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Study on Efficacy of Native *Trichoderma* spp. against Anthracnose of Soybean in Manipur, India

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Abstract: In order to determine the efficacy of native *Trichoderma* spp. against *Colletotrichum truncatum* causing anthracnose of soybean, *in vitro* and *in vivo* experiment were conducted during *kharif* season 2015-16 in Central Agricultural University, Imphal, Manipur. The pathogen was isolated and identified followed by pathogencity test. Effect of volatile compound produced by *Trichoderma* spp. was studied against the isolated pathogen. Two isolates of *T. harzianum* (KU933468) and *T. harzianum* (KU933474) recorded highest inhibition of 31.76% against *C. truncatum.* Effect of non-volatile compound produced by *Trichoderma* spp. was also studied on the pathogen at 7.5% *v/v* concentration and 15% v/v concentration. *T. harzianum* (KU933468) at 7.5% and 15.5% *v/v* concentration showed highest inhibition of 32.54% and 55.75% respectively. *In vivo* study of isolates of Trichoderma spp. under pot trials as seed treatment (@ 5g/kg seed) with foliar spraying (@ 5g/l of water) at 40 days after planting showed considerable reduction in disease incidence, increased production over control plot. The isolate *T. harzianum* (KU933468) showed highest yield under pot trial (29.70 g/pot).

Key words: Soybean, anthracnose, *Colletotrichum truncatum*, *Trichoderma* spp., Manipur

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I. INTRODUCTION

Soybean (*Glycine max* L.*)* belongs to a family leguminaceae, native to South East Asia. Soybean also called, 'The golden bean' is one of the fore most important oilseed crops in the world for its excellent protein (42-45%), oil (22%) and starch content (21%). It is a good source of vitamin-B complex, thiamine and riboflavin. Soybean protein is rich in valuable amino acids like Lysine (5%) in which, most of the cereals are deficient. Its oil is the largest component of the world's protein. It grows well in warm and moist climate and water logging is injurious to the crops (Sarabhoy and Agarwal, 1983).

Anthracnose caused by *Colletotrichum truncatum* is one of the most important seed-borne fungal pathogen of soybean (Sinclair and Backman, 1989). In India, yield losses of 16-100 per cent have been reported due to this disease (Sinclair, 1992; Anonymous, 1999). Higher physiological seed quality ensures healthy seedlings establishment under wider range of environmental conditions (Copeland and Mc Donald, 2001). Disease free quality seeds production in Soybean is utmost important to sustain the productivity and maintain the quality of the crop. Severe seed infection by *C. truncatum* may be able to inflict considerable damage to the seeds after harvest, consequently posing a serious problem to the economy in the world trade (Begum *et al. 2008). Trichoderma*, a filamentous soil borne saprophytic fungus is known to be one of the best candidate of biological agents for the management of 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; Claydon *et al.*1987). The growing interest in non-chemical methods of pest and disease management are solely due to environmental and health hazards.The use of *Trichoderma* based product is not only safe for the farmers and consumers but it is also good for the environment.

II. MATERIALS AND METHODS

Fungal identification

Disease samples were cut into small pieces of 2-3 mm size. The pieces were surface sterilized with 0.1% sodium hypochlorite solution for 1 minute and then rinsed three times with sterile distilled water. The sterilized pieces were then inoculated on potato dextrose agar (PDA) and incubated at 25 ± 2^0C. The isolated fungus was purified and identified as *C. truncatum* on the basis of morphological characteristics like shape and size of conidia and setae available in the literature Jagtap and Sontakke, (2009) in the laboratory, Department of Plant Pathology, College of Agriculture, CAU, Imphal. Pure culture was maintained on freshly prepared PDA slants inside refrigerator and periodically sub cultured to fresh medium during the investigation.

Pathogenecity test

The pathogenicity test of the isolated fungi was conducted by following the Koch's postulate. Soybean seedlings were raised in sterilized soil under pot condition. Spore suspension of the test pathogen was prepared by harvesting freshly sporulating 7-8 days old cultures in plates by flooding with 5-10 ml sterile distilled water. The resultant spore-cum-mycelial suspension was filtered through double layered muslin cloth and filtrate obtained was suitably diluted with sterile distilled water to get inoculums concentration of 3-5 x 10^6 spores/ml. Leaves of thirty days old seedlings of soybean (JS-335) were neatly pin-pricked and was artificially inoculated with the prepared suspension. High humidity (>80%) and optimum temperature were maintained by covering the tested pots with polyethylene bags for further development of anthracnose symptoms. Fungus from artificially induced diseased plants showing symptoms was re-isolated to confirm the pathogenecity of the causal organism in *in vitro*. It was similar with the findings of Jameel and Abdul, (2008) who isolated *C. truncatum* from plants and its pathogenecity was tested and proved individually. Initial symptoms of the disease in the form of yellowing and wilting of leaves were visible at 15-20 days after planting. From the diseased plants, re-isolation of the pathogens was made which yielded the fungus, *C. truncatum* identical with the original one that was inoculated.

***In vitro* antagonistic potential of *Trichoderma* isolates**

Some potent isolates of *Trichoderma* spp. *viz., T. ovalisporum* (KU904456), *T. harzianum* (KU933468), *T. atroviride* (KU933472), *T. harzianum* (KU933474), *T. asperellum* (KU933475), *Hypocrea lixii* (KX0113223) used in this study were collected from Department of Plant Pathology, COA, CAU, Imphal and were evaluated against the tested pathogen through dual culture technique, production of non-volatile and volatile compounds by Dennis and Webster, (1971a, 1971b). The radial mycelial growth of test pathogen was recorded daily and compared with control plates. The radial mycelial growth of test pathogen and antagonist were measured periodically and the per cent inhibition of mycelial growth of test pathogen by antagonists was calculated as per formulae adopted by Garcia (1991) as:.

Per cent Inhibition of Radial Growth (% IRG) = 100 [(R1-R2) / R1], where,

R1 - the farthest radial distance grown by the pathogen in the direction of the antagonist.

R2 - the distance grown on a line between inoculation positions of the pathogen and antagonist.

***In vivo* efficacy of *Trichoderma* isolates**

Six potent isolates of *Trichoderma* were evaluated against the tested pathogen under pot condition at College of Agriculture, Central Agricultural University, Imphal during 2015-16. Trials were taken up in randomized block design (RBD) with five replications. Soybean variety JS-335 (Jawahar Soybean-335) was used as test plants. Untreated plots served as control. Disease incidence was calculated at 15 days interval at 30, 60 and 90 days after planting. Observation was recorded on germination percentage, canopy, number of pods per plant, numbers of seeds per pod and yield. Six potent isolates of *Trichoderma* spp. and one chemical fungicide were used.

III. RESULTS

***In vitro* antagonistic potential of some isolates of *Trichoderma* spp.**

Effect of volatile compounds

Among the six isolates of *Trichoderma* spp. tested against *C. truncatum*, maximum percentage inhibition of 31.76% was recorded by *T. harzianum* (KU933468) and minimum percentage inhibition of 17.25% was recorded by *T. asperellum* (KU933475) as given in Table 1. The inhibition percentage shown by other isolates *viz., T. harzianum* (KU933474). *T. ovalisporum* (KU904456), *Hypocrea lixii* (KX0113223) and *T. atroviride* (KU933472) were 29.80%, 24.70%, 18.82% and 18.43% respectively. The present findings are in accordance with the findings of Kale and Barhate (2016) who reported that *T. harzianum and T. viride* significantly inhibited the growth of *C. truncatum*, of soybean *in vitro*. Begum *et al.* (2008) also reported that two fungal biocontrol agents namely *Trichoderma virens* isolate UPM23, *T. harzianum* isolate UPM40 and a bacterial biocontrol agent namely *Pseudomonas aerugina* isolate UPM13B8 strongly inhibited the growth of *C. truncatum* on dual culture and culture filtrate tests.

Effect of non-volatile compunds

The effect of non-volatile compounds produced by *Trichoderma* spp. at two different concentrations *viz.,* 7.5% (v/v) and 15% (v/v) were studied against *C. truncatum*. Results showed that per cent inhibition of radial growth of *C. truncatum* by six isolates of *Trichoderma* spp. ranged from 15.68% to 32.54% at 7.5% v/v concentration and from 29.41% to 53.72% at 15% v/v. The highest percent inhibition of 32.54% and 53.72% at 7.5% v/v and 15% v/v concentration were recorded by the isolate *T. harzianum* (KU933468) and *T. harzianum* (KU933474) respectively.

Effect of *Trichoderma* isolates on the yield and yield attributing characters of soybean under pot trials
Germination percentage

Results as given in Table 2. showed that the highest germination percentage of 92.00% was found in Carbendazim-50WP treated seeds, followed by *Trichoderma* isolates treated pot *viz., T. harzianum* - KU933468 (88%), *T. harzianum* - KU933474 (84%), *Hypocrea lixii* - KX0113223 (84%), *T. asperellum* - KU933475 (76%), *T. atroviride* - KU933472 (76%) and *T. ovalisporum* - KU904456 (72%) respectively. In untreated pot, the germination percentage was 60%.

Disease incidence

It is evident from Table 2. that percentage of disease incidence varied among the treatment at different dates of observations. At 30 days after planting, disease incidence was found to be non-significant. Pod blight incidence recorded at 45, 60, 75 and 90DAP was in the range from 2.22-6.44%, 5.11-14.44%, 10.00-23.11%, 17.55-33.77% at respectively. Average disease incidence was taken and the best treatment was found to be Carbendazim-50WP treated pots which recorded 9.14% of pod blight followed by *Trichoderma* isolates treated pots *viz., T. harzianum* - KU933468 (7.33%), *T. harzianum* - KU933474 (7.60%), *Hypocrea lixii* - KX0113223 (8.50%), *T. atroviride* - KU933472 (8.75%), *T. asperellum* - KU933475 (9.29%), *T. ovalisporum* - KU904456 (9.69%). However, in untreated control pot, the average disease incidence was found to be 14.31%.

Plant canopy

Plant canopy of each treatment of five randomly selected and tagged plants were measured at 30, 60 and 90 days after planting from East-West and North-South direction and result were presented in table 2. It is evident from the table that canopy area varied among the treatments. Results showed that canopy area of plants treated with Carbendazim-50WP was higher than those treated with *Trichoderma* isolates. Canopy area ranged from 23.72-42.02cm^2, 95.01-159.09cm^2, 169.32-343.10cm^2 at 30 DAP, 60 DAP and 90 DAP respectively.

Number of pods per plant

Effect of different treatment on the yield parameters of soybean are presented in Table 2. Among the different treatments, Carbendazim-50WP was found to have the highest numbers of pod per plant (33.06). Among the isolates of *Trichoderma* spp. *T. harzianum* - KU933468 was found to have the highest number of pods per plant (28.03) followed by *T. harzianum* - KU933474 (26.70), *T. atroviride* - KU933472 (25.18), *Hypocrea lixii* - KX0113223 (23.78), *T. asperellum* - KU933475 (22.80) and *T. ovalisporum* - KU904456 (22.58). However, in untreated control plot the number of pods per plant was 14.53.

Number of seeds per pod

There was no significant difference among the treatments on number of seeds per pod. However, highest number of seeds per pod i.e. 3 nos. of seeds per pod was found in *T. harzianum* (KU933468) and Carbendazim-50WP treated pots.

Yield

Effects of potent different treatments on the yield of soybean under pot experiment are presented in Table 2. Results indicated that highest yield was obtained in Carbendazim-50WP treated plot of 33.42 g/pot. Among the *Trichoderma* isolates treated plots, *T. harzianum* - KU933468 treated plot gave the highest yield (29.70 g/pot) followed by *T. harzianum* - KU933474 (28.54 g/pot), *T. atroviride* - KU933472 (26.00 g/pot), *Hypocrea lixii* - KX0113223 (25.07 g/pot), *T. asperellum* - KU933475 (23.82 g/pot) *T. ovalisporum* - KU904456 (22.95 g/pot) respectively. Lowest yield was observed in control plot of 16.85 g/pot.

Likewise there are several reports of biocontrol of plant pathogens *in vivo* and *in vitro* conditions against seed borne diseases (Patel and Joshi, 2001; Ramamoorthy and Samiyappan, 2001; Ingle *et al*. 2002; Raheja and Thakore, 2002; Rao and Narayana, 2005). The use of *Trichoderma* species in controlling seed-borne diseases in crops have been reported (Wright, 1956; Tyner and Mckinnon, 1964; Hadar *et al.* 1984). The increased germination and reduced disease incidence with *Trichoderma* treatment of infested seeds might be due to reduction of the inoculum of pathogen by the antagonist, or displacement of the pathogen at or near infection counts by antagonists (Schroth and Hancock, 1981). Thus the present findings are in the line of several workers with some new information in relation to reduce disease incidence, ecological behaviour and their use in biological management of anthracnose of soybean in Manipur.

Table 1. Effect of volatile and non volatile compounds of *Trichoderma* spp. on growth of *Colletotrichum truncatum*

Sl.no	*Trichoderma* spp.	Per cent inhibition over control *		
		Volatile compounds	Non volatile compounds	
			(7.5%)	(15%)
1.	*T. ovalisporum* (KU904456)	24.70 (5.01)	28.22 (5.34)	39.60 (6.33)
2.	*T. harzianum* (KU933468)	31.76 (5.68)	32.54(5.75)	53.72 (7.36)
3.	*T. atroviride* (KU933472)	18.82 (4.37)	25.88 (5.13)	32.15 (5.71)
4.	*T. harzianum* (KU933474)	29.80 (5.49)	29.02(5.43)	53.72 (7.36)
5.	*T. asperellum* (KU933475)	17.25 (4.20)	15.68 (4.01)	29.41 (5.46)
6.	*Hypocrea lixii* (KX0113223)	18.43(4.32)	20.78 (4.59)	34.11 (5.88)
S.E (d)+-		0.37	0.35	0.26
C.D. (5%)		0.82	0.77	0.57

*Mean of three replication

Values in parentheses are Square Root Transformed values.

Table 2. Effect of *Trichoderma* isolates on yield and yield attributing characters of pod blight of soybean under pot trial during 2015– 2016

Sl. No.	**Treatment (*Trichoderma spp.*)**	**Germination (%) ***	**Canopy area (cm^2) ***			**No. of pods/ plant ***	**No. of seeds/ pod ***	**Yield (q/ha) ***
			30 DAP	**60 DAP**	**90 DAP**			
1.	*T. ovalisporum* (KU904456)	72.00 (61.15)	29.95	129.69	233.28	22.58	2.52	22.95
2.	*T. harzianum* (KU933468)	88.00 (74.06)	40.60	154.23	317.54	28.03	2.66	29.70
3.	*T. atroviride* (KU933472)	76.00 (63.68)	34.53	139.98	288.76	25.18	2.64	26.00
4.	*T. harzianum* (KU933474)	84.00 (71.53)	38.66	145.48	302.44	26.70	2.42	28.54
5.	*T. asperellum (*KU933475)	76.00 (61.15)	29.84	130.37	239.05	22.80	2.50	23.82
6.	*Hypocrea lixii* (KX0113223)	84.00 (68.75)	35.16	134.11	254.50	23.78	2.54	25.07
7.	Carbendazim-50WP	92.00 (79.37)	42.02	159.09	343.10	33.06	2.74	33.42
8.	Control	64.00 (53.30)	23.72	95.01	169.32	14.53	2.48	16.85
S.E(d)±		11.95	4.65	9.92	18.13	2.04	0.28	2.74
C.D.(5%)		25.62	9.97	21.27	38.89	4.38	NS	5.87

*Mean of three replication
Values in parentheses are angular transformed values

Table 3. Effect of *Trichoderma* isolates on incidence of pod blight of soybean under pot trial during 2015-2016

Sl. No.	Treatment (*Trichoderma* spp.)	Disease incidence (%) under field experiment *					Average disease incidence (per cent)**
		30 DAP	45 DAP	60 DAP	75 DAP	90 DAP	
1.	*T. ovalisporum* (KU904456)	2.00 (1.56)	4.00 (2.10)	8.22 (2.95)	13.11 (3.68)	21.11 (4.65)	9.69 (3.00)
2.	*T. harzianum* (KU933468)	0.65 (1.00)	2.44 (1.71)	5.33 (2.41)	10.22 (3.27)	17.99 (4.30)	7.33 (2.56)
3.	*T. atroviride* (KU933472)	1.55 (1.37)	3.55 (1.95)	6.88 (2.65)	11.99 (3.52)	19.77 (4.49)	8.75 (2.84)
4.	*T. harzianum* (KU933474)	0.84 (1.12)	2.44 (1.70)	5.77 (2.48)	10.44 (3.30)	18.44 (4.35)	7.60 (2.61)
5.	*T. asperellum (*KU933475)	1.78 (1.45)	4.00 (2.06)	7.78 (2.81)	12.44 (3.56)	20.44 (4.56)	9.29 (2.94)
6.	*Hypocrea lixii* (KX0113223)	1.33 (1.31)	3.55 (1.99)	7.11 (2.71)	11.55 (3.45)	19.11 (4.42)	8.53 (2.80)
7.	Carbendazim-50WP	0.44 (0.93)	2.22 (1.62)	5.11 (2.35)	10.00 (3.24)	17.55 (4.25)	7.06 (2.50)
8.	Control	2.89 (1.83)	6.44 (2.63)	14.44 (3.86)	23.11 (4.86)	33.77 (5.85)	14.31 (3.63)
S.E(d)±		0.42	0.39	0.50	0.35	0.26	0.11
C.D.(5%)		NS	0.85	1.07	0.75	0.58	0.25

*Mean of five replication
** Mean of five replicatiom
Values in parentheses are square root transformed values

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