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Evaluation of *Trichoderma* spp. against seedling diseases of solanaceous vegetables

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Three crop seeds (chilli, tomato and brinjal) were primed with seven isolates of *Trichoderma* using both mycelial and conidial inocula and one bacterial antagonist, *Pseudomonas fluorescens*, to evaluate their potentialities in terms of improvement in per cent germination of seeds, vigour index and seedling biomass of respective crop. The results suggested that irrespective of nature of biocontrol agents (may be bacterial or fungal), an enhancement in the seed germination (%),vigour index and seedling biomass was noted. Among the forms of inocula, the mycelial form gave better responses with respect to enhanced germination of seeds and increasing seed vigour index either in terms of calculated vigour index or estimated biomass on dry weight basis.The biopriming of seeds suggested that the isolates ThrAN-5, TvAN-3, TvAN-5, ThrWB-1 and ThrAN-7 were most efficient isolates of fungal antagonists regardless of crop seeds and form of inocula used and the bacterial antagonist, *Ps. fluorescens* was found intermediate effect with respect to their potential in enhancing the per cent germination, vigour index and seedling biomass of the crop seeds used/tested. The isolate ThrAN-5 and TvAN-5 were equally effective in inducing germination of chilli (88.0%) but TvAN-5 was superior over ThrAN-5 with respcet to vigour index (915.2) and seedling biomass (415.2 mg). However, highest seedling biomass was recorded with TvAN-3 (416.4 mg). Highest percentage germination (90.0%) of tomato seed was obtained with the isolates ThrWB-1, ThrAN-5 and TvAN-5, but highest vigour index (963.0) and seedling biomass (395.0 mg) was recorded with the isolate TvAN-3 and ThrWB-1, respectively.The isolates ThrAN-5, ThrWB-1, TvAN-3 and TvAN-5 were statistically at par, but TvAN-3 was most effective in inducing germination (86.0%), vigour index (867.0) and seedling biomass (430.6 mg) of brinjal.All isolates of bioagents significantly suppressed the incidence seedling diseases of chilli, brinjal and tomato as compared to control, but the isolate ThrAN-5, TvAN-3, TvAN-5, ThrWB-1 and lone isolate of bacterial antagonist, *Ps. fluorescens* were very effective in suppression of damping off of seedlings (tomato. chilli and brinjal).

Key words: Andaman and Nicobar Islands, biopriming, solanaceous crops, *Trichoderma* spp.

INTRODUCTION

Seed treatment with bioagents for protection of seeds and control of seed borne diseases offers the growers/farmars 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 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.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 seedings (challan *et al.*,1997). Several researchers have reported the biological seed treatments for protection of seed and control of pathogens causing seedling diseases in greenhouse and under field condition (Dubey *et al.*, 2007 ; Bhagat and Pan, 2010). The pre and post-emergence damping off seedling is a major disease in solanaceous vegetables crops causing reduction of seedling population in the nursery bed (Singh, 1995). The seedling diseases in solanaceous vegetables are primarily caused by *Rhizoctonia solani, Sclerotium* *rolfsii, Fusarium solani, F.o.f.* sp *lycopersici, Pythium* and *Phytophthora* spp. (Singh.1995) which further aggravates the disease problems after transplanting in the main field. *S. rolfsii, R. solani* and *F. o. f.*sp *lycopersici* in brinjal, chilli and tomato, respctively, are the major pathogens causing seedling diseases in Andaman and Nicobar Islands (Bhagat *et al.,* 2006). Therefore, present investigation has been carried out to evaluate the biocontrol agents as biopriming of solanaceous vegetable seeds, *viz,* tomato, chilli and brinjal to improve their germination behaviour and greenhouse evaluation aganinst seedling diseases of solanaceous vegetables.

MATERIALS AND METHODS

Twelve isolates of *Trichoderma* spp were isolated from rhizosphere soil of chilli, brinjal and tomato from Island ecosystem of Andaman and Nicobar Islands, India. They were morphologically identified by following taxonomic keys of Rifai (1969) and Domsch *et al.*(1980). They were maintained and preserved in Potato dextrose agar (PDA) medium for subsequent use.

The pathogens, *S. rolfsii, R. solani* and *F.o.f.*sp *lycopersici* were isolated from infected plant parts of respective crop seedlings by following tissue segment method (Rangaswami, 1958) and pure culture was obtained by repeated subculture. These pathogens were mass multiplied in sand- maize meal medium and applied 2-3 cm deep in the earthen pot filled with mixture of FYM and soil (1:2 ratio) before inoculating the test isolates of *Trichoderma* in the same soil. The moisture holding capacity of soil was maintained at 60% by irrigation whenever required.

Preparation of inoculum

For mycelial preparation of test isolates of *Trichoderma,* mycelial plug (6 mm dia) from young growing region of 4 days old culture of teast isolates of *Trichoderma* was inoculated into Erlenmeyer flasks (250 ml) containing 100 ml potato dextrose broth medium (PDB). The inoculated flasks were incubated at 28 ± 1°C for 3-4 days into a BOD incubator. The mycelial mat was harvested by passing through the Whatman No. 42 filter paper and homogenized by a stirrer. For conidial inocula, same procedures was followed up to the inoculation of antagonist in the medium (PDB) but incubated for 9 days instead of 3-4 days as in case of mycelial preparation. The conidia of *Trichoderma* isolates was separated from the mycelial mat by shaking the conical flasks clockwise and anticlockwise, the conidial suspension was collected into sterilized conical flask and centrifuged it at 6000 rpm for 10 min. The supernatant was decanted out from the centrifuge tube and pellets formed in the bottom of centrifuge tube were again resuspended in sterilized distilled water.

Seed priming with bioagents

Seeds of test crops were thoroughly washed with distilled water, air dried and finally dipped into the suspension of bioagents for few min., stirred thoroughly to ensure uniform coverage of seeds with suspension of bioagents. The treated seeds were spreaded on a cleaned blotter paper and allowed to shade dry. The treated seeds were seeded into Petridishes lined with double layered moist blotter paper and covered with upper lid of Petriplate lined with moist blotter paper and incubated for one week at 28 ± 1°C. The germination of seeds was observed periodically and the root length, shoots length, roots and shoot weight under wet and dry condition was 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 (%).

Green house test

In vivo efficacy of test isolates of *Trichoderma* were evaluated against, damping off and collar rot of chilli, damping off and collar rot of brinjal and wilt of tomato under green house condition. The sclerotia of *S. rolfsii* were buried 2-3 cm depth into earthen pot duly filled with a mixture of well rotten FYM and soil (1:2 ratio) before transplanting of seedlings of brinjal.

The details of treatments in the green house test were as follows:

T_1 - Seed treatment with *T. harzianum* (ThrWB-1) @ 5 g wheat bran + mustard cake (1×10^8 cfu/g) /kg seed + 25 sclerotia of *S. rolfsii* .

T_2 - Soil treatment with *T. harzianum* (ThrWB-1) @ 25 g wheat bran + mustard cake (1x 10^8 cfu/g) / pot

T_3 - $T_1 + T_2$

T_4 - Seed treatment with *T. harzianum* (ThrAN-5) @ 5 g wheat bran + mustaed cake (1x10^8 cfu/g) / kg seed

T_5 - Seed treatment with *T. harzianum* (ThrAN-5) @ 25 g wheat bran + mustard cake (1 x 10^8 cfu/g) / pot

T_6 - T_4+T_5

T_7 - Seed treatment with *T. harzianum* (ThrAN-7) @ 5 g wheat bran + mustard cake (1 x 10^8 cfu/g) / kg seed

T_8 - Soil application of ThrAN-7 @ 25 g wheat bran + mustard cake (1x10^8 cfu/g) / pot

T_9 - $T_7 + T_8$

T_{10} - Seed treatment with TvAN-3 @ 5 g @ 5 g wheat bran + mustard cake (1x10^8 cfu / g) / kg seed

T_{11} - Soil application of TvAN-3 @ 25 g wheat bran + mustard cake (1 x 10_8 cfu/g)/ pot

T_{12} - $T_{11} + T_{12}$

T_{13} - Seed treatment with TvAN-5 @ 25 g wheat bran + mustard cake (1 x 10_8 cfu/g)/ pot

T_{14} - Soil application of TvAN-5 @ 25 g wheat bran + mustard cake (1 x 10_8 cfu/g)/ pot

T_{15} - T_{13}+T_{14}

T_{16} - Seed treatment with TvAN-10 @ 5 g @ 5 g wheat bran + mustard cake (1 x 10_8 cfu/g) /kg seed

T_{17} - Soil application of TvAN-10 @ 25 g wheat bran + mustard cake (1 x 10_8 cfu/g)/ pot

T_{18} - T_{16}+T_{17}

T19 - Without *Trichoderma* isolatrs (non-treated control).

RESULTS AND DISCUSSION

The results on biopriming of seeds suggested that ThrAN-5, TvAN-3, TvAN-5, ThrWB-1 and ThrAN-7 were the most efficient isolates of fungal antagonists regardless of crop seeds and form of inocula used. The bacterial antagonist, *Ps. fluorescens* was found to have intermediate effect with respect to their potential in enhancing the per cent seed germination, vigour index and seedling biomass of the crop seeds used/tested. Among the two forms of inocula the mycelial form gave better response with respect to enhanced germination of seeds and increasing seed vigour index either in terms of calculated vigour index or estimated biomass on dry weight basis. The lone bacterial inoculum, *Ps. fluorescens* used for seed treatment gave better performance in stimulating germination of seeds over some of the fungal biocontrol agents, viz. TvAN-10 and ThrAN-13 while it was equivalent to lone *Trichoderma* isolate, ThrWB-1 with respect to improvement of germination behaviour of crop seeds tested.

The isolate ThrAN-5 and TvAN-5 were equally effective in inducing germination of chilli (88.0%) but TvAN-5 was superior over ThrAN-5 with respect to vigour index (915.2) and seedling biomass (415.2 mg) (Table 1). However, highest seedling biomass was recorded with TvAN-3 (416.4 mg). The isolates ThrWB-1, ThrAN-7 and *Ps. fluorescens* were also found promising in their potentialities to induce germination behaviour of chilli seeds.

Highest percentage germination (90.0%) of tomato seed was obtained with the isolates ThrWB-1, ThrAN-5 and TvAN-5 (Table 2), but highest vigour index (963.0) and seedling biomass (395.0 mg) was the isolate TvAN-3 and ThrWB-1, respectively. The isolates ThrWB-1, ThrAN-5, ThrAN-3, TvAN-5 and *Ps. fluorescens* did not differed statistically in their ability to induce germination (%), vigour index and biomass of seedlings of tomato.

In case of brinjal seeds, the isolates ThrAN-5, ThrWB-1, TvAN-3 and TvAN-5 were statistically at par, but TvAN-3 was most effective in inducing germination (86.0%), vigour index (867.0) and seedling biomass (430.6 mg) of brinjal (Table 3). The lone bacterial antagonist *Ps. fluorescens* was also good inducer of seed germination of brinjal seeds as well as vigour index and seedling biomass.All antagonists including one bacterial isolate were only able to increased germination (%) but also increased root and shoot length as well as increased number of root hairs.The entire root systems were fully covered with whitish green mycelial mass of *Trichoderma* exhibiting as if was infected with fungal mass but it gave protection to germinated seeds and seedlings from other pathogenic fungi. Most of the seeds did not germinated and more often the germinated seedling were attacked by various seed mycoflora,causing rotting of both root and shoots and did not develop into a healthy seedlings in control.

In vivo test

In case of chilli (Table 4), all isolates isolates sig-

Table 1 : Effect of seed priming with bioagents on seed germination and seedling vigour of chilli

Isolates of *Trichoderma*	Germination' (%)		Root length (cm) *		Shoot length (cm) *		Seedling vigour index		Biomass of seedling (mg)	
	M.I.	C.I.	M.I.	C.I.	M.I.	C.I.	M.I.	C.I.	M.I.	C.I.
ThrWB-1	87.0 (68.86)	84.0 (66.42)	3.8	3.6	6.2	6.0	870.0	806.0	420.1	405.6
ThrAn-5	88.0 (69.73)	84.0 (66.42)	3.8	3.5	6.3	6.0	889.0	798.0	412.2	397.4
ThrAn-7	82.0 (64.89)	77.0 (61.34)	3.5	3.3	5.5	5.2	738.0	654.0	392.4	364.1
ThrAN-13	78.0 (62.03)	75.0 (60.00)	3.5	3.1	5.0	4.7	663.0	585.0	365.5	351.2
TvAN-3	86.0 (68.03)	83.0 (65.65)	3.9	3.5	6.5	6.2	894.4	805.1	416.4	409.2
TvAN-5	88.0 (69.73)	83.0 (65.65)	4.0	3.6	6.4	6.2	915.2	813.4	415.2	406.2
TvAN-10	79.0 (62.72)	75.0 (60.00)	3.7	3.5	5.5	5.0	727.0	637.5	369.0	351.7
Ps. fluorescens	85.0 (67.21) @	85.0 (67.21)	3.7	3.7	6.2	6.2	841.5	841.5	409.2	384.4
Control	60.0 (50.77)	60.0 (50.77)	2.0	2.0	3.5	3.5	450.0	450.0	250.0	250.0

	Germination			Root length			Shoot length		
	Isolate	Form of inocula	Isolate x Form of inocula	Isolate	Form of inocula	Isolate x Form of inocula	Isolate	Form of inocula	Isolate x Form of inocula
SE (±)	0.3272	0.1889	0.567	0.2384	0.1376	0.413	0.2384	0.1376	0.413
CD (0.05)	0.775	0.447	0.342	0.565	0.392	NS	0.565	0.392	NS

M.I. - Mycelial inoculum; C.I. - Conidial inoculum; @ Culture filtrate; ' Means of 100 seeds observed. *Means of four replications

Table 2 : Effect of seed priming with bioagents on seed germination and seedling vigour of tomato

Isolates of *Trichoderma*	Germination (%) †		Root length (cm) *		Shoot length (cm) *		Seedling vigour index		Biomass of seedling (mg)	
	M.I.	C.I.	M.I.	C.I.	M.I.	C.I.	M.I.	C.I.	M.I.	C.I.
ThrWB-1	90.0 (71.56)	88.0 (69.73)	4.0	3.8	6.5	6.3	945.0	889.0	395.5	355.4
ThrAn-5	90.0 (71.56)	86.0 (68.03)	4.0	3.7	6.4	6.0	936.0	834.2	380.2	345.4
ThrAn-7	84.0 (66.42)	80.0 (63.43)	3.7	3.5	5.5	5.1	773.0	688.0	320.5	294.6
ThrAN-13	78.0 (62.03)	75.0 (60.00)	3.4	3.3	5.0	4.6	655.2	592.5	299.1	277.5
TvAN-3	88.0 (69.73)	87.0 (68.86)	4.0	3.7	6.6	6.2	933.0	861.3	390.6	361.2
TvAN-5	90.0 (71.56)	86.0 (68.03)	4.1	3.8	6.6	6.3	963.0	869.0	386.6	351.4
TvAN-10	85.0 (67.21)	82.0 (64.89)	3.8	3.5	5.4	5.0	782.0	697.0	305.0	289.1
Ps. fluorescens	88.0 (69.73) @	88.0 (69.73)	3.6	3.6	5.6	5.6	809.6	809.6	347.8	347.8
Control	65.0 (53.73)	65.0 (53.73)	2.5	2.5	3.7	3.7	403.0	403.0	200.0	200.0

	Germination			Root length			Shoot length		
	Isolate	Form of inocula	Isolate x Form of inocula	Isolate	Form of inocula	Isolate x Form of inocula	Isolate	Form of inocula	Isolate x Form of inocula
SE (±)	0.3272	0.1889	0.5667	0.2384	0.1376	0.4129	0.2384	0.1376	0.4129
CD (0.05)	0.775	0.447	1.342	0.565	0.469	NS	0.565	0.469	NS

M.I. - Mycelial inoculum; C.I. - Conidial inoculum; @ Culture filtrate; † Means of 100 seeds observed; *Means of four replications

Table 3 : Effect of seed priming with bioagents on seed germination and seedling vigour of brinjal

Isolates of *Trichoderma*	Germination (%) †		Root length (cm) *		Shoot length (cm) *		Seedling vigour index		Biomass of seedling (mg)	
	M.I.	C.I.	M.I.	C.I.	M.I.	C.I.	M.I.	C.I.	M.I.	C.I.
ThrWB-1	85.0 (67.21)	83.0 (65.65)	4.2	4.0	5.8	5.4	850.0	780.2	438.0	406.7
ThrAn-5	86.0 (68.03)	83.0 (65.65)	4.1	3.9	5.9	5.5	860.0	780.2	427.2	389.4
ThrAn-7	80.0 (63.43)	78.0 (62.03)	3.4	3.1	4.8	4.5	656.0	593.0	398.2	359.7
ThrAN-13	76.0 (60.67)	74.0 (59.34)	3.3	3.0	4.4	4.1	585.2	525.4	382.1	347.8
TvAN-3	85.0 (67.21)	81.0 (64.16)	4.2	3.8	6.0	5.4	867.0	745.2	430.6	410.2
TvA-5	86.0 (68.03)	84.0 (66.42)	4.1	3.9	5.8	5.5	851.4	790.0	421.4	385.5
TvAN-10	78.0 (62.03)	75.0 (60.00)	3.5	3.3	4.4	4.0	616.2	547.5	375.0	335.8
Ps. fluorescens	84.0 (66.42) @	84.0 (66.42)	4.2	4.2	5.5	5.5	815.0	815.0	415.8	376.4
Control	64.0 (53.13)	64.0 (53.13)	2.1	2.1	3.5	3.5	358.4	358.4	350.0	350.0

	Germination			Root length			Shoot length		
	Isolate	Form of inocula	Isolate x Form of inocula	Isolate	Form of inocula	Isolate x Form of inocula	Isolate	Form of inocula	Isolate x Form of inocula
SE (±)	0.2384	0.1376	0.4129	0.2384	0.1376	0.4129	0.2384	0.1376	0.4129
CD (0.05)	0.565	0.469	NS	0.565	0.392	NS	0.565	0.392	NS

M.I. - Mycelial inoculum; C.I. - Conidial inoculum; @ Culture filtrate; † Means of 100 seeds observed; *Means of four replications

Table 4 : *In vivo* efficacy of *Trichoderma* isolates against damping off and collar rot (*R. solani*) of chilli

Isolates of *Trichoderma*	Germination (%) +	Per cent mortality of chilli seedling				% RDI
		15 DAS	30 DAS	45 DAS	60 DAS	
ThrWB (T_1)	86.0 (68.03)	9.5 (17.95)	16.1 (23.66)	24.4 (29.60)	27.2 (31.44)	56.4 EF
ThrWB (T_2)	84.0 (66.42)	7.0 (15.34)	12.2 (20.44)	17.2 (24.50)	19.1 (25.91)	69.4 bc
ThrWB (T_3)	89.0 (70.63)	5.9 (14.09)	9.5 (17.95)	14.8 (22.63)	15.6 (23.26)	75.0 A
ThrAN-5 (T_4)	85.0 (67.21)	10.0 (18.43)	16.8 (24.20)	24.0 (29.33)	27.4 (31.56)	56.1 EF
ThrAN- 5 (T_5)	83.0 (65.65)	7.0 (15.34)	12.5 (20.70)	17.5 (24.73)	19.6 (26.28)	68.6 BC
ThrAN-5 (T_6)	88.0 (69.73)	5.5 (13.56)	9.3 (17.76)	14.5 (22.38)	15.9 (23.50)	74.5 A
ThrAn-13 (T_7)	75.0 (60.00)	15.0 (22.79)	23.8 (29.20)	31.8 (34.33)	35.5 (36.57)	43.1 G
ThrAN-13 (T_8)	73.0 (58.69)	10.0 (18.43)	18.2 (25.25)	24.5 (29.67)	28.8 (32.46)	53.8 F
ThrAN-13 (T_9)	78.0 (62.03)	7.5 (15.89)	12.6 (20.79)	19.9 (26.49)	22.5 (28.32)	63.9 CD
TvAN-3 (T_{10})	84.0 (66.42)	9.5 (17.95)	17.0 (24.35)	25.0 (30.00)	28.0 (31.95)	55.1 F
TvAN-3 (T_{11})	81.0 (64.16)	7.2 (15.56)	13.0 (21.13)	18.0 (25.10)	20.5(26.92)	67.1 C
TvAN-3 (T_{12})	88.0 (69.73)	5.7 (13.81)	9.9 (18.34)	15.0 (22.79)	16.4 (23.89)	73.7 AB
TvAN-5 (T_{13})	86.0 (68.03)	9.8 (18.21)	17.5 (24.73)	24.8 (29.87)	27.5 (31.63)	55.9 F
TvAN-5 (T_{14})	82.0 (64.90)	7.5 (15.89)	13.0 (21.13)	17.7 (24.88)	19.5 (26.21)	68.7 BC
TvAN-5 (T_{15})	90.0 (71.56)	5.0 (12.92)	9.0 (17.46)	14.4 (22.30)	16.0 (23.58)	74.4 A
TvAN-10 (T_{16})	78.0 (62.03)	14.9 (22.71)	25.0 (30.00)	34.0 (35.67)	38.2 (38.17)	38.8 G
TvAN-10 (T_{17})	75.0 (60.0)	10.5 (18.91)	20.0 (26.57)	25.5 (30.33)	30.4 (33.46)	51.3 F
TvAN-10 (T_{18})	80.0 (63.43)	7.5 (15.89)	14.0 (21.97)	20.0 (29.33)	24.0 (29.33)	61.5 DE
Control (T_{19})	55.0 (47.87)	20.2 (26.71)	39.9 (39.17)	48.8 (44.31)	62.4 (52.18)	0.0

	Germination	Isolate	DAS	Treatment	Isolate x DAS	Isolate x Treatment	DAS x Treatment	Isolate x DAS x Treatment
SEm (±)	0.403	0.044	0.033	0.023	0.087	0.062	0.047	0.123
CD (0.05)	1.168	0.121	0.091	0.065	0.242	0.171	0.129	0.3342

+Means of 100 seeds observed; *Means of four replications: DAS -Days after sowing; RDI- Reduction in disease incidence

nificantly controlled the damping off chilli caused by *R . solani* over that of untreated control. However, highest germination (89.0%), lowest per cent mortality of chilli due to damping off disease and highest reduction in incidence (75.0%) of damping off of chilli was noted with ThrWB-1, foolowed by T_6 (88.0%, 74.5%), T_{15} (90.0%, 74.4%), T_{12} (88.0%, 73.7%) and lest effective isolate being the TvAN-10 with 87.0% germination and 61.5% reduction in damping off disease incidence in chilli.

It appeared (Table 5) that all isolates of *Trichoderma* (regardless of mode of application significantly suppressed the tomato wilt disease under the condition of artificial infestation with *F. o.* sp *lycopersici*, as compared to untreated control whereas lowest germination (60.0%) and highest percentage mortality of tomato plants at 60 DAS was recorded. The lowest percentage mortality of plants infected with wilt disease in tomato was noted with seed and soil application of TvAN-5 (19.2%) and highest control

Table 5 : *in vivo* efficacy of *Trichoderma* isolates against wilt (*F. o. f.* sp *lycopersici)* of tomato

Isolates of *Trichoderma*	Germination (%) *	Per cent mortality of tomato seedling				% RDI
		15 DAS	30 DAS	45 DAS	60 DAS	
ThrWB (T_1)	88.0 (69.73)	12.4 (20.62)	19.6 (26.28)	25.2 (30.13)	30.1 (33.27)	63.3 C
ThrWB (T_2)	86.0 (68.03)	11.5 (19.82)	18.0(25.10)	24.0 (29.33)	28.9 (32.52)	64.7 C
ThrWB (T_3)	90.0 (71.57)	7.5 (15.89)	11.4 (19.73)	15.5 (23.18)	19.0 (25.84)	76.8 A
ThrAN-5 (T_4)	89.0 (70.63)	12.0 (20.27)	20.0 (26.57)	24.0 (29.33)	29.6 (32.96)	63.9 C
ThrAN- 5 (T_5)	86.0 (68.03)	10.9 (19.28)	18.8 (25.70)	22.5 (28.32)	27.0 (31.31)	67.1 BC
ThrAN-5 (T_6)	91.0 (72.54)	7.0 (15.34)	11.0 (19.37)	15.0 (22.79)	18.4 (25.40)	77.6 A
ThrAn-13 (T_7)	75.0 (60.00)	18.8 (25.70)	26.6 (31.05)	32.5 (34.76)	39.2 (38.76)	52.2 DE
ThrAN-13 (T_8)	72.0 (58.05)	16.9 (24.27)	24.2 (29.47)	28.7 (32.79)	36.0 (36.87)	56.1 D
ThrAN-13 (T_9)	80.0 (63.43)	9.8 (18.24)	15.4 (23.11)	20.0 (26.57)	25.0 (30.00)	69.5 B
TvAN-3 (T_{10})	86.0 (68.03)	13.6 (21.64)	21.5 (27.62)	26.5 (30.98)	31.2 (33.96)	61.9 C
TvAN-3 (T_{11})	82.0 (64.89)	12.0 (20.27)	19.0 (25.84)	23.0 (28.66)	27.6 (31.69)	66.3 BC
TvAN-3 (T_{12})	89.0 (70.63)	8.0 (16.43)	12.0 (20.27)	16.0 (23.58)	19.5 (26.21)	76.2 A
TvAN-5 (T_{13})	87.0 (68.86)	12.8 (20.96)	20.5 (26.92)	27.0 (31.31)	30.0 (33.21)	65.0 BC
TvAN-5 (T_{14})	82.0 (64.89)	11.0 (19.37)	17.4 (24.65)	22.8 (28.52)	26.2 (30.79)	668.0 BC
Tv AN-5 (T_{15})	90.0 (71.57)	7.0 (15.34)	11.0 (19.37)	15.0 (22.79)	19.2 (25.99)	76.6 A
TvAN-10 (T_{16})	80.0 (63.43)	20.2 (26.71)	28.2 (32.08)	35.1 (36.33)	43.0 (40.98)	47.6 E
TVAN-10(T_{17})	77.0 (61.34)	17.0 (24.35)	25.0 (30.0)	30.8 (33.71)	37.9 (38.00)	53.8 D
TvAN-10 (T_{18})	83.0 (65.65)	12.0 (20.27)	17.2 (24.50)	24.5 (29.67)	29.8 (33.09)	63.6 C
Control (T_{19})	60.0 (60.00)	44.0 (41.55)	56.6 (48.79)	70.5 (57.10)	82.0 (64.90)	0.0

	Germination	Isolate	DAS	Treatment	Isolate x DAS	Isolate x Treatment	DAS x Treatment	Isolate x DAS x Treatment
SEm (±)	0.520	0.106	0.080	0.057	0.212	0.150	0.113	0.30
CD (0.05)	1.506	0.294	0.222	0.157	0.588	0.416	0.315	0.832

*Means of 100 seeds observed; *Means of four replications: DAS -Days after sowing; RDI- Reduction in disease incidence

was recorded in T_6 treatment (77.6%, by seed + soil application of ThrAN-5). The isolates TvAN-3 and ThrWB-1 were closely followed by TvAN-5 and ThrAN-5 in their ability to control the wilt of tomato under artificial sick soil condition.

Highest germination (89.0%) of seed was observed with seed and soil application of ThrAN-5 (T_6 treatment), followed by T_{12} (TvAN-3,87%), (ThrAN-5, 86.0%) and T_8 (72.0%, seed treament with ThrAN-13) being the least effective in its descending order of chronology (Table 6). Lowest collar rot incidence in brinjal was noted with ThrWB-1 (T_3, seed + soil application) at all the dates observed and highest reduction in disease incidence (77.1%) was also recorded with seed and soil application of same isolate. This isolate was closely followed by TvAN-3 (T_{12}), ThrAN-5 (T_6), TvAN-5 (T_{15}), whereas the isolate ThrAN-13 being the poorest performer in its potentiality to suppress the collar rot of brinjal, when it was applied as seed treatment only.

The addition of microbial biocontrol agents during biopriming allows for colonization of the seed prior

Table 6 : *in vivo* efficacy of *Trichoderma* isolates against collar rot (*S. rolfsii*) of brinjal

Isolates of *Trichoderma*	Germination (%) †	Per cent mortality of brinjal seedling				% RDI
		15 DAS	30 DAS	45 DAS	60 DAS	
ThrWB (T_1)	83.0 (65.65)	8.0 (16.43)	14.4 (22.30)	19.2 (25.99)	24.4 (29.53)	66.4 B
ThrWB (T_2)	80.0 (63.43)	7.5 (15.89)	13.5 (21.56)	17.0 (24.35)	22.5 (28.32)	69.0 B
ThrWB (T_3)	86.0 (68.03)	7.0 (15.34)	10.5 (18.91)	12.0 (20.27)	16.6 (24.04)	77.1 A
ThrAN-5 (T_4)	85.0 (67.21)	8.5 (16.95)	14.0 (21.97)	20.0 (26.57)	25.0 (30.00)	65.6 B
ThrAN-5 (T_5)	82.0 (64.89)	8.0 (16.43)	13.3 (21.39)	17.2 (24.50)	22.0 (27.97)	69.7 B
ThrAN-5 (T_6)	89.0 (70.63)	7.0 (15.34)	10.2 (18.63)	11.7 (20.00)	17.0 (24.35)	76.6 A
ThrAn-13 (T_7)	74.0 (59.34)	12.2 (20.44)	19.2 (25.99)	27.5 (31.63)	35.5 (36.57)	51.1 C
ThrAN-13 (T_8)	72.0 (58.05)	11.7 (20.00)	17.8 (24.95)	25.0 (30.00)	32.4 (34.70)	55.4 C
ThrAN-13 (T_9)	79.0 (62.73)	9.0 (17.46)	13.5 (21.56)	16.0 (23.58)	25.5 (30.33)	64.9 B
TvAN-3 (T_{10})	83.0 (65.65)	8.5 (16.95)	15.0 (22.79)	19.8 (26.42)	24.8 (29.87)	65.8 B
TvAN-3 (T_{11})	80.0 (63.43)	8.1 (16.54)	14.2 (22.14)	16.5 (23.97)	21.9 (27.90)	69.8 B
TvAN-3 (T_{12})	87.0 (68.87)	6.8 (15.12)	10.0 (18.43)	11.8 (20.00)	16.9 (24.27)	76.7 A
TvAN-5 (T_{13})	85.0 (67.21)	8.0 (16.43)	15.5 (23.18)	20.0 (26.57)	26.0 (30.66)	64.2 B
TvAN-5 (T_{14})	83.0 (65.65)	7.6 (16.00)	14.6 (22.46)	16.0 (23.58)	22.4 (28.25)	69.1 B
Tv AN-5 (T_{15})	88.0 (69.73)	6.5 (14.77)	10.0 (18.43)	12.0 (20.27)	17.1 (24.43)	76.4 A
TvAN-10 (T_{16})	76.0 (60.67)	13.0 (21.13)	22.0 (27.97)	30.1 (33.27)	36.0 (36.87)	50.4 C
TVAN-10(T_{17})	73.0 (58.69)	12.3 (20.53)	20.6 (26.99)	26.0 (30.66)	32.0 (34.45)	55.9 C
TvAN-10 (T_{18})	78.0 (62.03)	9.4 (17.85)	16.2 (23.73)	19.8 (20.42)	24.5 (29.67)	66.2 B
Control (T_{19})	60.0 (54.33)	22.5 (28.32)	41.6 (41.16)	56.6 (48.79)	72.6 (58.44)	0.0

	Germination	Isolate	DAS	Treatment	Isolate x DAS	Isolate x Treatment	DAS x Treatmen	Isolate x DAS x Treatment
SEm (±)	0.513	0.052	0.039	0.028	0.103	0.073	0.055	0.146
CD (0.05)	1.482	0.143	0.108	0.076	0.286	0.202	0.153	0.405

*Means of 100 seeds observed; *Means of four replications: DAS -Days after sowing; RDI- Reduction in disease incidence

to planting and adds a new dimension to seed priming treatment. Precolonization provides the biocontrol agent with a competitive advantage over seed attacking pathogens and often provides superior seed protection when compared to seed coating (Harman *et al.*, 1989; Callan *et al.*,1990; Harman, 1991; Kubik, 1995). In present investigation there were significant increases in per cent germination of seed, vigour index seedling biomass of treated seeds (tomato, brinjal and chilli). These results were consistent with the findings of Harman *et al.*(2004) where they concluded that the roots and shoots were larger (roots were nearly twice as long) in maize seed treated with T-22 strain of *T. harzianum* than in its absence. They also reported that both main and secondary roots increased in size and area and the root hair area was greater with bioprimed seeds. The increased secondary roots and hairs were observed in present investigation in all treated seed crops. Prasad *et al.* (2002) reported that biopriming with *Trichoderma* resulted into increased germination (%) root and shoot lenght of red gram under field condition. Increased plant fresh weigh (140%) and Foliar area (300%), as well as proliferation of secondary roots (300%) and true leaves (140%) were observed with tobacco seedlings when both tobacco and tomato seedlings transferred to Petridishes inoculated with *T. harzianum* conidia (Chacon *et al.,* 2007). the combined application of seed and soil of all the isolates of *Trichoderma* spp. gave better result than seed or soil application alone. This is because many isolates of *Trichoderma* do not readily proliferate in the soil and confined to seed coat/seed surface in case of seed treatment, while the *Trichoderma* spp. well establish in the soil system which can compete for root exudates, nutrient and/or space with other microorganisms, but relatively less number of antagonist population in the vicinity of seeds, which provide an ample chance of pathogen's attack. But in case of simultaneous application of both as seed priming and in soil *Trichoderma,* they make a protective cover in the seed coat by very fast multiplication in the spermatosphere applied in soil as the *Trichoderma* population applied to soil have enough strength to out compete the other microorganisms or directly parasitizing *in situ.* However, the soil application of antagonist have got edge over that of seed treatment in view of inducing seed germination and reducing the disease incidence as well in all test crops.

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