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Occurrence of *Fusarium oxysporum* (Schlecht. Emend. Snyder & Hansen) Causing Pod Blight of Soybean (*Glycine max* L.) and Its Suitable Management with Native *Trichoderma* spp. in Manipur, India

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A B S T R A C T

During 2015-16 survey was conducted in six different soybean growing areas of Manipur, India. The survey revealed that the maximum mean pod blight intensity was at Thoubal (21.23%) and minimum (13.82%) at Andro Research Farm, CAU. *Fusarium oxysporum* was isolated and identified by morphological characteristics and gene sequencing, MF-512000. The pathogen was found to be the causal organism of pod blight of soybean. Total genomic DNA of the fungal cultures was extracted by using HiPurA DNA isolation Kit (HiMedia, India). PCR amplification of internal transcribed spacer regions was done using specific ITS1 and ITS4 primers and was confirmed by 1.2% agarose gel electrophoresis which produced a fixed region length of approximately 600bp for *Fusarium oxysporum*. Effects of volatile compounds produced by the *Trichoderma* spp. against *Fusarium oxysporum* ranged from 26.27-42.40 per cent. The effects of non-volatile compounds produced by *Trichoderma spp*. ranged from 20.39-43.52 per cent at 7.5% v/v concentration and 29.41-53.72 per cent at 15% v/v. *In vivo* study of isolates of *Trichoderma spp.* under field trials as seed treatment (@ 5g/kg seed) with foliar spraying (@ 5g/l of water) at 40 days after planting of water showed considerable reduction in disease incidence and increased production over control plot. The isolate *T. harzianum* (KU933468) showed highest yield in field trial (17.44 q/ha).

Introduction

Soybean (*Glycine max* L.) is the most important oilseed crop 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).

In India, area, production and yield during 2015-2016 were 11.40 million per hectares, 7.38 million metric tonnes and 0.65 metric tonnes per hectare respectively (Anonymous, 2016). Soybean growing major states in the country are Madhya Pradesh, Maharashtra, Andhra Pradesh, Tamil Nadu, Rajasthan, Gujarat, Uttar Pradesh, Punjab and Haryana (Bhatnagar, 1997). Yield losses due to

Fusarium rot of soybean were estimated to be over 7300 metric tonnes (2.5%) in Canada (Wrather *et al.,* 2001).

In spite of its phenomenal increase in area and production, its productivity remains low due to lack of quality seeds, various diseases and insect-pests. More than 100 plant pathogens have been reported to affect soybean, but among them very few are economically important causing yield losses to the tune of 12-20 per cent (Mittal *et al.,* 1993). Soybean diseases cause reductions in yield to the tonnes of 10 to 30% in most of the areas (Sinclair, 1992). So far, not much previous research have been done regarding pod blight of soybean and its management in this region. Therefore, the present study deals with the study of occurrence pod blight of soybean caused by *Fusarium oxysporum* in some soybean growing areas of Manipur and to find suitable management with native *Trichoderma spp.*

Materials and Methods

Soybean disease survey

A detail survey was conducted at different valley areas of Manipur *viz.,* Andro Research Farm (CAU), Iroisemba (College campus), Imphal East, Thoubal, Bishnupur and Imphal West at different days intervals (30, 60 & 90 DAP) on intensity of pod blight of soybean. Disease severity was recorded by random sampling and diseased samples were collected separately for *in-vitro* study. Disease severity was assessed based on 0-9 scale [0 (No discolouration), 1 (1% area covered with discolouration), 3 (1.1-10% area covered with discolouration), 5 (10.1-25% area covered with discolouration), 7 (25.1-50% area covered with discolouration), 9 (25.1-55% area covered with discolouration)]. Percentage of disease index was calculated by following formula;

$$\text{Per cent Disease Index} = \frac{\text{sum of all numerical ratings}}{\text{Number of leaf sample x Maximum disease grade}} \text{x100}$$

Fungal identification and pathogenicity

Disease samples collected from the surveyed field were cutted 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 with sterile distilled water. The sterilized pieces were then inoculated on potato dextrose agar (PDA) and incubated at 25 ± 2^{0}C. The fungus was identified 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.

Molecular characterization

The pure culture of the fungus was grown on Potato Dextrose Broth 50 ml in100ml conical flask at temperature of 27±2°C in BOD incubator. After 3-4 days of fungal growth, the mycelia of all the isolates were harvested by filtering. The mass of mycelium thus obtained was pressed with blotting paper and dried slightly and used directly for DNA isolation. DNA of the fungal culture was isolated using the HiPurA DNA isolation Kit (HiMedia, India).

PCR amplification and electrophoresis

The above extracted genomic DNA of fungal isolates was amplified using ITS-1 primer (5' TCC GTA GGT GAA CCT GCC G3' (5V)) and ITS-4 primer (5' TCC TCC GCT TAT

TGA TAT GC 3'). The forward (ITS-1) and reverse (ITS-4) primers used in the PCR reactions performed in this study were developed by White *et al.*, (1990) and designed to amplify the ITS region of the rRNA operon. The PCR amplification was carried out in 0.2 ml PCR tubes with 25 µl reaction volumes by using PX2 thermal cycler (Thermo Electron Corporation) and with a temperature profile standardized for gene amplification.

The electrophoresis of the amplified DNA was carried out in 1.2% agarose gel stained with ethidium bromide. To each PCR amplified sample, 1µl of 6 X loading dye was added.

A DNA ladder marker (100bp) was used as standard and the gel was run at 70 V until the loading dye reached the gel front. The amplified DNA was viewed under the gel documentation system (BIORAD, Molecular Imager Gel DOCTM XR).

In vitro antagonistic potential of some isolates of *Trichoderma*

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 *Fusarium* sp. through dual culture technique, production of non-volatile and volatile 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) = $100\ [(R_1-R_2) / R_1]$, where,

R_1 - the farthest radial distance grown by the pathogen in the direction of the antagonist.

R_2 - the distance grown on a line between inoculation positions of the pathogen and antagonist.

Seed priming with bioagents

Biopriming of soybean seeds was done for 1 hour by potent isolates of *Trichoderma spp.* The germination of seeds was observed periodically and the root length, shoots length were measured. The vigour index of respective crop seedlings were calculated on the basis of root and shoot length as follows:

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

In vivo efficacy of *Trichoderma* isolates

Six potent isolates of *Trichoderma* were evaluated against *Fusarium sp.* under field condition. Field experiment to study the efficacy of *Trichoderma* isolates against pod blight of soybean caused by *Fusarium sp.* was conducted at College of Agriculture, Central Agricultural University, Imphal during 2015-16. Field trials were taken up in randomized block design (RBD) with three replications. Plot sizes were 2m x 2m in all the trials. 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 and the numbers of seeds per pod. Pods of soybean were harvested and pod yield of each plot were taken and converted into quintal per hectare. The six potent isolates of *Trichoderma spp.* and one chemical fungicide were used.

Results and Discussion

Incidence of pod blight of soybean in farmer's field

Field survey was conducted at six different areas of Manipur *viz*., Andro Research Farm (CAU), Iroisemba (College campus), Imphal East, Thoubal, Bishnupur and Imphal West for natural incidence of pod blight of soybean during kharif season 2015-16 at regular interval i.e. 30DAP, 45DAP, 60DAP, 75DAP, 90DAP and results are presented in table 1. Results indicated that the average incidence of pod blight of soybean ranged from 13.82 per cent to 21.23 per cent. The highest average disease incidence was found at Thoubal (21.23 per cent) and lowest at Andro Research Farm, CAU (13.82 per cent).The present findings are in accordance with the findings of Chavan and Dhutraj (2017) who conducted a survey studies during 2011-2012 throughout all the eight districts of the Marathwada region in Maharashtra during *kharif* season and found that during *kharif* 2011, average pod blight intensity in the eight districts surveyed ranged from 27.75 to 38.88 per cent and during *kharif* 2012, average disease intensity ranged from 29.85 to 38.98 per cent respectively.

Molecular characterization

Total genomic DNA of the isolated purified fungal cultures was extracted by using the HiPurA DNA isolation Kit (HiMedia, India). PCR amplification of internal transcribed spacer region was done using specific ITS1 and ITS4 primers for *Fusarium* sp. and approximately 600bp were amplified using PCR and were confirmed by 1.2% agarose gel electrophoresis which was shown in Figure 1. Devi *et al.*, (2016) reported that ITS region was successfully amplified from DNA from all *F. oxysporum* strains by the fungal-specific universal primer pairs ITS1-ITS4.

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

In vitro antagonistic potential of isolates of *Trichoderma* spp. were evaluated against *Fusarium sp.* through production of volatile and non-volatile compounds.

Effect of volatile compounds

The effects of volatile compounds produced by *Trichoderma* spp. against *Fusarium* sp. were examined and results are presented in table 2. Among the six isolates of *Trichoderma spp.* tested, maximum percentage inhibition of 42.74 per cent was recorded by isolate *T. harzianum* (KU933474) and minimum percentage inhibition of 26.27 per cent was recorded by isolate *T. asperellum* (KU933475). The inhibition percentage of other isolates *viz., T. harzianum* (KU933468), *T. ovalisporum* (KU904456), *Hypocrea lixii* (KX0113223) and *T. atroviride* (KU933472) were 41.17 per cent, 36.47 per cent, 28.62 per cent and 26.27 per cent respectively. The present findings are in accordance with the findings of Raza *et al.,* (2013) who reported that strain SQR-T037 of *T. harzianum* produced volatile compounds that can inhibit the growth of *F. oxysporum* up to 40%, while the non-volatile antifungal compounds extracted from the liquid culture significantly inhibited the growth of *F. oxysporum.*

Effect of non-volatile compounds

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 *Fusarium sp.* and results are presented in table 2. Results showed that per cent inhibition of radial growth of *Fusarium sp.* by six isolates of *Trichoderma* spp. ranged from 20.39 per cent to 43.52 per cent at 7.5 % v/v concentration and from 36.07 per cent to 77.65 per cent at 15 % v/v

respectively. The inhibition percentage shown by other isolates at 7.5 % were 34.11 per cent *(T. harzianum* - KU933468), 30.97 per cent (*T. atroviride* - KU933472), 22.74 per cent *(T. ovalisporum* - KU904456), 21.96 per cent (*Hypocrea lixii* - KX0113223), 20.39 per cent (*T. asperellum*- KU933475). The inhibition percentage shown by other isolates at 15 % v/v were 75.49 per cent (*T. harzianum* - KU933468), 54.51 per cent (*T. atroviride* - KU933472), 43.52 per cent (*T. ovalisporum* - KU904456), 40.39 per cent *(Hypocrea lixii* - KX0113223), 36.07 per cent (*T. asperellum* - KU933475). Altinok and Erdogan (2015) also reported that *T. harzianum* strains produced volatile and non-volatile metabolites that inhibited growth *of F. oxysporum* strains on PDA medium.

Seed priming with bioagents

Biopriming of soybean seeds was done with six potent isolates of *Trichoderma* spp. *viz., T. ovalisporum* (KU904456), *T. harzianum* (KU933468), *T. atroviride* (KU933472), *T.harzianum* (KU933474), *T. asperellum* (KU933475), *Hypocrea lixii* (KX0113223) and results are presented in table 3. The highest root length was observed in isolate *T. harzianum* - KU933468 (7.02 cm) and lowest in *Hypocrea lixii* - KX0113223 (4.33 cm). However, in untreated control the root length was only 3.18 cm. The highest shoot length was observed in isolate *T. harzianum* - KU933468 (1.10 cm) lowest in *Hypocrea lixii* - KX0113223 (0.80 cm) and in control it was 0.38 cm. Highest germination percentage was recorded in *T. harzianum* - KU933468 (86.67%) and lowest was in *T. asperellum* - KU933475 (46.67%). There was only 33.33% seed germination in untreated control. The highest vigour index was observed in *T. harzianum* - KU933468 (596.82) and lowest was in *T. asperellum*- KU933475 (251.52). However, in untreated control, vigour index was 143.89. All the isolates showed significant differences of vigour index among them. The present study is supported by several researchers who have reported the biological seed treatments for protection of seed and control of pathogens causing seedling diseases (Harman *et al.,* 1991; Bennett *et al.,* 1992; Chet and Inbar, 1994). Seed priming is reported to eliminate toxic substances from seeds; consequently it is possible to remove pathogen from seeds which contribute to disease reduction and subsequent enhancement (Doijode, 2006) (Fig. 2).

In vivo efficacy of *Trichoderma* isolates

In vivo efficacy of six potent isolates *of Trichoderma viz., T. ovalisporum* (KU904456), *T. harzianum* (KU933468), *T. atroviride* (KU933472), *T. harzianum* (KU933474), *T. asperellum* (KU933475) and *Hypocrea lixii* (KX0113223) were evaluated against *Fusarium sp.* under field conditions during 2015-2016.

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

Germination percentage

The data on germination percentage of soybean under different treatments are presented in table 4. Results showed that the germination percentage in Carbendazim-50WP treated seed was highest (91.00%), followed by *T. harzianum* - KU933468 (87.64%), *T. harzianum* - KU933474 (85.22%), *Hypocrea lixii* - KX0113223 (77.74%), *T. asperellum* - KU933475 (76.42%), *T. atroviride*- KU933472 (75.53%) and *T. ovalisporum* - KU904456 (74.60%) respectively. In untreated plots, the germination percentage was 60.64%.

Table.1 Incidence of pod blight of soybean in different areas of Manipur during 2015-2016

Location	Disease Incidence (%)*				Latitude	Longitude
	30DAP	60DAP	90DAP	Average disease incidence		
1. Iroisemba (CAU, College campus)	4.07 (2.13)	16.66 (4.14)	38.88 (6.28)	19.87 (4.19)	N-24^{0}78.070	E-093^{0}09.674
2. Imphal East	2.40 (1.70)	10.37 (3.29)	29.63 (5.49)	14.13 (3.50)	N-24^{0}24.001	E-093^{0}42.252
3. Thoubal	4.81 (2.30)	18.14 (4.32)	40.74 (6.42)	21.23 (4.35)	N- 24^{0}35.350	E-094^{0}01.489
4. Andro Research Farm, CAU	2.96 (1.84)	11.11 (3.40)	27.40 (5.28)	13.82 (3.52)	N-24^{0}45.827	E-093^{0}03.138
5. Bishnupur	3.70 (2.05)	16.29 (4.10)	37.40 (6.16)	19.13 (4.10)	N-24^{0}25.927	E-093^{0}43.074
6. Imphal West	4.44 (2.21)	17.77 (4.27)	39.99 (6.36)	20.73 (4.29)	N- 24º49.416	E-093º54.161
S.E(d)±	0.14	0.13	0.07	0.13		
C.D (5%)	0.31	0.30	0.16	0.29		

*Mean of three replications
Values in parentheses are square root transformed values

Table.2 Effect of volatile and non-volatile compounds of *Trichoderma* spp. on growth of *Fusarium* sp.

Sl. no	*Trichoderma* spp.	Percent inhibition over control*		
		Volatile compounds	Non-volatile compounds	
			(7.5)%	(15)%
1.	*T. ovalisporum* (KU904456)	36.47 (6.08)	22.74 (4.80)	43.52(6.63)
2.	*T. harzianum* (KU933468)	41.17 (6.45)	34.11 (5.86)	75.49 (8.71)
3.	*T. atroviride* (KU933472)	39.60 (6.33)	30.97 (5.60)	54.51 (7.42)
4.	*T. harzianum* (KU933474)	42.74 (6.57)	43.52 (6.63)	77.65 (8.84)
5.	*T. asperellum (*KU933475)	26.27 (5.16)	20.39 (4.56)	36.07 (6.04)
6.	*Hypocrea lixii* (KX0113223)	28.62 (5.39)	21.96 (4.74)	40.39 (6.37)
S.E(d)±		0.25	0.36	0.30
C.D. (5%)		0.54	0.77	0.65

*Mean of three replications
Values in parentheses are square root transformed values

Table.3 Biopriming of soybean seeds with *Trichoderma* spp.

Sl. no.	*Trichoderma* spp.	Root length (cm)*	Shoot length (cm)*	Germination (%)*	Vigour index
1.	*T. ovalisporum* (KU904456)	6.39	0.90 (1.18)	60.00 (50.76)	384.25
2.	*T. harzianum* (KU933468)	7.02	1.10 (1.26)	86.67 (72.08)	596.82
3.	*T. atroviride* (KU933472)	5.09	0.84 (1.15)	53.33 (46.92)	292.78
4.	*T. harzianum* (KU933474)	6.92	1.05 (1.24)	80.00 (63.43)	517.58
5.	*T. asperellum* (KU933475)	4.69	0.83 (1.15)	46.67 (43.07)	251.52
6.	*Hypocrea lixii* (KX0113223)	4.33	0.80 (1.14)	60.00 (51.14)	279.73
7.	Control	3.18	0.38 (0.93)	33.33 (35.01)	143.89

*Mean of three replications
Values in parentheses are angular and square root transformed values

Table.4 Effect of *Trichoderma* isolates on yield and yield attributing characters pod blight of soybean under field condition 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)	74.60 (59.79)	30.27	149.96	721.58	35.23	2.67	15.11
2.	*T. harzianum* (KU933468)	87.64 (69.52)	38.74	225.37	835.48	46.41	3.00	17.44
3.	*T. atroviride* (KU933472)	75.53 (60.42)	35.72	196.82	773.49	42.69	2.13	16.57
4.	*T. harzianum* (KU933474)	85.22 (67.92)	37.50	220.40	800.19	44.89	3.00	17.11
5.	*T. asperellum* (KU933475)	76.42 (61.05)	28.50	152.26	729.52	37.52	2.67	15.59
6.	*Hypocrea lixii* (KX0113223)	77.74 (61.87)	29.84	185.70	759.14	38.64	2.33	16.51
7.	Carbendazim-50WP	91.00 (72.54)	42.52	255.62	874.81	54.63	3.00	19.50
8.	Control	60.64 (51.15)	25.32	115.40	456.18	21.68	2.00	13.02
S.E(d)±		3.06	1.03	25.45	36.32	1.02	0.48	0.31
C.D. (5%)		6.56	2.21	54.60	77.91	2.20	NS	0.67

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

Table.5 Effect of *Trichoderma* isolates on incidence of pod blight of soybean under field condition 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)	2.40 (1.70)	5.92 (2.53)	12.03 (3.54)	19.63 (4.49)	29.62 (5.49)	13.92 (3.55)
2.	*T. harzianum* (KU933468)	1.18 (1.30)	4.44 (2.22)	8.51 (3.00)	15.92 (4.05)	24.81 (5.03)	10.97 (3.12)
3.	*T. atroviride* (KU933472)	2.13 (1.62)	5.00 (2.34)	9.63 (3.18)	17.77 (4.27)	27.77 (5.32)	12.46 (3.35)
4.	*T. harzianum* (KU933474)	1.51 (1.42)	4.63 (2.26)	9.07 (3.09)	16.66 (4.14)	25.92 (5.14)	11.56 (3.21)
5.	*T. asperellum* (KU933475)	1.89 (1.54)	5.90 (2.53)	11.11 (3.41)	18.88 (4.40)	28.51 (5.39)	13.26 (3.45)
6.	*Hypocrea lixii* (KX0113223)	1.85 (1.53)	5.18 (2.38)	10.00 (3.24)	18.51 (4.36)	28.14 (5.35)	12.73 (3.37)
7.	Carbendazim-50WP	0.92 (1.17)	3.33 (1.95)	7.59 (2.84)	13.88 (3.79)	20.00 (4.53)	9.14 (2.86)
8.	Control	3.33 (1.95)	7.22 (2.78)	17.40 (4.23)	29.25 (5.45)	43.33 (6.62)	20.11 (4.21)
S.E(d)±		0.08	0.04	0.02	0.04	0.05	0.20
C.D. (5%)		0.17	0.11	0.06	0.10	0.11	0.43

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

Fig.1 DNA amplification shown by ITS1 and ITS4 primers of *Fusarium sp.* Where M: Molecular marker (100bp ladder in size)

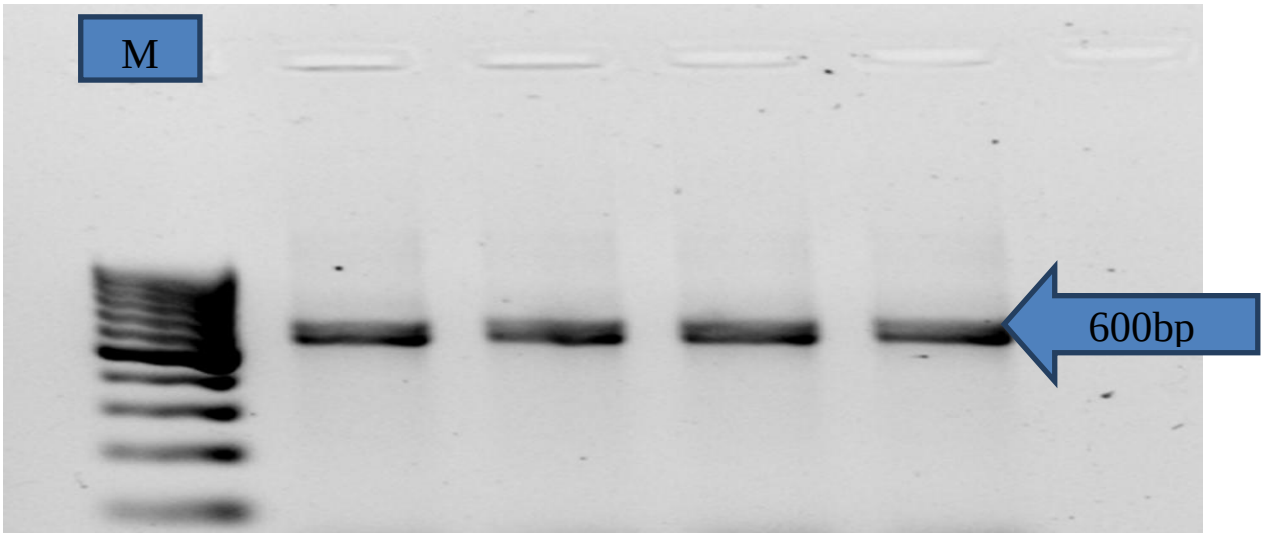

Fig.2 Biopriming of soybean seeds with *Trichoderma* isolates

0 - Control, 1 - *T. harzianum* (KU933468), 2 - *T. harzianum* (KU933474), 3 - *T. ovalisporum* (KU904456), 4 - *T. atroviride* (KU933472), 5 - *Hypocrea lixii* (KX0113223), 6 - *T. asperellum* (KU9334472).

Disease incidence

Data generated on the effect on *Trichoderma* isolates are presented in table 5. It is evident from the table that percentages of disease incidence were varied among the treatment. At 30 days after planting, disease incidence ranged from 0.92-3.33%. Disease incidence ranged from 3.33-7.22%, 7.59-17.40%, 13.88-29.25%, 20-43.33% at 45DAP, 60DAP, 75DAP, 90DAP respectively.

Average disease incidence was found to be lowest at Carbendazim-50WP (9.14%) followed by *Trichoderma* isolates *viz., T. harzianum* - KU933468 (10.97%), *T. harzianum*- KU933474 (11.56%), *T. atroviride*- KU933472 (12.46%), *Hypocrea lixii* - KX0113223 (12.73%), *T. asperellum* - KU933475 (13.26%), *T. ovalisporum* - KU904456 (13.92%). However, in untreated control plot, the average disease incidence was found to be 20.11%.

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 4. Results showed that canopy area of plants treated with Carbendazim-50WP was higher than those treated with *Trichoderma* isolates. Canopy area was ranged from 25.32-42.52cm^2, 115.40-255.62cm^2 and 456.18-874.81cm^2 at 30DAP, 60DAP and 90DAP respectively.

Number of pods per plant

Effect of different treatments on the yield parameters of soybean are presented in table 4. Among the different treatments, Carbendazim-50WP was found to have the highest numbers of pod per plant (54.63). Among the isolates of *Trichoderma* spp. *T. harzianum* (KU933468) was found to have the highest number of pods per plant (46.41)

and lowest in *T. ovalisporum* - KU904456 (35.23). However, in untreated control plot the number of pods per plant was 21.68.

Number of seeds per pod

There was no significant treatment difference among the treatment on the number of seeds per pod. However, highest number of seeds per pod i.e. 2.74 nos. of seeds per pod was found in Carbendazim-50WP treated plot as presented in table 4.

Yield

Results on effects of potent *Trichoderma* isolates and chemical on the yield of soybean under field experiment are presented in table 4. Results indicated that highest yield of 19.50 q/ha were obtained in Carbendazim-50WP treated plot. Among the *Trichoderma* isolates, *T. harzianum* - KU933468 treated plot gave the highest yield (17.44 q/ha) followed by *T. harzianum*- KU933474 (17.11 q/ha), *T. atroviride*- KU933472 (16.57 q/ha), *Hypocrea lixii* - KX0113223 (16.51 q/ha), *T. asperellum* - KU933475 (15.59 q/ha) *T. ovalisporum*- KU904456 (15.11 q/ha) respectively. Lowest yield of 13.02 q/ha was observed in control plot. 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) Similarly, Prasad *et al.*, (2002) reported that biopriming with *Trichoderma* resulted into increased germination percentage, root and shoot length of red gram under field condition.

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