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Native *Trichoderma* spp for the Management of Stem Rot of Groundnut Caused by *Sclerotium rolfsii* Sacc in Manipur, India

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A B S T R A C T

A total of seven isolates of two different *Trichoderma* spp. were isolated from the rhizosphere of groundnut from different locations of Manipur. *In vitro* study on the effect of *Trichoderma* isolates on the growth of *Sclerotium rolfsii* ranges from71.85 - 61.11per cent. Effect of volatile compound produced by *Trichoderma* spp. against *S. rolfsii* showed inhibition ranged from20.00 - 30.00 per cent and for non-volatile compound 11.47- 30.7 per cent at 7.5 % v/v concentrations and 20.77- 47.78 per cent at 15 % v/v. Experiment on ecological adaptability of two potent *T. viride* showed variation in the colonization of sclerotia under three different soil conditions. The rhizosphere competence of two potent isolates of *T. viride viz*., TvG1 andTvG2 at different days after sowing showed variation in the levels of colonization under three different soil conditions. *In vivo* study on efficacy of two potent isolates showed reduction in stem rot of groundnut among the different treatments (seed, soil and seed plus soil). The treatment with seed + soil of *Trichoderma* isolates TvG1 and TvG2 recorded the highest plant height, plant canopy, fresh weight, dry weight and yield, but no significant difference in chlorophyll content among treatments.

Introduction

Groundnut (*Arachis hypogeae* L.) is an important oilseed crop of tropical and sub-tropical region of the world. It is native of South America and belongs to annual legume group. Groundnut kernel is rich source of energy because of its oil (44-48%) and protein content (25-36%) than meat, about two and a half times than in eggs, and far more than any other vegetable food except soybean and yeast. In India it is grown mainly in Gujarat, Maharashtra, Andhra Pradesh, Uttar Pradesh, Tamil Nadu, Rajasthan, Karnataka and Madhya Pradesh. The crop is known to be attacked by number of fungal, bacterial and viral diseases.

The literature reveals that the yield losses caused by major fungal disease like leaf spot, rust and soil borne diseases like stem rot, root rot, collar rot and pod rot singly or in combination as high as 15-70% during both kharif and rabi-summer season (Subramanyam *et al.*, 1984).The stem rot of groundnut is caused by pathogen (*Sclerotium rolfsii* Sacc.) is a soil inhibitant, polyphagous, facultative fungal parasite. It is documented that the fungal has host range of 500 plant species including several cultivated field and vegetables crops and many others (Aycock 1966).The fungus survived in the soil for many years by producing sclerotial bodies

and causing the disease either in the form of stem rot or root rot in addition to blight on several of its host (Garret, 1956).

Trichoderma, a saprophytic fungus is known to be one of the best candidates of biocontrol agents for the management of soil borne plant pathogens. The antagonistic action of *Trichoderma* species against phytopathogenic fungi might be due to either by secretion of extra cellular hydrolytic enzyme (Di Pietro *et al.,* 1993; Schirmbock *et al.,* 1994) or by the production of antibiotics (Dennis and Webster, 1971a, b).

Manipur is likely to harbour useful *Trichoderma* isolates. So far not much previous research has been done on the use of native microbial agents for the management of stem rot disease of groundnut in this region.

Therefore, the present study was conducted to understand on the occurrence of stem rot of groundnut caused by *Sclerotium rolfsii* Sacc. and its management by native *Trichoderma* spp in Manipur.

Materials and Methods

Fungal identification and pathogenicity test

Collection of the disease samples and isolation of causal pathogen involved

Diseased plants were collected from the field and the samples were cut into small pieces of 1mm size, then surface sterilized with 0.1% sodium hypochlorite solution for 1 minute and rinsed with distilled water. The sterilized pieces were then inoculated on PDA slants. The inoculated slants were incubated at 28± 1^0C and fungal growth appearing on the sample was identified in the laboratory and culture was maintained on PDA slants for further use.

Pathogenicity test

Pathogenicity test of the isolated fungus was conducted. Soil inoculation with pathogen was done by means of rice seed inoculums technique of Weideman and Wehner (1993).

Biocontrol of *Sclerotium rolfsii* Sacc. by native *Trichoderma* spp.

Collection of soil sample from rhizosphere of groundnut

The soil samples were collected from the rhizosphere of groundnut plant of Manipur at a depth ranged in 5–6 cm by removing 2 cm surface soil.

The soil was air dried under shade and kept sealed in cloth bag inside refrigerator at 4°C for subsequent use whenever required.

Isolation of the antagonists

Different *Trichoderma* spp. were isolated from different areas by soil dilution plate technique (Dhingra and Sinclair, 1995) using *Trichoderma* specific medium (TSM) (Elad and Chet, 1983) modified by Saha and Pan (1997).

All the identified species of *Trichoderma* were maintained in potato dextrose agar (PDA) slants and preserved inside the refrigerator at 4°C for subsequent use.

In vitro antagonistic potential of some isolates of *Trichoderma*

In vitro antagonistic potential of isolates of *Trichoderma* spp. were evaluated against *Sclerotium rolfsii* Sacc. Through dual culture technique (Morton and Stroube, 1955), production of volatile (Dennis and Webster, 1971b) and non-volatile antibiotics (Dennis and Webster, 1971a).

Ecological fitness of *Trichoderma* isolates

Ecological fitness of *Trichoderma* spp. was carried out in laboratory and pot experiment using the soil of Manipur. The ecological studies included the competitive parasitic ability and rhizosphere colonization by isolates of *Trichoderma*. Three soil types, *viz.*, unsterilized, sun dried and sterilized soil were used by following the methods of Papavizas (1982).

In vivo Efficacy of *Trichoderma* isolates

Field experiment on efficacy of *Trichoderma* isolates against stem rot of groundnut was conducted at Sangaiprou Maning Leikai, Imphal. Field trails were taken up in randomized block design (RBD) with three replications with plot sizes of 1.5 m x 3m.

Each plot was treated with 200gm of pathogen mixture before planting of groundnut variety ICGS- 76 and untreated plots were served as control. The two potent isolates *viz.,* TvG1 and TvG2 were used with different treatment combination as seed, soil treatment and combination of two (seed and soil) and observation on yield parameters including chlorophyll contents (by SPAD meter at 30, 60 and 90 DAP) were recorded both pot and field experiments.

Results and Discussion

Fungal identification and pathogenicity test

Diseased samples collected from farmer's field during the survey were brought to laboratory and isolation was done and with repeated isolations, *Sclerotium rolfsii* was consistently found with the infected plant of groundnut. *S. rolfsii* cultures isolated from the infected groundnut were identified based on the types of fungal mycelium and mustard shaped of sclerotia as compared with the old cultures available in the laboratory, Department of Plant Pathology, COA, CAU, Imphal. Pathogenicity test of *S. rolfsii* was carried out as described in materials and methods.

Biocontrol of *Sclerotium rolfsii* Sacc.by native *Trichoderma* spp.

Isolation and identification of *Trichoderma* spp.

Soils collected from rhizosphere of different groundnut growing areas of Manipur were tested and *Trichoderma* isolates were identified based on morphology and taxonomic keys mentioned by Rifia (1969) and altogether seven *Trichoderma* isolates were isolated from different groundnut growing areas and among these, two were *Trichoderma viride* and five were *Trichoderma harzianum* and was reconfirmed from National Centre for Integrated Pest Management, New Delhi.

In vitro antagonistic potential of some isolates of *Trichoderma*

Mycelial growth inhibition

Mycelial growth inhibition of *Trichoderma* spp. against *S. rolfsii* Sacc. is presented in table 1. Among the isolates of *Trichoderma* spp. tested, maximum percentage inhibition was recorded with isolate TvG1 (71.85 per cent) and minimum was observed with the isolate ThrG4 (61.11 per cent).

Effect of volatile antibiotics

Effect of volatile compounds produced by *Trichoderma* spp. against *S.rolfsii*Sacc.is presented in table 1. Among the seven isolates of *Trichoderma* spp. tested, maximum percentage inhibition was recorded with isolate ThrG1 (30.00 per cent) and minimum was recorded with isolate ThrG7 (20.00 per cent).

Effect of non-volatile antibiotics

Effect of non-volatile compounds produced by *Trichoderma* spp. at two different concentrations *viz*., 7.5 % (v/v) and 15 % (v/v) are presented in table 1. Results showed that per cent inhibition of radial growth of *S. rolfsii* Sacc. by seven isolates of *Trichoderma* spp. ranged from 11.47 - 30.37 percent at 7.5 % v/v concentration and from 20.77 - 47.78 percent at 15 % v/v.

Competitive parasitic ability of potent *Trichoderma* spp. against *S. rolfsii*

Competitive parasitic ability of the two potential isolates of *Trichoderma* against *S. rolfsii* is presented in table 2. It is revealed from table that the colonization percentage of sclerotia of *S. rolfsii* by TvG1 and TvG2 in unsterilized soil was 86.67 percent and 73.33 percent, while in sundried soil colonization percentage were 73.33 percent and 66.67 percent.

The degrees of colonization in sterilized soil were 100 percent by the isolates.

In vivo efficacy of *Trichoderma* isolates

In vivo efficacy of two potent isolates *viz*., TvG1 and TvG2 *of Trichoderma* were evaluated against *S. rolfsii* under field and pot conditions with different treatments viz., seed treatment, soil treatment and combination of seed and soil.

Table.1 Effect of different *Trichoderma* isolates on growth of *S. rolfsii*

Sl No	*Trichoderma* spp.	Inhibition (%)	Percent inhibition over control		
			Volatile compounds	Nonvolatile compounds	
				(7.5%)	(15%)
1	TvG1	71.85	30.00(5.57)*	30.37(33.43)*	47.78(43.74)
2	TvG2	71.11	27.78(5.27)	20.47(26.90)	27.41(31.58)
3	ThrG3	64.07	23.33(4.82)	12.96(20.75)	20.97(27.25)
4	ThrG4	61.11	22.22(4.71)	13.33(21.38)	22.22(28.11)
5	ThrG5	97.78	27.04(5.20)	18.17(25.23)	27.04(31.35)
6	ThrG6	61.85	29.70(5.45)	11.47(19.79)	20.77(27.11)
7	ThrG7	61.48	20.00(4.47)	15.93(23.50)	20.93(27.22)
S.E(d)±		1.38	1.52	1.64	0.69
C.D.(5%)		3.01	3.30	3.58	1.50

*Mean of three replication
Values in parentheses are transformed values

Table.2 Competitive parasitic ability of *Trichoderma* spp. against sclerotia of *S. rolfsii*

Sl.No.	*Trichoderma* spp.	Colonisation (%)
1.	TvG1(UN)	86.67(9.32)
2.	TvG2(UN)	73.33(8.57)
3.	TvG1(SD)	73.33(8.57)
4.	TvG2(SD)	66.67(8.18)
5	TvG1(S)	100.00(10.02)
6	TvG2(S)	100.00(10.02)
7	Control	0.00(0.71)
S.E(d)±		0.45
C.D. (5%)		0.97

*Mean of three replications, UN= Unsterilized soil, SD= Sundried soil and S= Sterilized soil
Values in parentheses are Square Root Transformed values

Table.3 Effect of *Trichoderma* isolates on yield and yield attributing characters under field condition

Sl no.	*Trichoderma*spp	Treatments	Germination percentage	Disease incidence (%)	Plant height(cm) (DAS)			Plant canopy(cm^2) (DAS)			Fresh weight (g)	Dry weight (g)	Yield (g/plot)
					30	60	90	30	60	90			
1	TvG1	Seed	73.89(8.60)	5.55(2.43)	23.13	35.43	41.43	586.00	1883.37	2997.53	253.33	50.67	1333.33
		Soil	84.44(9.18)	3.33(1.93)	26.73	38.03	42.03	716.40	1979.13	3724.60	271.67	54.33	1416.67
		S+S	82.78(9.10)	1.11(1.13)	29.07	40.80	46.80	759.07	2113.13	3754.40	313.33	62.67	1633.33
2	TvG2	Seed	80.00(8.94)	7.78(2.81)	23.87	38.60	45.33	608.93	2061.63	2796.73	196.67	39.33	1233.33
		Soil	73.89(8.60)	5.55(2.43)	27.00	40.33	48.60	620.93	2134.21	2997.53	216.67	43.33	1300.00
		S + S	80.00(8.95)	0.56(0.96)	27.00	43.13	50.13	640.33	2304.98	3671.20	220.00	44.00	1433.33
3	Control		72.78(8.53)	9.44(3.10)	26.00	45.33	49.45	504.67	1631.53	1967.47	126.67	25.33	770.00
S.E(d)±			0.19	2.07	NS	2.47	0.87	NS	43.64	70.76	12.32	2.46	158.70
C.D. (5%)			0.42	4.51		5.38	1.90		95.08	154.18	26.85	5.37	345.80

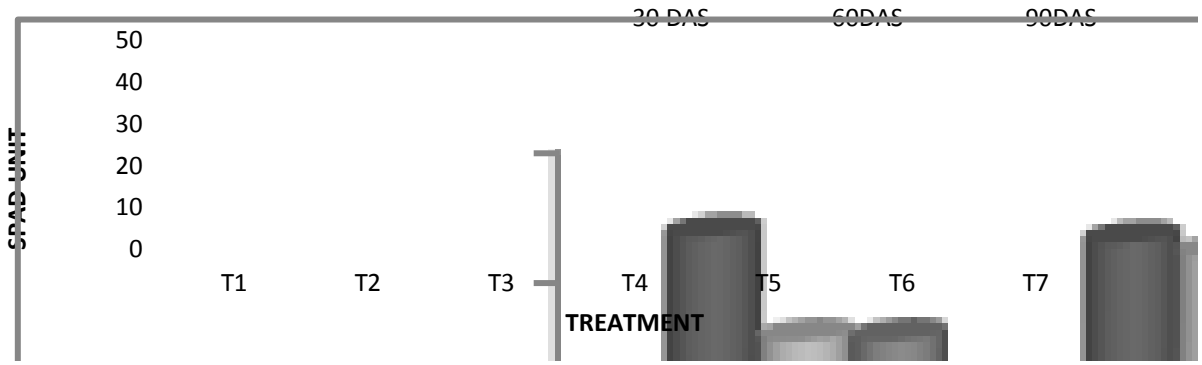
Values in parentheses are Transformed values, S+S= Seed + Soil

Table.4 Effect of *Trichoderma* isolates on yield and yield attributing characters under pot trails

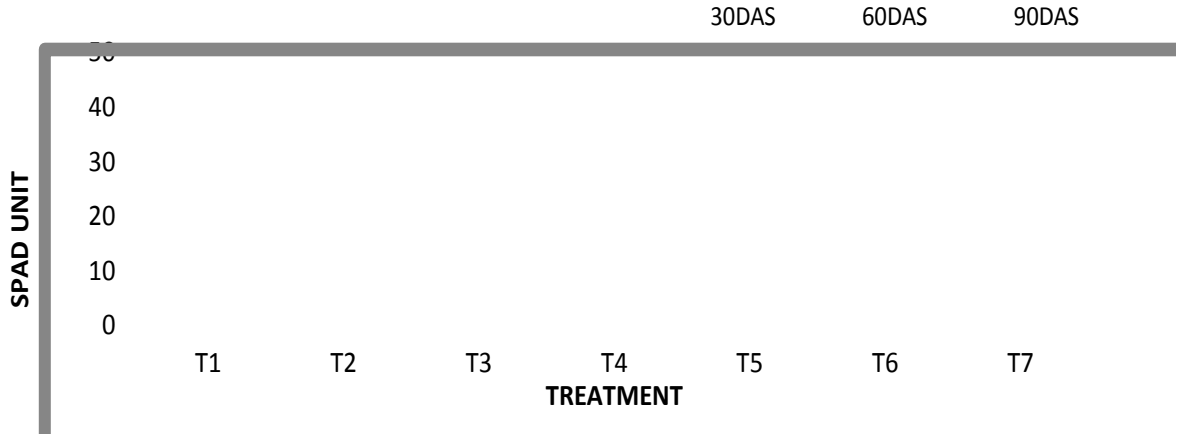
Sl no.	*Trichoderma*spp	Treatment	Germination percentage	Disease incidence (%)	Plant height(cm) (DAS)			Plant canopy(cm^2) (DAS)			Fresh weight (g)	Dry weight (g)	Yield(g/ pot)
					30	60	90	30	60	90			
1	TvG1	Seed	26.67(30.78)	2.00(1.41)	13.00	35.67	38.67	91.00	460.33	788.00	67.33	13.47	134.00
		Soil	26.67(30.78)	3.67(1.91)	13.00	38.99	41.33	176.33	492.33	896.67	73.33	14.80	155.00
		S+ S	60.00(50.77)	3.67(1.91)	14.33	40.00	44.67	181.33	630.00	937.67	74.00	14.67	162.67
2	TvG2	Seed	26.67(30.78)	3.00(1.71)	12.67	34.33	38.70	166.00	588.67	689.33	64.67	12.93	100.00
		Soil	26.67(30.78)	3.67(1.91)	13.33	36.67	38.82	211.33	623.33	860.67	67.00	13.40	136.00
		S + S	26.67(30.78)	3.67(1.91)	14.00	37.33	39.70	211.67	632.67	874.67	69.67	13.93	140.67
4	Control		20.00(26.58)	10.00(2.16)	13.00	31.33	35.37	152.67	290.33	417.00	34.00	6.80	96.67
S.E (d)±			5.66	0.31	NS	2.56	0.98	NS	71.01	80.95	4.86	1.00	9.07
C.D. (5%)			11.89	0.66		5.39	2.06		149.20	170.09	10.21	2.10	19.05

Values in parentheses are Transformed values. S+S= Seed + Soil

Graph.1 Effect of *Trichoderma* isolates on chlorophyll content (SPAD UNIT) of groundnut in field trial



Graph.2 Effect of *Trichoderma* isolates on chlorophyll content (SPAD UNIT) of groundnut in pot trial



Effect of *Trichoderma* isolates on the yield and yield attributing characters of groundnut under field conditions

Germination percentage

The data on germination percentage of groundnut under different treatments of *Trichoderma* spp are presented in table 3. The results showed that germination percentage in isolates TvG1 seed treatment, soil treatment and combination of seed and soil treatment were 73.89, 84.44and 82.78 percent respectively and in TvG2, it was 80.00, 73.89 and 80.00 percent respectively.

Disease incidence

Results on diseases incidence in isolates TvG1 seed, soil and combination of seed and soil treatments were 5.55, 3.33 and 1.11 per cent respectively and in TvG2 7.78, 5.55 and 0.56 per cent respectively (Table 3).

Chlorophyll content

Chlorophyll content of groundnut plants of different treatments are presented in graph 1 and it showed that the chlorophyll content in 30, 60 and 90 DAP were 42.07, 41.06 and 34.46, 34.14, 40.65 and 39.20 and 34.14, 40.65 and 39.30 (SPAD unit) in isolates TvG1

seed, soil and combination of seed and soil treatments respectively and in isolates TvG2, 40.12, 40.35 and41.37, 37.57, 37.93 and 36.45 and 37.57, 39.00 and 36.45 (SPAD unit) respectively.

Plant height

Plant height at 30, 60 and 90 DAP showed 23.13 - 29.07 cm, 35.43 - 40.80 cm and 41.43 - 46.80 cm respectively in isolate TvG1and in TvG2, 23.87 to 27.00 cm, 38.60 to 43.13 cm and 45.33 to 53.33 cm respectively in seed, soil and combination of seed and soil treatments, whereas untreated controlled plants height ranged from 26.00 -49.45cm (Table 3).

Plant canopy

Plant canopy at 30, 60 and 90DAP showed that in TvG1 seed, soil and seed plus soil treatments ranged from 586.00 - 759.07 cm^2, 1883.37 - 2113.13 cm^2 and 2997.53 - 3754.40 cm^2 respectively and in isolates TvG2 l it was 608.93 - 640.33 cm^2, 2061.63- 2304.98 cm^2 and 2796.73 - 3671.20 cm^2 respectively (Table 3).

Fresh weight, dry weight and yield of groundnut

The data on fresh weight of groundnut plants in isolate TvG1 treated plot showed highest 313.33g (seed + soil treated plot), followed by 271.67g (soil) and 253.33g (seed) treated plots. In isolate TvG2 treated plots, fresh weight showed moderately more or less similar among the treatments. The data on dry weight of groundnut plants in isolate TvG1 treated plot showed highest of 62.67g (seed + soil treated plot) followed by 54.33 g (soil), 50.67 g (seed) treated plots. Highest yield was obtained of 1630g/ plot to the TvG1 (seed + soil) treated plot (Table 3). The similar trends of the plot experiments were observed as filed experiments (Table 4). It is important to mention that *Trichoderma* spp. are known to produce a number of antibiotics such as Trichodermin, Trichodermol, Harzianum A, Hrazianolide (Kucuk and Kivanc, 2004) as well as some cell wall degrading enzymes such as chitinases, glucanases that break down polysaccharides, chitins and beta glucanase destroying cell wall (Elad, 2000). Noveriza and Quimio (2004) reported that *Trichoderma* spp. were able to cause 66.36% growth inhibition of *P. capsici* through dual culture technique and the pathogens like *R. solani*, *Pythium* sp., *S. rolfsii*, *Macrophamina phaseolina* and *Fusarium oxysporum f.* sp. *lycopersici* were also significantly inhibited by *Trichoderma* spp *in vitro* (Pan and Bhagat, 2008, Rajlakshmi and Bireswar 2014, Sandam *et al.,* 2016). Present investigation also found the different degrees of inhibition on radial growth of *Sclerotium rolfsii* by different volatile and non-volatile compounds produced by *Trichoderma* spp. Experiment on ecological adaptability of potent *Trichoderma* spp. showed variation in the colonization of sclerotia of *Sclerotium rolfsii* with highest colonization of 100 per cent. These findings are in accordance with Hennis *et al.,* (1983) who have shown that strains of *Trichoderma* spp. varied in their ability to colonize the sclerotia of *Sclerotium rolfsii* (Rashmi *et al.,* 2015). *Trichoderma* treated plants showed greater growth, vigour, chlorophyll content and yield than untreated pathogen inoculated control. Lo *et al.,* (2002) reported that the *Trichoderma* treated plant promote root growth of bitter gourd, loofah and cucumber. The experiment in greenhouse showed that the plant treated with *Trichoderma* spp significantly increased seedling height (26 to 61 %), root exploration (85 to 209 %) leaf area (27 to 38 %) and root dry weight (38 to 62 %) after 15 DAS of bitter gourd. Similarly these *Trichoderma* strains also increased the seedling growth of loofah and cucumber. Results of chlorophyll content of plants of

different treatments did not show any significant difference among themselves. However, Satyendra *et al.,* (2012) reported that the concentration of chlorophyll (mg/cm^2 of leaves) increased in plant treated with *Trichoderma*. Moradia *et al.,* (2011) reported that in groundnut, the pod yield was highest *i.e.* 1427 kg/ha in the treatment combination of seed treatment with vitavax + soil application of *Trichoderma viride* with neem cake where pod yield was increase by 35.1 per cent followed by seed and soil application of *Trichoderma viride* with neem or castor cake. Our present finding will give idea on the management of stem rot of groundnut by the application of native bio agent (*Trichoderma*) under filed conditions in Manipur.

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