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Combined Application of *Trichoderma longibrachiatum* T(SP)-20 and *Trichoderma asperellum* T(AR)-10 in the Management of Stem Rot of Groundnut

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ABSTRACT

Background: *Sclerotium rolfsii* is a soil borne notorious pathogen widely affecting pulses, vegetables, oil seeds, flowers and ornamentals recording high yield loss. In groundnut it causes stem rot. The current study is focused towards management of *S. rolfsii* using combined application of *Trichoderma* spp. under field condition.

Methods: Twenty-five isolates of *Trichoderma* spp. were screened initially against the stem rot pathogen through dual culture method. The effective *Trichoderma* spp. again assessed by agar well diffusion method and their secondary metabolites were identified using GC-MS.

Result: From the 25 isolates, *T. longibrachiatum* and *T. asperellum* were inhibitory to the growth of *S. rolfsii*. The isolate T(SP)-20 of *T. longibrachiatum* showed 84.44% inhibition of mycelial growth of pathogen followed by T(AR)-10 of *T. asperellum* (75.55%). The major compounds present in GC-MS analysis of *T. longibrachiatum* and *T. asperellum* are 2-Tricosenoic acid (3.29%), Hexadecane (3.12%) and Phenol (27.18%), 2,6,10-Trimethyltridecane (3.44%) respectively. At field level combined application of effective *T. longibrachiatum* and *T. asperellum* excelled well in reducing stem rot disease incidence (82.67%) when compared to individual species.

Key words: Biological control, GC-MS, Groundnut, *S. rolfsii*, *Trichoderma* spp.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is known as king of oil seeds and it is cultivated mainly in China, Indian, Nigeria, Sudan and Myanmar countries. In India, it occupies an area of 4.73 million ha with a production of 6.72 million tonnes (FAOSTAT, 2019). Gujarat ranks first in groundnut area (20 lakh ha) and production (26 lakh tonnes). The highest productivity of groundnut (1604 kg/ha) is recorded in the State of Tamil Nadu, while in Gujarat, productivity is about 1190 kg/ha. Groundnut is suffered by various fungal, bacterial and viral diseases. Among them, stem rot disease was caused by a necrotrophic, soil-borne fungal pathogen, *Sclerotium rolfsii* Sacc. (*Athelia rolfsii*), recording a range of yield losses of 10- 40% (Dodia *et al*., 2019). The symptoms are partial or complete wilting of the stem and branches. As the pathogen produces a very hard resting structure sclerotia having melanin pigment on the outer membrane and a long-lasting survival period it is too difficult to manage this disease by applying a single strategy. Due to undefined usage of systemic fungicides most of the pathogens develop resistance in addition to this, fungicides leave some toxic residual compounds in plant parts. Biocontrol is an alternative method under which *Trichoderma* spp. play a crucial role in the management of soil-borne diseases. *Trichoderma* spp. exhibit different mechanisms like competition, antibiosis, mycoparasitism, lysis and induced systemic resistance. Lytic enzymes such as chitinase and glucanase produced by the *Trichoderma* spp. effectively inhibited the *Sclerotium rolfsii (S. rolfsii*) (Kumar *et al*., 2012; Ponnusamykonar *et al*., 2011). Keeping the above points in view, this research paper deals with the molecular confirmation of *Trichoderma* spp. evaluation of their metabolites and checking their efficacy individually and in combination under field conditions.

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MATERIALS AND METHODS

Survey and isolation of *S. rolfsii*

The survey was conducted in major groundnut growing regions like Thiruvannamalai, Sivagangai, Theni, Salem and Madurai districts of Tamil Nadu. The percentage of the disease incidence was calculated based on the severity of the infection. The stem rot pathogen was isolated through a

tissue segmentation method (Kumar *et al.*, 2014). Totally ten isolates were maintained (Table 1). Mycelium proliferated well within 2 days and produced sclerotia on 12th day. The virulence of all the isolates of *S.rolfsii* was checked through pathogenicity test. It revealed that the isolate IS(BDI)-8 is the most virulent by recording 89.27 per cent incidence.

Isolation of *Trichoderma spp.*

During the survey, the soil samples were collected from the healthy groundnut rhizosphere region. *Trichoderma* spp. were isolated through soil dilution plating technique in *Trichoderma* selective medium (TSM) (Elad and Chet, 1983).

In-vitro* screening of *Trichoderma* spp. against *S. rolfsii

Twenty five isolates of *Trichoderma* spp. were evaluated *in vitro* against *S. rolfsii* by dual culture technique (Dennis and Webster, 1971). Seven days old cultures of *Trichoderma* spp. and *S. rolfsii* IS (BDI)-8 were used for the study. The culture disc (9 mm dia.) of the test pathogen and *Trichoderma* sp. were cut out and placed aseptically at equidistance and in opposite directions to each other on solidified PDA medium in Petri plates and then plates were incubated at 28±2°C. Three replications were carried out. The PDA plates inoculated only with the culture disc of the test pathogen were served as control. The inhibition percentage of radial growth of the pathogen by *Trichoderma* sp. was compared with control (Vincent, 1947).

Per cent inhibition (I) =

$$C - T/C \times 100$$

Dc = Average diameter of fungal growth (cm) in control.
Dt = Average diameter of fungal growth (cm) in treatment.

Molecular confirmation

The most virulent *S. rolfsii* IS(BDI)-8 isolate and the elite *Trichoderma* spp. isolates T(AR)-10, T(SP)-20 chosen based on dual culture and were confirmed using molecular tool. DNA extraction was carried out using CTAB method. The reaction mixture (10 µl) for PCR amplification comprises of master mix (5 µl), forward and reverse primer (each 1 µl), DNA template (2µl) and sterile water (1 µl). Master mix contains 0.25 mM dNTPs, 1.5 mM $MgCl_2$, Taq polymerase and buffer. The sequence of genomic DNA was amplified using the Forward primer: ITS 1(5′-TCCGTAGGTGAAC CTGCGG-3′) and Reverse primer : ITS4 (5′TCCTCCGCTTA TTGATATGC-3′) PCR was done in a master cycler with inbuilt program of initial denaturation at 94°C for 5 mins, continued by 35 cycles of denaturation at 94°C for 1 mins, annealing at 46°C for 1 mins, extension at 72°C for 1 mins and ended up after a final extension at 72°C for 10 mins (White *et al.*, 1990).The PCR products were subjected to gel electro phoresis on 1.5 per cent concentration. Then it was visualized and documented in gel documentation system.

The ITS gene sequences of the virulent *S.rolfsii* IS(BDI)-8 and proficient *Trichoderma* spp. T(SP)-20, T(AR)-10 were compared with NCBI blast search gene bank data base (http://www.ncbi,nlm.nih.g).The sequences were submitted to the gene bank and received the accession numbers namely MZ277282-(*Athelia rolfsii* IS(BDI)-8), MZ277326-(*Trichoderma asperellum* T(AR)-10) and MZ277378-(*Trichoderma longibrachiatum* T(SP)-20).

Agar well diffusion method and GC-MS analysis

Trichoderma spp. isolates with highest antagonistic activity (Table 4) were further investigated for their ability to produce inhibitory metabolites. The *Trichoderma* sp. was grown in a conical flask containing PDA broth which was kept at 125 rpm and 28±2°C in a shaker cum incubator for up to 7 days. After that, the content was centrifuged at 8000 rpm for 15 mins and the supernatant was filtered through Whatman no.1 filter paper to remove the residues of mycelia. Culture filtrate was extracted with ethyl acetate at the ratio of 1:1 and separated by a separation funnel. The extract was passed through anhydrous sodium sulfate to remove the excess water content. The extract was further concentrated in a rotary vacuum evaporator and used for agar well

Table 1: Survey of stem rot incidence of groundnut.

Isolates	Place of collection	District	Geo co-ordinates		Percent disease incidence (%)*
			Latitude (°N)	Longitude (°E)	
IS (AKP)-1	Anaikaraipatti	Sivagangai	10.0465	77.6486	42.65^{c} (40.77)
IS (SVP)-2	Servarayanpatti	Sivagangai	10.1840	78.4263	20.54^{f} (26.95)
IS (MDU)-3	AC and RI	Madurai	9.9699	78.2040	24.86^{e} (29.90)
IS (MAL)-4	Malligapuram	Theni	9.8410	77.1130	41.48^{c} (40.09)
IS (ANR)-5	Alanganallur	Madurai	10.6313	78.7666	33.45^{d} (35.33)
IS (SPM)-6	Soolapuram	Theni	9.8386	77.3181	18.46^{g} (25.44)
IS (SMI)-7	Silamalai	Theni	9.9356	77.9596	34.25^{d} (35.81)
IS (BDI)- 8	Bodi	Theni	10.9701	77 .8878	62.34^{a} (52.14)
IS (VVI)-9	Vinnavadi	Thiruvannamalai	12.9498	79.0879	54.86^{b} (47.78)
IS (EPI)-10	Edappadi	Salem	11.6228	77.4471	14.43^{h} (22.32)
CD (P=0.05)					1.56

*Means followed by the same letter differ non-significantly at P≤0.05 according to DMRT; values are mean of three replications, values in the parentheses are arc sine transformed values.

diffusion assay and GC/MS analysis. A nine mm mycelial disc of *S. rolfsii* was placed in the center of the Petri plate and then 100 µl of extract of effective *Trichoderma* sp. was dropped into the agar well 1 cm away from the edge at four sides on the periphery of Petri plate. The plates were incubated at room temperature and the plates were scored when the mycelium covered the entire Petri plate in control. Control was maintained with the sterile distilled water instead of crude extract.

Management of stem rot disease using elite *Trichoderma* spp. in field

A field experiment was conducted in a stem rot affected groundnut field in Salem district of Tamil Nadu, India. The groundnut variety used was VRI-2 to evaluate the *Trichoderma* spp. for the management of stem rot. The field, which was abandoned for commercial cultivation due to severe stem rot incidence, was selected for this purpose. The talc formulation of effective *Trichoderma* isolates *viz., T. longibrachiatum* T(SP)-20 and *T. asperellum* T(AR)-10 was applied through seed treatment 4 g/kg of seed and soil application (@ 2.5 kg/ha). Three replications per treatment were maintained. The observations such as per cent disease incidence, per cent reduction over control and yield (kg/ha) were taken at the time of harvest.

Statistical analysis

Mean differences of the treatment were evaluated with ANOVA at a significant level (P<0.05) and means were compared by duncan's multiple range test (DMRT) (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Survey and isolation of stem rot pathogen

During the survey, highest (62.34%) and least (14.43%) stem rot incidence were recorded in Bodi of Theni district and Edappadi of Salem district (Table 1). The isolate IS (BDI)-8 showed maximum incidence in groundnut field, grew very fast and produced maximum number of sclerotia in the Petri plate (162 per plate).The color of the isolates ranged from dull white to pure white (Table 2,). Sivakumar *et al.* (2016) conducted a survey in different locations of groundnut fields at Cuddalore district. Among them Adhivaraganullur village registered the maximum incidence of 32.0% followed by

Table 2: Morphological characters of *Sclerotium rolfsii.*

Isolates	Colony colour	Mycelial growth	Sclerotia production (Nos)
IS (AKP)-1	Dull white	Moderate	124de
IS (SVP)-2	Dull white	Fast	115^{f}
IS (MDU)-3	Dull white	Moderate	93^{h}
IS (MAL)-4	Pure white	Fast	102^{g}
IS (ANR)-5	Pure white	Moderate	143^{b}
IS (SPM)-6	Dull white	Moderate	118^{ef}
IS (SMI)-7	Pure white	Fast	73^{i}
IS (BDI)- 8	Dull white	Very Fast	162^{a}
IS (VVI)-9	Pure white	Very slow	130^{cd}
IS (EPI)-10	Dull white	Very slow	136^{c}
CD (P=0.05)	-	-	6.53

*Means followed by the same letter differ non-significantly at P≤0.05 according to DMRT; values are mean of three replications.

Table 3: *In-vitro* screening of *Trichoderma* spp. against *S. rolfsii.*

Isolate	Place of isolation	District	Percent inhibition over control (%)*
T (TH)-1	Thirumangalam	Madurai	46.66 (43.08)
T (KA)-2	Kanakiliyanallur	Trichy	52.22 (46.27)
T (VT)-3	VavvalThottam	Madurai	73.33 (58.90)
T (PA)-4	Palamedu	Madurai	35.55 (36.60)
T (VA)-5	Vandalaikudalur	Trichy	33.33 (35.26)
T (KK)-6	Kamayagoundanpatti	Theni	45.55 (42.44)
T (PU)-7	Pudhupatti	Theni	37.77 (37.92)
T (AY)-8	Ayanpannapatti	Trichy	34.44 (35.93)
T (VI)-9	Vilangudi	Madurai	65.55 (54.05)
T (AR)-10	Alanganallur	Madurai	75.55 (60.36)
T (CR)-11	CR Palayam	Trichy	38.88 (38.57)
T (AP)-12	Anaikaraipatti	Sivagangai	28.88 (32.50)
T (VI)-13	Vinnavadi	Thiruvannamalai	26.66 (31.08)
T (MD)-14	Madurai (AC and RI)	Madurai	33.33 (35.26)
T(ML)-15	Malligapuram	Theni	73.33 (58.90)
T (BI)-16	Bodi	Theni	33.33 (35.26)
T (SM)-17	Soolapuram	Theni	52.22 (46.27)
T (SI)-18	Silamalai	Theni	64.44 (53.39)
T (KO)-19	Kottampatti	Madurai	43.33 (41.16)
T (SP)-20	Servarayanpatti	Sivagangai	84.44 (66.76)
T (EI)-21	Edappadi	Salem	41.11 (39.88)
T (CH)-22	Cholavanthan	Madurai	65.55 (54.06)
T (TK)-23	Thiruparankundram	Dindugal	31.11 (33.90)
T (TM)-24	Tharamangalam	Salem	34.44 (35.93)
T (KK)-25	Kodaikanal	Dindugal	43.33 (41.16)
TNAU-TA	TNAU	TNAU	75.00 (60)
Control	-	-	0.0
CD (P=0.05)	-	-	2.42

*Mean of three replications.
Values in the parentheses are arc sine transformed values.

Table 4: Culture metabolites of *Trichoderma* spp. against *S. rolfsii.*

Isolates	Inhibition zone (cm)*
T(SP)-20	1.1^{a}
T(AR)-10	0.9^{b}
T(VT)-3	0.3^{e}
T(BI)-16	0.7^{c}
T(TK)-23	0.5^{d}
TNAU-TA	0.9^{b}
CONTROL	0^{f}
CD(P=0.05)	0.019

*Means followed by the same letter differ non-significantly at P≤0.05 according to DMRT; values are mean of three replications.

Ponveli, (29.56%) and least disease incidence was recorded in Rajkuppam (7.88%) and the highly virulent isolate produced the light brown colored mycelium and 346 sclerotia per plate.

Isolation of *Trichoderma* spp. from different location of Tamil Nadu

The 25 different *Trichoderma* spp. were isolated and confirmed based on morphological characters such as color and growth pattern of mycelium, shape of the conidia and phialids (Rifai, 1969).The Isolate T(AR)-10 produced green colored with ring-like zone of culture and T(SP)-20 produced slight yellowish ring like growth pattern of culture in the Petri plate.

Antagonistic activity of *Trichoderma* spp. against the stem rot pathogen

The *Trichoderma* sp. T(SP)-20 exhibited the maximum percentage inhibition (84.4%) followed by T(AR)-10 (75.5%) and TNAU TA (75.0%). (Table 3). Babu and Kumar (2008) reported that nine *Trichoderma* spp. (Th-1 to Th-9) were isolated from the microflora of the groundnut rhizosphere. Among them, the isolate Th-3inhibited the *S. rolfsii* mycelial growth up to 83% in the dual culture technique. Similarly, Hirpara *et al.* (2016) noticed that *Trichoderma virens* NBAII Tvs12 inhibited the mycelial growth of *S. rolfsii* by coiling surround and production of hook like structure inside the mycelium of test pathogen and exhibited the percent growth reduction of 76.37% and 87.91% at 6 DAI and 12 DAI respectively and also arrested the sclerotia production compared to the control.

Molecular confirmation of *Sclerotium rolfsii* and *Trichoderma* spp.

The virulent isolate IS (BDI)-8 was amplified at the specific size of 700 bp using ITS1 and ITS4 primers depicting the molecular based confirmation of *S. rolfsii*. Similar results were obtained by Prasad *et al.* (2010). These workers performed rRNA amplification of *S. rolfsii* with ITS1 and ITS4 primers which produced a fragment of approximate size between 650 to 700 bp.

The best antagonists T(SP)-20 and T(AR)-10 chosen based on dual culture when amplified using ITS1 and ITS4 primers yielded a product of approximately 650-700 bp. According to Shahid (2013), universal primers (ITS-1 and ITS4) were used for the amplification of 28S rRNA gene of *Trichoderma longibrachiatum* and a sharp band of about 700 bp was seen on the gel.

Agar well diffusion assay

The culture filtrate extracts of six isolates (T(SP)-20, T(AR)-10, T(VT)-3,T(BI)- 16, T(TK)-23, TNAU-TA) were assessed against the *S. rolfsii.* Among them T(SP)-20 exhibited the maximum radial growth inhibition (1.1 cm) followed by T(AR)-10 (0.9 cm) (Table 4).

GC-MS analysis of *Trichoderma* spp.

According to GC-MS analysis it is clear that the T(SP)-20 and T(SP)-10 produced various antifungal compounds. The major antimicrobial compounds detected from T(SP)-20 of peak area% and RT value are Pentadecafluorooctanoic acid (1.29) (15.186), Quinoline,1,2-dihydro2,2,4trimethyl (1.97) (19.191), Pentadecane (1.48) (20.561), Nonadecane (6.75) (23.081), Hexadecanoicacid (3.07) (30.421, Phthalic acid (1.61) (31.101), 2-Trichosenoic acid (3.29) (36.655) (Table 5a).

The isolate T(AR)10 produced the antifungal compounds such as phenol (27.18) (6.819), 2, 6, 10 trimethyltridecane (3.44) (16.261), nonadecane (5.36) (23.077), 9-undeconal, 2, 10 dimethyl (3.18) (26.625), heptacosanoic acid (2.56) (30.407), acetic acid (2.51) (31.190), erucic acid (3.46) (38.880) (Table 5b, Fig 7b). Similarly, Siddiquee *et al.* (2012) reported that *T. harzianum* produced 278 volatile compounds like alkanes, alcohols, ketones, pyrones (lactones) *etc*., possessing the antifungal activity against a wide range of soil borne pathogens. Lee *et al.* (2016) noticed that *Trichoderma* sp.produced many volatile compounds such ascedrane, isobutyl acetate, caryophyllene, pentadecane, p-xylene, benzoic acid, pyridine, acetic acid and butanoic acid having the antimicrobial activity and induced the growth promotion in plants.

Management of stem rot disease using elite *Trichoderma* spp. in field

The results depicted that all the modules consisting with *Trichoderma* sp. either alone or in combinations were found

Table 5a: Major secondary metabolites from *Trichoderma longibrachiatum.*

Name of the compound	RT	Peak area %	MW g/mole	Formula	Specific role	Reference
Pentadecafluorooctanoic acid	15.186	1.29	414.07	$CF_3(CF_2)_6COOH$	Antimicrobial Antifungal	(Yuef *et al.*, 2018)
Quinoline, 1,2-dihydro -2,2,4-trimethy	19.191	1.97	173.25	$C_{12}H_{15}$	Antimicrobial Antifungal	(Khamkhenshorngphanuch *et al.*, 2020)
Pentadecane	20.561	1.48	212.41	$C_{15}H_{32}$	Antifungal	(Lee *et al.*, 2016)
Hexadecane, 2,6,1 0,14-tetramethyl-	30.421	3.12	282.5475	$C_{20}H_{42}$	Antimicrobial Antifungal	(Yuef *et al.*, 2018)
Isopropyl myristate	30.648	1.44	270.5	$C_{17}H_{34}O_2$	Antimicrobial	(Ringertz and Ringertz, 1982)
Phthalic acid	31.101	1.61	306.4	$C_{18}H_{26}O_4$	Antimicrobial	(Beulah *et al.*, 2018)
2-Tricosenoic acid	36.655	3.29	352.6	$C_{23}H_{44}O_2$	Antibacterial	(Lee *et al.*, 2021)

Table 5b: Major secondary metabolites from *Trichoderma asperellum*.

Name of the compound	RT	Peak area %	MW g/mole	Formula	Specific role	Reference
Phenol	6.81	27.18	94.11	C_6H_6O	Antifungal Antibacterial	(Dini *et al.*, 2020)
Hexadecane	15.75	2.51	256.4241	$C_{16}H_{32}O_2$	Antifungal Antibacterial	(Akpuaka *et al.*, 2013)
Dodecane, 4, 6-dimethyl	16.03	2.99	582.8	$C_{39}H_{50}O_4$	Antimicrobial	(Dini *et al.*, 2021)
2,6,10-Trimethyltridecane	16.26	3.44	226.44	$C_{16}H_{34}$	Antibacterial	(Okla *et al.*, 2019)
Tetradecane	17.91	2.66	198.39	$C_{14}H_{30}$	Antifungal	(Bhardwaj and Kumar, 2017)
1-Dodecanol	19.91	1.85	186.33	$C_{12}H_{26}O$	Antimicrobial	(Wijekoon *et al.*, 2013)
9-Undecenal, 2,10-dimethyl-	26.62	3.18	198.34	$C_{13}H_{26}O$	Antifungal	(Li *et al.*, 2020)
Acetic acid, 10,11-dihydroxy	31.19	2.51	209.20	$C_{10}H_{11}NO_4$	Antimicrobial	(Yuef *et al.*, 2018)
Erucic acid	38.88	3.46	338.6	$C_{22}H_{42}O_2$	Antifungal	(Walters *et al.*, 2004)

Table 6: Efficacy of *Trichoderma* spp. Against*S.rolfsiiin-vivo* condition.

T. No.	Treatments	PDI*	Disease reduction over control (%)	Yield (kg/ha)
T_1	Seed treatment (ST) with *Trichoderma longibrachiatum* T(SP)-20 @ 4 g/kg of groundnut seed+Soil application (SA) of *Trichoderma longibrachiatum* T(SP)-20 @ 2.5kg/ha (B.S)	23.77^f (29.18)	55.33	1220
T_2	ST with *Trichoderma asperellum* T(AR)-10 @ 4 g/kg+SA of *Trichoderma asperellum* T(AR)-10 @ 2.5 kg/ha (B.S)	26.22^g (30.80)	50.73	1180
T_3	ST with (T(SP)-20 +T(AR)-10) @ 4 g/kg + SA of (T(SP)-20 +T(AR)-10) @ 2.5kg/ha (1:1) (B.S)	19.55^e (26.24)	63.26	1250
T_4	T1 + SA of T(SP)-20 @ 2.5kg/ha after 30 DAS	11.22^c (19.57)	78.91	1370
T_5	T2 + SA of T(AR)-10 @ 2.5kg/ha after 30 DAS	13.33^d (21.41)	74.95	1280
T_6	T3 + SA of T(SP)-20+T(AR)-10 @ 2.5 kg/ha after 30 DAS (1:1)	9.22^b (17.68)	82.67	1452
T_7	ST Carbendazim at 2g/kg+Soil drenching with Carbendazim @ 0.2% (Chemical check)	7.66^a (16.07)	85.60	1510
T_8	Control	53.22^h (46.85)	00.00	962
	CD(P=0.05)	1.42	3.62	49.54

B.S-Before sowing, DAS-Days after sowing.

*Means followed by the same letter differ non-significantly at P≤0.05 according to DMRT; values are mean of three replications, values in the parentheses are arc sine transformed values.

superior in reducing the stem rot disease incidence and resulted maximum pod yield as compared to control. The combined module treatment, T6 - ST with (T(SP)-20+T(AR)-10) @ 4 g/kg+SA of (T(SP)-20+T(AR)-10) @ 2.5 kg/ha before sowing+SA of (T(SP)-20+T(AR)-10) @ 2.5 kg/ha after 30 DAS (1:1) recorded the least disease incidence, highest disease control and pod yield (9.22%, 82.67% and 1452 kg ha^{-1}) respectively followed by T4 - ST with T(SP)-20 @ 4 g/kg+SA of T(SP)-20 @ 2.5 kg/ha before sowing+SA of T(SP)-20 @ 2.5kg/ha after 30 DAS (11.22%,78.91% and 1370 kg ha^{-1}) (Table 6). Conclusively, the combined application of two *Trichoderma* spp. used as seed treatment and soil application reduced the disease incidence more effectively as compared to seed treatment and soil application of *Trichoderma* sp. alone. The *Trichoderma* sp. treatments significantly enhanced the yield attributes and reduced the disease incidence. Similarly. Meena *et al.* (2018)reported that soil application of *T. harzianum* (Th-BKN) @ 10 kg ha^{-1} +FYM @ 10 tonnes.ha^{-1} gave highest disease control, pod yield in kg ha^{-1} and lowest disease incidence was 86.30%, 2173 kg ha^{-1} and 7.51% respectively.

CONCLUSION

From this experimental result, the *Trichoderma longibrachiatum* T(SP)-20, *Trichoderma asperellum* T(AR)-10

produced numerous organic compounds with antimicrobial activity against a wide range of pathogens including *S. rolfsii*. Combined talc-based application of the above two species through seed treatment and soil application is found highly effective reducing the stem rot incidence compared to individual application. In near future, commercially it will be exploited for the management of stem rot of groundnut.

Conflict of interest: None.

REFERENCES

Akpuaka, A., Ekwenchi, M., Dashak, D. and Dildar, A. (2013). Biological activities of characterized isolates of n-hexane extract of *Azadirachta indica* A. Juss (Neem) leaves. Nature and Science. 11: 141-147.

Babu, K. and Kumar, M. (2008). Mycoparasitic activity of *Trichoderma harzianum* against stem rot sclerotium rolfsii of groundnut. J. Mycol. Pl. Pathol. 38: 593-595.

Beulah, G., Soris, P.T. and Mohan, V. (2018). GC-MS determination of bioactive compounds of *Dendrophthoe falcata* (LF) Ettingsh: An Epiphytic Plant. Int. J. Health Sci. Res. 8: 261-269.

Bhardwaj, N.R. and Kumar, J. (2017). Characterization of volatile secondary metabolites from *Trichoderma asperellum*. Journal of Applied and Natural Science. 9: 954-959. DOI: 10.31018/jans.v9i2.1303.

Dennis, C. and Webster, J. (1971). Antagonistic properties of species-groups of *Trichoderma*: II. Production of volatile antibiotics. Transactions of the British Mycological Society. 57: 41-IN44. DOI:10.1016/S0007-1536(71) 80077-3.

Dini, I., Graziani, G., Fedele, F. L., Sicari, A., Vinale, F., Castaldo, L. and Ritieni, A. (2020). Effects of *Trichoderma biostimulation* on the phenolic profile of extra-virgin olive oil and olive oil by-products. Antioxidants. 9: 284. DOI: 10.3390/antiox 9040284.

Dini, I., Marra, R., Cavallo, P., Pironti, A., Sepe, I., Troisi, J.,Vinale, F. (2021). *Trichoderma* strains and metabolites selectively increase the production of volatile organic compounds (VOCs) in Olive Trees. Metabolites. 11: 213. DOI:10.3390/ metabo11040213.

Dodia, S.M., Joshi, B., Gangurde, S.S., Thirumalaisamy, P.P., Mishra, G.P., Narandrakumar, D., Sangh, C. (2019). Genotyping-by-sequencing based genetic mapping reveals large number of epistatic interactions for stem rot resistance in groundnut. Theoretical and Applied Genetics. 132: 1001-1016.

Elad, Y. and Chet, I. (1983). Improved selective media for isolation of *Trichoderma* spp. or *Fusarium* spp. Phytoparasitica. 11: 55. DOI: 10.1007/BF02980712.

FOASTAT. (2019). FAO Statistical Database. FAO, Rome, Italy; Available: http//:www.fao.org/faostat/en/#data/QC.

Gomez, K.A. and Gomez, A.A. (1984). Statistical Procedures for Agricultural Research: John Wiley and Sons.

Hirpara, D.G., Gajera, H., Bhimani, R. and Golakiya, B. (2016). The SRAP based molecular diversity related to antifungal and antioxidant bioactive constituents for biocontrol potentials of *Trichoderma* against *Sclerotium rolfsii* Scc. Current Genetics. 62: 619-641. DOI: 10.1007/s00294-0 16-0567-5.

Khamkhenshorngphanuch, T., Kulkraisri, K., Janjamratsaeng, A., Plabutong, N., Thammahong, A., Manadee, K., Khotavivattana, T. (2020). Synthesis and Antimicrobial Activity of Novel 4-Hydroxy-2-quinolone Analogs. Molecules. 25: 3059. DOI: 10.3390/molecules25133059.

Kumar, Amaresan, N., Bhagat, S., Madhuri, K. and Srivastava, R. (2012). Isolation and characterization of *Trichoderma* spp. for antagonistic activity against root rot and foliar pathogens. Indian Journal of Microbiology. 52: 137-144. DOI: 10.1007/s12088-011-0205-3.

Kumar, Santhoshi, M.M., Krishna, T.G. and Reddy, K.R. (2014). Cultural and morphological variability *Sclerotium rolfsii* isolates infecting groundnut and its reaction to some fungicidal. International Journal of Current Microbiology and Applied Sciences. 3: 553-561.

Lee, S., Yap, M., Behringer, G., Hung, R. and Bennett, J.W. (2016). Volatile organic compounds emitted by *Trichoderma* species mediate plant growth. Fungal Biology and Biotechnology. 3: 1-14. DOI: 10.1186/s40694-016-0025-7.

Lee, J.H., Kim, Y.G., Khadke, S.K. and Lee, J. (2021). Antibiofilm and antifungal activities of medium chain fatty acids against *Candida albicans via* mimicking of the quorum sensing molecule farnesol. Microbial Biotechnology. 14: 1353-1366. DOI: 10.1111/1751-7915.13710.

Li, X., Wang, X., Shi, X., Wang, B., Li, M., Wang, Q. and Zhang, S. (2020). Antifungal effect of volatile organic compounds from *Bacillus velezensis* CT32 against *Verticillium dahliae* and Fusarium oxysporum. Processes. 8: 1674. DOI: 10.3390/pr8121674.

Meena, A., Meena, P. and Meena, R. (2018). Management of stem root of groundnut incited by *S. Rolfsii* through important bioagents. Chemical Science Review and Letters. 7: 1012-1017.

Okla, M.K., Alamri, S.A., Salem, M.Z., Ali, H.M., Behiry, S.I., Nasser, R.A., Soufan, W. *et al.* (2019). Yield, phytochemical constituents and antibacterial activity of essential oils from the leaves/twigs, branches, branch wood and branch bark of Sour Orange (*Citrus aurantium* L.). Processes. 7: 363. DOI: 10.3390/pr7060363.

Ponnusamykonar, P., Venkitasamy, K. and Varatharajan, P. (2011). *In vitro* study of antagonistic effect of *Trichoderma* spp., on tea plant pathogen, *Phomopsis theae*. Archives of Applied Science Research. 3: 352-358.

Prasad, Basha, S.T., Peddanarappa, N. and Reddy, G.E. (2010). Molecular variability among the isolates of *Sclerotium rolfsii* causing stem rot of groundnut by RAPD, ITS-PCR and RFLP. Eur Asian Journal of BioSciences. 4: 80-87.

Rifai, M.A. (1969). A revision of the genus *Trichoderma*. Mycological Papers. 116: 1-56.

Ringertz, S. and Ringertz, O. (1982). Antimicrobial effect of isopropyl myristate when used as a solvent in sterility testing. Pharmaceutica Acta Helvetiae. 57: 193-195.

Shahid, D.M. (2013). Molecular characterization of *Trichoderma longibrachiatum* 21PP isolated from rhizospheric soil based on universal ITS primers. African Journal of Microbiology Research. 7: 4902-4906. DOI: 10.5897/AJ MR2013.5761.

Siddiquee, S., Cheong, B.E., Taslima, K., Kausar, H. and Hasan, M.M. (2012). Separation and identification of volatile compounds from liquid cultures of *Trichoderma harzianum* by GC-MS using three different capillary columns. Journal of Chromatographic Science. 50: 358-367. DOI: 10.1093/chromsci/bms012.

Sivakumar, T., Sanjeevkumar, K. and Balabaskar, P. (2016). Variability in *Sclerotium rolfsii* Sacc. causing Stem rot of groundnut. Bull. Env. Pharmacol. Life Sci. [Spl. Issue 2]. 92: 99. DOI: 05.2016-13212626.

Walters, D., Raynor, L., Mitchell, A., Walker, R. and Walker, K. (2004). Antifungal activities of four fatty acids against plant pathogenic fungi. Mycopathologia. 157: 87-90. DOI: 10.1023/B:MYCO.0000012222.68156.2c.

White, T.J., Bruns, T., Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: A Guide to Methods and Applications. 18: 315-322.

Wijekoon, M.J.O., Bhat, R., Karim, A.A. and Fazilah, A. (2013). Chemical composition and antimicrobial activity of essential oil and solvent extracts of torch ginger inflorescence (*Etlingera elatior* Jack.). International Journal of Food Properties. 16: 1200-1210. DOI: 10.1080/10942912. 201 1.579674.

Yuef, M., Inez-Padr, Hadassa, O., Ariel, T.C. J., Ra, Ul, R., Andez. (2018). Identification and evaluation of secondary metabolites by gas chromatography-mass spectrometry (GC-MS) in native strains of *Trichoderma* species. African journal of Biotechnology. 17: 1162-1171. DOI: 10.5897/ AJB2018.16546.