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Biological control of black pepper and ginger pathogens, *Fusarium oxysporum*, *Rhizoctonia solani* and *Phytophthora capsici*, using *Trichoderma* spp

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ABSTRACT

Target specific fungal biocontrol agents are considered ideal for plant pathogen management strategies in crop protection and it offsets the negative environmental impact of chemical pesticides. The present study was conducted to develop novel microbial biocontrol agents for the effective management of black pepper and ginger pathogens in an eco-friendly and sustainable manner. Several *Trichoderma* species were isolated from the forest rhizosphere soil of Palakkad and Idukki districts of Kerala during pre-monsoon season. After preliminary screening, four isolates were identified by biochemical and molecular characterization, and later, they were studied for their anti-fungal activity. The novel isolates, *T. asperellum* strain AFP, *T. asperellum* strain MC1, *T. brevicompactum* MF1 and *T. harzianum* strain CH1 were tested for their efficacy in managing various soil-borne phytopathogens such as *F. oxysporum*, *R. solani* and *P. capsici*. The results of antimycotic activity of these isolates showed that *T. harzianum* exhibited maximum of mycelial growth inhibition over *Fusarium oxysporum* (78.3%) and *Phytophthora capsici* (65.3%) than *T. asperellum* (Strain AFP and MC1) and *T. brevicompactum* (MF1). Dual culture test results over mycelial growth of *Rhizoctonia solani* was found to be maximum in *T. asperellum* strain AFP (62.3%) followed by *T. asperellum* strain MC1 (56%), *T. brevicompactum* strain MF1 (49%) and *T. harzianum* strain CH1 (45.3%). The analyses of growth inhibition assay of all the four *Trichoderma* isolates were suggestive of their use as effective microbial biocontrol agents.

1. Introduction

Production of black pepper (*Piper nigrum* L.) and ginger (*Zingiber officinale* Rosc.) depends heavily on agro-climatic factors, insects, pests and various diseases caused by soil-borne phytopathogens. Among the diseases reported of black pepper, *Phytophthora* foot rot caused by *Phytophthora capsici* is one of the most devastating soil-borne diseases, and it causes severe economic loss (Anandaraj and Sarma, 1995). The development of foot rot disease is noticed mainly during south-west monsoon period (June-September), where the activity of fungus is higher because of fungus favorable weather conditions. On global scale, annual crop loss due to *Phytophthora* foot rot has been estimated to be US$ 4.5–7.5 million (De Waard, 1979). In Kerala, crop loss due to foot rot has been reported to a range of 3.4–9.4% of the crop (Anandaraj et al., 1989).

Ginger (*Zingiber officinale* Rosc.) occupies an important position among the cultivated spices in the world and India is one of the leading producers and exporters of ginger. In Kerala, it is one of the most valuable cash crops grown. Ginger is now going through a host of problems besetting its farming, though the productivity of ginger was not affected too badly from market point of view (Dohroo et al., 2012). However, during cultivation the crop may get severely affected with rhizome or soft rot caused by *Fusarium oxysporum f.sp zingiberi* and occasionally, by *Rhizoctonia solani*. It drastically reduces the productivity of ginger causing loss to farmer; thus, an eco-friendly pest management strategy is required for the increased crop protection and production. Biological control of soil-borne pathogens is an eco-friendly, consistent and cost-effective alternative to chemical pesticides and insecticides. Fungal biocontrol agents, such as *Trichoderma harzianum* and *Trichoderma virens* are effective in controlling pathogens of black pepper, cardamom, and ginger (Prakash et al., 1999). The present study was undertaken to evaluate the efficacy of different *Trichoderma* isolates, obtained from rhizosphere soil of ginger and black pepper, to suppress fungal disease incidences.

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2. Materials and methods

2.1. Sample collection

Rhizosphere soil samples of pepper and ginger fields were collected from different regions of Palakkad and Idukki districts, Kerala for the isolation of *Trichoderma* strains. Samples were taken from 15 to 30 cm deep soil in sterilized bottles and brought to laboratory, and stored at 4 °C for further study.

2.2. Isolation of Trichoderma strains

Trichoderma strains were isolated from soil by serial dilution method. Six-step serial dilutions (10^{-1} to 10^{-6}) of each samples were prepared in sterilized distilled water and 0.1 mL of the diluted sample was spread on the surface of *Trichoderma* Specific Medium ($MgSO_4 \cdot 7H_2O$-0.2 g, K_2HPO_4-0.9 g, KCl-0.15 g, NH_4NO_3-3.0 g, glucose-3.0 g, agar-15 g, rosebengal-0.15 g, chloramphenicol-0.25 g, distilled water-1000 mL, pH-6.5; Elad et al., 1980). The plates were incubated at 28 ± 2 °C for 3–5 days. Morphologically different colonies appearing on plates were subcultured on fresh potato dextrose agar (PDA Hi Media). Pure cultures of *Trichoderma* strains were preserved on PDA slants at 4 °C.

2.3. Plant pathogens for study

The black pepper and ginger pathogenic strains, *Fusarium oxysporum*, *Rhizoctonia solani* and *Phytophthora capsici*, were a generous gift of Plant pathology laboratory, Kerala Agricultural University, Mannuthy, Thrissur, Kerala.

2.4. Morphological characterization of Trichoderma strains

Pure cultures of *Trichoderma* were grown on PDA medium for microscopic and macroscopic examinations. Macroscopic identification was performed by observing the colony morphology, surface and reverse coloration of colony, texture and sporulation pattern of *Trichoderma* strains on PDA.

Examination of conidiophores and conidia were done under light microscope (Radical RMH-4B) from slide preparations stained with lacto-phenol cotton blue.

2.5. Molecular characterization of isolated Trichoderma strains

2.5.1. Genomic DNA extraction of Trichoderma sp

Genomic DNA was extracted by modified CTAB method (Moller et al., 1992). The fungal mass was scraped out from the ten days old fungal cultures. The fresh mycelium was manually ground in 1.5 mL microfuge tubes with liquid nitrogen by micropestle. 500 µL of pre-warmed (60 °C) TES lysis buffer (100 mM Tris. HCl, pH 8.0; 10 mM EDTA; pH 8.0; 2% SDS) was added and allowed it to thaw for 30 min. 50 µg of proteinase K was added to the ground material. Samples were incubated at 60 °C for 60 min. To that suspension, 140 µL of 5 M NaCl and 64 µL of 10% (w/v) CTAB were added and incubated at 65 °C for 10 min. DNA extraction was done by adding equal amount of phenol:-chloroform: isoamyl alcohol (25:24:1) and centrifuged at 14,000 rpm for 10 min. The aqueous phase was collected in a fresh tube and equal amount of chloroform: isoamyl alcohol (24:1) was added and centrifuged at 14,000 rpm for 10 min. DNA was precipitated by adding 0.6 mL of cold isopropanol and 0.1 mL of 3 M sodium acetate, pH 5.2 and maintained at −20 °C overnight. The DNA was pelleted by centrifuging at 12,000 rpm for 10 min at 4 °C and washed twice with 70% ethanol. The pellets were suspended in 50 µL TE buffer (100 mM Tris. HCl, pH 8.0; 1 mM EDTA; pH 8.0). RNA was digested by adding 10 mg/mL of RNase and incubated at 37 °C for 45 min and stored at −20 °C for further use.

2.5.2. PCR amplification and sequencing of ITS region

Polymerase Chain Reactions (PCRs) were performed in a total volume of 25 µL by mixing 12.5 µL Emerald Amp® GT PCR Master Mix (TAKARA BIO INC) with 1 µL of 10 µM concentration of each of the primer combinations ITS1 (5′-GGAAGTAAAAGTCGTAAC-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (Integrated DNA Technologies, Inc., USA) and 1 µL of 40 ng of genomic DNA.

PCR reactions were carried out in Bio-Rad thermal cycler (S 1000™) with the following PCR profile: an initial denaturation for 5 min at 97 °C, followed by 40 cycles of 1 min at 97 °C, 1 min at 48 °C and 2 min at 72 °C and a final extension at 72 °C for 5 min. Aliquots were analyzed by electrophoresis with 1% (w/v) agarose gel in 1X TAE buffer (40 mM Tris, 20 mM Acetic acid, 1 mM EDTA, pH 8.0). The resulting PCR product was purified by electro elution method.

Sequencing of the nuclear ribosomal DNA region containing 18 S ribosomal RNA gene; ITS 1; 5.8 S ribosomal RNA; ITS 2 and 28 S ribosomal RNA were amplified by PCR (SciGenom, Kochi, India)

2.5.3. Phylogenetic analysis

Phylogenetic tree was obtained by sequence analysis of nuclear ribosomal DNA region of four *Trichoderma* isolates and the sequences of other *Trichoderma* spp obtained from sequence data bank (NCBI, GenBank) (https://www.ncbi.nlm.nih.gov).

Phylogenetic dendrogram was constructed by Neighbor Joining method (Saitou and Nei, 1987) and evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013). The reliability of the branches in the NJ tree was assessed by using bootstrap test (1000 replicates). Evolutionary distances were analyzed by Jukes-Cantor method (Jukes and Cantor, 1969).

2.6. Growth inhibition assay by dual culture method

In vitro dual culture method (Morton and Stroube, 1955) was adopted to study the biocontrol activity of *Trichoderma* strains against black pepper and ginger pathogens such as *Fusarium oxysporum, Phytophthora capsici* and *Rhizoctonia solani*. Four different strains of *Trichoderma* spp and pathogens were grown on PDA for a week at room temperature (28 ± 2 °C). A 5 mm diameter mycelial disc of 7 days old culture of *Trichoderma* strains and phytopathogens were placed on opposite side of a PDA plate with 4 cm gap from each other. A completely randomized design was used with four replicates for each *Trichoderma* isolate. Control plates were inoculated with pathogen at one side and a sterile agar disc was placed opposite to it. The plates were incubated at room temperature for 5–7 days. Growth inhibition by *Trichoderma* strains against phytopathogens were calculated by the following formula (Vincent, 1927).

Percentage inhibition (PI) = C-T/C x 100 where, C = Growth of test pathogen in absence of antagonist (cm), T = Growth of test pathogen in presence of antagonist (cm).

2.7. PDA well diffusion assay

In vitro biocontrol activity of the total of potent antifungal metabolites present in *Trichoderma* species culture filtrate was determined by the above method in potato dextrose broth at 28 ± 2 °C. After incubating for 7 days, 10 mL of culture was centrifuged at 10,000 rpm for 20 min. The supernatant was filtered by Whatman No.1 filter paper and passed through 0.34 µM Millipore filter.

Inoculated cultures of four *Trichoderma* strains were incubated on orbital shaker at 150 rpm for 3 days. Different culture filtrate concentrations (0.05, 0.1, 0.15, 0.2 and 0.25 mg/µL of mycelial extract) were dispensed into four wells, separated at a distance of 2 cm from the centrally placed phytopathogens such as *F. oxysporum*, *P. capsici*, *R. solani* disc (5 mm) inoculated on a potato dextrose agar plate. Control plate without culture filtrate was incubated at 28 ± 2 °C for 7 days. The inhibition percentage over mycelial growth of phytopathogens was

compared with the control plate data. Assay was repeated twice, with four replications for each treatment. The growth inhibition percentage (PI) of the radial mycelial growth of phytopathogens was calculated by the formula of Vincent (1927).

2.8. Antifungal efficiency of volatile metabolites

In vitro antifungal activity of *Trichoderma* volatile metabolites was evaluated according to the method described by Dennis and Webster (1971). Petri plates containing PDA medium were centrally inoculated with a 5 mm diameter disc of *Trichoderma* strain and phytopathogen individually. The plates were incubated for 3 days at 28 °C. The upper lid of each petri plate was removed aseptically and lower plate containing pathogen was placed over a plate containing antagonist strain. The plates were wrapped by para film to prevent the loss of volatile substances

from sides of a petri plate, and incubated for 5 days at 28 °C. The petri plate containing PDA without antagonist served as control. Each assay was performed in quadruplicates. The percent inhibition was obtained using the formula of Vincent (1927).

2.9. Statistical analysis

Statistical significance of antagonistic effect of *Trichoderma* isolates were compared by one-way ANOVA and means separated by Fischer's protected least significant difference (LSD). The significance of *Trichoderma* on mycelial growth characteristics was determined by the magnitude of F- value ($p < 0.05$).

3. Results and discussion

3.1. Isolation and morphological characterization of Trichoderma isolates

In this study, four different strains of *Trichoderma* were isolated from rhizosphere soil of Palakkad and Idukki districts, Kerala during pre-monsoon seasons. The fungal cultures were examined under light microscope for morphological characteristics. All the four fungal colonies have thick and dark green conidial pigments. All these isolates were identified according to the identification key (Rifai, 1969) based on morphological characteristics such as arrangement, development and branching of conidiophores, shape of phialides, and shape and emergence of phialospores. The isolates identified were of three species, viz; two isolates of *T. asperellum* and one each of *T. brevicompactum* and *T. harzianum* (Table 1). Microscopic features of *T. asperellum* conidia are subglobose to ovoidal in shape with tuft green in color whereas *T. brevicompactum* has white to green conidia which is subglobose round in shape. In the case of *T. harzianum*, conidia are smooth and obovoid in shape and yellow to pale green in color.

3.2. Molecular identification of Trichoderma isolates

Based on the sequence analysis of the nuclear ribosomal regions, four isolates were identified as *Trichoderma asperellum* strain AFP, *Trichoderma asperellum* strain MC1, *Trichoderma brevicompactum* strain MF1 and *Trichoderma harzianum* strain CH1.

Phylogenetic dendrogram was constructed using data obtained by sequence analysis of ITS1 and ITS4 of four different *Trichoderma* isolates and the reference sequence obtained from NCBI deposited as Supplementary materials (ESM_1–4). Phylogenetic analysis of strain AFP was done with the 24 nucleotide sequences and it showed 99% sequence similarity to *T. asperellum* strain MMCC 1532.2, *T. asperellum* isolate NBAll Ta-12 and strain MC1 was with the 23 nucleotide sequences for phylogenetic analysis and it showed 99% sequence similarity with *T. asperellum* strain MMCC 1532.2 and *T. asperellum* isolate NBAll Ta-12. The strain MF1 was analyzed with the sequence of 21 nucleotides for phylogenetic study. It showed 97% sequence similarity to *T. brevicompactum* strain CEN509. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (ESM_1–4). The strain CH1 showed 95% sequence similarity with *T. harzianum* voucher UMAS SD14.

Sequence data of four of the *Trichoderma* isolates have been submitted to the Genbank (KX712092, KX712093, KX756616 and KX756617).

3.3. Growth inhibition assay by dual culture technique

The results of dual culture test showed that all the four *Trichoderma* isolates had significant antagonistic activity against various soil-borne phytopathogens such as *Fusarium oxysporum*, *Rhizoctonia solani*, *Phytophthora capsici* (Table 2). *F. oxysporum* radial growth was inhibited by *Trichoderma harzianum* strain CH1, *T. brevicompactum* strain MF1, *T. asperellum* strain AFP, and *T. asperellum* strain MC1 with 78.3%, 55.8%, 62% and 65.3% inhibition respectively after 5 days of incubation. Maximum pathogen inhibition was recorded by *T. harzianum* compared to the other three isolates (Fig. 1). The results are in match with previous reports (Rajappan and Ramaraj, 1999; Dennis and Webster, 1971; Sid Ahmed et al., 1999)

Among the four potential antagonists, antimycotic activity against *R. solani* was found maximum with *T. asperellum*. It grew over the mycelia of the *R. solani* and sporulated abundantly on its surface. The inhibition against *R. solani* by *T. harzianum* was found to be 45.3%, while that of *T. brevicompactum* strain MF1, *T. asperellum* strain AFP and *T. asperellum* strain MC1 were 49%, 62.3% and 56% respectively (Fig. 2).

A reduction in mycelial growth of *Phytophthora capsici* was seen during dual culture method. It indicated that all the four strains of *Trichoderma* tested for inhibition against soil-borne phytopathogens were efficient biocontrol agents. Among them, *T. harzianum* was found to be the most effective with 65.3% mycelial growth inhibition than *T. asperellum* strain AFP (42.1%), *T. asperellum* strain MC1 (53.1%) and *T. brevicompactum* strain MF1 (45%) (Fig. 3). The present investigation is in agreement with previous studies, that *T. harzianum* had significant antagonistic activity against *P. capsici* (Shashidhara et al., 2008).

In the present study, *T. harzianum* showed maximum inhibition of mycelial growth against selected soil-borne phytopathogens in all the treatments, but there was no definite trend. In the present investigation, maximum antimycotic activity was observed against *F. oxysporum* followed by *P. capsici* and least for *R. solani*. Antifungal activities of the reported strains are provided as Supplementary data (Supplementary table). Comparative analysis shows that the present isolates are superior to the previously reported strains (Aswini et al., 2016; Durak,

Table 1
Isolation and identification of *Trichoderma* isolates.

Sl. No	Identified Species	Isolate designation	Place of collection	Isolation source	Accession number	Macro/Microscopic characteristics
1	*T. asperellum*	AFP	Palakkad	Soil	KX712092.1	Dark green colony and conidia subglobose to ovoidal.
2	*T. asperellum*	MC1	Marayur	Coffee husk	KX712093.1	Dark green colony and conidia subglobose to ovoidal.
3	*T. brevicompactum*	MF1	Marayur	Forest soil	KX756616.1	White to green conidia. Conidia smooth and subglobose round in shape.
4	*T. harzianum*	CH1	Chittur	Wood waste	KX756617.1	Watery white to dark green colony color. Concentric rings present.

Table 2
Mycelial growth inhibition by *Trichoderma* isolates after 7 days of inoculation in dual culture. Values are mean of four replicates ± standard error. Data were analyzed using one-way ANOVA and means separated by LSD (values with different lower case letters are significantly different; $p < 0.05$. a, b, c, d are in decreasing order).

Plant pathogen	*T. harzianum* strain CH1 Inhibition %	*T. brevicompactum* strain MF1 Inhibition %	*T. asperellum* strain AFP Inhibition %	*T. asperellum* strain MC1 Inhibition %
Fusarium oxysporum	78.3 ± 0.2^{a}	55.8 ± 0.2^{d}	62.0 ± 0.4^{bc}	65.3 ± 0.6^{ab}
Rhizoctonia solani	45.3 ± 0.4^{d}	49.0 ± 0.0^{bc}	62.3 ± 0.4^{a}	56.0 ± 0.0^{ab}
Phytophthora capsici	65.3 ± 0.2^{a}	45.0 ± 0.4^{bc}	42.1 ± 0.1^{cd}	53.1 ± 0.1^{ab}

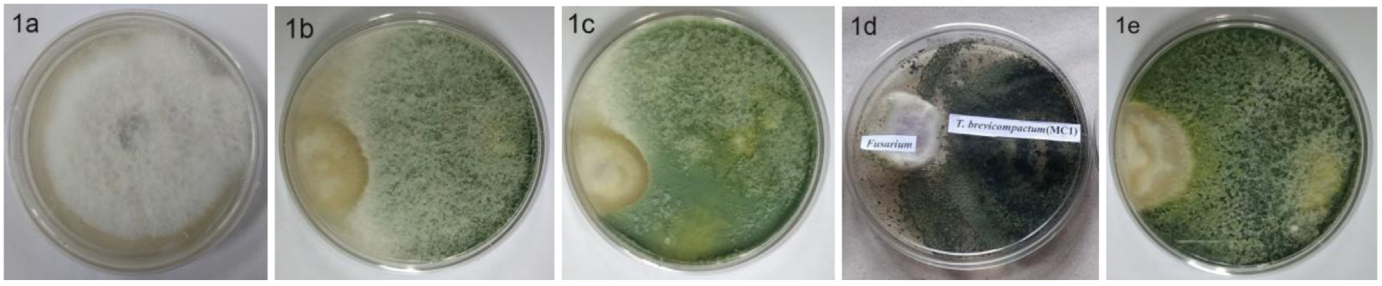

Fig. 1. Growth inhibition of F. oxysporum by isolates of Trichoderma strains by dual culture method (a) F. oxysporum (b) F. oxysporum and T. asperellum AFP (c) F. oxysporum and T. asperellum MC1 (d) F. oxysporum and T. brevicompactum MF1(e) F. oxysporum and T. harzianum CH1.

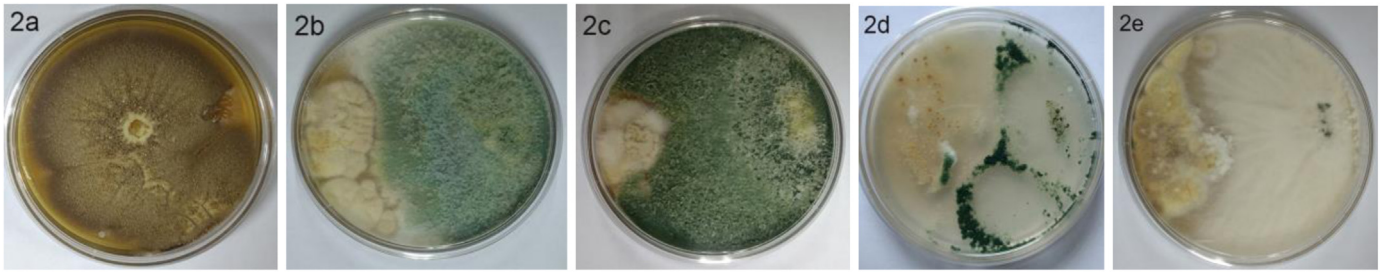

Fig. 2. Growth inhibition of *R. solani* by isolates of *Trichoderma* strains by dual culture method (a) *R. solani* (b) *R. solani* and *T. asperellum* AFP (c) *R. solani* and *T. asperellum* MC1 (d) *R. solani* and *T. brevicompactum* MF1 (e) *R. solani* and *T. harzianum* CH1.

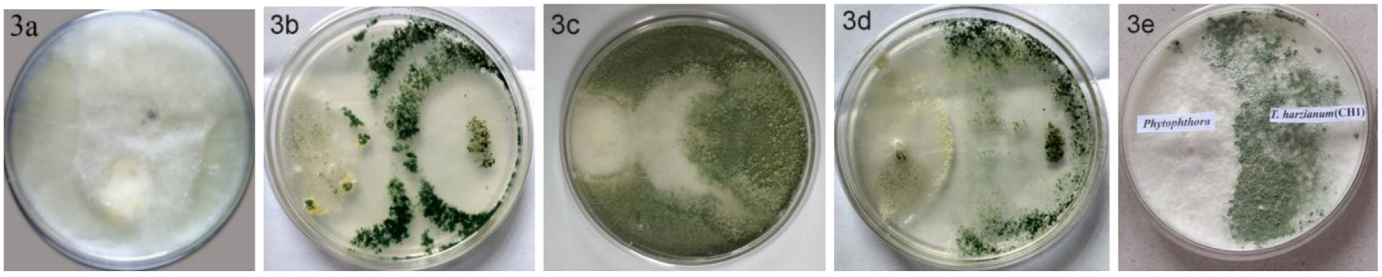

Fig. 3. Growth inhibition of P. capsici by isolates of Trichoderma strains by dual culture method (a) P. capsici (b) P. capsici and T. asperellum AFP (c) P. capsici and T. asperellum MC1 (d) P. capsici and T. brevicompactum MF1 (e) P. capsici and T. harzianum CH1.

Table 3
Antagonistic activity of potent antifungal metabolites present in *Trichoderma* culture filtrate against *F. oxysporum.* Values are mean of four replicates ± standard error. Data were analyzed using one-way ANOVA and means separated by LSD (values with different lower case letters are significantly different; $p < 0.05$. a, b, c, d are in decreasing order).

Concentration of *Trichoderma* filtrate (mg/μL)	Percentage inhibition of the growth of *F. oxysporum* by isolates of *Trichoderma*			
	T. asperellum strain MC1 Inhibition %	*T. asperellum* strain AFP Inhibition %	*T. brevicompactum* strain MF1 Inhibition %	*T. harzianum* strain CH1 Inhibition %
0.1	61.5 ± 0.5^{bc}	70.3 ± 0.2^{ab}	45.3 ± 0.2^{cd}	76.3 ± 0.4^{a}
0.15	63.8 ± 0.2^{bc}	76.5 ± 0.2^{ab}	56.0 ± 0.4^{cd}	81.8 ± 0.2^{a}
0.2	68.8 ± 0.2^{bc}	85.1 ± 0.4^{ab}	63.5 ± 0.2^{cd}	88.8 ± 0.2^{a}
0.25	70.5 ± 0.2^{bc}	Total inhibition	66.0 ± 0.0^{cd}	Total inhibition

Table 4
Antagonistic activity of potent antifungal metabolites present in *Trichoderma* culture filtrate against *P. capsici*. Values are mean of four replicates ± standard error. Data were analyzed using one-way ANOVA and means separated by LSD (values with different lower case letters are significantly different; $p < 0.05$. a, b, c, d are in decreasing order).

Concentration of *Trichoderma* filtrate (mg/μL)	Percentage inhibition of the growth of *P. capsici* by isolates of *Trichoderma*			
	T. asperellum strain MC1 Inhibition %	*T. asperellum* strain AFP Inhibition %	*T. brevicompactum* strain MF1 Inhibition %	*T. harzianum* strain CH1 Inhibition %
0.1	50.8 ± 0.1^{c}	68.1 ± 0.4^{ab}	48.1 ± 0.04^{cd}	70.9 ± 0.3^{a}
0.15	60.5 ± 0.2^{c}	71.0 ± 0.4^{ab}	52.0 ± 0.4^{cd}	73.8 ± 0.2^{a}
0.2	65.3 ± 0.2^{c}	80.8 ± 0.4^{ab}	55.0 ± 0.4^{cd}	82.8 ± 0.2^{a}
0.25	80.5 ± 0.2^{c}	Total inhibition	59.0 ± 0.4^{cd}	Total inhibition

Table 5
Antagonistic activity of potent antifungal metabolites present in *Trichoderma* culture filtrate against *Rhizoctonia solani*. Values are mean of four replicates ± standard error. Data were analyzed using one-way ANOVA and means separated by LSD (values with different lower case letters are significantly different; $p < 0.05$. a, b, c, d are in decreasing order).

Concentration of *Trichoderma* filtrate (mg/μL)	Percentage inhibition of the growth of *R. solani* by isolates of *Trichoderma*			
	T. asperellum strain MC1 Inhibition %	*T. asperellum* strain AFP Inhibition %	*T. brevicompactum* strain MF1 Inhibition %	*T. harzianum* strain CH1 Inhibition %
0.1	45.8 ± 0.2^{bc}	50.3 ± 0.6^{a}	35.6 ± 0.3^{d}	62.3 ± 0.6^{ab}
0.15	51.8 ± 0.4^{bc}	67.0 ± 0.4^{a}	42.0 ± 0.4^{d}	63.3 ± 0.2^{ab}
0.2	65.8 ± 0.2^{bc}	77.5 ± 0.2^{a}	45.0 ± 0.4^{d}	70.3 ± 0.2^{ab}
0.25	70.8 ± 0.2^{bc}	82.0 ± 0.0^{a}	57.1 ± 0.1^{d}	75.3 ± 0.2^{ab}

2016; Siameto et al., 2010; Ramirez-Delgado et al., 2018).

3.4. PDA well diffusion assay

Different concentration of fungal mycelial extract was distributed over 50 cm^2 of potato dextrose agar plates (PDA) and tested for plant pathogen inhibition. 0.25 mg/μL mycelial extract of *T. harzianum* filtrate showed complete inhibition over mycelial growth from visibly turbid inoculum of *F. oxysporum* and *P. capsici* on day 5 and inhibiting *R. solani* mycelial growth up to 75% signifying its antifungal and biocontrol activity (Tables 3–5 and Figs. 4–6). The results are in agreement with previous reports (Srideepthi and Krishna, 2015; Mathew and Gupta, 1998; Ezziyyani et al., 2009).

Similar inhibition of colony growth of *F. oxysporum*, *P. capsici* and *R.* solani by *T. asperellum* was reported earlier (El Komy et al., 2015; Jiang et al., 2016; Asad et al., 2014). Culture filtrate of *T. asperellum* AFP and MC1 showed significant inhibition over three phytopathogens. 0.25 mg/μL mycelial extract of *T. asperellum* strain AFP had completely arrested *F. oxysporum* and *P. capsici* growth and showed good biocontrol potential against *R. solani* (82%) whereas strain MC1 inhibiting *Phytophthora* mycelial growth up to 80.5% and showed a significant level of reduction in radiant growth of *Fusarium* (70.5%) and *R. solani* (70.8%).

The PDA well diffusion assay revealed that *T. brevicompactum* culture filtrate was effectively inhibiting the mycelial growth of phytopathogen in the following order, *F. oxysporum* > *P. capsici* > *R. solani*. Results of PDA well diffusion assay revealed that the increase in concentration of *Trichoderma* culture filtrate resulted in decrease in the mycelial growth of phytopathogens. Among the four *Trichoderma* isolates, *T. harzianum* and *T. asperellum* may be considered to be potent biocontrol agents, as observed by inhibiting the radial growth of phytopathogens. On the whole, all the organisms had showed significant levels of inhibition needed be designated as biocontrol agents.

3.5. Growth inhibition by volatile metabolites

Volatile metabolites present in the culture filtrate of *T. asperellum* AFP and MC1 revealed its potent antimycotic activity by inhibiting *P. capsici* at 49% & 42%, *F. oxysporum* growth by 40% & 44% and least inhibition on *R. solani* 38% and 33%. Whereas, *T. harzianum* restricted growth of *P. capsici* by 40%, that of *F. oxysporum* by 37% and *R. solani by* 34% (Table 6). The volatile assay revealed that *T. brevicompactum* culture filtrate was effectively inhibiting the mycelial growth of phytopathogens in the following order, *F. oxysporum* > *P. capsici* > *R. solani*. Results of volatile assay revealed that volatile compounds present in the culture filtrate inhibited mycelial growth of pathogens effectively.

4. Conclusions

The soil and climate, from which the *Trichoderma* isolates were found, are comparable to the pepper and ginger cultivated regions. Generally the pathogens of black pepper and ginger are controlled by chemical pesticides. Biocontrol methods are undoubtedly better than chemical pesticides. Hence, all the four *Trichoderma* isolates reported here may be considered as efficient biocontrol agents for black pepper and ginger. *T. harzianum* CH1 showed maximum biocontrol property against plant pathogens studied. Further research on mass

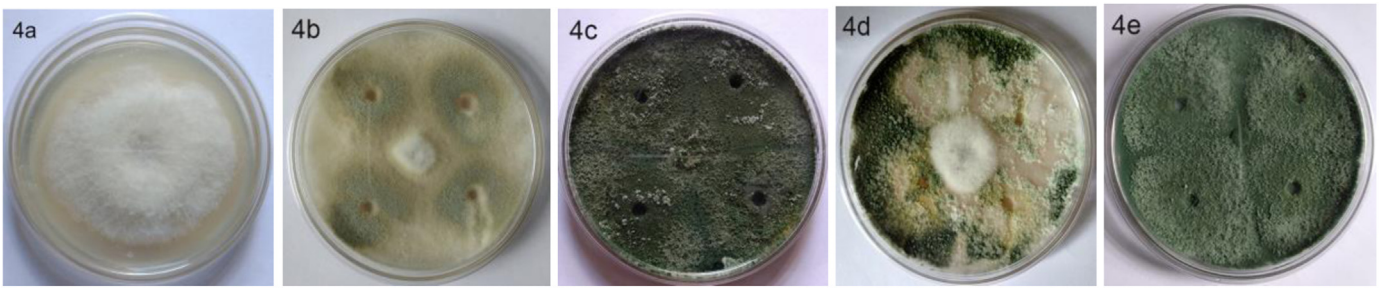

Fig. 4. PDA well diffusion assay: Inhibition efficacy of *Trichoderma* isolates over soil-borne phytopathogens **a**) *F. oxysporum* – control **b**) *F. oxysporum* and *T. asperellum* MC1 **c**) *F. oxysporum* and *T. asperellum* AFP **d**) *F. oxysporum* and *T. brevicompactum* MF1 **e)** *F. oxysporum* and *T. harzianum* CH1.

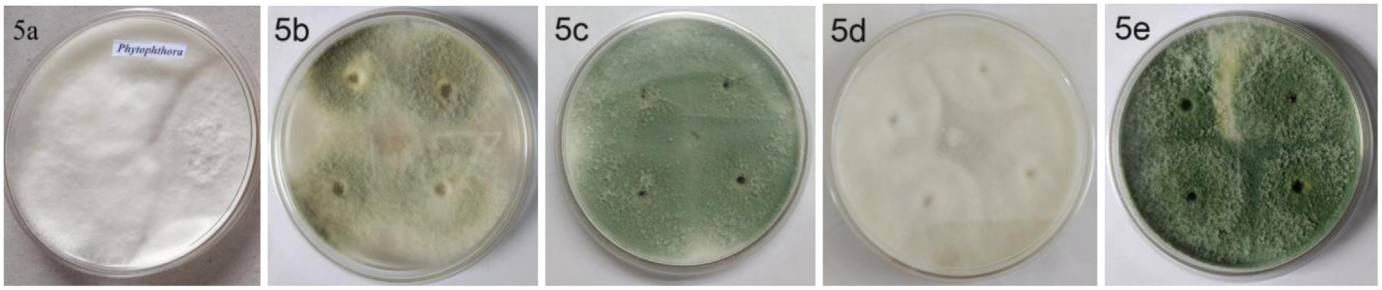

Fig. 5. PDA well diffusion assay- Inhibition efficacy of *Trichoderma* isolates over soil-borne phytopathogens **a**) *P. capsici*- control **b**) *P. capsici* and *T. asperellum* MC1 **c**) *P. capsici* and *T. asperellum* AFP **d**) *P. capsici* and *T. brevicompactum* MF1 **e)** *P. capsici* and *T. harzianum* CH1.

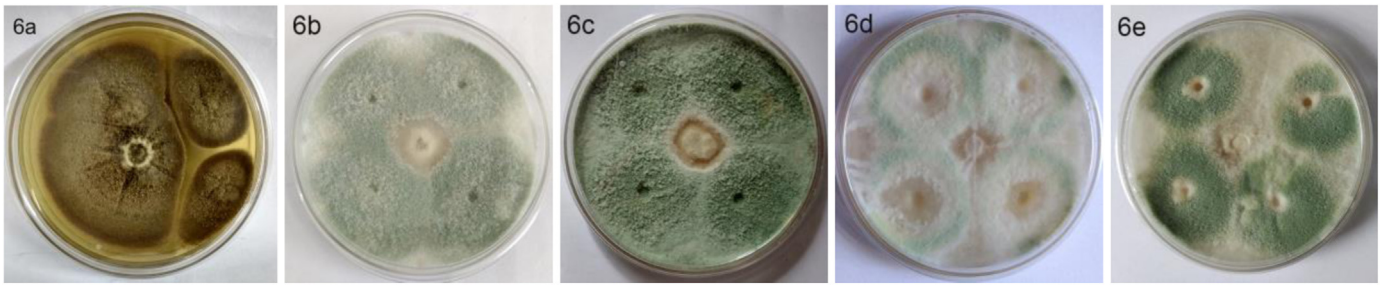

Fig. 6. PDA well diffusion assay- Inhibition efficacy of *Trichoderma* isolates over soil-borne phytopathogens **a**) *R. solani* - control **b**) *R. solani* and *T. asperellum* MC1 **c**) *R. solani* and *T. asperellum* AFP **d**) *R. solani* and *T. brevicompactum* MF1 **e)** *R. solani* and *T. harzianum* CH1.

Table 6
Effect of volatile metabolites of *Trichoderma* spp. Values are mean of four replicates ± standard error. Data were analyzed using one-way ANOVA and means separated by LSD (values with different lower case letters are significantly different; $p < 0.05$. a, b, c, d are in decreasing order).

Plant pathogen	*T. harzianum* strain CH1 Inhibition %	*T. brevicompactum* strain MF1 Inhibition %	*T. asperellum* strain AFP Inhibition %	*T. asperellum* strain MC1 Inhibition %
Fusarium oxysporum	37.3 ± 0.2^{bc}	35.3 ± 0.2^{cd}	40.3 ± 0.2^{ab}	44.3 ± 0.2^{a}
Rhizoctonia solani	34.3 ± 0.2^{ab}	30.3 ± 0.2^{cd}	38.3 ± 0.4^{a}	33.0 ± 0.0^{bc}
Phytophthora capsici	40.8 ± 0.04^{bc}	32.0 ± 0.4^{cd}	49.8 ± 0.2^{a}	42.3 ± 0.2^{ab}

multiplication by large scale fermentation is proposed for field trials. Isolation and identification of antibiotics and their field level application in crop fields may prove to be advantageous.

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Conflict of interest

The authors declare that they have no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bcab.2018.11.021.

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