puntoža	86.00 ± 0.00 a	86.00 ± 0.00 a
Istarska bjelica	86.00 ± 0.00 a	86.00 ± 0.00 a
Leccino	86.00 ± 0.00 a	86.00 ± 0.00 a
Rosinjola	86.00 ± 0.00 a	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	0.00 ± 0.00 d	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	0.00 ± 0.00 d	86.00 ± 0.00 a
Vanilic acid (0.1)	31.33 ± 4.51 c	71.67 ± 7.09 b
Vanilic acid (0.5)	5.00 ± 2.00 d	14.33 ± 2.31 c
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 d
Control	56.67 ± 8.32 b	86.00 ± 0.00 a
MSD	8.52	6.57

37

Table S8. Inhibition percentage (%) of mycelial growth of *Nigrospora gorlenkoana* under different treatment conditions. 38 39

Treatment	Concentration	Day 2	Day 7
Buža	0.2	-51.76	0.00
	0.5	-51.76	0.00
	2	-51.76	0.00
	6	-51.76	0.00
	10	-51.76	0.00
Buža puntoža	0.2	-51.76	0.00
	0.5	-51.76	0.00
	2	-51.76	0.00
	6	-51.76	0.00
	10	-51.76	0.00
	0.2	-51.76	0.00
	0.5	-51.76	0.00

Istarska bjelica	2	-51.76	0.00
	6	-51.76	0.00
	10	-51.76	0.00
Leccino	0.2	-51.76	0.00
	0.5	-51.76	0.00
	2	-51.76	0.00
	6	-51.76	0.00
	10	-51.76	0.00
Rosinjola	0.2	-51.76	0.00
	0.5	-51.76	0.00
	2	-51.76	0.00
	6	-51.76	0.00
	10	-51.76	0.00
Hydroxytxrosol	0.1	100	0.00
	0.5	100	0.00
Vanillic acid	0.1	44.70	16.66
	0.5	91.17	83.33
Nativo 75WG	0.02	100	100

Table S9. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Nigrospora osmanthi* under different treatments. Values within the same row marked with identical letters are not significantly different, based on Tukey's Honest Significant Difference (HSD) test at a significance level of $p < 0.05$. The concentrations of the OMWWs are shown above the values, while the concentrations of hydroxytyrosol and vanillic acid are indicated in parentheses next to their names.

Treatment	Day 2	Day 7
Concentration of OMWW = 0.2		
Buža	40.67 ± 0.58 c	45.67 ± 4.62 b
Buža puntoža	60.00 ± 1.73 b	86.00 ± 0.00 a
Istarska bjelica	75.00 ± 2.65 a	86.00 ± 0.00 a
Leccino	72.00 ± 4.00 a	86.00 ± 0.00 a
Rosinjola	34.00 ± 6.24 c	36.33 ± 5.03 c
Hydroxytyrosol (0.1)	62.00 ± 0.00 b	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	56.33 ± 2.08 b	86.00 ± 0.00 a
Vanilic acid (0.1)	10.67 ± 0.58 d	45.00 ± 0.00 b
Vanilic acid (0.5)	0.00 ± 0.00 e	0.00 ± 0.00 e
Nativo 75WG	0.00 ± 0.00 e	21.33 ± 1.15 d
Control	71.67 ± 6.35 a	86.00 ± 0.00 a
MSD	9.24	6.16
Concentration of OMWW = 0.5		
Buža	37.67 ± 2.89 d	40.00 + 3.61 c
Buža puntoža	58.00 ± 6.08 c	86.00 ± 0.00 a
Istarska bjelica	67.67 ± 1.53 ab	86.00 ± 0.00 a
Leccino	72.33 ± 1.53 a	86.00 ± 0.00 a
Rosinjola	30.33 ± 1.53 d	33.33 + 1.53 d
Hydroxytyrosol (0.1)	62.00 ± 0.00 bc	86.00 ± 0.00 a

Hydroxytyrosol (0.5)	56.33 ± 2.08 c	86.00 ± 0.00 a
Vanilic acid (0.1)	10.67 ± 0.58 e	45.00 ± 0.00 b
Vanilic acid (0.5)	0.00 ± 0.00 f	0.00 ± 0.00 f
Nativo 75WG	0.00 ± 0.00 f	21.33 ± 1.15 e
Control	71.67 ± 6.35 a	86.00 ± 0.00 a
MSD	8.68	3.69
Concentration of OMWW = 2		
Buža	15.00 ± 3.61 e	20.00 ± 1.00 e
Buža puntoža	47.67 ± 2.89 d	50.33 ± 0.58 b
Istarska bjelica	62.67 ± 6.03 bc	86.00 ± 0.00 a
Leccino	79.00 ± 4.58 a	86.00 ± 0.00 a
Rosinjola	15.00 ± 1.00 e	20.00 ± 2.65 e
Hydroxytyrosol (0.1)	62.00 ± 0.00 bc	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	56.33 ± 2.08 cd	86.00 ± 0.00 a
Vanilic acid (0.1)	10.67 ± 0.58 e	45.00 ± 0.00 c
Vanilic acid (0.5)	0.00 ± 0.00 f	0.00 ± 0.00 f
Nativo 75WG	0.00 ± 0.00 f	21.33 ± 1.15 d
Control	71.67 ± 6.35 ab	86.00 ± 0.00 a
MSD	9.83	2.87
Concentration of OMWW = 6		
Buža	16.00 ± 1.00 e	18.67 ± 2.08 e
Buža puntoža	31.33 ± 0.58 d	38.67 ± 1.15 c
Istarska bjelica	62.67 ± 3.05 c	86.00 ± 0.00 a
Leccino	82.33 ± 3.79 a	86.00 ± 0.00 a
Rosinjola	11.00 ± 1.73 e	12.33 ± 0.58 f
Hydroxytyrosol (0.1)	62.00 ± 0.00 c	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	56.33 ± 2.08 c	86.00 ± 0.00 a
Vanilic acid (0.1)	10.67 ± 0.58 e	45.00 ± 0.00 b
Vanilic acid (0.5)	0.00 ± 0.00 f	0.00 ± 0.00 g
Nativo 75WG	0.00 ± 0.00 f	21.33 ± 1.15 d
Control	71.67 ± 6.35 b	86.00 ± 0.00 a
MSD	7.52	2.54
Concentration of OMWW = 10		
Buža	12.67 ± 2.08 e	17.33 ± 0.58 e
Buža puntoža	31.00 ± 1.00 d	37.67 ± 1.15 c
Istarska bjelica	59.33 ± 2.08 c	86.00 ± 0.00 a
Leccino	84.67 ± 1.53 a	86.00 ± 0.00 a
Rosinjola	10.67 ± 1.53 e	13.33 ± 1.53 f
Hydroxytyrosol (0.1)	62.00 ± 0.00 c	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	56.33 ± 2.08 c	86.00 ± 0.00 a
Vanilic acid (0.1)	10.67 ± 0.58 e	45.00 ± 0.00 b
Vanilic acid (0.5)	0.00 ± 0.00 f	0.00 ± 0.00 g
Nativo 75WG	0.00 ± 0.00 f	21.33 ± 1.15 d
Control	71.67 ± 6.35 b	86.00 ± 0.00 a
MSD	6.78	2.21

47

Table S10. Inhibition percentage (%) of mycelial growth of *Nigrospora osmanthi* under different treatment conditions.

Treatment	Concentration	Day 2	Day 7
Buža	0.2	43.25	46.89
	0.5	47.44	53.48
	2	79.06	76.74
	6	77.67	78.29
	10	82.32	79.84
Buža puntoža	0.2	16.27	0.00
	0.5	19.06	0.00
	2	33.48	41.47
	6	56.27	55.03
	10	56.74	56.20
Istarska bjelica	0.2	-4.65	0.00
	0.5	5.58	0.00
	2	12.55	0.00
	6	12.55	0.00
	10	17.20	0.00
Leccino	0.2	-0.46	0.00
	0.5	-0.93	0.00
	2	-10.23	0.00
	6	-14.88	0.00
	10	-18.13	0.00
Rosinjola	0.2	52.55	57.75
	0.5	57.67	58.52
	2	79.06	76.74
	6	84.65	85.65
	10	85.11	84.49
Hydroxytyrosol	0.1	13.48	0.00
	0.5	21.39	0.00
Vanillic acid	0.1	85.11	47.67
	0.5	100	100
Nativo 75WG	0.02	100	75.19

Table S11. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Nigrospora philosophiae-doctoris* under different treatments. Values within the same row marked with identical letters are not significantly different, based on Tukey's Honest Significant Difference (HSD) test at a significance level of $p < 0.05$. The concentrations of the OMWWs are shown above the values, while the concentrations of hydroxytyrosol and vanillic acid are indicated in parentheses next to their names.

Treatment	Day 2	Day 7
Concentration of OMWW = 0.2		
Buža	0.00 ± 0.00 d	86.00 ± 0.00 a
Buža puntoža	1.00 ± 0.00 d	86.00 ± 0.00 a
Istarska bjelica	21.67 ± 2.52 b	86.00 ± 0.00 a
Leccino	3.00 ± 1.00 d	86.00 ± 0.00 a

Rosinjola	13.33 ± 2.52 c	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	9.33 ± 1.15 c	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	8.33 ± 1.15 c	86.00 ± 0.00 a
Vanilic acid (0.1)	35.33 ± 1.53 a	86.00 ± 0.00 a
Vanilic acid (0.5)	1.00 ± 1.73 d	3.00 ± 2.65 b
Nativo 75WG	4.33 ± 0.58 cd	86.00 ± 0.00 a
Control	25.00 ± 3.61 b	86.00 ± 0.00 a
MSD	5.21	2.33
Concentration of OMWW = 0.5		
Buža	0.00 ± 0.00 f	86.00 ± 0.00 a
Buža puntoža	0.00 ± 0.00 f	86.00 ± 0.00 a
Istarska bjelica	15.67 ± 3.21 c	86.00 ± 0.00 a
Leccino	5.00 ± 0.00 ef	86.00 ± 0.00 a
Rosinjola	13.00 ± 1.00 cd	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	9.33 ± 1.15 de	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	8.33 ± 1.15 de	86.00 ± 0.00 a
Vanilic acid (0.1)	35.33 ± 1.53 a	86.00 ± 0.00 a
Vanilic acid (0.5)	1.00 ± 1.73 f	3.00 ± 2.65 b
Nativo 75WG	4.33 ± 0.58 ef	86.00 ± 0.00 a
Control	25.00 ± 3.61 b	86.00 ± 0.00 a
MSD	5.03	2.33
Concentration of OMWW = 2		
Buža	0.00 ± 0.00 e	86.00 ± 0.00 a
Buža puntoža	0.00 ± 0.00 e	86.00 ± 0.00 a
Istarska bjelica	14.33 ± 4.93 c	86.00 ± 0.00 a
Leccino	5.00 ± 1.00 de	86.00 ± 0.00 a
Rosinjola	10.67 ± 3.06 cd	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	9.33 ± 1.15 cd	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	8.33 ± 1.15 cd	86.00 ± 0.00 a
Vanilic acid (0.1)	35.33 ± 1.53 a	86.00 ± 0.00 a
Vanilic acid (0.5)	1.00 ± 1.73 e	3.00 ± 2.65 b
Nativo 75WG	4.33 ± 0.58 de	86.00 ± 0.00 a
Control	25.00 ± 3.61 b	86.00 ± 0.00 a
MSD	6.59	2.33
Concentration of OMWW = 6		
Buža	0.00 ± 0.00 f	86.00 ± 0.00 a
Buža puntoža	0.00 ± 0.00 f	86.00 ± 0.00 a
Istarska bjelica	13.00 ± 1.00 c	86.00 ± 0.00 a
Leccino	10.50 ± 3.50 c	86.00 ± 0.00 a
Rosinjola	3.67 ± 0.58 ef	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	9.33 ± 1.15 cd	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	8.33 ± 1.15 cde	86.00 ± 0.00 a
Vanilic acid (0.1)	35.33 ± 1.53 a	86.00 ± 0.00 a
Vanilic acid (0.5)	1.00 ± 1.73 f	3.00 ± 2.65 b
Nativo 75WG	4.33 ± 0.58 def	86.00 ± 0.00 a
Control	25.00 ± 3.61 b	86.00 ± 0.00 a
MSD	5.2	2.33

Concentration of OMWW = 10		
Buža	0.00 ± 0.00 g	86.00 ± 0.00 a
Buža puntoža	0.00 ± 0.00 g	86.00 ± 0.00 a
Istarska bjelica	11.33 ± 0.58 cd	86.00 ± 0.00 a
Leccino	13.33 ± 0.58 c	86.00 ± 0.00 a
Rosinjola	0.00 ± 0.00 g	86.00 ± 0.00 a
Hydroxytyrosol (0.1)	9.33 ± 1.15 cd	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	8.33 ± 1.15 de	86.00 ± 0.00 a
Vanilic acid (0.1)	35.33 ± 1.53 a	86.00 ± 0.00 a
Vanilic acid (0.5)	1.00 ± 1.73 fg	3.00 ± 2.65 b
Nativo 75WG	4.33 ± 0.58 ef	86.00 ± 0.00 a
Control	25.00 ± 3.61 b	86.00 ± 0.00 a
MSD	4.13	2.33

57

Table S12. Inhibition percentage (%) of mycelial growth of *Nigrospora philosophiae-doctoris* under different treatment conditions. 58 59

Treatment	Concentration	Day 2	Day 7
Buža	0.2	100	0.00
	0.5	100	0.00
	2	100	0.00
	6	100	0.00
	10	100	0.00
Buža puntoža	0.2	96	0.00
	0.5	100	0.00
	2	100	0.00
	6	100	0.00
	10	100	0.00
Istarska bjelica	0.2	13.33	0.00
	0.5	37.33	0.00
	2	42.66	0.00
	6	48.00	0.00
	10	54.66	0.00
Leccino	0.2	88.00	0.00
	0.5	80.00	0.00
	2	80.00	0.00
	6	58.00	0.00
	10	46.66	0.00
Rosinjola	0.2	46.66	0.00
	0.5	48.00	0.00
	2	57.33	0.00
	6	85.33	0.00
	10	100	0.00
Hydroxytyrosol	0.1	62.66	0.00
	0.5	66.66	0.00
Vanillic acid	0.1	-41.33	0.00
	0.5	96.00	96.51

Nativo 75WG	0.02	82.66	0.00

Table S13. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Phaeoacremonium iranianum* under different treatments. Values within the same row marked with identical letters are not significantly different, based on Tukey's Honest Significant Difference (HSD) test at a significance level of $p < 0.05$. The concentrations of the OMWWs are shown above the values, while the concentrations of hydroxytyrosol and vanillic acid are indicated in parentheses next to their names.

Treatment	Day 2	Day 7
Concentration of OMWW = 0.2		
Buža	2.00 ± 2.65 cde	4.50 ± 1.50 d
Buža puntoža	3.67 ± 0.58 bcd	5.67 ± 1.15 d
Istarska bjelica	0.67 ± 0.58 e	22.67 ± 1.15 a
Leccino	5.67 ± 0.58 ab	17.33 ± 0.58 ab
Rosinjola	0.00 ± 0.00 e	0.00 ± 0.00 d
Hydroxytyrosol (0.1)	2.00 ± 1.00 cde	18.67 ± 2.08 ab
Hydroxytyrosol (0.5)	2.00 ± 0.00 cde	21.33 ± 3.06 a
Vanilic acid (0.1)	4.67 ± 1.15 bc	13.00 ± 3.46 c
Vanilic acid (0.5)	0.00 ± 0.00 e	0.00 ± 0.00 d
Nativo 75WG	1.33 ± 0.58 de	5.00 ± 1.73 d
Control	7.67 ± 0.58 a	22.33 ± 4.04 a
MSD	2.92	6.24
Concentration of OMWW = 0.5		
Buža	3.33 ± 1.53 bcd	7.00 ± 1.00 cd
Buža puntoža	4.00 ± 2.00 bc	6.00 ± 2.65 cd
Istarska bjelica	0.67 ± 0.58 de	19.00 ± 6.08 ab
Leccino	4.00 ± 0.00 bc	15.67 ± 0.58 ab
Rosinjola	0.00 ± 0.00 e	0.00 ± 0.00 d
Hydroxytyrosol (0.1)	2.00 ± 1.00 bcde	18.67 ± 2.08 ab
Hydroxytyrosol (0.5)	2.00 ± 0.00 bcde	21.33 ± 3.06 ab
Vanilic acid (0.1)	4.67 ± 1.15 b	13.00 ± 3.46 bc
Vanilic acid (0.5)	0.00 ± 0.00 e	0.00 ± 0.00 d
Nativo 75WG	1.33 ± 0.58 cde	5.00 ± 1.73 cd
Control	7.67 ± 0.58 a	22.33 ± 4.04 a
MSD	2.74	8.36
Concentration of OMWW = 2		
Buža	3.00 ± 0.00 bcd	6.33 ± 2.08 c
Buža puntoža	3.67 ± 1.53 bc	5.00 ± 1.00 c
Istarska bjelica	0.33 ± 0.58 e	14.33 ± 2.31 b
Leccino	4.00 ± 0.00 bc	16.00 ± 1.00 ab
Rosinjola	0.00 ± 0.00 e	0.00 ± 0.00 c
Hydroxytyrosol (0.1)	2.00 ± 1.00 cde	18.67 ± 2.08 ab
Hydroxytyrosol (0.5)	2.00 ± 0.00 cde	21.33 ± 3.06 a
Vanilic acid (0.1)	4.67 ± 1.15 bc	13.00 ± 3.46 b
Vanilic acid (0.5)	0.00 ± 0.00 e	0.00 ± 0.00 c
Nativo 75WG	1.33 ± 0.58 de	5.00 ± 1.73 c

Control	7.67 ± 0.58 a	22.33 ± 4.04 a
MSD	2.09	6.62
Concentration of OMWW = 6		
Buža	2.33 ± 0.58 cd	6.67 ± 0.58 cd
Buža puntoža	3.00 ± 1.73 bcd	5.33 ± 3.21 d
Istarska bjelica	0.00 ± 0.00 e	14.33 ± 2.31 b
Leccino	4.00 ± 0.00 bc	16.00 ± 0.00 ab
Rosinjola	0.00 ± 0.00 e	0.00 ± 0.00 d
Hydroxytyrosol (0.1)	2.00 ± 1.00 cde	18.67 ± 2.08 ab
Hydroxytyrosol (0.5)	2.00 ± 0.00 cde	21.33 ± 3.06 a
Vanilic acid (0.1)	4.67 ± 1.15 b	13.00 ± 3.46 bc
Vanilic acid (0.5)	0.00 ± 0.00 e	0.00 ± 0.00 d
Nativo 75WG	1.33 ± 0.58 de	5.00 ± 1.73 d
Control	7.67 ± 0.58 a	22.33 ± 4.04 a
MSD	2.22	6.87
Concentration of OMWW = 10		
Buža	2.67 ± 2.52 bcd	7.67 ± 1.53 de
Buža puntoža	2.33 ± 1.53 bcd	6.67 ± 1.15 e
Istarska bjelica	0.00 ± 0.00 d	15.67 ± 1.53 cd
Leccino	4.00 ± 0.00 bc	16.00 ± 0.00 cd
Rosinjola	0.00 ± 0.00 d	0.00 ± 0.00 f
Hydroxytyrosol (0.1)	2.00 ± 1.00 bcd	18.67 ± 2.08 bcd
Hydroxytyrosol (0.5)	2.00 ± 0.00 bcd	21.33 ± 3.06 ab
Vanilic acid (0.1)	4.67 ± 1.15 ab	13.00 ± 3.46 cd
Vanilic acid (0.5)	0.00 ± 0.00 d	0.00 ± 0.00 f
Nativo 75WG	1.33 ± 0.58 cd	5.00 ± 1.73 ef
Control	7.67 ± 0.58 a	22.33 ± 4.04 a
MSD	3.01	6.28

67

Table S14. Inhibition percentage (%) of mycelial growth of *Phaeoacremonium iranianum* under different treatment conditions. 68 69

Treatment	Concentration	Day 2	Day 7
Buža	0.2	73.91	79.85
	0.5	56.52	68.65
	2	60.86	71.64
	6	69.56	70.14
	10	65.21	65.67
Buža puntoža	0.2	52.17	74.62
	0.5	47.82	73.13
	2	52.17	77.61
	6	60.86	76.11
	10	69.56	70.14
	0.2	91.30	-1.49
	0.5	91.30	14.92

Istarska bjelica	2	95.65	35.82
	6	100	35.82
	10	100	29.85
Leccino	0.2	26.08	22.38
	0.5	47.82	29.85
	2	47.82	28.35
	6	47.82	28.35
	10	47.82	28.35
Rosinjola	0.2	100	100
	0.5	100	100
	2	100	100
	6	100	100
	10	100	100
Hydroxytyrosol	0.1	73.91	16.41
	0.5	73.91	4.47
Vanillic acid	0.1	39.13	41.79
	0.5	100	100
Nativo 75WG	0.02	82.60	77.61

Table S15. The results of the one-way ANOVA (mean ± standard deviation, in mm) for the mycelial growth of *Sordaria fimicola* under different treatments. Values within the same row marked with identical letters are not significantly different, based on Tukey's Honest Significant Difference (HSD) test at a significance level of $p < 0.05$. The concentrations of the OMWWs are shown above the values, while the concentrations of hydroxytyrosol and vanillic acid are indicated in parentheses next to their names.

Treatment	Day 2	Day 7
Concentration of OMWW = 0.2		
Buža	74.33 ± 1.53 a	86.00 ± 0.00 a
Buža puntoža	65.67 ± 6.11 b	86.00 ± 0.00 a
Istarska bjelica	74.33 ± 2.89 a	86.00 ± 0.00 a
Leccino	60.33 ± 3.79 b	66.33 ± 4.16 b
Rosinjola	18.67 ± 2.31 d	32.00 ± 6.93 c
Hydroxytyrosol (0.1)	66.00 ± 1.00 b	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	64.67 ± 1.15 b	86.00 ± 0.00 a
Vanilic acid (0.1)	34.33 ± 2.08 c	78.33 ± 6.81 a
Vanilic acid (0.5)	0.00 ± 0.00 e	0.00 ± 0.00 d
Nativo 75WG	0.00 ± 0.00 e	0.00 ± 0.00 d
Control	77.67 ± 0.58 a	86.00 ± 0.00 a
MSD	7.6	9.29
Concentration of OMWW = 0.5		
Buža	72.33 ± 1.53 ab	86.00 ± 0.00 a
Buža puntoža	67.33 ± 3.51 bc	86.00 ± 0.00 a
Istarska bjelica	74.67 ± 2.52 a	86.00 ± 0.00 a
Leccino	55.00 ± 1.73 d	56.33 ± 1.15 c
Rosinjola	13.00 ± 2.65 f	31.67 ± 1.53 d
Hydroxytyrosol (0.1)	66.00 ± 1.00 c	86.00 ± 0.00 a

Hydroxytyrosol (0.5)	64.67 ± 1.15 c	86.00 ± 0.00 a
Vanilic acid (0.1)	34.33 ± 2.08 e	78.33 ± 6.81 b
Vanilic acid (0.5)	0.00 ± 0.00 g	0.00 ± 0.00 e
Nativo 75WG	0.00 ± 0.00 g	0.00 ± 0.00 e
Control	77.67 ± 0.58 a	86.00 ± 0.00 a
MSD	5.43	6.22
Concentration of OMWW = 2		
Buža	74.00 ± 0.00 ab	86.00 ± 0.00 a
Buža puntoža	73.33 ± 2.52 ab	86.00 ± 0.00 a
Istarska bjelica	68.00 ± 8.00 ab	86.00 ± 0.00 a
Leccino	38.33 ± 6.43 c	41.33 ± 8.33 b
Rosinjola	6.67 ± 2.31 d	28.33 ± 4.04 c
Hydroxytyrosol (0.1)	66.00 ± 1.00 b	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	64.67 ± 1.15 b	86.00 ± 0.00 a
Vanilic acid (0.1)	34.33 ± 2.08 c	78.33 ± 6.81 a
Vanilic acid (0.5)	0.00 ± 0.00 d	0.00 ± 0.00 d
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 d
Control	77.67 ± 0.58 a	86.00 ± 0.00 a
MSD	9.79	10.11
Concentration of OMWW = 6		
Buža	78.33 ± 6.66 a	86.00 ± 0.00 a
Buža puntoža	73.33 ± 2.08 ab	86.00 ± 0.00 a
Istarska bjelica	67.00 ± 3.61 bc	86.00 ± 0.00 a
Leccino	27.67 ± 3.51 d	24.33 ± 10.26 b
Rosinjola	5.67 ± 0.58 e	24.00 ± 2.65 b
Hydroxytyrosol (0.1)	66.00 ± 1.00 bc	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	64.67 ± 1.15 c	86.00 ± 0.00 a
Vanilic acid (0.1)	34.33 ± 2.08 d	78.33 ± 6.81 a
Vanilic acid (0.5)	0.00 ± 0.00 e	0.00 ± 0.00 c
Nativo 75WG	0.00 ± 0.00 e	0.00 ± 0.00 c
Control	77.67 ± 0.58 a	86.00 ± 0.00 a
MSD	7.94	11.09
Concentration of OMWW = 10		
Buža	81.33 ± 8.08 a	86.00 ± 0.00 a
Buža puntoža	75.33 ± 1.53 ab	86.00 ± 0.00 a
Istarska bjelica	45.33 ± 14.01 c	86.00 ± 0.00 a
Leccino	9.67 ± 3.51 d	12.00 ± 5.29 d
Rosinjola	2.67 ± 0.58 d	26.00 ± 0.00 c
Hydroxytyrosol (0.1)	66.00 ± 1.00 b	86.00 ± 0.00 a
Hydroxytyrosol (0.5)	64.67 ± 1.15 c	86.00 ± 0.00 a
Vanilic acid (0.1)	34.33 ± 2.08 c	78.33 ± 6.81 b
Vanilic acid (0.5)	0.00 ± 0.00 d	0.00 ± 0.00 e
Nativo 75WG	0.00 ± 0.00 d	0.00 ± 0.00 e
Control	77.67 ± 0.58 ab	86.00 ± 0.00 a
MSD	14.82	7.59

Table S16. Inhibition percentage (%) of mycelial growth of *Sordaria fimicola* under different treatment conditions.

Treatment	Concentration	Day 2	Day 7
Buža	0.2	4.29	0.00
	0.5	6.86	0.00
	2	4.72	0.00
	6	-0.85	0.00
	10	-4.72	0.00
Buža puntoža	0.2	15.45	0.00
	0.5	13.30	0.00
	2	5.57	0.00
	6	5.57	0.00
	10	3.00	0.00
Istarska bjelica	0.2	4.29	0.00
	0.5	3.86	0.00
	2	12.44	0.00
	6	13.73	0.00
	10	41.63	0.00
Leccino	0.2	22.31	22.86
	0.5	29.18	34.49
	2	50.64	51.93
	6	64.37	71.70
	10	87.55	86.04
Rosinjola	0.2	75.96	62.79
	0.5	83.26	60.46
	2	91.41	67.05
	6	92.70	72.09
	10	96.56	69.76
Hydroxytyrosol	0.1	15.02	0.00
	0.5	16.73	0.00
Vanillic acid	0.1	55.79	8.91
	0.5	100	100
Nativo 75WG	0.02	100	100

Table S17. MIC and MFC values of the applied treatments.

Treatment	Species	MIC	MFC
		%	
Buža	*Biscogniauxia mediterranea*	0.2	/
	Biscogniauxia nummularia	0.2	/
	Cytospora pruinosa	0.2	/
	Nigrospora gorlenkoana	/	/
	Nigrospora osmanthi	0.2	/
	Nigrospora philosophiae-doctoris	0.2	/
	Phaeoacremonium iranianum	0.2	/
	Sordaria fimicola	0.2	/

Buža puntoža	*Biscogniauxia mediterranea*	0.2	/
	Biscogniauxia nummularia	2	/
	Cytospora pruinosa	0.2	6
	Nigrospora gorlenkoana	/	/
	Nigrospora osmanthi	0.2	/
	Nigrospora philosophiae-doctoris	0.2	/
	Phaeoacremonium iranianum	0.2	/
	Sordaria fimicola	0.2	/
Istarska bjelica	*Biscogniauxia mediterranea*	0.2	/
	Biscogniauxia nummularia	/	/
	Cytospora pruinosa	0.2	/
	Nigrospora gorlenkoana	/	/
	Nigrospora osmanthi	0.5	/
	Nigrospora philosophiae-doctoris	0.2	/
	Phaeoacremonium iranianum	0.2	/
	Sordaria fimicola	0.2	/
Leccino	*Biscogniauxia mediterranea*	6	/
	Biscogniauxia nummularia	/	/
	Cytospora pruinosa	0.2	/
	Nigrospora gorlenkoana	/	/
	Nigrospora osmanthi	/	/
	Nigrospora philosophiae-doctoris	0.2	/
	Phaeoacremonium iranianum	0.2	/
	Sordaria fimicola	0.2	/
Rosinjola	*Biscogniauxia mediterranea*	0.2	/
	Biscogniauxia nummularia	0.2	2
	Cytospora pruinosa	0.2	/
	Nigrospora gorlenkoana	/	/
	Nigrospora osmanthi	0.2	/
	Nigrospora philosophiae-doctoris	0.2	/
	Phaeoacremonium iranianum	/	0.2
	Sordaria fimicola	0.2	/
Hydroxyty-rosol	*Biscogniauxia mediterranea*	0.1	/
	Biscogniauxia nummularia	0.1	/
	Cytospora pruinosa	0.1	/
	Nigrospora gorlenkoana	0.1	/
	Nigrospora osmanthi	0.1	/
	Nigrospora philosophiae-doctoris	0.1	/
	Phaeoacremonium iranianum	0.1	/
	Sordaria fimicola	0.1	/
Vanillic acid	*Biscogniauxia mediterranea*	0.1	0.5
	Biscogniauxia nummularia	0.1	/
	Cytospora pruinosa	0.1	/
	Nigrospora gorlenkoana	0.1	/
	Nigrospora osmanthi	0.1	/

	Nigrospora philosophiae-doctoris	0.5	/
	Phaeoacremonium iranianum	0.1	/
	Sordaria fimicola	0.1	0.5
Nativo 75WG	*Biscogniauxia mediterranea*	0.02	/
	Biscogniauxia nummularia	/	0.02
	Cytospora pruinosa	/	0.02
	Nigrospora gorlenkoana	/	0.02
	Nigrospora osmanthi	0.02	/
	Nigrospora philosophiae-doctoris	0.02	/
	Phaeoacremonium iranianum	0.02	/
	Sordaria fimicola	/	0.02

Table S18. Growth inhibition category (GIC) scale results. B_RB (from OMWW Buža) and R_RB (from OMWW Rosinjola) are isolates of *Rhodotorula mucilaginosa*, R_BB (from OMWW Rosinjola) is an isolate of *Nakazawaea molendiniolei*, B_BB (from OMWW Buža) is an isolate of *Bacillus velezensis*, and BP_P (from OMWW Buža puntoža), BJ_P (from OMWW Istarska bjelica), and L_P (from OMWW Leccino) are isolates of *Penicillium crustosum*.

	Pathogen							
Treatment	*Biscogniauxia mediterranea*	*Biscogniauxia nummularia*	*Cytospora pruinosa*	*Nigrospora gorlenkoana*	*Nigrospora osmanthi*	*Nigrospora philosophiae -doctoris*	*Phaeoacremonium iranianum*	*Sordaria fimicola*
B_RB	0	3	3	3	3	2	2	3
B_BB	0	4	2	3	3	2	2	3
R_RB	0	3	1	2	2	0	1	0
R_BB	0	4	3	3	3	0	2	3
BJ_P	4	0	4	4	3	3	3	3
L_P	3	3	0	3	3	4	0	3
BP_P	3	4	4	0	2	3	0	3

Naslov izvornog znanstvenog rada broj 8: The Antifungal Efficacy of Olive Mill Wastewater on Phytopathogenic Fungi from the Class Sordariomycetes

Prošireni sažetak:

Provedeno je istraživanje s ciljem ispitivanja antifungalnog učinka otpadnih biljnih voda masline (OBVM) protiv fitopatogenih gljiva iz razreda Sordariomycetes izoliranih iz maslina (*Olea europaea* L.). S obzirom na rastuću potrebu za održivim alternativama kemijskim fungicidima i valorizacijom industrijskog otpada, istraživanje je obuhvatilo procjenu antifungalnog djelovanja OBVM dobivenih od različitih sorata maslina te fenolnih spojeva prisutnih u OBVM.

Testirane su OBVM sorata Buža, Buža puntoža, Istarska bjelica, Rosinjola i Leccino, zajedno s fenolnim spojevima hidroksitirosol i vanilinska kiselina. Evaluacija antifungalne aktivnosti provedena je protiv osam fitopatogenih gljiva: *Biscogniauxia mediterranea* (De Not.) Kuntze, *Biscogniauxia nummularia* (Bull.) Kuntze, *Cytospora pruinosa* Défago, *Nigrospora gorlenkoana* Novobr., *Nigrospora osmanthi* Mei Wang & L. Cai, *Nigrospora philosophiae-doctoris* M. Raza, Qian Chen & L. Cai, *Phaeoacremonium iranianum* L. Mostert, Grafenhan, W. Gams & Crous i *Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not. Paralelno su ispitana i antagonistička svojstva mikroorganizama izoliranih iz OBVM, tj. *Bacillus velezensis* Ruiz-Garcia et al., *Rhodotorula mucilaginosa* (A. Jörg.) F.C. Harrison, *Nakazawaea molendiniolei* (N. Cadez, B. Turchetti & G. Peter) C. P. Kurtzman & C. J. Robnett i *Penicillium crustosum* Thom.

Rezultati su pokazali da je OBVM sorte Rosinjola pokazala najveću antifungalnu učinkovitost, dok je OBVM sorte Leccino bila najmanje učinkovita. Među gljivama, *Nigrospora philosophiae-doctoris* bila je najosjetljivija na sve tretmane, dok je *N. gorlenkoana* pokazivala stimulativni odgovor na OBVM. Fenolni spojevi, posebno vanilinska kiselina, pokazali su antifungalnu aktivnost, pri čemu je potpuna inhibicija rasta zabilježena već pri koncentraciji od 0,5%.

Minimalne inhibicijske koncentracije (MIC) za OBVM varirale su ovisno o vrsti gljive i sorti masline od koje su OBVM potjecale, dok su minimalne fungicidne koncentracije (MFC) postignute samo za određene kombinacije tretmana i patogena.

Ispitivanje antagonističkih mikroorganizama pokazalo je da su izolati *P. crustosum* pokazali najveću inhibiciju rasta patogena, s posebnim naglaskom na izolat iz OBVM sorte Istarska

bjelica. Suprotno tome, bakterije i kvasci poput *R. mucilaginosa* i *N. molendiniolei* pokazali su slabije ili stimulativne učinke na rast nekih patogena.

Ovi nalazi ukazuju na veliki potencijal korištenja OBVM i komponenti kao prirodnih sredstava u zaštiti bilja, što otvara mogućnost razvoja novih, ekološki prihvatljivih proizvoda za suzbijanje bolesti maslina. Daljnja istraživanja su nužna kako bi se optimizirala primjena, utvrdile optimalne koncentracije i istražile mogućnosti sinergijskog djelovanja OBVM s drugim biološkim agensima.

Ključne riječi: antagonizam, bakterija, ekologija, zaštita bilja, kvasci

SAŽETAK

Gljive su iznimno važni organizmi na Zemlji, s ključnim ulogama u ekosustavima i ljudskom životu. Iako se procjenjuje da postoji između 2,2 i 3,8 milijuna vrsta gljiva, znanstveno je opisano tek oko 150 tisuća. Gljive razgrađuju organsku tvar, tvore korisne simbioze s biljkama, proizvode važne kemijske spojeve koji se koriste u farmaceutske svrhe, ali i uzrokuju bolesti kod biljaka, ljudi i životinja. U poljoprivrednoj proizvodnji je čak 70–80% biljnih bolesti prouzročeno gljivama. Precizna identifikacija gljiva ključna je zbog njihove ekološke važnosti i raznolikosti, pri čemu se danas koriste kombinacije morfoloških i molekularnih metoda. Ipak, izazovi kao što su preklapanje morfoloških karakteristika i nedovoljno razvijene baze genetičkih podataka i dalje otežavaju točnu klasifikaciju gljiva. Biljni patogeni, osobito gljive, uzrokuju velike ekonomske gubitke i ugrožavaju zdravlje ljudi kroz proizvodnju mikotoksina. Klimatske promjene i intenzivna poljoprivreda pogoduju širenju fitopatogena, a Mediteran se smatra osobito ranjivim. Maslina, kao važna kultura Mediterana, pogođena je sve većim brojem bolesti koje uzrokuju patogene gljive, među kojima prednjače vrste iz porodice Botryosphaeriaceae. U Hrvatskoj je istraživanje patogenih gljiva na maslini još uvijek u začecima, a većina dosadašnjih istraživanja fokusirala se na nekoliko poznatih patogena. Problem kontrole bolesti dodatno otežava otpornost gljiva na fungicide i štetan učinak kemijskih sredstava na okoliš. Europski zeleni plan i Strategija bioraznolikosti naglašavaju potrebu smanjenja uporabe pesticida te promoviraju razvoj alternativnih metoda, uključujući biološku kontrolu. Ovo istraživanje imalo je nekoliko glavnih ciljeva: identifikaciju gljiva s maslina, ispitivanje njihove patogenosti, procjenu antifungalnog učinka eteričnih ulja (EtU) i otpadnih biljnih voda masline (OBVM) te utvrđivanje osjetljivosti različitih sorata maslina na gljivične bolesti. Hipoteze su uključivale mogućnost pronalaska novih vrsta gljiva, varijacije u njihovoj patogenosti te učinkovitost alternativnih tretmana poput EtU i OBVM u suzbijanju patogena.

Terenskim istraživanjem u Istri i na Kvarneru, Hrvatska, prikupljeni su brojni uzorci zaraženih maslina. Iz njih su izolirane i identificirane različite vrste gljiva, pri čemu su korištene morfološke i molekularne metode. Ukupno je identificirano šest vrsta iz porodice Botryosphaeriaceae i osam vrsta iz razreda Sordariomycetes, od kojih su mnoge prvi put zabilježene na maslini u Hrvatskoj i svijetu. Testovi patogenosti pokazali su da su svi izolati gljiva patogeni za maslinu, dok su testovi otpornosti sorata pokazali da sorte pokazuju različite razine otpornosti na zarazu. Antifungalni testovi s EtU pokazali su da su EtU kineskog cimeta, origana i njihove glavne komponente (e-cinamaldehid i karvakrol) iznimno učinkoviti protiv

svih testiranih gljiva. S druge strane, EtU limuna i paprene metvice, kao i njihove komponente (limonen i mentol), pokazala su slabije djelovanje. EtU su u mnogim slučajevima pokazala veću učinkovitost od komercijalnih fungicida. Analizom OBVM utvrđeno je da se one, nakon određene obrade, mogu koristiti kao održiva alternativa kemijskim sredstvima. OBVM su sadržavale brojne fenolne spojeve poput vanilinske kiseline i hidroksitirozola, koji su pokazali antifungalna svojstva, posebno protiv gljiva poput *Dothiorella iberica* A.J.L. Phillips, J. Luque & A. Alves. Međutim, kod nekih gljiva, poput *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, učinak je bio slab. Tijekom istraživanja provedena je izolacija mikroorganizama iz OBVM, pri čemu su identificirane bakterije, kvasaci i filamentozne gljive. Odabrani izolati testirani su na antagonističku aktivnost protiv fitopatogenih gljiva identificiranih u istraživanju, primjenom metode dvostruke kulture na KDA podlozi. Rezultati su pokazali da su određeni izolati ostvarili značajnu inhibiciju rasta fitopatogena. Posebno se istaknula bakterija roda *Bacillus*. Neki izolati gljiva, izolirani iz OBVM, također su iskazali kompetitivnu prednost u suzbijanju rasta patogena, što ukazuje na mogućnost njihove primjene u biološkoj kontroli. Dobiveni rezultati potvrđuju potencijal mikroorganizama iz OBVM kao prirodnih bioloških sredstava u zaštiti bilja, čime se dodatno pridonosi valorizaciji otpada maslinarske industrije kroz ekološki prihvatljive tehnologije. Zaključno, ovo istraživanje ističe važnost kombinirane primjene morfoloških i molekularnih metoda za identifikaciju gljiva, potrebu za razvojem održivih metoda zaštite bilja i važnost istraživanja autohtonih sorata zbog njihove otpornosti na bolesti. U kontekstu klimatskih promjena i ekoloških izazova, upotreba prirodnih sredstava poput EtU i OBVM predstavlja važnu alternativu konvencionalnim kemijskim tretmanima.

SUMMARY

Fungi are extremely important organisms on Earth, playing key roles in ecosystems and human life. Although it is estimated that there are between 2.2 and 3.8 million fungal species, only about 150,000 have been scientifically described. Fungi decompose organic matter, form beneficial symbioses with plants, and produce important chemical compounds used for pharmaceutical purposes, but they also cause diseases in plants, humans, and animals. In agricultural production, as much as 70–80% of plant diseases are caused by fungi. Accurate identification of fungi is crucial due to their ecological significance and diversity, and today this is achieved through a combination of morphological and molecular methods. However, challenges such as overlapping morphological characteristics and underdeveloped genetic databases still hinder precise fungal classification.

Plant pathogens, especially fungi, cause significant economic losses and threaten human health through the production of mycotoxins. Climate change and intensive agriculture contribute to the spread of phytopathogens, with the Mediterranean considered particularly vulnerable. The olive tree, a key crop of the Mediterranean, is increasingly affected by diseases caused by pathogenic fungi, particularly species from the Botryosphaeriaceae family. In Croatia, research on pathogenic fungi in olives is still in its early stages, with most studies so far focused on a few known pathogens. Disease control is further complicated by fungal resistance to fungicides and the harmful effects of chemical agents on the environment. The European Green Deal and Biodiversity Strategy emphasize the need to reduce pesticide use and promote the development of alternative methods, including biological control. This research had several main objectives: to identify fungi from olive trees, test their pathogenicity, assess the antifungal effects of essential oils (EOs) and olive mill wastewaters (OMWWs), and determine the susceptibility of different olive cultivars to fungal infections. The hypotheses included the possibility of discovering new fungal species, variations in their pathogenicity, and the effectiveness of alternative treatments such as EOs and OMWWs in controlling pathogens. Field research in Istria and the Kvarner region, Croatia, resulted in the collection of numerous samples from infected olive trees. Various fungal species were isolated and identified using morphological and molecular methods. A total of six species from the Botryosphaeriaceae family and eight species from the class Sordariomycetes were identified, many of which were recorded on olive trees in Croatia and even globally for the first time. Pathogenicity tests showed that all fungal isolates were pathogenic to olive, while variety resistance tests demonstrated varying levels of

resistance to infection among different olive varieties. Antifungal tests with EOs revealed that EOs from Chinese cinnamon and oregano, as well as their main components (e-cinnamaldehyde and carvacrol), were extremely effective against all tested fungi. On the other hand, EOs from lemon and peppermint, along with their components (limonene and menthol), showed weaker effects. In many cases, EOs proved more effective than commercial fungicides. OMWWs analysis revealed that, after certain treatment, they could be used as a sustainable alternative to chemical agents. OMWWs contained numerous phenolic compounds such as vanillic acid and hydroxytyrosol, which demonstrated antifungal properties, particularly against fungi like *Dothiorella iberica* A.J.L. Phillips, J. Luque & A. Alves. However, for some fungi, such as *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, the effect was weaker. During the research, microorganisms were isolated from OMWWs, including bacteria, yeasts, and filamentous fungi. Selected isolates were tested for antagonistic activity against phytopathogenic fungi identified in the study, using the dual culture method on PDA medium. Results showed that certain isolates significantly inhibited the growth of phytopathogens, with *Bacillus* species particularly standing out. Some fungal isolates isolated from OMWWs also demonstrated competitive advantages in suppressing pathogen growth, indicating their potential use in biological control. The results confirm the potential of microorganisms from OMWWs as natural biocontrol agents in plant protection, further contributing to the valorization of olive industry waste through environmentally friendly technologies.

In conclusion, this research highlights the importance of combining morphological and molecular methods for fungal identification, the need to develop sustainable plant protection methods, and the significance of studying native varieties due to their disease resistance. In the context of climate change and environmental challenges, the use of natural agents such as EOs and OMWWs represents an important alternative to conventional chemical treatments.

ŽIVOTOPIS

Elena Petrović rođena je 03. svibnja 1993. godine u Rijeci, gdje je završila osnovnu školu te srednju Medicinsku školu u Rijeci. Na Veleučilištu u Požegi je, 2016. godine, završila preddiplomski stručni studij Prehrambena tehnologija. Na Fakultetu agrobiotehničkih znanosti Osijek, Sveučilišta Josipa Jurja Strossmayera u Osijeku, 2016. godine upisuje sveučilišni diplomski studij, smjer Ekološka poljoprivreda. Fakultetsko vijeće joj, zbog odličnog uspjeha tijekom studiranja, omogućuje paralelan upis na drugi diplomski studij te u listopadu 2017. godine upisuje sveučilišni diplomski studij, smjer Biljna proizvodnja. U travnju 2018. godine sudjeluje na Festivalu znanosti. Oba diplomska studija završila je s prosječnom ocjenom 5,0 u rujnu 2019. godine. U Visokoj poslovnoj školi PAR u Rijeci, 2021. godine, završava program „Voditeljica izrade i provedbe projekata financiranih iz EU fondova“. Tijekom diplomskog studija nagrađena je s „Dekanovom nagradom za postignuti uspjeh na diplomskom sveučilišnom studiju za akademsku godinu 2017./2018.“ te je dobila „Pohvalu za uspješnost u studiranju po godinama za akademsku godinu 2017./2018.“. U ožujku 2020. godine je nagrađena poveljom "Najbolji student generacije". Poslijediplomski doktorski studij Poljoprivredne znanosti, smjer Zaštita Bilja, upisuje 2021./2022. akademske godine na Fakultetu agrobiotehničkih znanosti Osijek. Tijekom poslijediplomskog doktorskog studija nagrađena je nagradom za najbolji poster u sekciji Zaštita bilja na Simpoziju agronoma u Dubrovniku, 2024. godine te nagradom Rad po izboru urednika – MPDI Horticulturae.

Od kolovoza 2021. godine zaposlena je na projektu Hrvatske zaklade za znanost DOK-2021-02-2882 kao asistentica, na Institutu za poljoprivredu i turizam u Poreču. Suradnica-doktorandica je na projektu Hrvatske zaklade za znanost UIP-2020-02-7413 (Anti-Mikrobi-OL, AMO). Prisustvovala je na devet usavršavanja. U rujnu i listopadu 2021. i rujnu 2022. godine se usavršavala u Hrvatskoj agenciji za poljoprivredu i hranu, Zagreb, iz područja Laboratorijske dijagnostike i Molekularne biologije i dijagnostike. U lipnju i listopadu 2022. i u studenom 2023. boravila je na Fakultetu agrobiotehničkih znanosti Osijek na usavršavanju iz područja Tehnika ispitivanja antimikrobne aktivnosti eteričnih ulja i komponenti, biljnih voda i komponenti i fungicida te Morfološke determinacije gljiva. U lipnju 2023. se usavršavala na Kemijskom institutu u Ljubljani, Slovenija, iz područja Metoda i tehnika testiranja kemijskog profila složenih bioaktivnih sastojaka. U listopadu 2023. se usavršavala na Victor Babes University of Medicine and Pharmacy u Temišvaru, Rumunjska, iz područja Primjene inovativnih metoda korištenja medicinskih biljaka u farmaciji, poljoprivredi i prehrani. U travnju 2024. se usavršavala na Slovenska Polnohospodarska Univerzita v Nitre, Nitra,

Slovačka iz područja identifikacije bakterija primjenom MALDI-TOF i testiranja antifungalne učinkovitosti preparata. Od veljače do kolovoza 2025. usavršavala se na Universidade de Aveiro, Portugal, iz područja molekularne biologije.

Sudjelovala je na Simpoziju agronoma u Srbiji 2021. godine. u Hrvatskoj 2022., 2023. i 2024. godine te u Bosni i Hercegovini 2024. godine. Prezentirala je prihvaćenu temu svoje doktorske disertacije na Danima doktorata na Fakultetu agrobiotehničkih znanosti Osijek u listopadu 2023. godine, na događaju PhD Café Hrvatske zaklade za znanost u veljači 2024. godine i znanstvenoj konferenciji Mutimir 2024. godine. Prezentirala je rezultate znanstvenog istraživanja na Kongresu NZZJZ Angrija Štampar "Sigurna hrana, danas i sutra" 2024. godine. Sudjelovala je na Festivali znanosti na Fakultetu agrobiotehničkih znanosti Osijek 2024. i 2025. godine. Također, recenzirala je radove za časopise Plant Disease i International Journal of Plant Biology. Održala je radionicu „Molekularna identifikacija gljiva" povodom obilježavanja 150. obljetnice Instituta za poljoprivredu i turizam Poreč. Članica je Hrvatske agronomske komore te je upisana u Popis ovlaštenih agronoma i članica je udruge Penkala.

Kao autor ili koautor do sada je objavila 23 rada, od toga jedanaest znanstvenih radova kategorije A1, četiri znanstvena rada kategorije A2, šest znanstvenih radova kategorije A3, dva stručna rada i devet sažetaka.

CURRICULUM VITAE

Elena Petrović was born on May 3, 1993, in Rijeka, where she completed elementary school and attended the Secondary Medical School. In 2016, she earned her undergraduate professional degree in Food Technology from the Polytechnic in Požega. That same year, she enrolled in the University Master's Program in Ecological Agriculture at the Faculty of Agrobiotechnical Sciences in Osijek, part of Josip Juraj Strossmayer University of Osijek. Due to her outstanding academic performance, the Faculty Council given the opportunity her to concurrently enroll in a second master's program, and in October 2017, she began the Master's Program in Plant Production. In April 2018, she participated in the Science Festival. She completed both master's programs with a perfect GPA of 5.0 in September 2019.

In 2021, she completed the program "Project Manager for the Design and Implementation of Projects Funded by EU Funds" at PAR Business School in Rijeka. During her graduate studies, she received the "Dean's Award for Academic Excellence in the 2017/2018 Academic Year" and a "Commendation for Academic Success per Year for 2017/2018." In March 2020, she was awarded the "Best Student of the Generation" certificate. In the 2021/2022 academic year, she enrolled in the Postgraduate Doctoral Program in Agricultural Sciences, specializing in Plant Protection, at the Faculty of Agrobiotechnical Sciences in Osijek.

During her doctoral studies, she received the award for Best Poster in the Plant Protection section at the Agronomist Symposium in Dubrovnik in 2024, and the "Editor's Choice Paper Award" from MDPI Horticulturae journal.

Since August 2021, she has been employed as an assistant on the project of the Croatian Science Foundation DOK-2021-02-2882 at the Institute of Agriculture and Tourism in Poreč. She is a doctoral researcher on Croatian Science Foundation project UIP-2020-02-7413 (Anti-Mikrobi-OL, AMO). She has participated in nine professional trainings. In September and October 2021, and again in September 2022, she attended training at the Croatian Agency for Agriculture and Food in Zagreb in the fields of Laboratory Diagnostics and Molecular Biology and Diagnostics. In June and October 2022 and November 2023, she underwent training at the Faculty of Agrobiotechnical Sciences in Osijek in techniques for testing the antimicrobial activity of essential oils and components, plant waters and components, fungicides, and morphological identification of fungi. In June 2023, she attended training at the National Institute of Chemistry in Ljubljana, Slovenia, focused on methods and techniques for testing the chemical profile of complex bioactive compounds. In October 2023, she participated in training at Victor Babes University of Medicine and Pharmacy in Timișoara, Romania, on the application of innovative

methods in the use of medicinal plants in pharmacy, agriculture, and nutrition. In April 2024, she attended training at the Slovak University of Agriculture in Nitra, Slovakia, in bacterial identification using MALDI-TOF and antifungal efficacy testing of formulations. From February to August 2025, she participated the training at the University of Aveiro, Portugal, in molecular biology.

She participated in the Agronomist Symposium in Serbia in 2021, in Croatia in 2022, 2023, and 2024, and in Bosnia and Herzegovina in 2024. She presented her accepted doctoral dissertation topic at the Doctoral Days of the Faculty of Agrobiotechnical Sciences in Osijek in October 2023, at the PhD Café event organized by the Croatian Science Foundation in February 2024, and at the scientific conference Mutimir 2024. She also presented research findings at the Congress of the Andrija Štampar Teaching Institute of Public Health, titled "Safe Food, Today and Tomorrow" in 2024. She participated in the Science Festival at the Faculty of Agrobiotechnical Sciences Osijek in 2024 and 2025. Additionally, she reviewed papers for the journals Plant Disease and International Journal of Plant Biology.

She conducted a workshop on "Molecular Identification of Fungi" to mark the 150th anniversary of the Institute of Agriculture and Tourism in Poreč. She is a member of the Croatian Chamber of Agronomists, listed as an authorized agronomist, and a member of the Penkala Association.

As author or co-author, she has published 23 papers to date: 11 A1 scientific papers, four A2 scientific papers, six A3 scientific papers, two professional papers, and nine abstracts.