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RESEARCH ARTICLE

In vitro biocontrol mechanism of *Trichoderma* spp. against crown rot pathogens in banana

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Abstract

Crown rot, caused by various fungal pathogens, is a major post-harvest disease of bananas, leading to significant storage losses. Biocontrol by antagonistic microorganisms is a promising alternative to synthetic fungicide application. *Trichoderma* spp. are well-known biological control agents due to their strong antagonistic properties. Soil samples were collected from banana-cultivated orchards in the districts of Mayurbhanj, Jagatsinghpur, Jajpur and Ganjam. A total of 4 *Trichoderma* species were isolated using the serial dilution method: *T. asperellum* (8 isolates), *T. atroviride* (4 isolates), *T. harzianum* (5 isolates) and *T. hamatum* (6 isolates). Based on their micro-morphological and cultural characteristics, bioagents were identified. *Trichoderma* isolates were selected for *in vitro* testing against the *Fusarium* species by conidia germination assay and dual culture assay. Among the tested isolates, *T. asperellum* showed the highest inhibition of conidial germination (88.24 %) and mycelial radial growth (91.22 %) of *F. equiseti*. *T. atroviride* showed the lowest level of inhibition of conidia germination and mycelial radial growth against the *F. equiseti*. Solid-phase microextraction (SPME) was applied to trap volatiles emitted by *T. asperellum*. The GC/MS profiling revealed the presence of antifungal compounds, including azetidine, 1-Methylideneindene, phenylethyl alcohol and fluoro(trinitro)methane, which are involved in antifungal activity and the dominant compound was tentatively identified as phenylethyl alcohol (PEA), making up 21.79 % of the peak area with 96.24 % match in 7.15 retention time. This study indicates that *T. asperellum* is an effective antagonistic biocontrol agent and can produce volatile antifungal compounds that involve major mechanisms against *Fusarium* spp. *in vitro* conditions.

Keywords: crown rot; *Fusarium* spp.; GC-MS analysis; phenylethyl alcohol; *Trichoderma* spp.

Introduction

India is the world's leading banana grower, accounting for 25-27 % of global output, followed by China and Indonesia with 9.6 % and 6.4 %, respectively (1). *Fusarium* crown rot and fruit rot are common post-harvest banana diseases that cause significant problems (2). Crown rot, a disease complex produced by different fungi, is one of the most serious post-harvest banana diseases. The most common fungi responsible for banana crown rot disease include *Fusarium* spp., *Lasiodiplodia theobromae*, *Thielaviopsis paradoxa* and *Colletotrichum musae* (3). Other *Fusarium* species associated with crown and fruit rot include *F. camptoceras*, *F. concentricum*, *F. concolor*, *F. fujikuroi*, *F. equiseti*, *F. incarnatum*, *F. oxysporum*, *F. proliferatum*, *F. pseudocircinatum*, *F. sacchari*, *F. solani* and *F. verticillioides* (2, 4–6). Typical symptoms include dark brown to black necrotic lesions on the peel, which vary in shape and size.

Although chemical fungicides are commonly used to manage post-harvest fungal diseases, their overuse poses environmental and health risks and contributes to the development of resistant pathogen strains (7). These limitations have increased the demand for sustainable alternatives such as biological control methods. *Trichoderma* species have various beneficial properties, such as antibiosis, competition with the pathogens for nutrients and space, mycoparasitism and induction of systemic resistance to control disease pathogens and promote plant growth (8-14). Endophytes are well known for producing unique biologically active secondary metabolites that provide significant ecological benefits to their host plants. Antibiosis is one of the strongest defensive mechanisms of *Trichoderma* species and its endophytic interaction in nature provides an effective combination of defense responses against various plant pathogenic fungi (15). Among the mechanisms, antibiosis is particularly important, often mediated through the production of volatile organic compounds (VOCs) and cell wall-degrading enzymes. VOCs from *Trichoderma* can inhibit fungal growth and may trigger host defense responses (16-19). *Trichoderma* species are significant biocontrol agents in managing disease across various plants, yet the exact mechanisms behind their antagonistic effects are not fully understood. The production of VOCs and their biological effects vary significantly among species and even among strains within a

species and are influenced by substrate and environmental conditions. This study investigates the potential of volatile organic compounds released by endophytic *Trichoderma* as a biofumigant against post-harvest crown rot disease in bananas caused by *Fusarium* species. The isolates were screened for antagonistic activity, with a focus on elucidating the underlying mechanisms responsible for pathogen suppression.

Materials and Methods

Isolation of fungal pathogens

In the disease management study, three *Fusarium* species were isolated from various banana cultivars (Champa: AAB, Patakapura: AAB and Grand Naine: AAA) exhibiting crown rot symptoms across ten districts of Odisha. The agar plate and standard paper blotter techniques were used to isolate pathogens associated with banana crown rot disease, followed by purification using the hyphal tip or single spore methods. The isolated fungi were identified based on the morphological characteristics of their colonies and conidium morphological characters (20). Molecular analysis confirmed the fungal pathogens as *F. verticillioides* (Accession no. OQ363325), *F. equiseti* (Accession no. OP735534) and *F. oxysporum* (Accession no. OQ438654). We amplified the internal transcribed spacer (ITS) sections, including the 5.8S rDNA gene of four fungal isolates, using universal ITS1 and ITS4 primers. These cultures were grown on potato dextrose agar (PDA), incubated in the dark at 28 ± 2 °C for 3-4 days and used for various experiments.

Soil collection and transport

In this investigation, *Trichoderma* isolates were collected from the soil of banana orchards and gardens in four districts of Odisha: Mayurbhanj, Jagatsinghpur, Jajpur and Ganjam (Table 1). Each sample contained around 200 g of soil collected at a depth of about 20 cm. The samples were placed in sterile polyethene bags, transported to the laboratory and kept at 4 °C until they were isolated.

Isolation and culturing procedure

Trichoderma was isolated from soil samples using the procedure described in the previous study with minor modifications (21). This approach is simple, inexpensive and appropriate for large samples. *Trichoderma* colonies were cultured using the serial dilution method. To make a stock solution, 10 g of the soil sample was dissolved in 100 mL of distilled water in a 250 mL conical flask and mixed thoroughly on a rotary shaker (MaxQ Mini 4450) set to 210 rpm for 30 min. 1 mL of the solution was combined with 9 mL of sterile distilled water to make a 10^{-1} dilution. The soil suspension was further diluted to 10^{-4} and 10^{-5} concentrations to calculate colony-forming units (CFUs). 1 mL of each diluted solution was pipetted onto a petri dish with 9 mL of *Trichoderma* selective medium (TSM), which was then swirled manually until solidification. The plates were then incubated at 25 ± 2 °C for 7 days to facilitate the growth of fungal colonies, which appeared as small white spots. These colony-forming units (CFUs) were subsequently transferred to fresh potato dextrose agar (PDA) for further culturing. The pure culture was stored using the lyophilisation method for future use as stock cultures.

Morphological identification of bioagents

Trichoderma strains were aseptically re-cultured from slant agar and transferred to fresh PDA medium petri dishes. They were incubated at room temperature (25 ± 2 °C) for 7 days. The experiment was conducted in triplicate. All isolates with similar traits were selected for morphological and microscopic identification. Regular observations focused on colony morphology, pigmentation, conidia size and conidiophore branching patterns. Following a modified procedure, the slide culture technique was used for the microscopic evaluation of *Trichoderma* strains (22). Fungal hyphae were cut and immersed in 1 % lactophenol cotton blue before being examined under the microscope. After 3 days, the slide culture was examined under a light compound microscope at 40X magnification. *Trichoderma* isolates were identified at the species level using microscopic observation of fungal structures and morphological characteristics (21, 23-27).

Antagonistic activity of *Trichoderma* spp. against *Fusarium* isolates

To evaluate the antagonistic activity of *Trichoderma* spp. against the *Fusarium* strain, conidial germination and dual culture assay were conducted.

Conidia germination assay

The conidia germination assay was carried out using the procedure developed by previous research with minor changes (28). The bio-control isolates were cultured separately for one week at 28 ± 2 °C in 250 mL conical flasks with potato dextrose broth (PDB). After incubation, fungal cells were extracted by filtering the culture through two layers of muslin cloth, followed by a 0.2 µm millipore filter. Aliquots of *Trichoderma* culture filtrate (125 µL) were mixed in sterile Eppendorf tubes with 125 µL of *Fusarium* strain spore suspension (1 ×10^6 CFU/mL) in PDB broth (1:1 ratio). Each mixture was then placed onto a cavity microscope slide with sterile distilled water and thoroughly mixed. The slides were placed in petri dishes within a glass container and incubated for 72 hr at 28 °C before being viewed under a microscope. Conidial suspensions of the *Fusarium* strain in sterile distilled water were used as controls. The number of germinated conidia was determined by inspecting the first 100 spores randomly under a light microscope. Conidia germination was evaluated until at least 80 % of the control conidia germinated (29). Conidia germination inhibition was expressed as a percentage of mm (% CGI) and calculated using the following formula (28):

$$\%\ \text{inhibition}\ (\%\ \text{I}) = (1 - \%\ \text{St} / \%\ \text{Sc}) \times 100 \qquad \text{(Eqn. 1)}$$

where % St is the percentage of spores germinating in the treatment and % Sc is the percentage of spores germinating in the control. Each treatment contained ten replicates and the

Table 1. The denotation of soil samples

Place of collection of soil sample	Sample code	Treatments	Latitude and longitude
Udala, Mayurbhanj	MT1	T_1	21.5772° N, 86.5653° E
Panikoili,Jajpur	MT2	T_2	20.9122°N, 86.2286° E
Bhanjanagar,Ganjam	MT3	T_3	19.9358°N, 84.5825° E
Paradip, Jagatsinghpur	MT4	T_4	20.3166° N, 86.6114° E

experiments were repeated twice.

Dual culture assay

Four *Trichoderma* spp. strains were investigated for antifungal activity against *Fusarium* isolates by evaluating their effect on mycelial growth using a dual-culture experiment on PDA plates (30). A 5 mm agar plug from a 7-day-old *Fusarium* colony was placed on one side of 9 cm petri dishes, with a 5 mm agar plug from each *Trichoderma* strain placed on the opposite side, 5 cm away from the pathogens. PDA plates containing just the *Fusarium* strain served as the control. The experiment used a completely randomised design (CRD) with ten replicates. Plates were incubated at room temperature (25 ± 2 °C) for 10 days. The colony radial growth of *Fusarium* isolates was measured and the percentage inhibition was calculated using the method given in equation (31).

$$\text{Percentage inhibition (\%)} = (R_1 - R_2)/R_1 \times 100 \qquad \text{(Eqn. 2)}$$

where R_1 is the radial growth of *Fusarium* spp. in the control and R_2 is the radial growth of *Fusarium* spp. with treatment.

Volatile organic compounds and solid phase microextraction (SPME) gas chromatography-mass spectrometry (GC/MS) analysis

The study aimed to identify the volatile and semi-volatile antifungal compounds produced by *Trichoderma* species using gas chromatography-mass spectrometry (GC-MS). The most effective *Trichoderma* species was selected for GC-MS analysis. For the experiments, the selected *Trichoderma* strain was cultivated in a 20 mL chromatography vial (20 mm in diameter) and incubated at 28 ± 2 °C for 14 days (32). Volatile organic compounds (VOCs) were extracted using solid-phase microextraction (SPME) following the protocol, with slight modifications. A 50/30 µm DVB/CAR/PDMS-coated SPME fiber (Supelco, USA) was exposed to the headspace above the fungal culture for 45 min (33). GC-MS analysis was carried out using the protocols followed by previous studies with minor changes (13, 34). The SPME fiber captured the volatiles emitted by *Trichoderma*, which were then injected into the SQ8 gas chromatograph (PerkinElmer Co. Ltd., Thailand). This investigation used an AT-5MS capillary column (5 % phenylmethylpolysiloxane) with dimensions of 30 m × 0.25 mm inner diameter and 0.25 mm film thickness. The column temperature was initially set at 45 °C and gradually increased at a rate of 7 °C per min until it reached 230 °C. Purified helium was used as the carrier gas with a flow rate of 1 mL/min. Electron impact (EI) mass spectra were obtained at an ionization voltage of 70 eV, covering the m/z range of 29-550. The ion source and quadrupole were both kept at 200 °C. The total volatiles released by *Trichoderma* were determined by comparing the chromatographic data to the Wiley NBS and the NIST mass spectral library search.

Statistical analysis

The laboratory experiment used the Completely Randomized Design (CRD) design. The recorded data were analyzed using the Analysis of Variance (ANOVA) techniques. The critical difference (CD) test or least significant difference (LSD) test was used as a post hoc test to compare the treatments and determine the significant difference between the treatments. R Software Version V 4.2.2 was used to analyze the data. The LSD values were calculated at a 5 % probability level of significance (P=0.05).

Results and Discussion

Isolation of *Trichoderma* isolates from rhizosphere soils and morphological identification

Out of 38 fungal colonies isolated from a soil sample, 23 were recognized as *Trichoderma* species. Isolates with similar traits were randomly selected for morphological identification. Four *Trichoderma* species were isolated from rhizosphere soils: *T. asperellum*, *T. atroviride*, *T. harzianum* and *T. hamatum*. The colonies were identified mostly based on their morphological traits. Different *Trichoderma* species have varying colony colours, from whitish and yellowish to dark green.

T. asperellum produced 8 pure cultures out of 23 species. Following 2 days of incubation on PDA plates at 25-30 °C, *T. asperellum* colonies produced a dark green centre and a colourless reverse side. The colonies turned light green at 35 °C. Colonies grew rapidly. Green conidia were found around the plate, but were heavier near the centre. Concentric rings, one or two in number, were produced near the inoculation zone, with no visible colour diffusion or pigmentation beyond that area. The colonies emitted a faintly aromatic smell, like pine pitch. The conidia were subglobose and measured between 3.4 - 4.2 µm. The *T. asperellum* phialides were solitary or in 2-3 whorls. The phialides in the middle were slightly wider, ranging from 1.3 to 2.5 µm. The primary branches of the conidiophore system extended from the base to the tip, giving it a pyramidal form (Fig. 1). The similar morphological and cultural identification of *T. asperellum* was described in the previous study (21). Four pure cultures of *Trichoderma* exhibited consistent phenotypic traits, initially glaucous to dark green colonies and greenish mycelia around the plate's margins and centre. Additionally, the cultures also emitted a coconut-like aroma. The opposite side was usually dull yellowish or drab with age. Colonies also proliferated. The conidia were dark green, smooth and subglobose when mature, measuring 3.8 - 4.4 µm. Additionally, solitary or 2-4 verticillate phialids were also observed, which were more or less lageniform and frequently curved, along with septate conidiophores. Research indicates that, based on detailed comparable morphological and cultural aspects of this species, the species was identified to be *T. atroviride* (Fig. 1) (35, 36).

Five pure cultures of *T. harzianum* grew rapidly and showed yellowish to green mycelium. Mature colonies displayed yellow to dark brown pigmentation across the entire PDA plate, forming 3 to 4 concentric rings with dense conidials (Fig. 1). The reverse side was colourless to dull yellowish, buff, or drab and the odour was faintly earthy. The globose to subglobose, smooth-walled, subhyaline to pale green conidia of *T. harzianum* ranged in size from 2.0 to 3.7 µm. Phialides were flask-shaped and ranged in length from 6.8 to 11.0 µm, which was shorter than that of other species. The conidiophores were strongly branched, with the tips of the main branches taking on a conical or pyramidal shape. These morphological and cultural characteristics align with previous studies of *T. harzianum* (21, 36). Six pure cultures of *T. hamatum* exhibited bluish-green to dark-green mycelium and showed moderate growth rates. Conidiation occurred in compact pustules, often arranged concentrically or clustered near the colony edge. The surface of these pustules appeared velvety due to numerous, delicate, flexuous, sterile conidiophore tips, which were bluish-green in colour. The reverse side of the colony was colourless and the odour was indistinct. The conidiophores

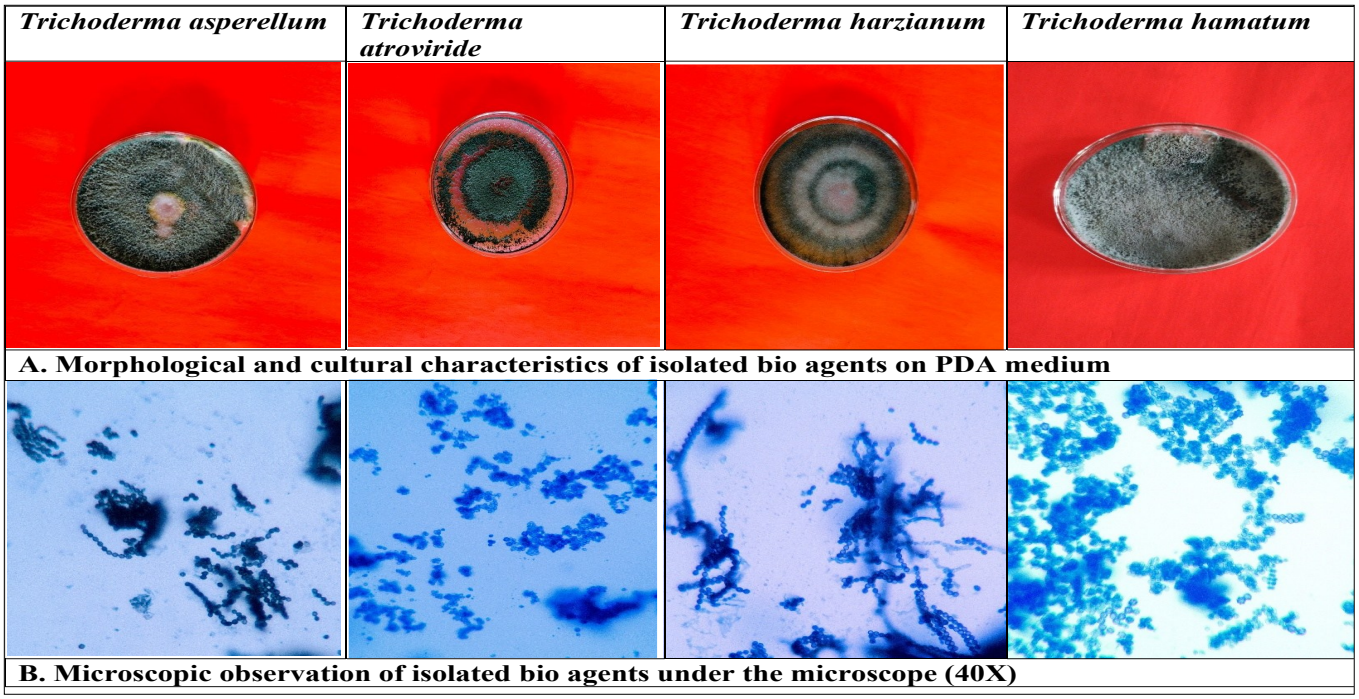

Fig. 1. A. Morphological and cultural characteristics of isolated *Trichoderma* spp. on PDA medium; **B.** Microscopic observation of isolated *Trichoderma* spp. under the microscope (40X).

displayed typical features for this species with an upper portion that was sinuous, undulate or hamate and irregular branching. These conidiophores remained sterile for approximately 100 µm towards the apex. Phialides were subglobose to ellipsoidal or ampulliform, measuring 3.5-5.4 µm and usually found in dense whorls of 4-6. The conidia were smooth, thin-walled, diluted green, oblong to ellipsoidal and measured 4.3-5.7 µm (Fig. 1). These morphological and cultural traits correspond closely to the descriptions of *T. hamatum* (36).

Antagonistic activity of *Trichoderma* spp. on conidial germination and mycelial growth of *Fusarium* spp. *in vitro* conditions

Four *Trichoderma* spp. were isolated and tested for their ability to inhibit spore germination and mycelial growth of *Fusarium* species. *T. asperellum* demonstrated the best inhibition value against the *Fusarium* strain (Table 2-3 & Fig. 2-3). *T. asperellum* bioagents inhibited conidial germination by 88.24 % and mycelial growth by 91.22 %, followed by *T. hamatum,* which inhibited 83.88 % of conidial germination and 87.26 % of mycelial growth against *F. equiseti*. *T. asperellum* also inhibited conidial germination (83.44 % and 78.52 %, respectively) and mycelial growth (89.68 % and 85.44 %, respectively) of *F. verticillioides* and *F. oxysporum*, followed by *T. hamatum*. *T. atroviride* had the lowest level of inhibition of conidia germination (72.66 %, 69.37 % and 64.86 %, respectively) and colonization (79.24 %, 76.46 % and 71.28 %, respectively) against *F. equiseti*, *F. verticillioides* and *F. oxysporum*, followed by *T. harzianum*. ANOVA revealed highly significant differences ($P < 0.05$) among treatments for both conidial germination and mycelial growth. The bioagents most efficiently inhibited conidial germination and mycelial growth in *F. equiseti*, followed by *F. verticillioides* and *F. oxysporum*.

Table 2. Mean conidial germination inhibition of *Fusarium* spp. and proportional reductions (compared with controls) for different introduced *Trichoderma* spp. isolates tested *in vitro* conditions

Treatments	Sample code	Antagonists	Conidial germination inhibition (%)		
			F. equiseti	*F. verticillioides*	*F. oxysporum*
T_1	MT1	*T. asperellum*	88.24	83.44	78.52
T_2	MT2	*T. atroviride*	72.66	69.37	64.86
T_3	MT3	*T. harzianum*	79.49	74.25	71.64
T_4	MT4	*T. hamatum*	83.88	80.36	75.44
	SE(m)		**1.06**	**1.64**	**2.18**
	CD 5 %		**3.07**	**3.82**	**4.72**

*Mean (± SD) of 10 replications. Numerical values followed by the same letter significantly differ at the 5 % level ($p < 0.05$)

Table 3. Mean Mycelial growth inhibition of *Fusarium* spp. and proportional reductions (compared with controls) for different introduced *Trichoderma* isolates tested *in vitro* conditions

Treatments	Sample code	Antagonists	Mycelial growth inhibition (%)		
			F. equiseti	*F. verticillioides*	*F. oxysporum*
T_1	MT1	*T. asperellum*	91.22	89.68	85.44
T_2	MT2	*T. atroviride*	79.24	76.46	71.28
T_3	MT3	*T. harzianum*	84.66	81.84	75.58
T_4	MT4	*T. hamatum*	87.26	83.42	78.22
	SE(m)		**0.74**	**0.96**	**1.21**
	CD 5 %		**2.21**	**2.68**	**4.03**

*Mean (± SD) of 10 replications. Numerical values followed by the same letter significantly differ at the 5 % level ($p < 0.05$)

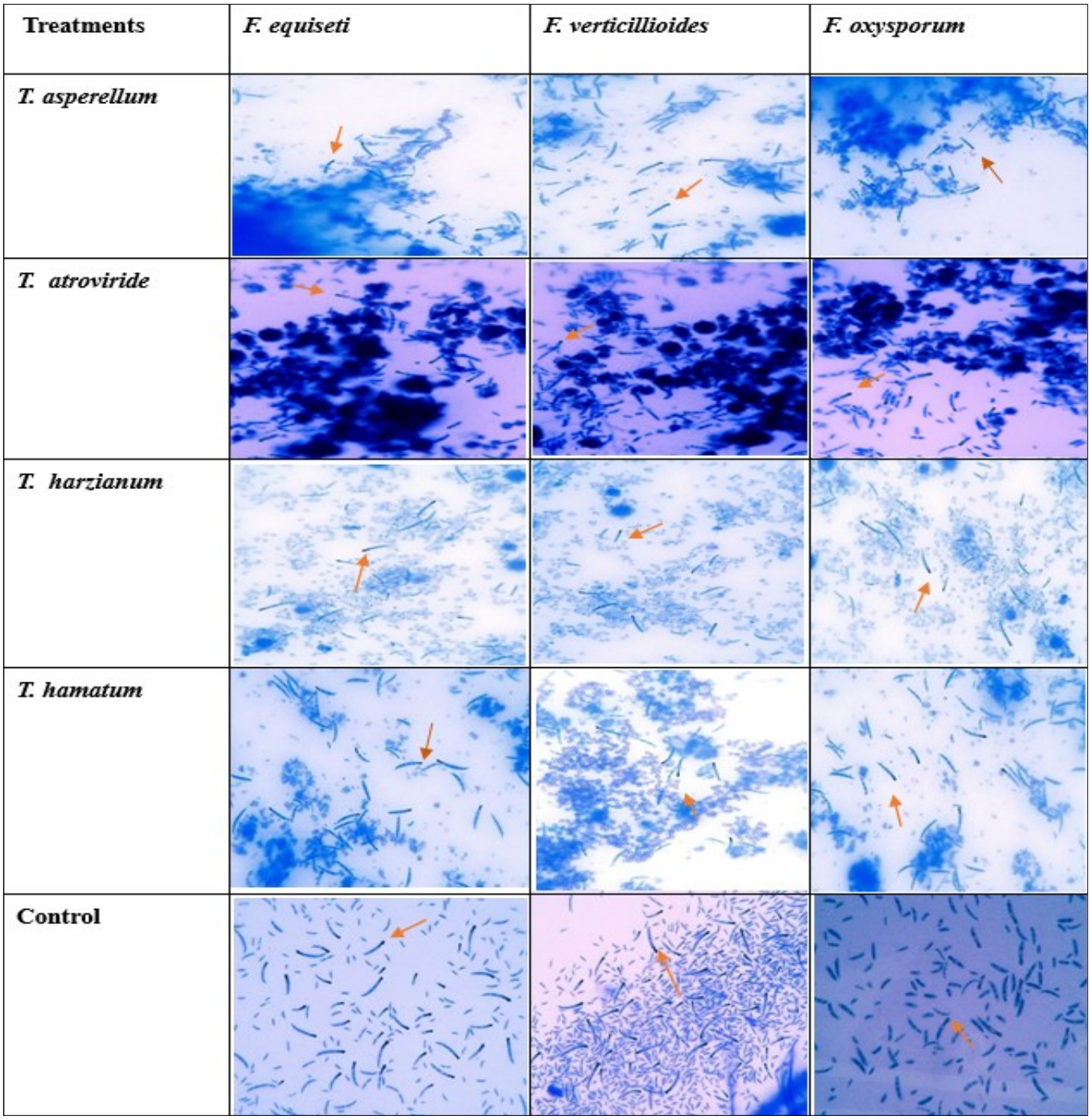

Fig. 2. Antagonistic effect of fungal bio-agent against different *Fusarium* spp. in conidia germination (Orange colour mark indicated the conidia germination).

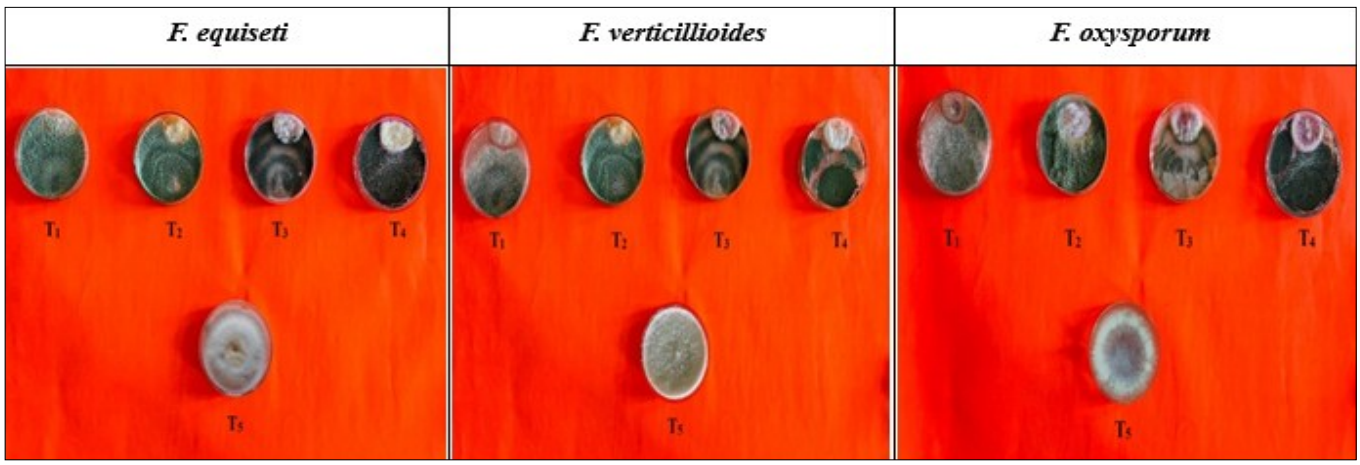

Fig. 3. Antagonistic effect of fungal bio-agent (T_1: *T. asperellum,* T_2: *T. hamatum,* T_3: *T. harzianum* , T_4: *T. atroviride* and T_5 : Control) against different *Fusarium* spp. in radial growth.

Trichoderma species are used as biocontrol agents against banana crown rot pathogens by using dual culture and conidial germination assays (37). The efficiency of several biocontrol agents evaluated against postharvest banana fruit rot caused by *F. oxysporum*, *C. musae* and *L. theobromae* (38). Researchers found that *Trichoderma* species were recovered on the plated portions of rotted fruit tissues, which had been inoculated with pathogens and antagonists. Consistent with the present findings, *T. asperellum* T76-14 demonstrated significant antagonistic activity (81 % inhibition) against *F. incarnatum* in muskmelon fruit rot (34). Similarly, another study reported that three *T. asperellum* isolates, B1902 (84.85 %), T2007 (77.78 %) and C1667 (75.76 %), demonstrated high levels of radial growth inhibition, effectively suppressing the growth of *F. oxysporum* f. sp. *cubense,* followed by a dual culture test (39). These reports corroborate the high efficacy of *T. asperellum* observed in the present investigation.

Solid phase microextraction GC-MS analysis and identification of volatile organic compounds

GC/MS analysis was performed to profile the volatile organic compounds (VOCs) produced by *T. asperellum* with antifungal activity against *Fusarium* species (Table 4). Solid-phase microextraction was used to collect the volatile organic compounds (VOCs) produced by *T. asperellum*, which were then analyzed by GC/MS. A total of 19 compounds were identified using the NIST library. Three VOCs, phenylethyl alcohol, fluoro (trinitro)methane and 1-Methyleneindene, matched with over 90 % accuracy. Other prominent compounds included 1-Methylideneindene (14.83 %, at 2.42 min) and fluoro(trinitro) methane (12.02 %, at 6.44 min). Additional VOCs with > 80 % match included azetidine, 3-Methylpentane, zingiberenol, pentan-1-ol, cubenol, octadec-9-yne and various terpenes. These belonged to major chemical classes such as alcohols, alkanes and sesquiterpenes. The structure of PEA and the mass spectra of key volatiles are shown in Fig. 4.

Numerous *Trichoderma* species have been found to produce and emit a range of volatile organic compounds (VOCs) (40). The study revealed that *T. asperellum* produces VOCs that inhibit the growth of a *Fusarium* strain, which is mediated by an antibiosis mechanism within the *Trichoderma* species. It has been shown that specific *Trichoderma* species produce volatile antifungal compounds that inhibit pathogen growth (32, 41). In this study, *T. asperellum* produced 19 VOCs, with PEA being the most dominant compound, accounting for 21.79 % of the peak area (Table 4). Phenylethyl alcohol has been reported as a compound produced by different *Trichoderma* species. 2-Phenylethanol produced by *T. asperellum* T76-14 has been shown to control postharvest fruit rot in muskmelon (34).

PEA produced by *Trichoderma* sp., *T. harzianum* and *T. virens* have been shown to have antifungal efficacy against several plant diseases (42-45). Phenylethyl alcohol, a type of aromatic alcohol, is an organic compound that appears as a colourless liquid with a rose fragrance. It is typically found as an essential oil in plants such as roses, jasmine, carnations and hyacinths (42). PEA has been shown to have antimicrobial effects. PEA inhibits fungal growth and interferes with RNA, DNA

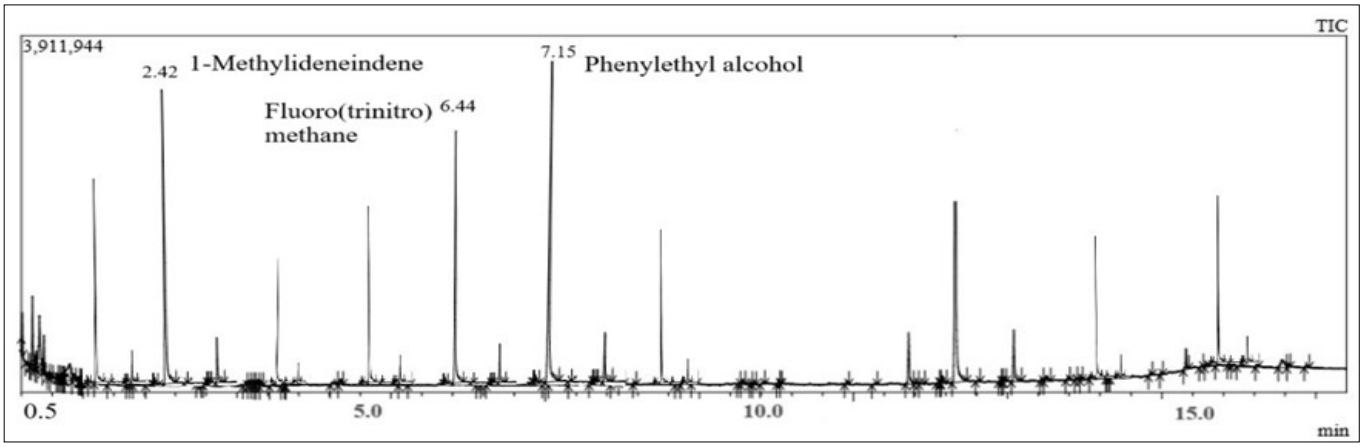

Fig. 4. Total ion chromatogram of volatile compounds identified from *T. asperellum.*

Table 4. Volatile compounds produced by *T. asperellum* were tentatively identified through Solid- phase microextraction (SPME) GC/MS analysis

Peak no.	Name of compound	Retention time	% match	Percentage area
1	3-Methylpentane	1.36	83.17	1.25
2	azetidine	2.21	89.52	4.07
3	1-Methylideneindene	2.42	93.26	14.83
4	(4-nitrophenyl) heptanoate	3.12	77.94	2.68
5	Zingiberenol	3.36	83.86	5.04
6	Pentan-1-ol	4.23	87.64	3.73
7	2,4,6-Trimethyloctane	4.47	85.29	1.59
8	Cubenol	5.28	82.96	7.05
9	Octadec-9-yne	5.58	76.51	2.03
10	Fluoro(trinitro)methane	6.44	91.48	12.02
11	Phenylethyl alcohol	7.15	96.24	21.79
12	3-Isopropyl-6,8a-dimethyl-1,2,4,5,8,8a-hexahydroazulene	8.54	87.96	0.94
13	Sesquisabinene B	9.49	84.24	1.39
14	5,7-Dimethylundecane	11.46	81.97	0.79
15	α-Bisabolene	12.58	84.71	1.88
16	β-Curcumene	13.05	86.25	6.22
17	Undeca-3,4-diene-2,10-dione, 5,6,6-trimethyl	13.51	77.83	2.33
18	cis-Z-α-Bisabolene epoxide	14.46	74.57	2.75
19	Allyldimethyl(prop-1-ynyl)silane	15.38	79.23	0.38

and protein production in *Neurospora crassa in vitro* conditions (46). PEA has been reported to exhibit strong antifungal activity against fungal growth, extending strawberries' postharvest life (47) and controlling blue mould decay caused by *Penicillium digitatum* and *P. italicum* in citrus fruits (45). These findings support the conclusion that *T. asperellum* VOCs, particularly PEA, contribute significantly to the inhibition of *Fusarium* spp. through antibiosis, thereby enhancing its potential as a postharvest biocontrol agent.

Conclusion

This study revealed that *T. asperellum* was the most effective isolate and showed its ability to inhibit the growth of the *Fusarium* strain under *in vitro* conditions. Its antagonistic potential was primarily attributed to competition for nutrients and space, as well as the production of antifungal volatile organic compounds (VOCs), particularly phenylethyl alcohol (PEA). *T. asperellum*-based biopesticides and VOC-based biofumigants, future studies should focus on the field-level validation of VOC effects, formulation development and large-scale application strategies for sustainable management of banana postharvest crown rot disease.

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Authors' contributions

MR and SSM[1] planned the design of the study and wrote the original draft. MR had done all the experiments in the laboratory of the Department of Plant Pathology, Institute of Agricultural Sciences, Sikhsha 'O' Anusandhan (Deemed to be) University. SSM[2] and GM guided the overall experiments and reviewed the manuscript. SKB and AM performed statistical analysis, editing and coordination. All authors read and approved the final manuscript. [SM[1] stands for Shyama Sundar Mahapatra and SM[2] stands for Sushree Suparna Mahapatra].

Compliance with ethical standards

Conflict of interest: The Authors do not have any conflict of interest to declare.

Ethical issues: None

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