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Analysis of Volatile Metabolic Compounds for Tracking Symptomatic Infection of *Fusarium solani* infection causing *Fusarium* Wilt in Brinjal Plant

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ABSTRACT: Brinjal (*Solanum melongina* L.) is an economically important crop grown in India, however the plant is susceptible to different of fungal diseases, resulting in low crop production. The pathogenic fungus was isolated from diseased plant parts in this research and identified as *Fusarium solani* f. sp. *melongenae* based on morphological and cultural features. To control the soil-borne diseases in brinjal with the use of chemicals under in field condition is hazardous. For eco-friendly and sustainable management of the disease the excellent potential of effective Bio control *Trichoderma* were used. Potential antagonists *Trichoderma's* antifungal properties have been observed in various of contexts as a means of controlling a wide range of soil-borne diseases. *Trichoderma* secrete chitinases, proteases which degrade *Fusarium solani* fungal cell walls to liberate the oligomers, causing exochitinases and to begin myco parasitism strategies. To examine the antagonistic activity of *Trichoderma* dual culture assay, paired petri dish technique, and GC-MS analysis were conducted. Under *in vitro* condition of dual culture assay revealed that *Trichoderma* isolate T(AB)-8 was found to be effectively inhibiting the radial mycelial growth of the pathogen by 83.11 % by the production of volatile compounds. Further, secondary metabolites from *Trichoderma asperellum* (T(AB)-8) were identified as 31-Hexadecanol (14.33%), were n-Nonadecyl trifluroacetate, 1-Tetradecene, 1,2Benzedicarboxylic acid, by Gas chromatography mass spectrometry (GC-MS). In conclusion, these findings suggest that *T. asperellum* (T(AB)-8) could be used as a biocontrol agent for Fusarium wilt disease caused by *Fusarium solani* f. sp. *melongena.*

Keywords: Eggplant, *Fusarium solani*, *Trichoderma* spp, Antagonism, secondary metabolites.

INTRODUCTION

Brinjal (*Solanum melongena* L.) is a commercially important vegetable crop grown in India and around the world. Where it is grown in green houses, polytunnels, and fields over an area of approximately 20,000 acres. Despite their origins in South East Asia, they are now grown in tropical, subtropical, and temperate regions such as India, China, Sri Lanka, the Philippines, Africa, and Australia, among others (Cericola *et al.*, 2013). It is extremely productive and serves as the poor man's vegetable (Som, 2002). India has a massive brinjal production of 12,779.54 thousand tonnes. Similarly, Tamil Nadu ranks eleventh in India in terms of brinjal production (Apeda, 2020). Brinjal is grown on 728.00 thousand hectares, yielding a yearly yield of 12,660.00 thousand metric tonnes and a productivity of 17.7 metric tonnes per hectare (Indiastat, 2019). They are well known for their therapeutic properties and, as a result, have been used in traditional medicine since ancient times. The presence of fungal phytopathogens, on the other hand, is critical throughout brinjal production because these organisms can cause dreadful wilt disease. In recent years, soil-borne pathogenic fungi such as *Fusarium solani* f. sp. *melongenae* have

been discovered in brinjal crops as the cause of fusarium wilt of brinjal. Dark to slight yellowing of foliage and lower leaves, wilting of upper leaves, underground stems becoming dry and brown as a result of cortical decay, roots appearing soft and water soaked, drooping of the apical portion, diminutive growth, withering of undeveloped fruits, and eventually the entire plant drying were all symptoms caused by these pathogens in eggplant. When dissected transversely, the vascular tissues of the stem and root show reddish-brown discoloured striations. Wilting of seedlings is another common symptom of Fusarium wilt, as is a reduction in the size of leaves and fruits, both of which have an impact on yield and quality (Singh *et al*., 2014). Mycotoxin is a secondary metabolite produced by *Fusarium solani* f. sp. *melongenae* that poses a serious threat to plants and animals (Prasad *et al*., 2018). Various studies have documented the use of various control measures for the management of diseases in brinjal, including chemical and biological control.

Chemical fungicides and pesticides are currently being restricted in a number of countries due to their harmful effects on human health and the environment, which have been widely discussed. Different plant rhizodeposition attracts beneficial microbiota to the rhizosphere. It has also been reported that rhizospheric fungi act as an effective antagonist against pathogens of the same plant. (Berg *et al*, 2005). Biocontrol agents are the most effective way to manage *Fusarium* wilt in brinjal, according to research conducted over the last few decades. Biological control agents are increasingly being used, particularly against soil-borne pathogens. Antagonist research of biological control agents has become one of the most fascinating and rapidly emerging fields in plant pathology due to its enormous potential to solve many agricultural and environmental problems (Andleeb *et al*, 2017). *Trichoderma* species, particularly *Fusarium* species, have been used as BCAs to control plant pathogenic fungus and manage plant diseases (Abbas *et al*., 2017; Al-Ani, 2018). They can act indirectly (Vinale *et al*., 2008; Ajitha and Lakshmedevi 2010) by competing for nutrients and space, modifying environmental conditions, or promoting plant growth, plant defensive mechanisms, and antibiosis, or directly. *Trichoderma* filamentous fungi have gained the attention of researchers due to their diverse action against a wide range of plant pathogenic fungi, including *Fusarium* species (Saravanakumar *et al*., 2016). As a result, the goal of this study was to assess the antagonistic potentiality of some native *Trichoderma* spp. *in vitro* against the pathogen that causes brinjal *Fusariual* wilt.

MATERIALS AND METHODS

Isolation, maintenance and identification. All the pathogenic fungal strains used in the experiments brought from the Farmers field. During 2021, samples of wilted eggplant plants with peculiar symptoms were collected in various parts of Tamil Nadu. The research was conducted in the Anton Debary lab, Department of Plant Pathology, Faculty of Agriculture, Agriculture College and Research Institute, Madurai. Infected eggplant roots and stems with redish - brown discoloured vascular tissues were used to isolate fungal strains. The wilted root and vascular stem tissues were dissected, cleaned, surface sterilised, and blotted dry. Stem segments were incubated for five days at 25°C in PDA. The single hyphal tip method was used to purify it in plain agar. The resulting single spore cultures were stored at 4°C for future use.

Isolation and Identification of antagonistic fungi from the rhizosphere region. Sandy Soil samples were taken from the rhizosphere of healthy brinjal plants in different brinjal-growing regions in Tamil Nadu, India. Plants were gently and carefully uprooted for rhizosphere soil, and soil tightly adhering to the roots was collected, randomly selected, mixed, and one-fourth part was used as a composite rhizosphere soil sample of the region. The pH of the soil was measured in a 1:2 (soil: water) ratio with a 30-minute equilibration time. The serial dilution technique was used to isolate soil samples after they were collected and air dried for four hours. The *Trichoderma* selective medium (TSM) was used to isolate *Trichoderma* isolates. 1 mL soil suspension was obtained and placed onto the TSM-seeded Petri plate using a 5 mL sterilized pipette. The plates were incubated for 5 days at 282°C. Observations on the emergence of colonies were kept from the third to the fifth day. Individual colonies were chosen for future research and kept in pure culture. On the basis of their cultural and morphological characteristics, the *Trichoderma* species were identified and examined under a compound microscope, and the cultures were kept on PDA slants at 4°C for further study.

Evaluation of antagonistic activity of different isolates of *Trichoderma* spp. *Trichoderma* spp was tested for antagonistic activity against *F. solani* f. sp. *melongenae* in a preliminary screening study. *Trichoderma* spp. (BCA) antagonistic activity was assessed on PDA medium using the direct confrontation method described by (Comporta, 1985). Briefly, two 5 mm discs obtained from one-week old *Trichoderma* sp. and *F. solani* f. sp. *melongenae* cultures on PDA were placed at opposite points on the same diagonal line, 1 cm from the edge. For each fungus-fungus interaction and the biological control potential of each fungus, three plates were considered. The fungus linear growth was measured daily for 10 days in both the dual and individual cultures, which were incubated at 25±2°C. The percentage of growth inhibition was calculated using the formula given below (Gaigole *et al*., 2011)

$$\text{Per cent inhibition (I)} = \frac{C - T}{C} \times 100$$

Where, C – Growth of pathogen in control plates
T – Growth of pathogen in dual culture plates
I – Per cent inhibition in mycelial growth

Efficacy of volatile compounds eluted from antagonistic fungi against *F. solani* f. sp. *melongenae* in vitro. The effect of antagonistic microorganisms' volatile metabolites on pathogens and mycelial growth was examined using the Paired Petri dish Technique (Laha *et al*., 1996). On the last day of incubation *F. solani* f. sp. *melongenae* resistance to volatile organic compounds produced by *Trichoderma* spp. The antagonistic fungi were inoculated in the centre of the PDA plate by placing a 9 mm diameter mycelia disc from a one-week old culture on the plate and incubating it at 26±2°C for two days. The top of each Petri dish was replaced with the bottom of a PDA plate that had been inoculated with the pathogen in the middle. Two plates were taped together and incubated at 25°C. *T. asperellum* isolates were replaced with a 5 mm inoculum of sterile PDA medium only in the control treatment. At 5 and 7 days after incubation, the diameter of the pathogen colonies was measured, and the inhibition of mycelial growth was calculated. As a control, PDA plates were incubated with the pathogen alone and paired with PDA plates without biocontrol agents. Using equation, the percent growth inhibition was calculated (Dolatabadi *et al*., 2012)

$$PI = \frac{Dc - Dt}{Dc} \times 100$$

D_c = average diameter of fungal growth (cm) in control
D_t = average diameter of fungal growth (cm) in treatment

Extraction of crude metabolites from *Trichoderma* spp. The crude antibiotics were extracted from *T. asperellum* based on the results of the dual plate assay as per the proposal by (Yin *et al*., 2010). A fungal mycelim disc of 8mm from a 5 days old culture of *Trichoderma* maintained on PD (Potato dextrose) broth. Centrifugation was used to collect filtered filtrates from a 20-day-old culture of both strains grown in potato dextrose broth. An equal volume of ethyl acetate was added to the supernatant and incubated at 150 rpm for 12 hours in an orbital shaker. A separating funnel was used to separate the solvent phase, which was then concentrated using a rotary flask evaporator. The crude extracts were then air-dried before being redissolved in 1 mL HPLC grade methanol. At the Centre of Innovation, Dept. of Biotechnology AC&RI, Madurai, the extract was subjected to GC-MS for compound detection (Trace GC UltraDSQ II, Thermo Scientific, made in Germany). Computer searches on the NIST Version 2005 MS data library were used to identify the compounds (Vinodkumar *et al.*, 2017)

Gas chromatography mass spectrometry analysis of crude antibiotics. By using GC-MS, secondary metabolites can be identified. *Trichoderma* spp. were found to inhibit growth in studies. With a Shimadzu Gas chromatography equipped with a mass detector Turbo mass gold containing an Elite-1 (100 percent Dimethyl Poly Siloxane), 30 m 0.25 mm ID 1 mM df, extracts were selected and chemical constituents were determined. The following conditions were used: Helium (1 ml/min) as the carrier gas; oven temperature programme 110°C (2 min) to 280°C (9 min); injector temperature (250°C); total GC time (45 min). In 1.0 ml aliquots, the ethyl acetate extract was injected into the chromatograph. The major constituents were identified using a computer-driven algorithm and then by comparing the mass spectrum of the analysis to that of a library from the National Institute of Standards and Technology (NIST) (Version. 2.0, year-2005). Turbo mass-5.1 was used for gas chromatography mass spectroscopy (GC-MS).

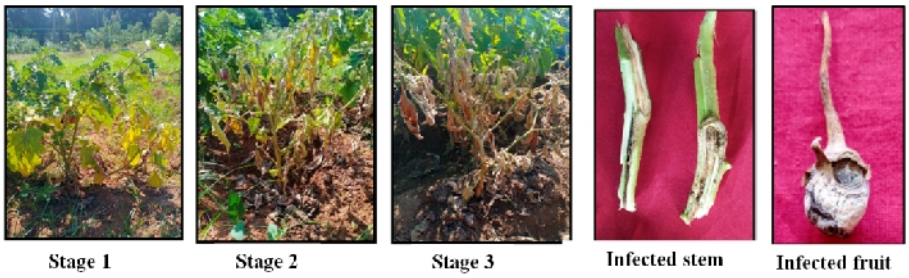
Estimation of extra cellular enzymes of *Trichoderma* spp. All the fifteen isolates of *Trichoderma* spp were tested for enzyme production. Isolates of *Trichoderma asperellum* were grown in a 100 mL liquid mineral synthetic medium (MSM) containing the following ingredients (in g/l): $MgSO_47H_2O$, 0.2; K_2HPO_4, 0.9; KCl, 0.2; NH_4NO_3, 1.0; $FeSO_47H_2O$, 0.002; $MnSO_4$, 0.002; and $ZnSO_4$, 0.002, supplemented with 0.1 percent FOL cell walls to induce cell wall enzyme production, or 0.1 percent glucose as a control (Mondejar *et al.*, 2011). The cultures were grown for 6 days at 25°C on a rotary shaker at 150 rpm. Mycelia were obtained by filtering them through Whatman No. l filter paper and centrifuging the filtrate at 4°C for 10 minutes at 5000g. The supernatant was decanted and stored at -20°C until enzyme activity was determined (El-Katatny *et al*., 2000). The activity of chitinase was determined using a colorimetric method with a Jenway 6715 spectrophotometer, as described by (Molano *et al.*, 1977) with minor modifications. In a 1.5 ml micro-centrifuge tube, 500 l of 0.5 percent chitin (suspended in 50 mM acetate buffer pH 5.2) and 500 l of the supernatant were used in the assay. The mixture was shaken and incubated at 37°C for 4 hours. The tubes were placed in a boiling water bath for 5 minutes to stop the reaction, and then 500 l of dinitrosalicylate was added to each tube. Based on standard curves of N-acetyl-D-glucosamine (GlcNAc) measured as absorbance at 540 nm, the amount of released reducing sugars due to enzyme activity was calculated. The activity of the enzyme was measured in pmol/s/ml. The amount of reducing sugars was measured with dinitrosalicylate (DNS) after incubating 200 l of the supernatant with 500 l of 5.0 percent (w/v) laminarin (suspended in 50 mM acetate buffer pH 4.8) in a 1.5 ml micro-centrifuge tube at 45°C for 60 minutes (Miller, 1959). Based on glucose standard curves measured as absorbance at 540 nm, the amount of released reducing sugars due to enzyme activity was calculated. The activity of the enzyme was measured in nmol/s/ml.

Statistical analysis. The treatment's mean differences were assessed statistics ANOVA was used to assess treatment mean differences, and the calculated means were subjected to Duncan's multiple range test at P00.05. For statistical analysis, SPSS version 17.0 was used (SPSS, Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Isolation of *Fusarium solani*

Pathogenic fungal strain was isolated and identified, on the basis of morphological and cultural characteristics as *Fusarium solani* f. sp. *melongenae* (Plate 1-3). The results of this study agree with those of (Faruq *et al.*, 2014), who found that *Fusarium* wilt of brinjal caused by *Fusarium solani* f. sp. *melongenae* can result in severe yield loss. (Arshi *et al*., 2021) found a similar result, indicating that *Fusarium solani* is the fungal pathogen that reduces eggplant production.



Stage 1: Stunting of infected plants and yellowing of older leaves. **Stage 2:** Browning of vascular tissues and dropping of leaves. **Stage 3:** The death of the plant, without producing fruit.

Plate 1. Symptoms of *Fusarium solani* f. sp. *melongenae.*

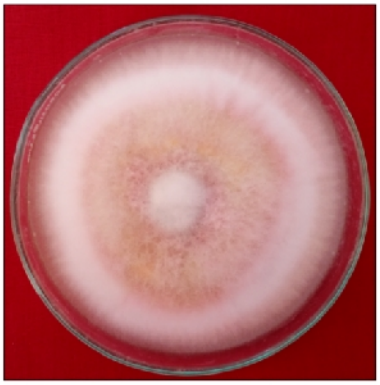


Plate 2. Auxenic culture of *Fusarium solani* f. sp. *melongenae.*

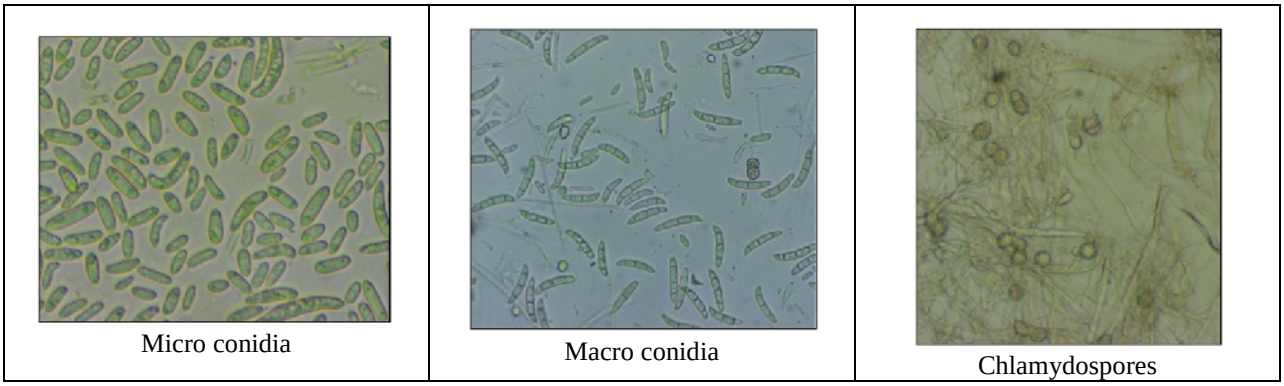


Plate 3. Microscopic features of *Fusarium solani* f. sp. *melongenae.*

Isolation and Identification of *Trichoderma* isolates from the rhizosphere region.

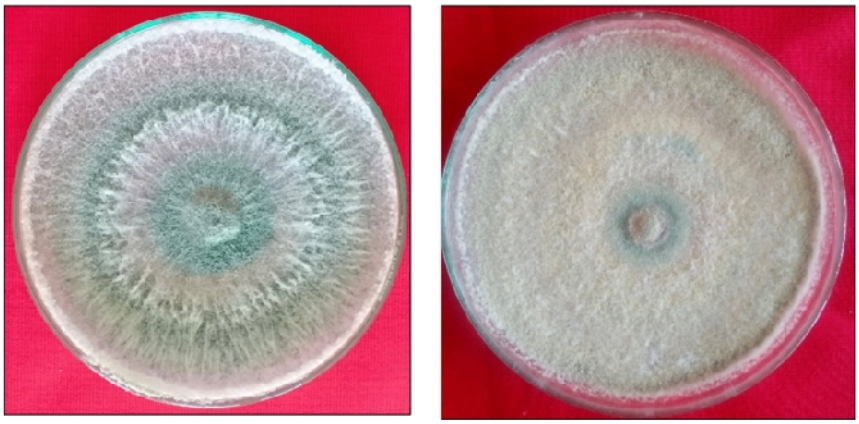
A total of fifteen isoates of *Trichoderma* spp. were isolated from different regions of rhizosphere soil of healthy brinjal plants. The obtained isolates are identified based on morphology and conidia characters such as *Trichoderma asperellum* and *Trichoderma longibrachiatum* were named as isolated code of T(AB)-1, T(AB)-2, T(LB)-3, T(LB)-4, T(LB)-5, T(AB)-6, T(AB)-7, T(AB)-8, T(LB)-9, T(LB)-10, T(LB)-11, T(LB)-12, T(AB)-13, T(LB)-14, and T(AB)-15 (Table 1, 2) (Plate 4). The distribution of isolates, on the other hand, was discovered to differ between districts. *In vitro* experiments proclaim, *Trichoderma asperellum* had effective biological control activity against *Fusarium solani* f. sp. *melongenae* and a variety of plant diseases.

Table 1: *Trichoderma* spp. isolated from brinjal rhizosphere soil of Tamil Nadu.

Sr. No.	Place of collection	Isolate code	District	Latitude	Longitude
1.	Thirumangalam	T(AB)-1	Madurai	9°82′16″N	77°98′24″E
2.	Kamayagoundanpatti	T(AB)-2	Theni	9° 73′86″N	77°31′81″E
3.	Chempatti	T(LB)-3	Dindugal	10°28′20″N	77°87′24″E
4.	Kathiripatti	T(AB)-4	Dindugal	10°36′55″N	77°97′65″E
5.	chekkanurani	T(LB)-5	Madurai	10°02′38″N	78°22′35″E
6	Vandalaikudalur	T(AB)-6	Trichy	10°97′01″N	78° 88′78″E
7.	Kodarankulam	T(AB)-7	Tirunelveli	8°69′58″N	77°42′78″E
8.	Pullimankombai	T(AB)-8	Madurai	10°23′83″N	78°22′35″E
9.	Ayanpannapatti	T(LB)-9	Trichy	9°63′13″N	77°76′66″E
10.	Omalur	T(AB)-10	Salem	11°74′29″N	78°04′73″E
11.	Aruppukottai	T(LB)-11	Virudunagar	9°56′80″N	77°96′24″E
12.	Kottampatti	T(LB)-12	Madurai	10°21′97″N	78°37′92″E
13.	Podumbu	T(AB)-13	Madurai	9° 98′51″N	78° 08′44″E
14.	Puliyarai	T(LB)-14	Tirunelveli	9°00′41″N	77°18′73″E
15.	Gudiyattam	T(AB)-15	Vellore	12°97′00″N	79°31′05″E

Table 2: Morphological characteristics of *Trichoderma* spp.

Sr. No.	Place of collection	Fungal Native Antagonists	Isolates of *Trichoderma*	Colony marphology
1.	Thirumangalam	*Trichoderma asperellum*	T(AB)-1	Mycelial growth looks to be white with bright green culture
2.	Kamayagoundanpatti	*Trichoderma asperellum*	T(AB)-2	Dark green with sluggish growth
3.	Chempatti	*Trichoderma longibrachiatum*	T(LB)-3	Light green to yellowish in colour, mycelial proliferation.
4.	Kathiripatti	*Trichoderma asperellum*	T(AB)-4	Dark green mycelial growth
5.	chekkanurani	*Trichoderma asperellum*	T(AB)-5	Fully dull green mycelium spikes with papule sprouting
6.	Vandalaikudalur	*Trichoderma asperellum*	T(AB)-6	Dark green to dull green with fringed culture
7.	Kodarankulam	*Trichoderma hamatum*	T(LB)-7	Green to yellow mycelial growth
8.	Pullimankombai	*Trichoderma asperellum*	T(AB)-8	Sunrise like Hyphae growth on bright green culture
9.	Ayanpannapatti	*Trichoderma longibrachiatum*	T(LB)-9	Greenish yellow with green ring-like zones
10.	Omalur	*Trichoderma asperellum*	T(AB)-10	Light to dark green mycelial growth
11.	Aruppukottai	*Trichoderma longibrachiatum*	T(LB)-11	Dark green with clumsy yellowish ring
12.	Kottampatti	*Trichoderma longibrachiatum*	T(LB)-12	Sparse ring like growth with slight grey color
13.	Podumbu	*Trichoderma asperellum*	T(AB)-13	Dark green mycelial growth
14.	Puliyarai	*Trichoderma longibrachiatum*	T(LB)-14	A yellowish - green culture is made up of white growth on the peripheral.
15.	Gudiyattam	*Trichoderma asperellum*	T(AB)-15	Dark green mat like growth



(a) *Trichoderma asperellum* (b) *Trichoderma longibrachiatum*

Plate 4. Auxenic culture of *Trichoderma* spp.

Antagonistic activity of *Trichoderma* spp. against *F. solani* f. sp. *melongenae* under *in vitro*.

In a dual culture of *Fusarium solani* f. sp. *melongenae* on PDA medium, the effectiveness of local *Trichoderma* isolates in suppressing the mycelial growth of *Fusarium solani* f. sp. *melongenae* was investigated. The results of dual culture assays showed that all identified *Trichoderma* spp. inhibited *F. solani* radial development to varying degrees (Table 3), (Plate 5) (Fig. 1). These *Trichoderma* isolates inhibited *F. solani* mycelial growth in the range of 53.00 to 83.11 percent. *F. solani* colony mycelial growth was significantly slowed by fifteen isolates, with the most promising isolates showing more than 50% suppression. T(AB)-8 had the largest inhibition zone (83.11 percent), followed by T(AB)-6 (78.11 percent), and T(AB)-2 (72.66 percent), with T(LB)-3 having the smallest (53.00 percent). The antagonists slowed *Fusarium solani* f. sp. *melongenae* mycelial growth, then the pathogen outgrew them after three to four days. However, the *Trichoderma* had outgrown the pathogen and had completely taken over the medium five days later. The consequences of this study are similar to those of a number of other researchers, such as (Ramaraju *et al*., 2017), who tested *Trichoderma* spp. isolates for antifungal activity against *F. oxysporum* f. sp. *melongenae* and found that the maximum extent of inhibition was 81.11 percent. The findings are also consistent with the findings of (Montaser *et al.*, 2017), who found that the *Trichoderma* strain inhibited by 76.25 percent in dual culture. Competition for nutrients and space, mycoparasitism, and the production of antibiotic substances and hydrolytic enzymes are the main mechanisms by which *Trichoderma* sp. hinder *F. solani* mycelial growth.

Table 3: Antifungal activity of different isolates of *Trichoderma* spp. against the mycelia growth of *Fusarium solani*.

Sr. No.	Isolates	Mycelial growth of the pathogen (cm)*	Percent reduction over control
1.	T(AB)-1	3.19	64.55 (53.46)
2.	T(AB)-2	2.46	72.66 (58.47)
3.	T(LB)-3	4.23	53.00 (46.72)
4.	T(AB)-4	3.85	57.66 (49.15)
5.	T(LB)-5	3.04	66.22 (54.46)
6.	T(AB)-6	1.97	78.11 (62.10)
7.	T(AB)-7	3.64	59.55 (50.51)
8.	T(AB)-8	1.52	83.11 (65.73)
9.	T(LB)-9	2.18	75.77 (60.51)
10.	T(AB)-10	3.92	56.44 (48.70)
11.	T(LB)-11	3.47	61.44 (10.74)
12.	T(LB)-12	2.71	69.88 (9.48)
13.	T(AB)-13	2.24	75.22 (8.61)
14.	T(LB)-14	2.69	70.33 (9.44)
15.	T(AB)-15	3.35	62.77 (10.55)
	Control	9.00	0.00
	CD (P=0.5)	0.14	2.51

*Mean of three replications; Values in parentheses are arcsine transformed values

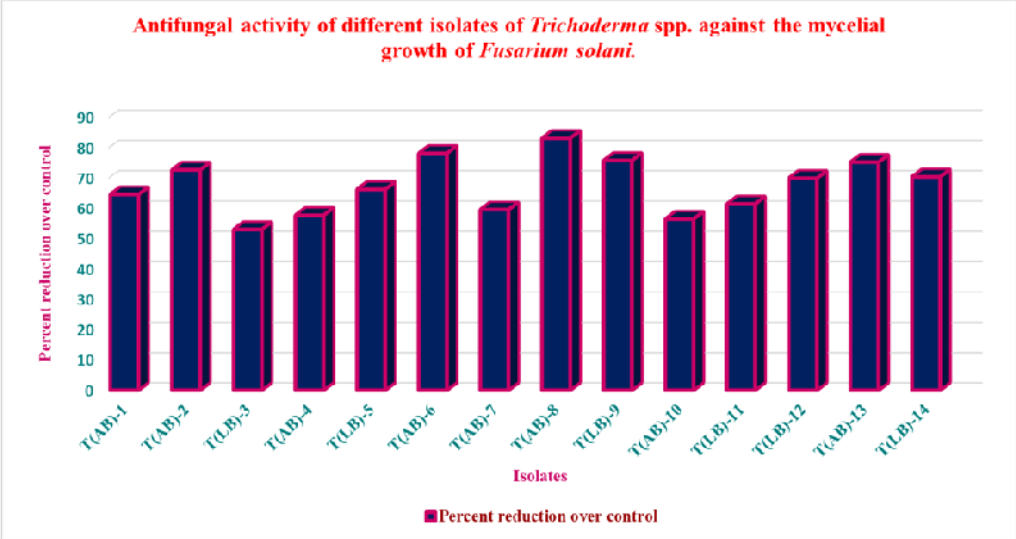
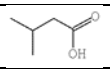
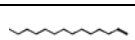
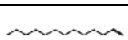
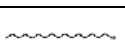
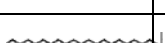
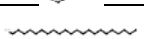
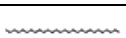
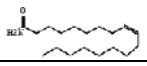


Fig. 1. Antifungal activity of different isolates of *Trichoderma* spp. against the mycelia growth of *Fusarium solani.*

Plate 5. Antagonistic activity of *Trichoderma asperellum* against *Fusarium solani.*

Efficacy of VOCs produced by selected *Trichoderma* spp. inhibit the growth of *F. solani* f. sp. *melongenae* *in vitro*. The chromatograms of *Trichoderma asperellum*, chloroform, and ethyl acetate fractions revealed the presence of 4 and 9 peaks of volatile compounds, respectively, according to the GC-MS analysis. (Table 4) (Plate 6, 7) lists the compounds in the chloroform fraction, along with their percent peak areas and retention times. The compound present in the highest concentration in the ethyl acetate fraction mounted 1-Hexadecanol (14.33 percent). The compounds n-Nona decyl trifluoroacetate (13.67 percent) and 1-Tetradecene were moderately abundant in this fraction (11.50). Less abundant compounds included 1-Heptacosanol (6.91 percent), 1,2 Benzedicarboxylic acid, bis (2-mrthyl propyl) (5.19 percent), and 1-Tridecene (2.16 percent). The antifungal, antibacterial, and antioxidant effects of these compounds, such as Hexadecanol, 2-methyl, have been attributed to the results of Wonglom *et al.*, (2020). 1-Methoxy-2-propyl acetate, 2, 2-Dideutero Octadecanal, 9,12- Octadecadienoic acid (Z, Z), 9-octadecenoic acid (z)-methyl ester, and 1-Docosene are some of the other compounds. (Preetisonkar, 2019) also stated that *Trichoderma asperellum* also stated that *Trichoderma asperellum* secreted a variety of volatiles and secondary metabolites that play a role in antifungal, antagonistic, and antibacterial activity. The observed peak of compound was named Phenol, 3, 5-bis (1,1-dimethylethyl), Pentadecanoic Acid, 14-methyl, methyl ester, and Benzenepropanoic acid, 3, 5, -bis (1,1-dimethyl ethyl)-4-hydroxy-methyl ester respectively.

Table 4: Antimicrobial volatile compounds identified from *Trichoderma asperellum* (TB3) through GC/MS.

Peak	RT	Compound name	Structure	Molecular formula	Molecular weight (g/mol)	Peak Area%	Activity	References
1	5.02	Butanoic acid, 3-methyl-		C5H10O2	102	2.41	Antimicrobial	Hayashida-Soiza *et al.*, (2008)
2	12.13	1-Dodecanol		C12H260	186	6.82	Antifungal activity	Teresa *et al.*, (2014)
3	17.73	1-Tetradecene		C14H28	196	11.50	Antifungal activity	Jiang *et al.*, (2014)
4	17.94	1-Tridecene		C13H26	182	2.16	Antifungal activity	Ahsan *et al.*, (2017)
5	22.94	1-Hexadecanol		C16H340	242	14.33	Antimicrobial activity	Susanti *et al.*, (2013)
6	23.10	Henicosane		C10H20O	296	2.29	Antimicrobial activity	Ertürk *et al.*, (2016)
7	27.64	n-Nonadecyl trifluroacetate		C19H400	284	13.67	Antimicrobial and cytotoxic properties	Kuppuswamy *et al.*, (2013)
8	29.12	1,2 Benzedicarboxylic acid, bis (2-mrthyl propyl)		C16H2204	278	5.19	Antifungal activity	Kim *et al.*, (2004)
9	30.15	Pentanoic acid, 3-methyl-		C6H1202	116	8.89	Antifungal activity	Wheatley *et al.*, (1997)
10	31.16	Dibutyl phthalate		C16H2204	278	6.19	Antimicrobial activity	Seddek *et al.*, (2019)
11	31.51	Phthalic acid, butyl 2-pentyl ester		C17H2404	292	2.64	Antimicrobial activity	Matysiak *et al.*, (2019)
12	31.91	Behenic alcohol		C22H460	326	10.55	Antimicrobial activity	Kai *et al.*, (2009)
13	36.56	1-Heptacosanol		C27H560	396	6.91	Antifungal	Kim *et al.*, (2004)
14	40.41	9-octadecenamide(z)-		C18H35N0	281	2.69	Antifungal and antibacterial activity	Hossain *et al.*, (2016)
15	41.08	1-Hexacosanol		C26H540	382	3.75	Antimicrobial activity	Matysiak *et al.*, (2019)

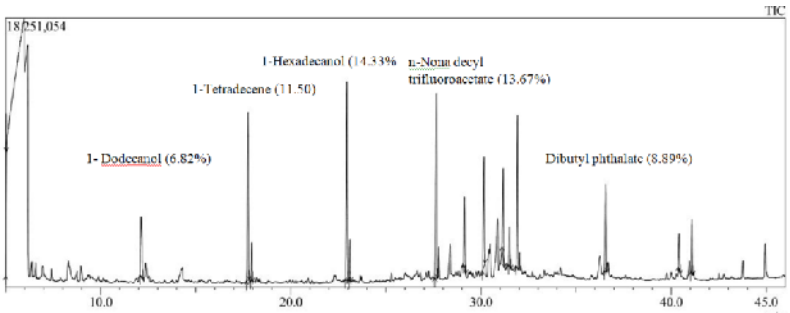


Plate 6. Antimicrobial volatile compounds identified from *Trichoderma asperellum* T(AB) -8 through GC/MS.

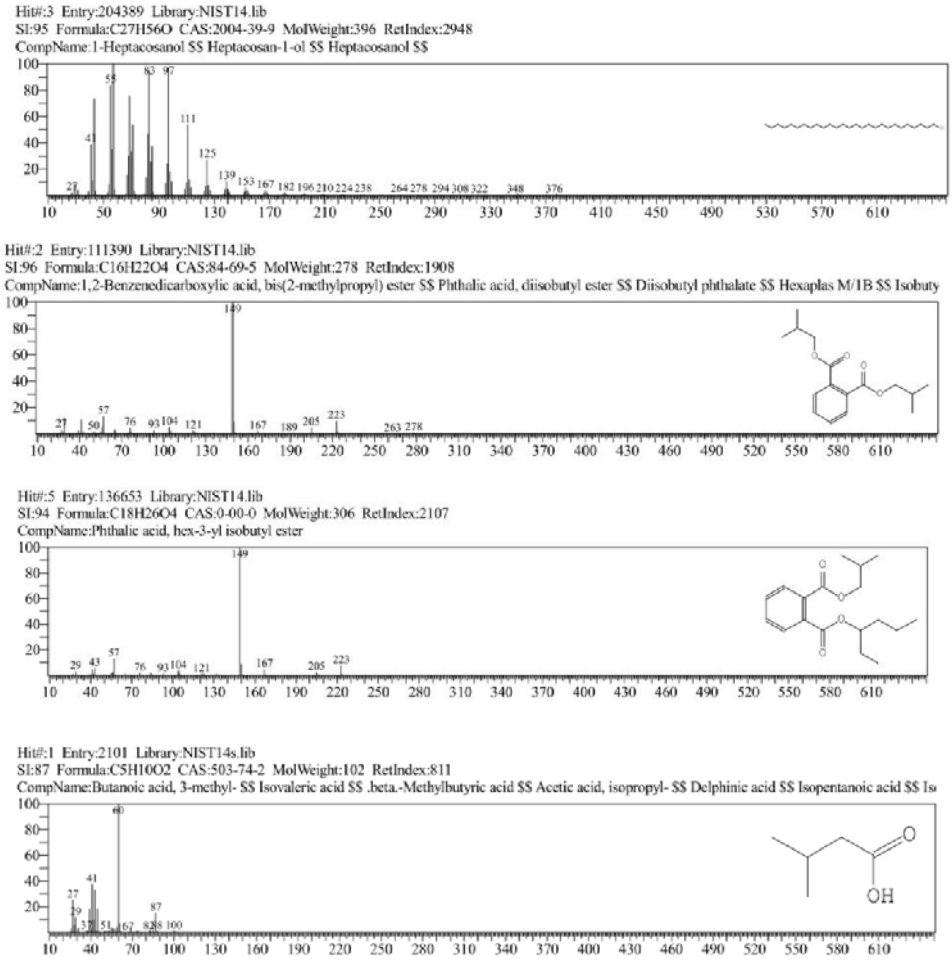


Plate 7. Secondary metabolites produced by *Trichoderma asperellum.*

In vitro* evaluation of crude metabolites from antagonistic fungi found against the mycelial growth of *Fusarium solani* f sp *melongenae

The results of the radial growth of *Fusarium solani* due to the production of volatile compounds by *Trichoderma* spp. isolates revealed that T(AB)-8 had the highest reduction of mycelial growth (3.02 cm) at 15 days after incubation, followed by *Trichoderma* spp. isolate T(AB)-6, which had the lowest reduction of mycelial growth (3.47 cm) (Table 5) (Fig. 2). Antifungal volatile molecules have shown to be highly effective against a variety of pathogens in the past. Volatile metabolites from several *Trichoderma* spp. inhibited the growth of *Fusarium solani* f sp *melongenae*, with inhibitory zones ranging from 33 to 71 percent (Qualhato *et al.*, 2013). *Fusarium solani* and *Fusarium oxysporum* mycelial growth have been shown to be inhibited by the volatile compounds produced by *Trichoderma* spp (Cristina *et al.*, 2017). According to this study, the metabolites produced by these *Trichoderma* species are toxic and fungistatic to *Fusarium.*

Table 5: Efficacy of volatile compounds of effective *Trichoderma* spp. against the growth of *Fusarium solani.*

Sr. No.	*Trichoderma* isolates	Mycelium growth (cm)*	Per cent reduction over control (%)
1.	T(AB)-8	3.02	66.44 (54.60)
2.	T(AB)-2	4.06	54.88 (47.80)
3.	T(LB)-5	4.64	48.44 (44.11)
4.	T(LB)-12	4.21	53.22 46.85()
5.	T(AB)-1	4.79	46.77 (43.15)
6.	T(AB)-7	5.24	41.77 (40.26)
7.	T(LB)-11	4.95	45.00 (42.13)
8.	T(AB)-4	5.65	37.22 (37.60)
9.	T(LB)-3	6.03	33.00 (35.06)
10.	T(AB)-6	3.47	61.44 (51.61)
11.	Control	9.00	0.00
12.		CD (P=0.5) 0.57	2.08

*Mean of three replications; Values in parentheses are arcsine transformed values

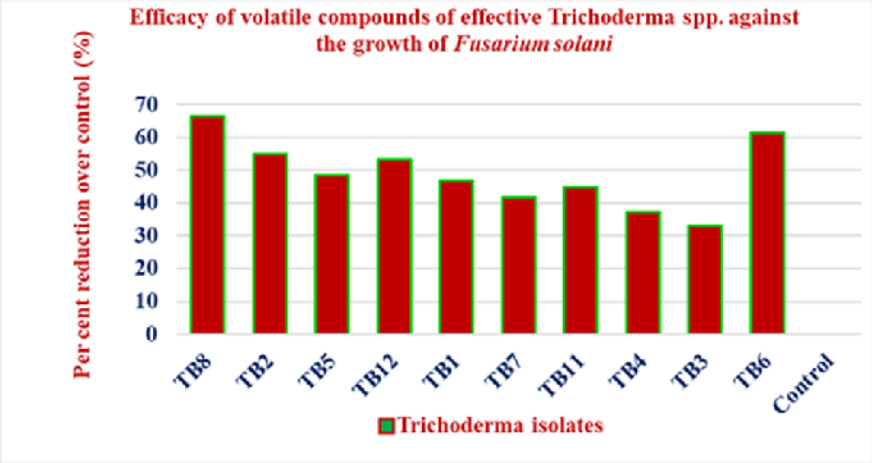


Fig. 2. Efficacy of volatile compounds of effective *Trichoderma* spp. against the growth of *Fusarium solani* f. sp. *melongenae.*

Screening the extra cellular enzymes of *Trichoderma* spp. The ability of the fungal antagonistic strains to produce cell wall degrading enzymes was tested. *T. asperellum* strains grown in liquid cultures containing *Fusarium solani* cell walls secreted more enzymes than those grown with glucose as a carbon source in general. (Table 6) (Fig. 3) shows that isolates of *T. asperellum* have a diverse range of hydrolytic enzyme activities. Chitinase (8.72-10.5 pmol/s/ml) and β-1,3-glucanases (1.23-1.97 nmol/s/ml) activities were highest in isolates T(AB)-8, T(AB)-6, T(AB)-2, T(LB)-12, T(LB)-5, and T(AB)-1. The isolates T(LB)-11, T(AB)-7, T(AB)-4, and T(LB)-3 had the lowest chitinase (3.83-4.45pmol/s/ml) and β-1,3-glucanases (0.8-0.9 nmol/s/ml) activities. However, the majority of the remaining isolates had moderate lytic enzyme activity.

Table 6: Hydrolytic enzymes activities of *Trichoderma* strains grown in liquid cultures media supplemented with 0.1% cell walls of FSM after six days of incubation at 25±1°C.

Trichoderma isolates	Chitinase activity (pmol/s/ml)	β-1,3-glucanase activity (nmol/s/ml)
T(AB)-6	9.88	1.50
T(AB)-1	7.57	0.86
T(AB)-7	5.53	1.23
T(AB)-8	10.5	1.97
T(LB)-12	8.72	0.74
T(LB)-3	4.45	0.65
T(LB)-11	7.51	0.92
T(LB)-5	8.40	0.76
T(AB)-4	3.83	0.95
T(AB)-2	9.65	0.89

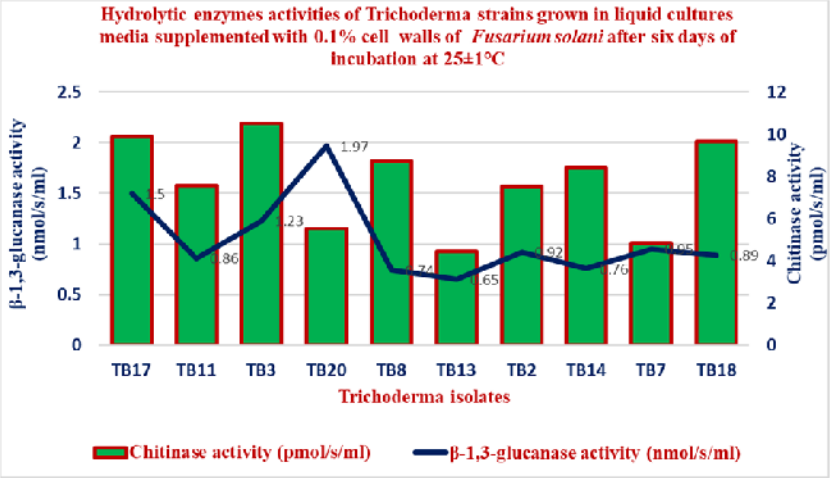


Fig. 3. Hydrolytic enzymes activities of *Trichoderma* strains grown in liquid cultures media supplemented with 0.1% cell walls of *Fusarium solani* after six days of incubation at 25±1°C.

Mycoparasitism is a complex process in which antagonistic *Trichoderma* strains produce hydrolytic enzymes (such as chitinases and β -1,3-glucanases) that hydrolyze chitin and -glucan, the primary structural components of fungal cell walls (Woo *et al.*, 2004; Mausamverma *et al*., 2007). Affording to Mohammad *et al*., 2015, the number of generated cell-wall disintegrating enzymes secreted by *Trichoderma* strains is related to their ability to suppress plant pathogenic fungi. The levels of enzymatic activity varied significantly among the recovered *T. asperellum* isolates. This finding could be explained by the induction and variation of *T. asperellum* hydrolytic enzyme genes in response to the presence of FOL cell wall components in the culture medium.

CONCLUSION

Under laboratory conditions, all antagonist species of *Trichoderma* isolates were found to be effective in controlling *Fusarium solani* f. sp. *melongenae*, *T. asperellum* was identified as the isolate with the highest chitinase activity. It has the ability to stop *Fusarium solani* f sp *melongenae* from growing and causing fusarium wilt disease in brinjal plants. It makes the enzymes chitinase, protease and β-1,3 glucanase, which break down pathogen cell walls. Many bioactive compounds were found in the crude extract from *T. asperellum* culture broth. *T. asperellum* could be used to protect crops from plant pathogens as a biological agent.

FUTURE SCOPE

In future aspects, *Trichoderma* strains have been identified as an internationally recognised biocontrol fungus due to their effective broad-spectrum antimicrobial activity. Some are already being used to resist fusarium wilt and soil-borne pathogens. The widespread use of selected metabolites produced by *Trichoderma* spp. to induce host resistance and promote crop yield is still limited due to their low potency in disease management when compared to synthetic pesticides, and their performance can be adversely affected by a wide range of biotic and abiotic agents. As a consequence, studying the characteristics of *Trichoderma* species, as well as their interactions with pathogens, plants, and biocontrol mechanisms, can help to improve *Trichoderma* spp capability. The current study may motivate and inspire farmers to give special attention to biocontrol agents in brinjal cultivation.

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Conflict of Interest. None.

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