

Research Article

## Antagonistic potential of *Trichoderma hamatum* against *Alternaria porri* causing purple blotch disease of onion through Gas chromatography-mass spectrometry (GCMS) analysis

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### Abstract

*Alternaria porri* causing purple blotch disease of onion is a destructive phytopathogen which causes severe loss in productivity. The present study aimed to unravel the antagonistic potential and efficacy of volatile organic compounds produced by various *Trichoderma* spp. against *A. porri* causing purple blotch disease of onion through Gas chromatography-mass spectrometry (GCMS) analysis. Ten isolates of *Trichoderma* species were isolated from the rhizospheric soil of healthy onion plants. Upon paired plate technique, the *in vitro* efficacy of ten *Trichoderma* isolates were tested against virulent isolate of *Alternaria porri* isolated from purple blotch disease infected onion plants. The *Trichoderma* isolate TIM2 showed 76.29 per cent inhibition on mycelial growth of pathogen. The effective *Trichoderma* isolate was identified as *Trichoderma hamatum* through the analysis of the rDNA of internal transcribed spacers (ITS) region and it was subjected to GC-MS analysis. The result of GCMS analysis indicated the highest peak area and retention time with major antimicrobial bioactive compounds like Tetradecane, 2,6,10-trimethyl (20.327), (1.22) and Dodecane, 2-cyclohexyl (20.079), (2.14), Heptadecane (21.222), (9.50), Octadecane (22.379), (3.58), Eicosane, 9-cyclohexyl (22.578), (1.84), 2-Propenoic acid, pentadecyl ester (23.400), (10.37), 2,6,10,14-tetramethyl (23.567), (10.37), Eicosane (27.311), (2.34), Hexadecanoic acid, methyl ester (27.918), (4.43), n-Hexadecanoic acid (29.156), (3.59) and Tetrapentacontane, 1,54-dibromo (31.906), (3.33). These bioactive compounds identified through GCMS analysis from the crude extracts of *Trichoderma hamatum* exhibited a stronger antifungal activity against *A. porri*. Hence the application of *T. hamatum* for the management of purple blotch disease highly suppress growth of the pathogen and reduce the disease incidence.

**Keywords:** *Alternaria porri*, *Trichoderma hamatum*, Purple blotch, GC-MS, Volatile organic compounds

### INTRODUCTION

Onion (*Allium cepa* L.), a bulbous, biennial herb rightly called "Queen of kitchen" is an important crop cultivated in India and used as a vegetable. It is not only used for

domestic consumption but also serves as the highest foreign exchange earner among the vegetables exported (Alpona Roy *et al.*, 2016). Onion is well known for its pungency, flavour, and eco-friendly protectant for stored grains. It is a temperate crop grown worldwide

under a wide range of climatic conditions. Medicinal values of onion are innumerable. The bulb contains diuretic and heart stimulant properties (Ravichandran *et al.*, 2012). Onion contains quercetin, a flavonoid help in delaying or slowing the oxidative damage to cells and tissue of the body. In India, onion is grown in an area around 14.05 lakh ha with a production of around 224.27 lakh tonnes and productivity of 17.18 t/ha (Mahajan *et al.*, 2017). Both biotic and abiotic stresses affects the productivity of onion. One of the major constraints in the production of onion is purple blotch disease caused by *Alternaria porri* which causes severe loss in productivity in field conditions. Purple blotch disease causes 50-70 % yield loss and it extends more than 70% under favourable environmental condition (Resti and Liswarni 2021). It causes huge loss in seed production of onion as it mostly affects the seed crops rather the bulb crops. Chemical fungicides achieve the successful management of disease but due to the hazardous impact of agrochemicals on the environment, development of resistant mutants, escalating cost of pesticides and frequent breakdown of resistance strongly demand a sustainable alternative management approach. Hence recent studies have been devoted the use of biological control as an alternative method for effective and sustainable disease management (Jayapradha and raja 2017). *Trichoderma* is an effective bio control agents used against a many foliar pathogens. *Trichoderma* evades the pathogens directly by competing for nutrients and space, it produce antibiotic and lytic enzymes and thereby inactivates the growth of pathogen. It indirectly produces various biochemical and morphological changes in host plants and induces resistance against pathogen attack (Heimpel and Mills 2017). The aim of the present research was to focus on the antagonistic potential of various *Trichoderma* against *Alternaria porri* and unravel the volatile organic compounds (VOC) responsible for the inhibition of *A. porri* through GC-MS gas chromatography spectrometry analysis.

## MATERIALS AND METHODS

### Isolation of *A. porri* causing purple blotch disease

Onion leaves with typical symptoms of circular to oval, purple sunken lesions were collected. The pathogen was isolated by tissue segmentation method in Petri plates containing potato dextrose agar (PDA) medium. The pure culture of *A. porri* was obtained by single hyphal tip method. The isolated pathogen was morphologically confirmed by observing conidia under light microscope (the light to dark brown muriform conidia with transverse and longitudinal septum) and molecularly confirmed as *A. porri* (API1) with the accession number ON908900 obtained from National Center for Biotechnology Information (NCBI).

### Isolation of *Trichoderma* spp. from the rhizospheric soil

Soil samples were collected from rhizosphere 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* specific medium (TSM). The TSM plates were incubated at (28±2°C) for two days. Growth of numerous fungal colonies were 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 against *A. porri*.

Paired plate technique was followed to assess the antifungal potentiality of *Trichoderma* isolates. The ten different isolates of *Trichoderma* isolated from the soil where inoculated on the PDA medium and the mycelial plug of 9mm from the virulent pathogen was inoculated in the separate Petri plates containing PDA medium and they were subjected to paired plate technique i.e., the two plates were placed face to face cautiously in such a way that bottom plate contains the biocontrol agent (*Trichoderma* spp.) and the upper plate contains the pathogen (*Alternaria porri*). The growth reduction of pathogen was measured periodically. The Petri plate containing pathogen without biocontrol agent served as control. The percent growth inhibition of the pathogen was calculated by using the formula

$$PI = C-R/C \times 100 \quad \text{Eq.1}$$

Where,

PI - Percent inhibition,

C - Mean linear growth of pathogen in control,

R - Mean linear growth of pathogen in treatments

### Preparation of crude extracts of *Trichoderma* sp.

The crude extracts of the effective *Trichoderma* sp. was prepared by transferring the mycelial disc of 9mm from the actively growing effective *Trichoderma* isolate (TIM2) into 200ml of potato dextrose broth and incubated at 25°C for seven days. The culture filtrates were obtained by filtering the extracts using Whatmann no.1 filter paper and then centrifuged for 15mins at 9000 rpm. The metabolites from the culture filtrates were extracted using solvent ethyl acetate. The solvent containing VOC were concentrated using rotary evaporator until complete evaporation of solvent. The final output was diluted with 2ml ethyl acetate and filtered using 0.4µ bacterial filter.

### Gas chromatography–mass spectrum analysis (GCMS) of crude extracts of *Trichoderma* sp.

Various 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 were injected at 1.0µl into the chromatography. The major volatile organic compounds present were identified using a computer algorithm. The analysis was matched with National Institute of Standards Technology's library database (NIST) and the software Turbo mass version 5.1. This GC-MS analysis was carried out at centre of innovation for excellence, Agricultural College & Research Institute, Tamil Nadu Agricultural University, Madurai.

### Statistical analysis

The data were tabulated as the mean of three replicates and the data were statistically analysed with the help of SPSS version 16.0. data were subjected to ANOVA at a significant level ( $p < 0.05$ ) and by using DMRT, the means were compared.

## RESULTS AND DISCUSSION

### Isolation and identification of *Trichoderma* Spp.

Totally ten *Trichoderma* isolates were successfully isolated from the soil samples collected from rhizospheric region of onion plants using *Trichoderma* specific medium (TSM). Ten isolates of *Trichoderma* spp. were named as TIM1, TIM2, TIT1, TITR1, TIR2, TIS1, TIS2, TID1, TID2 and TIV1. All ten isolates exhibited morphological and cultural variability. Their conidiophore morphology, arrangement of phialides and conidia were

observed under the microscope. They formed well branched conidiophores containing primary conidiophores and secondary conidiophores. Three phialides are formed at right angles at the end of the secondary conidiophore in a triangle manner. This is the typical morphology of *Trichoderma* type conidiophore (Various *Trichoderma* spp. produces this type of conidiophore except *T. virens*). Molecular confirmation was done only for the effective isolate obtained from the paired plate technique.

### Antifungal efficacy of volatile organic compounds

The results of *in vitro* assay evaluating antifungal activity of ten different *Trichoderma* isolates against *Alternaria porri* revealed that the isolate (TIM2) inhibited the mycelial growth of the pathogen at 76.29 percent followed by the isolate (TID1) with 74.80 per cent reduction over control which was shown in Table 1 and Fig 1. The genomic DNA from the effective isolate was isolated and further amplified by polymerase chain reaction (PCR). The DNA sequence obtained was submitted to the NCBI and identified as *Trichoderma hamatum*. Accession no. of ON920706 was obtained for the effective isolate by NCBI. The inhibition of mycelial growth of *Alternaria porri* was due to the emission of various (VOC) volatile organic compounds produced by the *Trichoderma hamatum*. Propyl benzene is the key compound for the inhibition of various foliar pathogens, including *A. brassicae*, *Alternaria alternata*, *A. solani*, *Fusarium oxysporum* (Meena et al., 2016). Besides the antifungal activity, the volatile compounds produced by the *Trichoderma* induce resistance in plants against various diseases and helps in growth promotion (Narayan et al., 2006, Siddiquee et al.,

**Table 1.** Efficacy of volatile organic compounds produced by *Trichoderma* spp. against *Alternaria porri* by paired plate technique

S. No.	<i>Trichoderma</i> isolates	8 <sup>th</sup> Day		15 <sup>th</sup> Day
		Mycelial growth (cm)	Mycelial growth (cm)	Per cent growth reduction over control (%)
1.	TIM1	2.0	2.7	69.36 (56.39) <sup>d</sup>
2.	TIM2	1.0	2.1	76.29 (60.86) <sup>a</sup>
3.	TIT1	2.1	2.9	67.40 (55.18) <sup>e</sup>
4.	TIR1	1.9	2.6	70.74 (57.25) <sup>c</sup>
5.	TIR2	1.9	2.7	69.34 (56.39) <sup>d</sup>
6.	TIS1	1.8	2.5	71.84 (57.95) <sup>c</sup>
7.	TIS2	2.2	3.1	65.18 (53.84) <sup>f</sup>
8.	TID1	1.4	2.3	74.80 (59.87) <sup>b</sup>
9.	TID2	2.0	2.8	67.99 (55.54) <sup>e</sup>
10.	TIV1	2.4	3.3	63.70 (52.95) <sup>g</sup>
11.	control	4.5	9.0	00.00
CD(0.05)			0.737	

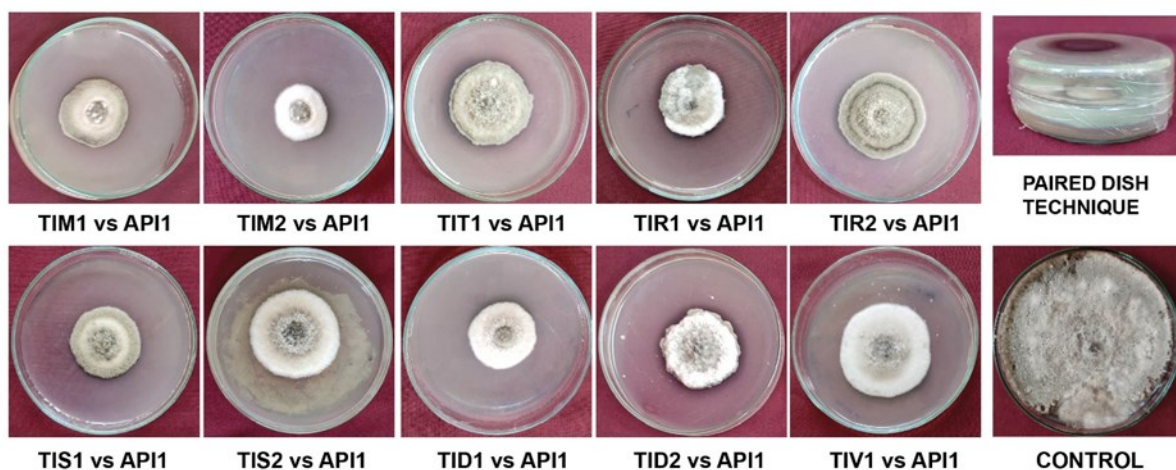
\*Mean of three replications; Values with different superscripts are significantly differ from each other at  $p < 0.05$ ; Values in the parenthesis are arc sine transformed values

2012).

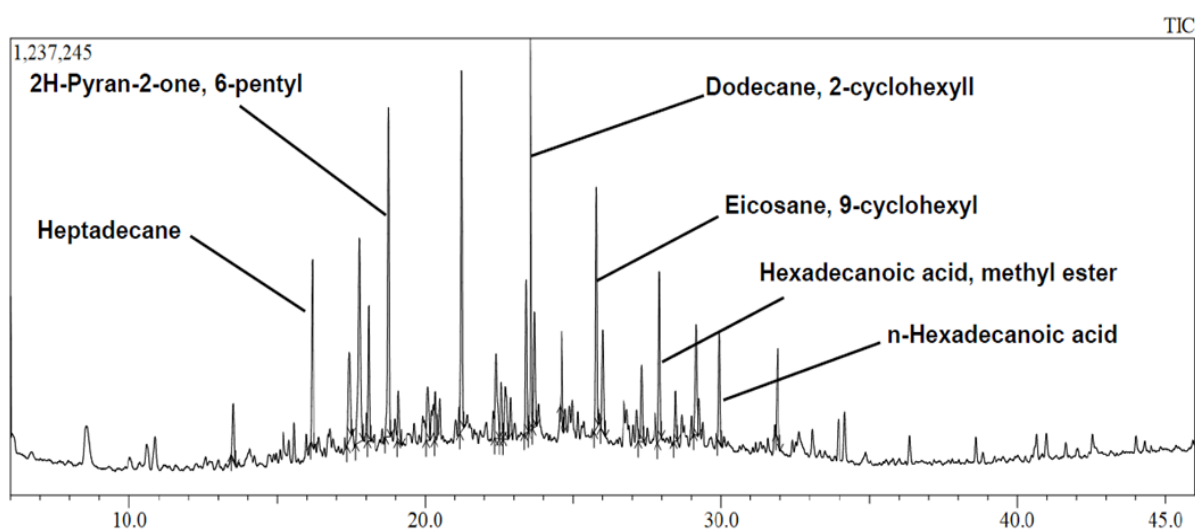
### GC-MS analysis of *Trichoderma hamatum*

The crude extracts of *T. hamatum* was reported to have the following important bioactive compounds such as Tetradecane, 2,6,10-trimethyl (20.327), (1.22), and Dodecane, 2-cyclohexyl (20.079), (2.14), Heptadecane (21.222), (9.50), Octadecane (22.379), (3.58), Eicosane, 9-cyclohexyl (22.578), (1.84), 2-Propenoic acid, pentadecyl ester (23.400), (10.37), 2,6,10,14-tetramethyl (23.567), (10.37), Eicosane (27.311), (2.34), Hexadecanoic acid, methyl ester (27.918), (4.43), n-Hexadecanoic acid (29.156), (3.59) and Tetrapentacontane, 1,54-dibromo (31.906), (3.33) which are shown in Fig. 2 and antimicrobial containing compounds like Tridecane (13.519), (1.74), 1,2-Dimethyltryptamine (17.445), (3.43), 2H-Pyran-2-one, 6-pentyl (17.780), (8.11), 1-Dodecanol (18.089), (3.52) and 2,4-Di-tert-butylphenol (19.086), (1.48) with their

peak area, retention time (RT) and molecular formula along with molecular weight are listed in Table 2 and Fig. 3 and the chemical structures are in Fig. 4. Besides the antimicrobial activity, the compounds like 6-n-pentyl-2-H-pyran-2-one has the ability to reduce the production of mycotoxin deoxynivalenol produced by *F. graminearum* (Bharose and Gajera 2018a, b). Hexadecenoic acid is a compound produced by various *Trichoderma* spp. which shows both antibacterial and antifungal activity against numerous plant pathogens. The mechanism of action of hexadecenoic acid damages the cell wall of the targeting organisms (Al-Marzoqi et al., 2015) noted the antibacterial activity of hexadecenoic acid which damaged the cell wall and cause lysis of *Salmonella typhi*. Similarly the heptadecenoic acid has a strong antifungal activity against *Sclerotinia sclerotiorum* under *in vitro* condition (Vinodkumar et al., 2017). The findings of present study are in accordance with (Aldakheel et al., 2020), who reported that culture



**Fig 1.** Efficacy of volatile organic compounds produced by *Trichoderma* spp. against *Alternaria porri* by paired plate technique.



**Fig. 2.** Gas chromatogram of antifungal compounds identified from crude extracts of *Trichoderma hamatum*

**Table 2.** GC-MS compounds of *Trichoderma* sp. with their nature

S.No	Name of the compound	RT	Peak area %	MW g/mol	Molecular formula	Specific role	Reference
1.	Tridecane	13.519	1.74	184	C13H28	Antifungal activity	(Yassin <i>et al.</i> , 2021)
2.	1,2-Dimethyltryptamine	17.445	3.43	188	C12H16N2	Antifungal	(Chen <i>et al.</i> , 2020)
3.	2H-Pyran-2-one, 6-pentyl-	17.780	8.11	212	C15H32	Antifungal activity	(Rao <i>et al.</i> , 2022)
4.	1-Dodecanol	18.089	3.52	186	C12H26O	Antifungal activity	(Sangeetha <i>et al.</i> , 2018)
5.	2,4-Di-tert-butylphenol	19.086	1.48	206	C14H22O	Antifungal activity	(Sangeetha <i>et al.</i> , 2018)
6.	Dodecane, 2-cyclohexyl-	20.079	2.14	252	C18H36	antibacterial activity	(Pucot <i>et al.</i> , 2021)
7.	Tetradecane, 2,6,10-trimethyl-	20.327	1.22	240	C17H36	Antifungal activity	(de Brito <i>et al.</i> , 2022)
8.	Heptadecane	21.222	9.50	240	C17H36	Antifungal and antibacterial activity	(Jishma <i>et al.</i> , 2017),
9.	Octadecane	22.379	3.58	254	C18H38	Antifungal activity	(Rajaofera <i>et al.</i> , 2019)
10.	Eicosane, 9-cyclohexyl-	22.578	1.84	364	C26H52	Antifungal activity	(Alsultan <i>et al.</i> , 2019)
11.	2-Propenoic acid, pentadecyl ester	23.400	4.01	282	C18H34O2	antibacterial activity	(Aldakheel <i>et al.</i> , 2020)
12.	2,6,10,14-tetramethyl-	23.567	10.37	240	C17H36	Antifungal activity	(Sheoran <i>et al.</i> , 2015)
13.	Eicosane	25.794	6.20	282	C20H42	Antifungal activity	(Alsultan <i>et al.</i> , 2019)
14.	1,2-Benzenedicarboxylic acid, bis(2-methylprop	26.006	3.10		C24H38O4	Antifungal	(Chen <i>et al.</i> , 2020)
15.	Eicosane	27.311	2.34	280	C20H40	Antifungal activity	(Alsultan <i>et al.</i> , 2019)
16.	Hexadecanoic acid, methyl ester	27.918	4.43	278	C16H22O4	Antifungal activity	(Zohair <i>et al.</i> , 2018, Chowdhury <i>et al.</i> , 2020)
17.	n-Hexadecanoic acid	29.156	3.59	270	C17H34O2	Antifungal activity	(Masiulionis and Pagnocca 2020)
18.	Eicosane	29.945	2.84	366	C26H54	Antifungal activity	(Alsultan <i>et al.</i> , 2019)
19.	Tetrapentacontane, 1,54-dibromo-	31.906	3.33	914	C54H108Br2	antibacterial activity	(Samy <i>et al.</i> , 2013)

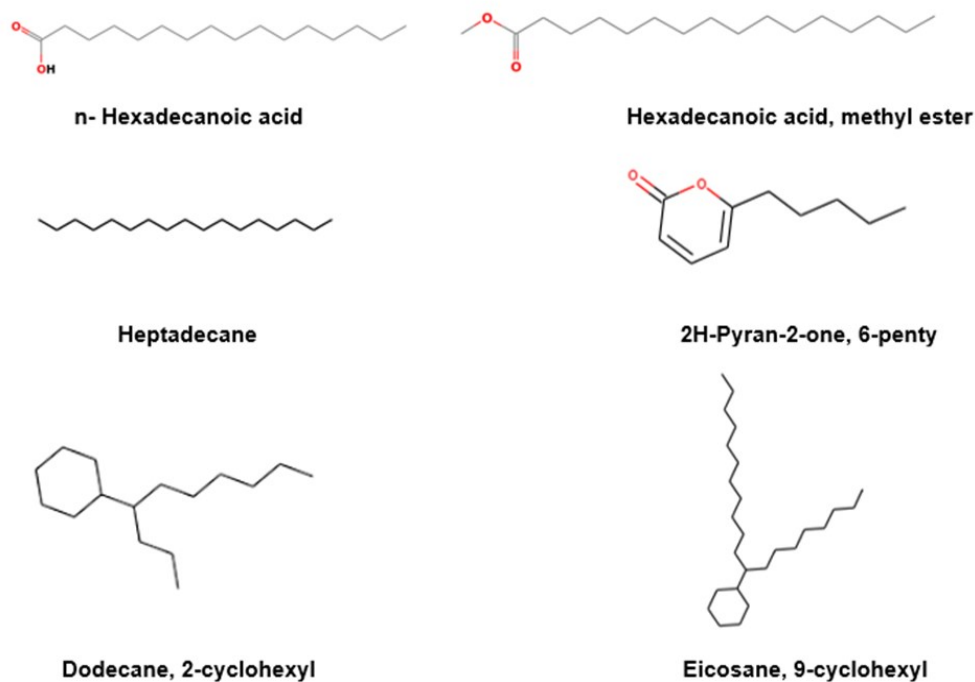


Fig. 3. Chemical structure of important antifungal compounds produced by *Trichoderma hamatum*

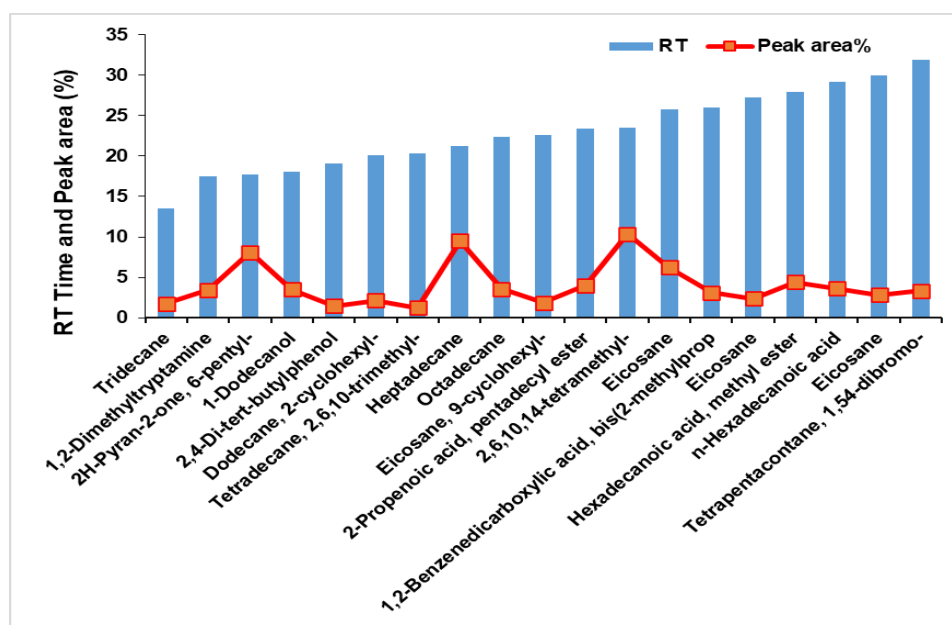


Fig. 4. RT and Peak area of major antifungal compounds

filtrates of *T.harzianum* suppressed the mycelial growth of *Fusarium* spp. (Tomah et al., 2020) proved that various bioactive compound like quinolone, heptadecane, heneicosane, 6-pentyl-2H-pyran-2, phenol, 2-(6-hydrazino-3pyridazinyl), 17-methoxy-4-methyl-d-homo-18-norandrosta one, eicosane, nonadecane, benzene propianoic acid, dibutyl phthalate and hexadecane produced by *Trichoderma citrinoviride* has a strong antifungal activity and showed an growth inhibition of 77.8% against fungal pathogens. Dodecane a bioactive volatile produced by *Trichoderma* spp. which showed

higher level of mycelial growth inhibition against *aspergillus niger* and some plant pathogenic bacteria (Pucot et al., 2021).

### Conclusion

The present study indicated that the bioactive compound such as Tetradecane, 2,6,10-trimethyl (20.327), (1.22), Heptadecane (21.222), (9.50), Octadecane (22.379), (3.58), n-Hexadecanoic acid (29.156), (3.59), Dodecane, 2-cyclohexyl (20.079), (2.14), Eicosane, 9-

cyclohexyl (22.578), (1.84) and 2H-Pyran-2-one, 6-pentyl (17.780), (8.11) present in the extracts of *T. hamatum* possess a strong antifungal activity against *A. porri*. These compounds are highly known for fungistatic activity on *A. porri*. As a result, the talc or liquid-based application of *Trichoderma hamatum* under field conditions is highly effective against the purple blotch disease of onion caused by *Alternaria porri*. The soil application of *T. hamatum* was effective against many soil-borne and foliar pathogens.

### Conflict of interest

The authors declare that they have no conflict of interest.

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