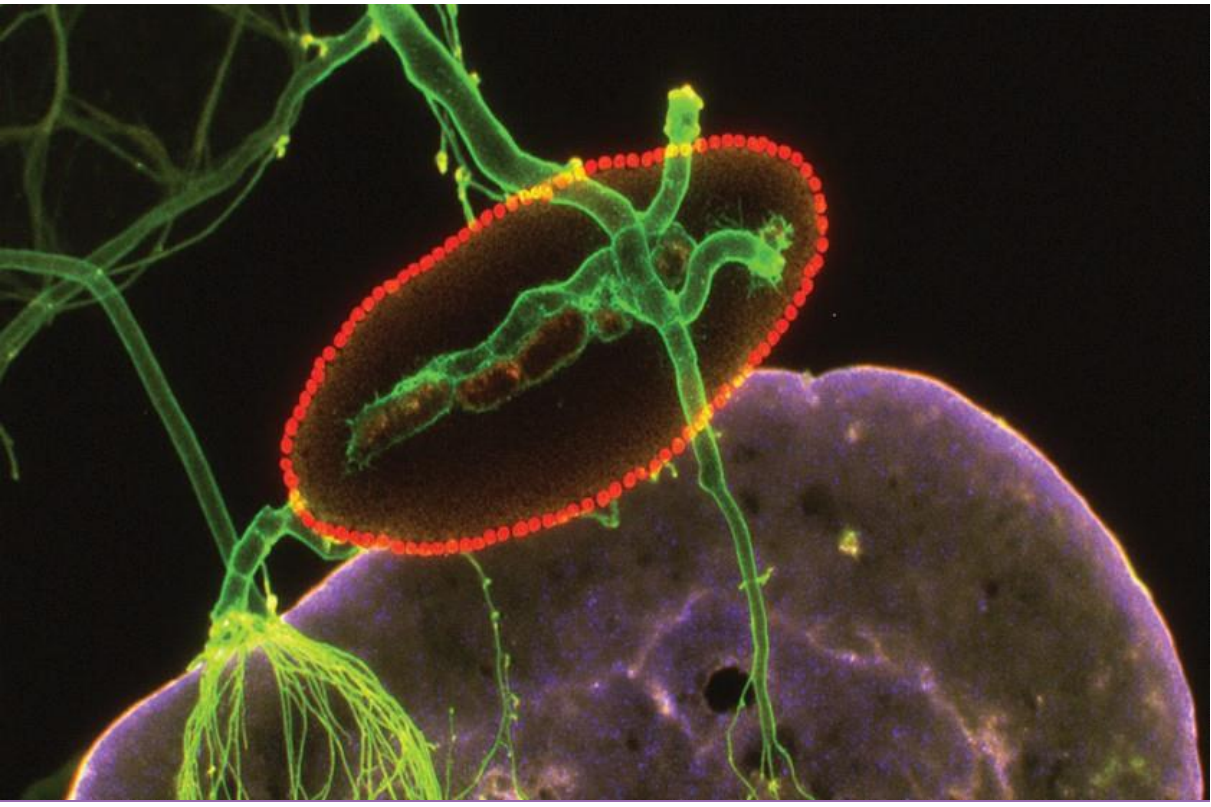

FUNGAL BIOCONTROL AGENTS IN THE MANAGEMENT OF PLANT DISEASES

Editors

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PREFACE

The world is facing significant challenges to crop protection in agricultural production under increasing population pressure and climate change. In this context, biological protection methods offer substantial potential for sustainable agriculture and ecosystem management. In addition, it will be possible to eliminate the concerns and threats regarding the effects of chemical control, which has become a prominent issue in the world, on human and environmental health. Although biological control of pests seems to be at the forefront, scientists' close observation of nature and their intensive studies based on the knowledge they have gained through observation have shown that it is possible to control plant diseases with biological methods. Studies on fungi and oomycetes, which constitute an important group of microorganisms used in biological control, are progressing rapidly. This book aims to pave the way for further research in this challenging but promising field and contribute to further exploring the potential of fungal biocontrol agents in plant protection. We thank all the researchers, authors, and contributors who contributed to its preparation. We hope it will contribute to the body of knowledge in the field and inspire readers to further consider fungal biological control agents.

Editors

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CHAPTER 1

HARNESSING *CHAETOMIUM* FUNGI FOR SUSTAINABLE BIOLOGICAL CONTROL OF CROP DISEASES

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INTRODUCTION

Endophytic fungi constitute a distinctive group of microorganisms that inhabit the internal tissues and intercellular spaces of plants without producing visible disease symptoms (Arnold & Lutzoni, 2007). Although their evolutionary adaptation and colonization strategies within plant hosts remain under investigation, numerous studies have emphasized their important contribution to plant growth, development, and resistance under both biotic and abiotic stress conditions (Jha et al., 2023). Among these endophytes, several genera including *Trichoderma*, *Chaetomium*, *Piriformospora*, *Curvularia*, *Fusarium*, *Epicoccum*, and *Penicillium* have been documented to enhance plant health by improving resistance to diverse environmental challenges (Rjani et al., 2021).

Within this diverse community, the genus *Chaetomium* occupies a key position. It is taxonomically classified under the division Ascomycotina, class Pyrenomycetes, order Sordariales, and family Chaetomiaceae. Members of this genus are darkly pigmented (dematiaceous) fungi commonly found in decaying organic matter soil, plant debris and air as well as in marine habitats such as coral reefs and seaweeds (Abdel-Azeem, 2019; Waill et al., 2021). These fungi are frequently isolated from cellulose-based materials like wood, paper, straw, and drywall, and are generally recognized as soft rot fungi due to their ability to decompose both hardwood and softwood (Waill et al., 2021). Although most *Chaetomium* species exhibit a saprophytic or endophytic lifestyle, some can act as opportunistic human pathogens, with certain species demonstrating thermo-tolerant or neurotropic properties (Abdel-Azeem et al., 2020).

To date, over 400 species of *Chaetomium* have been identified. Their colonies typically develop rapidly on potato dextrose agar (PDA), initially appearing white and cottony, later turning gray to olive green as they mature. Microscopically, they form asci, and ascospores septate hyphae, perithecia. The perithecia are usually large, spherical, dark and or flask shaped, covered with characteristic setae. Each perithecium contains 4–8 asci, which open through ostioles to release cylindrical asci, each enclosing unicellular, lemon shaped, olive-brown ascospores (Wang et al., 2016; Waill et al., 2021). Frequently encountered species include *C. atrobrunneum*, *C. funicola*, *C. globosum*, and *C. strumarium*, with *C. globosum* designated as the type species of the genus (Wang et al., 2016).

More than 200 secondary metabolites have been reported from *Chaetomium* species, including anthraquinones, chaetoglobosins, steroids, tetramic acids terpenoids, xanthones, diketopiperazines, azaphilones, pyranones, and orsellides. Many of these compounds possess antimicrobial, cytotoxic, anticancer, antimalarial, and antiviral properties (Dwibedi et al., 2023). Several *Chaetomium* species are well known for their strong antagonistic potential against phytopathogens, particularly those responsible for soil and seed-borne diseases (Aggarwal et al., 2004; Soytong et al., 2021). Derived from these fungi, biofungicides such as Ketomium®, which has been successfully commercialized in Thailand, Laos, Vietnam, Cambodia, and China have demonstrated broad spectrum efficacy in plant disease management.

The biocontrol efficiency of *Chaetomium* is largely linked to its ability to produce extracellular enzymes, including formate dehydrogenase, cellulases, L-methioninase, laccases, polysaccharide monooxygenases (PMOs), β-1,3-glucanases, dextranases, pectinases, lipases, amylases, proteases, and chitinases (Soytong et al., 2021; Seethapathy et al., 2023; Dwibedi et al., 2023). These enzymes underline the genus's biotechnological importance, suggesting potential use in biorefinery, organic waste degradation, and crop protection. Nevertheless, further investigation into their structure, catalytic mechanisms, and substrate specificity is essential to maximize their industrial application potential.

Ecologically, *Chaetomium* species act as saprophytic decomposers, thriving in organic matter–rich environments. Their strong cellulolytic and ligninolytic activities enable them to decompose complex biopolymers such as cellulose and lignin, contributing significantly to organic matter recycling and soil nutrient balance (Darwish & Abdel-Azeem, 2020).

Numerous investigations have confirmed that *Chaetomium* species are capable of suppressing plant pathogens through several biological mechanisms, such as antibiosis (Kumar & Kaushik, 2013), competition for nutrients and colonization sites (Madbouly et al., 2020), mycoparasitic interactions (Gao et al., 2005), and activation of host defense signaling pathways (Elshahawy & Khattab, 2022). Microscopic examinations have shown that *Chaetomium* spp. can cause visible alterations in pathogen structures, including conidial deformation, cell wall breakdown, hyphal lysis, and formation of pores, which collectively result in restricted spore germination and inhibited mycelial growth.

These morphological disruptions interfere with pathogen development and reproduction, positioning *Chaetomium* as an effective biocontrol genus in sustainable plant disease management (Mandal et al., 1999; Biswas et al., 2000).

In recent years, attention has shifted toward ecofriendly strategies for disease management, particularly biopesticides and nanopesticides. In this regard, biodegradable nanoparticles derived from *Chaetomium*-based metabolites have been identified as innovative agents that strengthen plant defense mechanisms and enhance resistance to a variety of pathogens (Tann & Soytong, 2016; Song et al., 2020a, b).

1. USE OF *CHAETOMIUM* SPECIES IN THE CONTROL OF FIELD CROPS DISEASES

Research exploring the involvement of *Chaetomium* species in the control of field crops diseases dates back to the early decades of the twentieth century, with most early efforts focusing on pathogens of rice and maize. Initial experiments carried out in Thailand revealed that *Chaetomium cupreum* and *C. globosum* exhibited pronounced antagonistic effects against key crop pathogens, notably those responsible for *Curvularia lunata* (corn leaf spot), *Pyricularia oryzae* (rice blast), and *Rhizoctonia oryzae* (sheath blight) (Soytong et al., 1989). Subsequent investigations by Kanokmedhakul et al. (2002) demonstrated that *C. globosum* strain KMITL-N0802 was capable of synthesizing a diverse range of bioactive molecules such as chetomanone, ergosterol, ergosteryl chrysophanol, isoketoglobosin D, alternariol monomethyl ether, chaetoglobosin C echinoaline, and palmitate, highlighting the genus's potential for anti- biotic and antifungal activity. Likewise, *C. cupreum* displayed inhibitory effects on various seed-borne rice pathogens, including *Fusarium moniliforme, Curvularia lunata*, and *Pyricularia oryzae*, *Drechslera oryzae* (Soytong et al., 1992a, b, c).

Park et al. (2005) reported the purification of chaetoviridin A, an anti-fungal metabolite isolated from *C. globosum* strain F0142 originating from *Echinochloa crus-galli*. This compound demonstrated remarkable disease control efficiency exceeding 80% against *Pyricularia oryzae* (rice blast) and *Puccinia recondita* (wheat brown rust). Endophytic *C. globosum* isolates obtained from wheat roots were also shown to produce gliotoxin, a secondary metabolite exhibiting strong inhibitory activity toward *Fusarium graminearum* (wheat head and root blight) (Li et al., 2011).

Furthermore, Biswas et al. (2012) analyzed ethyl acetate extracts of *C. globosum* and identified several compounds chaetomin, BHT, mollicelin G, and chaetoglobosin via GC–MS and NMR characterization. Bioassay evaluations revealed that both chaetomin and chaetoglobosin substantially restricted the mycelial growth of *Macrophomina phaseolina, Pythium ultimum, Rhizoctonia solani* and *Bipolaris sorokiniana*. At a concentration of 1000 ppm, chaetoglobosin reduced *F. graminearum* colony diameter from 33 mm to 1.6 mm, while the same dose of chaetomin decreased *B. sorokiniana* growth from 69 mm to 13 mm, confirming their antifungal potential (Aggarwal et al., 2011; Aggarwal, 2015; Ahammed et al., 2012).

Over 200 biologically active compounds have now been identified from *Chaetomium* spp., exhibiting cytotoxic, enzymatic inhibitory, and antimicrobial effects (Yang et al., 2011a, b). Among them, *C. globosum* stands out as a powerful biocontrol agent, producing metabolites such as orsellides, globosumones A–C (Bashyal et al., 2005), ketoviridins A and C (Qin et al., 2009), cytoglobosins A–G (Cui et al., 2010), ketoglobosins (Zhang et al., 2010), and pyrones and ketoglosins A–B (Ge et al., 2011). In further experiments, Park et al. (2005) purified two antifungal compounds chaetoviridin A and B that effectively inhibited rice blast (*Magnaporthe grisea*) and wheat rust (*Puccinia recondita*). Application of 62.5 µg/mL chaetoviridin A reduced disease severity by over 80%. Similarly, *C. globosum* (strain Chg-1) decreased maize late wilt caused by *Cephalosporium maydis* by 70–90%, enhancing yield both in greenhouse and field trials, through direct antagonism and defense induction (Elsha- Hawy & Khattab, 2022). Moreover, *C. globosum* CEF-082 alleviated *Verticillium dahliae* infection in cotton by activating MAPK and phenylpropanoid defense pathways, while *chaetoviridin A* played a key biochemical role in this protection (Zhang et al., 2020).

Leaf inhabiting *Chaetomium* strains also contribute significantly to pathogen control. Isolates from healthy wheat leaves reduced pustule formation by *Puccinia recondita f. sp. tritici* in *in vivo* and *in vitro* trials (Dingle & McGee, 2003). Similarly, Istifadah et al. (2006) observed that various *Chaetomium* isolates suppressed *Pyrenophora tritici-repentis* in wheat leaves. Mitra et al. (2013) demonstrated that metabolites from *C. globosum* combined with *Trichoderma viride, Aspergillus niger*, and *Alternaria alternata* altered host defense responses and minimized foliar disease symptoms.

Moreover, *C. aureum* effectively controlled *Magnaporthe grisea* and *Rhizoctonia solani* in greenhouse experiments. In another study, *C. globosum* (strain no. 05) completely prevented northern leaf blight symptoms caused by *Setosphaeria turcica* on maize leaves; the main active agents were ketoglobosin A and C, which also showed antibacterial and phytotoxic properties (Jiao et al., 2004; Zhang et al., 2013).

Subsequent studies have further verified that *C. cochliodes, C. globosum* and *C. cupreum* exhibit strong antagonistic effects against multiple phytopathogens, including *Phomopsis* spp., *Rhizoctonia oryzae, Pyricularia oryzae* and *Curvularia lunata* (Manandhar et al., 1986; Soytong, 2014). Among these, *Chaetomium* isolates designated as C2 and C5 originally recovered from barley seedlings displayed inhibition levels of approximately 36–40% against *Drechslera teres* and 30–31% against *Bipolaris sorokiniana*. Microscopic observations revealed evident morphological alterations such as spore deformation and plasmolysis, providing visual confirmation of antagonistic activity (Moya et al., 2016). These outcomes support mycoparasitism, competition for resources, and antibiosis as the principal modes of pathogen suppression. Additionally, the hydrolytic enzyme systems produced by *Chaetomium* metabolites have been shown to decompose the cell walls of cereal pathogens including *Rhizoctonia solani*, *Septoria tritici*, and *Bipolaris sorokiniana* (Liu et al., 2008; Yue et al., 2018). Likewise, *C. globosum* isolates effectively limited root rot in sugar beet caused by *Pythium ultimum* (Di Pietro et al., 1991), while *C. cupreum* derived metabolites demonstrated suppression of *Pythium aphanidermatum* infections in sugarcane (Soytong et al., 2005). More recently, Linkies & Jacob (2021) observed that *C. globosum* was particularly potent against *Pyrenophora graminea, Bipolaris sorokiniana, Botrytis cinerea* whereas *C. aureum* and *C. cochliodes* displayed comparatively stronger inhibitory activity toward *F. culmorum* and *P. infestans*. The most promising isolates have been formulated into commercial biofungicides, particularly Ketomium®, which demonstrated high efficacy against *Sclerotium rolfsii* the agent of corn root rot under greenhouse, field and laboratory conditions (Soytong et al., 2001). Likewise, *C. cochliodes* exhibited strong antagonism against *Drechslera oryzae*, the cause of rice brown leaf spot, with crude metabolite, powder, and liquid formulations successfully inhibiting pathogen development (Soytong, 2014).

Seed treatment with *Chaetomium* spores has proven particularly effective. *C. cochliodes* and *C. globosum* suppressed *Helminthosporium*, *Fusarium*, and other seed-borne pathogens. Coating maize seeds with *C. globosum* spores prevented *F. roseum* f. sp. *cerealis graminearum* and reduced soilborne infections (Kommedahl & Mew, 1975). Treated wheat seeds showed enhanced germination, decreased *F. pseudograminearum* root rot, and increased yield (Feng et al., 2023). Similarly, alfalfa seeds coated with *C. globosum* conidia or mycelia were protected against *Pythium spinosum*, preventing pre-emergence damping-off (Maghazy et al., 2008). Comparable results were observed against *Bipolaris sorokiniana, Macrophomina phaseolina*, *Rhizoctonia solani* and *Pythium ultimum* (Biswas et al., 2012).

Recent advances have focused on nanotechnology-based formulations. Copolymer nanoparticles loaded with *Chaetomium* -derived metabolites, such as nano CGM, CGE, and CGH synthesized from *C. globosum* KMITL N0805, exhibited potent inhibition against *Curvularia lunata* the rice leaf spot pathogen in Cambodia reducing spore production by over 90% with ED_{50} values of 1.2–1.9 μg/mL (Soytong et al., 2011). Similarly, *Chaetomium* -based nanoparticles inhibited *Pyricularia* spp. (Song & Soytong, 2018), while nano-formulated extracts of *C. cupreum* reduced *Curvularia lunata* sporulation and field disease severity by 40–58% (Tann & Soytong, 2017).

Ongoing studies emphasize the need to optimize nanoparticle-based delivery systems using *Chaetomium* metabolites to boost plant immunity and achieve long term, sustainable protection against cereal crop pathogens.

2. USE OF *CHAETOMIUM* SPECIES IN THE CONTROL OF VEGETABLE DISEASES

Chaetomium species rank among the most widespread endophytic fungi inhabiting the rhizosphere of numerous vegetable crops. Early investigations on their biological control capabilities revealed that *C. globosum* and *C. cupreum* could effectively suppress *Pseudomonas solanacearum* and *F. oxysporum* f. sp. *lycopersici* on tomato (Soytong et al., 1992b; Soytong & Soytong, 1997). During the same period, studies conducted in Japan demonstrated that *Chaetomium* isolates inhibited the mycelial growth of *Sclerotinia sclerotiorum*, implying the production of antifungal metabolites, which were later identified and characterized (Nakashima et al., 1991).

Likewise, Pereira & Dhingra (1997) and Kay & Stewart (1994) reported that *C. globosum* significantly reduced infections caused by *Diaporthe phaseolorum* f. sp. *meridionalis*, *P. ultimum Sclerotium cepivorum*, and the same species was also applied successfully in controlling *Phytophthora palmivora* (black pepper root rot) and *F. oxysporum* f. sp. *lycopersici* (tomato bottom rot) (Zhang et al., 2010).

During the following decade, researchers identified a novel cytotoxic compound, chaetomugilin, from *C. globosum* isolated from *Ginkgo biloba* (Qin et al., 2009). Subsequently, Zhao et al. (2017) assessed the antifungal efficacy of *C. globosum* strain CDW7 (endophytic in *G. biloba*) against *S. sclerotiorum*, the pathogen responsible for rape seedling rot. The fermentation broth exhibited an inhibition level similar to the chemical fungicide carbendazim (59.8% at 250 μg/mL). Among the detected metabolites, ketoglobosin A and ketoglobosin D displayed strong inhibitory activities with IC_{50} values of 0.35 and 0.62 μg/mL, respectively, approaching that of carbendazim (0.17 μg/mL). Similarly, extracts of *C. globosum* EF18 derived from *Withania somnifera* showed remarkable control over *S. sclerotiorum*, the causative agent of vegetable white rot (Kumar et al., 2013).

Antifungal compounds produced by *C. lucknowense* CLT01*C* and *C. elatum* ChE01, two isolates obtained from Thai soils, exhibited pronounced inhibitory activity against *F. oxy.* f. sp. *lycopersici*, the pathogen responsible for tomato wilt. The purified metabolite chaetoglobosin-C demonstrated an ED_{50} value of 5.98 μg/mL. Tomato seedlings treated with this compound remained symptom-free, and microscopic examination confirmed morphological alterations and collapse of pathogen conidia, resulting in complete loss of pathogenicity. Correspondingly, Biswas et al. (2012) identified *C. globosum* metabolites including chetomin, BHT, mollicelin G, and chaetoglobosin via GC–MS and NMR analysis, all of which exhibited substantial inhibitory effects against *Macrophomina phaseolina Bipolaris sorokiniana*, *Pythium ultimum* and *R. solani*.

Fierro-Cruz et al. (2017) isolated 355 endophytic fungi from *Trattinnickia rhoifolia* and *Protium heptaphyllum* and screened them for antifungal activity against *F. oxysporum*. Among them, ethyl acetate extracts of *Meyerozima* sp. F281 and *C. globosum* F211 UMNG UMNG showed exceptional inhibition.

Chemical profiling revealed active compounds such as cladosporin, ketoatrosin A, and ketoviridin A (identified through RP-HPLC-DAD-ESI-MS). Gophna et al. (2003) proposed that such biosynthetic abilities may be linked to horizontal gene transfer between plants and endophytic fungi, explaining the occurrence of plant-like metabolites. Furthermore, several *Chaetomium*-derived molecules were reported to degrade the cell walls of significant vegetable pathogens including *Phytophthora sojae, S. sclerotiorum*, *F. oxysporum*, *Valsa sordida* and *R. solani* (Liu et al., 2008).

Endophytic *C. globosum* isolated from *Houttuynia cordata* (a leafy vegetable consumed widely in East Asia) exhibited potent *in vitro* antifungal activity against *B. cinerea*. The highest inhibition was achieved when the cultures were grown in potato dextrose broth (pH 7.5) for 4–8 days prior to extraction (Pan et al., 2016). Although *Chaetomium* species are prolific producers of bioactive enzymes and metabolites (Dwibedi et al., 2023), relatively limited research has focused on their nanotechnological utilization for vegetable disease management (Servin et al., 2015).

Joselito et al. (2014) synthesized copolymer nanoparticles encapsulating metabolites from *C. globosum* and *C. cupreum*. Likewise, *C. globosum* isolated from healthy bean plants was utilized for enzyme-mediated synthesis of zinc nanoparticles, yielding xylanase, pectinase, and chitinase (Sherien et al., 2017). These nanoparticles produced inhibition zones of 17.0 mm against *Sclerotium rolfsii*, 15.0 mm against *R. solani*, and 13.0 mm against *F. solani.* Additionally, chaetoviridin A (125 μg/mL), purified from *C. globosum* F0142, reduced tomato late blight by 50%, with suppression increasing to 87% when the concentration was doubled (Park et al., 2005). The zinc nanoparticles demonstrated optimal antifungal efficiency at pH 8.0, with microscopy revealing extensive hyphal and conidial deformation.

Overall, these studies indicate that *Chaetomium* species represent highly promising biocontrol agents for vegetable crops and serve as essential microbial resources for nanoparticle-based disease control. The metabolites from these fungi also act as biostimulants, strengthening plant health and defense systems (Park et al., 2005; Phong et al., 2016). In Türkiye, Turhan et al. (1995) reported that *C. jodhpurense* reduced *Verticillium* wilt in eggplant by approximately 75–80% under pot culture conditions.

Field experiments with the commercial biofungicide Ketomium® in Chiang Mai, Thailand where *Phytophthora infestans* caused severe potato blight showed a 38% decline in disease incidence compared with untreated controls, chieving efficacy comparable to chemical fungicides (Soytong & Ratanacherdchai, 2005). Follow-up investigations by Shanthiyaa et al. (2013) revealed that *C. globosum* Cg-6 strongly inhibited *P. infestans*, exhibiting elevated exo- and endo-glucanase activities. The researchers highlighted that *Chaetomium* strains producing high levels of glucanase and cellulase typically display enhanced antagonism against *Phytophthora* spp., whose cell walls consist mainly of glucans and cellulose. Formulated as a liquid bioagent, *C. globosum* Cg-6 achieved nearly complete disease suppression through foliar, tuber and soil applications, either alone or with *P. infestans*, providing up to 100% protection and significantly increasing tuber yield. These observations align with the findings of Soytong & Ratanacherdchai (2005) and Ahammed et al. (2008).

Furthermore, *C. globosum* W7 metabolites containing ketoglobosin A as the key bioactive compound proved highly effective against *F. sporotrichioides*, the causal organism of potato dry rot. The compound showed MIC values of 9.45–10.50 μg/mL and an IC_{50} of 4.34 μg/mL, ensuring substantial protection under laboratory and field conditions (Jiang et al., 2017). Since most *P. infestans* management programs depend on single bioagents, Shanthiyaa et al. (2013) suggested that combining multiple *Chaetomium* strains with complementary activities could enhance pathogen suppression and ensure broader, more stable crop protection throughout growth and harvest stages.

3. USE OF *CHAETOMIUM* SPECIES IN THE CONTROL OF FRUIT AND FOREST TREES DISEASES

In vitro and *in vivo* research has revealed that several *Chaetomium* strains exhibit varying degrees of antifungal activity against numerous phytopathogens, including species of *Fusarium*, *Alternaria*, *Botrytis*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Phomopsis*, and *Colletotrichum*, which are known to cause diseases in both fruit and forest trees (Soytong et al., 2001; Soytong et al., 2021). *Chaetomium* spp. has been effectively employed in managing citrus and black pepper root rot, although its antagonistic efficacy can be influenced by factors such as soil temperature, moisture content, and pH levels during application (Tomilova & Shternshis, 2006).

Among these, *Chaetomium globosum* is one of the most prevalent species, acting as a saprophyte in the rhizosphere and phyllosphere of trees. This species has successfully controlled several fruit diseases under *in vivo* conditions, including tangerine root rot caused by *Phytophthora parasitica*, strawberry root rot caused by *P. cactorum*, and black pepper root rot due to *P. palmivora* (Zhang et al., 2010).

C. globosum is known to synthesize diverse antifungal metabolites. Cullen & Andrews (1984) demonstrated that a foliar spray of *C. globosum* (strain NRRL 6296), applied under controlled growth conditions, effectively managed *Venturia inaequalis* (apple scab). When this strain was applied alone or combined with cellulose formulations, scanning electron microscopy (SEM) revealed that cellulose amendments enhanced the growth and persistence of *C. globosum* on apple leaves. This improvement was attributed to the nutritional support from cellulose, which fostered fungal colonization and improved biocontrol efficacy. The compound responsible for inhibiting *V. inaequalis* was identified as the metabolite chetomin.

In a study from Thailand, *C. cupreum* (str. CC3003), *C. lucknowense* (str. CL01) and *C. globosum* (str. CG05) exhibited potent inhibition against *Phytophthora palmivora*, the pathogen responsible for pomelo (*Citrus maxima*) root rot. These *Chaetomium* species lysed the pathogen's mycelia and reduced sporangia formation by 92–99%. Additionally, methanol extract of *C. globosum* (str. CG05) significantly restricted mycelial growth and sporangia development with effective doses of 26.5 μg/mL and 2.3 μg/mL, respectively. Hung et al. (2015a) also reported that *C. lucknowense*, *C. cupreum* and *C. globosum* inhibited *Phytophthora nicotianae* isolated from infected pomelo roots by 50–56% *in vitro* and parasitized its hyphae, leading to mycelial fragmentation after 30 days of incubation. Greenhouse trials showed that the application of conidia and methanol extracts of these species reduced root rot severity by 66–71% and enhanced seedling biomass by 72–85% compared to untreated controls. Follow-up studies by Hung et al. (2015b) confirmed that these species also controlled *P. palmivora* in citrus plants.

Crude extracts of *C. cupreum* (str. CC3003) prepared using methanol, ethyl acetate, and hexane displayed strong inhibition against *Colletotrichum gloeosporioides*, the causal agent of anthracnose on coffee, with ED50 values of 28, 13 and 11ppm, respectively.

Bioactive compounds from this isolate deformed pathogen conidia, causing abnormal morphologies. Applications of bioformulated *C. cupreum* (str. CC3003), nano-trichotoxin, and nano-rothiorinol reduced anthracnose severity by 54.77%, 46.23%, 42.71% and 18.59%, respectively (Vilavong & Soytong, 2017). Moreover, *Chaetomium*-based formulations have shown promising potential in controlling *Thielaviopsis* bud rot in *Hyophorbe lagenicaulis* (bottle palm) under field conditions (Soytong et al., 2005).

Powder and pellet formulations of Ketomium® a commercial *Chaetomium*-based biofungicide have been registered as broad-spectrum biological agents capable of decomposing organic matter, stimulating plant immunity, and promoting growth (Soytong et al., 2001). The Thai-developed Ketomium® was highly effective against raspberry thorn blight (*Didymella applanata*) and was also found to suppress potato root rot and black wart caused by *Rhizoctonia solani*, ultimately improving yield (Shternshis et al., 2005). Formulations of *C. globosum* and *C. cupreum* successfully controlled root rot caused by *Phytophthora palmivora* in durian trees, significantly lowering pathogen levels in the soil of *Monthong* variety durians (Prechaprome et al., 1997). Similarly, *Chaetomium*-based biofungicides effectively reduced *Phytophthora parasitica* infection in citrus fields one of Thailand's most serious disease challenges (Soytong et al., 2001).

In Cambodia, a biofungicide containing *Chaetomium* successfully controlled citrus root rot, marking the first identification of *Pythium ultimum* as a citrus pathogen in the country (Soytong et al., 2021). Prior to intervention, the disease had devastated more than 90% of citrus trees. Field trials comparing chemical fungicides, *Chaetomium*, and *Trichoderma* showed remarkable recovery in treated trees within 3–4 months, characterized by new leaf and root growth. Combined applications of *Chaetomium* and *Trichoderma* with metalaxyl fungicide achieved superior control (Kean et al., 2010).

A biofungicide containing *C. bostrychodes* BN08 and *C. cupreum* RY202 was also tested against white root disease (*Rigidoporus microporus*) in *Hevea brasiliensis* (rubber trees). These strains inhibited pathogen growth by over 50% *in vitro*. Crude extracts of *C. cupreum* RY202 displayed the highest antifungal activity, with ED50 values of 1220, 402, 170 and µg/L, while rothiorinol one of its bioactive compounds exhibited an ED50 value of 26 µg/L (Kaewchai & Soytong, 2010). Powder and oil formulations of *C. cupreum* RY202 further inhibited *R. microporus* growth by 75% (Kaewchai et al., 2010).

Applications of *Chaetomium* spp. and their metabolites significantly reduced pomelo root rot by 66–71% and improved plant biomass by 72–85% compared to controls (Khan et al., 2019). As an endophytic biological agent, *C. aureum* HP047 markedly reduced pitch canker in *Pinus radiata* seedlings caused by *Fusarium circinatum* (Martínez-Álvarez et al., 2016). In subsequent experiments, over 150 endophytic isolates were screened against *F. circinatum*; six with the highest antagonistic potential were tested on various pine species (*P. radiata*, *P. sylvestris*, *P. pinaster*, *P. nigra*, and *P. pinea*) under field conditions. Both *C. aureum* and *Alternaria* sp. suppressed disease progression in *P. radiata*, validating their potential as biocontrol agents for pitch canker.

Thiep & Soytong (2015) identified three effective *Chaetomium* spp. *C. cochliodes*, *C. bostrychodes*, and *C. gracile* with strong antagonistic effects against *Fusarium roseum* (coffee and tea wilt) and *Colletotrichum gloeosporioides* (coffee anthracnose). Dual culture assays revealed that all three isolates inhibited pathogen colony expansion and spore formation. Solvent extracts of *C. cochlioides* (hexane, ethyl acetate, and methanol) reduced spore production by 60.87–78.16% for *F. roseum* and 67.63–76.50% for *C. gloeosporioides*.

In another investigation, *C. cupreum* CC3003, *C. globosum* CG05, and *C. lucknowense* CL01 exhibited moderate antagonistic effects against *F. oxysporum* (tea wilt and root rot), suppressing mycelial growth by 31.69–34.03%. After 30 days, conidial production decreased by 67.25–75.92%. The methanol extract of *C. cupreum* CC3003 inhibited conidial germination with an ED50 of 85.30 μg/mL, while ethyl acetate and hexane extracts of *C. lucknowense* (str. CL01) and *C. globosum* (str. CG05) showed ED50 values of 62.17 and 49.32 μg/mL, respectively (Phong et al., 2016).

A *Chaetomium elatum* ChE01-based biofungicide was also tested against banana anthracnose caused by *Colletotrichum musae*, inhibiting fungal growth by over 60% and reducing spore formation by 57%. Metabolites from *C. elatum* (str. ChE01) including, crude hexane, ethyl acetate, and methanol extracts demonstrated ED50 values of 19, 7 and 5, μg/mL, respectively (Soutong et al., 2019). The strain was found to produce a novel chaetoglobosin V, two new compounds (prochaetoglobosin IIIed and prochaetoglobosin III), and six known chaetoglobosins (B–D, F, G, and isochaetoglobosin D), indicating an antibiosis-based mechanism.

4. CONCLUSION AND RECOMMENDATIONS

Cumulative evidence from prior research unequivocally demonstrates that *Chaetomium* species utilize diverse biological mechanisms contributing to their efficiency as biocontrol agents. Their antagonistic strength stems from both direct mechanisms, such as antibiosis and enzymatic lysis, and indirect pathways, including the activation of plant defense systems. These multiple strategies make *Chaetomium* spp. highly adaptable and effective under various environmental and agricultural contexts. Unlike chemical fungicides that target specific molecular sites and frequently lead to pathogen resistance, *Chaetomium*-derived metabolites act through multiple pathways, reducing the chance of resistance development. Moreover, the endophytic behavior of many *Chae- tomium* strains enables them to colonize plant roots and persist through the entire life cycle, ensuring systemic and long-lasting protection. Several studies have also emphasized the synergistic benefits when *Chaetomium* is integrated with other beneficial microorganisms like *Trichoderma* and *Bacillus* species. These combinations not only improve nutrient uptake and root architecture but also enhance plant resilience to stress, boosting overall productivity and crop stability. However, despite this great potential, the practical implementation of *Chaetomium*-based biocontrol still faces limitations. Environmental factors such as temperature, pH, and moisture can significantly influence colonization and metabolite production. Therefore, formulating optimized delivery systems such as encapsulated granules, nanoemulsions, or liquid inoculants is essential to guarantee stability, shelf life, and consistent field performance under different climatic conditions. Future research should prioritize molecular level studies to better understand the genetic and biochemical regulation of secondary metabolite biosynthesis in *Chaetomium*. Integrated omics approaches (genomics, transcriptomics, metabolomics) could identify elite strains with superior biocontrol potential and environmental resilience, paving the way for the commercial expansion of *Chaetomium*-based biofungicides and their incorporation into sustainable, eco-friendly plant disease management systems.

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CHAPTER 2

CHITINOLYTIC MICROORGANISMS: ECO-FRIENDLY AGENTS FOR THE BIOLOGICAL CONTROL OF PLANT DISEASES

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INTRODUCTION

Plant pathogens including fungi, bacteria, and nematodes represent major biotic stressors responsible for considerable economic damage in global crop production. Although chemical pesticides remain a common method for managing harmful organisms, their intensive use leads to several negative consequences, such as environmental contamination, reduced soil productivity, polluted water resources, and chemical residues in food products (Fravel, 2005).

In contrast, biological control offers a more specific and environmentally sustainable strategy by utilizing beneficial microorganisms and natural antagonists. This method lowers reliance on chemical inputs, maintains ecological stability, and supports the principles of sustainable agriculture (Whipps and Lumsden, 2001).

Chitin, a linear polymer composed of N-acetylglucosamine (GlcNAc) monomers, is a nitrogen-containing and structurally rigid polysaccharide that occurs abundantly in insects, crustaceans (e.g., shrimp, crab, and lobster), and the cell walls of many phytopathogenic fungi. Although plants themselves lack chitin, its abundance in fungal pathogens makes it a suitable biological control target.

Chitinolytic microorganisms, including certain bacteria and fungi, are capable of synthesizing chitin-degrading enzymes known as chitinases. Notable examples include *Bacillus spp.*, *Streptomyces spp.*, *Pseudomonas spp.*, *Trichoderma spp.*, and *Serratia marcescens*. These enzymes are essential in the decomposition of chitin and the suppression of pathogenic organisms in plant–microbe interactions.

As biocontrol agents, such as *Trichoderma* and *Bacillus* species, they can activate plant immune pathways including systemic acquired resistance (SAR) and the production of pathogenesis-related proteins (PRs). They are widely employed in seed coating, foliar spraying, and soil inoculation practices. In this context, chitinolytic microorganisms constitute pivotal elements of biological disease management, as their chitinases enzymatically break down fungal cell walls, limiting pathogen proliferation.

During soil, seed, and foliar treatments, chitin-degrading bacteria and fungi promote plant vigor not only by directly inhibiting pathogens but also by triggering induced systemic resistance (ISR), which reduces the dependence on

synthetic agrochemicals (Harman et al., 2004; Kloepper et al., 2004). Consequently, their application forms the basis of sustainable and environmentally compatible crop production, diminishing pesticide reliance (Gooday, 1990; Brzezińska et al., 2014). Thus, chitinolytic microorganisms serve as vital components of biocontrol, functioning through dual modes direct pathogen inhibition and activation of host defenses while contributing to ecological balance and lowering chemical usage in agriculture.

1. CHITIN AND CHITINASE ENZYMES

Chitin was first recognized nearly two centuries ago as a structural constituent of insect exoskeletons (Khoushab and Yamabhai, 2010). It represents the second most widespread polysaccharide in nature, surpassed only by cellulose, and is built from N-acetylglucosamine (GlcNAc) units connected by β-(1→4) glycosidic linkages. This compound is found within fungal cell walls, arthropod outer shells, crustaceans, and several algal taxa. It has additionally been identified in nematode eggshells, pharyngeal membranes, and even in marine as well as freshwater sponges (Rinaudo, 2006; Ehrlich et al., 2013). As a biological polymer, chitin provides mechanical rigidity and structural protection, safeguarding organisms from environmental pressures and microbial invasion.Because of these characteristics, chitin has found widespread applications in medicine, agriculture, food processing, and biotechnology, particularly in biological control systems and bioactive compound production (Hamid, 2013; Rathore, 2015).

Chitinases are enzymatic proteins that catalyze the hydrolysis of chitin, converting it into N-acetylglucosamine monomers or short-chain oligomers. Owing to this biochemical capability, they are regarded as valuable agents for eco-friendly management of plant pathogens and insect pests. These enzymes are naturally produced by a wide range of living organisms, including bacteria (*Bacillus spp., Streptomyces spp.*), fungi (*Trichoderma spp.*), plants, and even certain animals (Hamid et al., 2013; Suginta et al., 2000).

Depending on how they interact with their substrate, chitinases are generally divided into two functional categories: Endochitinases, which randomly cleave internal β-1,4 linkages within the chitin polymer, and Exochitinases, such as chitobiosidases and β-1,4-N-acetylglucosaminidases,

which act on the terminal regions of the chain, releasing diacetylchitobiose or single GlcNAc residues (Sahai and Manocha, 1993).

Based on amino acid sequence similarity and structural motifs, chitinases are grouped into glycoside hydrolase (GH) families GH18, GH19, and GH20. The GH18 family, found in bacteria, fungi, and mammals, includes both endo- and exo-chitinases and typically features a TIM-barrel (α/β-barrel) structure. GH19 chitinases, primarily present in plants and occasionally in fungi, act as endochitinases with a β-sheet conformation and function as defense-related enzymes during biotic stress; PR-3 proteins in plants represent this class. Members of the GH20 family, common in bacteria and animals, display β-N-acetylglucosaminidase activity, performing the terminal step of chitin degradation by releasing GlcNAc monomers.

These enzymes contribute not only to biological control through the lysis of fungal cell walls but also to the natural recycling of organic matter and ecosystem nutrient cycles. Therefore, they hold significant importance in biopesticide development, biofermentation, enzyme biotechnology, and biomedical research. Chitinase-producing microorganisms serve as eco-friendly biocontrol agents in agriculture, and among them, Trichoderma, Bacillus, and Streptomyces species are regarded as prominent candidates for sustainable plant disease management (Herrera-Estrella, 1999; Akanksha et al., 2025).

2. BACTERIAL CHITINASES

Bacterial chitinases are enzymes that hydrolyze chitin, a widespread polymer found in fungal cell walls, insect exoskeletons, and crustacean shells. These enzymes degrade chitin into smaller units *N*-acetylglucosamine (GlcNAc) oligomers or monomers and mainly belong to glycoside hydrolase families GH18 and GH19, with some bacteria also producing GH20-type enzymes. The most common chitinase-producing bacteria include *Serratia marcescens*, *Bacillus spp.*, *Streptomyces spp.*, *Chitinibacter spp.*, and *Aeromonas spp.* (Patil et al., 2000). Among these, *Serratia marcescens* is the most extensively studied species with high chitinase activity, particularly of the GH18 type (Table 1).

Bacillus spp. are widespread soil bacteria exhibiting both endo- and exo-chitinase activities, while *Streptomyces spp.* are notable for their strong chitin-

degrading capacity and potential as biocontrol agents. Aquatic and soil bacteria such as *Chitinibacter* and *Aeromonas* also exhibit chitinolytic activity. Most bacterial chitinases are active at neutral or slightly alkaline pH, with optimal activity typically between 25–37 °C (Unuofin, 2024). These enzymes target plant pathogenic fungi in biological control, contribute to the degradation of crustacean and fungal waste, and serve as raw materials for *N*-acetylglucosamine and chitooligosaccharide production. Additionally, they help reduce biofilm formation by certain pathogenic bacteria (Rinaudo, 2006).

Table 1. Chitinase producing bacteria and their target pathogens

Chitinase-Producing Bacterium	**Chitinase Type**	**Target Pathogen(s)**	**Main Findings**	**Reference**
Pseudomonas fluorescens CHA0	Chitinase + β-1,3-glucanase	*Fusarium oxysporum*, *Rhizoctonia solani*	Controlled seedling damping-off; increased seedling vigor	Downing et al., 2000
Paenibacillus chitinolyticus	Extracellular chitinase	*Sclerotium rolfsii*	Strong antifungal effect against soil-borne fungal pathogen	Chang et al., 2003
Lysobacter enzymogenes C3	Lytic chitinase	*Fusarium graminearum*, *Bipolaris sorokiniana*	Conferred resistance against root pathogens in cereals	Palumbo et al., 2005
Bacillus thuringiensis	Chitinase (ChiA74)	*Rhizoctonia solani*	Inhibited mycelial growth and sclerotia formation	Barboza-Corona et al., 2008
Burkholderia cepacia	Chitinase gene cluster	*Sclerotinia sclerotiorum*, *Botrytis cinerea*	Provided rhizosphere protection; 70% biocontrol efficiency	Shanmugaiah et al., 2010
Serratia marcescens JPP1	Multiple chitinases (ChiA/B/C)	*Rhizoctonia*, *Fusarium* spp.	Strong *in vitro* degradation and greenhouse antagonism; potential BCA	Wang et al., 2013
Streptomyces halstedii AJ-9	Chitinase complex (ChiA, ChiB)	*Rhizoctonia solani*, *Fusarium moniliforme*	Fungal cell wall degradation; inhibited	Nagpure et al., 2014

			mycelial growth	
Chitinolytic microorganisms (general)	–	Multiple fungal pathogens	Discovery and application potential of chitinase-producing bacteria discussed	Brzezińska et al., 2014
–	Roles of bacterial chitinases	General	Review of bacterial chitinases in plant protection, insect control, and biotechnological use	Veliz et al., 2017
Bacillus sp. 30VD-1	Chitinase + volatiles + antifungal metabolites	*Fusarium* spp.	Multiple antagonistic mechanisms; hyphal deformations observed	Khan et al., 2018
Chitinolytic *Bacillus* isolates	Chitinase + other antifungal products	Wheat rhizosphere pathogens	Identified rhizospheric *Bacillus* isolates with chitinase activity; potential BCAs	Brzezińska et al., 2020
Bacillus subtilis FJ3	Chitinase production; PGP activities	Soil-borne fungi	Promoted plant growth and suppressed fungal pathogens; application potential	Jan et al., 2023
Serratia marcescens (B8) etc.	Chitinase production	*Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*	Some isolates showed >30 mm inhibition zones; toxicity tested	Pereira et al., 2023
Bacillus spp.	Chitinase + lipopeptides + ISR	Various	Discussed multiple biocontrol mechanisms and commercialization examples of *Bacillus*	Zhang et al., 2023

Bacillus cereus C1	Chitinase + protease	*Fusarium oxysporum, Colletotrichum gloeosporioides*	Reduced root rot in tomato and cotton	Singh et al., 2010
–	Chitinolytic microorganisms	General	Updated summary of new research and trends; chitinase engineering approaches	Das et al., 2024
Bacillus chitinase-secreting strains	Chitinase	Soil-borne fungal pathogens	*B. subtilis* chitinases reported to reduce disease incidence by 20–35%	Haq et al., 2024

3. FUNGAL CHITINASES

Fungal chitinases are enzymes synthesized by fungi that hydrolyze and degrade the polysaccharide chitin. Chitin is a fundamental structural component of fungal cell walls and is also found in the exoskeletons of insects and crustaceans. Therefore, fungal chitinases play important roles in both cell wall remodeling and biological control. These enzymes are typically classified within the glycosyl hydrolase (GH) families, predominantly belonging to the GH18 family, and are directly involved in cell wall degradation processes. Fungal chitinases are further categorized as endochitinases and exochitinases: endochitinases randomly cleave internal β-(1→4) linkages within the chitin chain, while exochitinases release *N*-acetylglucosamine (GlcNAc) monomers or dimers from the terminal ends of the polymer. Some fungal chitinases function in cell wall remodeling, facilitating fungal growth, sporulation, and hyphal elongation by reshaping the wall during development. Others act as biocontrol chitinases, attacking the chitin-containing cell walls of pathogenic fungi or the exoskeletons of insects this is especially evident in *Trichoderma* species (Thakur et al., 2023). In addition, certain fungi utilize chitinous substrates as a source of energy and carbon (Gooday, 1990). Chitinolytic fungi such as *Trichoderma harzianum* and *Beauveria bassiana* are widely used for the biological control of plant pathogens and insect pests (Table 2). In biotechnology, fungal chitinases are valuable for chitin and chitosan

production, biodegradable material synthesis, and antimicrobial agent development (Singh et al., 2020). In waste management, they enable the conversion of crustacean shell waste into high-value bioproducts through enzymatic chitin hydrolysis. Common fungal chitinase producers include: *Trichoderma harzianum* extensively used in biological control and agricultural applications. *Aspergillus niger* exploited industrially for large-scale chitinase production. *Beauveria bassiana* applied as a biocontrol agent against insect pests. *Penicillium* species involved in chitin hydrolysis and antibiotic production (Gooday, 1990; Patil et al., 2021; Singh et al., 2020).

Table 2. Chitinase producing fungi and their target pathogens

Chitinase-Producing Fungus	**Target Pathogen(s)**	**Main Findings**	**Reference**
Trichoderma spp., *Aspergillus* spp.	*Fusarium, Rhizoctonia, Alternaria*	Chitinases degraded fungal cell walls and inhibited pathogen growth; biocontrol potential demonstrated through meta-analysis.	Das, 2024
Trichoderma harzianum, T. viride	*Fusarium oxysporum, Botrytis cinerea*	Trichoderma-derived chitinases inhibited spore germination; identified as key enzymes in biocontrol mechanisms.	Yao et al., 2023
Trichoderma spp.	*Rhizoctonia solani, Sclerotium rolfsii*	High chitinase activity in *Trichoderma* strains correlated with antifungal effects.	Chung et al. 2022
Trichoderma longibrachiatum	*Fusarium solani, Pythium ultimum*	Partially purified enzyme was heat-stable; reduced root rot by 70% under greenhouse conditions.	Anwar, et al., 2023
Trichoderma asperellum	*Botrytis cinerea, Alternaria alternata*	Purified chitinase showed optimal activity at pH 5.5 and 40°C; inhibited pathogen growth by 80%.	Ornela, 2024
Trichoderma asperellum PQ34	*Colletotrichum gloeosporioides*	Extracellular chitinase exhibited chitosan-like inhibitory effect; high enzyme stability observed.	Loc et al., 2019
Aspergillus niger	*Fusarium oxysporum, Rhizoctonia solani*	Antifungal activity of *A. niger* chitinase identified; optimal pH	Brzezińska, 2012

		and temperature parameters established.	
Trichoderma spp.	Multiple fungal pathogens	Synergistic effects demonstrated between chitinase and glucanase enzyme production.	Guzmán-Guzmán, 2023
Penicillium chrysogenum, *Trichoderma harzianum*	*Fusarium oxysporum*	Microbial chitinases highlighted as effective and eco-friendly biocontrol agents.	Haq, 2024
Trichoderma atroviride, *Beauveria bassiana*	*Rhizoctonia solani*, *Phytophthora capsici*	Metagenomic analysis revealed diversity of chitinase genes; enhanced biocontrol potential.	Gomez-Lama Cabanás, 2025
Trichoderma asperellum PQ34	*Colletotrichum gloeosporioides*	Extracellular chitinase exhibited chitosan-like inhibitory effect; high enzyme stability observed.	Loc et al., 2019
Trichoderma and *Aspergillus*	Multiple fungal pathogens	Transfer of chitinase genes to plants shown to confer resistance; discussed from a genetic engineering perspective.	Unuofin, 2024
Trichoderma spp.	*Dematophora necatrix*, *Sclerotium rolfsii* (soil-borne pathogens)	Field application of *Trichoderma*-derived chitinase on apple seedlings significantly suppressed white root rot and seedling diseases; optimal pH = 5, optimum temperature ≈ 30 °C.	Walia et al., 2025
Trichoderma spp.	—	Purification, optimization, and characterization of chitinase enzyme from *Trichoderma* isolates were reported.	Sharma et al., 2025
Trichoderma spp.	Various fungal pathogens	Mycoparasitic mechanisms involving chitinase and glucanase enzymes were examined.	Singh et al., 2024
Trichoderma spp.	Soil-borne pathogens	Discussed the role of *Trichoderma* in managing soil pathogens through its biocontrol capacity and enzyme contribution (e.g., chitinase).	Nassary et al., 2025

4. PLANT CHITINASES

Plant chitinases are enzymes found in plants that can hydrolyze chitin. Although plants do not produce chitin themselves, these enzymes are typically expressed as part of their defense mechanisms, degrading the chitin present in the cell walls of fungal pathogens and thereby neutralizing them. Thus, plant chitinases are considered an essential component of the plant's natural biological defense system against pathogenic fungi. Most plant chitinases belong to the glycoside hydrolase families GH18 and GH19, which are capable of degrading fungal cell wall chitin, thereby inhibiting fungal growth. When a plant is exposed to pathogen attack, the expression of chitinase genes increases significantly. These enzymes can also be used in biological control applications or in transgenic plants to enhance resistance to fungal diseases (Hilooğlu, 2017). A study by Shobade et al. (2024) examined the structural characteristics of root-associated plant chitinases and their roles in defense against pathogens. Their research demonstrated that these enzymes inhibit fungal growth by degrading chitin and contribute to nutrient cycling in the soil ecosystem. In a review by Grover et al. (2012), the genetic diversity and physiological roles of plant chitinases in pathogen defense were discussed, emphasizing that these enzymes constitute a crucial part of the plant's biological defense mechanisms. Similarly, a study conducted by Silva et al. (2025) explored the potential of chitinases in enhancing resistance to fungal pathogens in coffee plants, revealing their significant role in plant–fungus interactions (Table 3). Furthermore, Li et al. (2024) analyzed 26 different FtCHI genes (chitinase-related genes), evaluating their expression patterns and biochemical characteristics under salt stress conditions. The findings indicated that these genes play an important role in plant responses to abiotic stress. A study by Bulut et al. (2023) also showed that the application of chitosan to tomato plants mitigated the adverse effects of salt stress and improved the plants' physiological stress responses, suggesting a close relationship between chitinase activity, stress adaptation, and plant health.

Table 3. Chitinase producing plants and their target pathogens

Plant Species	Chitinase Source / Type	Target Pathogen	Main Findings	Reference
Tobacco (*Nicotiana tabacum*)	PR-3 type chitinase	*Rhizoctonia solani*, *Alternaria alternata*	Reduced fungal growth and fewer leaf lesions	Broglie et al., 1991
Rice (*Oryza sativa*)	*OsCHIT1* gene	*Magnaporthe oryzae* (rice blast pathogen)	Fungal development inhibited at infection site	Nishizawa et al., 1999
Arabidopsis (*Arabidopsis thaliana*)	*AtChiC* gene family	*Botrytis cinerea*	Decreased infection severity; antifungal defense activated	Hu et al., 2015
Wheat (*Triticum aestivum*)	*TaChi* gene	*Fusarium graminearum*	Growth of *Fusarium* suppressed; disease severity reduced	Kumar et al., 2012
Tomato (*Solanum lycopersicum*)	*Chit42* type chitinase	*Verticillium dahliae*, *Alternaria solani*	Reduction in leaf spot and wilt symptoms	Dana et al., 2006

5. ARCHAEA CHITINASES

Archaeal chitinases are enzymes produced by members of the domain Archaea that are capable of degrading chitin. These enzymes differ in several biochemical and structural aspects from bacterial or fungal chitinases. Archaea are microorganisms that thrive in extreme environments, including high temperatures, elevated salinity, and acidic or alkaline conditions. Archaeal chitinases typically belong to the glycosyl hydrolase (GH) families, particularly exhibiting activity similar to GH18 and GH19 chitinases. They hydrolyze chitin into oligosaccharides or monomeric glucosamine units, which can be utilized as energy sources. These enzymes remain active at high temperatures, tolerate elevated salt concentrations, and possess a broad pH stability range. Because of these characteristics, archaeal chitinases are promising biocatalysts for industrial processes conducted under harsh conditions. They can be applied in the bioconversion of chitin-rich wastes such as crustacean shells and agricultural residues (e.g., straw) (Chen et al., 2019). Current research mainly focuses on their role in chitin waste bioconversion and enzyme production

rather than their direct application against fungal pathogens. In biotechnology, archaeal chitinases are used in the production of bioplastics, biofuels, and in food and agricultural industries for chitin degradation. Their remarkable resistance to high temperature, acidic pH, and salinity allows their use in environmentally friendly industrial processes. The biological degradation of organic wastes like crustacean shells and straw through these enzymes contributes to eco-friendly bioplastic production. Moreover, archaeal chitinases hold potential for use in biological control of plant pathogens and food processing technologies (Chen et al., 2019; Dukariya and Kumar, 2025).

6. INSECT CHITINASES

Chitin is an essential structural polysaccharide present in the exoskeleton of insects. Insect-derived chitinases are enzymes produced either by the insects themselves or by entomopathogenic microorganisms associated with them. These enzymes belong mainly to the GH18 and GH19 glycosyl hydrolase families, representing the most common types of insect chitinases. Insect chitinases exhibit variable stability under different pH and temperature conditions and display distinct substrate specificities, targeting chitin linkages with high precision. Some insect chitinases also possess proteolytic activity, enhancing their efficiency in degrading chitin-containing structures. These enzymes can be used directly against plant pathogens, as many phytopathogenic fungi have chitin in their cell walls (Table 4). Furthermore, insect or microbial chitinase genes can be transferred into plants through genetic engineering to enhance resistance. For example, transgenic lines of tobacco, rice, and tomato expressing insect chitinase genes have demonstrated increased resistance to fungal pathogens. Microorganisms producing insect chitinases (such as entomopathogenic bacteria and fungi) can be applied to plants via spore inoculation or foliar spraying, effectively suppressing pathogens. Purified insect chitinases may also be applied directly to plant tissues, particularly for seedling protection or in sensitive plant tissues prone to fungal infection. These enzymes thus represent valuable tools for integrated pest and disease management. The widespread use of insect chitinases as biological control agents is currently being investigated. The development of more stable and specific chitinases through CRISPR and genetic engineering

technologies is being planned. These enzymes represent promising alternatives for reducing chemical pesticide use in sustainable agricultural practices.

Table 4. Chitinase producing insects and their target pathogens

Chitinase Source	Target Pathogen	Main Findings	Reference
Tenebrio molitor	*Botrytis cinerea*	Cell wall degradation and inhibition of fungal growth	Wang et al., 2018
Galleria mellonella	*Fusarium oxysporum*	Enhanced resistance observed in transgenic plants	Chen et al., 2020
Insect entomopathogenic fungal chitinase	*Rhizoctonia solani*	Foliar application reduced fungal development	Li et al., 2021

7. MAMMALIAN CHITINASES

Chitinases found in humans and other mammals primarily belong to the GH18 glycosyl hydrolase family and are closely associated with the immune system. The most well-characterized mammalian chitinases are chitotriosidase (CHIT1) and acidic mammalian chitinase (AMCase). These enzymes play important roles in antimicrobial defense, immune regulation, and degradation of chitin-containing structures such as fungal cell walls or parasitic exoskeletons (Table 5). Although mammalian chitinases are not directly applied to plants, they hold potential for biotechnological and transgenic applications. For example, transferring CHIT1 or AMCase genes into plants such as tobacco or Arabidopsis has been proposed to enhance resistance to fungal pathogens. Purified mammalian chitinases can also be experimentally applied to plant tissues, providing biological protection, especially in seedlings and sensitive plant organs. Furthermore, when combined with plant- or microbe-derived chitinases, mammalian chitinases may produce synergistic effects, strengthening the plant's overall defense response against pathogenic fungi.

Table 5. Chitinase producing mammals and their target pathogens

Chitinase Source	Target Pathogen	Main Findings	Reference
Human CHIT1	*Fusarium oxysporum*	Inhibited fungal growth in transgenic plant systems	Reese et al., 2007
AMCase (Mouse)	*Botrytis cinerea*	Purified enzyme provided pathogen suppression in foliar application	Zhu et al., 2015
Human CHIT1	*Rhizoctonia solani*	Increased resistance observed in transgenic *Arabidopsis* plants	Hartl et al., 2019
Mammalian GH18 chitinases	Various fungal pathogens	Demonstrated potential for biotechnological applications	van Aalten et al., 2021

8. THE POTENTIAL OF CHITINOLYTIC ENZYMES AS BIO-PESTICIDES

Chitolytic enzymes have high potential in sustainable biological control with the transfer of purified enzymes, enzyme genes to plants, microorganism applications and various combination strategies (Table 6).

Table 6. Application Methods of Chitolytic Enzymes as Bio-Pesticide

Method	Description
Transgenic plants	Chitinolytic enzyme genes are introduced into plants, enhancing resistance against fungal and insect pathogens.
Microorganism application	Chitinase-producing bacteria or fungi are applied to soil or foliage as sprays to control pathogens biologically.
Purified enzymes	Direct application of purified enzymes to leaf or root tissues provides biological control against pathogens.
Combination strategies	Plant- and microbe-derived chitinases are combined to achieve a synergistic antifungal and pest-suppressive effect.

Their eco-friendly characteristics, low risk of resistance development, and specificity toward target organisms such as fungi, insects, and nematodes have made them a central focus in biological control research (Abdel-Rahman and El-Din, 2022). Moreover, pathogens develop resistance to these biological agents much more slowly than to chemical pesticides. The target organisms of these enzymes include fungal pathogens, insect pests, and nematodes. The mechanisms of action involve the degradation of chitin and glucan structures in

cell walls, which leads to weakening of the cell wall, inhibition of pathogen growth, and activation of plant defense mechanisms, such as pathogenesis-related (PR) proteins and antifungal metabolites. These multifunctional properties make chitinolytic enzymes promising candidates for the next generation of bio-based pest management systems, aligning with the goals of eco-friendly and sustainable crop protection (Dahiya et al., 2006; Gomaa and El-Morsi, 2018). Some licensed biopesticides, their ingredients and application methods are given in Table 7.

Table 7. Application Methods of Chitolytic Enzymes as Bio-Pesticide

Product Name	Manufacturer	Active Ingredient	Target Pathogens/Pests	Registration Status / Country	Application Areas
Tidal Grow®	Tidal Vision Products	Chitosan (chitin derivative)	Nematodes, fungal pathogens	USA (EPA approved)	Agricultural crops, greenhouse plants
Ketomium®	Bioglobal	*Chaetomium cupreum* fungus	*Rhizoctonia solani*, *Fusarium oxysporum*	China, Russia, Vietnam, Thailand	Fruit trees, vegetables
Chitinase 48 SC	Doğal Kimya	Chitinase enzyme	Insects (e.g., cotton leafworm)	Türkiye (Registered by the Ministry of Agriculture and Forestry)	Apple, pear, cotton, and other crops

9. CONCLUSION

Considering the environmental and health risks associated with chemical pesticides, chitinolytic microorganisms are emerging as sustainable and ecologically safe alternatives for plant protection. The future prospects for microbial and plant-derived chitinase enzymes are highly promising and multifaceted, encompassing both fundamental scientific research and practical applications in agriculture and biotechnology. Chitinases are increasingly used as biological control agents and present viable alternatives to chemical fungicides. In the future, the use of chitinase-based biopesticides is expected to

expand within organic and sustainable farming systems. Due to their target-specific action, these enzymes enable the development of environmentally friendly and safer crop protection products. Through the transfer of chitinase genes into plants or their optimization in microbial production systems, it will be possible to obtain more stable and efficient enzymes. Moreover, CRISPR and other genome-editing technologies are anticipated to enhance the specificity and potency of chitinases against various pathogens. Metagenomic and microbial genome analyses will facilitate the discovery of new microorganisms with superior chitinase production capacity. Using genetic engineering, the chitinase output of existing strains can be increased, and their efficacy against multiple pathogens can be optimized. Chitinases are effective not only against fungal pathogens but also against bacteria and insects, making them excellent candidates for broad-spectrum biological control products. Future research should focus on developing multi-functional bioformulations that provide combined effects against different pests and pathogens. Chitinases can be used alone or in synergistic combinations with other biocontrol agents to enhance their overall effectiveness. Additionally, further emphasis should be placed on developing practical chitinase-based formulations for soil applications, seed coatings, and foliar sprays, contributing significantly to eco-friendly and sustainable agricultural practices.

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CHAPTER 3

DETERMINATION OF THE EFFECT OF SOME *Trichoderma* Pers ON PLANT GROWTH PARAMETERS IN PISTACHIO

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INTRODUCTION

The scientific name of the pistachio is *Pistacia vera* L., derived from Latin. This name has Greek origins and ultimately stems from the Persian root "pistak." The pistachio is native to Asia Minor (modern-day Turkey), Lebanon, Syria, Iran, the Caucasus region, Afghanistan, and Turkmenistan. Wild or semi-wild varieties of pistachios have been cultivated for centuries in Turkey, India, Afghanistan, Syria, Iran, and other countries in the Near East and North Africa (Eskalen et al., 2001). The primary global producers of pistachios include the United States, Iran, and Turkey, with additional significant contributions from Syria, India, Greece, and Pakistan.

Currently, numerous biological control agents have been approved for commercialization and are available for application in agricultural production systems. Among these agents, species of *Trichoderma* occupy a prominent position due to their efficacy and adaptability. *Trichoderma* spp. are soil- borne, filamentous fungi that exhibit facultative anaerobic metabolism and possess a cosmopolitan distribution. They are commonly found in agricultural soils as well as on organic substrates such as decaying wood (Abbas et al. 2017). It exhibits strong antagonistic activity against numerous phytopathogenic fungi. These species are widely distributed across the globe and occur in both agricultural and natural ecosystems (Papavizas, 1985). The agricultural application of *Trichoderma* provides several benefits that vary according to the strain used. *Trichoderma* rapidly colonizes the plant rhizosphere, effectively competing with other microorganisms and establishing a favorable microbial balance. It also suppresses plant diseases through various antagonistic mechanisms and promotes both plant and root development (Harman et al. 2004). *Trichoderma* species are reported to exhibit strong antagonistic activity against plant diseases caused by soil-borne pathogens. This antagonism operates through multiple mechanisms, including mycoparasitism, the production of antifungal metabolites, and competition for space and nutrients (Kredics et al. 2003). *Trichoderma* species not only promote plant growth but also activate plant defense responses (Abbas et al., 2017). Druzhinina et al. (2011) reported that the genus *Trichoderma* (teleomorph: *Hypocrea*) supports plant growth and plays an important role in the biological control of *Rhizoctonia solani*. Moreover, it induces plant defense mechanisms and synthesizes a range of enzymes and antibiotic

compounds. Through its indirect interactions with the host plant, *Trichoderma* contributes to enhanced growth and development, increased resistance to pathogens, and improved yield performance (Sani et al., 2020).

The present study aimed to evaluate the effects of different *Trichoderma* species on specific growth parameters of pistachio (*Pistacia vera* L.) sapling.

MATERIAL AND METHOD

The *Trichoderma* isolates used in the study included *T. harzianum* TUZ16 and *T. viride* VG18, which were obtained from the isolate collection of the Plant Pathology Laboratory at the Faculty of Agriculture, Siirt University. Additionally, *Trichoderma* sp. FT1 and *T. virens* İB1 were isolated from the root zone (rhizosphere) of pistachio trees.

The setup procedures of the pot experiment were carried out as detailed below.

Preparation and sterilization procedures for potting soil

Potting soil was prepared by mixing equal proportions of garden soil, sand, and peat (1:1:1, v/v/v). The mixture was sterilized in autoclavable bags at 121 °C for 15 minutes. After sterilization, the soil was aerated, thoroughly mixed, and then filled into pots.

Preparation of Trichoderma spore suspension

Trichoderma isolates were cultured on potato dextrose agar (PDA) plates and incubated for one week. After incubation, sterile distilled water was added to the plates, and the surface of the colonies was gently scraped with a sterile spatula. The resulting suspension was filtered through a double layer of sterile cheesecloth to separate the spores from the mycelial fragments and culture medium. The filtrate was diluted with sterile water, and the spore concentration was adjusted to 1×10^7 spores mL^{-1} using a haemocytometer under a light microscope.

To improve adhesion of the suspension to plant surfaces, 0.05% (w/v) carboxymethyl cellulose (CMC) and three drops of Tween 20 per litre were added. The suspensions were then homogenized on a shaker for 30 minutes to ensure uniform distribution of the spores.

The design and evaluation of the experiment

The Siirt pistachio (*Pistacia vera* L.) cultivar, commonly grown in the Siirt region, was used as plant material to assess the effects of antagonistic fungi on specific plant growth parameters. Pistachio seeds harvested in the same year were stored in a dark environment at +4 °C for 3–4 months. Before sowing, the seeds were soaked in water for 12 hours and then dried on blotting paper to promote uniform germination. Surface sterilization was carried out by immersing the seeds in 2% NaOCl solution for 5 minutes, followed by two rinses with sterile distilled water. Subsequently, the seeds were immersed in the prepared *Trichoderma* spore suspensions for approximately 60 minutes. Treated seeds were sown at a rate of three seeds per 5-L pot containing sterilized soil that had been prepared one day prior to sowing. The experiment was conducted using four *Trichoderma* treatments and one negative control (sterilized soil + untreated seeds). The trial was established on 14 April 2023 in the Phytopathology Laboratory under room conditions (25–30 °C). A randomized block design was employed, consisting of five treatments with four replicates each. Watering and routine maintenance were carried out periodically throughout the experimental period.

Evaluation

On 25 July 2023, the pistachio seedlings were carefully uprooted and evaluated according to the following morphological parameters.

Measurement of root and stem length (mm)

The length of each plant was measured from the point of soil contact to the apical meristem using a digital caliper. Root length was determined as the distance between the root–shoot junction and the tip of the longest vertically oriented root.

Determination of fresh and dry weight in plants (g)

The plants were cut at the root collar, and the root and shoot parts were weighed separately on a precision balance to determine their fresh weights. After recording the fresh weights, the samples were placed in aluminium foil containers and dried in an oven at 70 °C for 48 hours. The dry weights of the

roots and shoots were then measured to determine biomass values (Kaçar, 2008).

The significance of differences in morphological parameters was determined by analysis of variance (ANOVA), and treatment means were compared using the Least Significant Difference (LSD) test at $P < 0.01$ and $P < 0.05$. All statistical analyses were conducted using JMP 13 software.

RESULTS AND DISCUSSION

Morphological parameters of plants

Determination of root and stem length, fresh, and dry weight of plants

Certain morphological development parameters of pistachio seedlings grown from seeds treated with *Trichoderma* in sterile soil were measured and are presented in Table 1. Statistical analyses were conducted separately for each morphological development parameter.

Table 1. Morphological development parameters of pistachio sapling treated with *Trichoderma*

Applications	PRL(mm)	PSL (mm)	PFW(g)	PDW (g)
Trichoderma virens **İB1**	150,44 ^{c*}	128,10 ^{a}	1,97 ^{bc}	0,75 ^{b}
Trichoderma sp. **FT1**	172,97 ^{bc}	143,80 ^{a}	1,93 ^{bc}	0,65 ^{b}
Trichoderma viride **VG18**	184,30 ^{b}	134,05 ^{a}	2,71 ^{b}	1,32 ^{a}
Trichoderma harzianum **TUZ16**	265,00 ^{a}	134,12 ^{a}	3,77 ^{a}	1,75 ^{a}
Control (-)	172,67 ^{bc}	124,65 ^{a}	1,75 ^{c}	0,65 ^{b}

PRL: Plant Root length, PSL: Plant Stem length , PFW: Plant Fresh Weight, PDW: Plant Dry Weight,

* Differences between means followed by the same letter within a column are not significant at the $p < 0.01$ level.

Upon examination of Table 1, four distinct groups were identified based on plant root length (PRL). Among these, the *T. harzianum* TUZ16 isolate, with a root length of 265.00 mm, belonged to group a and exhibited the best development. This was followed by *T. viride* VG18 in group b, *Trichoderma* sp. FT1 in Group bc, and *T. virens* IB1 in group c. In terms of plant stem length (PSL), no significant differences were observed among the treatments, and all were grouped together. *Trichoderma* sp. FT1, with a stem length of 143.80 mm, showed the best development, followed by *T. harzianum* TUZ16, *T. viride* VG18, and *T. virens* IB1. Differences were observed among the treatments in terms of plant fresh weight (PFW) and plant

dry weight (PDW), with *T. harzianum* TUZ16 identified as the most effective treatment.

It was followed by *T. viride* VG18, *T. virens* IB1, and *Trichoderma sp.* FT1. The appearance of some treated and untreated plants is shown in Figure 1.

Figure 1. (A) Growth of plants treated with *T. viride* VG18 and the negative control; (B) Growth of plants treated with *Trichoderma sp.* FT1 and the negative control.

Morphological examination of Figure 1 shows that seedlings treated with the antagonist exhibited better development after emergence compared to those in the control pots.

This study investigated the effects of *T. harzianum* TUZ16, *T. virens* IB1, *T. viride* VG18, and *Trichoderma sp.* FT1 on selected morphological parameters of pistachio. Examination of Table 1 shows that *T. harzianum* TUZ16 was the most effective isolate for enhancing root length, stem length, fresh weight, and dry weight. *T. harzianum* is the most extensively studied species within the genus *Trichoderma* for controlling plant diseases and is

commonly used in bioformulations and production processes (Aydın, 2015). *Trichoderma* species have been reported to influence host plant interactions by promoting root and shoot development, enhancing resistance to biotic and abiotic stresses, and altering the plant's nutritional status. Recent studies have shown that certain *Trichoderma* species positively affect seedling emergence by modulating plant-secreted compounds that promote pre-emergence damping-off caused by fungal pathogens (Howell, 2003). For example, the application of *T. harzianum* (T-22) to maize seeds in nitrogen-deficient soils produced seedlings that were greener and taller during early growth, with higher grain and silage yields at harvest compared to untreated controls. No such differences were observed in soils with sufficient nitrogen, which has been attributed to the strong interaction between T-22 and nitrogen-fixing bacteria (Harman, 2000). Similarly, the application of *T. harzianum* (T-203) to cucumber plants was reported to significantly increase root area, dry weight, and leaf area (Yedidia et al., 2001). In addition, Windham et al. (1986) reported that certain strains of *T. harzianum* and *T. koningii* enhanced germination rates, seedling emergence, and dry weight in maize, tomato, tobacco, and carrot plants. n another study, Mastouri et al. (2010) reported that the application of *T. harzianum* to tomato seeds increased germination rates and enhanced seedling tolerance to water deficiency. Recent research has also shown that *Trichoderma* species are not only present on plant root surfaces but can colonize various plant tissues endophytically (Druzhinina et al., 2011). These endophytic *Trichoderma* species have been reported to promote plant growth and protect plants from both biotic and abiotic stresses (Bae et al., 2009). The findings from these studies are consistent with the results of our experiment, confirming that *Trichoderma* species positively influence the development of plant morphological parameters.

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CHAPTER 4

DETERMINATION OF THE EFFECT OF *Clonostachys rosea* (Sch.) Schroers & Samuels ON PLANT GROWTH PARAMETERS IN PISTACHIO

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INTRODUCTION

The pistachio (*Pistacia vera* L.) is a nut-bearing species of the family Anacardiaceae, naturally distributed between the 30° and 45° latitudes (Tekin et al., 2001). It is native to Asia Minor (Turkey), Iran, Syria, Lebanon, Turkmenistan, the Caucasus, and Afghanistan. According to FAO (2021), global pistachio production reached approximately 915,718 tonnes in 2021, with the United States (40.49%), Iran (28.69%), and Turkey (16.13%) being the leading producers, collectively accounting for about 85% of the world's total production.

Along with periodicity, several factors limit pistachio production, including environmental conditions, improper fertilization and irrigation, physiological disorders, variety selection, and diseases and pests. Addressing these constraints is essential to ensure healthy plant growth and achieve optimal yield.

Today, numerous biological control agents have been licensed as commercial products and are actively used in agriculture. Among them, *Clonostachys rosea* (Syn. *Gliocladium roseum*) has emerged as one of the most prominent agents in recent years. *C. rosea* was first reported as an aggressive mycoparasite in the late 1950s (Barnett and Lilly, 1962). Following this discovery, research on its potential use in the biological control of plant diseases began (Shigo, 1958). The mechanisms by which *C. rosea* suppresses plant pathogens include the secretion of cell wall-degrading enzymes, the production of secondary metabolites such as antibiotics and toxins, and the stimulation of plant growth (Chatterton and Punja, 2009; Fatema et al., 2018). This species can typically be isolated from soil, other fungi, plant debris, and plant parts such as roots, leaves, and flowers (García et al., 2003; Nobre et al., 2005). Owing to its endophytic ability, the pathogen is reported to colonize plant organs near potential entry points and, in some cases, to activate plant defence mechanisms by inducing induced systemic resistance (ISR) (Lahoz et al., 2004; Saraiva et al., 2015; Wang et al., 2019; Kamou et al., 2020). Çevik et al. (2022) reported that *Clonostachys rosea* was effective against *Verticillium* wilt and early blight diseases in tomato plants. They also observed that the fungus had a positive influence on certain morphological characteristics of the tomato plants. Accordingly, it has been noted that biological control agents exert both direct effects on pathogens and

indirect effects on the host plants promoting growth and development through their indirect interactions and thereby enhancing resistance against pathogens (Sani et al., 2020).

In this study, the effectiveness of *Clonostachys rosea* on pistachio seedlings was evaluated in terms of root length, stem length, and fresh and dry weights.

MATERIAL AND METHOD

The *Clonostachys rosea* LO41 isolate used in this study was obtained from the isolate collection of the Plant Pathology Laboratory, Faculty of Agriculture, Siirt University. The other isolates, *C. rosea* FG8, FG9, and FG10, were isolated from the roots of pistachio trees. Morphological identification of the isolates was performed based on colony colour and growth characteristics, conidiophore branching, and the shape and size of conidia, following the criteria described by Schroers et al. (1999) and Schroers (2001).

In the experimental setup, the potting soil mixture was prepared by combining garden soil, sand, and peat moss in a 1:1:1 ratio. The prepared mixture was then sterilised by autoclaving at 121 °C for 15 minutes in autoclavable bags.

In this study, seeds of Siirt pistachio variety from the same harvest year were stored in a refrigerator at +4 °C in a dark environment for 3–4 months. Prior to sowing, the seeds were soaked in water to promote germination and subsequently dried on filter paper. Surface sterilisation was performed by immersing the seeds in 2% NaOCl for 5 minutes, followed by two rinses with sterile distilled water. The disinfected seeds were then immersed in a spore suspension of *Clonostachys rosea* isolates, adjusted to a concentration of 1×10^7 conidia/ml, for approximately 60 minutes. After treatment, three seeds were sown per 5-litre pot containing clean soil prepared one day in advance.

The experiment was established on 14 April 2023 in the Phytopathology Laboratory under room conditions (25–30 °C) using four antagonist isolates and a clean soil control, arranged in a randomised block design with five treatments and four replications. Watering and maintenance were performed periodically throughout the experiment.

Evaluation

The pistachio saplings were uprooted on 31 July 2023 and evaluated based on the following morphological parameters.

To determine the root and stem length (mm) of the plants, measurements were taken using a digital caliper. Stem length was measured from the point where the plant meets the soil to the shoot apex, while root length was measured from the soil surface to the tip of the longest vertical root.

To determine the fresh and dry weights (g) of the plants, they were cut at the root collar and weighed on a precision balance to record their fresh weights. The samples were then placed in aluminium foil containers, dried in an oven at 70 °C for 48 hours, and subsequently weighed again to determine their dry weights (Kaçar, 2008).

The significance of differences in morphological parameters was determined through analysis of variance (ANOVA), and the least significant difference (LSD) test was used to compare treatment means at the $P < 0.01$ level. All statistical analyses were conducted using JMP 13 statistical software.

RESULTS AND DISCUSSION

In this study, the effects of the biological control agent *Clonostachys rosea* on certain morphological development parameters of pistachio seedlings were evaluated. Root and stem lengths, as well as fresh and dry weights of pistachio saplings grown from seeds treated with *C. rosea* in sterilised soil, were measured and are presented in Table 1. Statistical analyses were conducted separately for each morphological parameter.

Table 1. Some morphological development parameters of pistachio seedlings treated with *Clonostachys rosea*

Applications	PRL(mm)	PSL (mm)	PFW(g)	PDW(g)
C. rosea F10	255,00[a*]	190,00 [a]	3,20 [a]	1,29 [a]
C. rosea F8	192,75[ab]	180,50 [a]	2,30 [b]	1,32 [a]
C. rosea LO41	167,50 [b]	171,75 [a]	1,82 [b]	0,58 [b]
C. rosea F9	153,75 [b]	163,75 [ab]	2,12 [b]	0,94 [ab]
Control (-)	147,00 [b]	154,50 [ab]	1,64 [b]	0,44 [b]

PRL: Plant Root length, PSL: Plant Stem length, PFW: Plant Fresh weight, PDW: Plant Dry weight,

* Differences between means followed by the same letter within a column are not significant at the $p < 0.01$ level.

Upon examination of Table 1, statistically significant differences were observed among the treatment groups in terms of the plants' morphological development parameters. Based on root length comparisons, three distinct groups were formed. The isolate *C. rosea* F10, with a root length of 255.00 mm, was identified as the most effective in promoting root development, followed by *C. rosea* F8, *C. rosea* LO41, and *C. rosea* F9. The control exhibited a root length of 147.00 mm and was grouped together with *C. rosea* LO41 and *C. rosea* F9. In terms of stem length, two groups were identified, with *C. rosea* F10 showing the greatest stem length (190.00 mm), followed by *C. rosea* F8, *C. rosea* LO41, and *C. rosea* F9. When examining the fresh weight data of the plants, two groups were identified. The isolate *C. rosea* F10, with a fresh weight of 3.20 g, showed the best development and was placed in group "a". It was followed by *C. rosea* F8, *C. rosea* F9, and *C. rosea* LO41. The fresh weight of the control plant was 1.64 g and was placed in group "b" together with *C. rosea* F8, *C. rosea* F9, and *C. rosea* LO41. Regarding dry weight, three groups were identified. The isolate *C. rosea* F8, with a dry weight of 1.32 g in group "a", exhibited the highest value, followed by *C. rosea* F10, *C. rosea* F9, and *C. rosea* LO41. The control plant showed a dry weight of 0.44 g. Overall, the results indicate that *C. rosea* isolates positively influenced the morphological development of pistachio plants. Figure 1 presents the visual appearance of the treated plants along with the negative control.

Figure 1. Comparison of the development of *Clonostachys rosea* F10 (left) and the control plant (right) (A), and *C. rosea* F8 (left) and the control plant (right) (B).

In the experiment shown in Figure 1, which was conducted with antagonist isolate + clean soil, it can be observed that the development of the plants in the pots treated with the antagonist, as well as the uprooted plants, was better than that of the control plants.

In agriculture, chemical control methods are commonly used as the most effective and rapid means of managing plant diseases and pests. However, many soil-borne pathogens are polyphagous and capable of infecting a wide range of hosts, which makes chemical control challenging. Consequently, alternative control strategies have been increasingly investigated, with biological control emerging as one of the most important options. Various biological control agents have been identified to date, and numerous *Clonostachys rosea* isolates have been obtained from plant tissues in different studies. This fungus is recognised as an effective antagonist in biological control (Barnett and Lilly, 1962). In a study by Aydın and Turhan (2009), *Gliocladium roseum* isolates were reported to exhibit relatively slow growth on PDA medium compared to pathogen colonies; however,

microscopic observations revealed that they were highly aggressive, actively coiling around and penetrating the pathogen hyphae.

In conclusion, this study demonstrated that *Clonostachys rosea* positively influences morphological characteristics such as root and stem length, as well as fresh and dry weight, in pistachio plants. The primary objective of this study was to highlight the effectiveness of *C. rosea*, a species well known for its strong antagonistic properties.

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CHAPTER 5

Rhizopus spp. IN PLANT GROWTH PROMOTION AND BIOCONTROL

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1. INTRODUCTION

Plant diseases are an important global risk to agriculture, decreasing food yields and quality, also producing toxins that harm humans and animals (Attia et al., 2022a). Modern agriculture primarily uses chemical fertilizers and pesticides to increase crop yields and protect plants against pathogens. However, the overuse of these agrochemicals has caused severe ecological imbalances, including soil degradation, groundwater contamination, and the emergence of resistant phytopathogens (El-Saadony et al., 2022; Nath et al., 2015). As a result, there is a growing emphasis on sustainable and eco-friendly solutions that reduce chemical dependency while supporting global food security. Researchers worldwide are focusing on using natural alternatives, like beneficial microorganisms, instead of chemicals to protect the environment. These microorganisms offer mutual benefits to both plants and soil. They are valuable for sustainable agriculture due to their ability to produce beneficial secondary metabolites that support plant growth and protection, while also enhancing soil fertility (Swamy & Sandhu, 2020; Zohair et al., 2018).

Endophytic fungi, among these helpful microbes, have received increased attention due to their unique ability to colonize plant tissues without harming the host (Morales-Vargas et al., 2024). They often contribute to stress tolerance and disease resistance through the production of bioactive compounds (Wen et al., 2022).

These symbiotic fungi promote plant growth by secreting phytohormones, solubilizing nutrients, and producing a wide range of bioactive secondary metabolites like antibiotics and siderophores that help plants withstand both biotic and abiotic stress. These secondary metabolites also help in boosting the immune defense of the plants (Sharma & Singh, 2021; Swamy & Sandhu, 2020). They also contribute to soil health by enhancing nutrient cycling and organic matter decomposition, increasing crop quality and quantity without causing environmental pollution (Attia et al., 2022a). These fungi also help control harmful plant pathogens by producing antioxidant compounds that reduce mycotoxin production (Abdelaziz et al., 2022). Therefore, they represent a promising strategy for sustainable agriculture and maintaining productivity. Several fungal genera—including *Trichoderma, Penicillium, Aspergillus, Fusarium*, and *Rhizopus*—have been recognized for their plant

growth-promoting and biocontrol potential (Sharma & Singh, 2021; Zohair et al., 2018).

Moreover, plant growth-promoting fungi (PGPF) also play a vital role in sustainable agriculture and are typically found in the rhizosphere, unlike endophytes. However, PGPF do not necessarily establish an internal symbiotic relationship with the host plant. PGPF, such as *Trichoderma, Penicillium, Aspergillus,* and arbuscular mycorrhizal fungi, play an eco-friendly and effective role in enhancing crop productivity. They promote plant growth by improving root and shoot development, seed germination, chlorophyll production, and overall yield. PGPF functions through nutrient mineralization, phytohormone production, and activation of plant defense mechanisms, including induced resistance. These fungi help protect plants like tomato, apple, and cucumber from biotic and abiotic stress, reducing the need for agrochemicals. They also exhibit biocontrol activity, support nutrient uptake, produce ACC deaminase to regulate ethylene levels, and enhance systemic resistance in plants (Adedayo & Babalola, 2023).

Rhizopus, in specific, is a multipurpose fungus group that serves as a saprotrophic decomposer as well as a helpful endophyte. *Rhizopus* species are widely distributed in soil and plant environments and are known for their versatile metabolic profile. They are capable of producing a variety of extracellular enzymes, such as lipases and cellulases, that assist in organic matter breakdown and nutrient mineralization (Agbor et al., 2021; Torres et al., 2003). Moreover, *Rhizopus spp.* exhibit strong phosphate-solubilizing ability, enhancing the bioavailability of phosphorus—a key nutrient often limited in agricultural soils (Agbor et al., 2021). This trait directly contributes to improved root development, chlorophyll synthesis, and photosynthetic efficiency in host plants (Attia, et al., 2022a). Due to these abilities, *Rhizopus* spp. are classified as PGPF, with their phosphate-solubilizing potential playing a central role in supporting plant nutrition and development.

In addition to their nutrient-cycling capabilities, *Rhizopus* isolates have demonstrated the ability to synthesize phytohormones such as indole-3-acetic acid (IAA) and gibberellic acid (GA), which play essential roles in root elongation, seed germination, and overall plant growth (Nath et al., 2015; Sharma & Singh, 2021). These fungi also produce antioxidant compounds and secondary metabolites that reduce oxidative stress and inhibit mycotoxin

production in plants, thereby reinforcing their biocontrol potential (Adedayo & Babalola, 2023; Attia et al., 2022b). Through mechanisms such as competition for nutrients and space, production of antifungal metabolites, and activation of induced systemic resistance, *Rhizopus spp.* can suppress a variety of phytopathogenic fungi, making them promising microbial biocontrol agents (Adedayo & Babalola, 2023; El-Saadony et al., 2022).

Because of these multifunctional attributes, *Rhizopus* species represent a promising group of PGPF with applications as both biofertilizers and biopesticides. Despite their potential, the diversity and functional mechanisms of *Rhizopus*-associated endophytes remain underexplored compared to other fungal genera such as *Trichoderma* or *Aspergillus*. Therefore, further investigation into the biochemical and physiological traits of *Rhizopus* strains—particularly their enzyme production profiles, phosphate solubilization ability, and antagonistic activity against phytopathogens—will provide valuable insights into their potential use as sustainable biological control agents and plant growth promoters (Agbor et al., 2021; Liu et al., 2009).

This review provides a comprehensive overview of the taxonomy, morphology, and ecological roles of *Rhizopus* species, emphasizing their multifunctional contributions to plant growth promotion and biocontrol. Furthermore, it highlights the mechanisms through which these fungi enhance nutrient availability, produce bioactive metabolites, and mitigate biotic and abiotic stresses, underscoring their potential applications in sustainable agriculture and integrated pest management strategies.

2. *Rhizopus spp.*: TAXONOMY, MORPHOLOGY, AND GENERAL CHARACTERISTICS

Rhizopus is a genus of saprotrophic zygomycete fungi (Mucoromycotina, Mucoromycota) commonly found in soil, animal feces, and decaying plant matter. *Rhizopus* species produce a variety of industrial products by submerged fermentation and biotransformation including enzymes (lipases, proteases, glucoamylase and celluloytic enzymes), organic acids (lactic acid, fumaric acid) and steroids, terpenoids and alkoloids, pesticides and herbicides, antibiotics (Londoño-Hernández et al., 2017; Meussen et al., 2012). Certain species within this genus can act as plant pathogens, affecting crops, while others are utilized as fermentation agents in traditional foods or as producers of

industrially relevant enzymes and metabolites (Muszewska et al., 2014). Some of them create a considerable risk to post-harvest agricultural products by affecting the appeal and flavor quality of crops, particularly sweet potatoes and strawberries (Tournas, 2005).

Throughout history, *Rhizopus* species have been crucial in the process of food fermentation. *R. oryzae* and *R. oligosporus* have been utilized for generations to produce traditional Asian dishes, including tempeh, ragi, sufu, and even fermented drinks (Londoño-Hernández et al., 2017). Commercial inocula are regarded as safe; no toxins have been reported for *R. oryzae*, and the US Food and Drug Administration (FDA) classifies its products as "generally recognized as safe" (GRAS) for human consumption (Faisal Peeran et al., 2018; Liu et al., 2009; Sharma & Singh, 2021; Xing et al., 2011). *Rhizopus* species—especially those belonging to the *R. oryzae* —have been used in bioindustrial applications to produce organic acids, ethanol, carotenoids, and hydrolytic enzymes (Meussen et al., 2012).

Some of *Rhizopus* species were studied according to cultural growth, temperature performance, acid production, assimilation of carbon and nitrogen sources, fermentation of carbohydrates, amylase activity and % G + C values among three distinct morphological groups: stolonifer, arrhizus, and microsporus. Morphologically, *Rhizopus* species are filamentous fungi characterized by a network of branching hyphae, including stolons, rhizoids, and sporangiophores (Figure 1 and 2) (Gryganskyi et al., 2018; Jerez Lazo et al., 2024). Colonies typically grow rapidly on PDA, exhibiting a fluffy, cotton-like appearance within five days, initially whitish and gradually turning brown as sporangiospores mature. Pigmented rhizoids, sporangiophores, apophyses, and columellae often collapse to form an umbrella-like structure, with sporangiospores being short-ellipsoidal. The mycelial network formed by these hyphae is essential for nutrient absorption and distribution, and *R. oryzae* forms longer networks than *R. oligosporus*, allowing it to thrive on a wider range of substrates (Gryganskyi et al., 2018; Jerez Lazo et al., 2024).

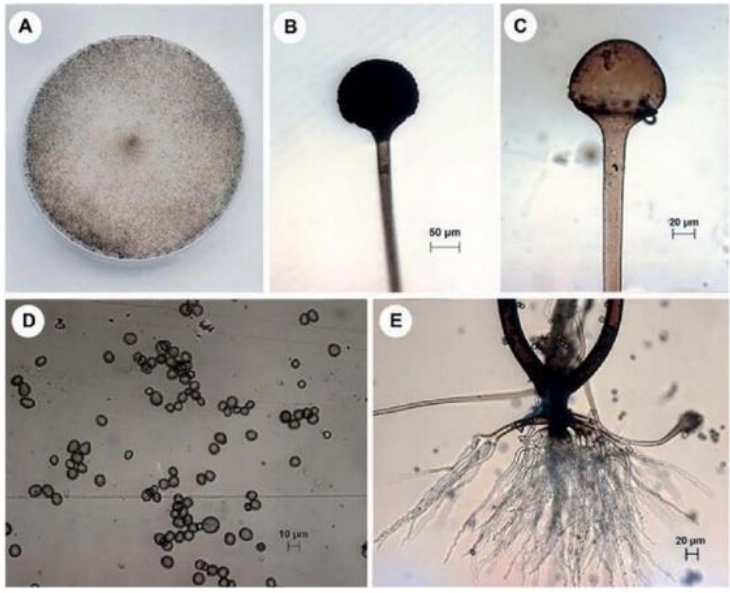

Figure 1. Morphology of *Rhizopus stolonifer* (A: Colonization appearance on PDA after a 2 day incubation, B: Sporangium and sporangiophore, C: Columella, D: Sporangiospores, E: Rhizoids and stolons) (Liu et al., 2024).

Figure 2. Morphology of *Rhizopus oryzae*. (A: Colonization appearance on PDA after a 7 day incubation, B: Sporangium and sporangiophore, C: Columella, D: Sporangiospores, E: Rhizoids) (Kwon et al., 2011).

Ecologically, *Rhizopus* species thrive in warm and humid environments, growing between 7 and 45 °C, with an optimum around 37 °C (Swamy & Sandhu, 2020). As saprophytes, they can survive without host organisms, which allows them to colonize diverse environments, including coastal and nutrient-

poor soils. Their hyphae form mesh-like networks that bind soil particles together, enhanced by extracellular polymeric substances that act as natural adhesives—a feature that distinguishes *Rhizopus* from microbially induced calcite precipitation (MICP) by bacteria (Jerez Lazo et al., 2024).

Importantly, several *Rhizopus* species have been reported as PGPF due to their ability to enhance nutrient availability, produce phytohormones such as indole-3-acetic acid (IAA) and gibberellic acid (GA), and stimulate plant growth (Agbor et al., 2021; Swamy & Sandhu, 2020). Their extracellular enzymes contribute to soil nutrient cycling, while secondary metabolites and antioxidant compounds can reduce oxidative stress and suppress phytopathogens, functioning as a biocontrol mechanism (Agbor et al., 2021; Jerez Lazo et al., 2024). These traits position *Rhizopus* spp. as multifunctional microbes capable of improving plant productivity while promoting soil health, highlighting their potential use as eco-friendly biofertilizers and biopesticides.

Overall, *Rhizopus* represents a versatile genus with important roles in agriculture, industry, human health, and sustainable plant growth promotion (Agbor et al., 2021; Gryganskyi et al., 2018; Jerez Lazo et al., 2024; Leewijit et al., 2016).

3. PLANT GROWTH-PROMOTING AND BIOCONTROL POTENTIAL OF *Rhizopus* spp.

Several studies have highlighted the role of *Rhizopus* species as PGPF and endophytic microorganisms with substantial potential in sustainable agriculture. PGPF, including *R. microsporus* and *R. oryzae*, have been reported to enhance plant growth by producing IAA and GA, increasing chlorophyll and carotenoid content, and improving photosynthetic efficiency (Agbor et al., 2021; Attia et al., 2023; Attia et al., 2022b).

Attia et al. (2023) aimed to develop a biological control method against root-knot nematode (*Meloidogyne incognita*), which is one of the most destructive pathogens affecting eggplant, leading to severe yield losses. In this study, six PGPF isolates were evaluated for their potential to enhance biochemical defenses and improve physiological performance in eggplant seedlings under nematode infection. The isolates were tested *in vitro* for siderophore and hydrogen cyanide (HCN) production, as well as their antagonistic activity against *M. incognita*. Among them, *R. microspores* and

Aspergillus oryzae demonstrated the highest HCN production and nematode mortality rates (74.20% and 60.35%, respectively), along with strong antioxidant activity (IC_{50} values of 145 µg/mL and 350 µg/mL). Moreover, the application of *R. microsporus*, either individually or in combination with *A. oryzae*, significantly improved chlorophyll a and b as well as carotenoid content, indicating enhanced photosynthetic efficiency and overall plant vitality. When applied together in vivo, these two isolates significantly reduced nematode populations by 68.78–95.23% across different developmental stages. These findings suggest that the ethyl acetate extracts of *A. oryzae* and *R. microsporus* could serve as effective, eco-friendly biocontrol agents and plant growth stimulants for managing *M. incognita* in eggplant cultivation (Attia et al., 2023).

Endophytic fungi, including *Rhizopus* spp., have also been isolated from roots, stems, and leaves of various plants such as saffron, coriander, and *Adiantum* species (Chamkhi et al., 2018; Leewijit et al., 2016; Parvez et al., 2023). Chamkhi et al. (2018) made a study on *Crocus sativus* (saffron) roots and identified sixty endophytic fungal isolates: *R. oryzae*, *A. fumigatiaffinis*, and *A. niger* being the most abundant species. Extracts from these endophytes showed notable antibacterial and antioxidant activities, with *R. oryzae* demonstrating the strongest bactericidal effect and *A. niger* showing the highest antioxidant activity in the DPPH assay. Overall, these findings suggest that saffron-associated endophytic fungi, especially *R. oryzae* and *A. niger*, represent valuable sources of bioactive compounds with potential pharmaceutical applications (Chamkhi et al., 2018).

The capacity of *Rhizopus* spp. to alleviate biotic and abiotic stresses has been well documented. For instance, a study explored the effects of *Rhizopus* spp. on the growth, physiology, and defense mechanisms of tomato plants, both healthy and infected with *Phytophthora infestans*. Application of *Rhizopus* spp. significantly enhanced plant height, leaf number, chlorophyll (a, b, and total) content, photosynthetic efficiency, and biochemical parameters such as total protein, flavonoids, phenolics, antioxidants, and vitamin C. These improvements were attributed to the fungus's ability to solubilize insoluble phosphate through organic acid and enzyme production, thereby increasing nutrient availability. Moreover, *Rhizopus* spp. inoculation markedly reduced disease incidence and severity of late blight by stimulating the plant's defense

responses, including the activation of antioxidant enzymes (CAT, APx, SOD) and the accumulation of protective metabolites. Overall, the findings indicate that *Rhizopus* spp. not only promotes growth and photosynthetic activity but also enhances antioxidant and enzymatic defenses, providing an eco-friendly approach to improving tomato health and resistance against pathogens (Agbor et al., 2021).

Similarly, a different study examined the ability of the endophytic fungus *R. oryzae* to alleviate heat stress (40°C) in sunflower and soybean plants. *R. oryzae* was found to secrete key compounds such as indole-3-acetic acid (IAA), salicylic acid (SA), flavonoids, and phenolics, which regulate stress-responsive genes and strengthen the plant's defense against abiotic stress. Under heat stress, plants inoculated with *R. oryzae* showed increased levels of proline, phenolics, antioxidant enzymes (CAT, AAO, SOD), and soluble sugars, while exhibiting reduced abscisic acid (ABA) accumulation compared to uninoculated controls. These biochemical adjustments improved photosynthesis, protein and lipid synthesis, and protection against reactive oxygen species. Overall, the results demonstrate that *R. oryzae* enhances the antioxidant defense system, osmotic balance, and metabolic stability of sunflower and soybean, thereby providing significant protection against heat-induced oxidative damage and improving plant tolerance under thermal stress (Ismail et al., 2020).

Espinoza et al. (2008) evaluated the fungistatic and fungicidal activities of culture filtrates from five fungal isolates against several plant pathogenic fungi. The culture filtrates of *Rhizopus* spp. have demonstrated fungistatic and fungicidal activity comparable to commercial fungicides such as Captan and Benlate against various plant pathogens. *Rhizopus* spp. was also effective against a wide range of pathogens, including *Alternaria, Cochliobolus, Pestalotia*, and *Setosphaeria* species. These results suggest that *Rhizopus* spp. produce potent antifungal metabolites—such as tyrosol, geraniol, curvularine, rhizoxin, and others—making it a promising candidate for the development of biological control agents in plant disease management (Espinoza et al., 2008).

Another study investigated the ability of *R. oryzae* and *Trichoderma reesei* to degrade aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2, and AFM_1) under controlled culture conditions. Both fungi used extracellular enzymes—such as peroxidases and oxidoreductases—to transform aflatoxins into more polar, less

toxic derivatives through oxidation and ring cleavage of their molecular structures. After 96 hours, *R. oryzae* achieved almost complete degradation (>98%) of AFB_1 and AFG_1, while *T. reesei* required 120 hours for similar results. However, when exposed to all five toxins simultaneously, *R. oryzae*'s efficiency decreased, achieving reductions ranging from 14% (AFM_1) to 98% (AFB_1). The transient increase in AFM_1 suggested that it was formed as a hydroxylated metabolite of AFB_1, which is less toxic. Biomass analysis revealed that *R. oryzae* grew faster, reaching peak levels of ergosterol and glucosamine at 72–96 hours, while *T. reesei* peaked at 120 hours. The higher growth rate of *R. oryzae* corresponded with its superior detoxification performance. Overall, the findings demonstrate that *R. oryzae* is an efficient biological agent for aflatoxin degradation, capable of enzymatically transforming highly toxic compounds into less harmful forms, offering a promising eco-friendly strategy for mycotoxin detoxification in contaminated substrates (Hackbart et al., 2014).

The endophytic lifestyle of *Rhizopus* spp. allows these fungi to persist in host plants for extended periods, contributing to nutrient solubilization, growth promotion, and disease suppression. For example, *R. oryzae* isolated from coriander roots was shown to produce auxin and positively influence spinach growth through synergistic interactions between fungal metabolites and plant hormones (Parvez et al., 2023).

Furthermore, *R. oryzae* and related species can improve soil properties in challenging environments, such as coastal sands, by forming extensive mycelial networks that enhance soil stability and long-term strength, underscoring their potential role in soil bioengineering. Jerez Lazo et al. (2024) showed the potential of *R. oryzae* for soil improvement in coastal environments (Jerez Lazo et al., 2024). Previous research showed that *R. oligosporus*, from the same family, could enhance sand properties but had limited durability, losing cohesive strength within 10 days (Lim et al., 2020). In contrast, sand treated with *R. oryzae* retains substantial strength for an extended length of time without extra water or nutrients due to its more widely branching and interwoven mycelial network. The findings suggest that soil improvement can be tailored by selecting between the fast-acting *R. oligosporus* and the longer- lasting *R. oryzae*. Experiments on Miami Beach sand, which has previously been subjected to salty conditions, indicated the potential efficacy of this

therapy. Overall, *R. oryzae* emerges as an effective and durable biological agent for coastal soil stabilization (Jerez Lazo et al., 2024).

Collectively, these studies demonstrate that *Rhizopus* spp. can serve as multifunctional PGPF and biological control agents. Their ability to enhance nutrient availability, stimulate plant growth, produce bioactive metabolites, mitigate biotic and abiotic stresses, and improve soil structure establishes them as promising candidates for sustainable agriculture and integrated pest management strategies.

4. CONCLUSION

Overall, the literature demonstrates that *Rhizopus* species represent a highly versatile and ecologically significant group of fungi with substantial potential in sustainable agriculture. Their multifunctional attributes—including nutrient solubilization, phytohormone production, antioxidant and antimicrobial metabolite synthesis, and the activation of plant defense mechanisms—enable them to promote plant growth while simultaneously protecting crops against a wide range of biotic and abiotic stresses. As both PGPF and endophytes, *Rhizopus spp*. contribute to improved photosynthetic efficiency, enhanced stress tolerance, and reduced pathogen incidence, offering an eco-friendly alternative to chemical fertilizers and pesticides. In addition, their robust extracellular enzyme systems and extensive mycelial networks support soil nutrient cycling and structural stability, extending their applications into areas such as soil bioengineering and mycotoxin detoxification.

Despite these promising findings, the functional diversity and underlying mechanisms of many *Rhizopus* strains remain insufficiently explored compared to more extensively studied genera such as *Trichoderma* or *Aspergillus*. Future research focusing on strain-specific metabolite profiles, enzymatic pathways, and interactions with host plants will be crucial to fully harnessing their potential as biofertilizers, biopesticides, and soil-enhancing agents. In conclusion, *Rhizopus spp.* emerge as powerful candidates for the development of sustainable agricultural practices and integrated pest management strategies, contributing to environmentally responsible crop production and long-term food security.

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CHAPTER 6

BIOCONTROL STRATEGIES AGAINST GRAY MOLD (*Botrytis cinerea* Pers.) IN GERANIUM (*Pelargonium* spp.) UNDER IN VIVO CONDITIONS

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INTRODUCTION

Ornamental plants have played an important role in human societies from ancient civilizations to the present day, serving aesthetic, cultural, and economic purposes worldwide (Atalay, 2023). They are generally categorized into four main groups: cut flowers, indoor (potted) ornamental plants, outdoor (landscape) ornamental plants, and flower bulbs (Ay, 2009; Kazaz, 2012). In Turkey, ornamental plant cultivation is largely centered in the Marmara, Aegean, and Mediterranean regions, where the favorable climate supports high levels of production intensity This sector contributes significantly to the national economy, particularly due to its strong export potential and its role in the development of the floriculture industry (Kazaz et al., 2015).

Table 1. Ornamental Plant Production Statistics in Turkey for 2023 and 2024 (Anonymous, 2024)

Ornamental Plants	**2023**	**2024**
Cut flowers	1 413 978 778	1 413 208 458
Carnation	912 485 536	889 334 480
Gerber daisy	103 296 420	105 294 120
Rose (cut)	105 771 175	123 359 282
Chrysanthemum	78 763 020	79 674 550
Freesia	15 403 060	15 236 560
Tulip	4 485 000	4315000
Goldenrod	33 829 000	32228000
Gypsophilla	46 121 940	45991940
Daffodil	36 742 302	38360046
Gladiolus (sword lily)	3 043 800	2696800
Lisianthus	15 909 500	15695350
Lilium	8 166 850	8459875
Hyacinth	1 058 000	908000
Gillyflower	8 689 150	10170400
Anemone (windflower Manisa lalesi)	288 000	288000
Iris	40 000	40000
Orchids	2 870 300	2870300
Statice	301 000	247000
Other cut flowers	36 714 725	38038755
Other ornamental plants	749 769 771	619390856
Total Production	2 163 748 549	2 032 599 314

In Turkey, ornamental plant production constitutes an important component of the horticulture sector. According to national production data, the total number of ornamental plants produced was 2,163,748,549 (number) in

2023, showing a slight decline to 2,032,599,314 (number) in 2024 (Anonymous, 2024; Table 1).

The geranium (*Pelargonium* spp.) is a widely cultivated ornamental plant grown both indoors and outdoors across the world, including in Turkey, and is known for its rich diversity. Belonging to the family Geraniaceae (cranesbill), the genus Pelargonium comprises approximately 280 species that vary in form, ranging from annual herbaceous plants to dwarf shrubs (Alp, 2017). Among these, the most commonly preferred varieties are upright geraniums (*Pelargonium hortorum* × *Pelargonium zonale*) and ivy or cretan geraniums (*Pelargonium peltatum*). Geraniums are frequently utilized in garden landscaping due to their attractive foliage and colorful flowers, which may be single or double and occur in shades of pink, red, purple, or white. Propagation of geraniums is generally achieved through vegetative methods or by seed.

However, despite their economic and aesthetic value, ornamental plants are frequently affected by various biotic and abiotic stresses, among which fungal diseases are particularly significant. The most commonly observed diseases in geraniums are leaf spot (*Alternaria alternata*), rust in Pelargonium species (*Puccinia pelargonii*), root rot (*Pythium* and *Rhizoctonia species*), wilt (*Fusarium* sp. and *Verticillium dahliae*), and the widespread gray mold (*Botrytis cinerea*), bacterial stem rot, leaf spot, and wilt disease. Among the fungal pathogens that threaten ornamental plant production, *B. cinerea*, the causal agent of gray mold disease, is considered one of the most destructive due to its wide host range and adaptability (Uchneat et al., 1999).

Botrytis Blight (Root, Leaf, and Flower Blight or gray mold), which causes geraniums to become diseased and lose their market value, is most commonly seen in greenhouses, but it can also be seen in outdoor geranium plants depending on environmental conditions. It can develop at any stage of the plant's physiological development or on any part of the plant (Hausbeck and Pennypacke, 1991).

The infection caused by *B. cinerea* growes quite rapidly on the weak leaves, stems, and especially the flowering parts of the sardunya plant that are close to falling off. The damage caused by the disease to the flowers reduces their visual appeal, thereby lowering their market value and causing financial losses (Elad and Stewart, 2007; Elmhirst, et al., 2011).

The high sensitivity of ornamental plants to chemically synthesized fungicides has considerably restricted the number of such products licensed for the management of fungal infections. In our country, no chemical or biological fungicidal agents are currently registered for the management of grey mould in geraniums, despite their extensive use as ornamental plants both indoors and outdoors owing to their diverse colour varieties.

Table 2 demonstrates that biological preparations currently licensed for plant disease management in our country consist predominantly of fungicides based on species of the *Trichoderma* genus. Various formulations of *Trichoderma asperellum*, *T. gamsii*, *T. harzianum*, and *T. viride* have been authorised and are primarily recommended for the biological suppression of specific soil-borne fungal pathogens and grey mould infections affecting crops such as tomato, strawberry, cotton, parsley, cress, rocket, mint, and cucumber. This limited diversity of registered biological products highlights the need for expanded research and development to broaden the range of effective biocontrol agents available for sustainable disease management (Anonymous, 2025).

In this study, the effects of a biological preparation active ingredient with *Trichoderma harzianum* Rifai strain Krl–AG2, a bacterial-based biological product derived from *Bacillus amyloliquefaciens* strain MBI 600 ($>5.5 \times 10^{10}$ cfu/g), and tea tree extract (222.5 g/L tea tree oil) were investigated against *Botrytis cinerea*, the causal agent of grey mould disease that infects geranium flowers and significantly reduces their market value.

Table 2. Registered Fungal-Based Biological Fungicides in Türkiye

Active Ingredient	Plant	Disease	Trade Name	Licence Status
$1x10^8$ cfu/g *Trichoderma aspellerum* strain ICC 012+ *Trichoderma gamsii* strain ICC 080	Strawberry	*Fusarium oxysporum*, *Macrophomina phaseolina*, *Phythium* spp., *Rhizoctonia solani*	REMEDIER® (IMPORTED) 300 g / da	The licence is still valid
Trichoderma harzianum Rifai Strain T22,$1x10^9$ CFU/g	Tomato	*Fusarium* spp., *Phytophthora* spp.	TRIANUM P (IMPORTED) 30 g/1000 plants (drenching) 40 g/da (drip irrigation)	The licence is still valid
Trichoderma harzianum rifai strain KRL-AG2 (T 22) 400 million spor/gr	Tomato	*Botrytis cinerea*	T-22 PLANTER BOX (IMPORTED) 60 g /100 l su	The licence is still valid
Trichoderma harzianum rifai strain KRL-AG2 (T 22) 400 million spor/gr	Cotton	*Pythium* spp., *Rhizoctonia* spp., *Fusarium* spp., *Scierotinia* spp.	T-22 PLANTER BOX (IMPORTED) 7,5 g/ 1 kg seed	The licence is still valid
Trichoderma viride $1x10^8$ kob/ml min.	Parsley Watercress Rocket-arugula Mint	*Fusarium* spp., *Macrophomina* spp., *Phytophthora* spp., *Pythium* spp., *Rhizoctonia* spp.	BIO-CURE F (IMPORTED) 300 ml/da (with drip irrigation)	Only for Parsley until 31 December 2025. The licence authorisati-on for Watercress and Mint has expired.
Trichoderma viride $1x10^8$ kob/ml min.	Hıyar	*Fusarium* spp., *Macrophomina* spp., *Phytophthora* spp., *Pythium* spp., *Rhizoctonia* spp.	BIO-CURE F (IMPORTED) 300 ml/da (with drip irrigation)	The licence is still valid

MATERIAL AND METHOD

Material

The study was conducted using upright geranium (*Pelargonium zonale*) varieties cultivated under controlled greenhouse conditions in the Central District of Yalova Province, Turkey, for ornamental plant seedling production. These varieties had previously been identified as susceptible to gray mold disease (*Botrytis cinerea*). *P. zonale* originates from South Africa and has gradually spread to Mediterranean countries due to its ornamental value and strong adaptability to regional climatic conditions (Nameth et al.,1999). It is commonly grown as an ornamental species, particularly in coastal areas. The upright geranium is characterized by slightly hairy, soft leaves and can reach heights exceeding 1 meter. Under favorable temperature conditions, it continuously produces flowers in various colors, including red, white, and pink (Figure 1).

Figure 1. The red upright geranium variety used in the experiment

B. cinerea fungus isolate isolated from the geranium plant was used (Figure 2). Whether the gray mold isolate was pathogenic was determined by a preliminary pathogenicity test conducted on an upright geranium variety.

Figure 2. Geranium plant infected with *Botrytis cinerea* (a) and re-isolation of the pathogen in a PDA-containing Petri dish (b).

Preparations Used in the Application

The plant protection products used in the trial were selected from those licensed for gray mold in vegetables in the Ministry of Agriculture and Forestry's Plant Protection Products database. The selected plant protection products Switch 62.5 WG, Timorex Gold, T-22 Planter Box, and Serifel were used at the dose applied to gray mold in tomatoes in the established trial as the application dose (Anonymous, 2024b). The treatments were applied as foliar sprays to the geranium (*Pelargonium* spp.) plants.

Table 3. Chemical and Biological Fungicides and Their Dosages for Use in Applications

Fungicides	**Application Dosages**
Switch 62.5 WG (%37,5 Cyprodinil + %25 Fludioxonil)	60g/100 l water
Timorex Gold(222,5 g/l Tea tree oil)	150 ml/100 l water
T-22 Planter Box (*Trichoderma harzianum Rıfai* Strain Krl – AG2 (T 22) 400 million spore /gr)	60g/100 l water
Serifel (*Bacillus amyloliquefaciens* strain MBI 600 >5.5x10 ^{10} cfu/g)	50g/100 l water

In the trials, the commercial chemical fungicide **Switch** (62.5 WG; 37.5% cyprodinil + 25% fludioxonil, 60 g/100 L water), marketed by Syngenta Agricultural Industry and Trade Co., was applied to compare its efficacy with that of biological fungicides. The plant-based preparation **Timorex Gold** (222.5 g/L tea tree oil, 150 mL/100 L water), sold by Nufarm Turkey Chemicals Import and Trade Co., Ltd., was also included. Additionally, the biofungicide **T-22 Planter Box** (*Trichoderma harzianum* Rıfai strain Krl–AG2, 400 million spores/g, 60 g/100 L water), supplied by Hasel Agricultural Products Industry and Trade Ltd., and the biofungicide **Serifel** (*Bacillus amyloliquefaciens* strain MBI 600, >5.5 × 10^{10} cfu/g, 50 g/100 L water), marketed by BASF Turkish Chemical Industry and Trade Co., Ltd., were tested in the study (Table 3).

Location of the Experiment

The experiment was conducted in the Central District of Yalova Province using 3-liter plastic pots, which were placed inside an unheated greenhouse covering approximately 300 m².

Method

The trial was arranged using a randomized block design with four replicates per treatment, each containing ten plants. To prevent cross-contamination during spraying, a 1-meter safety strip was maintained between treatment blocks, and plastic sheets were placed as barriers. The treatments were applied according to the instructions provided on the product labels. All spraying operations in the experimental area were conducted using a hand-operated, pressurized, two-liter plastic sprayer to ensure uniform distribution and accurate targeting. A separate sprayer was used for each treatment throughout the experiment to prevent cross-application.

Figure 3. General view of the geranium plants used in the experiment

Temperature and relative humidity data were obtained from the Çiftlikköy–Çukurköy early warning station of the Yalova Provincial Directorate of Agriculture and Forestry (Figure 3). The recorded data covered the period from April 23, 2023, when the experiment was established, to May 31, 2023, when the results were collected. On April 23, 2023, the average temperature and relative humidity were 9.58°C and 84.5%, respectively, whereas on May 31, 2023, the average temperature and relative humidity were 17.5°C and 87.41%, respectively. During the experimental period, the maximum temperature and relative humidity recorded were 22.75°C and 99.84%, respectively. These environmental conditions were considered suitable for the development and spread of *Botrytis cinerea* (Figure 4).

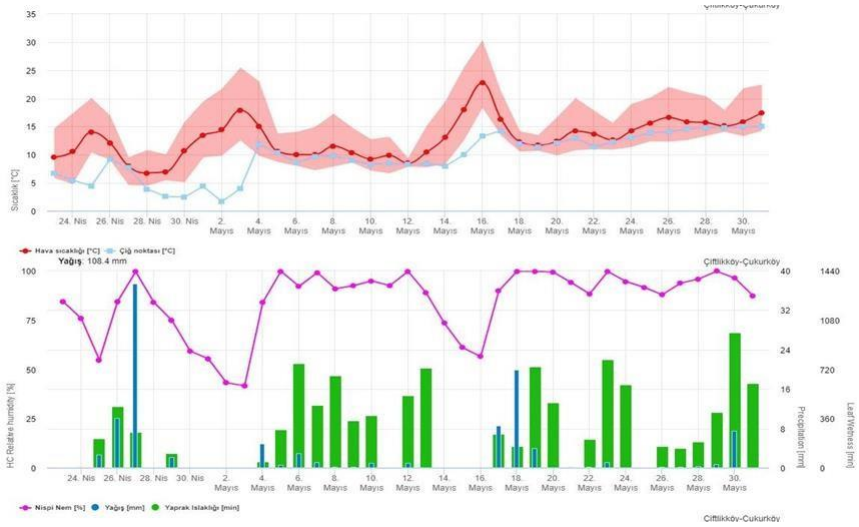

Figure 4. Humidity and temperature values between the start and end dates of the trial in Yalova province

The trial was established on April 24, 2023, and arranged according to a randomized block design with four replicates, each consisting of ten plants (pots). Ten-day-old pure *Botrytis cinerea* cultures, developed on PDA medium, were moistened with 2 mL of sterile distilled water. The spores were scraped using a sterile glass spatula and collected in sterile beakers, after which the inoculum was filtered through sterile gauze to remove mycelial fragments. The spore suspension was quantified using a Thoma counting chamber and adjusted to a density of 5×10^5 spores/mL. Tween 20 was added as a surfactant, and the volume of water required for uniform plant coverage was calibrated prior to application. The pathogenic fungus was inoculated onto geranium plants in all plots except the negative control, followed by the first spraying of the treatments. Subsequent sprayings were applied at one-week intervals, with a total of five applications performed during the trial period. Spraying continued until the disease incidence in the control plots reached 20%. Pesticides were applied at the manufacturer-recommended dosages, expressed as g or mL of formulation per decare in 100 L of water, and the appropriate application rate was selected. For each replicate, the volume of water required per application was determined through calibration prior to spraying (Anonymous, 2022).

All plants in the plot were assessed according to the following 0–10 scale (Elmhirst et al., 2011, Table 4). The % disease severity calculation according to the Elmhirst et al. (2011) scale was performed using the Tawsend and Heuberger (1943) formula, and the % effect ratio calculation was performed using the Abbott formula (Karman 1971).

$$\% \text{ Disease severity} = [\Sigma (n \times v) \div V \times N] \times 100$$

$$\% \text{ Effect} = [\text{Index in control} - \text{Index in application} \div \text{Index in control}] \times 100$$

Table 4. Gray mold disease assessment scale

Scale Value	Definition	Scale Value	Definition
0	No disease in the plant		
1	1–10% of the plant is infected		
2	11–20% of the plant is infected		
3	21–30% of the plant is infected		
4	31–40% of the plant is infected		
5	41–50% of the plant is infected		
6	51–60% of the plant is infected		
7	61–70% of the plant is infected		
8	71–80% of the plant is infected		
9	81–90% of the plant is infected		
10	91–100% of the plant is infected and the leaves have fallen off		

Disease severity was assessed when the incidence in the positive control plots reached at least 20% (Anonymous, 2022). The percentage efficacy of the fungicides was calculated using Abbott's formula. Disease rates were determined based on the scale values obtained from the plant counts required for the formula. Statistical analyses were performed using the SPSS software package, and differences between treatments were evaluated using the LSD (Least Significant Difference) multiple comparison test.

RESULTS AND DISCUSSION

Gray mold symptoms on geranium plants were first observed on May 14, 2023. Disease severity was evaluated on May 30, 2023, using a 0–10 scale (Elmhirst et al., 2011). Negative control plants remained symptom-free (scale value = 0), whereas the positive control plants reached a scale value of 6, corresponding to 51–60% of the plant being infected (Figure 5). The application of commercial fungicides was halted after disease incidence in the positive control exceeded 20% (Anonymous, 2022).

Figure 5. Plant score according to the gray mold assessment scale

As shown in Table 5, the average disease severity of gray mold in positive control plants is 46%, followed by the application of the biological preparation derived from *Trichoderma harzianum* (strain Krl – AG2 400 Million spores/g) with an average disease severity value of 36.25%. The disease severity in plants treated with *T. harzianum* (strain Krl – AG2 400 million spores/g) was statistically different from the disease severity in the control group according to the LSD test, and this difference was statistically significant ($p \leq 0.01$). In the application of the biological preparation derived from the antagonist bacterium *Bacillus amyloliquefaciens* (MBI 600 $>5.5x10^{10}$ cfu/g strain), the disease severity value was 33.5%, which was approximately the same level of effectiveness as the previously mentioned biological preparation.

Table 5. Evaluation of disease severity caused by gray mold in geranium plants under biological control appliactions

Replications	(-) Control	(+) Control	cyprodinil (%37,5) + fludioxonil, (%25)	Tea tree oil (222,5 g/l)	*T.harzianum Rıfai* str. Krl – AG2	*B.amyloliquefaciens* MBI 600 str.
1	0[1]	44	10	18	38	37
2	0	48	7	21	35	32
3	0	46	6	18	36	34
4	0	46	12	23	36	31
Mean	0	46a	8,75d	20c	36,25b	33,5b

[1]The difference between values shown with different letters in different columns is significant at p≤0.01. Each value is the average of four replicates, with 10 plants evaluated in each replicate.

The biological fungicide derived from *T. harzianum* (strain Krl – AG2 400 million spores/g) was statistically in the same group as the biological fungicide derived from *B. amyloliquefaciens* (strain MBI 600 >5.5 x10^{10} cfu/g) (p≥0.01) and were effective against gray mold at rates of 21.19% and 27.16%, respectively (Table 6, Figure 8, and Figure 9).

Table 5 indicates that the positive control group recorded the greatest mean gray mold severity at 46%. This was followed by the treatment with the *Trichoderma harzianum*–based biological preparation (strain Krl–AG2, 400 million spores/g), which resulted in an average disease severity of 36.25%. According to the LSD test, the disease severity observed in plants treated with *T. harzianum* (strain Krl–AG2, 400 million spores/g) was statistically different from that of the control group, and this difference was highly significant ($p \leq 0.01$). In the application of the biological preparation derived from the antagonist bacterium *Bacillus amyloliquefaciens* (strain MBI 600, >5.5 × 10^{10} cfu/g), the disease severity was determined as 33.5%, indicating an efficacy level comparable to that of the previously mentioned biological treatment. The biological fungicide formulated from *T. harzianum* (strain Krl–AG2, 400 million spores/g) was statistically in the same group as the *B. amyloliquefaciens*-based fungicide (strain MBI 600, >5.5 × 10^{10} cfu/g) ($p \geq 0.01$), showing control efficiencies of 21.19% and 27.16%, respectively, against gray mold (Table 6, Figure 8, and Figure 9).

Table 6. Effectiveness of treatments against grey mould disease in the trial (%)

replication	cyprodinil (%37,5) + fludioxonil, (%25)	Tea tree oil (222,5 g/l)	*T.harzianum Rıfai* Str. Krl – AG2	*B.amyloliquefaciens* MBI 600 str.
1	78,26[1]	60,86	17,39	19,56
2	84,78	54,34	23,91	30,43
3	86,95	60,86	21,73	26,08
4	73,91	50,0	21,73	32,60
Mean	80,97	56,51	21,19	27,16

[1]Each value is the average of four replicates, with 10 plants evaluated in each replicate.

Figure 6. Comparison with control plants following Switch 62.5 WG application

Figure 7. Comparison with control plants following Timorex Gold application

Figure 8. Comparison with control plants following T-22 Planter Box application

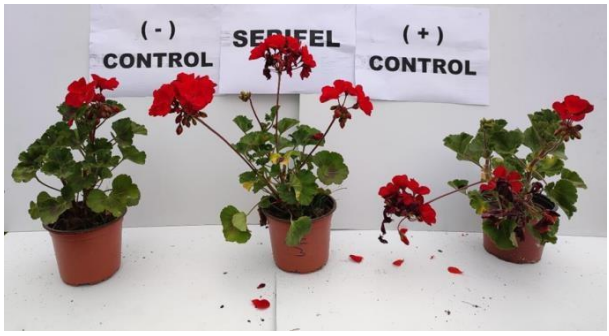

Figure 9. Comparison with control plants following Serifel application

In the prevention of gray mold disease in geranium plants, the plant-based preparation, tea tree extract, exhibited higher efficacy (56.51%) compared to both biological preparations tested in the experiment (Table 6; Figure 7). Among all treatments, the chemical fungicide included as a reference to compare the effectiveness of the biologically derived preparations demonstrated the highest control, reducing disease severity to 8.75% and achieving an efficacy of 80.97% (Table 6; Figure 6).

Among the treatments applied, the chemical fungicide combination cyprodinil (37.5%) + fludioxonil (25%) exhibited the highest efficacy, controlling the disease at 80.97%. The plant-based preparation, tea tree oil (222.5 g/L), achieved 56.51% control, while the biological fungicides *Trichoderma harzianum* (strain Krl–AG2) and *Bacillus amyloliquefaciens* (strain MBI 600) showed control rates of 21.19% and 27.16%, respectively.

Previous studies conducted worldwide on gray mold disease in ornamental plants have reported the presence of fungicide-resistant *B. cinerea* isolates derived from various hosts. It has been suggested that the overexpression of transporter genes is predominant in these isolates compared to low-resistant and sensitive ones; nevertheless, this resistance does not appear to extend to the active ingredient fludioxonil (Acosta et al. 2024). Abbey et al. (2024) aimed to characterize the resistance profile of *B. cinerea* isolated from burnt flowers and fruits in their research. It was determined that more than 80% of the isolates were susceptible to fludioxonil, fluopyram, and fenhexamid. Pyraclostrobin and boscalidin showed the lowest susceptibility frequencies against *B. cinerea*. As a chemical control test fungicide, the chemical preparation containing 37.5% cyprodinil + 25% fludioxonil active ingredient was preferred (Table 3). In another study on geranium plants against *B. cinerea*, the effect of a preparation containing the fungus *Ulocladium atrum* as a biocontrol agent was compared with that of Euparene M fungicide treatment (Gerlagh et al. 2001). In the study, in the first trial, although *U. atrum* was less effective than the fungicide, its application reduced the mortality of 4-week-old cuttings (mortality rates were 1.2%, 20%, and 38% for fungicide application, *U. atrum* application, and control, respectively). In the second trial, only the fungicide was found to reduce cutting loss. The study discussed the effect of integrating *U. atrum* into the control system for *B. cinerea* in geraniums.

The study discussed the effect of integrating *U. atrum* into the control system of *B. cinerea* in geraniums. In our study, the biological preparations applied were similarly effective in suppressing the disease as the *U. atrum* biological preparation used by Gerlagh et al. (2001) (Table 6). Shrestha (2020) examined petunia varieties in terms of their susceptibility to Botrytis blight and the biological preparations that limit Botrytis blight. Thirteen traditional and spreading (wave) petunia varieties were selected for the study, ten biorational products were evaluated for the control of Botrytis blight, and they were compared with the standard fungicide fenhexamid and untreated controls. When evaluated on the 'Shock Wave Red' petunia, *Aureobasidium pullulans* (Botector) and *Gliocladium catenulatum* (Prestop) provided similar control of *B. cinerea* to the standard fungicide fenhexamid (Decree) in both trials. Except for the area under the disease progression curve data in Trial 1 with

Pseudomonas chlororaphis (Zio) applications, disease severity ratings and area values similar to the fungicide standard were determined in both trials.

At the end of the trial, based on disease severity assessment, it was determined that treatment with soybean and corn oil (Pure Crop 1) and *Ulocladium oudemansii* (Botry Stop) and *Bacillus mycoides* (Life Gard) provided a control similar to that of the fungicide fenhexamide in test 30, but was not significantly different from the untreated control. The results obtained from this study showed that some biological preparation products can limit *B. cinerea* when used in combination with a disease-resistant variety. Among the biologically derived fungicides applied in the trial, the plant preparation containing tea tree extract yielded the most effective result after the chemical fungicide. In the study, the two biological preparations applied against the disease, tea tree oil and chemical fungicide, were similar in their effectiveness, being less effective than each other. According to our trial findings, the effectiveness of the preparation containing tea tree oil extract was found to be higher than in these studies (Table 6). In their study, Xu et al. (2017) aimed to investigate the antifungal mechanisms underlying the strong inhibitory effect of tea tree oil on *B. cinerea*, which they had previously identified, at the molecular level. The research results show that tea tree oil inhibits glycolysis, disrupts the tricarboxylic acid cycle, and induces mitochondrial dysfunction, thereby disrupting energy metabolism.

In their study, Safa et al. (2024) investigated the antifungal potential of *Cupressus sempervirens* organic extracts against *B. cinerea* in crops. Extracts obtained from various phenological stages were evaluated for their antifungal activities. The dichloromethanoic extract obtained from the flowering stage showed the highest efficacy, completely inhibiting *B. cinerea* mycelial growth at 250 μg/ml and preventing conidia germination at 500 μg/ml. In our study, the efficacy of the tea tree oil extract preparation was found to be similar to that reported in these studies (Table 6). Contreras et al. (2022) analyzed the activity of *in vitro* cultured *Colobanthus quitensis* extracts against *B. cinerea*. The research results determined that *C. quitensis* extracts are a good alternative for controlling *B. cinerea*. In their research, Köse and Soylu (2023) obtained essential oils from different types of thyme and oregano to control *B. cinerea* and investigated the effectiveness of these oils. The study found that essential oils obtained from thyme species could be an environmentally friendly method

for controlling fungal diseases. In their study, Zaldúa et al. (2010) evaluated the effectiveness of antagonistic fungi in controlling *B. cinerea* in *Eucalyptus globulus* mini cuttings. Five fungal species were tested in the study, two *Clonostachys* and three *Trichoderma* (5×10^6 conidia ml^{-1}), and applied weekly. Absolute control negative (water) and fungicide application were also used as comparison treatments. The results of the study indicate that Clonostachys is effective as a control agent against gray mold disease in *E. globulus* mini-cuttings, with efficacy comparable to the data obtained in this study (Table 6). Continued research on bio-based and biological fungicide management is essential to determine the extent to which these alternatives can decrease the dependence on conventional pesticides in ornamental plant cultivation.

CONCLUSIONS

Geranium (*Pelargonium* spp.) is an ornamental plant of considerable commercial importance, cultivated both indoors in pots and outdoors in landscape areas such as parks and gardens. The absence of a registered fungicide for the control of gray mold disease in geraniums in Turkey, along with the high sensitivity of ornamental plants to chemical fungicides, constituted the main rationale for this study. Previous research on *Botrytis cinerea* has provided valuable contributions to the existing body of knowledge; however, the findings of the present study demonstrate that biological formulations based on plant extracts, antagonistic fungi, and bacteria can also serve as effective and environmentally friendly control methods against gray mold disease. Moreover, future studies should focus on evaluating the combined application of biological preparations and tea tree extract–based formulations to enhance disease management in geraniums. It is considered that future studies should investigate combinations of biopesticides and fungicides containing plant-based active ingredients for the biological control of *B. cinerea*. It is expected that the results obtained in this study will contribute to the ongoing registration and licensing processes of biofungicides in biological control studies against grey mould disease in ornamental plants.

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CHAPTER 7

BIOLOGICAL CONTROL POTENTIAL OF OOMYCETES

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1. INTRODUCTION

Oomycetes, commonly referred to as water molds, are a phylogenetically distinct group of eukaryotic microorganisms that belong to the kingdom Straminipila (Lévesque, 2011). Although they exhibit filamentous growth and spore production similar to true fungi, molecular and ultrastructural studies have confirmed that Oomycetes are more closely related to brown algae and diatoms (Beakes et. al 2012). These organisms have evolved diverse ecological strategies, enabling them to colonize soil, water, and plant surfaces, and to function as saprophytes, pathogens, or occasionally, as biocontrol agents.

Oomycetes have long been of scientific and practical importance due to their profound impacts on global agriculture and forestry. Within this group, some of the most destructive plant pathogens are found, notably *Phytophthora infestans*, the causal agent of the Irish potato famine in the mid-nineteenth century, and *Phytophthora ramorum*, responsible for sudden oak death outbreaks in Europe and North America (Erwin & Ribeiro, 1996; Jung et al., 2000; Rizzo et al 2005). The global spread of invasive Phytophthora species has also been facilitated by modern trade, nursery practices, and climate change, raising concerns about biodiversity and ecosystem stability (Judelson & Blanco, 2005; Meentemeyer et al., 2008; Jung et al., 2018; Bourret et al., 2023).

In recent years, taxonomic revisions have refined our understanding of related genera. The genus *Phytopythium* (family Peronosporaceae s. lat., order Peronosporales) was established to incorporate species formerly placed within *Pythium* clade K. These pathogens are associated with stem and root rots across a broad spectrum of hosts, including perennial woody plants, annual field crops, forest and fruit trees, and ornamentals, and are regarded as significant contributors to crop and yield losses worldwide (Bala et al., 2010; Baten et al., 2014; de Cock et al., 2015; Baysal-Gurel & Ghimire, 2023). Species of Pythium affect a wide range of crops and ornamental plants, causing damping-off, root rots, and wilts, resulting in significant yield losses worldwide (Bielenin et al., 1976; Al-Sheikh, 2010; Moein, 2016; Astapchuk et al., 2020; Zhou et al., 2022).

Despite their destructive nature as plant pathogens, certain members of the Oomycetes exhibit remarkable potential for biological control applications. Several species of Pythium have been reported to suppress the growth of plant-pathogenic fungi and oomycetes, including *P. nunn* (Laing & Deacon, 1991);

P. acanthicum and *P. periplocum* (Ribeiro & Butler, 1995); *P. lycopersicum* (Karaca et al., 2008); and *P. oligandrum* (Deacon, 1976; Ribeiro & Butler, 1995; Belonozníkova, 2022). Among them, *Pythium oligandrum* is well recognized as a mycoparasitic species capable of parasitizing a range of pathogenic fungi and Oomycetes while simultaneously inducing systemic resistance mechanisms in host plants (Benhamou et al, 2012). Through this synergistic effect, pathogen growth is restricted while plant defence mechanisms are stimulated, resulting in greater tolerance to subsequent infections. Beyond *P. oligandrum*, several non-pathogenic or weakly pathogenic Oomycetes produce diverse secondary metabolites—such as elicitins, enzymes, and volatile compounds—with antifungal and antibacterial properties that actively promote the suppression of of soil-borne diseases.

The application of beneficial Oomycetes constitutes an innovative and environmentally sound strategy for plant disease management, coherently integrated within the framework of integrated pest management (IPM). Owing to their capacity to establish stable associations within the rhizosphere, to effectively compete with pathogenic microorganisms for ecological niches and nutrient resources, and to elicit host defense mechanisms, these organisms represent highly promising candidates for sustainable use in both agricultural and forest ecosystems. In addition, recent advancements in molecular biology and biotechnology have facilitated a more precise characterization, functional assessment, and optimization of these taxa as biological control agents, thereby providing effective and ecologically compatible alternatives to conventional chemical pesticides and supporting the transition toward sustainable and resilient production systems.

2. BIOLOGICAL CONTROL MECHANISMS OF OOMYCETES

Among them, *Pythium oligandrum* stands out as a model species that integrates multiple biocontrol strategies. It contributes to the suppression of plant pathogens both directly, through mycoparasitic interactions, and indirectly, by activating induced systemic resistance (ISR) in host plants. Moreover, its ability to rapidly colonize substrates, produce antibiotic-like secondary metabolites, and emit volatile organic compounds (VOCs) further enhances its ecological competitiveness and biocontrol efficacy.

2.1 Mycoparasitism

In fungal interactions, the process by which one fungus acquires nutrients by directly invading and exploiting another is referred to as *mycoparasitism*, a phenomenon first conceptualized by Viterbo and Horwitz (2010). At one extreme of this interaction, the host mycelium remains alive while the parasitic fungus extracts nutrients internally, maintaining a relatively stable association known as *biotrophic mycoparasitism* (van West et al., 2003). *Pythium oligandrum* represents a remarkable example of an opportunistic and beneficial oomycete that has successfully evolved from a saprophytic to a mycoparasitic and plant-associated lifestyle. As described by Benhamou et al. (2012), this species exerts its biocontrol potential through a multifaceted strategy combining direct antagonism against plant pathogens and indirect stimulation of host defense responses. Microscopic and ultrastructural observations revealed that *P. oligandrum* parasitizes several phytopathogenic fungi, including *Phytophthora*, *Pythium*, and *Fusarium* species, by adhering to and penetrating their hyphae through specialized appressoria-like structures. This interaction involves the secretion of cell wall-degrading enzymes such as β-1,3-glucanases, chitinases, and cellulases, leading to the lysis and collapse of the host mycelium. This study established that *P. oligandrum* employs a multifactorial biocontrol strategy, integrating direct mycoparasitism, rapid niche colonization, and secondary metabolite-mediated suppression. While these early experiments were primarily descriptive and *in vitro*, they laid the groundwork for subsequent mechanistic studies, including those of Benhamou et al. (2012), which elucidated the molecular underpinnings of plant defense activation and ecological persistence in soil environments. Consistently, Horner (2012) found that *Pythium oligandrum* upregulates putative effector genes in the course of mycoparasitism against *Phytophthora infestans*. The study highlighted that *P. oligandrum* is amenable to genetic transformation, allowing detailed investigation of the molecular mechanisms underlying its mycoparasitic interactions. Their findings indicate that specific molecular responses are triggered in the pathogen during parasitism, providing insights into the molecular basis of biocontrol activity and emphasizing the potential of *P. oligandrum* as a model organism for studying oomycete–oomycete interactions.

The evidence gathered from *P. oligandrum* studies provides a valuable framework for understanding similar interactions in other *Pythium* species, such as *P. nunn*, which has also been shown to parasitize and suppress pathogenic counterparts.

Kobayashi et al. (2010) conducted a detailed morphological and physiological characterization of *Pythium nunn*, a species recorded in Japan, and investigated its mycoparasitic potential against the plant-pathogenic *Pythium ultimum* var. *ultimum*. The study employed dual culture assays on potato dextrose agar (PDA) plates to assess hyphal interactions between the two species under controlled conditions. Microscopic observations revealed that *P. nunn* hyphae exhibited coiling and close adherence to those of *P. ultimum*, followed by partial degradation of the host hyphae, indicating a parasitic relationship rather than mere competition. Growth inhibition zones surrounding *P. nunn* colonies were evident, suggesting the secretion of diffusible inhibitory compounds into the medium. Importantly, *P. nunn* demonstrated no pathogenicity toward healthy plant tissues in vivo assays, supporting its potential as a non-pathogenic mycoparasitic suitable for biological control applications.

2.2 Antagonism and competition

Antagonism and competition refer to the strategies by which one microorganism inhibits the growth or establishment of another by competing for nutrients, space, or other essential resources. *Pythium oligandrum* has been extensively studied as a biocontrol oomycete exhibiting strong antagonistic activity against a wide range of phytopathogenic fungi. Brožová (2002) provided a comprehensive review summarizing early research on the antagonistic and competitive abilities of *P. oligandrum*, emphasizing its capacity to rapidly colonize substrates and suppress soilborne pathogens such as *Fusarium*, *Rhizoctonia*, *Sclerotinia*, and *Alternaria* spp. Later experimental studies confirmed that *P. oligandrum* restricts pathogen growth both by competition for nutrients and by secreting antimicrobial compounds and cell wall-degrading enzymes (Benhamou & Rey, 2012). Recent findings have further expanded the understanding of such interactions, highlighting that biocontrol oomycetes employ diverse mechanisms including nutrient depletion, space exclusion, and antibiosis to effectively suppress pathogenic fungi (van

West et al., 2003). Collectively, these strategies underline the ecological adaptability of *P. oligandrum* and its potential as a sustainable biological control agent.

As described by Alabouvette et al. (2006), microbial competition involves the regulation of population dynamics within a shared ecological niche under limited resource availability. Biocontrol oomycetes employ this strategy to suppress plant pathogens by competing for nutrients and space. The mycoparasitic species *Pythium oligandrum* is particularly noted for its strong competitive capacity against co-occurring microorganisms (Gerbore et al., 2014). Environmental and edaphic factors can modulate this interaction; for instance, Martin and Hancock (1986) demonstrated that elevated soil chloride concentrations increased *P. oligandrum* propagule density, enhancing its ability to outcompete *P. ultimum* and reduce pre-emergence damping-off in cotton. This may involve chloride-induced metabolic adaptations that improve nutrient uptake and utilization efficiency (Benhamou et al., 2012). Moreover, altered plant root exudation under such conditions could favor *P. oligandrum* activity, contributing to the overall soil suppressiveness against pathogenic oomycetes.

2.3 Induced systemic resistance (ISR)

Induced systemic resistance (ISR) is a plant defense mechanism activated by non-pathogenic microorganisms, including certain Oomycetes, which primes the plant immune system for enhanced resistance against subsequent pathogen attacks. This process involves the modulation of signaling pathways, particularly those mediated by jasmonic acid and ethylene, leading to the activation of defense-related genes and the strengthening of structural barriers.

Picard et al. (2000) provided one of the first detailed molecular insights into the elicitor-mediated biocontrol mechanisms of *Pythium oligandrum* against *Phytophthora parasitica*. The primary aim of the study was to identify and characterize molecules produced by *P. oligandrum* that could trigger systemic resistance in tomato plants, thereby limiting pathogen infection. The researchers focused on a 10 kDa protein, later named oligandrin, which was isolated from the culture filtrates of *P. oligandrum* grown under laboratory conditions. Oligandrin was purified using classical protein fractionation techniques, including ammonium sulfate precipitation and chromatographic

separation. Its molecular weight and proteinaceous nature were confirmed through SDS-PAGE and protease sensitivity assays. Tomato seedlings were treated with purified oligandrin or *P. oligandrum* culture filtrates and subsequently challenged with *P. parasitica* inoculation. Disease progression was monitored through lesion development and pathogen colonization assays. Microscopy techniques, including light and electron microscopy, were used to observe cellular responses in root and leaf tissues. The accumulation of phenolic compounds, lignin deposition, and hypersensitive-like reactions were assessed as markers of induced defense. Although limited by the year of the study, early markers of plant defense such as pathogenesis-related proteins (PR proteins) were monitored to provide molecular evidence of elicitor-triggered responses. Oligandrin treatment significantly reduced *P. parasitica* infection in tomato plants. The effect was observed both locally at the root level and systemically in above-ground tissues, indicating activation of induced systemic resistance (ISR). Histochemical analyses revealed accumulation of phenolic compounds and lignin in cell walls, as well as plasma membrane modifications in root cortical cells, which are typical hallmarks of plant defense activation. The inhibitory effect on pathogen infection was lost upon protein denaturation, confirming that oligandrin itself, rather than other secondary metabolites, was responsible for defense elicitation. While *P. oligandrum* is capable of direct mycoparasitism (as shown in later studies), Picard et al. demonstrated that its secreted molecules alone can prime the host plant, providing protection against pathogens without requiring physical contact.

These findings are supported by Benhamou and Rey (2012), who demonstrated in detail that *P. oligandrum* triggers Induced Systemic Resistance (ISR), activating cellular responses and defense signaling pathways that enhance the plant's ability to resist pathogen infection. ISR elicited by oligandrin primes the plant, enabling faster and stronger defense responses upon pathogen challenge, thereby restricting pathogen spread in both root and above-ground tissues. Consequently, ISR-inducing molecules of *P. oligandrum* represent a promising strategy for plant protection not only under laboratory conditions but also in field applications, offering an environmentally friendly and sustainable approach to biological control.

2.4 Volatile organic compounds

Volatile organic compounds (VOCs) are low-molecular-weight organic chemicals that easily vaporize at ambient temperatures and can mediate interactions between microorganisms or between microorganisms and plants. These compounds can act as signaling molecules, antimicrobial agents, or growth modulators, influencing microbial communities and plant health without requiring direct physical contact. Sheikh et al. (2023) explored the contribution of volatile organic compounds (VOCs) to the antagonistic activity of the soil-dwelling oomycete *Pythium oligandrum*, a known parasite and predator of various fungi and oomycetes. Their findings revealed that VOCs released by *P. oligandrum* markedly suppressed the development of *Pythium myriotylum*, its natural host or prey, resulting in nearly 80% inhibition of mycelial growth and around 60% reduction in zoospore production. Through gas chromatography–mass spectrometry (GC–MS), 23 VOCs were identified, with compounds such as methyl heptenone, d-limonene, 2-undecanone, and 1-octanal exhibiting the strongest inhibitory activity. Microscopic examinations demonstrated pronounced structural damage in *P. myriotylum* hyphae, including shrinkage and membrane disintegration, accompanied by elevated reactive oxygen species (ROS) accumulation. Transcriptomic analyses further indicated a temporary activation of detoxification-related genes during early exposure, which declined over time. Growth inhibition continued even after the removal of VOCs and led to diminished infection rates in plant hosts. Overall, these observations suggest that VOC emission represents an additional strategy, complementing hydrolytic enzyme activity, through which *P. oligandrum* exerts parasitic pressure and contributes to its effectiveness as a biological control agent in soilborne pathogen management.

2.5 Exudation of lytic enzymes

Biocontrol oomycetes employ lytic enzymes as a key mechanism to suppress plant pathogens by degrading their cell walls (Bělonožníková et al., 2022; Faure et al., 2020). Fungal cell walls, composed of polysaccharides and proteins, exhibit species- and cell-specific variations, yet share a conserved primary structure (Bowman & Free, 2006). *Pythium oligandrum*, a well-studied mycoparasitic species, secretes cellulases, β-1,3-glucanases, β-glucosidases, and diverse proteases to disrupt hyphal integrity and inhibit pathogen growth.

Additional enzymes, including α-mannosidase and β-galactosidase, may target glycoproteins, contributing to efficient mycoparasitism (Bělonožníková et al., 2022).

These enzymes act synergistically to weaken the pathogen cell wall, facilitating direct hyphal penetration and nutrient acquisition. The enzymatic cocktail produced by *P. oligandrum* varies depending on the host species and environmental conditions, suggesting a tightly regulated secretion system responsive to pathogen-derived signals. Genomic analyses of the mycoparasitic species *P. oligandrum* and *P. periplocum* indicate that gene duplication and horizontal transfer events in carbohydrate-active enzyme families are associated with their mycoparasitic capabilities (Liang et al., 2020). In *P. periplocum*, secreted proteases and glycoside hydrolases have been detected at higher levels during contact with *Phytophthora infestans*, emphasizing a host-induced expression pattern. Furthermore, the enzymatic degradation of pathogen cell walls can release pathogen-associated molecular patterns (PAMPs), which may activate plant immune responses and enhance overall biocontrol efficacy.

3. BIOCONTROL OF *PYTHIUM* SPP. IN SEVERAL CROPS

3.1 Biocontrol by *Pythium oligandrum*

3.1.1 Grapevine *(Vitis vinifera)*

Grapevine *(Vitis vinifera)* trunk diseases, including black foot and Botryosphaeria dieback, pose major challenges to vineyard productivity. Recent studies have demonstrated the efficacy of *Pythium oligandrum* as a biological control agent against these pathogens, highlighting both direct antagonistic effects and systemic induction of host defense mechanisms. Yacoub et al. (2020) conducted greenhouse experiments with grapevine cuttings, in which roots were inoculated with *P. oligandrum* and subsequently challenged with *Neofusicoccum parvum*. Treatments included controls, pathogen-only, *P. oligandrum*-only, and combined applications. Disease severity was quantified by measuring wood necrosis, and defense responses were assessed through real-time PCR analysis of 62 genes related to pathogenesis-related proteins, cell wall reinforcement, and hormone-mediated signaling. Root colonization by *P. oligandrum* reduced wood necrosis by approximately 65% and primed the plants for enhanced systemic defense

responses upon pathogen attack. Complementing these findings, Lopez et al. (2025) evaluated the action of a *P. oligandrum* biopesticide under vineyard conditions, assessing both disease control and impacts on rhizosphere microbial communities. *Pythium oligandrum* application significantly reduced necrosis caused by major GTD pathogens including *Neofusicoccum parvum* and *Phaeomoniella chlamydospora* by approximately 47–67%. Interestingly, the treatment did not disrupt the overall structure of rhizospheric microbial communities but rather promoted beneficial taxa such as plant growth-promoting rhizobacteria (PGPR) and other biocontrol-associated microorganisms. Collectively, these studies underscore the dual functionality of *P. oligandrum* in grapevine trunk disease management, combining root colonization, pathogen antagonism, systemic host defense activation, and positive modulation of the rhizosphere microbiome, thereby positioning it as a promising agent for integrated vineyard disease management strategies.

3.1.2 Apple (*Malus domestica*)

Rubák et al. (2023) evaluated the efficacy of a *Pythium oligandrum* biocontrol product as a postharvest treatment to control fruit rot in apples. In the study, harvested apples were treated with a formulated *P. oligandrum* preparation and stored under cold conditions conducive to pathogen development. Disease incidence and severity were monitored over the storage period, and treated fruits were compared to untreated controls. The results demonstrated a significant reduction in fruit rot in treated apples, indicating that *P. oligandrum* can effectively suppress pathogenic microorganisms through competition for colonization sites and possible production of antifungal metabolites. These findings highlight the potential of *P. oligandrum* as a sustainable postharvest biocontrol strategy, reducing reliance on chemical fungicides and enhancing fruit storage quality.

3.1.3 Tomato

The colonization dynamics of *Pythium oligandrum* in the tomato rhizosphere have been thoroughly investigated to elucidate its role in the biological control of bacterial wilt caused by *Ralstonia solanacearum* (Takenaka et al., 2008). In their experiments, tomato seedlings were inoculated with *P. oligandrum* oospores and subsequently challenged with the bacterial

pathogen under greenhouse conditions. Quantitative real-time PCR (qPCR) assays were used to monitor the population dynamics of *P. oligandrum* in the rhizosphere and to quantify its persistence in association with tomato roots. In parallel, confocal laser-scanning microscopy enabled the visualization of hyphal colonization patterns and interactions between *P. oligandrum*, plant roots, and the bacterial pathogen.

The study demonstrated that *P. oligandrum* effectively colonizes tomato roots, forming dense hyphal networks along the rhizoplane and within intercellular spaces. This stable colonization was associated with a significant reduction in *R. solanacearum* populations and a marked decrease in bacterial wilt incidence. Furthermore, *P. oligandrum* induced systemic resistance in tomato plants, as evidenced by the upregulation of defense-related genes and enhanced expression of pathogenesis-related proteins. These findings confirmed that the successful establishment of *P. oligandrum* in the rhizosphere, coupled with its ability to activate plant immune responses, underlies its strong biocontrol efficacy.

3.1.4 Ginger soft-rot

Daly et al. (2021) investigated the biocontrol potential of a newly isolated *Pythium oligandrum* strain, GAQ1, against *Pythium myriotylum*, the causative agent of Pythium soft rot in ginger. In laboratory assays, *P. oligandrum* exhibited antagonistic behavior by coiling around and penetrating the hyphae of *P. myriotylum*, leading to the pathogen's loss of viability. Dual RNA sequencing revealed that *P. myriotylum* upregulated genes encoding protease inhibitors, cellulases, elicitin-like proteins, and DNA repair enzymes in response to the interaction, indicating a defensive strategy. Conversely, *P. oligandrum* upregulated genes related to proteases, cellulases, and peroxidases, suggesting an offensive mechanism. In greenhouse trials, *P. oligandrum* effectively suppressed soft rot symptoms in ginger, demonstrating its potential as a biocontrol agent against this pathogen.

3.2 Pythium nunn

3.2.1 Azalea and sweet orange

Fang and Tsao (1995) assessed the efficacy of *Pythium nunn* as a prospective biological control organism targeting *Phytophthora* root rots in

azalea (*Rhododendron spp.*) and sweet orange (*Citrus sinensis*). The study involved inoculating plant seedlings with *P. nunn* prior to challenge with pathogenic *Phytophthora* species *(P. cinnamomi, P. parasitica, P. citrophthora)* under controlled greenhouse conditions. Assessments included disease incidence, severity scoring, and root development measurements to determine both protective efficacy and potential growth-promoting effects. The results demonstrated that *P. nunn* effectively reduced the severity of *Phytophthora* root rot in both hosts, while also promoting early root growth and seedling vigor. These findings suggest that *P. nunn* exerts its biocontrol activity through mechanisms such as competition for root colonization sites and possible production of inhibitory metabolites, highlighting its potential as a sustainable agent for managing soil-borne Oomycete pathogens in ornamental and citrus crops.

4. LIMITATIONS AND OPTIMIZATION STRATEGIES FOR BIOCONTROL OOMYCETES

Although substantial progress has been made in elucidating the biocontrol mechanisms of oomycetes, their practical implementation in agriculture still faces several constraints. Field performance often varies because of environmental fluctuations, including differences in temperature, moisture, and soil pH. The activity and persistence of these organisms can also be influenced by interactions with indigenous microbial communities, which may compete for space and nutrients (Sullam & Musa, 2021). Moreover, variations in soil composition and regional climatic factors greatly impact the consistency of biocontrol efficacy, complicating the standardization of their use in diverse production systems.

Despite the growing recognition of biocontrol agents (BCAs) as sustainable and eco-friendly alternatives to chemical pesticides, the number of commercially available products effective against oomycete pathogens remains limited. Many formulations still struggle to match the consistency and reliability of conventional agrochemicals under field conditions. Therefore, accelerating the development of efficient biocontrol products requires the identification and optimization of BCAs with robust, reproducible performance across varying environmental conditions (Taboadela-Hernanz et al, 2025).

Another major limitation concerns formulation stability and storage. The survival of biocontrol oomycetes during transportation and long-term storage remains problematic, as many preparations rapidly lose viability before field application (Brožová, 2002). However, recent advances demonstrate that optimized formulations and application technologies can significantly improve performance and reliability. For instance, a *Pythium oligandrum*-based product (Polyversum®, Biopreparáty, spol. s r.o.) has been successfully developed and commercialized, with innovative fogging application methods introduced by Bioagris (Poland). Its efficacy was confirmed in postharvest trials on apples, where treatments markedly reduced fruit rot under both cold storage and ULO (ultra-low oxygen) conditions across two seasons (Rubák et al., 2023). Such progress highlights the importance of improving formulation technology and delivery systems to enhance the stability and field effectiveness of biocontrol oomycetes.

Even though most biocontrol oomycetes are regarded as environmentally benign, potential side effects on non-target organisms—such as beneficial soil microbes, insects, or neighboring plants—cannot be entirely ruled out. Thorough ecological risk assessments are therefore essential to confirm that their use does not unintentionally disturb native ecosystems (Bailey et al., 2010). Furthermore, the regulatory procedures for approving new microbial biocontrol agents remain time-consuming and demanding, involving extensive testing for efficacy, environmental safety, and risk evaluation (Glare et al., 2012).

Finally, the widespread adoption of oomycete-based products by growers is limited by factors such as low awareness, skepticism toward biological alternatives, and higher initial costs relative to chemical pesticides. Increasing farmer engagement through extension programs, on-site demonstrations, and effective communication of economic and environmental benefits could significantly enhance acceptance and utilization (Chandler et al., 2011).

5. CONCLUSION

Oomycetes represent a phylogenetically distinct and ecologically versatile group of microorganisms that play dual roles as both devastating plant pathogens and promising biological control agents. Among them, *Pythium oligandrum* has emerged as a model organism for studying mycoparasitism,

antagonism, competition, and induced systemic resistance (ISR), offering a multifaceted approach to sustainable disease management. Its ability to parasitize a wide range of plant-pathogenic fungi and oomycetes, coupled with its capacity to stimulate host defense pathways, underlines its biotechnological potential for integration into modern crop protection systems.

Despite these advances, translating laboratory successes into consistent field performance remains challenging. Environmental variability, formulation instability, and regulatory complexities continue to limit the large-scale application of biocontrol oomycetes. Future research should therefore focus on improving formulation technologies, enhancing stress tolerance and shelf life, and unraveling the molecular basis of ecological adaptation and host interaction. Integrating omics-based tools, advanced bioformulation techniques, and precision agriculture approaches could substantially enhance the predictability and robustness of these agents under field conditions.

Ultimately, the successful incorporation of beneficial oomycetes into integrated pest management (IPM) frameworks will depend not only on scientific innovation but also on effective communication, farmer education, and the establishment of clear regulatory guidelines. With continued interdisciplinary collaboration, the development of stable, efficient, and environmentally safe oomycete-based biocontrol products can become a cornerstone of sustainable agriculture and forest health management in the face of climate change and global biosecurity challenges.

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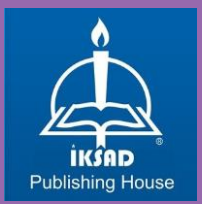

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