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Research Article

Gas Chromatography Mass Spectrometry (GCMS) analysis of the antagonistic potential of *Trichoderma hamatum* against *Fusarium oxysporum* f. sp. *cepae* causing basal rot disease of onion

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ABSTRACT: *Fusarium oxysporum* f. sp. *cepae* causing basal rot disease of onion is a destructive phytopathogen resulting in 30-50% yield loss and remains as a major constraint in onion productivity. The management of disease through application of fungicide is not feasible and economically viable. Hence, the present study is focused on investigation of effective *Trichoderma* sp. and identifying the effective volatile organic compounds produced by it against the basal rot pathogen in onion. A total of ten *Trichoderma* spp. were isolated from rhizospheric soil of healthy onion plants and tested against virulent *Fusarium oxysporum* f. sp. *cepae* isolate FCIM1. The *Trichoderma* isolate (TIM2) showed 77.40% inhibition on mycelial growth of pathogen followed by the isolate (TIV1) with 70.36% inhibition. The molecular identification of effective *Trichoderma* isolate through the analysis of the rDNA of Internal Transcribed Spacers (ITS) region revealed isolate TIM2 as *Trichoderma hamatum.* The GC-MS analysis of *Trichoderma hamatum* unravelled the important volatile organic compounds like Methyl stearate, n-Hexadecanoic, Eicosane, 9-cyclohexy, Heptadecane, Dodecane, 2-cyclohexyl, to 2H-Pyran-2-one, 6-pentyl, 5-Hydroxymethylfurfural, Tetrapentacontane, 1-Dodecanol, 2-Propenoic acid, pentadecyl ester, Benzene, (2-methylbutyl) and 1,2-Dimethyltryptamine with peak area and retention time. These bioactive compounds exert a strong antifungal activity against *Fusarium oxysporum* f. sp. *cepae.* The scanning electron micrographs of *Fusarium* paired with effective *Trichoderma* (TIM2) showed the swollen hyphae with cell wall damage which is clear evident of antagonistic interaction of volatile compounds produced by *Trichoderma hamatum*.

KEY WORDS: Dodecane, *Trichoderma hamatum,* Internal Transcribed Spacers, rhizospheric soil, volatile organic compound

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INTRODUCTION

Onion (*Allium cepa* L.), an important biennial vegetable crop, bulbous in nature known as Queen of kitchen and has been used as spice and medicine for thousands of years. Besides the domestic consumption it earns highest foreign exchange (Jaggi, 2005). It exhibits numerous therapeutic properties like antifungal, antibacterial, antiseptic, anti-inflammatory, antispasmodic, antihelmintic etc. It is highly rich in vitamin C, vitamin B6, sulfur, iron, potassium, anthocyanin and antioxidant particularly quercetin. Flavonoids and quercetin present in onions reduce the oxidative damage of cells (Ravichandran *et al*., 2012). In India, the average production of onion is around 224.27 lakh tonnes and productivity is about 17.18 t/ha (Mahajan *et al*., 2017). The productivity of onion is pretended by various fungal and bacterial diseases such as basal rot, purple blotch, anthracnose and stem blight which cause severe loss in the productivity both in field and in storage conditions. Among which basal rot disease caused by *Fusarium oxysporum* f. sp. *cepae* (Hans.) Snyder and Hansen (FOC) cause severe yield loss in production and storage. The basal rot disease occurs in all stages of crop growth. Yield loss up to 50% has been recorded in susceptible cultivars (Coşkuntuna and Özer, 2008). Approximately basal rot disease caused 45% loss in yield and 12-30% of bulb loss in the storage (Rajput and Patel, 2006). So far, the successful management of disease is achieved by use of chemical fungicides but due to the hazardous impact of agrochemicals on the environment, higher cost of pesticides, development of resistant mutants, frequent breakdown of resistance etc. strongly demands an alternate sustainable management approach. Hence recent researches have been improvised the use of biological control as an alternative method for effective and sustainable

plant disease management. *Trichoderma* species are the effective biocontrol agent commonly found in the all types of soils, rhizospheric and phylloplane region and have been known to control various soil-borne fungal diseases caused by genera of *Fusarium, Sclerotinia, Gaeumannomyces, Pythium, Rhizoctonia* etc. (Howell, 2003). *Trichoderma* evades the pathogens directly by competing for nutrients and space, it produces antibiotic and lytic enzymes and thereby inactivates the growth of pathogen. It indirectly produce various biochemical and morphological changes in host plant and induce resistance against pathogen attack (Ramamoorthy *et al*., 2001). Application of biocontrol agent induced the defence related proteins in tomato and suppressed the growth of *Fusarium oxysporum f. sp. lycopersici* (Ramamoorthy *et al*., 2002). The beneficial effect of *Trichoderma* last for a longer period when compared with chemical fungicides by rapid multiplication in soil and protecting the plant throughout all the crop growth stages. Various *Trichoderma* spp. produce different types of volatile and non-volatile antibiotics that are inhibitory to the growth of fungi and bacteria (Dennis and Webster, 1971a, 1971b). There are several volatile organic compounds like Dodecanol, Heptadecane, Eicosane, 2H-Pyran-2-one, 6-pentyl-, thymine, ribitol etc and non-volatile secondary metabolites *viz*., konginginin, trichodermadione, trichodermin, viridian, viridiol, atroviridin, gliotoxin, gliovirin etc. (Jayalakshmi *et al.,* 2021) which are highly known for their antifungal and antibacterial activity against many phytopathogens. (Harris and Lumsden, 1997; Highley, 1997; Wright, 1956). Hence the aim of the present research was focused on evaluating the antagonistic potential of various *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *cepae* and to unravel the Volatile Organic Compounds (VOC) responsible for the inhibition of *Fusarium oxysporum* f. sp. *cepae* through GC-MS gas chromatography spectrometry analysis.

MATERIALS AND METHODS

Collection of the sample

During *kharif* 2020, onion leaf with typical symptoms of yellowing, curling and necrosis at the tip of leaf blades, stunted growth and orange to salmon coloured spore masses around the rotted basal plate were observed on the onion cultivars grown in southern districts of Tamil Nadu. A rowing survey was conducted and disease incidence of 53% was recorded. The onion bulb showing typical symptoms were collected from the infected field for the isolation of pathogen causing basal rot disease.

Isolation of *Fusarium oxysporum* f. sp. *cepae* causing basal rot disease

Onion leaf with typical symptoms were collected and the pathogen was isolated by tissue segmentation method in Petri plates containing Potato Dpkextrose Agar (PDA) medium for which the infected bulb samples were washed with tap water to remove the soil particles and swabbed with 70% ethanol solution to eliminate the surface microbial contaminants. The infected region was cut into small pieces and surface sterilized with 0.1% sodium hypochlorite for 30 seconds followed by washing in sterile distilled water (thrice). The infected tissues were placed on the Petri plate containing solidified PDA medium. The plates were incubated at (25 ± 2°C). Once the growth of fungal colonies was observed, the pure culture was obtained and stored at 4°C. The morphological identification of the pathogen was done by observing the cultural and conidial characters under compound microscope.

Isolation of *Trichoderma* spp. from the rhizospheric soil

Soil samples were collected from rhizospheric region of healthy onion plants. Isolation of *Trichoderma* spp. were carried out from the soil by serial dilution of the soil samples and plated on *Trichoderma*s Elective Medium (TSM). The TSM plates were incubated at (28 ± 2°C) for two days. Growth of numerous fungal colonies was observed and *Trichoderma* spp. were identified based on the morphology of conidiophores and arrangement of phialides. The fungal colonies of *Trichoderma* spp. were transferred to sterile Petri plates containing PDA medium.

Efficacy of volatile organic compounds produced by *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *cepae*

Paired plate technique was followed to test the efficacy of volatiles produced by *Trichoderma* isolates in suppressing the growth of *Fusarium oxysporum* f. sp. *cepae.* Ten *Trichoderma* isolates were inoculated on the PDA medium separately and incubated at 27°C for 3 days. The lid of all Petri plates were removed and replaced with the bottom plate containing PDA medium inoculated with the virulent pathogen (*Fusarium* isolate FCIM1). Both plates were sealed together with paraflim. The Petri plate with *Fusarium* isolate FCIM1 alone was kept as control and incubated at (25 ± 2°C) for 7 days. The fungal mycelial diameter of the pathogen was measured at different intervals and the percent growth inhibition was calculated by using a formula:

PI = Dc-Dt/Dc ×100
Where,
Pl - Percent inhibition,
Dc–Mycelial growth of pathogen in control,
Dt - Mycelial growth of pathogen in treatments.

Scanning electron microscopic study on effect of VOC of effective *Trichoderma* on the mycelial growth of *Fusarium oxysporum* f. sp. *cepae*

To test the effect of volatile compounds exerted by the effective *Trichoderma* isolate. a mycelial strand of *Fusarium*

oxysporum f. sp. *cepae* from the Petriplate containing the least mycelial diameter of *Fusarium* isolate under Paired plate technique with effective *Trichoderma* isolate (i.e., *Fusarium* isolate FCIM1 paired with *Trichoderma* isolate TIM2) was placed in a cover slip. Similarly, few mycelial strands of *Fusarium* isolate from the control plate were also placed in a cover slip. Cover slips with mycelium was fixed with 2.5% glutaraldehyde and washed three times with graded ethanol followed by drying of samples in a vacuum drier under Co_2. Finally, the samples were examined and micrographed under versatile tungsten thermionic emission Scanning Electron Microscope (SEM) system equipped with magnification range upto 1, 50,000X, detectors SE, w filament, BSE and resolution of 2 nm located in Central Instrumentation Centre at Madurai Kamaraj University.

Molecular characterization of the effective *Trichoderma* isolate TIM2

For molecular identification of the effective *Trichoderma* isolate TIM2, the fungal culture was grown on Potato Dextrose broth (PDB) for 7 days at 27 $\pm$ 1°C. The fungal mycelial mat was filtered and ground to into fine powder. The genomic DNA was extraction was done using CTAB method. The universal primers (internal Transcribed Spacer region) ITS1-5' TCCGTAGGTGAACCTGCGG 3' (forward primer) and ITS4 -5' TCCTCCGCTTATTGATATGC3' (reverse primer) were used for the PCR amplification. Amplification was done using PCR conditions of initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 sec, annealing temperature at 50°C for 30 sec and extension at 72°C for 60 sec and a final extension at 72°C for 10 min. The reaction was carried out in an Eppendorf master cycler gradient PCR machine. The PCR products was analysed on agarose gel of 0.8% stained with ethidium bromide. The amplicon was viewed under UV Trans illuminator. The ITS product was sequenced at Eurofins genomics India Pvt. Ltd. Bangalore. The obtained DNA sequence was trimmed at 5' and 3' region. Then DNA sequences, in which clear chromatogram obtained, were made in Fasta format. This was used as input sequence (Query sequence) in nucleotide blast analysis program at NCBI database. The output was retrieved from the bioinformatics analysis tool and the organism showing major score from the output is considered as the closely related species to the effective isolate.

Preparation of crude extracts of *Trichoderma* sp. for GC-MS

For the preparation of the crude extracts, mycelial disc of 9 mm from the actively growing effective *Trichoderma* isolate (TIM2) was inoculated into potato dextrose broth of 250 ml and incubated at 25°C for seven days. PDB with well grown fungus mycelia was filtered twice using Whatmann no.1 filter paper and centrifuged at 9000 rpm for 15 mins. Equal volume of solvent ethyl acetate was added to the filtrates and incubated in a shaker at 25°C, 150 rpm overnight. The solvent phase was filtered using a separating fungal to collect the volatile metabolites. The solvent containing VOC were concentrated using rotary evaporator until complete evaporation of solvent. The final output was diluted with 2 ml ethyl acetate and filtered using 0.4 µ bacterial filter.

Gas Chromatography Mass Spectrum Analysis (GCMS) of crude extracts of *Trichoderma* sp.

Potential VOC of effective *Trichoderma* sp. were identified with Shimadzu Gas chromatography equipped with mass detector turbo mass gold containing an Elite -1 (100% Dimethyl Poly Siloxane), 30 m x 0.25 mm ID x one mM df. The conditions employed were the following: Carrier gas, helium (1 ml/ min), Oven temperature program 110°C (2 min) to 280°C (9 min), Injector temperature (250°C), Total GC time (45 min), the final output ethyl acetate extracts was injected at 1.0 µl into the chromatography. The major volatile organic compounds present were identified using a computer algorithm. The analysis was matched with library database of National Institute of Standards Technology (NIST) and with the software Turbo mass version 5.1. This GC-MS analysis was carried out at centre of innovation for excellence, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai.

Statistical analysis

The data were statistically analyzed with the help of SPSS version 160. Data were subjected to the ANOVA at a significant level (p 0.05) and by using DMRT, the means were compared.

RESULTS AND DISCUSSION

Isolation and identification of *Fusarium oxysporum* f. sp. *cepae*

Pathogen associated with basal rot disease of onion was isolated from the symptomatic infected samples. The infected plants mainly show yellowing, curling and necrosis of the leaf blades. On lateral stages whole leaf blades show symptoms and eventually wither and decay. The entire aerial potion of the plant collapses. Decay often appears on one side of the bulb base, causing bulbs to appear irregularly shaped. When an infected bulb is cut vertically, a watery, brown discoloration of the stem plate tissue is apparent. The stem plate tissue becomes pitted and shows a dry rot. Under dry conditions, a whitish fungal growth appears on the base of the bulb scales followed by semi watery rot. The tissue segmentation method was followed for the isolation of pathogen and the cultures were grown on PDA plates. Initially the fungal colony was thin and dull white to pure white in colour. Later the mycelium was thick and the central surface

of the culture exhibits pink to purple colour pigmentation. Few isolates were devoid of this pink pigmentation. The morphological confirmation was made by observing the spores under light microscope. The fungus produces macroconidia and microconidia. The microconidia were oval to reniform in shape while the macroconidia were falcate to straight and slightly point. The length of macroconidia varied from 22.13 to 27.83µm and the width ranged from 2.27 to 3.14 µm. The length of microconidia varied from 7.12 to 8.47µm and the width ranged from 2.16 to 2.62 µm (Figure 1). Similarly Gowda *et al*. (2020) noticed the fluffy white cottony growth with pinkish purple pigment patches. The virulent *Fusarium* isolate was identified by pathogenicity test and it was molecularly confirmed as *Fusarium oxysporum* f. sp. *cepae.*

Isolation and identification of *Trichoderma* spp.

Totally ten *Trichoderma* isolates namely TIM1, TIM2, TIT1, TITR1, TIR2, TIS1, TIS2, TID1, TID2 and TIV1 were successfully isolated from the soil samples collected from rhizospheric region of onion plants using *Trichoderma*s Elective Medium (TSM). All the ten isolates exhibited morphological and cultural variability (Fig. 2a). Their conidiophore morphology, arrangement of phialides and conidia were observed under light microscope. All *Trichoderma* isolates except TIV1 have well branched conidiophores containing primary and secondary conidiophores. Three phialides are formed at right angle to each other at the end of the secondary conidiophore in triangle manner. This is the typical morphology of *Trichoderma* type conidiophore. Isolate TIV1 have typical *Penicillium* type conidiophore without production of secondary conidiophores and the phialides were grouped at the end of the conidiophore in acute angle resembling *Gliocladium* type conidiophore which clearly indicates that Isolate TIV1 was*Trichoderma virens* (Fig. 2b). Molecular confirmation was done only for the effective isolate obtained from the paired plate technique.

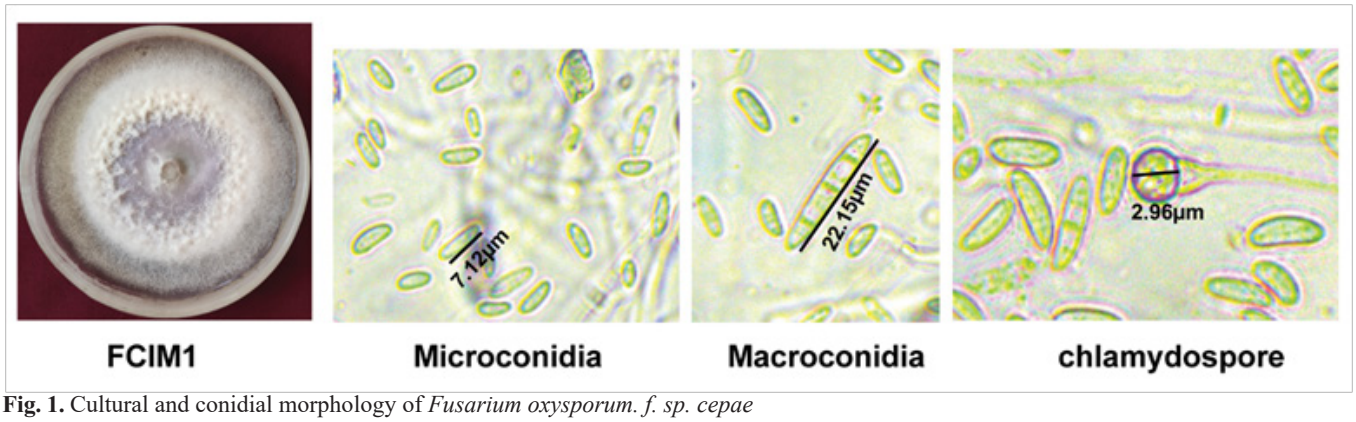

Fig. 1. Cultural and conidial morphology of *Fusarium oxysporum. f. sp. cepae*

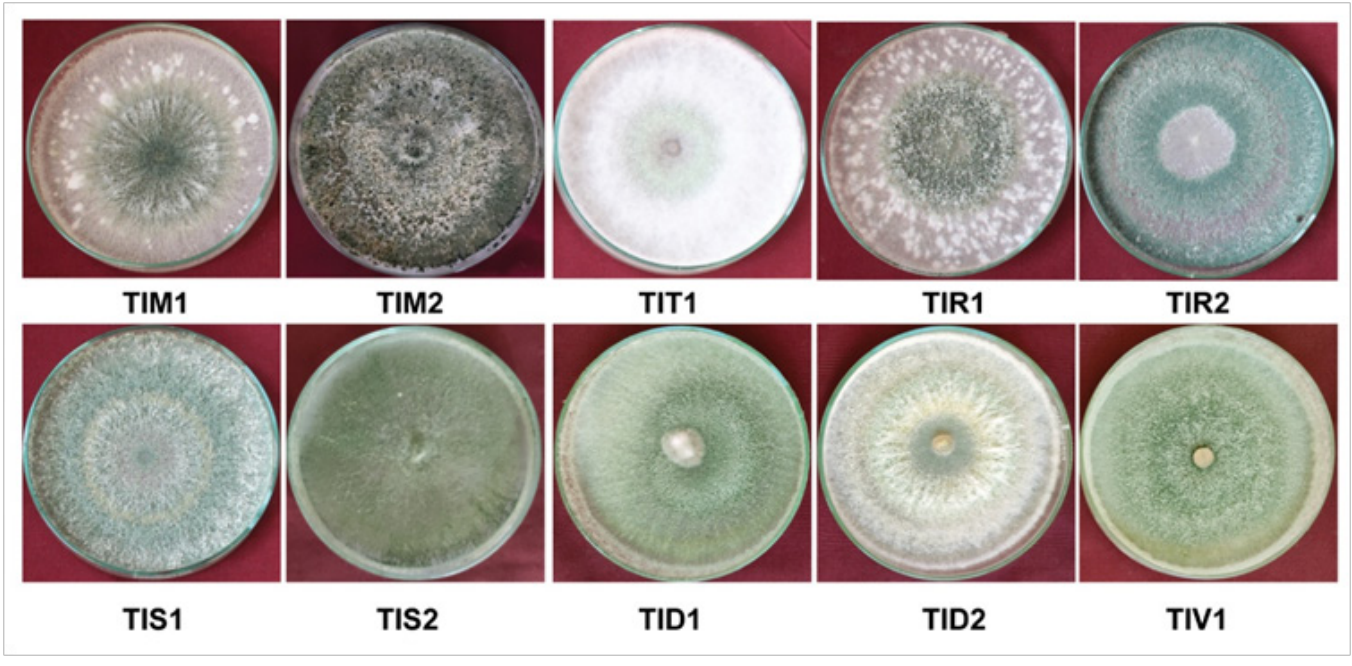

Fig. 2a. Cultural variability of different *Trichoderma* spp. isolated from rhizospheric region of healthy onion plants.

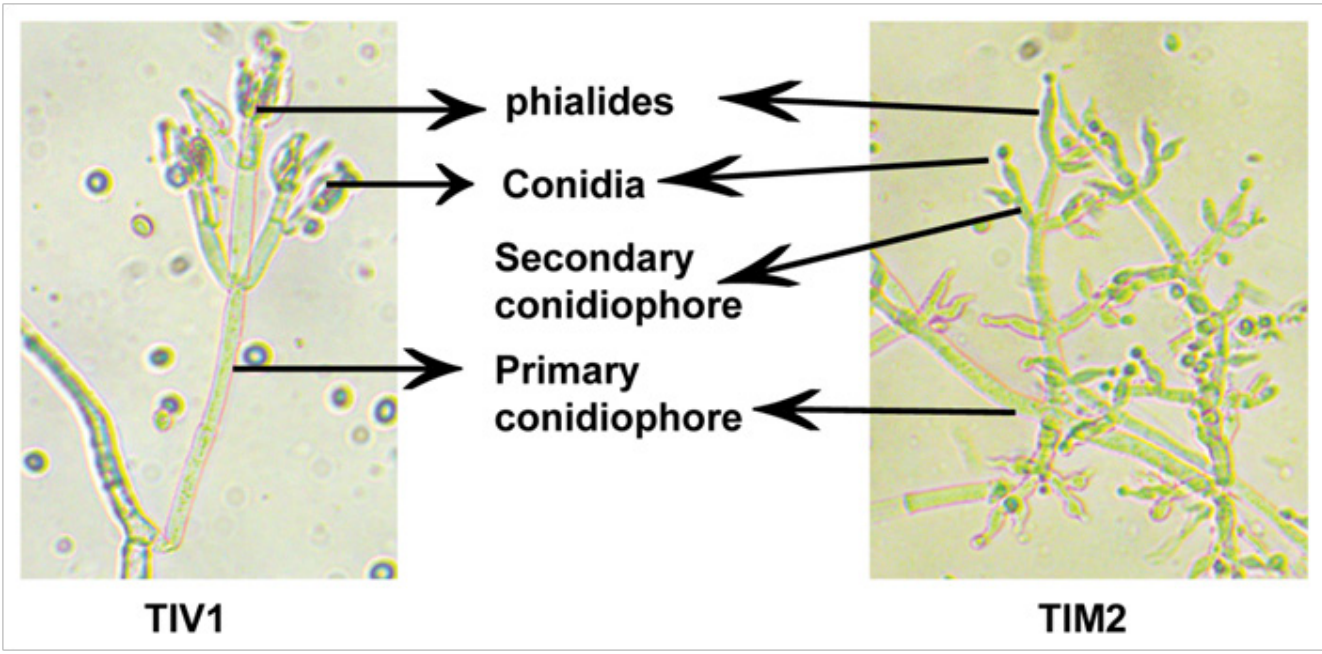

Fig. 2b. Conidial morphology of effective *Trichoderma* spp.

TIM2 - (*Trichoderma* type conidiophore) phialides are formed at right angle to each other at the end of the secondary conidiophores.

TIV1 - (*Gliocladium* type conidiophore) phialides were grouped at the end of the conidiophore in acute angle.

Antifungal efficacy of volatile organic compounds produced by *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *cepae*

Among the ten *Trichoderma* isolates tested against *Fusarium oxysporum* f. sp. *cepae,* the isolate (TIM2) inhibited the mycelial growth of the pathogen at 77.40% followed by the isolate (TIV1) with 70.36% reduction over control (Table 1, Fig. 3). The *Fusarium* isolate corresponding to the effective isolate in paired plate technique exhibited constricted mycelial growth with pale orange to light brown colour pigmentation showing the antagonistic interaction of different volatile organic compound exerted by effective *Trichoderma* isolates. The inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *cepae* was due to the emission of various Volatile Organic Compounds (VOC) produced by the *Trichoderma hamatum*. Key compounds like propyl benzene produced by *Trichoderma* sp. was highly effective against *Fusarium oxysporum, A. solani*, *Alternaria alternata* etc. (Meena *et al.,* 2016). Sesquiterpenes an antifungal compounds produced by *Trichoderma* sp. inhibited the mycelial growth of *F. oxysporum* and functions as a plant growth regulators (Kottb *et al.,*2015) . Gliotoxin a secondary metabolite produced by *Trichoderma virens* has the strong antifungal activity against *Fusarium, Ralstonia* and *Pythium*. The solvent ethyl acetate effectively extracted the gliotoxin (Jayalakshmi *et al.,* 2021). Similarly, in the present study the isolate TIV1 resembling Gliocladium like conidial type and morphologically confirmed as *Trichoderma virens* inhibited the mycelial growth *Fusarium.* The volatile compounds produced by *T. harzianum* and *T. virens* possess a strong antifungal activity. Li *et al*. (2018) studied the antifungal activity of four *Trichoderma* species which were antagonistic against *F. oxysporum* and also noted that *F. oxysporum* recognizes *Trichoderma* spp. by sensing their VOCs and released VOCs that inhibited *Trichoderma*, suggesting that both types of VOC-mediated interaction were common among fungi. Narayan *et al.* (2006); Siddiquee *et al.* (2012) reported that the volatile compounds produced by the *Trichoderma* not only induce resistance in plants but also helps in growth promotion.

Effect of VOC of by *Trichoderma* spp. against *Fusarium oxysporum f.* sp. *cepae* observed by Scanning Electron Microscope

Upon paired plate technique, Fusarium plates paired with effective *Trichoderma* isolates TIM2 and TIV1 showed the least mycelial growth with modified cultural characters. The changes occurred in mycelium and spores of *Fusarium oxysporum* f. sp. *cepae* due to the interaction of VOC produced by *Trichoderma* was examined under scanning electron microscope. The control plate without the biocontrol agent showed well developed falcate shaped macroconidia and oval shaped microconidia found with hyaline profused mycelium (Figure 4a). The mycelium of *Fusarium* isolate paired with *Trichoderma* were shortened and abnormally

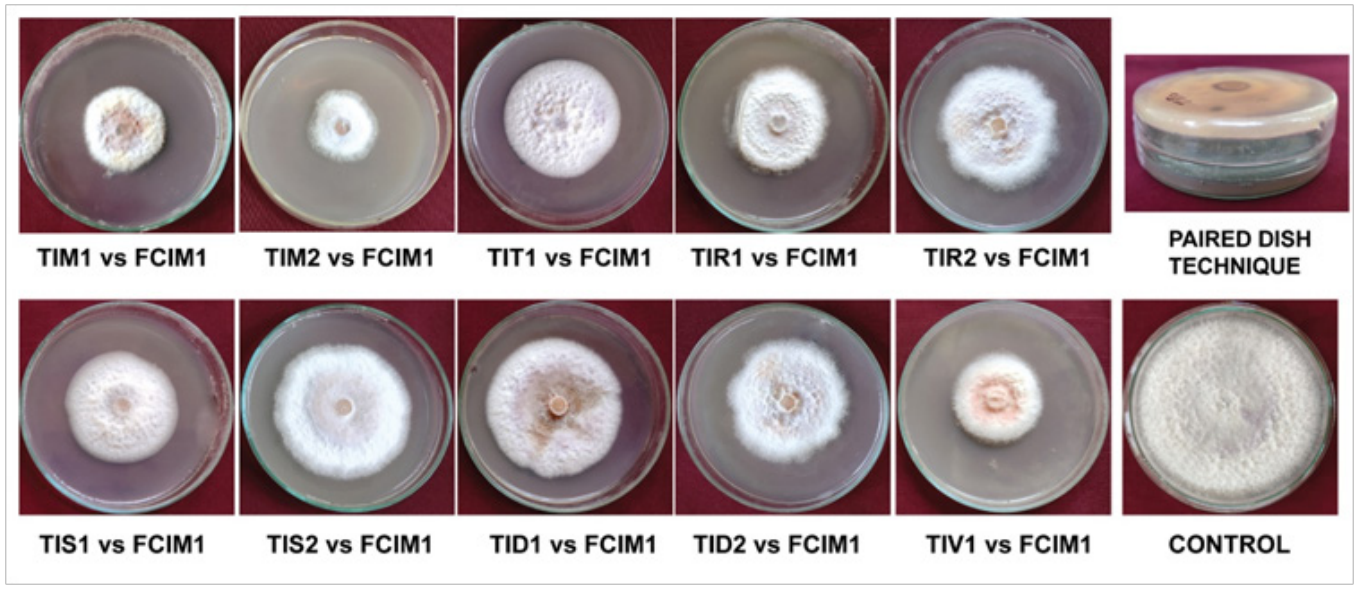

Fig. 3. Efficacy of volatile organic compounds produced by *Trichoderma* spp. against *Fusarium oxysporum.* F. sp. *cepae* by paired plate technique.

Table 1. Efficacy of volatile organic compounds produced by *Trichoderma hamatum* against *Fusarium oxysporum. f. sp. cepae* by paired plate technique

S. no	Trichoderma isolates	8th Day	15th Day	
		Mycelial growth (cm)	Mycelial growth (cm)	Per cent growth reduction over control (%)*
1.	TIM1	2.0	2.7	69.25 (56.32)[b]
2.	TIM2	1.0	2.0	77.40 (61.61)[a]
3.	TIT1	2.1	5.7	36.29 (37.04)[e]
4.	TIR1	2.4	4.4	50.74 (45.42)[c]
5.	TIR2	2.9	6.0	32.96 (35.05)[f]
6.	TIS1	2.7	5.2	42.22 (40.52)[d]
7.	TIS2	4.6	7.9	12.59 (20.77)[h]
8.	TID1	4.2	7.5	16.29 (23.80)[g]
9.	TID2	3.0	6.1	32.59 (34.81)[f]
10.	TIV1	1.5	2.6	70.36 (57.02)[b]
11.	control	4.5	9.0	00.00
CD($P \leq 0.05$)	0.821			

*Mean of three replications
Values with different superscripts are significantly differ from each other at $p<0.05$
Values in the parenthesis are arc sine transformed values.

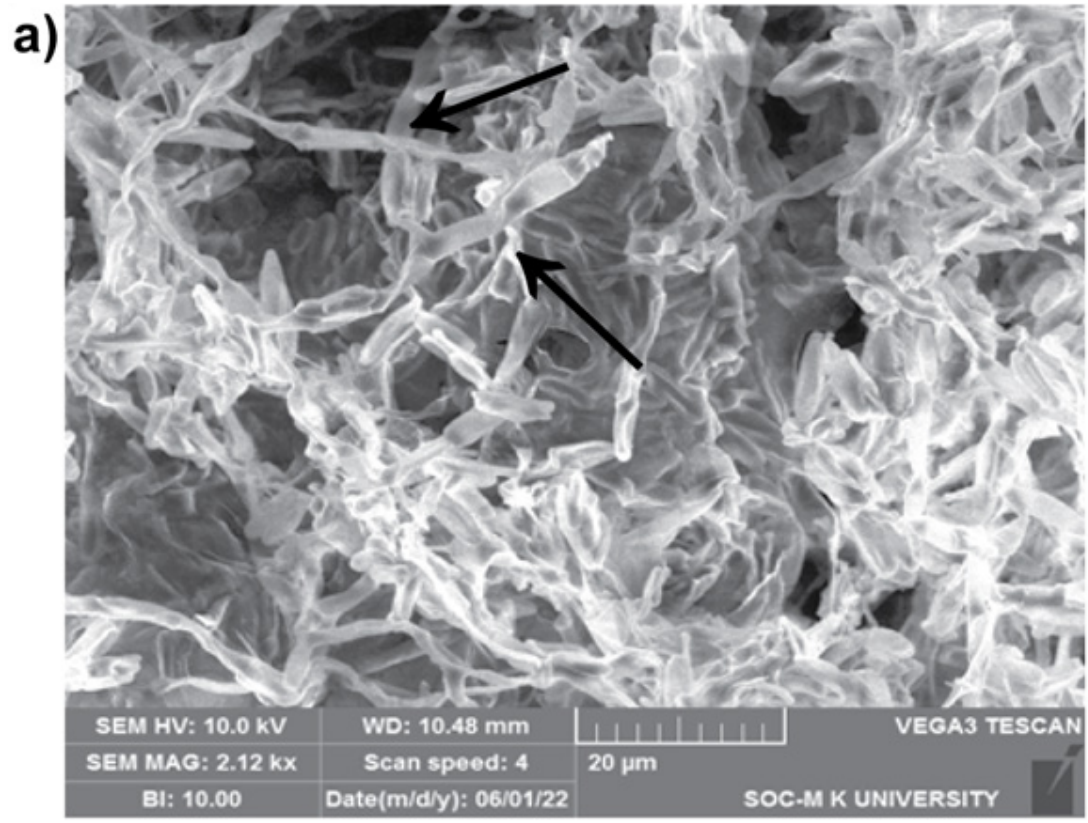

Fig. 4a. Scanning Electron Micrographs **(SEM) of** *Fusarium oxysporum f. sp. cepae.*
Arrow indicates the normal profused mycelia of *Fusarium oxysporum f. sp*. *cepae* in control plate.

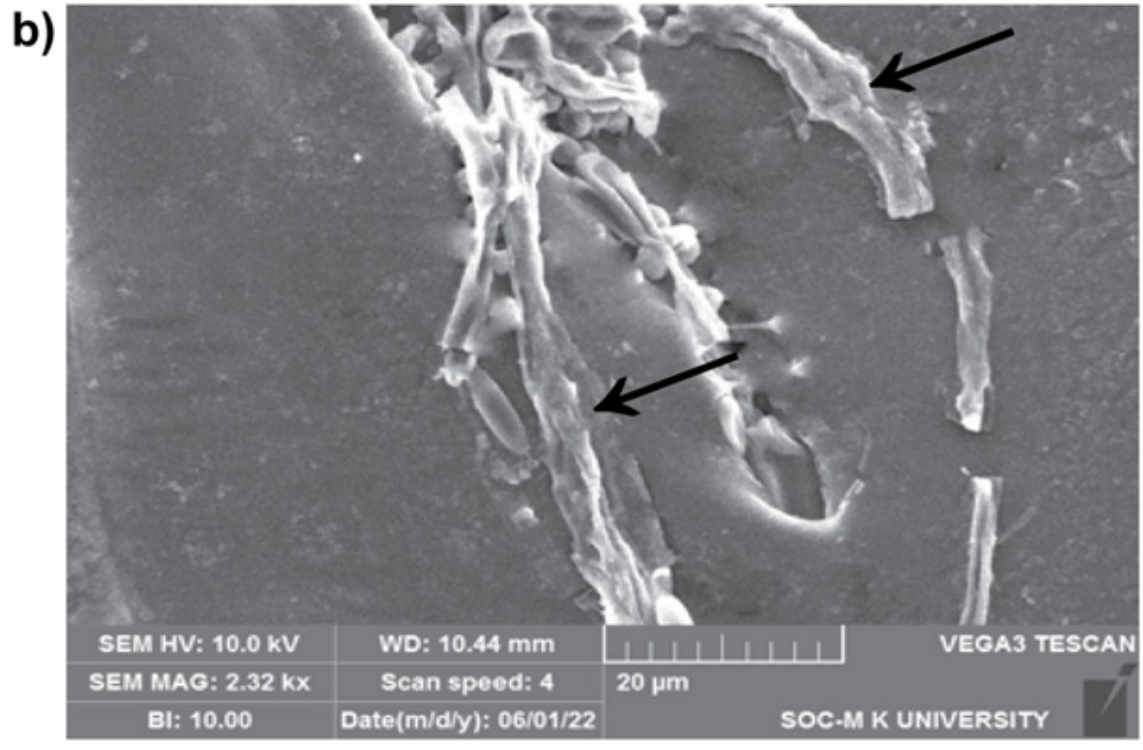

Fig. 4b. Scanning Electron Micrographs (SEM) showing the effect of volatile compounds of *Trichoderma hamatum* on *Fusarium oxysporum*. *f. sp. cepae*. Arrow indicates shortened, abnormally swollen and cell wall damaged mycelia of *Fusarium oxysporum. f. sp. cepae*

swollen. The cell wall was shrunken, collapsed and loses its impermeability and integrity (Figure 4b). The results were in accordance with Wu *et al.* (2015) who reported that VOC such as 2-pentylfuran,4-methoxystyrene and anisole have the antifungal activity against *Fusarium oxysporum* and *Sclerotinia sclerotiorum.* The SEM observation of the mycelium treated with volatile compounds showed the shrunken and swollen appearance. Upon staining with trypan blue, the damaged cell wall and dead hyphae were dark blue in colour indicating the loss of cellular impermeability.

Molecular identification of the effective *Trichoderma isolate*

ITS sequence analysis is one of the commonly used molecular methods for the identification of fungi at species level. Genomic DNA from isolate TIM2 was isolated using CTAB method. The DNA was amplified with ITS 1 and ITS 4 primer pair using a thermo cycler, Single band of intact genomic DNA was visualised on the agarose gel. The size of the PCR fragments was approximately 650 bp length confirming it as *Trichoderma hamatum* (Fig. 5). The full length of ITS sequences obtained from *T. hamatum* strain TIM2 were BLAST searched in the nucleotide database of National Centre for Biotechnology information (NCBI). The output data showed matching sequences of *T. hamatum* already in the database. Thus, *Trichoderma* strain TIM2 used in the present study was confirmed as *T. hamatum* and obtained with the accession number (ON920706).

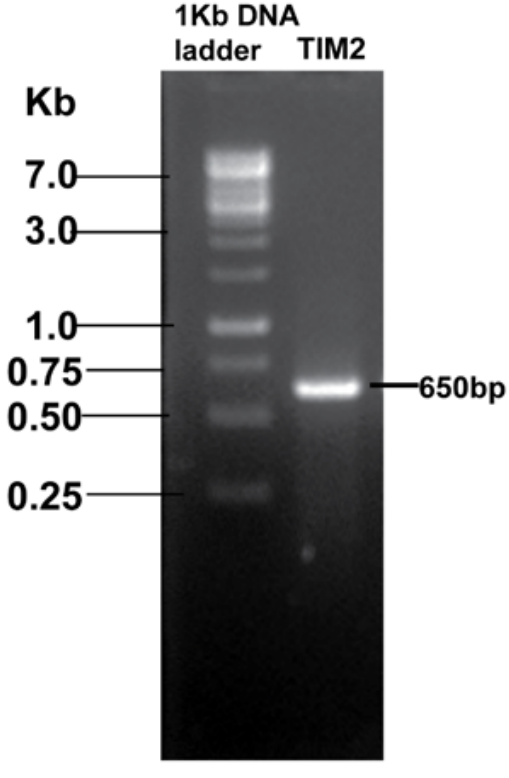

Fig. 5. PCR amplification of ITS region of effective *Trichoderma* isolate TIM2.

GCMS analysis of *Trichoderma hamatum*

The crude extract of *Trichoderma hamatum* isolate (TIM2) containing volatile organic compounds, extracellular antifungal compounds and antibiotics was analysed through GC-MS. It detected the secondary metabolites and novel volatile compounds responsible for antifungal activity. In total 20 compounds were detected among these 8 compounds were selected based on the Retention Time (RT) and relative abundance of peak. The peaks with retention time 33.764 min corresponds to Methyl stearate, n-Hexadecanoic acid with 3.59% of peak area, 29.156 of RT,27.918 min represent to Hexadecanoic acid, methyl ester, 2 Eicosane, 9-cyclohexyl- with 22.578 min of RT, 21.222 min RT and 9.50% peak area pertaining to Heptadecane, Dodecane, 2-cyclohexyl with 2.14% of peak area and 20.079 min RT, 17.780 min corresponds to 2H-Pyran-2-one, 6-pentyl- with 8.11% of peak area, 5-Hydroxymethylfurfural with RT of 12.572 min and peak area of 6.07% (Figures 6 to 8). Other antimicrobial compounds like Tetrapentacontane, 1,54-dibromo- with 31.906 min RT, Eicosane with 29.945 RT and peak area of 2.84%, 1,2-Benzenedicarboxylic acid, bis (2-methylprop) with 26.006 min RT and 4.43% of peak area, 2,6,10,14-tetramethyl- with peak area of 10.37% and RT of 23.567 min, 2-Propenoic acid, pentadecyl ester with peak area of 4.01% and 23.400 min RT, Benzene, (2-methylbutyl) with peak area of 2.55% and RT of 23.166, 1-Dodecanol with peak area of 3.52% and RT of 17.780 min and 3-Acetoxy-3-hydroxypropionic acid, methyl ester, 1,2-Dimethyltryptamine were also found in the crude extracts of effective *Trichoderma* isolate TIM2. Biological activity and chemical structure of phyto compound were identified and tabulated (Table 2, Figures 6, 7 and 8). Specific volatile namely 6-n-pentyl-2 H-pyran-2-one has the ability to reduce the mycotoxin productivity. Production of Mycotoxin deoxynivalenol by *F. graminearum* was significantly lowered by the application of synthetic form of 6-n-pentyl-2 H-pyran-2 (Bharose and Gajera, 2018a). the findings of Aldakheel *et al.* (2020) who reported that the culture filtrates of *T. harzianum* possessing many antifungal volatile compounds suppressed the mycelial growth of *Fusarium* spp. Vinodkumar *et al*. (2017) proved the antifungal activity of volatile compound heptadecenoic acid against *Sclerotinia sclerotiorum* under *in vitro* condition. *Trichoderma citrinoviride* produce various bioactive compound like heneicosane, quinolone, 6-pentyl-2H-pyran-2, heptadecane, phenol, 2-(6-hydrazino- 3pyridazinyl), eicosane, , benzene propianoic acid, hexadecane and dibutyl phthalate which showed 77.8% of inhibition against fungal pathogens (Tomah *et al*., 2020). Similarly Dodecane produced by *Trichoderma* spp. inhibited the growth of some plant pathogenic bacteria and restricted the mycelial growth of *Aspergillus niger* (Pucot *et al*.,2021).

Table 2. GCMS compounds of effective *Trichoderma* spp.

S.NO	Name of the compound	Molecular formula	MW g/mol	Peak area%	RT	Specific role	Reference
1.	Methyl stearate	$C_{19}H_{38}O_2$	298	0.37	33.764	Antifungal activity	(Marques *et al.,* 2018)
2.	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	914	3.33	31.906	antibacterial activity	(Samy *et al.,* 2013)
3.	Eicosane	$C_{26}H_{54}$	366	2.84	29.945	Antifungal activity	(Alsultan *et al.,* 2019)
4.	n-Hexadecanoic acid	$C_{17}H_{34}O_2$	270	3.59	29.156	Antifungal activity	(Masiulionis and Pagnocca, 2020)
5.	Hexadecanoic acid, methyl ester	$C_{16}H_{22}O_4$	278	4.43	27.918	Antifungal activity	(Chowdhury *et al.,* 2018)
6.	1,2-Benzenedicarboxylic acid, bis(2-methylprop	$C_{24}H_{38}O_4$	278	3.10	26.006	Antifungal	(Chen *et al.,* 2020)
7.	2,6,10,14-tetramethyl-	$C_{17}H_{36}$	240	10.37	23.567	Antifungal activity	(Sheoran *et al.,* 2015)
8.	2-Propenoic acid, pentadecyl ester	$C_{18}H_{34}O_2$	282	4.01	23.400	antibacterial activity	(Aldakheel *et al.,* 2020)
9.	Benzene, (2-methylbutyl)	$C_{11}H$	148	2.55	23.166	Antifungal activity	(Wei *et al.,* 2020)
10.	Eicosane, 9-cyclohexyl-	$C_{26}H_{52}$	364	1.84	22.578	Antifungal activity	(Alsultan *et al.,* 2019)
11.	Octadecane	$C_{18}H_{38}$	254	3.58	22.379	Antifungal activity	(Rajaofera *et al.,* 2019)
12.	Heptadecane	$C_{17}H_{36}$	240	9.50	21.222	Antifungal and antibacterial activity	(Jishma *et al.,* 2017),
13.	- Dodecane, 2-cyclohexyl	$C_{18}H_{36}$	252	2.14	20.079	antibacterial activity	(Pucot *et al.,* 2021)
14.	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206	1.48	19.086	Antifungal activity	(Sangeetha *et al.,* 2018)
15.	1-Dodecanol	$C_{12}H_{26}O$	186	3.52	18.089	Antifungal activity	(Sangeetha *et al.,* 2018)
16.	2H-Pyran-2-one, 6-pentyl-	$C_{15}H_{32}$	212	8.11	17.780	Antifungal activity	(Rao *et al.,* 2022)
17.	1,2-Dimethyltryptamine	$C_{12}H_{16}N_2$	188	3.43	17.445	Antifungal	(Chen *et al.,* 2020)
18.	3-Acetoxy-3-hydroxypropionic acid, methyl ester	C_6H_{1005}	162	0.60	13.041	Antifungal activity	(Zhang *et al.,* 2018)
19.	5-Hydroxymethylfurfural	$C_5H_6N_2O_2$	126	6.07	12.572	Antifungal activity	(El-Benawy *et al.,* 2020)
20.	Thymine	$C_5H_6N_{202}$	126	3.19	8.347	Antifungal activity	(Sridharan *et al.,* 2021)

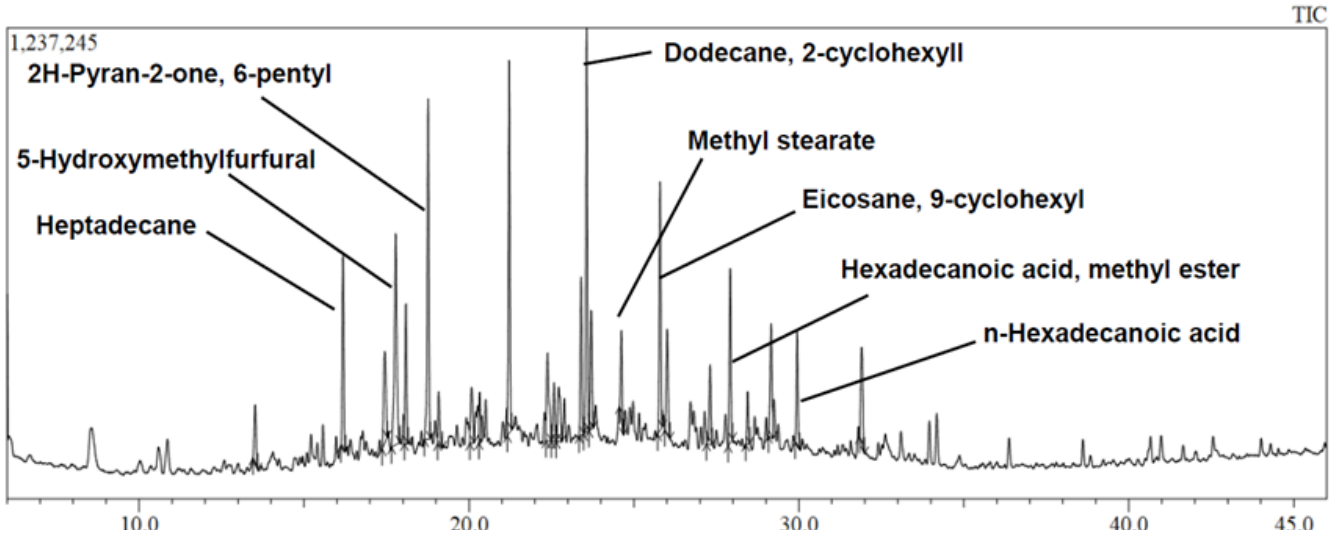

Fig. 6. Gas chromatogram of volatile compounds identified from crude extracts of *Trichoderma hamatum*

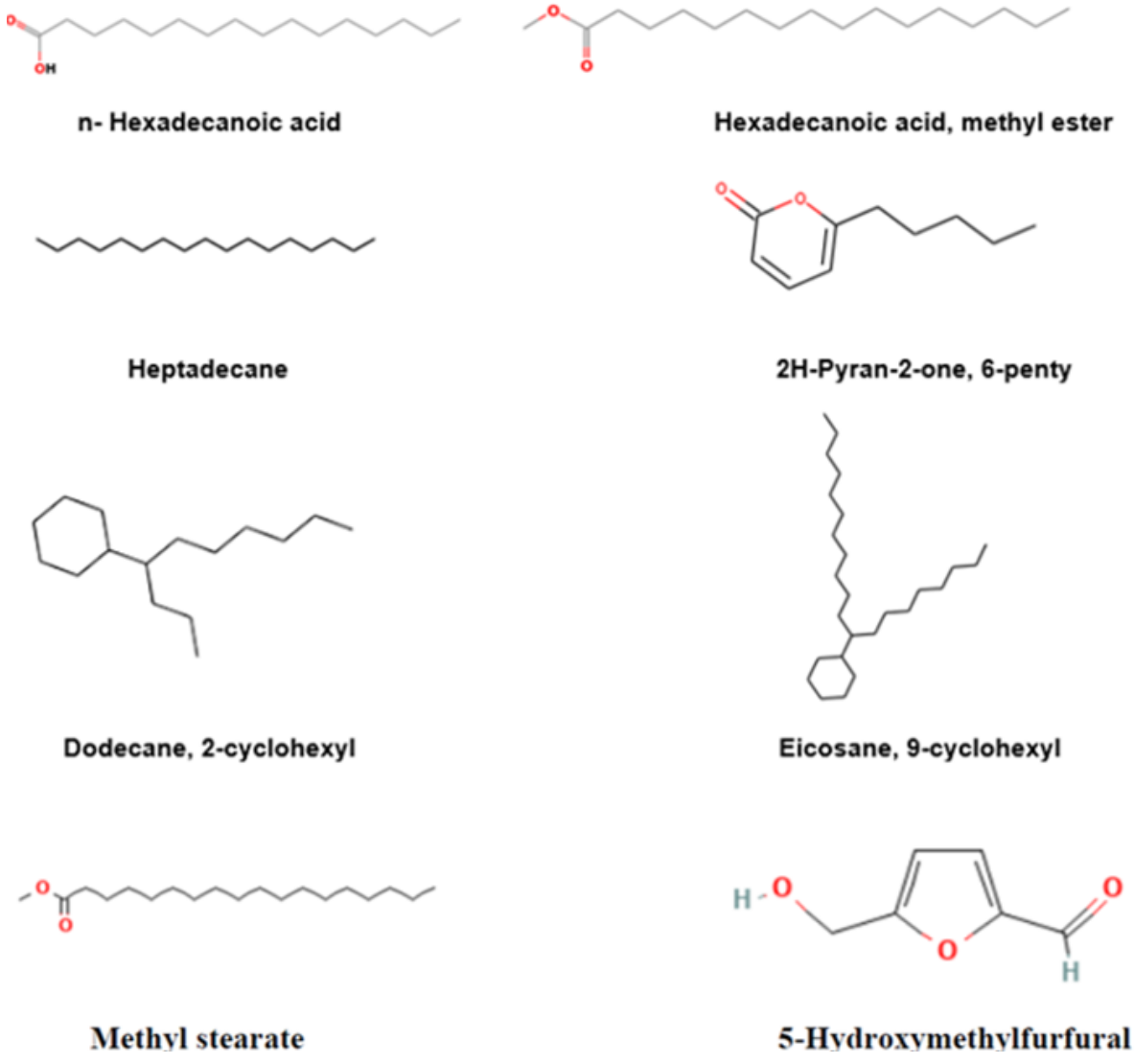

Fig. 7. Chemical structure of various antifungal compounds produced by *Trichoderma hamatum.*

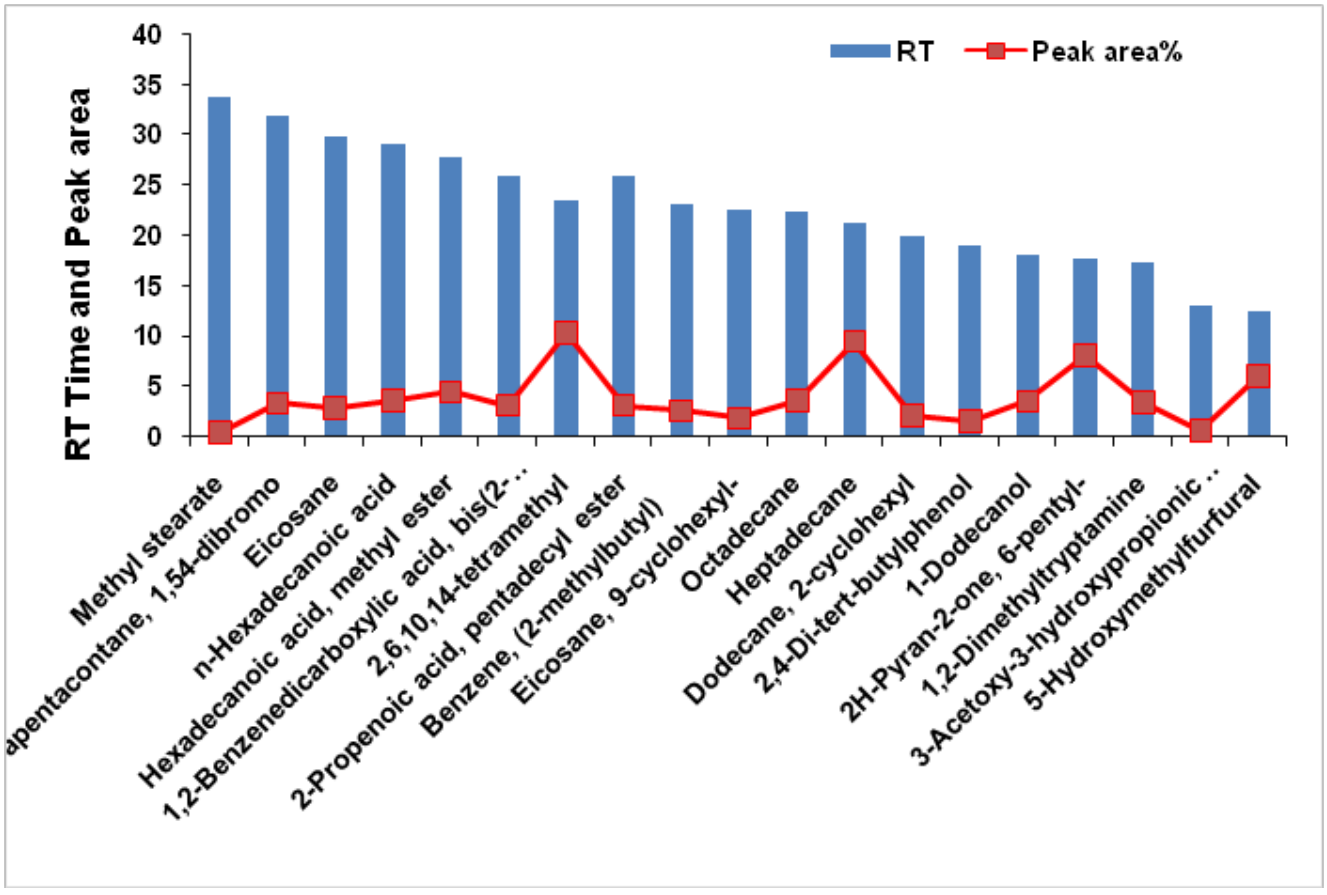

Fig. 8. RT and Peak area of major antifungal compounds.

CONCLUSION

The present investigation indicated that the pathogen *Fusarium oxysporum f. sp. cepae* inciting basal rot disease of onion is highly suppressed by fungal bioagent *Trichoderma hamatum* (TIM2) followed by *Trichoderma virens* (TIV1) which showed mycelial growth inhibition of 77.40% and 70.36%, respectively. The antifungal potential of *Trichoderma hamatum* is due to the production of major volatile metabolites such as Methyl stearate, n-Hexadecanoic, Eicosane, 9-cyclohexy, Heptadecane, Dodecane, 2-cyclohexyl, to 2H-Pyran-2-one, 6-pentyl, 5-Hydroxymethylfurfural, Tetrapentacontane, 1-Dodecanol, 2-Propenoic acid, pentadecyl ester, Benzene, (2-methylbutyl) and 1,2-Dimethyltryptamine. These compounds are highly known for fungistatic activity against many plant pathogenic fungus. The present study paves the better way to manage the basal rot disease of onion through talc or liquid based application of *Trichoderma hamatum* under field condition or by application of commercially available effective volatile compounds.

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