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Antagonistic activities of bioagent fungi *Trichoderma harzianum* and *Pleurotus ostreatus* against three species of *Fusarium* in cucumber plants

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Abstract. Many bioagent fungi have promising potential as eco-friendly alternatives to fungicides, with considerable antagonistic activity against various phytopathogenic fungi. The present study aimed to investigate the antagonistic activity of *Trichoderma harzianum* and *Pleurotus ostreatus* isolates against *Fusarium* spp., the causative agents of wilt disease in cucumber plants, through a dual plate assay of volatile and nonvolatile compounds from these bioagent fungi. The results showed significant ($P < 0.05$) antagonistic activities of *T. harzianum* against the growth of *F. solani* AJA2 (62.3%), followed by *F. oxysporum* AJA (55.2%), *F. incarnatum* AJA (53.2%), and *F. solani* AJA1 (50.8%). The effectiveness of *P. ostreatus* against the four *Fusarium* species was notably less than that of *T. harzianum*. In contrast, in the dual culture assay, the bioagent fungal filtrate exhibited inhibitory effects on the growth of all pathogens at 25% concentration. The highest inhibition rate (85%) was shown by *T. harzianum* against *F. incarnatum*. The percent of inhibition caused by *P. ostreatus* was substantially lower than that caused by *T. harzianum*, which reached 35% in *F. incarnatum* followed by other pathogens. The volatile compounds of *T. harzianum* led to a high percentage of inhibition of all the three *Fusarium* species, while the highest percentage of inhibition due to the compounds of *P. ostreatus* was observed only for *F. solani* AJA1 (41.5%). From these results, we concluded that despite the diverse inhibitory effects of both bioagent fungi against *Fusarium* species, they exhibited successful antagonistic activity and the ability to compete against these species.

Keywords: *Fusarium* species, *Trichoderma harzianum*, *Pleurotus ostreatus*, antagonistic activities.

INTRODUCTION

Endophytic fungi have been shown to possess several advantages in terms of reducing plant diseases, supporting rich biodiversity, serving as a bioresource for bioremediation of organic pollutants and heavy metals, and promoting agricultural sufficiency; thus, making them an important component of sustainable development in community ecology (Mishra & Sarma, 2018; Chen *et al.,* 2020). Several companies are developing biopesticides and biofertilizers that can be used instead of synthetic chemicals, which could reduce the amount of chemical residues in the environment. *Trichoderma* is a well-known fungus with a wide distribution. Previous studies on fungal interactions mediated by *Trichoderma*-produced volatile organic compounds (VOCs) (Oszako *et al.,* 2021; Rajani *et al.,* 2021) have demonstrated that *Trichoderma* species play a significant role in antagonizing and suppressing the growth of several plant pathogenic fungi.

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Moreover, it has been hypothesized that VOCs may play a role in this inhibitory effect of *Trichoderma*. Understanding the key drivers of VOC production during fungal coculture could be useful for leveraging this knowledge in using endophytes to combat plant pathogenic fungi. A previous study investigated the potential of four *Trichoderma* species to produce VOCs and examined their inhibitory effects on the growth of *Fusarium oxysporum*, while another study investigated the synthesis of bioactive VOCs from *T. atroviride* under a wide range of conditions and assessed their activity against the pathogenic fungus *Rhizoctonia* (Kottb *et al.,* 2015). The crude extract of *Trichoderma* contains a wide range of secondary metabolites that may possess antimicrobial capabilities, and it has been used to assess the potential of this fungus in biocontrol and other industrial applications (Li *et al.,* 2018). Speckbacher *et al.* (2020) investigated the antifungal activities of culture filtrates obtained from four *Pleurotus* spp. in liquid and solid media against a variety of phytopathogenic fungi. The authors concluded that *Pleurotus ostreatus* culture filtrate had the highest antifungal activity against pathogenic fungi. Marques *et al.* (2018) demonstrated that *Pleurotus salmoneostramineus* liquid filtrate shows high antimicrobial activity against *Pseudomonas aeruginosa* and *Candida parapsilosis*. *Pleurotus cornucopiae* mycelia suppressed the growth of *Enterococcus faecalis* and *C. parapsilosis* by 5.21% and 29.19%, respectively. Thus far, chemical fungicides have been used excessively and continuously for crop protection, thereby posing major health risks to humans, animals, and the environment. Therefore, alternative crop protection approaches must be investigated, and one of the potential methods that is both consumer and environmental friendly is the use of biological control agents and their secondary metabolites (Owaid *et al.,* 2015; Owaid *et al.,* 2017). The present study aimed to investigate the antagonistic potential of *T. harzianum* and *P. ostreatus* isolates against *Fusarium solani*, *Fusarium oxysporum*, and *Fusarium incarnatum*, the causative agents of root rot and wilt in cucumber plants, through an in vitro antagonist assay and assessment of the effect of volatile metabolites and fungal filtrate of these bioagent fungi under laboratory conditions.

MATERIALS AND METHODS

Source of bioagent and pathogenic fungal isolates

P. ostreatus isolate was kindly given and identified by Prof. Dr. Majeed M. Dewan, College of Agriculture, University of Kufa, Iraq. *T. harzianum* AJA, *F. incarnatum* AJA, *F. solani* AJA1, *F. solani* AJA2, and *F. oxysporum* AJA were morphologically and molecularly characterized and described in previous studies (Heydari *et al.,* 2010). In this experiment, *P. ostreatus* and *T. harzianum* AJA were used as bioagent fungi, while the four strains of *Fusarium* spp. were used as pathogenic fungi.

According to the type of experiment, fungal isolates were recultured on potato dextrose agar (PDA) by taking a 0.5 cm (diameter) mycelial disc from the edge of the most recent colony of each fungus and placing it on the center of a Petri plate containing sterilized PDA supplemented with chloramphenicol (0.05 g/L) at pH 5.6; the plate was incubated at 25±1°C for 7 days and then stored at 5°C.

In vitro antagonist assay

The antagonistic potential of the bioagent fungi against the fungal pathogens (*F. incarnatum* AJA, *F. solani* AJA1, *F. solani* AJA2, and *F. oxysporum* AJA) was determined using the dual culture method (Khan *et al.,* 2020). A mycelial disc (5 mm diameter) was taken from the edge of a 5-day-old culture of each pathogen and placed 1 cm away from the periphery of the Petri plate (90 mm), and a disc of the same size for each tested bioagent fungus was placed in the same way but at the opposite end of the pathogen sample. The mycelium plug of each *Fusarium* strain was inserted in the periphery of the Petri plates in the control treatment. The plates were incubated at 25±1°C in triplicate for each pairing. The average of two crossing lines in the plate's center was used to calculate radial growth (Kuzmanovska *et al.,* 2018; Nasir *et al.,* 2021). These tests were conducted when the pathogen colonized the entire surface of the medium in the control treatment.

The percent growth inhibition (PGI) was determined on the 7th day of the test by using the following equation (Miyashira et al., 2010):

PGI = [(C - T)/C X 100)]

Where PGI=Percent Growth Inhibition (%); C=Pathogen Radial Growth (Control); T=Pathogen Radial Growth (Test).

Preparation of T. harzianum and P. ostreatus extracellular culture filtrate

The potential of culture filtrates of *T. harzianum* and *P. ostreatus* isolates against the plant pathogenic fungi *F. incarnatum* AJA, *F. solani* AJA1, *F. solani* AJA2, and *F. oxysporum* AJA was evaluated using the method proposed by Fokkema (1978) and Tamur *et al.* (2018). Both bioagents and the pathogenic fungi were cultivated on PDA in plastic Petri plates. The cultures were incubated for 7 days at 25±1°C. *T. harzianum* and *P. ostreatus* isolates were grown in potato dextrose broth (PDB) by inoculating the medium with a 0.5 mm mycelial disc of a 5-day-old culture followed by incubation at 25±1°C on a shaker at 140 rpm in dark for 10 days to obtain the liquid phase with nonvolatile metabolites.

After twice filtration through a Whatman No. 1 filter paper, the fungal culture was centrifuged and sterilized using a membrane filter with a pore size of 0.22 mm. The filtrate was kept in the refrigerator in a dark bottle until required for analysis (Ali *et al.,* 2020).

Antagonistic efficacy of extracellular culture filtrates

The culture filtrates of both bioagent fungi were tested as antifungal agents in this study. For this purpose, 5-mm-diameter agar discs were cut from the cultures of *F. incarnatum* AJA, *F. solani* AJA1, *F. solani* AJA2, and *F. oxysporum* AJA and placed in the center of each Petri plate containing (25% v/v) culture filtrate. A similar procedure was performed in the control treatment by using distilled water instead of culture filtrate. The experiment was replicated thrice. Radial mycelial growth and percentage inhibition of the pathogens were assessed as mentioned in earlier (section: In vitro antagonist assay).

Volatile compound interaction assay

The effect of volatile compounds produced by *T. harzianum* and *P. ostreatus* on the growth of the four strains of *Fusarium* species was investigated using the sandwiched Petri plate method (Fokkema, 1978), with some modifications. On the top surface of *T. harzianum* and *P. ostreatus* Petri plates, each *Fusarium* strain was placed individually. Three layers of parafilm were used to seal the plates, and the plates were then incubated at 25±1°C. Each plate of pathogenic fungus was sandwiched between two uninoculated PDA plates (control treatment). The colony diameter and the percentage growth inhibition of *Fusarium* species were measured as described in section: In vitro antagonist assay.

Statistical analysis

Statistical data are presented as mean and standard deviation (SD). A one-way analysis of variance (ANOVA) was used to analyze the data, and Duncan's Multiple Range Test was used to assess whether differences in mean values were significant at α level of 0.05 (IBM SPSS Statistics software version 20.0).

RESULTS

Antagonistic activity of T. harzianum and P. ostreatus against the growth of Fusarium species

As shown in Figure 1-G1 (A1 to D1), the growth of *F. incarnatum* AJA (A1), *F. solani* AJA1 (B1), F. solani AJA2 (C1), and *F. oxysporum* AJA (D1) treated with *T. harzianum* was substantially diminished, and *T. harzianum* mycelia mostly covered the Petri plates of all pathogenic fungi after 7 days of incubation (A2 to D2). This antagonistic fungus grew rapidly and had a substantial inhibitory effect on the growth of the four *Fusarium* strains.

A similar tendency was noted for the treatment of *F. incarnatum* AJA, *F. solani* AJA1, *F. solani* AJA2, and *F. oxysporum* AJA with P. ostreatus (A3 to D3). The antagonistic fungus covered the entire Petri plate within 7 days of incubation and grew very rapidly. It was able to grow over the mycelial growth of the pathogens with an increase in the incubation period. All pathogens were susceptible to *P. ostreatus* but to a varying degree. Both *P. ostreatus* and *T. harzianum* covered the entire Petri plate and did not allow the pathogenic fungi to grow as they actively

colonized the plate inoculated with *Fusarium* species.

The PGI of pathogenic fungi in a solid culture medium was determined by dual culture testing of the interaction between *T. harzianum* and pathogenic fungi mycelia. As shown in Figure 1-G2 (S1), the highest antagonistic activities were observed against *F. solani* AJA2 (62.3%), followed by *F. oxysporum* AJA (55.2%), *F. incarnatum* AJA (53.2%), and *F. solani* AJA1 (50.8%).

P. ostreatus (Figure 1-S2) showed the highest inhibitory activity against *F. solani* AJA2 (58.4%), followed by *F. oxysporum* AJA (50.7%), *F. solani* AJA1 (47.2%), and *F. incarnatum* AJA (44.6%). The results showed a significant difference ($P<0.05$) in the PGI of the four *Fusarium* strains under the antagonistic activity of *T. harzianum* and *P. ostreatus*.

Antagonistic effect of nonvolatile compounds on the growth of Fusarium species

The results shown in Figure 2-G1 (A2 to D2) revealed that the *T. harzianum* filtrate completely inhibited the colony growth of all the four *Fusarium* strains.

Despite using an appropriate filtration procedure to obtain a pure *T. harzianum* fungal filtrate, many spores passed through the filter, which increased the antagonistic ability of this bioagent fungus to cover the entire plate and prevent pathogen growth. Therefore, a high inhibitory effect on the fungal pathogens was observed due to the direct growth of *T. harzianum* spores from the filtrate, which completely dominated the colony development of *Fusarium* species. In contrast, the mycelial growth of all the four *Fusarium* strains covered the entire surface of the untreated Petri plates during the same incubation period. The filtrate of *P. ostreatus* greatly affected the morphological characteristics such as the color, texture, and nature of colonies of all *Fusarium* species (Figure 2 right side). The filtrate of *P. ostreatus* inhibited the growth of all the four *Fusarium* strains, and the maximum inhibition was observed for *F. incarnatum*, followed by *F. oxysporum* AJA, *F. solani* AJA2, and *F. solani* AJA1. In contrast, the mycelial growth of *Fusarium* species in control samples covered the entire plate. In the dual culture assay, the fungal filtrate of *T. harzianum* and *P. ostreatus* showed varying inhibitory effects on the growth of all pathogens (Figure 2-G2 (S1 and S2)). The fungal filtrate at 25% (v/v) concentration caused substantial changes in the colony morphology of the pathogens, with a significant reduction ($P\leq0.05$) in the growth of the pathogenic fungi. The percentage inhibition varied according to the type of bioagent fungus and the strain of *Fusarium* species. The highest inhibition was caused by *T. harzianum* (Figure 2-G2 S1) in *F. incarnatum* AJA (85%), followed by *F. solani* AJA1 and *F. solani* AJA2 (76%) and *F. oxysporum* AJA (68%). In contrast, the percent inhibition caused by *P. ostreatus* (Figure 2-G2S2) was substantially lower than that caused by *T. harzianum*; the percent inhibition was 35% in *F. incarnatum* AJA, followed by *F. oxysporum* AJA (18.3%), *F. solani* AJA1 (13.2%), and *F. solani* AJA2 (12.6%).

Antagonistic effects of volatile compounds on the growth of Fusarium species

Figure 3-G1 (A2 to D2) shows that the pathogens *F. incarnatum* AJA, *F. solani* AJA1, *F. solani* AJA2, and *F. oxysporum* AJA not treated with the bioagent fungi grew very rapidly and covered the entire plates in control cultures in 7 days, whereas the growth of these pathogenic fungi that came into contact with volatile compounds released from the bioagent fungi (*T. harzianum* (A2 to D2) or *P. ostreatus* (A3 to D3)) was restricted with substantial morphological changes.

Despite differences in the inhibitory effect of *T. harzianum* and *P. ostreatus* on the growth of the Pathogenic *Fusarium* strains (Figure 3-G2 (S1 and S2)), the results revealed that both bioagent fungi produce potentially toxic volatiles that significantly affected the radial growth of the pathogenic fungi. The volatile compounds of *T. harzianum* (G2:S1) increased the percentage inhibition of all the pathogenic fungi; the highest inhibition was observed for *F. incarnatum* (50%), followed by *F. oxysporum* (35.1%), *F. solani* AJA2 (27.2%), and *F. solani* AJA1 (12.5%). The volatile compounds of *P. ostreatus* (G2:S2) caused the highest inhibition of *F. solani* AJA1 (41.5%), followed by *F. oxysporum* (36.2%), *F. solani* AJA2 (31.2%), and *F. incarnatum* (18.1%).

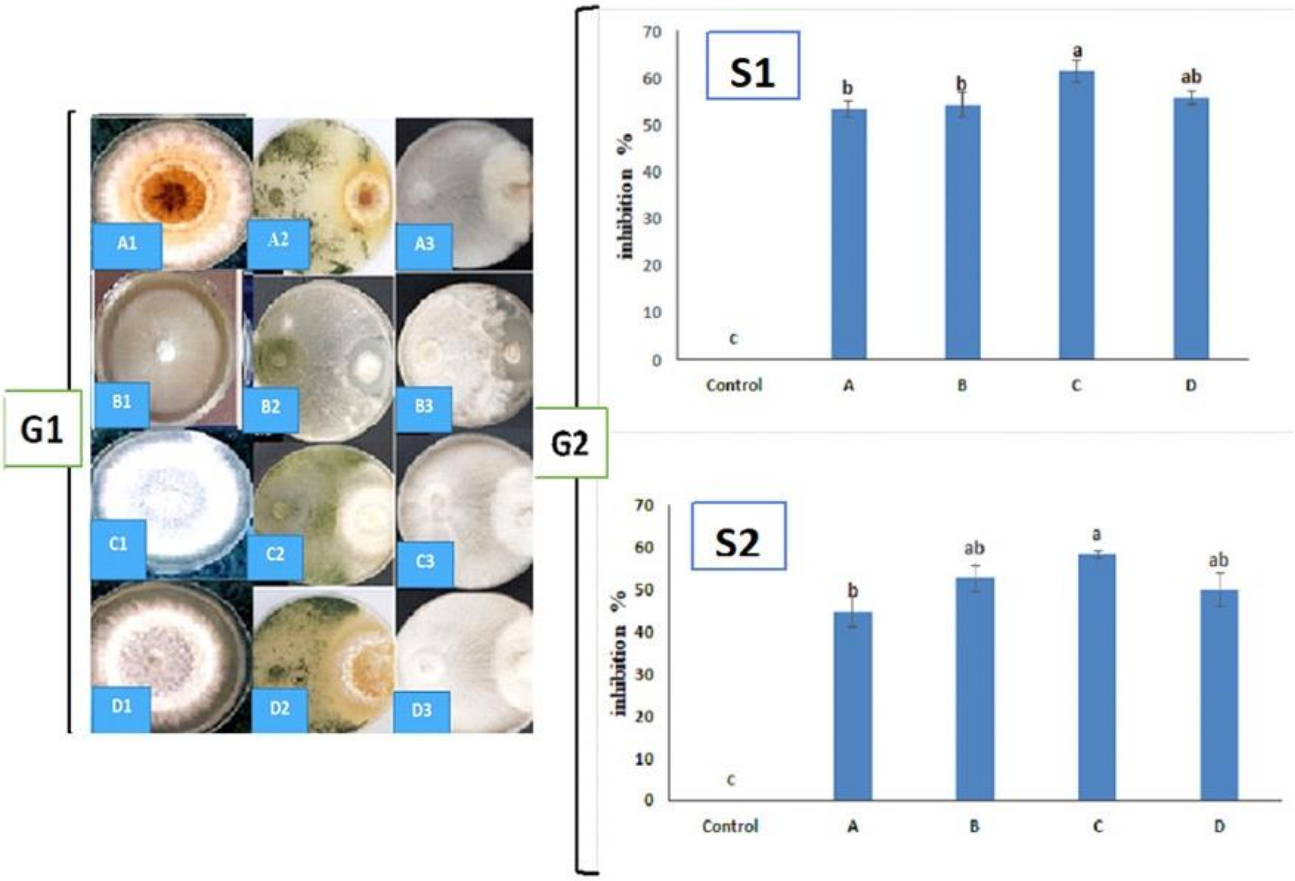

Figure 1. G1: Colony morphology of bioagent fungi (*T. harzianum* and *P. ostreatus*) and four strains of *Fusarium* species. Control treatment (left from A1 to D1): A1: *F. incarnatum* AJA, B1: *F. solani* AJA1, C1: *F. solani* AJA2, and D1: *F. oxysporum* AJA. Antagonistic activity using a dual plate assay of *T. harzianum* (middle from A2 to D2) and *P. ostreatus* (right from A3 to D3) grown on PDA medium (dual culture). G2: The percentage growth inhibition of *Fusarium* species, *F. incarnatum* AJA (A), *F. solani* AJA1 (B), *F. solani* AJA2 (C), and *F. oxysporum* AJA (D), under the antagonistic activity of *T. harzianum* (S1) and *P. ostreatus* (S2) in dual culture. The data were analyzed by comparison with control after 7 days of growth on PDA at 25±1°C. Values are expressed as mean±SE of three replicates for each treatment. Similar letters indicate no differences at P≤0.05. For the control plate, the pathogenic fungi were individually inoculated at the center of the Petri plate, but they were not treated with the bioagent fungi.

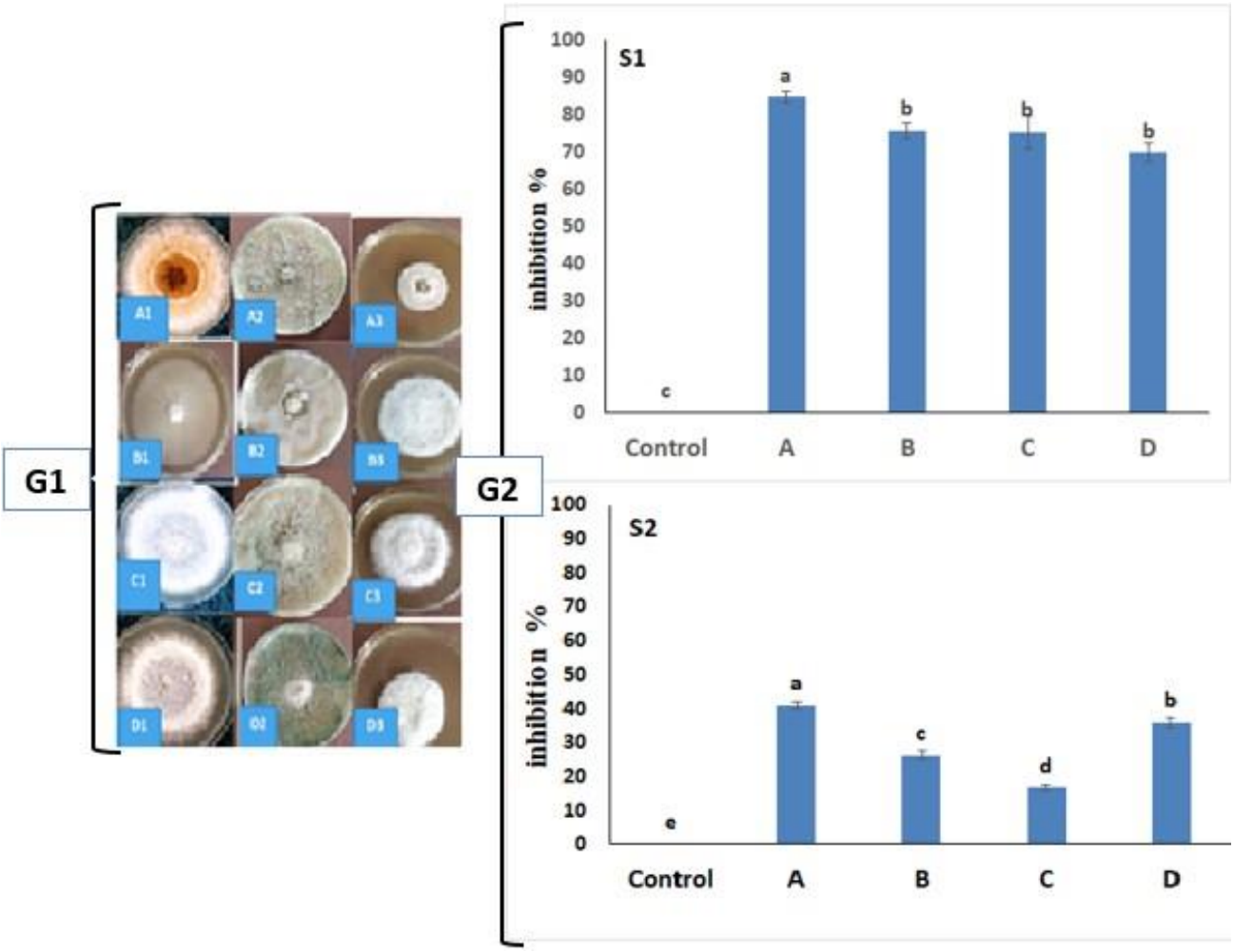

Figure 2. G1: Colony morphology of bioagent fungi (*T. harzianum* and *P. ostreatus*) and *Fusarium* species. Control treatment (left from A1 to D1): A1: *F. incarnatum* AJA, B1: *F. solani* AJA1, C1: *F. solani* AJA2, and D1: *F. oxysporum* AJA. Antagonistic activity using the fungal filtrate of *T. harzianum* (middle from A2 to D2) and *P. ostreatus* (right from A3 to D3) grown on PDA medium (dual culture). G2: The percentage growth inhibition of *Fusarium* species, *F. incarnatum* AJA (A), *F. solani* AJA1 (B), *F. solani* AJA2 (C), and *F. oxysporum* AJA (D), treated with filtrates of *T. harzianum* (S1) and *P. ostreatus* (S2). The data were analyzed by comparison with the control after 7 days of growth on PDA at 25±1°C. Values are expressed as mean±SE of three replicates for each treatment. Similar letters indicate no differences at P≤0.05. For the control plate, the pathogenic fungi were individually inoculated at the center of the Petri plate, but they were not treated with the filtrates of the bioagent fungi.

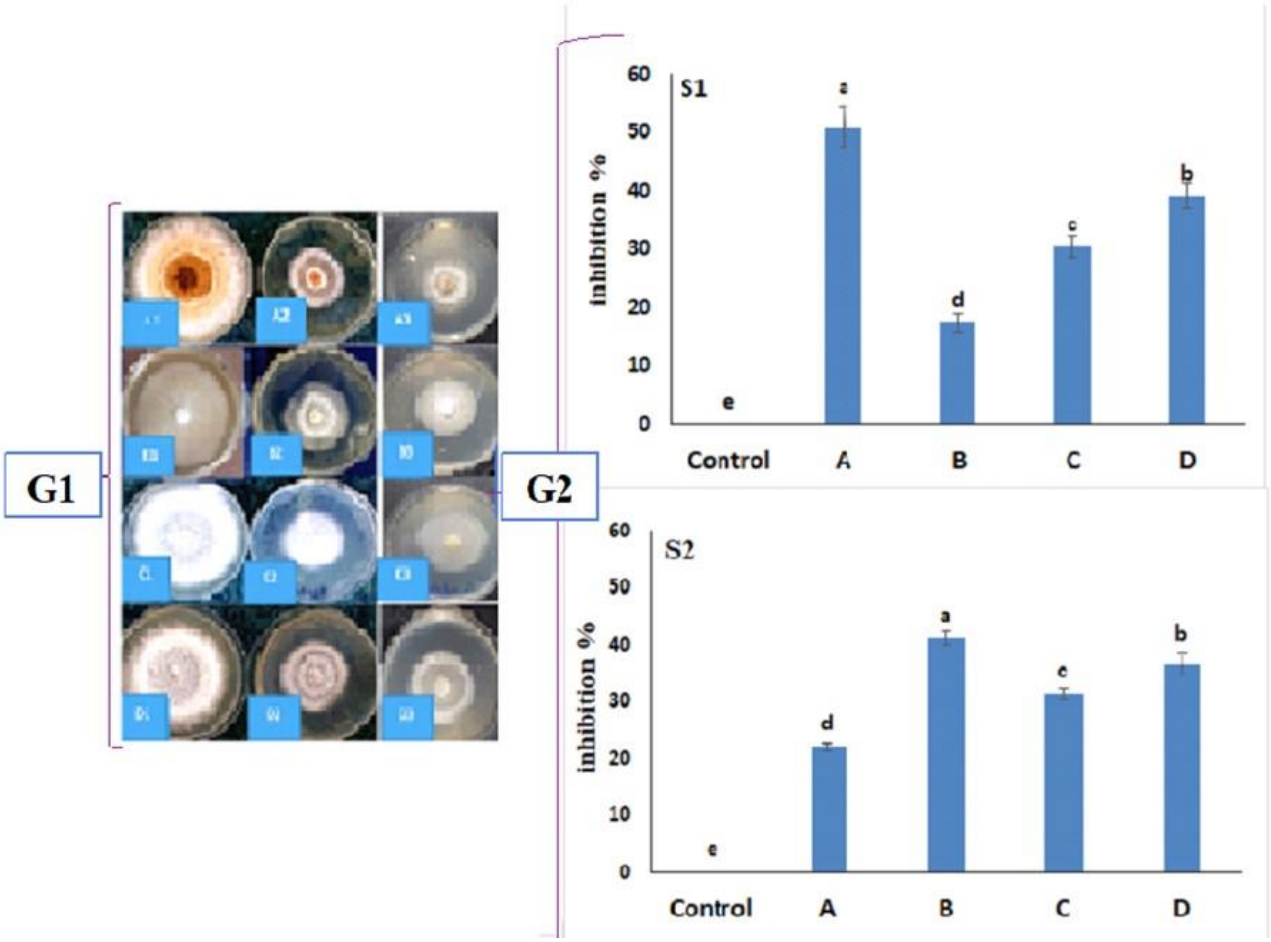

Figure 3. G1: Colony morphology of bioagent fungi (*T. harzianum* and *P. ostreatus*) and *Fusarium* species. Control treatment (left from A1 to D1): A1: *F. incarnatum* AJA, B1: *F. solani* AJA1, C1: *F. solani* AJA2, and D1: *F. oxysporum* AJA. Antagonistic activity using volatile compounds of *T. harzianum* (middle from A2 to D2) and *P. ostreatus* (right from A3 to D3) grown on PDA medium (dual culture). G2: The percentage growth inhibition of *Fusarium* species, *F. incarnatum* AJA (A), *F. solani* AJA1 (B), *F. solani* AJA2 (C), and *F. oxysporum* AJA (D), treated with filtrates of *T. harzianum* (S1) and *P. ostreatus* (S2). The data were analyzed by comparison with the control after 7 days of growth on PDA at 25±1°C. Values are expressed as mean±SE of three replicates for each treatment. Similar letters indicate no differences at P≤0.05. For the control plate, the pathogenic fungi were individually inoculated at the center of the Petri plate, but they were not treated with the filtrates of the bioagent fungi.

DISCUSSION

Several studies have reported the antagonistic activity of *Trichoderma* species against a variety of fungal phytopathogens (Dennis & Webster, 1971; Sanjay *et al.,* 2008; Kamala & Indira, 2011; Abd El-Hai *et al.,* 2019). In the present study, the antagonistic activity of *T. harzianum* and *P. ostreatus* was tested in vitro against the pathogenic fungal strains *F. incarnatum* AJA, *F. solani* AJA1, *F. solani* AJA2, and *F. oxysporum* AJA through the dual culture assay and by assessing the effect of volatile and nonvolatile compounds synthesized by *T. harzianum* and *P. ostreatus* on the growth of these fungal strains.

Antagonistic activity

The antagonistic fungi *T. harzianum* and *P. ostreatus* caused a substantial reduction in the growth of the pathogenic *Fusarium* species. The dual plate culture method showed high antagonistic activity of *T. harzianum* and *P. ostreatus* against the four *Fusarium* strains. The overgrowth of both bioagent fungi over the mycelial growth of the pathogenic fungi was observed, but sporulation

occurred only for *T. harzianum*. The colonies of *F. incarnatum* AJA, *F. solani* AJA1, *F. solani* AJA2, and *F. oxysporum* AJA treated with *T. harzianum* or *P. ostreatus* were considerably diminished and mostly covered with the mycelia of the bioagent fungi, as both bioagent fungi grew rapidly and could inhibit the growth of the four *Fusarium* isolates after 7 days of culture. The PGI of the pathogenic fungi ranged from 58.0% to 62.3% for *T. harzianum* and from 44.6% to 58.4% for *P. ostreatus*. This finding revealed that the presence of both antagonistic fungi is required for suppressing the growth of pathogenic fungal mycelia (Naglot *et al.,* 2015).

In the current study, both *T. harzianum* and *P. ostreatus* showed interesting antagonistic interactions such as overgrowth and sporulation over the four *Fusarium* strains. They also exerted a high inhibitory effect and reduced the radial growth of the pathogenic fungi. Because of their ability to produce mucolytic enzymes, both *T. harzianum* and *P. ostreatus* are used as biofertilizers, plant growth promoters, sources for bioremediation, and tools for increasing agricultural productivity (Rahman *et al.,* 2009 Stracquadanio *et al.,* 2020). Moreover, they also have novel potential as biocontrol agents (Hyder *et al.,* 2017).

Nonvolatile compounds

The fungal filtrate of both *T. harzianum* and *P. ostreatus* showed a strong inhibitory effect on the fungal pathogens, as the colony growth of all the four *Fusarium* strains was entirely suppressed by the filtrates of both bioagent fungi. In the control Petri plates, the growth of all *Fusarium* species covered the entire plate during the same incubation period. The filtrate of *P. ostreatus* greatly affected the morphological characteristics such as the color, texture, and nature of colonies of all *Fusarium* species. This change in colony characteristics might be due to the diffusion of metabolites synthesized by the bioagent fungi, while pigmentation occurred due to mycelial phenoloxidase or peroxidase activity (Rai *et al.,* 2020).

The PGI varied depending on the type of bioagent fungus and the pathogenic *Fusarium* strain. *T. harzianum* induced highest inhibition ranging from 75% in *F. oxysporum* AJA to 85% in *F. incarnatum* AJA. *P. ostreatus* also considerably inhibited the growth of *Fusarium* species, but showed a lesser degree of inhibition than *T. harzianum*; the percentage inhibition ranged from 13.2% in *F. solani* AJA1 to 35% in *F. incarnatum* AJA ($P = 0.05$).

The secondary metabolic products of *P. ostreatus* mycelia are important in preventing the growth of pathogenic fungi (Marques *et al.,* 2018). The increased activity of *P. salmoneostramineus* filtrate is because the chemical composition of the oyster mushroom broth differs depending on the type of fungal product (Adedeji *et al.,* 2016). Pathogenic fungi are inhibited by secondary metabolic products such as polysaccharides, proteins, enzymes, and triterpenoids (Chaudhary & Tripathi, 2016).

Antibiosis is an antagonistic effect induced by specific or nonspecific microbial metabolites, lytic enzymes, volatile compounds, and other inhibitory substances (Parameswari & Chinnaswamy, 2011). According to Akyuz & Kirbag (2009), *Trichoderma* spp. produce antibiotics such as trichodermin, trichodermol, and herzianolide, which show antagonistic properties against various microorganisms. Ali *et al.* (2020) observed that the culture filtrate of *Bacillus siamensis* S3 and *Bacillus tequilensis* S5 sustained their antifungal activity following thermal treatment by autoclaving and after storage at 4°C.

Volatile compounds

Volatile compounds of *T. harzianum* and *P. ostreatus* exhibited different inhibitory effects on Fusarium species. Both these fungal species produced toxic volatile compounds that significantly affected the radial growth of the fungal pathogens but to a varying degree; this was mostly related to the production of volatile organic compounds (VOCs) (Sharma *et al.,* 2013). These secreted metabolites exert an inhibitory effect on various *Fusarium* species (Küçük & Kivanç, 2005), and some such metabolites are volatile (Azevedo *et al.,* 2020). In previous investigations, bioagent fungi were found to significantly suppress the growth of *F. oxysporum, Rhizoctonia solani, Sclerotium rolfsii, Sclerotinia sclerotiorum,* and *Alternaria brassicicola* (Zeilinger *et al.,* 2016; Meena *et al.,* 2017).

Trichoderma produces a variety of volatile secondary metabolites such as ethylene, hydrogen cyanide, aldehydes, and ketones, which are

important in plant disease control (Amin *et al.,* 2010; Bhagat *et al.,* 2014). In an earlier study, six isolates of *Trichoderma* spp. showed potential to produce volatile compounds with activity against seven different fungal plant diseases. Among the six *Trichoderma* isolates studied, *T. viride* was found to be the most effective in suppressing the mycelial growth of *F. oxysporum* (41.8%). The mycelial growth and sclerotia formation in *S. rolfsii* were reduced by 40% and 48.1%, respectively. Other studies have shown that *Trichoderma* produces volatile compounds such acetaldehyde, ethylene, acetone, and carbon dioxide (Siddiquee *et al.,* 2014) as well as antibiotics such as trichodermin (Sharma *et al.,* 2016), gliotoxin, viridian (Vargas *et al.,* 2014), and ergokonin (Rai *et al.,* 2016).

Ebadzadsahrai *et al.* (2020) reported that VOCs of *Chromobacterium vaccinii* showed the strongest fungal growth inhibition against *Phoma* sp. and *Coleophoma* sp. Also, found that the cocultures of bacteria and fungi have emergent volatile metabolome properties.

CONCLUSION

The antagonistic activity of *T. harzianum* and *P. ostreatus* in reducing the growth of three pathogenic *Fusarium* species that cause wilt disease in cucumber plants is insufficiently reported in the literature. In the present study, we found that both *T. harzianum* and *P. ostreatus* isolates effectively suppressed the growth of *Fusarium* species. To manage wilt diseases caused by *F. incarnatum* AJA, *F. solani* AJA1, *F. solani* AJA2, and *F. oxysporum* AJA in cucumber plants, more research on this disease is needed in this field. Although *T. harzianum* and *P. ostreatus* showed differences in their inhibitory effects on *Fusarium* species, both these species showed effective antagonistic activity and the ability to compete with *Fusarium* species. To the best of our knowledge, this is the first study to reveal the antagonistic potential of both *T. harzianum* and *P. ostreatus* against *Fusarium* species, and the findings of this study may act as a catalyst for further research.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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