Volatile organic compounds emitted by *Trichoderma* spp. for growth promotion and management of rice diseases

SHANTI PRAVA BEHERA, ARUP K. MUKHERJEE, TOTAN ADAK, HAREKRUSHNA SWAIN, TORIT B. BAGCHI AND PRADEEP KUMAR CHAND

J. Mycopathol. Res. 61(4) : *465-479, 2023;*
ISSN 0971-3719

Department of Botany,
University of Calcutta,
Kolkata 700 019, India

J.Mycopathol.Res. 61(4) : 465-479, 2023, ISSN : 0971-3719 (Print), 2583-6315 (Online) doi.10.57023/JMycR.61.4.2023.465

Volatile organic compounds emitted by *Trichoderma* spp. for growth promotion and management of rice diseases

SHANTI PRAVA BEHERA[1,2], ARUP K. MUKHERJEE[1]*, TOTAN ADAK[1], HAREKRUSHNA SWAIN[1], TORIT B. BAGCHI[3] AND PRADEEP KUMAR CHAND[2]

[1]Crop Protection Division, ICAR-National Rice Research Institute, Cuttack 753006,Odisha
[2]PG Department of Botany, Utkal University, Bhubaneswar751004, Odisha
[3]Crop Physiology and Biochemistry Division, ICAR-National Rice Research Institute, Cuttack 753006, Odisha

Received : 06.08.2023 Accepted : 09.10.2023 Published : 25.12.2023

This study was conducted to evaluate the antagonistic potential of volatiles emitted from seven *Trichoderma* spp.against 4 major rice diseases and to investigate their role in enhancement of growth and vigour of rice plants. Isolates were identified based on both morphological characteristics as well as molecular assay. Their biocontrol potential was evaluated and *T. erinaceum* completely inhibited the growth of *Pyricularia oryzae* and *Bipolaris oryzae.* It also inhibited growth of *Ceratorhiza oryzae-sativae* and *Rhizoctonia solani* by 64 and 50%, respectively. Sandwich plate method showed that volatiles emitted from *T. erinaceum* could reduce the mycelial growth of all four pathogens. GC-MS analysis indicated emission of 6-Pentyl- 2H-pyran-2-one from *T. erinaceum* in large quantity. Seed treatment with *Trichoderma* enhanced vegetative and reproductive growth of rice cultivar, Sahbhagidhan. Over-expression of defense enzymes like catalase and superoxide dismutase in shoot and peroxidase in both root and shoot were observed in *Trichoderma* treated plants. Results of this study suggest that *T. erinaceum* obtained from the rice rhizosphere can be used as a potential biocontrol agent against major rice diseases and as a growth inducer. It can also be utilized as an efficient source to produce 6-Pentyl-2H-pyran-2-one for further use.

Keywords: 6-pentyl-2H-pyran-2-one, induced resistance, Sahbhagidhan, *Trichoderma erinaceum,* VOCs

INTRODUCTION

Rice (*Oryza sativa* L.) provides food for more than half of the world's population. Major challenges for sustainable rice productions are different biotic and abiotic stresses which cause severe loss to rice productivity. To prevent biotic stress, rice cultivation relies upon chemical pesticides, which further deteriorate soil health by doing harm to beneficial soil microflora and causing emergence of resistant pathogens (Bhattacharyya *et al*. 2015). To prevent such limitations, biocontrol agents (BCAs) could be a suitable alternative.*Trichoderma* is a diverse and ubiquitous genus of fungi that is commonly found in various habitats, including soil, water, and plant surfaces (Mukherjee *et al.* 2013, Gal-Hemed*et al*. 2011, Harman *et al.* 2004).

The genus comprises over 200 species, which can be classified into several morphological and molecular groups (Atanasova *et al.* 2013). *Trichoderma* spp. are free-living opportunistic symbionts that are commonly found in rhizosphere of many plants such as rice (Naeimi *et al*.2010; Khalili *et al*. 2012), maize (Harman *et al*. 2004), avocado (Ruano-Rosa and Hererra, 2009), tomato (Tsahouridou and Thanassoulopoulos, 2002; Harman *et al*. 2004), *Acacia* (Mukherjee *et al*. 2013) etc. They were also isolated from tree barks (Doni *et al*. 2014; Swain *et al*. 2021).

Trichoderma spp. are among the most studied organisms in the field of biocontrol agents, and they are also intensively explored for plant growth promotion. They have been reported as effective biocontrol agents against *Bipolaris oryzae* (Khalili *et al*. 2012), *Fusarium oxysporum* (Sundaramoorthy and Balabaskar, 2013), *Rhizoctonia solani* (Kamal and Devi., 2012, Chen *et al*. 2015), *Aspergillus flavus* (Anjaiah*et al*. 2006) and many other phytopathogens.*Trichoderma* spp. have been reported recently to be biocontrol agents in

*Correspondence: titirtua@gmail.com

rice (Swain *et al*. 2018, 2021). Many traits are possessed by *Trichoderma* that make them suitable biocontrol agents (Howell, 2003). They show mycoparasitism (Siameto*et al.* 2011; Gajera *et al.* 2012; Herath *et al.* 2015) and rhizosphere competence (Tsahouridou and Thanas-soulopoulos, 2002). Production of cell wall degrading enzymes (Marco and Felix, 2007; Gajera *et al.* 2012; Agrawal and Kotasthane, 2012), antibiotics (Swain and Mukherjee, 2020) and volatile compounds (Li *et al.* 2018) also explains successful disease control by *Trichoderma.* Mechanisms such as induction of defense responses (Swain *et al*. 2021) and metabolism of germination stimulants (Howell, 2003) are add-on to the biocontrol potential of *Trichoderma.*

Several studies have reported the growth-promoting ability of *Trichoderma* in various plants (Harman *et al.* 2004;Srivastava *et al.* 2006;Vinale *et al*. 2008; Salas-Marina *et al.* 2011;Bharti *et al.* 2012;Nagaraju*et al*. 2012). The authors attributed the growth-promoting effects to the production of auxin-like compounds.*Trichoderma* spp. execute their growth promoting activity by producing phytohormones (Harman *et al*. 2004; Contreras-Cornejo *et al.* 2009; Shoresh*et al.* 2010; Harman, 2011) and mobilizing plant nutrients which in return provides higher yield (Kapriand Tewari, 2010).

In addition to the above mechanisms, *Trichoderma* species have genes that code for the generation of secondary metabolites such as volatile organic compounds (VOCs), which have biocontrol properties (Kubicek *et al.* 2011; Mukherjee *et al.* 2012).Few studies indicated the role of VOCs for the biocontrol activity of *Trichoderma* spp. (Contreras-Cornejo *et al*. 2014; Li *et al*. 2018). *Trichoderma* VOCs had shown strong inhibition of plant pathogenic fungi such as *Fusarium oxysporum, Rhizoctonia solani, Sclerotium rolfsii, Sclerotinia sclerotiorum,* and *Alternaria brassicicola* (Amin *et al*. 2010; Meena *et al*. 2017). *Trichoderma* spp. emit VOCs differently depending on the species, strains, cultivation environment, and substrate composition (Stoppacher *et al*. 2010). More than 480 different VOCs have been found in *Trichoderma* species, and hydrocarbons, heterocycles, aldehydes, ketones, alcohols, phenols, thioalcohols, thioesters, and thioesters are being the most common (Siddiquee, 2014). In addition, several *Trichoderma* VOCs have been observed to promote plant growth directly (Hung *et al*. 2013; Lee *et al*. 2016, 2019; Nieto-Jacobo*et al*. 2017). Even in the absence of pathogen, *Arabidopsis thaliana* exposed to VOCs emitted by *T. viride* had enhanced fresh weight, root growth, and chlorophyll content (Hung *et al*. 2013). One of the major compounds detected in diverse *Trichoderma* species is 6-pentyl-2H-pyran-2-one (6-PP). Application of 6-PP resulted in growth promotion and a reduction in disease symptoms (Hung *et al*. 2013). It is also reported that all *Trichoderma* species did not synthesize 6-PP and yet they are able to induce plant growth promotion (Atanasova *et al*. 2013, Kottb *et al*. 2015). This suggests there may be other molecules responsible for such actions.

In this study, VOCs of seven different *Trichoderma* isolates were tested for their biocontrol and plant growth promotion activities. Among the seven isolates, two isolates were chosen for chemical characterisation of VOCs. This experiment aims to justify the role of volatile compounds produced by *Trichoderma* in growth promotion and biocontrol in rice.

MATERIALS AND METHODS

Isolation and characterisation

Seven antagonistic *Trichoderma* strains were isolated from rice rhizospheric soil of National Rice Research Institute, Cuttack, Odisha, India by soil dilution technique (Johnson *et al.* 1959). Single spore technique was used to isolate pure colonies (Khalili *et al.,*2012; Mukherjee *et al.* 2014). The isolates were stored on PDA slants in refrigerator as well as in deep freezer at -80 °C (Swain *et al.* 2018).The fungal pathogens used in this study were *Rhizoctonia solani*, CRRI-RS-8 (MTCC-12232) causing sheath blight of rice, *Ceratorhiza oryzae-sativae*, CRRI-RS-4 (MTCC-12231) causing aggregate sheath spot, *Pyricularia oryzae*, CRRI-PO-2 (KX881382.1) causing rice blast and *Bipolaris oryzae*, CRRI-BS-2 (ACC:KX881383.1.) causing brown-spot of rice.

The cultural and morphological characteristics of the isolated *Trichoderma* species were studied by growing them on Potato Dextrose Agar medium (PDA) at 27°C. Identification was based on the morphological and taxonomic keys provided by Gams and Bissett (1998). Genomic DNA was isolated from the pure cultures of *Trichoderma* strains (Mukherjee *et.al*., 1995). The ITS regions of 5.8 S r RNA gene were amplified using the primers ITS1F and ITS4R (Toju *et al*. 2012). The sequences were compared with those from GenBank using the BLAST program and a phylogenetic tree was constructed through http://www.phylogeny.fr. (Swain *et al*.2018). The DNA sequences of the 6*Trichoderma* isolates,and four pathogens have been submitted to NCBI GenBank (Table1). Four isolates were deposited at Microbial Type Culture Collection (MTCC),IMTech, Chandigarh.

Confrontation assay

Preliminary investigation on antagonistic potential of the *Trichoderma* isolates were carried out by confrontation assays against all the four pathogenic strains. Actively growing culture disc of the pathogen and that of antagonists were placed opposite to each other on the petri dish (diameter of 9 cm) containing PDA. Plates inoculated with pathogen alone were considered as control.

The percentage of inhibition was calculated using formula:

RI= 100* (R2-R1)/ R2.
Where RI is the percentage of ratio inhibition (Kumar et al., 2012), R1 was the growth of pathogen from the pathogen inoculums towards the inoculums of *Trichoderma* isolate and R2 was the growth of pathogen in control plates.

Effect of volatiles from Trichoderma on pathogens

Radial inhibition of pathogens by volatiles from *Trichoderma* was studied by sandwich plate method. Oatmeal agar plates were inoculated centrally with agar discs of pathogens, *Pyricularia oryzae*, *Bipolaris oryzae*. *Ceratorhiza oryzae-sativae, Rhizoctonia solani* and all the seven *Trichoderma* were multiplied on PDA plates. The bottom petri plates containing the *Trichoderma* covered by another petri plate with pathogens were sealed together using parafilm and was incubated keeping the pathogen at top. The effect of volatile was recorded by measuring the percentage of radial growth inhibition (Anees *et al.* 2010; Li *et al*. 2018).

Effect of volatiles as plant growth promoters

One petriplate (diameter of 9 cm) containing *Trichoderma* culture on PDA was placed in the bottom of a closed chamber (2.5 L). In the same chamber, Karuna and Sahabagidhan seeds were placed on different petriplates containing water-soaked blotting paper. The chamber was closed with an air-tight lid and placed at 27°C for 8 days under a photoperiod of 10 h light/14 h dark. Uninoculated PDA plates were used as control. Three replications were performed for each treatment. In this study, we have used two *Trichoderma* isolates namely, TS-1 and TS-10 based on their maximum antagonistic activity (as studied in sandwich plate method), and fresh weight, dry weight and root and shoot lengths were measured (Tahir *et al.* 2017).

Characterization of volatile compounds

The fungus was cultured in 20 mL head space vial. After 48 h incubation, the vial was sealed using a crimper and was used for volatile characterization using a GC-MS (Trace 1300-TSQ9000, Thermo Scientific, USA) equipped with HS-SPME auto sampler (TriPlus RSH, ThermoScientific, USA) and a capillary column (TG-5MS; 30 m*0.25 mm, film thickness: 0.25 µm,Thermo Scientific, USA). For extraction of volatiles in headspace, the vials were incubated in the in-built head space agitator at 40 °C for 60 min with the agitator on/off for 10 seconds each. SPME fibre (1 cm, 50/30 ìm Divinylbenzene/ Carbon Wide Range/Polydimethylsiloxane, DVB/ CarbonWR/PDMS, Dark Gray, Thermo Scientific, USA), was used for concentration of the volatiles.The volatiles were concentrated for 30 mins on the fibre. The needle speed inside the vial was 20mm sec^{-1}. After the equilibrium time, the fibre was directly injected into split-split less injector port for 2 mins. The injector temperature

was kept at 250°C with a split flow of 5 mL min $^{-1}$and split ratio of 5:1. The fibre was conditioned at 250 °C for 1 min before the concentration and for 15 min post injection. The following oven temperature program was followed; temperature was increased from 35 to 140°C at a ramp rate of 3°C min $^{-1}$ and hold for 1 min, temperature was increased to 230°C at the ramp rate of 10°C min^{-1} and hold for 3 min. Samples were ionized by the positive electron impact (EI) mode using electron energy of 70 eV. Helium (99.999 % purity) at the flow rate of 1 mL min $^{-1}$ was used as the carrier gas. Both the MS transfer line temperature and ion source temperatures were set at 230 °C. The MS data was acquired for them as range of m/z of 50-500. The compounds were identified using NIST library and AMDIS software (NIST 2017).

Field Experiments

Treatment

Trichoderma strains were grown on PDA for 5 days and conidial spores were separated using 0.5 % sterile carboxy methyl cellulose (CMC). The spore concentration was adjusted to 10^7 spores mL^{-1}. Seeds of Sahbhagidhan were treated with the spore suspension and left to air dry for 2 h. The control seeds were treated with 0.5 % CMC instead of spore suspension. Seeds were sown in 1m^2 plots with 15cmX15cm spacing in Randomized Block Design experiment with four replications.

Plant growth promotion

Plants were collected 8 weeks after sowing. Agronomical parameters such as shoot length, root length, fresh and dry weight of shoot and root was taken. Plants were harvested at maturity. Grains were threshed and dried to 15 % moisture content. Days to 50% flowering, panicle length, grain weight per hill, yield per plot, thousand grain weight and fertility percentage were noted.

Defence enzyme activity

Peroxidase (POD) activity was assayed by studying biotransformation of guaiacol spectrophotometrically (Nounjana *et al.* 2012). Catalase activity was determined by estimating the amount of enzyme required to break down H_2O_2 under the assay conditions described by Kar and Mishra (1976).The ability of superoxide dismutase to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) was measured as the enzyme activity according to the method of Beauchamp and Fridovich (1971).

Statistical Analysis

Statistical analysis was done using R-software. Analysis of variance (ANOVA) was calculated and tabulated. Treatment means of all the parameters were compared through Tukey's HSD test at probability at 5%.

RESULTS AND DISCUSSION

Isolation and characterization

Pyricularia oryzae and *Bipolaris oryzae* were isolated from infected rice leaves at NRRI, Cuttack and cultures of *Rhizoctonia solani* and *Ceratorhiza oryzae-sativae* available in the laboratory were used for this study. The *Trichoderma* isolates were identified using both morphological and molecular characters (Figs.1 & 2). The PCR amplified products of ITS sequences of 5.8 S r RNA gene (Fig.3)were compared with those from GenBank using the BLAST program (Altschul *et al.,* 1990). The DNA sequences of the 5 *Trichoderma* isolates were submitted to NCBI GenBank(Table1). Four isolates have been deposited at the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh. These are CRRI-TS-1 (*T. pleuroticola*) as MTCC 12407, CRRI-TS-2 (*T. pleurotum*) as MTCC 12408, CRRI-TS-5(*T. longibrachiatum*)as MTCC 12409 and CRRI-TS-6 (*T. harzianum*) as MTCC 12410. Their phylogeny has been depicted in Fig.4.

Confrontation assays

Confrontation assays were performed using all the seven *Trichoderma* isolates against four plant pathogen strains, which exhibited varying degrees of antagonistic potential (Fig.5).Growth inhibition of the pathogens *viz.Pyricularia oryzae, Bipolaris oryzae, Ceratorhiza oryzae-sativae and Rhizoctonia solani* by all the seven *Trichoderma*

Table 1: Rice rhizopheric*Trichoderma* isolates from ICAR-NRRI, Cuttack with respective NCBI accession numbers

Isolate Name	Species Name	Isolated from	Location	NCBI accession no
CRRI-TS-1	*T. pleuroticola*	Rice plant rhizosphere	CRRI, Cuttack	KR069050
CRRI-TS-2	*T. pleurotum*	Rice plant rhizosphere	CRRI, Cuttack	KR069051
CRRI-TS-3	*T. koningiopsis*	Rice plant rhizosphere	CRRI, Cuttack	-
CRRI-TS-5	*T. longibrachiatum*	Rice plant rhizosphere	CRRI, Cuttack	KR069052
CRRI-TS-6	*T. harzianum*	Rice plant rhizosphere	CRRI, Cuttack	KR069053
CRRI-TS-9	*T. harzianum*	Rice plant rhizosphere	CRRI, Cuttack	KR069054
CRRI- TS-10	*T. erinaceum*	Rice plant rhizosphere	CRRI, Cuttack	-

Table 2: Confrontation assay showing percentage of growth inhibition of pathogen by different *Trichoderma* isolates (BCA)

	Growth inhibition (%)			
Trichoderma Isolates	*P. oryzae*	*B. oryzae*	*C. oryzae-sativae*	*R. solani*
CRRI-TS-1	50.00^{d}	68.26^{cd}	56.14^{de}	73.59^{b}
CRRI-TS-2	64.65^{c}	62.72^{d}	50.88^{e}	42.78^{d}
CRRI-TS-3	100.00^{a}	65.67^{d}	70.24^{b}	54.03^{cd}
CRRI-TS-5	83.86^{b}	60.55^{d}	65.90^{bc}	46.67^{d}
CRRI-TS-6	87.66^{b}	82.21^{b}	98.25^{a}	86.15^{a}
CRRI-TS-9	100.00^{a}	76.08^{bc}	59.54^{cd}	65.85^{bc}
CRRI-TS-10	100.00^{a}	100.00^{a}	63.92^{bcd}	49.99^{d}
CV (%)	2.89	4.40	4.22	7.31
Tukey's HSD at 5%	6.76	9.04	7.82	12.19

Means with same letter are not significantly different at p d" 0.05.

Table. 3: Percentage of Inhibition of Pathogens by Volatile Compounds Produced by *Trichoderma* spp.

Trichoderma isolates	*P. oryzae*	*B. oryzae*		*R. solani*
CRRI-TS-1	9.41^{b}	46.94^{b}	0.00^{b}	0.00^{b}
CRRI-TS-2	15.38^{b}	14.11^{c}	0.00^{b}	0.00^{b}
CRRI-TS-3	17.07^{b}	1.96^{d}	0.00^{b}	0.00^{b}
CRRI-TS-5	9.76^{b}	15.77^{c}	0.00^{b}	0.00^{b}
CRRI-TS-6	15.85^{b}	15.03^{c}	0.00^{b}	0.00^{b}
CRRI-TS-9	15.85^{b}	2.45^{d}	0.00^{b}	0.00^{b}
CRRI-TS-10	65.88^{a}	59.59^{a}	76.08^{a}	20.39^{a}
CV (%)	9.43	8.47	2.36	9.63
Tukey's HSD at 5%	12.73	5.26	0.72	1.43

Means with same letter are not significantly different at $p \leq 0.05$.

species varied from 50.00-100.00%, 60.55-100.00%, 50.88-98.25% and 42.78-86.15% respectively (Table2).Complete inhibition of *Pyricularia oryzae* (CRRI-PO-2) was observed in confrontation to CRRI-TS-3, CRRI-TS-9 and CRRI-TS-10. CRRI-TS-6 inhibited both the radial growth of C.*oryzae-sativae* and *R. solani,*and it showed more than 80 % inhibition in case of all the four pathogens. CRRI-TS-10 was found to be most effective with 100% inhibition to foliar pathogens *viz.Pyricularia oryzae* and *Bipolaris oryzae*.

Trichoderma is a genus of fungi that has dual role in plant growth promotion and biocontrol. Strong antagonistic potential of different *Trichoderma*

Table. 4: Effect of VOCs on growth promotion of Sahbhagidhan & Karuna seedlings

Treatments Sahbhagidhan	Shoot length (cm)	Root length (cm)	Fresh root weight (g)	Fresh shoot weight (g)	Dry root weight (g)	Dry shoot weight (g)
T-0	5.94[b]	4.90[b]	0.027[a]	0.065[ab]	0.017[a]	0.034[a]
CRRI-TS-1	7.62[a]	5.15[b]	0.032[a]	0.072[a]	0.019[a]	0.039[a]
CRRI-TS-10	6.83[ab]	5.61[a]	0.034[a]	0.062[b]	0.016[a]	0.023[a]
CV (%)	7.87	2.45	9.17	4.63	8.66	9.01
Tukey's HSD at 5%	1.34	0.32	0.008	0.007	0.003	0.017
Karuna						
T-0	5.85[a]	3.83[a]	0.023[b]	0.049[b]	0.014[a]	0.032[ab]
CRRI-TS-1	5.57[a]	4.31[a]	0.028[a]	0.061[a]	0.015[a]	0.040[a]
CRRI-TS-10	5.88[a]	5.06[a]	0.029[a]	0.061[a]	0.016[a]	0.025[b]
CV (%)	6.38	9.56	3.32	6.99	8.55	9.29
Tukey's HSD at 5%	0.92	1.49	0.002	0.01	0.008	0.009

Means with same letter are not significantly different at p d" 0.05.

species has been reported against *Fusarium oxysporum* and *Rhizoctonia solani* (Kamala and Devi, 2012;Sundaramoorthy and Balabaskar, 2013), *Sclerotinia* (Garcia-Nunez *et al.* 2012), *Sclerotium delphinii* (Mukherjee *et al*. 2013), *Pyricularia oryzae* (Aravindan *et al*. 2016) and *Bipolaris oryzae* (Khalili *et al.*2012). This antifungal activity is known to be exhibited through several mechanisms such as antibiosis, utilization of available space, production of cell wall degrading enzymes, mycoparasitism, induction of systemic resistance (Harman *et al*. 2004; Saba *et al*. 2012; Mukherjee *et al*. 2022).

Assay of Trichoderma volatiles

The VOCs from *Trichoderma* isolates inhibited radial growth of the test pathogens *Pyricularia oryzae and Bipolaris oryzae* (Table3). CRRI-TS-10 was the best candidate as it showed growth inhibition all the pathogens. Out of the seven isolates, only CRRI-TS-10 was able to inhibit *R. solani*. Growth of *Bipolaris oryzae* was markedly inhibited by CRRI-TS-1(Fig 6).

Assay of volatiles as plant growth promoters

In the effect of volatiles emitted from CRRI-TS-10,Sahbhagidhan seedlings showed better shoot and root growth as compared to control (Table4), whereas, CRRI-TS-1 VOCs had positive effect on shoot length.In case of Karuna, seedlings, shoot and root biomass was increased significantly with CRRI-TS-1and CRRI-TS-10 VOCs treatment.

Volatile collection and characterisation

As confirmed from the confrontation assays,CRRI-TS-10 caused maximum inhibition of pathogen growth followed by CRRI-TS-1which was most effective against *Bipolaris oryzae.* So, VOCs were collected from these two*Trichoderma* spp. and analysed using GC-MS (Figs.7 and 8). The compound, 6-pentyl- 2H-Pyran-2-one was constituted 84.10 % of the total area of VOCs produced by CRRI-TS-10.(Z)- Ocimene, 1,3-Octadiene, 1-Octen-3-ol, 3-Octanone, (Z)-Ocimene & 3-(hydroxymethyl)-2-nonanone, were detected in both the isolates(Tables 5, 6).

Production of volatile compounds is one of the mechanisms executed by *Trichoderma* species for growth promotion and antagonism. So, the isolates were tested for the effect of volatiles emitted from them.CRRI-TS-1 and CRRI-TS-10being the best pathogen inhibitors were further analysed for VOCs composition by GC-MS technology. 6-Pentyl-2H-pyran-2-one was found to be the most abundant compound (84.10%) from CRRI-TS-10 i.e., *Trichoderma erinaceum* which is already reported to have strong

Table. 5: HS-SPME/gas chromatography-mass spectrometry (GC-MS) profile of volatile organic compounds (VOCs) produced by *Trichoderma pleuroticola*

RT	Compound Name	Calculated RI	Literature RI	RSI	Area (%)	Activity & Reference
2.39	1-Propanol, 3-amino	739.2	761	854	0.07	-
6.16	1,3-Octadiene	825	820	837	0.61	-
8.08	Heptan-2-one	868.3	898	909	0.07	Metabolite in many plants & E. coli (Pubchem), *T. viride* (Carballo *et al.,* 2017)
10.71	1-Octen-3-ol	1028.3	960	907	3.24	Antimicrobial in *Bignonia venusta* (L.) (Xiong *et al.,* 2017) & *T. atroviride* (Sharma *et al.,* 2017)
10.92	3-Octanone	1033.2	963	894	15.83	Antibacterial and antifungal in *Atta* ant (de Lima Mendonça *et al.,* 2009), *T. atroviride* (Sharma *et al.,* 2017)
12.24	Ocimene <(Z)-, beta->	1063.2	1035	775	0.19	Antifungal in *Cnidiummonnieri* (L.) (Sun *et al.,* 2020) Antimicrobial in Cleopatra mandarin (*Citrus reshni*) (Hamdan *et al.,* 2013)
14.18	2-Nonanone,3-(hydroxymethyl)-	1106	1093	882	0.10	-
17.34	Dodecane	1173	1200	880	0.20	3-(Prop-2-enoyloxy)dodecane has antibiotic properties in mangrove *Glutamicibacter mysorens* (Karthik *et al.,* 2023)
19.87	Cyclohexanol,2-methyl-5-(1-methylethyl)-(1α,2α,5β)-	1227	1219	783	0.11	-
22.77	Tridecane <n->	1289.6	1300	822	0.10	-
23.54	Isogermacrene D	1306.3	1447	918	0.87	Found in roots of *Leonurus sibiricus* L. (Sitarek *et al.,* 2017)
25.84	Alaskene<beta->	1357.2	1494	864	0.25	-
28.87	Cuprenene<gamma>	1425	1530	900	0.17	-
29.24	Anthracene, tetradecahydro-	1434	1561	871	0.38	Antimicrobial (Kim *et al.,* 2009)
30.44	4-phenyl-2-butanol, TBDMS derivatives	1462	1601	858	0.04	-

RT: Retention time RI: Retention index, RSI: Reverse similarity index

antimicrobial action in *Trichoderma koningii* (Ismaieland Ali, 2017) and *Trichoderma atroviride* (Jin *et al.*2020). Both strains, CRRI-TS-1 and CRRI-TS-10 produced 3-octanone and 1-octen-3-ol, which are fungicides reported from *T. atroviride* (de Lima Mendonça *et al.*2009;Xiong *et al.* 2017; Sharma *et al.,* 2017). (Z)- Ocimene is also a common volatile antifungal compound (Hamdan *et al.*2013, Sun *et al.* 2020) in both the species which does not have any previous report from *Trichoderma.*Tetradecahydro-anthracene, from CRRI-TS-1; Propanoic acid, Furan, 2-pentyl, Acetophenone, Benzoic acid, methyl ester,Furan <2-heptyl->&Neomethyl acetate from CRRI-TS-10 are some antimicrobial agents that were found in this study (Wang *et al.* 2011;Saharkhiz *et al.*2012; Ma *et al.* 2013; Wu *et al.* 2015; Sosa *et al.* 2016; Dias *et al.* 2017; Parra Amin *et al.* 2021). These compounds have not been earlier reported from *Trichoderma* to the best of our knowledge.

Field Experiment for evaluation of growth parameters

Trichoderma conidial suspension treatments resulted tremendous changes in both vegetative and reproductive growth of the rice cultivar Sahbhagidhan. The yield parameters like panicle length, no of panicles per square meter,1000 grain weight and percentage fertility were also

Table 6: HS-SPME/gas chromatography-mass spectrometry (GC-MS) profile of volatile organic compounds (VOCs) produced by *Trichoderma erinaceum*

RT	Compound Name	Calculated RI	Literature RI	RSI	Area (%)	Activity and References
2.65	Propanoic acid	745	739	856	0.02	Antimicrobial in *Euphorbia lathyrus* (Sosa *et al.,* 2016)
4.18	Butane, 2-cyclopropyl-	779	811	880	0.02	-
6.16	1,3-Octadiene	825	820	861	0.22	-
8.06	2-Hexanone, 5-methyl-	868	838	817	0.04	-
8.74	Acetate <pentyl->	883	915	815	0.05	-
10.69	1-Octen-3-ol	1027	960	892	1.01	Antimicrobial in *Bignonia venusta*(L.) (Xiong *et al.,* 2017) & *T. atroviride* (Sharma *et al.,* 2017)
10.92	3-Octanone	1033	963	855	0.85	antibacterial and antifungal in *Atta* ant(de Lima Mendonça *et al.,* 2009), *T. atroviride* (Sharma *et al.,* 2017)
11.07	Furan, 2-pentyl-	1036	977	912	1.64	Antifungal in *Streptomyces spp.* (Wu *et al.,* 2015, Dias *et al.,* 2017)
11.57	Propanoic acid, pentyl ester	1048	988	843	0.12	-
12.23	Ocimene<(Z)-beta>	1062	1035	875	0.03	Antifungal in *Cnidium monnieri* (L.) (Sun *et al.,* 2020) Antimicrobial in Cleopatra mandarin (*Citrus reshni*) (Hamdan *et al.,* 2013)
13.39	Acetophenone	1089	1068	855	0.02	Acetophenone derivatives are antifungal (Ma *et al.,* 2013)
14.17	2-Nonanone, 3-(hydroxymethyl)-	1106	1093	897	0.01	-
14.25	Benzoic acid, methyl ester	1108	1094	900	0.06	Benzoic acid derivative - Antifungal in *Piper cumanense* (Parra Amin *et al.,* 2021)
17.14	Furan <2-heptyl->	1169	1193	891	0.06	Antiphytopathogenic in *Pseudomonas spp.* (Wang *et al.,* 2011)
18.88	Neomethyl acetate	1206	1272	816	0.04	Antifungal in *Mentha piperita* L. (Saharkhiz*et al.,* 2012)
19.47	Acetophenone, 2-chloro	1219	1283	884	0.11	-
23.77	Bergamotene<alpha-, cis->	1311	1416	945	0.35	-
24.34	2H-Pyran-2-one, 6-pentyl-	1324	1425	925	84.10	Antimicrobial in *Trichoderma koningii* (Ismaiel& Ali, 2017), *Trichoderma atroviride* (Jin*et al.,* 2020)
26.02	Menth-1-en-9-ol acetate <para>	1361	1426	823	1.14	-

RT: Retention time RI: Retention index, RSI: Reverse similarity index

increased considerably. In comparison to untreated ones. CRRI-TS-9 treated plants yielded highest with 836.64g/m^2 followed by CRRI-TS-6 (757.5 g/m^2, CRRI-TS-3 (744.04 g/m^2) and CRRI-TS-10 (738.48 g/m^2 (Table7).

Plant growth promotion activity of this genus has been reported in many plants, such as rice (Swain *et al.*2018), sunflower (Nagaraju *et al.* 2012), sugarcane (Srivastava *et al.* 2006), tomato (Bharti *et al.* 2012) etc. Various physiological activities contribute towards growth promotion. These are production of phytohormones, such as auxin (Contreras-Cornejo *et al.* 2009), modification of factors affecting nutrient uptake, such as mineral solubilization (Kapriand Tewari, 2010) and protection of plants against phytopathogens (Khalili *et al.*2012; Kamala and Devi, 2012; Garcia-Nunez *et al.*2012). The *Trichoderma* isolates which have been characterized in our

Table 7: Effect of *Trichoderma* treatments on vegetative growth of rice cultivar Sahbhagidhan

Treatment	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	DFF	No of panicle per m^2	Panicle length (cm)	Grain weight Per hill (g)	Grain weight per plot (g)	1000 grain weight (g)	Fertility %
T-0	57.30^{c}	13.44^{c}	12.73^{d}	3.87^{e}	3.77^{d}	0.73^{c}	75.75^{a}	416.50^{a}	18.98^{e}	12.96^{d}	330.01^{c}	21.96^{a}	52.09^{a}
CRRI-TS-1	77.98^{ab}	19.57^{a}	37.14^{a}	11.59^{b}	5.38^{c}	1.47^{a}	72.00^{a}	575.75^{a}	23.69^{d}	20.04^{bc}	699.81^{b}	23.14^{a}	58.73^{a}
CRRI-TS-2	70.79^{b}	15.83^{bc}	21.83^{c}	7.34^{d}	6.97^{ab}	1.45^{ab}	72.50^{a}	553.70^{a}	26.07^{b}	23.11^{ab}	709.50^{ab}	22.78^{a}	65.20^{a}
CRRI-TS-3	72.88^{ab}	16.64^{abc}	20.23^{c}	5.23^{e}	5.43^{c}	1.37^{ab}	74.25^{a}	485.10^{a}	26.02^{b}	18.33^{c}	744.04^{ab}	22.84^{a}	61.23^{a}
CRRI-TS-5	76.38^{ab}	19.00^{ab}	30.89^{b}	6.75^{d}	4.95^{cd}	0.79^{c}	73.50^{a}	619.85^{a}	24.75^{c}	19.61^{bc}	695.84^{b}	22.00^{a}	61.20^{a}
CRRI-TS-6	79.84^{a}	17.33^{ab}	32.22^{ab}	13.15^{a}	8.11^{a}	1.57^{a}	74.75^{a}	600.25^{a}	23.98^{cd}	22.97^{ab}	757.50^{ab}	23.80^{a}	59.01^{a}
CRRI-TS-9	76.63^{ab}	18.76^{ab}	35.34^{ab}	8.98^{c}	6.20^{bc}	1.25^{ab}	71.00^{a}	600.25^{a}	27.35^{a}	23.77^{a}	836.64^{a}	21.46^{a}	59.23^{a}
CRRI-TS-10	72.66^{ab}	16.93^{ab}	32.48^{ab}	7.88^{cd}	6.04^{bc}	1.04^{bc}	73.50^{a}	612.50^{a}	24.77^{c}	22.03^{ab}	738.48^{ab}	23.70^{a}	60.52^{a}
CV (%)	4.60	8.17	8.22	7.93	9.52	9.72	3.70	9.35	1.64	7.75	8.31	6.045	9.68
Tukey's HSD at 5%	7.86	3.28	5.35	1.50	1.44	0.41	6.36	252.84	0.94	3.69	134.0	3.21	14.92

Means with same letter are not significantly different at $p \leq 0.05$.

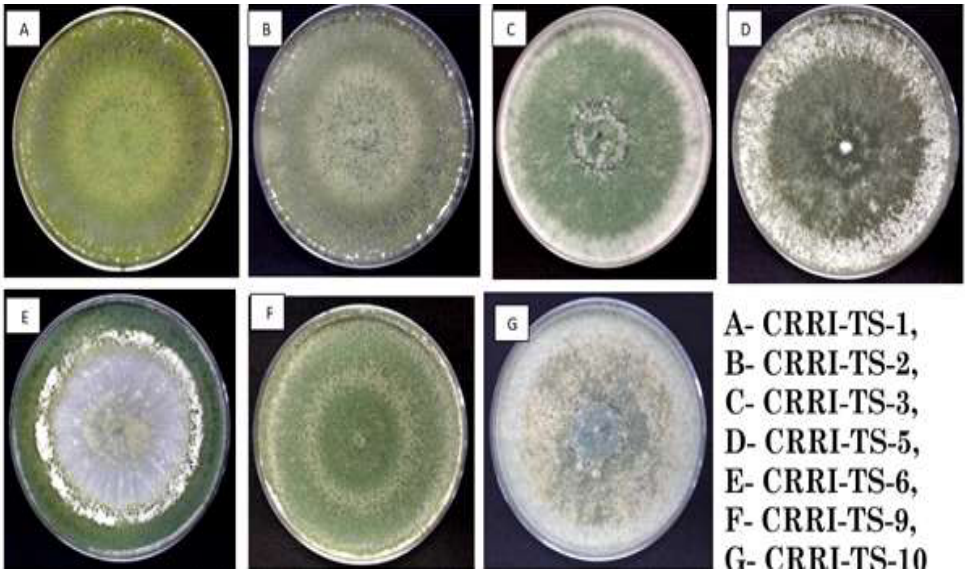

Fig.1: Morphological growth patterns of isolates(5 days after inoculation on PDA)

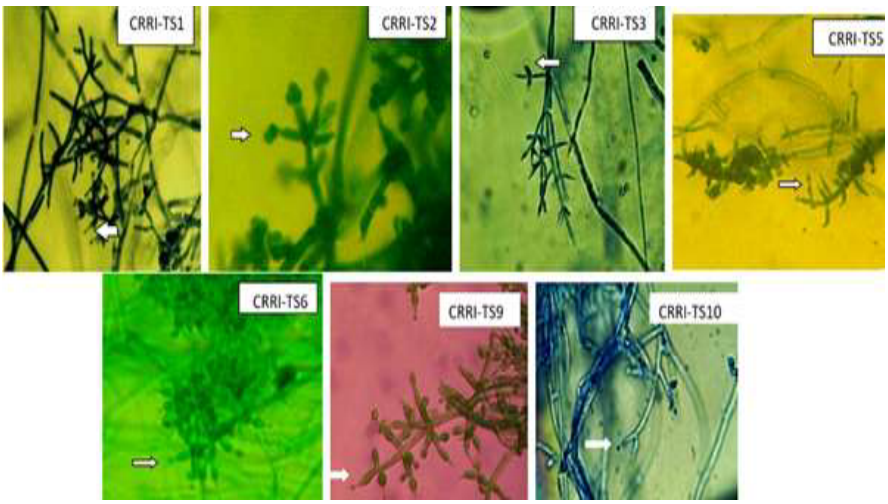

Fig. 2: Conidiophore structure bearing conidia (indicated by arrow mark) as observed under light microscope

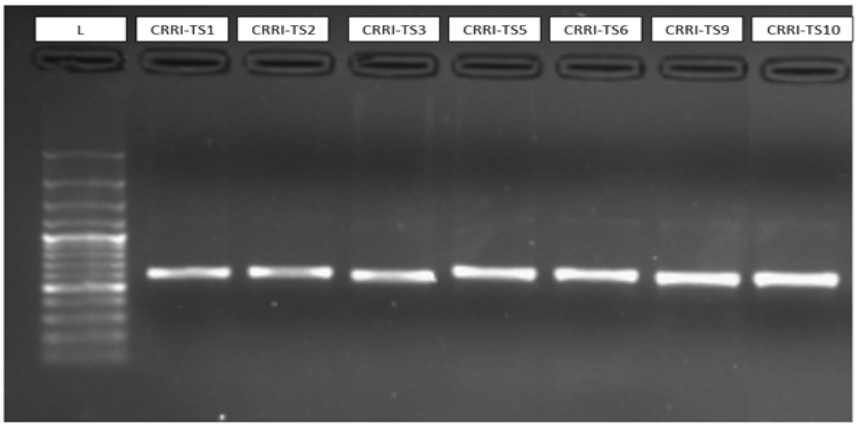

Fig. 3: ITS amplification of genomic DNA of *Trichoderma* spp.

Table. 8: Expression of stress related enzyme activities in the rice cultivar Sahbhagidhan

Treatment Name	Catalase activity (in unit/min/g)		Peroxidase activity (in unit/min/g)		Superoxide Dismutase activity (in unit/min/g)	
	Root	Shoot	Root	Shoot	Root	Shoot
T-0	4.00^{c}	5.00^{h}	0.27^{d}	0.28^{d}	0.82^{f}	1.91^{f}
CRRI-TS-1	6.00^{a}	18.00^{b}	0.48^{b}	0.65^{a}	7.52^{a}	4.60^{d}
CRRI-TS-2	6.00^{a}	12.00^{e}	0.33^{cd}	0.36^{cd}	2.83^{d}	3.98^{e}
CRRI-TS-3	5.00^{b}	6.00^{g}	0.32^{cd}	0.39^{bc}	1.83^{e}	5.03^{c}
CRRI-TS-5	5.00^{b}	10.00^{f}	0.40^{bc}	0.30^{d}	4.82^{b}	10.03^{a}
CRRI-TS-6	6.00^{a}	13.00^{d}	0.35^{cd}	0.47^{b}	2.67^{d}	4.72^{cd}
CRRI-TS-9	5.00^{b}	15.00^{c}	0.25^{d}	0.42^{bc}	2.87^{d}	4.94^{cd}
CRRI-TS-10	6.00^{a}	33.00^{a}	0.77^{a}	0.65^{a}	3.72^{c}	9.02^{b}
CV (%)	1.02	3.12	8.74	6.95	5.46	2.65
Tukey's HSD at 5%	0.55	0.23	0.097	0.08	0.52	0.41

Means with same letter are not significantly different at $p \leq 0.05$.

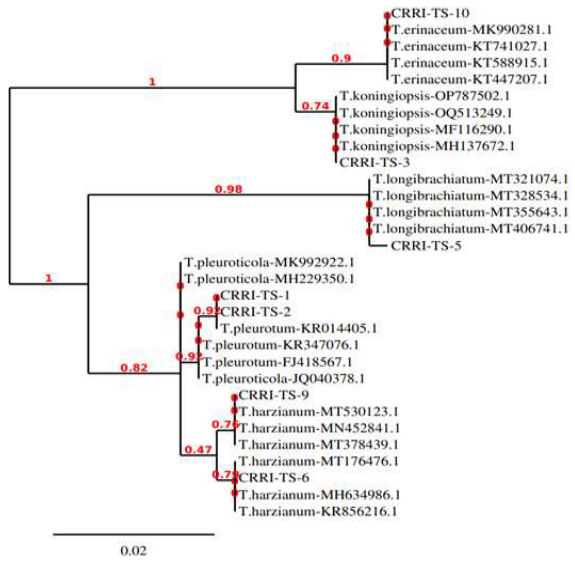

Fig. 4: Phylogeny of the isolated rhizospheric *Trichoderma* spp. (CRRI-TS-1 to CRRI-TS-10)

study exhibited growth promotion in rice in terms of enhanced biomass and yield. Positive effect on rice plant growth by *Trichoderma* species has been reported by Doni *et al.* (2014), Swain *et al*. (2018), Woo *et al.*(2023) and many others.

Defence enzyme activity

Experimental results revealed that the *Trichoderma* seed dressing could activate defence enzymes in rice plants. Catalase activity in Sahbhagidhan shoots was maximum increased by seed treatment with CRRI-TS-10 followed by CRRI-TS-1. Similarly, Both CRRI-TS-1 &CRRI-TS-10 were best peroxidase activity enhancers in shoot and root. In case of SOD activity in Sahbhagidhan roots, CRRI-TS-1 showed highest potential followed by CRRI-TS-5 and CRRI-TS-

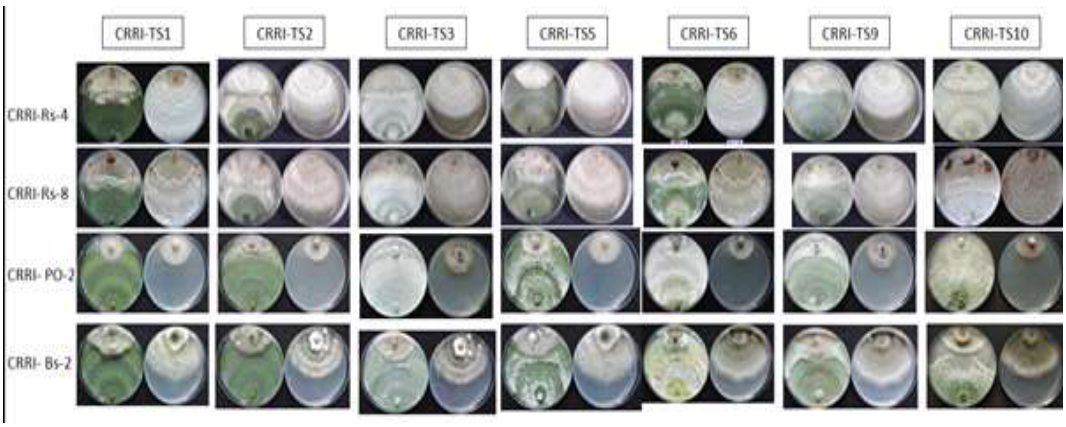

Fig. 5: Confrontation assay showing inhibition of pathogen by *Trichoderma* spp.

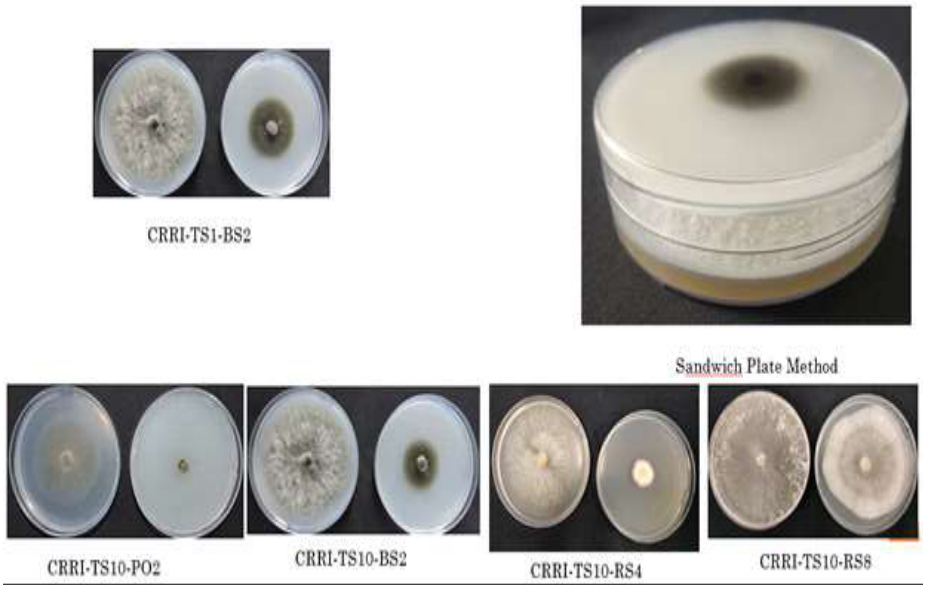

Fig.6 : Inhibition of Pathogens by Volatile Compounds Produced by *Trichoderma* spp.

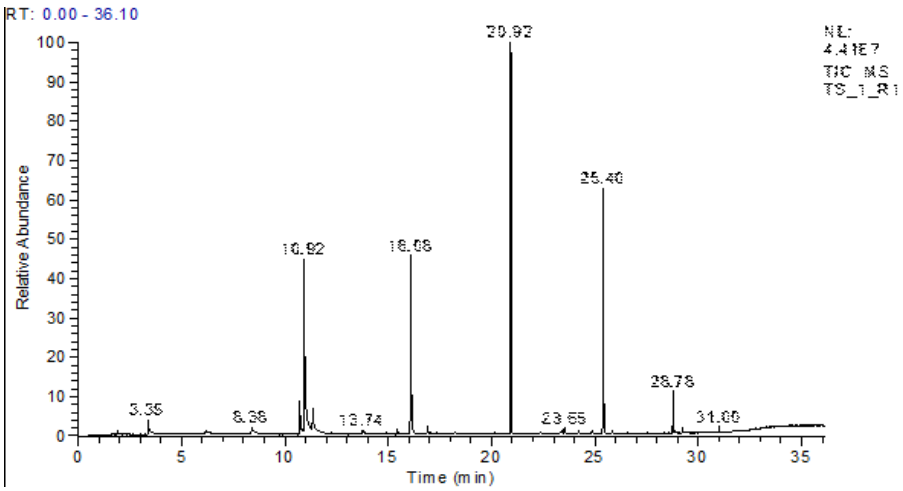

Fig. 7: Gas chromatography-mass spectroscopy profiling of volatile compounds from *Trichoderma pleuroticola*

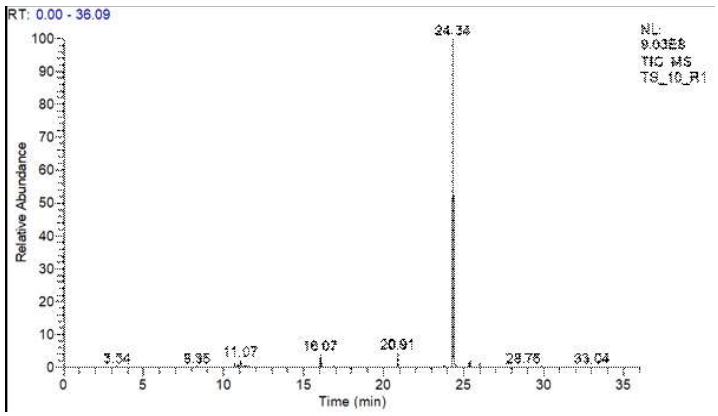

Fig. 8: Gas chromatography-mass spectroscopy profiling of volatile compounds from *Trichoderma erinaceum*

10. But in shoots, CRRI-TS-5 & CRRI-TS-10 performed significantly well (Table 8).

CONCLUSION

All the seven *Trichoderma* isolates were effective against four plant pathogens. However, the VOCs emitted by these *Trichoderma* isolates were not equally effective against plant pathogens. Among the isolates, the VOCs emitted by *Trichoderma erinaceum* had antagonistic potential against all four pathogens, whereas VOCs released by other isolates were not effective. The compound, 6-Pentyl-2H-pyran-2-one released by *Trichoderma erinaceum* is already reported to have both biocontrol and growth promotion action. This isolate improved the growth of rice plants under field conditions. *Trichoderma erinaceum* could be promoted for use in rice disease management as an effective, environment-friendly benign tool. The VOCs emitted by the effective *Trichoderma spp.* can be utilized for the proper management of paddy health.

ACKNOWLEDGEMENT

The authors duly acknowledge the Director, ICAR-National Rice Research Institute, Cuttack for providing research facilities and the Department of Science and Technology, Government of India, New Delhi for providing INSPIRE Fellowship. Authors are also thankful to HOD, PG Department of Botany, Utkal University, Bhubaneswar for providing necessary guidance and support.

REFERENCES

Abbas, A., Yli-Mattila, T. 2022. Biocontrol of *Fusarium graminearum*, a causal agent of Fusarium head blight of

Agrawal, T. Kotasthane, A. 2012. Chitinolytic assay of indigenous Trichoderma isolates collected from different geographical locations of Chhattisgarh in Central India. *SpringerPlus*. **1**. 73. 10.1186/2193-1801-1-73.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215**:403-410.

Amin, F.,Razdan, V. K.,Mohiddin, F. A., Bhat, K. A., Sheikh, P. A. 2010. Effect of volatile metabolites of *Trichoderma* species against seven fungal plant pathogens *in-vitro*. *J. Phytol.***2**: 34–37.

Anees, M., Tronsmo, A., Edel-Hermann, V., Hjeljord, L.G., He´Raud, C., Steinberg, C., 2010. Characterization of field isolates of *Trichoderma* antagonistic against *Rhizoctonia solani*. *Fungal Biol.***114**:691-701.

Anjaiah, V., Thakur, R.P., Koedam, N. 2006. Evaluation of bacteria and Trichoderma for biocontrol of pre-harvest seed infection by Aspergillus flavus in groundnut, *Biocontr. Sci. Technol.***16**:431-436

Aravindan, S., Yadav, M.K., Sharma, P. 2016.Biological control of rice blast disease with *Trichoderma* spp. under upland rice System. *Oryza* **53**: 167-173.

Atanasova, L., Crom, S.L., Gruber, S. *et al*. Comparative transcriptomics reveals different strategies of *Trichoderma* mycoparasitism. *BMC Genomics* **14**:121 2013. https://doi.org/ 10.1186/1471-2164-14-121

Beuchamp, C., Fridovich, I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analyt. Biochem.* **44**:276–287.

Bharti, M.K., Sharma, A.K., Pandey, A.K. *et al.* 2012. Physiological and Biochemical Basis of Growth Suppressive and Growth Promotory Effect of *Trichoderma* Strains on Tomato Plants. *Natl. Acad. Sci. Lett.* **35**: 355–359. https://doi.org/10.1007/s40009-012-0058-2

Bhattacharyya, R., Ghosh, B.N., Mishra, P.K., Mandal, B., Rao, C.S., Sarkar, D., Das, K., Anil, K.S., Lalitha, M.,Hati, K.M.,Franzluebbers, A.J. 2015. Soil Degradation in India: Challenges and Potential Solutions. *Sustainability*. **7**: 3528-3570. https://doi.org/10.3390/ su7043528

Carballo, R., Patrón Vázquez, J., Cáceres-Castillo, D., Quijano-Quiñones, R., Herrera-España, A., Moo-Puc, R., Chale, J., Mena Rejón, G. 2017. Synthesis and in vitro antiprotozoal activity of some 2- amino-4-phenyloxazole derivatives. *Trop. J.Pharmaceut. Res.***16**: 1951. 10.4314/tjpr.v16i8.27.

Chen, L.H., Zhang, J., Shao, X.H., Wang, S.S., Miao, Q.S., Mao, X.Y.,Zhai, Y.M., She, D.L. 2015. Development and evaluation of Trichoderma asperellum preparation for control of sheath blight of rice (Oryza sativa L.). *Biocont. Sci. Technol*. **25** :316–328.

Contreras-Cornejo, H.A., Macías-Rodríguez, L., Cortés-Penagos, C., López-Bucio, J. 2009. *Trichoderma virens*, a Plant Beneficial Fungus, Enhances Biomass Production and Promotes Lateral Root Growth through an Auxin-Dependent Mechanism in *Arabidopsis*. *Plant Physiol*. **149**: 1579-92. 10.1104/pp.108.130369.

Contreras-Cornejo H. A., Macias-Rodriguez L., Herrera-Estrella A., Lopez-Bucio J. 2014. The 4-phosphopantetheinyl transferase of *Trichoderma virens* plays a role in plant protection against *Botrytis cinerea* through volatile organic compound emission. *Plant Soil* **379**: 261–274. 10.1007/s11104-014-2069-x

de Lima Mendonça, A., da Silva, C.E., de Mesquita, F.L.T. *et al.* 2009. Antimicrobial activities of components of the glandular secretions of leaf cutting ants of the genus *Atta* . *Antonie van Leeuwenhoek*. **95**: 295–303. https://doi.org/10.1007/s10482-009-9312-0

Dias, M.P., Bastos, M.S., Xavier, V.B., Cassel, E., Astarita, L.V., Santarém, E.R. 2017. Plant growth and resistance promoted by Streptomyces spp. in tomato. *Plant Physiol. Biochem.***118**: 479-493. https://doi.org/10.1016/j.plaphy.2017.07.017.

Doni, F., Isahak, A., Che Mohd Zain, C.R. *et al.* 2014. Physiological and growth response of rice plants (*Oryza sativa* L.) to *Trichoderma spp*. inoculants. *AMB Expr* **4**: 45. https://doi.org/10.1186/s13568-014-0045-8

Gajera, H.P., Bambharolia, R.P., Patel, S.V., Khatrani, T.J., Goalkiya, B.A. 2012. Antagonism of *Trichoderma* spp. against *Macrophomina phaseolina*: Evaluation of Coiling and Cell Wall Degrading Enzymatic Activities. *J. Plant Pathol.Microb.* **3**:149. doi:10.4172/2157-7471.1000149

Gal-Hemed, I., Atanasova, L., Komon-Zelazowska, M., Druzhinina, I. S., Viterbo, A., Yarden, O. 2011. Marine isolates of *Trichoderma* spp. as potential halotolerant agents of biological control for arid-zone agriculture. *Appl. Environ. Microbiol*. **77**: 5100–5109. https://doi.org/10.1128/AEM.00541-11

Gams, W., Bissett, J. 1998. Morphology and Identification of *Trichoderma*. In: *Trichoderma and Gliocladium: Basic Biology, Taxonomy and Genetics* (Eds.G.E. Harman and C.P.

Kubicek). Vol. **1**, Taylor and Francis, London, UK., ISBN-13: 9780203483558, pp:3-34.

García-Núñez, H.G., Romero Gomez, S., González-Esquivel, C., Nava-Bernal, G., Campos, M. 2012. Isolation of native strains of *Trichoderma* spp, from horticultural soils of the valley of toluca, for potential biocontrol of sclerotinia. *Tropic. Subtropic. Agroecosystems*. **15**. 357-365.

Hamdan, D.I., Abdulla, R.H.,Mohamed, M.E. & El Sahzly, A.M. 2013. Chemical composition and biological activity of essential oils of Cleopatra mandarin (Citrus reshni) cultivated in Egypt. *J. Pharmacogn. Phytotherapy*. **5**. 83 - 90. 10.5897/JPP2013.0277.

Harman, G.E., Howell, C., Viterbo, A. *et al*. 2004. *Trichoderma* species — opportunistic, avirulent plant symbionts. *Nat. Rev.Microbiol.* **2**: 3–56.https://doi.org/10.1038/nrmicro797

Harman, G. E. 2011. Multifunctional fungal plant symbionts: new tools to enhance plant growth and productivity. *The New Phytol*. **189**: 647–649. https://doi.org/10.1111/j. 1469-8137.2010.03614.x

Herath, H.H.M.A.U., Wijesundera, R.L.C., Chandrasekharan, N.V., Wijesundera, W.S.S., Kathriarachchi, H.S. 2015. Isolation and characterization of *Trichoderma erinaceum* for antagonistic activity against plant pathogenic fungi. *Curr. Res. Environ. Appl. Mycol.***5**: 120–128.https://doi 10.5943/cream/5/2/5

Howell C. R. 2003. Mechanisms Employed by *Trichoderma* Species in the Biological Control of Plant Diseases: The History and Evolution of Current Concepts. *Plant Dis*. **87**: 4–10. https://doi.org/10.1094/PDIS.2003.87.1.4

Hung, R., Lee, S., Bennett, J.W. 2013. *Arabidopsis thaliana* as a model system for testing the effect of *Trichoderma* volatile organic compounds. *Fungal Ecol*. **6** : 19-26. 10.1016/j.funeco.2012.09.005

Ismaiel, A.A., Ali, D.M.I. 2017. Antimicrobial properties of 6-pentyl-á-pyrone produced by endophytic strains of *Trichoderma koningii* and its effect on aflatoxin B1 production. *Biologia.* **72**: 1403–1415 https://doi.org/10.1515/biolog-2017-0173

Jin, X., Guo, L., Jin, B., Zhu, S., Mei, X., Wu, J., Liu, T., & He, X. 2020. Inhibitory mechanism of 6-Pentyl-2H-pyran-2-one secreted by *Trichoderma atroviride* T2 against *Cylindrocarpon destructans*. *Pesticide Biochem. Physiol*. **170**:104683. https://doi.org/10.1016/j.pestbp.2020.104683

Johnson, L., Curl, E., Bond, J., Fribourg, H. 1959. *Methods for Studying Soil Mycoflora. – Plant Disease Relationships*. Burgess Publishing Company, Minneapolis.

Kamala T, Devi Indira S. 2012. Biocontrol properties ofindigenous*Trichoderma* isolates from North-east India against *Fusarium oxysporum* and *Rhizoctonia solani*. *Afr. J.Biotechnol*. **11**:8491-8499

Kapri, A., Tewari, L. 2010. Phosphate solubilization potential and phosphatase activity of rhizospheric *Trichoderma* spp. *Braz. J.Microbiol.* **41**: 787–795. https://doi.org/10.1590/S1517-83822010005000031

Kar, M., Mishra, D. 1976. Catalase, Peroxidase, and Polyphenoloxidase activities during Rice Leaf Senescence. *Plant Physiol.***57**: 315-319.

Karthik, Y., Ishwara Kalyani, M., Krishnappa, S., Devappa, R., Anjali Goud, C., Ramakrishna, K., Wani, M. A., Alkafafy, M., HussenAbduljabbar, M., Alswat, A. S., Sayed, S. M., & Mushtaq, M. 2023. Antiproliferative activity of antimicrobial peptides and bioactive compounds from the mangrove *Glutamicibacter mysorens*. *Front.Microbiol*. **14**:1096826. https://doi.org/10.3389/fmicb.2023.1096826

Khalili, E., Sadravi, M., Naeimi, S., Khosravi, V. 2012. Biological control of rice brown spot with native isolates of three *Trichoderma* species. *Braz. J.Microbiol.* **43**: 297-305.

Kim, M., Jeong, E., Lee, H. 2009. Antimicrobial effects of anthracene and its derivatives against intestinal bacteria. *Bioactive Materials* **52**: 327-330.

Kottb, M., Gigolashvili, T., Großkinsky, D. K.,Piechulla, B. 2015. *Trichoderma* volatiles effecting *Arabidopsis*: from inhibition to protection against phytopathogenic fungi. *Front.Microbiol*. **6**: 995. https://doi.org/10.3389/fmicb.2015.00995

Kubicek, C. P., Baker, S., Gamauf, C., Kenerley, C. M., Druzhinina, I. S. 2008. Purifying selection and birth-and-death evolution in the class II hydrophobin gene families of the ascomycete *Trichoderma/Hypocrea*. *BMC Evol.Biol*. **8**: 4. https://doi.org/10.1186/1471-2148-8-4.

Kumar, K., Amaresan, N., Bhagat, S., Madhuri, K., & Srivastava, R. C. 2012. Isolation and Characterization of *Trichoderma* spp. for Antagonistic Activity Against Root Rot and Foliar Pathogens. *Ind. J.Microbiol*. **52**: 137–144. https://doi.org / 10.1007/s12088-011-0205-3

Lee, S., Yap, M., Behringer, G., Hung, R., Bennet, J. W. 2016. Volatile organic compounds emitted by *Trichoderma* species mediate plant growth. *Fungal Biol. Biotechnol*. **3**: 1-14. 10.1186/s40694-016-0025-7

Lee, S., Behringer, G., Hung, R., Bennet, J. 2019. Effects of fungal volatile organic compounds on Arabidopsis thaliana growth and gene expression. *Fung. Ecol*. **37**: 1-9. https://doi.org/10.1016/j.funeco.2018.08.004

Li, N., Alfiky, A., Wang, W., Islam, M.,Nourollahi, K., Liu, X., Kang, S. 2018. Volatile Compound-Mediated Recognition and Inhibition Between*Trichoderma* Biocontrol Agents and *Fusarium oxysporum*. *Front. Microbiol.* **9**:2614.doi: 10.3389/fmicb.2018.02614

Ma, Y.T., Fan, H.F., Gao, Y.Q., Li, H., Zhang, A.L., Gao, J.M. 2013. Natural Products as Sources of New Fungicides (I): Synthesis and Antifungal Activity of Acetophenone Derivatives Against Phytopathogenic Fungi. *Chem. Biol. Drug Design* **81**: 545-552. https://doi.org/10.1111/cbdd.12064

Marco, J.,Felix, C. 2007. Purification and characterization of a beta-Glucanase produced by *Trichoderma harzianum* showing biocontrol potential. *Braz. Arch. Biol. Technol.* 50: 21-29. DOI 10.1590/S1516-89132007000100003.

Meena, R. K., Jangra, S., Wadhwa, Z., Leela Wati, M. 2017. Role of plant volatiles in defense and communication. *Int. J. Curr. Microbiol. Appl. Sci.***6**: 300–313.

Mukherjee, P.K., Mukhopadhyay, A., Sarmah, D., Shrestha, S. 1995. Comparative antagonistic properties of *Gliocladium virens* and *Trichoderma harzianum* on *Sclerotium rolfsii* and *Rhizoctonia solani*: its relevance to understanding the mechanisms of biocontrol. *J. Phytopathol.***143**:275–79

Mukherjee, P. K., Horwitz, B. A.,Kenerley, C. M. 2012. Secondary metabolism in *Trichoderma*—a genomic perspective. *Microbiology (Reading, England)*.**158**: 35–45. https://doi.org/10.1099/mic.0.053629-0

Mukherjee, A.K., Sampath Kumar, A., Kranthi, S., Mukherjee, P.K. 2013. Biocontrol potential of three novel *Trichoderma* strains: isolation, evaluation and formulation. *3Biotech.***4:** 275–281.

Mukherjee, P.K., Mendoza, A.M., Zeilinger, S., Horwitz, B.A. 2022. Mycoparasitism as a mechanism of *Trichoderma*-mediated suppression of plant diseases. *Fung. Biol. Reviews* **39**: 15-33. https://doi.org/10.1016/j.fbr.2021.11.004.

Naeimi, S., Okhovvat, S. M., Javan-Nikkhah, M., Vágvölgyi, C., Khosravi, V., Kredics, L. 2010. Biological control of *Rhizoctonia solani* AG1-1A, the causal agent of rice sheath blight with *Trichoderma* strains. *Phytopathologia Mediterranea*.**49**: 287–300. http://www.jstor.org/stable/26458654

Nagaraju, A., Sudisha, J., Murthy, S.M. *et al.* 2012. Seed priming with *Trichoderma harzianum* isolates enhances plant

growth and induces resistance against *Plasmopara halstedii*, an incitant of sunflower downy mildew disease. *Australasian Plant Pathol.* **41**, 609–620. https://doi.org/10.1007/s13313-012-0165-z

Nieto-Jacobo, M.F., Steyaert, J.M., Salazar-Badillo, F.B., Nguyen, D.V., Rostás, M., Braithwaite, M., De Souza, J.T., Jimenez-Bremont, J.F., Ohkura, M., Stewart, A., Mendoza-Mendoza, A. 2017.Environmental Growth Conditions of *Trichoderma* spp. Affects Indole Acetic Acid Derivatives, Volatile Organic Compounds, and Plant Growth Promotion. *Front. Plant Sci.***8**:102. doi: 10.3389/fpls.2017.00102

Nounjan, N., Nghia, P. T., Theerakulpisut, P. 2012. Exogenous proline and trehalose promote recovery of rice seedlings from salt-stress and differentially modulate antioxidant enzymes and expression of related genes. *J. Plant Physiol*. **169**: 596–604. https://doi.org/10.1016/j.jplph.2012.01.004

Parra Amin, J. E., Cuca, L. E., González-Coloma, A. 2021. Antifungal and phytotoxic activity of benzoic acid derivatives from inflorescences of *Piper cumanense*. *Natur. Prod.Res*. **35**: 2763–2771. https://doi.org/10.1080/14786419.2019.1662010

R project, 2023. Statistical database. https://www.r-project.org

Ruano-Rosa, D., Herrera, C. 2009. Evaluation of *Trichoderma* spp. As biocontrol agents against avocado white root rot. *Biol. Cont*. **51:** 66-71. 10.1016/j.biocontrol.2009.05.005.

Saharkhiz, M. J., Motamedi, M., Zomorodian, K., Pakshir, K., Miri, R., Hemyari, K. 2012. Chemical Composition, Antifungal and Antibiofilm Activities of the Essential Oil of *Mentha piperita* L. *ISRN Pharmaceutics* **2012**: 718645. https://doi.org/10.5402/2012/718645

Salas-Marina, M.A., Silva-Flores, M.A., Uresti-Rivera, E.E. *et al.* 2011. Colonization of *Arabidopsis* roots by *Trichoderma atroviride* promotes growth and enhances systemic disease resistance through jasmonic acid/ethylene and salicylic acid pathways. *Eur. J. Plant Pathol.* **131**: 15–26. https://doi.org/10.1007/s10658-011-9782-6

Sawant, I.S. 2014. *Trichoderma*- Foliar Pathogen Interactions. *The Open Mycol. J*. **8**: 58-70.

Sharma, M.K., Khichar, M.K., Agrawal, A.R. 2017. Isolation and identification of phytosterols from *Bignonia venusta* (L.). *Asian J.Pharmaceut. Clin. Res*. **10**: 247-251.

Shoresh, M., Harman, G. E.,Mastouri, F. 2010. Induced systemic resistance and plant responses to fungal biocontrol agents. *Annu.Rev.Phytopathol*. **48**: 21–43. https://doi.org/10.1146/annurev-phyto-073009-114450

Siameto, E., Okoth, S., Amugune, N., Chege, N. 2011. Molecular characterization and identification of biocontrol isolates of *Trichoderma harzianum* from Embu District, Kenya. *Tropic. Subtropic. Agroecosystems*. **13**: 81-90.

Siddiquee, S. 2014. Recent advancements on the role and analysis of volatile compounds (VOCs) from *Trichoderma*. *Biotechnol. Biol. Trichoderma, Elsevier B.V, Amsterdam*. 139-175.

Sitarek, P., Rijo, P., Garcia, C., Ska³a, E., Kalemba, D., Bia³as, A. J., Szemraj, J., Pytel, D., Toma, M., Wysokiñska, H.,Œliwiñski, T. 2017. Antibacterial, Anti-Inflammatory, Antioxidant, and Antiproliferative Properties of Essential Oils from Hairy and Normal Roots of *Leonurus sibiricus* L. and Their Chemical Composition. *Oxidative Med.Cellul.Longevity*. **2017**: 7384061. https://doi.org/10.1155/2017/7384061

Sosa, A. A., Bagi, S. H.,Hameed, I. H. 2016. Analysis of bioactive chemical compounds of Euphorbia lathyrus using gas chromatography-mass spectrometry and Fourier-transform infrared spectroscopy. *J.Pharmacog.Phytother.***8**: 109-126.

Srivastava, S.N., Singh, V., Awasthi, S.K. 2006. *Trichoderma* induced improvement in growth, yield and quality of sugarcane. *Sugar Tech.* **8**: 166–169 https://doi.org/10.1007/BF02943654

Stoppacher, N., Kluger, B., Zeilinger, S., Krska, R.,Schuhmacher, R. 2010. Identification and profiling of volatile metabolites of the biocontrol fungus *Trichoderma atroviride* by HS-SPME-GC-MS. *J. Microbiologic.Methods*. **81**:187–193. https://doi.org/10.1016/j.mimet.2010.03.011

Sundaramoorthy, S., Balabaskar, P. 2013. Biocontrol efficacy of Trichoderma spp. against wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici.J. Appl. Biol.Biotechnol*. **1**: 036-040

Sun, Y., Yang, A. W. H.,Lenon, G. B. 2020. Phytochemistry, Ethnopharmacology, Pharmacokinetics and Toxicology of *Cnidiumm onnieri* (L.) Cusson. *Inter.JMol.Sci*. **21**: 1006. https://doi.org/10.3390/ijms21031006

Swain, H., Adak, T., Mukherjee, A. K., Mukherjee, P. K., Bhattacharyya, Behera, S. P.et al. 2018. Novel *Trichoderma* strains. isolated from tree barks as potential biocontrol agents and biofertilizers for direct seeded rice. *Microbiol. Res*. **214**: 83–90. doi: 10.1016/j.micres.2018.05.015

Swain, H., and Mukherjee, A. K. 2020. Host–pathogen–*Trichoderma* interaction.In:*Trichoderma* (Eds A. Sharma and P. Sharma) (Singapore: Springer), 149–165. doi: 10.1007/978-981-15-3321-1_8

Swain, H., Adak, T., Mukherjee, A.K., Sarangi, S., Samal, P., Khandual, A., Jena, R., Bhattacharyya, P., Naik, S.K., Mehetre, S.T., Baite, M.S., Kumar, M.S. and Zaidi, N.W. 2021. Seed Biopriming With *Trichoderma* Strains Isolated From Tree Bark Improves Plant Growth, Antioxidative Defense System in Rice and Enhance Straw Degradation Capacity. *Front. Microbiol.***12**:633881. doi: 10.3389/fmicb.2021.633881

Tahir, H. A., Gu, Q., Wu, H., Raza, W., Hanif, A., Wu, L., Colman, M. V., & Gao, X. 2017. Plant Growth Promotion by Volatile Organic Compounds Produced by *Bacillus subtilis*SYST2. *Front.Microbiol*. **8**: 171. https://doi.org/10.3389/fmicb.2017.00171

Toju, H., Tanabe, A.S., Yamamoto, S., Sato, H. 2012. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. *PLoS One*. **7**:e40863. doi: 10.1371/journal.pone.0040863.

Tsahouridou, P., Thanassoulopoulos, C. 2002. Proliferation of *Trichoderma koningii* in the tomato rhizosphere and the suppression of damping-off by *Sclerotium rolfsii*. *Soil Biol.Biochem*. **34**: 767-776. 10.1016/S0038-0717(02)00006-8.

Vinale, F., Sivasithamparam, K., Ghisalberti, E.L., Marra, R., Barbetti, M.J., Li, H., Woo, S.L., Lorito, M. 2008 A novel role for *Trichoderma* secondary metabolites in the interactions with plants. *Physiol. Mol. Plant Pathol*. **72**: 80-86

Wang, X. Y., Xu, Y. Q., Lin, S. J., Liu, Z. Z.,Zhong, J. J. 2011. Novel antiphytopathogenic compound 2-heptyl-5-hexylfuran-3-carboxylic acid, produced by newly isolated *Pseudomonas* sp. strain SJT25. *Appl.Environ.Microbiol*. **77:** 6253–6257. https://doi.org/10.1128/AEM.00443-11

Woo, S.L.,Ruocco, M.,Vinale, F., Nigro, M.,Marra, R., Lombardi, N., Pascale, A.,Lanzuise, S., Manganiello, G.,Lorito, M. 2014. *Trichoderma*-based Products and their Widespread Use in Agriculture. *Open Mycol. J*.**8**: 71–126.

Woo, S.L., Hermosa, R., Lorito, M. *et al.* 2023. *Trichoderma*: a multipurpose, plant-beneficial microorganism for eco-sustainable agriculture. *Nat Rev Microbiol.* **21**: 312–326. https://doi.org/10.1038/s41579-022-00819-5

Wu, Y., Jun,Y., Yaoyao,E., Raza, Wa., Qirong,S.,Huang, Q. 2015. Effects of volatile organic compounds from *Streptomyces albulus* NJZJSA2 on growth of two fungal pathogens: Effects of VOCs from Streptomyces albulus NJZJSA2. *J. Basic Microbiol*. **55:** 10.1002/jobm.201400906.

Xiong, C., Li, Q., Li, S., et al. 2017. *In vitro* Antimicrobial Activities and Mechanism of 1-Octen-3-ol against Food-related Bacteria and Pathogenic Fungi. *J. Oleo Sci.*. **66**:1041-1049. DOI: 10.5650/jos.ess16196. .