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Original article

Antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* strains against some fusarial pathogens causing stalk rot disease of maize, *in vitro*

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ABSTRACT

Objectives: A high incidence of stalk rot disease of maize causes huge economic losses as well as deleterious effects on the environment and human health resulting from fungicide use. Against this background, the current study was established to evaluate the antagonistic efficacy of *Trichoderma viride* and *Trichoderma harzianum* strains against *Fusarium proliferatum* and *Fusarium verticillioides* strains, the most common causative agents of stalk rot disease of maize.

Methods: Dual culture assay was performed to determine the antagonistic efficacy of *Trichoderma* strains against some fusarial pathogens of maize. Mycoparasitic relationships of the antagonistic fungal strains against fungal pathogens were investigated using a slide culture technique. Furthermore, a food poisoning technique was performed to detect the antimycotic efficacy of *Trichoderma* culture filtrates against fusarial pathogens of maize. Antifungal activity of organic solvent extracts of *T. harzianum* and *T. viride* was evaluated using the disc diffusion method. GC–MS analysis was used to detect the active components of these extracts.

Results and conclusion: Trichoderma viride showed antagonistic activity against *F. proliferatum* and *F. verticillioides* with mycelial inhibition rates of 80.17% and 70.46%, while *T. harzianum* exhibited rates of 68.38% and 60.64%, respectively. The culture filtrates of *T. viride* and *T. harzianum* strains exhibited antifungal activity against *F. verticillioides* strain with suppressive rates of 56.7% and 32.2%, while the mycelial inhibition rates against *F. proliferatum* strain were 44.09% and 23.50%, respectively. Mycoparasitic action of *T. harzianum* strain against fusarial strains was detected, while no mycoparasitism was observed between *T. viride* and fungal pathogens. The fungicidal concentration of carbendazim fungicide against *F. proliferatum* was 2.00 ppm, while *F. verticillioides* strain exhibited resistance to carbendazim fungicide. Moreover, the acetonic extracts of *T. viride* and *T. harzianum* strains exerted the highest antifungal potency against *F. proliferatum* strain, recording minimum inhibitory concentrations of 0.25 and 0.50 mg/ml, respectively. The main bioactive constituents of the acetonic extracts of *T. viride* and *T. harzianum* strains were palmitic acid (22.87%) and acetic acid (21.36%), respectively. In conclusion, the antagonistic strains could be a potential source of novel biological fungicides, especially against carbendazim-resistant *F. verticillioides* strain, avoiding side effects of chemical fungicides.

1. Introduction

Maize is considered to be a key crop for food security, which is cultivated globally across an area of 160 million hectares. It has high nutritional value, which is due to its levels of carbohydrates, lipids, vitamins, proteins, and minerals (Da Silva et al., 2017). Fusarial stalk rot disease reduces the yield of maize crops in severely affected areas by 30%–50%. This disease appears as white

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or light pink mold on the kernels of maize crops (Oldenburg et al., 2017). *Fusarium verticillioides* is considered one of the major causative agents of fusarial rot disease of maize (*Zea mays* L.). This fungus was reported to produce a group of mycotoxins called fumonisins, which are toxic to humans and animals (van Rensburg et al., 2016). Recently, fumonisins were reported to exert deleterious effects on crop yield, resulting in huge economic losses (Silva et al., 2017). Furthermore, Pfordt et al. (2020) reported that *F. verticillioides* and *F. proliferatum* were the most common fungal pathogens causing stalk rot disease of maize. The application of fungicides to manage fungal diseases is potentially associated with health hazards due to the deleterious environmental impacts of these fungicides on terrestrial and aquatic ecosystems. Several harmful effects of fungicides threaten human health have been reported, including neurological, gastrointestinal, dermatological, and carcinogenic effects (Thakur et al., 2014). Recently, *Trichoderma* spp. have been reported to be eco-friendly biological control agents for managing plant diseases, which enable the use of chemical fungicides to be minimized (Puyam, 2016). *Trichoderma* species are abundant in all types of soil and are considered as potential antagonistic agents against parasitic soil-borne microorganisms (Shahid et al., 2014). Biological control agents were reported to play a key role in the successful management of fungal plant pathogens (Zhang et al., 2013). The antagonistic activity of *Trichoderma* sp. against different plant pathogens occurs through different mechanisms of action, including antibiosis, mycoparasitism, and competition for nutrients and space (Ghazanfar et al., 2018). *Trichoderma harzianum* and *Trichoderma viride* strain have been reported to possess antagonistic activity against *Fusarium oxysporum* strain, demonstrating mycelial growth inhibition rates of 75.7% and 67.7%, respectively (Singh et al., 2018). Given the harmful effects of chemical fungicides on human and animal health, in addition to the high economic losses due to the high incidence of stalk rot disease of maize, alternative biological control agents are needed. Against this background, the current study was established to evaluate the antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* strains against *Fusarium proliferatum* and *Fusarium verticillioides*, the most common fusarial pathogens causing stalk rot disease of maize.

2. Materials and methods

2.1. Fungal strains

Fusarial pathogens, *Fusarium verticillioides* ATTC 204499 and *Fusarium proliferatum* ATTC 76097, were used in the present study. Two antagonistic strains, *Trichoderma harzianum* ATTC 52443 and *Trichoderma viride* 26802, were tested for their antagonistic potency against fusarial pathogens. Fungal strains were cultured on freshly prepared potato dextrose agar (PDA) medium and incubated for 5 days at 28 °C. The fresh cultures were subcultured on PDA slants and kept in a refrigerator until use.

2.2. Dual culture assay

Evaluation of the antagonistic potency of *T. harzianum* and *T. viride* strains against fusarial strains was performed using a dual culture technique. Mycelial discs (8 mm in diameter) were cut from the margins of a 3-day-old culture of each strain using a cork borer. Mycelial discs of both antagonistic strains and fungal pathogens (*F. verticillioides*, *F. proliferatum*) were inoculated over freshly prepared PDA medium and placed 3 cm away from both edges of the plates. In the control group, PDA plates were inoculated only with mycelial discs of fungal pathogens. Both treated and control plates were incubated at 25 °C for 7 days. The mycelial inhibition rate was calculated as follows: Percentage of mycelial inhibition = (A – B)/A × 100, where A refers to the mycelial growth of fungal pathogens in control plates and B refers to the mycelial growth of fungal pathogens in dual culture plates (Awad et al., 2018).

2.3. Mycoparasitic relationships of Trichoderma sp. against fungal pathogens

A slide culture technique was used to evaluate the mycoparasitic behavior of *Trichoderma* strains against fusarial pathogens. A cube of PDA medium was cut using a sterile blade and placed over a sterile glass slide. The cube was inoculated with fusarial pathogens from one side and with antagonistic strains from the other. The glass slide was inoculated at 25 °C for 5 days. After incubation, the agar cube was removed and a sterile cover slip was placed over the mycelium. Finally, the mycelial interactions between antagonistic strains and fusarial pathogens were inspected using a light microscope (40×) (Naglot et al., 2015).

2.4. Antifungal potency of the culture filtrates of Trichoderma sp. against fungal pathogens

A food poisoning technique was performed to detect the antimicrobial potency of culture filtrates of *T. harzianum* and *T. viride* strains against different fungal pathogens. Mycelial agar discs (8 mm in diameter) were inoculated into freshly prepared potato dextrose broth medium and the inoculated flasks were incubated over a rotatory shaker (150 rpm) at 28 °C for 7 days. The cell-free filtrates were attained through filtration using double layers of muslin followed by centrifugation at 9000 rpm for 10 min to discard fungal spores that could potentially disrupt the membrane sterilization. The *Trichoderma* filtrates were further sterilized using Millipore filters (22 μm) and then the filtrates were added to PDA medium to obtain a final concentration of 25% v/v. Mycelial discs (8 mm in diameter) of fusarial pathogens were inoculated into PDA plates supplemented with *Trichoderma* sp. filtrates. Control PDA plates were inoculated with 8 mm mycelial discs and then both treated and control groups were incubated at 28 ± 1 °C for 7 days. The mycelial diameters of fungal pathogens in control and treated plates were measured using Vernier calipers and the mycelial inhibition rate was calculated as follows:

$$\%\ inhibition\ =\ (A\ -\ B)/A\ \times\ 100$$

here, A refers to the radial growth of the fusarial pathogens in the control group and B refers to the mycelial growth diameter of the fungal pathogens in the treated group (Sreedevi et al., 2011).

2.5. Antifungal efficacy of standard fungicide (carbendazim)

The antifungal activity of carbendazim fungicide against the tested fusarial pathogens was detected using a food poisoning technique. Different concentrations of carbendazim fungicide (5.0, 1.00, 1.50, 2.00, 2.50, and 3.00 ppm) were added to the freshly prepared PDA medium. Eight millimeter mycelial discs of fungal pathogens were inoculated into the center of the treated plates. Control PDA plates were also inoculated with 8 mm mycelial discs of fungal strains. Both control and treated groups were incubated at 28 ± 1 °C for 7 days. The radial growth diameter of fungal mycelium was measured using Vernier calipers and the rate of mycelial growth inhibition was calculated as follows:

$$\%\ inhibition\ =\ (A\ -\ B)/A\ \times\ 100.$$

here, A is the mycelial growth diameter of fusarial pathogens in the control group and B is the radial growth diameter of mycelial phytopathogens in PDA plates treated with carbendazim fungicide (Anand et al., 2010).

2.6. Preparation of Trichoderma crude extracts

Mycelial agar discs (8 mm in diameter) of the *Trichoderma* strains were cultured on 250 ml of potato dextrose broth medium and incubated over a rotatory shaker (150 ppm) at 28 °C for 7 days. Filtration of the fungal cultures was performed using Whatman filter paper no. 1 for the removal of *Trichoderma* spores and mycelia. The culture filtrates were centrifuged at 9000 rpm for the complete separation of cell pellets. The fungal metabolites were extracted from the cell pellets using methanol solvent. Furthermore, the antifungal metabolites were extracted from the culture filtrates using hexane, n-butanol, and acetone with different polarities of 0.1, 3.9, and 5.1, respectively, to ensure the extraction of all active constituents. The extracts were concentrated using rotatory evaporator to eliminate the solvents (Jantarach and Thanaboripat, 2010).

2.7. Antifungal activity of Trichoderma extracts against fusarial pathogens

A disc diffusion method was used to detect the antifungal efficacy of different *Trichoderma* extracts against *F. proliferatum* and *F. verticillioides* strains. The dried extracts were dissolved in their corresponding organic solvents to attain a final concentration of 10 mg/ml. Ten milliliters of PDA medium was poured into sterile Petri dishes as a basal medium, followed by the addition of 15 ml of seeded medium. The seeded medium was prepared by mixing 1 ml of fungal spore suspension of different *Fusarium* strains (10^6 spores/ml) with 100 ml of PDA medium. Sterile filter paper discs (8 mm in diameter) were loaded with the extracts (10 mg/disc) and placed over the seeded plates. Fluconazole antifungal discs (125 μg/disc) were used as positive controls. The plates were incubated at 28 °C for 5 days and the diameters of the inhibition zone were measured using Vernier calipers (Mostafa et al., 2020).

2.8. Determination of minimum inhibitory concentration of the acetonic extracts of T. harzianum and T. viride

The minimum inhibitory concentration (MIC) was determined for the acetonic extracts as these extracts exhibited the highest antifungal activity. Ten milliliters of PDA medium was poured into sterile Petri dishes as a basal medium, followed by the addition of 15 ml of seeded medium. The sterile filter paper discs (8 mm in diameter) were loaded with different concentrations (0.25, 0.50, 1.00, 2.00, 4.00, and 8.00 mg/ml) of the acetonic extracts of *T. harzianum* and *T. viride* strains then the discs were dried and placed over the seeded plates. The plates were incubated at 28 °C for 5 days and the diameters of the inhibition zone were measured using Vernier calipers. The lowest concentration of the extract exhibiting antimicrobial activity was recorded as MIC (Yassin et al., 2020).

2.9. GC–MS analysis of the acetonic extracts of T. harzianum and T. viride strains

Chemical analysis of the acetonic extracts of *T. viride* and *T. harzianum* strains exhibiting the highest antimicrobial activity was performed using GC–MS using GCMS-QP2010 Plus (Shimadzu, Japan). The analytical conditions were adjusted as described by Yassin et al. (2020). Identification of active ingredients was conducted by comparing the results of GC–MS analysis with reference standards in the NIST database.

2.10. Statistical analysis

The antagonistic activity data were statistically analyzed with GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) using one-way analysis of variance and Tukey's test. The data were tabulated as mean of triplicates ± standard error and will be considered statistically significant when the ($P < 0.05$).

3. Results and discussion

3.1. Dual culture assay

Trichoderma viride and *T. harzianum* strains showed antagonistic efficacy against fusarial pathogens causing stalk rot disease of maize, as shown in Fig. 1. *Trichoderma viride* strain showed greater inhibition against the *Fusarium* strains than *T. harzianum* strain. *Trichoderma harzianum* strain suppressed the mycelial growth of fusarial pathogens (*F. proliferatum* and *F. verticillioides*) by 68.38% and 60.64%, while *T. viride* strain did so at 80.17% and 70.46%, respectively, as shown in Fig. 2. Our results accord with those of (Nwankiti and Gwa, 2018), who confirmed the antagonistic potency of *T. harzianum* strain against *F. oxysporum*, with a mycelial inhibition rate of 45.69%. In addition, (Abhiram and Masih, 2018) reported that *T. viride* strain suppressed the mycelial growth of *F. oxysporum* strains demonstrating inhibition rates in the range of 62.50%–71.00%. Furthermore, the antagonistic potential of *T. harzianum* strain was evaluated by (Gwa and Nwankiti, 2017), who stated that *T. harzianum* suppressed the mycelial growth of *Fusarium moniliforme* strain, recording an inhibition rate of 58.70%.

3.2. Mycoparasitic relationships of Trichoderma sp. against fungal pathogens

Trichoderma harzianum strain exhibited mycoparasitic action against *F. proliferatum* and *F. verticillioides* strains, while *T. viride* showed no mycoparasitism. Mycoparasitism of *T. harzianum* strain was detected in the form of adhesion, coiling, penetration, and lysis of the fusarial mycelium, as shown in Fig. 3. Several mechanisms of action have been reported to enhance the effectiveness of *Trichoderma* spp. as biological control agents, including mycoparasitism, antibiosis, and competition for nutrients and space (Druzhinina et al., 2011). Lysis of the fusarial mycelium may be attributable to the ability of *Trichoderma* spp. to produce cell wall-degrading enzymes including β-(1,6)-glucanases, chitinases, and proteases (Sood et al., 2020). Ojha and Chatterjee (2011) confirmed that *T. harzianum* strain exerted mycoparasitic behavior against *F. oxysporum* strain through coiling, formation of aspersorium, and lysis of the fungal mycelium.

3.3. Antifungal potency of the culture filtrates of Trichoderma sp. against fungal pathogens

Culture filtrates of *T. viride* suppressed mycelial growth of *F. proliferatum* and *F. verticillioides*, recording inhibition rates of 32.3% and 56.7%, while *T. harzianum* recorded mycelial inhibition rates of 23.5% and 44.09%, respectively, as seen in Fig. 4. Our findings are consistent with those of Aswini et al. (2016), who reported the antimicrobial efficiency of culture filtrates of *T. viride* and *T. harzianum* strains at a concentration of 5% v/v against *F. oxysporum* strain, recording mycelial inhibition rates of 51.53% and 24.71%, respectively. Marques et al. (2018) confirmed the antifungal potency of culture filtrates of *Trichoderma* isolates against *F. oxysporum* strains and attributed the potent activity of these filtrates to the production of active secondary metabolites.

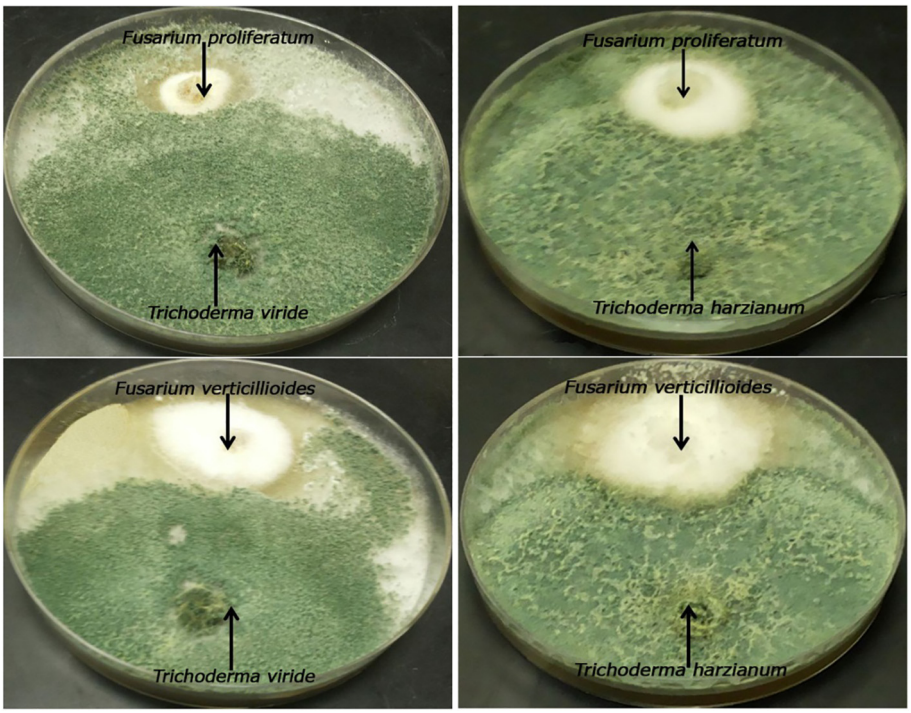

Fig. 1. Dual culture assay of antagonistic strains (*T. harzianum* and *T. viride*) against fusarial pathogens (*F. proliferatum* and *F. verticillioides*).

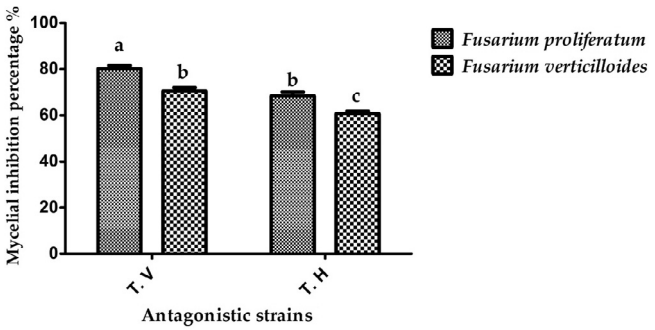

Fig. 2. Antagonistic activity of *T. harzianum* and *T. viride* strains against fusarial pathogens causing stalk rot disease of maize.

3.4. Antifungal efficacy of standard fungicide (carbendazim)

At a concentration of 2.00 ppm, carbendazim showed fungicidal activity against *F. proliferatum* strain, while *F. verticillioides* exhibited resistance to this fungicide at all tested concentrations, as demonstrated in Table 1. Carbendazim belongs to a group of broad-spectrum fungicides called the methyl benzimidazole carbamate (MBC) fungicides, which play important roles in combating fungal diseases of many agricultural crops (Karuppaiyan et al., 2015). Resistance of fusarial strains to MBC fungicides was reported in previous studies (Zhang et al., 2016). MBC fungicides bind to β-tubulin, resulting in the inhibition of tubulin biosynthesis and suppression of fungal cell mitosis (Ma and Michailides, 2005).

3.5. Antifungal activity of Trichoderma extracts against fusarial pathogens

The acetonic extract of *T. harzianum* strain exhibited the highest antifungal efficacy against *F. proliferatum* and *F. verticillioides* strains, with suppressive zones of 27.6 and 22.4 mm, respectively, as seen in Fig. 5. In contrast, the hexanic extract of *T. harzianum* strain exerted the lowest anti-fusarial activity against *F. proliferatum* and *F. verticillioides*, with suppressive zones of 13.3 and 10.5 mm, respectively. These findings accord with those of (Jantarach and Thanaboripat, 2010), who evaluated the antifungal potency of organic solvent extracts of four different *Trichoderma* isolates. These researchers stated that the hexane extracts showed the lowest antimicrobial efficiency against *Aspergillus flavus* IMI 242684 strain, with a suppressive zone diameter of 6 mm, while the ethyl acetate extracts exhibited the highest efficacy with inhibition zone diameters ranging from 7.6 to 37 mm. Moreover, the methanolic extract of *T. viride* showed the lowest antimicrobial efficacy against *F. proliferatum* and *F. verticillioides*, with inhibition zone diameters of 14.7 and 10.4 mm, while the acetonic extract exerted the highest activity, with suppressive zones of 33.2 and 27.8 mm, respectively, as shown in Fig. 6.

3.6. Determination of minimum inhibitory concentration of Trichoderma acetonic extracts against F. proliferatum strain

The minimum inhibitory concentration (MIC) was detected for the acetonic extracts of *T. viride* and *T. harzianum* strains against *F. proliferatum* strain, which exhibited the highest susceptibility to these extracts. The acetonic extracts of *T. viride* and *T. harzianum* strains demonstrated antifungal efficiency against *F. proliferatum* strain, recording MIC values of 0.250 and 0.50 mg/ml, respectively, as seen in Fig. 7. The MIC data contradicted those reported in a previous study, which indicated that the alcoholic mycelial extract of *T. viride* showed anti-fusarial potency against *Fusarium solani* and *Fusarium oxysporum* strains with an MIC value of 100 μg/ml. (Jantarach and Thanaboripat, 2010) reported that the MIC value of the ethyl acetate extracts of *Trichoderma* isolates against *A. flavus* strain was 1.0 mg/ml, recording suppressive zones ranging from 6 to 13.8 mm in diameter. The difference in MIC values between our findings and the previous studies is attributed to the variation in sensitivity of different fungal strains.

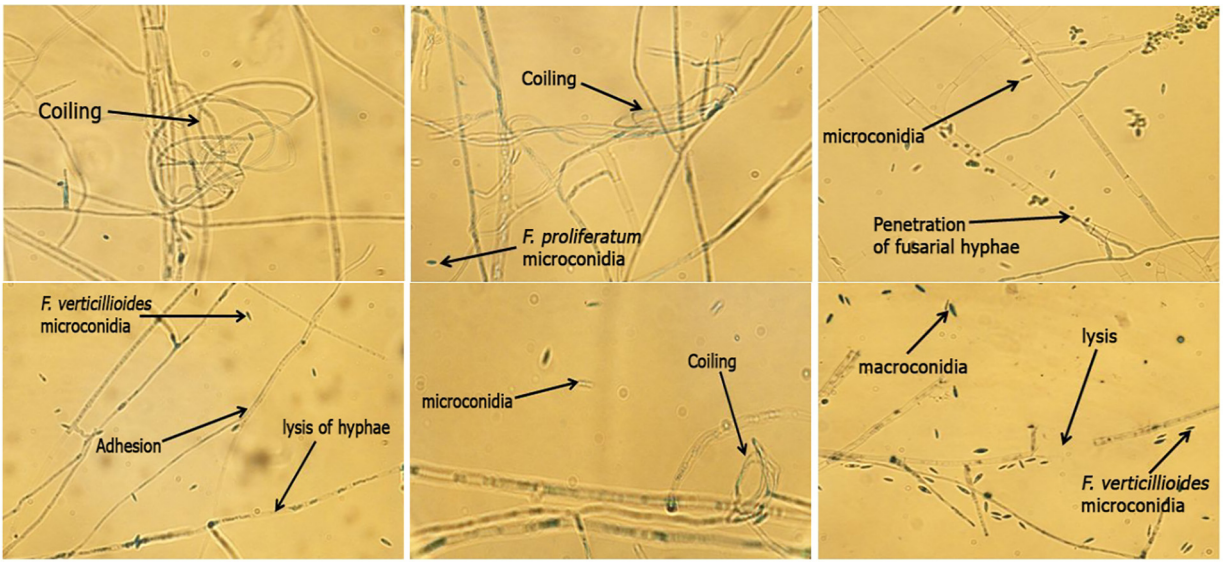

Fig. 3. Mycoparasitism of *T. harzianum* strain against *F. proliferatum* and *F. verticillioides* strains.

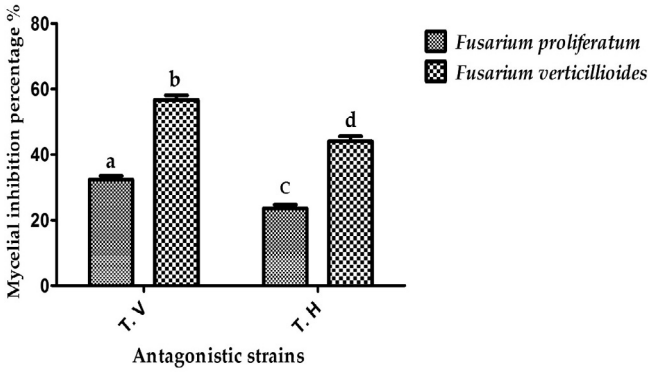

Fig. 4. Anti-mycotic potency of culture filtrates of *T. harzianum* and *T. viride* strains against fusarial pathogens.

Table 1
Antifungal activity of carbendazim fungicide against *F. proliferatum* and *F. verticillioides* strains.

Carbendazim Concn., ppm	Radial growth diameter (Mycelial inhibition percentage %)	
	F. proliferatum	*F. verticillioides*
0.00	75.24 ± 0.15^{a} (0.00%)	57.81 ± 0.32^{a} (0.00%)
0.50	46.27 ± 0.27^{b} (38.5%)	72.14 ± 0.54^{b} (0.00%)
1.00	21.18 ± 0.19^{c} (71.9%)	69.42 ± 0.47^{b} (0.00%)
1.50	12.67 ± 0.34^{d} (83.2%)	65.76 ± 0.26^{b} (0.00%)
2.00	0.00 ± 0.00^{e} (100%)	59.14 ± 0.28^{c} (0.00%)
2.50	0.00 ± 0.00^{e} (100%)	54.17 ± 0.17^{c} (6.3%)
3.00	0.00 ± 0.00^{e} (100%)	49.18 ± 0.58^{c} (14.9%)

* Different superscript letters in a column indicated that values were significantly different at ($P < 0.05$).

3.7. GC–MS analysis of the acetonic extracts of T. viride and T. harzianum strains

The acetonic extracts of *T. viride* and *T. harzianum* strains were analyzed to determine their active chemical constituents. Active constituents of *T. viride* acetonic extract were palmitic acid (22.87%), propyl benzene (12.75%), oleic acid (10.85%), caryophyllene oxide (9.32%), limonene (8.91%), β-eudesmol (7.14%), propanoic acid (6.78%), 1-pentanol (5.21%), cholic acid (4.93%), α-bisabolol (3.48%), octadecenoic acid (2.98%), ethyl benzene (2.54%), and chavicol (1.87%) as demonstrated in Table 2. GC–MS results were in accordance with those of Ali and El-Ghonemy (2014), demonstrating that the main active ingredient of the n-hexane extract of *T. viride* was palmitic acid (30.01%). Previous studies indicated that palmitic acid, octadecenoic acid, cholic acid, propanoic acid, β-caryophyllene, limonene, and β-eudesmol compounds inhibited the mycelial growth of different pathogenic fungal strains (Yun and Lee, 2016; Mohy El-Din and Mohyeldin, 2018; Cai et al., 2019). The antifungal activity cannot be attributed to the main constituent (palmitic acid) only but also to the presence of other bioactive constituents which were reported to have antifungal potency. On the other hand, bioctivity may be attributed to the synergistic effect between different bioactive components (Liu et al., 2016). Additionally, the main active component of *T. harzianum* acetonic extract was acetic acid (21.36%), followed by 2-phenylethyl alcohol (14.61%), hexadecanoic acid (12.98%), diisooctyl phthalate (10.67%), harzianic acid (9.45%), 6-pentyl-alpha-pyrone (7.98%), dihydroxyacetone (5.95%), 1-hexadecanol (4.78%), xylene (3.26%), 2H-pyran-2-one (2.39%), and 9-eicosane (2.14%), as shown in Table 3. The anti-fusarial potency of *T. harzianum* acetonic extract may be attributable to the presence of many bioactive ingredients as acetic acid, harzianic acid, 6-pentyl-alpha-pyrone and 2H-pyran-2-one, 2-phenylethyl alcohol, dihydroxyacetone, hexadecanoic acid, and 9-eicosane respectively. The antifungal potency of the previous extract may also be referred to the synergism between different bioactive components (Khan et al., 2020). The above conclusion was confirmed by Vinale et al. (2009) who reported that harzianic acid was one of the main components of *T. harzianum* extract and proved the effectiveness of this acid as an anti-mycotic agent against *Pythium irregulare*, *Sclerotinia sclerotiorum*, and *Rhizoctonia solani* strains. On the other hand, the bioactive alkyl pyrones produced by *T. harzianum* strain, such as 6-pentyl-alpha-pyrone and 2H-pyran-2-one, were reported to possess antifungal properties (Zeilinger et al., 2016). Moreover, Ismaiel and Ali (2017), showed that 6-pentyl-alpha-pyrone suppressed the mycelial growth of filamentous phytopathogenic fungal strains by 93.5% at a concentration of 250 μg/ml. Furthermore, 2-phenylethyl alcohol, dihydroxyacetone, hexadecanoic acid (Liu et al., 2014), and 9-eicosane compounds were reported to have antimicrobial potency against different fungal strains (Ahsan et al., 2017).

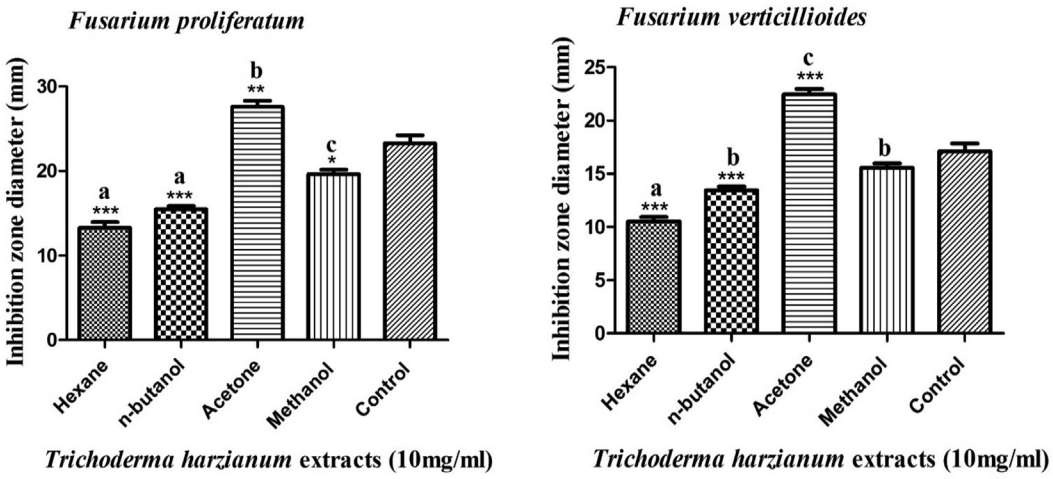

Fig. 5. Antifungal activity of different organic extracts of *T. harzianum* strain against *F. proliferatum* and *F. verticillioides* strains.

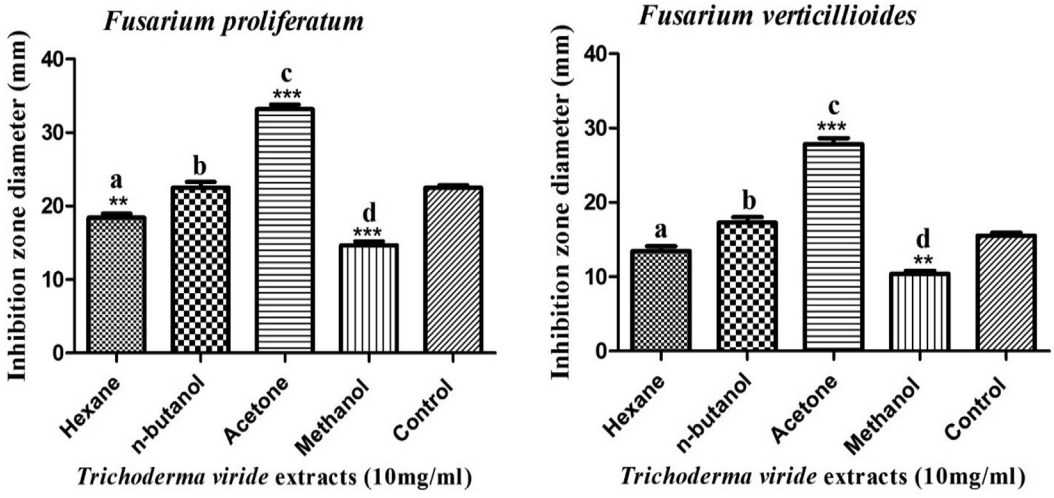

Fig. 6. Antifungal activity of different organic extracts of *T. viride* strain against *F. proliferatum* and *F. verticillioides* strains.

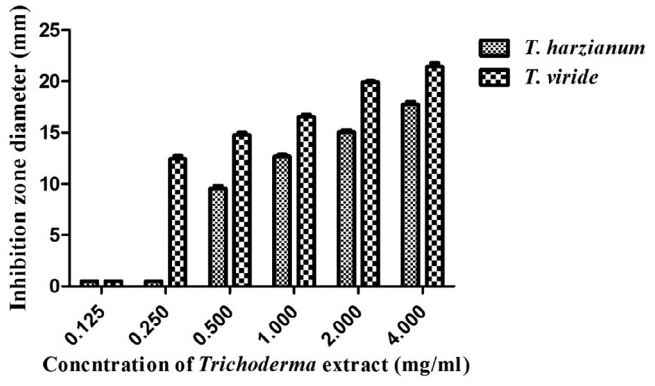

Fig. 7. Minimum inhibitory concentration (MIC) of *T. viride* and *T. harzianum* acetonic extracts against *F. proliferatum* strain.

4. Conclusion

The antagonistic strains *T. harzianum* and *T. viride* exhibited potential antimycotic activity against fusarial phytopathogens of maize. The potent antagonistic potency of culture filtrates and organic solvent extracts against fungal pathogens of maize highlights the ability to apply novel and safe biofungicides in order to avoid the harmful impacts of chemical fungicides on the environment and human health. The acetonic extracts exhibited the highest antifungal efficiency, especially against carbendazim-resistant *F. verticillioides* strain, highlighting the potential for using these bioagents for controlling resistant fungal phytopathogens.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 2
GC–MS analysis of the acetonic extract of *Trichoderma viride* strain.

% of Total	RT (min)	M.W.	Chemical formula	Compounds
6.78	6.812	74.08	$C_3H_6O_2$	Propanoic acid
5.21	7.248	88.15	$C_5H_{12}O$	1-Pentanol
2.54	7.921	106.16	C_8H_{10}	Ethyl benzene
12.75	9.453	120.19	C_9H_{12}	Propyl benzene
1.87	10.342	134.18	$C_9H_{10}O$	Chavicol
8.91	11.708	136.23	$C_{10}H_{16}$	Limonene
9.32	12.075	220.35	$C_{15}H_{24}O$	Caryophyllene oxide
3.84	13.734	222.37	$C_{15}H_{26}O$	α-Bisabolol
7.14	14.281	222.37	$C_{15}H_{26}O$	Beta-Eudesmol
22.87	16.167	256.42	$C_{16}H_{32}O_2$	Palmitic acid
2.98	21.389	282.47	$C_{18}H_{34}O_2$	Octadecenoic acid
10.85	24.981	282.47	$C_{18}H_{34}O_2$	Oleic acid
4.93	33.076	408.57	$C_{24}H_{40}O_5$	Cholic acid

Table 3
GC–MS analysis of the acetonic extract of *Trichoderma harzianum* strain.

% of Total	RT (min)	M.W.	Chemical formula	Compounds
21.36	7.365	60.05	$C_2H_4O_2$	Acetic acid
5.95	8.174	90.08	$C_3H_6O_3$	Dihydroxyacetone
2.39	9.268	96.08	$C_5H_4O_2$	2H-pyran-2-one
3.26	10.798	106.16	C_8H_{10}	Xylene
14.61	11.243	122.16	$C_8H_{10}O$	2-Phenylethyl alcohol
7.98	13.945	166.22	$C_{10}H_{14}O_2$	6-pentyl-alpha-pyrone
4.78	15.243	242.44	$C_{16}H_{34}O$	1-Hexadecanol
12.98	18.964	256.42	$C_{16}H_{32}O_2$	Hexadecanoic acid
4.56	21.435	280.45	$C_{18}H_{32}O_2$	Linoleic acid
2.14	23.675	282.50	$C_{20}H_{42}$	9-Eicosane
9.45	26.783	365.40	$C_{19}H_{27}NO_6$	Harzianic acid
10.67	31.483	390.60	$C_{24}H_{38}O_4$	Diisooctyl phthalate

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