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VARIABILITY OF NATIVE *TRICHODERMA* SPECIES ISOLATED FROM RHIZOSPHERE OF GROUNDNUT (*ARACHIS HYPOGEAE* L.) IN MANIPUR

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ABSTRACT
The present study was conducted to understand the variability of *Trichoderma* spp isolated from groundnut rhizosphere from six different groundnut growing areas of Manipur *viz*., Moirang, Chanung, Ningthoukhong, Andro, Churchandpur and Yambem during kharif 2013 and altogether seven isolates of two different *Trichoderma* spp. were isolated, among these, five were identified as *T.harzianum*, two were *T.viride*. The radial growth of *Trichoderma* isolates tested on three different media showed that PDA was found to be the best (8.42 cm). Maximum growth was obtained with isolate TvG2 in all the three media and lowest with TvG7. The growth pattern of *T. viride* was light green with fluffy growth and produced coconut like aroma in all the three media whereas growth of *T. harzianum* was fast to very fast without any significant aroma. Anamorphic characters *viz*., size of phialides, phialospore and conidiophores of all *Trichoderma* spp were ranges from 3.6–8.5X1.2-3.6μm, 1.5-5.3X1.2-2.8μm and 4.1-35.8X2.1-5.4μm respectively. The biopriming of groundnut seed showed increase in vigour index over control (TvG2- 454.00, TvG1-410.13, control - 222.80). The rhizosphere colonization of the two isolates of *Trichodrma* spp. were found significantly differences upto 30 days of sowing.

INTRODUCTION

Groudnut (*Arachis hypogeae* L.) is an important oilseed crop of tropical and sub-tropical region of the world. Its kernel is a rich source of energy because of its oil content (44-48%) and protein content (25-30%). 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 (Ghewande *et al*., 1983; Subramanyam *et al.,* 1984). The yield loss upto 75-80 per cent has been reported in New Mexico (Aycock, 1966). Yield losses usually ranged from 10 to 25% in India, Thailand, Indonesia, Taiwan, and the Philippines but may reach 80% in severely infested fields (Mayee and Datar 1988). Among soil borne diseases of groundnut, stem rot caused by *Sclerotium rolfsii* Sacc. is economically important one.

Trichoderma, a saprophytic fungus is known to be one of the best candidates of biocontrol agents for the management of soil borne plant pathogens. Mode of action of this fungus include mycoparasitism, antibiosis, competition for nutrient and space, tolerance to stress through enhanced root and plant development, solubilization and sequestration of inorganic nutrients and induced resistance. 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; Claydon *et al.,* 1987). Manipur is likely to harbour useful *Trichoderma* isolates. The present works has done to find the variability of *Trichoderma* species isolated from rhizosphere of Groundnut (*Arachis hypogeae* L.) in Manipur.

MATERIALS AND METHODS

Collection of soil sample

Altogether 20 soil samples were collected from rhizosphere of groundnut at depth of 5-6 cm from various locations of valley areas of Manipur for the presence of *Trichoderma* spp during 2012-13.

Isolation and identification of *Trichoderma* spp.

Soils collected from rhizosphere of different groundnut growing areas of Manipur were tested for the presence of *Trichoderma* isolates 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 4ºC for subsequent use.and they were identified based on morphologyand taxonomic keys mentioned by Rifia (1969). Altogether seven *Trichoderma* isolates were isolated from different groundnut growing areas and among these, two were *Trichoderma viride* and five were *Trichoderma harzianum* (Table 1) and was reconfirmed from National Centre

for Integrated Pest Management, New Delhi.

Cultural characteristics of *Trichoderma* spp.

The cultural characteristics of isolates of *Trichoderma* viz. colony, growth rate, presence or absence of pigments, hyphae and presence of any distinguishing odour were studied in three media, *viz.,* potato dextrose agar (PDA), *Trichoderma* specific media (TSM) and oat meal agar (OMA) and experiment was replicated three times.

Anamorphic characterization of *Trichoderma* isolates

Anamorphic characterization of *Trichoderma* isolates was done by growing in potato dextrose agar medium. Anamorphic characteristics, *viz*., conidiophores length and width, phialides length and width, phialospores length and width were recorded by observing under Binocular microscope (AHN, Germany) at 40 xs with the help of bio-wizard image analysis software with at least 50 observations of each.

Rhizosphere colonization

Studies on ecological fitness of *Trichoderma* spp. of rhizosphere colonization were done by three soil types, *viz.,*unsterilized, sun dried and sterilized soil following the methods of Papavizas (1982).

Seed priming with bioagents

The biopriming of seeds of groundnut was done with two potent isolates (TvG1 and TvG2) of *Trichoderma* spp. The vigour index of respective crop seedlings were calculated on the basis of root and shoot length as

Vigour index of seedlings = [Root length (cm) + shoot length (cm)] x germination (%)

RESULTS AND DISCUSSION

Growth and cultural characteristics of *Trichoderma* spp.

Growth of different *Trichoderma* isolates on three different culture media viz., potato dextrose agar (PDA), *Trichoderma* specific medium (TSM) and oat meal agar (OMA) showed that mean growth rate of seven isolates on PDA media ranged from 8.20 to 8.97 cm, on TSM ranged from 6.50 to 7.57 cm and on OMA ranged from 6.20 cm to 8.20 cm at 3 days after inoculation. The mean radial growth on PDA was found to be the highest (8.42 cm) followed by OMA (7.10 cm) and TSM (7.09 cm) (Table 2).

The cultural characteristics of seven isolates of *Trichoderma* of two different species viz., *T. viride* and *T. harzianum* three media *viz*., PDA, TSM and OMA are presented in table 3 and plate 1(a, b). It was observed that there was variation among the isolates on the cultural characters such as colony, growth rate, pigmentation and odor.

Anamorphic characterization of *Trichoderma* isolates

Anamorphic characteristics of *Trichoderma* isolates viz., conidiophores length and width, phialospores length and width were studied by growing them in potato dextrose agar medium and found that size of *T. viride* phialides ranged from 4.0-7.9x1.2-1.6 μm while phialospores ranged from 1.4-3.7x1.7-2.3 μm and conidiophores ranged from 4.7-28.9x2.4-4.7 μm.The size of *T. harzianum*phialides ranged from 3.6-8.4x2.2-3.7 μm while phialospores ranged from 1.8-3.3x1.3-2.8 μm and conidiophores ranged from 4.7-30.4x2.7-5.6 μm (Table 4).

Seed priming with bioagents

Biopriming of groundnut seeds with two potent isolates of *Trichoderma* spp. *viz*., TvG1 and TvG2 showed that highest root length was observed in isolate TvG2 (4.77 cm) followed by TvG1 (4.03 cm) whereas in control it was only 3.13 cm. The highest shoot length was observed in isolates TvG1 (1.5 cm) followed by TvG2 (1.29 cm) and in control it was (0.98 cm).The higher germination percentage of groundnut seeds was found same in both the *Trichoderma* isolate (73.33 percent) as compare to untreated one (53.33 per cent). The vigour index was highest in TvG2 (454.00) followed by TvG1 (410.13) and lowest (222.80) in untreated control (Table 5, plate 3). The biopriming of groundnut seeds were found to be significantly differences among the potent isolates of *Trichoderma* spp.

Rhizosphere colonization

The rhizosphere competence of two potent isolates of *Trichoderma* spp. *viz.,* TvG1 and TvG2 were conducted inside the net house with three different soil *viz*., unsterilized,sundried and sterilized soils found that in all the types of soil, colonization of two potential isolates of *Trichoderma*were found to be increased upto 30 days of sowing and then decreases. The rhizosphere colonization by isolate TvG1 at 15, 30, 45, 60, 75 and 90 days after sowing were 1.17x10^8cfu/g, 1.60 x10^8cfu/g, 1.27 x10^8cfu/g, 0.53 x10^8cfu/g, 0.07 x10^8cfu/g and 0.03 x10^8cfu/g in unsterilized soil,1.17 x10^8cfu/g, 1.57 x10^8cfu/g, 0.83 x10^8cfu/g, 0.17 x10^8cfu/g, 0.10 x10^8cfu/g and 0.07 x10^8cfu/g in sundried soil and 1.13 x10^8cfu/g, 1.80 x10^8cfu/g,0.77x10^8cfu/g, 0.07 x10^8cfu/g, 0.07 x10^8cfu/g and

Table 1: List of *Trichoderma* isolates from the rhizosphere of groundnut plants collected from different locations of Manipur

Sl. No.	Location	*Trichoderma* isolates
1.	Moirang	*Trichoderma viride* (TvG1)
2.	Chanung	*Trichoderma viride* (TvG2)
3.	Ningthoukhong	*Trichoderma harzianum* (ThrG3)
4.	Andro	*Trichoderma harzianum* (ThrG4) and *Trichoderma harzianum* (ThrG5)
5.	Pearsonmun	*Trichoderma harzianum* (ThrG6)
6.	Yambem	*Trichoderma harzianum* (ThrG7)

Table 2: Comparison on growth of *Trichoderma* isolates in different culture media*

Sl.no.	*Trichoderma* isolates	Radial growth on different media(cm)		
		PDA	OMA	TSM
1.	TvG1	8.20**	7.10	7.23
2.	TvG2	8.97	7.57	8.20
3.	ThrG3	8.47	7.10	6.20
4.	ThrG4	8.40	7.20	7.57
5.	ThrG5	8.20	7.13	6.90
6.	ThrG6	8.37	7.13	6.83
7.	ThrG7	8.33	6.50	6.70
Mean		8.42	7.10	7.09
S.E(d) ±		0.15	0.23	0.43
C.D.(5%)		0.33	0.50	0.94

* PDA - Potato dextrose agar, TSM - *Trichoderma* specific media, OMA- Oat meal agar
**Mean of three replications

Table 3: Cultural characteristics of different *Trichoderma* isolates

Sl. No.	*Trichoderma* spp.	Cultural characteristics	Medium PDA	OMA	TSM
1	TvG1	Colony	Concentric ring	Concentric ring	Concentric ring
		Growth rate	Fast	Medium	Fast
		Pigment	Dark green	Dark green	Dark green
		Hyphae	Hyaline	Hyaline	Hyaline
		Odour	No smell	No smell	No c smell
2	TvG2	Colony	Sparse growth	Sparse growth	Sparse growth
		Growth rate	Fast	Fast	Fast
		Pigment	White	White	White
		Hyphae	Hyaline	Hyaline	Hyaline
		Odour	Coconut smell	Coconut smell	Coconut smell
3	ThrG3	Colony	Concentric ring	Concentric ring	Concentric ring
		Growth rate	Fast	Fast	Fast
		Pigment	Light green	Lime green	Dark green
		Hyphae	Hyaline	Hyaline	Hyaline
		Odour	No smell	No smell	No smell
4	ThrG4	Colony	Dense	Doubled layer	Dense growth periphery
		Growth rate	Fast	Fast	Fast
		Pigment	Dark green	Dark green	Light green
		Hyphae	Hyaline	Hyaline	Hyaline
		Odour	No smell	No smell	No smell
5	ThrG5	Colony	Compact	Sparse growth	Irregular growth
		Growth rate	Fast	Medium	Medium
		Pigment	Dark green	Dark green	Dark green
		Hyphae	Hyaline	Hyaline	Hyaline
		Odour	No smell	No smell	No smell
6	ThrG6	Colony	Sparse growth	Sparse growth	Sparse growth
		Growth rate	Fast	Slow	Slow
		Pigment	Dark green	Light green	Dark green
		Hyphae	Hyaline	Hyaline	Hyaline
		Odour	No smell	No smell	No smell
7	ThrG7	Colony	Dense	Compact	Sparse growth
		Growth rate	Fast	Fast	Slow
		Pigment	Dark green	Dark green	Dark green
		Hyphae	Hyaline	Hyaline	Hyaline
		Odour	No smell	No smell	No smell

Table 4: Anamorphic characteristics of *Trichoderma* isolates

Sl.no.	*Trichoderma* spp.	Phialides(μm)	Phialospores(μm)	Conidiophores(μm)
1.	TvG1	4.0-7.7x 1.2-1.6*	1.4-2.4.1x1.8-2.3	4.7-28.9x3.1-4.2
2.	TvG2	4.0-7.9x1.3-1.6	2.1-3.7x1.7-2.1	5.3-27.1x2.4-4.7
3.	ThrG3	5.3-8.4x2.7-3.5	2.0-3.1x1.6-2.4	5.9-30.6x3.2-4.6
4.	ThrG4	4.2-7.6x2.2-3.4	2.0-2.9x1.4-2.8	5.2-26.8x3.1-4.7
5.	ThrG5	4.0-7.9x2.3-3.7	2.2-3.3x1.3-2.1	5.3-32.4x2.7-3.6
6.	ThrG6	3.6-8x1.3-1.6	1.9-2.4x1.7-2.5	4.7-28.1x3.0-4.4
7.	ThrG7	3.9-8.1x2.4-3.6	1.8-2.7x1.5-2.4	4.8-29.5x4.3-5.6

*Mean of 50 replication

0.03 x10^8cfu/g respectively in sterilized soil. The colonization of isolate TvG2 were 1.17 x10^8cfu/g, 1.53 x10^8cfu/g, 1.17 x10^8cfu/g, 0.23 x10^8cfu/g, 0.13 x10^8cfu/g and 0.03 x10^8cfu/g respectively in unsterilized soil, 0.70 x10^8cfu/g,0.70 x10^8cfu/g, 0.73 x10^8cfu/g, 0.30 x10^8cfu/g, 0.10 x10^8cfu/g and 0.03 x10^8cfu/g respectively in sundried soil and 0.57 x10^8cfu/g, 1.37 x10^8cfu/g, 0.60 x10^8cfu/g, 0.13 x10^8cfu/g, 0.33 x10^8cfu/g respectively in sterilized soil and no colonization was found in 90 days of sowing in sterilized soil. However no colonization was observed in untreated control (Table 6). The rhizosphere colonization of the two isolates of *Trichodrma* spp. were found significantly differences upto 30 days of sowing and did not show any significant differences at 45, 60, 75 and 90 days of sowing.

The present research works found that association of two different *Trichoderma* spp. from different rhizosphere of groundnut, five were *T. harzianum* and two were *T. viride*. It is well known the ecological preferences of *Trichoderma* (Papavizas, 1985), the prolonged dry condition of soil reduced the population of *Trichoderma* and *Gliocladium* as a group (Danielson and Davey, 1973) and also certain strains of *T. hamatum* adapted to excessive soil moisture. *T. viride* was restricted to areas where low temperature prevails, whereas *T. harzianum* is most commonly found in warm climatic

Table 5: Biopriming of groundnut seed with two potent Trichoderma isolates

Sl.no.	*Trichoderma* spp.	Root Length(cm)Mean ± S.D.	Shoot Length(cm)Mean ± S.D.	Germination Percentage	Vigour index
1	TvG1	4.03 ± 1.1*	1.5 ± 0.26	73.33	410.1
2	TvG2	4.77 ± 0.79	1.29 ± 0.22	73.33	454
3	Control	3.13 ± .69	0.98 ± 0.22	53.33	222.8

*Mean of three replication

Table 6: Rhizosphere colonization of two potent *Trichoderma* isolates

Sl.no.	*Trichoderma* spp.	Population of *Trichoderma* isolates at different days after sowing (DAS)(x 10^8 cfu/g soil)					
		15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
1	TvG1(UN)	1.17*(1.28)	1.60(1.45)	1.27(1.32)	0.53(1.02)	0.07(0.76)	0.03(0.76)
2	TvG2(UN)	1.17(1.29)	1.53(1.41)	1.17(1.28)	0.23(0.85)	0.13(0.79)	0.03(0.73)
3	TvG1(SD)	1.17(1.28)	1.57(1.43)	0.83(1.15)	0.17(0.83)	0.10(0.77)	0.07(0.75)
4	TvG2(SD)	0.70(1.10)	0.70(1.10)	0.73(1.10)	0.30(0.89)	0.10(0.78)	0.03(0.73)
5	TvG1(S)	1.13(1.27)	1.80(1.51)	0.77(1.12)	0.07(0.76)	0.07(0.76)	0.03(0.73)
6	TvG2(S)	0.57(1.03)	1.37(1.34)	0.60(1.02)	0.13(0.76)	0.33(0.73)	0.00(0.71)
7	Control	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)
S.E(d) ±		0.06	0.13	0.08	0.14	0.04	0.03
C.D.(5%)		0.13	0.28	0.26	NS	NS	NS

*Mean of three replication; UN = Unsterilized soil, SD = Sundried soil and S = Sterilized soil; Values in parentheses are square root transformed values

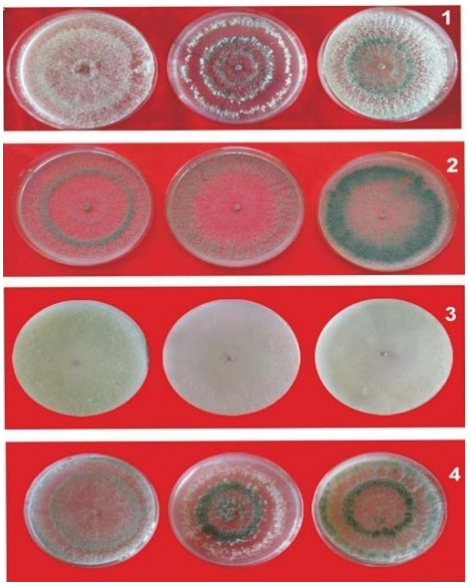

Plate1(a): Cultural characteristics of *Trichoderma* on PDA,OMA and TSM (1. Growth of ThrG4 on PDA,OMA and TSM, 2. Growth of ThrG5 on PDA,OMA and TSM, 3. Growth of TvG2 on PDA,OMA and TSM , 4. Growth of TvG1 on PDA,OMA and TSM).

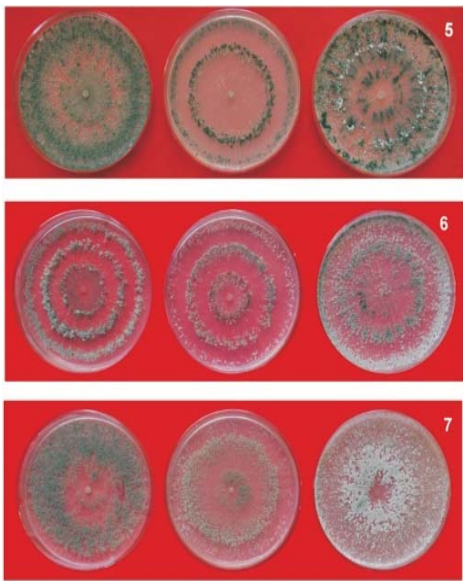

Plate 1(b): Cultural characteristics of *Trichoderma* on PDA,OMA and TSM (5. Growth of ThrG3 on PDA,OMA and TSM, 6. Growth of ThrG6 on PDA,OMA and TSM, 7. Growth of ThrG7 on PDA,OMA and TSM).

condition. Similar observations were found by Noveriza and Quimio (2004) in Indonesia where the presence of organic matter in soil largely determined the presence of promising antagonistic fungi. In Indonesia Quimio (2001) successfully isolated the *Trichoderm* spp. including *T. viride, T. hamatum* and *T.harzianum*. The possible reason of presence of of *T.harzianum* in comparatively less warm climate (Manipur) may be due to the diverse climatic conditions with high rainfall and comparatively high organic matter content than normal soil with high soil microbial diversity and complex interaction among the soil micro-organism.

The growth of *T. viride* was light greenish with fluffy growth and concentric ring with dense growth at the margin of the colony and one isolate produced coconut like aroma in all the three media. The growth of *T. harzianum* was fast to very fast with whitish greenish to green color, concentric ring, and hyaline hyphae without any significant aroma. Identification of the *Trichoderma* spp. based on cultural characters is similar with the findings of Bissett (1991a-c) who characterized that *T. harzianum* as fast growing colonies, white to greyish or sometimes yellowish exudates colourless to amber or greenish yellow, odour indistinct or faintly earthy and hyphae hyaline. Similarly, he also described that *T. viride* as rapidly growing fungus, aerial mycelium usually limited, side of the growth was colourless to dull yellowish, some isolates with distinctive aromatic odour resembling coconut like aroma (á - pyrone)

Plate 2: Biopriming of groundnut seedling with *Trichoderma* isolates (1.TvG1, 2.TvG2 and 3.Control).

and *T. hamatum* was characterized as moderately rapid growing fungus, white to grayish mycelium, indistinct odour, hyaline hyphae. Similar observations were made by Rifai, 1969; Samuel, 1996; Pan and Bhagat, 2008, Rajlakshmi and Bireswar, 2015) where they comprehensively reported that *T. harzianum* and *T. viride* were fast growing green coloured mycoparasitic fungi with distinct coconut or faintly earthy aroma.

Results of the present findings showed variation in the anamorphic characters viz., size of phialides, phialospore and conidiophores among the isolates and also species level studied in the present investigation. In general identification of *Trichoderma* spp at species level depends on cultural and anamorphic characteristics which are the major determinants. It is not an easy task to identify different *Trichoderma* spp. at species level. These findings are duly supported by earlier observations (Rifai, 1969; Domsch *et al.,* 1980, Bissett, 1991a-c; Samuel, 1996; Samuel, 2006) where they characterized different species of *Trichoderma*. They have also reported that *T. viride* and its related species are able to secrete á – pyrone, a sweet coconut like aroma.

The biopriming of seeds of groundnut was done by potent isolate of *Trichoderma* spp. *viz*., TvG1 and TvG2 which showed considerable increase vigour index over control. Seed treatment with bioagents for protection of seeds and control of seed borne diseases offers the growers/farmers an alternative means of chemical fungicides. The biological seed treatment can be highly effective, it must be recognized that they differ from chemical seed treatment by their utilization of living microorganisms. Storage and application conditions are more critical than with chemical seed protectants and differential reaction to host and environmental conditions may cause biological seed treatment to have a narrower spectrum of use than some chemicals. Conversely, some biocontrol agents applied as seed treatment are capable of colonizing the rhizosphere, potentially providing benefits to the plant beyond the emergence stage of the seedlings (Callan *et al.,* 1997). Effective biological control agents have been developed for control of seed and seedling pathogens such as *Pythium* spp., *S. rolfsii*, *M. phaseolina* and *Fusarium* spp. Several researchers have reported the biological seed treatments for protection of seed and control of pathogens causing seedling diseases and also disease caused by *R. solani* (Harman *et al*., 1991; Bennett *et al.,* 1992; Chet and Inbar, 1994; Bireswar and Padamini, 2015). The present findings will give information about the occurrence, availability and distribution of the *Trichoderma* in the groundnut growing areas of Manipur. In the future the isolated potent isolates of Trichoderma can be used for the management of various soil borne groundnut diseases.

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