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Biological management of root and collar rot (*Rhizoctonia solani*) of Frenchbean (*Phaseolus vulgaris*)

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ABSTRACT

Twelve isolates of *Trichoderma* were screened *in vitro* during 2006–08 against *Rhizoctonia solani* Kuhn. causing root and collar rot of Frenchbean *(Phaseolus vulgaris* L.) by dual culture tests and production of volatile and non-volatile antibiotics and it was found that all the isolates significantly inhibited the mycelial growth of *R. solani*. The isolate ThrAN-5 was most effective in suppression of mycelial growth of test pathogen, followed by TvAN-3, ThrAN-7, ThrWB-1 and ThrWB-2. ThrAN-5 was most effective in view of percentage inhibition of mycelial growth of *R. solani* (73.8 and 83.9%) by production of non-volatile substances at variable concentrations (7.5 and 15.0% respectively). Highest per cent germination (91.0%) vigour index (1287.0) and seedling biomass (772.5 mg) of Frenchbean seeds were recorded when the seeds were primed with TvAN-5, followed by ThrAN-5 and ThrWB-1. The bacterial antagonist *Ps. fluorescens* was also very effective in inducing germination behaviour of Frenchbean seeds. The collar rot of Frenchbean (c.o.-*R. solani*) was most effectively controlled in greenhouse test by seed and soil application of ThrWB-1 (79.0%), followed by TvAN-5 and ThrAN-5 (78.2% each), TvAN-3 (77.6%) while the isolate TvAN-10 was the least effective isolate with only 67.4% reduction in disease incidence. The highest germination (%), per cent reduction in disease incidence and increase in green pod yield of Frenchbean was recorded with combination of seed and soil application of ThrWB-1 (348.2%) over control under *R. solani* sick field condition, followed by T_{15} (TvAN-5, 345.8%), T_6 (ThrAN-5, 343.4%), T_{12} (TvAN-3, 339.7%) while lowest (239.7%) increase in yield was noted with seed application of TvAN-10 (T_{16} treatment).

Key words: Frenchbean, *Trichoderma* spp, Root rot, *Rhizoctonia solani*

Frenchbean *(Phaseolus vulgaris* L.) is an important legume vegetable crop grown throughout India. The crop suffers from several pests and diseases leading to heavy loss in yield. Among the diseases, root and collar rot is the most devastating disease causing considerable losses in yield. The disease is caused by *Rhizoctonia solani* Kuhn. The disease can be effectively controlled by many chemical and biological pesticides (Nath *et al*. 2004, Joshi *et al*. 2007). *Trichoderma* spp are among the most frequently isolated soil fungi and present in plant root systems. These fungi are opportunistic, avirulent plant symbionts (Harman *et al.* 2004) and functions as parasites and antagonists of many phytopathogenic fungi, thus protecting plants from disease. So far, *Trichoderma* spp are among the most studied fungal biocontrol agents and commercially marketed as biopesticides, biofertilizers and soil amendments (Harman *et al*. 2004). Depending upon the strain, the use of *Trichoderma* in agriculture can provide numerous advantages: (i) colonization of the rhizosphere by biocontrol agents rhizosphere competence allowing rapid establishment within the stable microbial communities in the rhizosphere, (ii) control of pathogenic and competitive/ deleterious microflora by using a variety of mechanisms, (iii) improvement of the plant health, and (iv) stimulation of root growth (Harman *et al*. 2004, Vinale *et al*. 2008). *Trichoderma,* a filamentous soil, inhabiting mycoparasite, has been used in commercial preparation for biological control of many fungal induced plant diseases (Jash and Pan 2004, Bhagat and Pan 2007). The present investigation was carried out to screen out some superior strain of *Trichoderma* spp and their evaluation against root and collar rot of French-bean in greenhouse and field condition.

Based on a part of Ph D thesis of the first author submitted to BCKV, Mohanpur during 2008

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MATERIALS AND METHODS

The pathogen, *R. solani* Kuhn was isolated from the infected plant part of Frenchbean from West Bengal. The biocontrol agents, viz *Trichoderma* isolates were isolated from rhizosphere soil of Frenchbean from Andaman and Nicobar Islands and West Bengal (ThrWB-1 and ThrWB-2). The isolates of *Trichoderma* from Andaman and Nicobar Islands, ThrAN-5, ThrAN-7, TvAN-3 and TvAN-5 were isolated before tsunami and ThrAN-13, ThrAN-16, TvAN-8 and TvAN-10 were isolated after tsunami on 26 December 2004. The lone isolate of *Pseudomonas fluorescens* was obtained from biocontrol laboratory of Plant Pathology, BCKV, West Bengal.

The chitin adaptation of *Trichoderma* isolates was performed by growing (28±1°C for 10 days) them in chitin amended potato dextrose agar broth medium (1.0% pure chitin) for at least 5 successive generations. The chitin adapted *Trichoderma* cultures were maintained in potato dextrose medium and preserved in refrigerated condition for subsequent use.

Both pathogens *(R. solani)* and antagonists were inoculated at peripheral region opposite to each other in sterilized petri plates (90 mm dia) containing 20 ml sterilized potato dextrose agar (PDA) medium and incubated at 28±1°C for one week. The PDA medium inoculated either with pathogen and antagonist only served as control. The experiment was replicated thrice. The radial mycelial growth of test pathogens and antagonist were measured periodically and the per cent inhibition of mycelial growth of test pathogens by antagonists was calculated as per formula adopted by Garcia (1991)

The volatile and non-volatile antibiotic produced by antagonistic fungi, *Trichoderma* was studied by following methods of Dennis and Webster (1971 a b) and Quimio and Cumagun (2001). The test isolates of *Trichoderma* were centrally inoculated (6 mm dia) into Petri-plates containing solidified PDA medium and incubated at 28±1°C for 3–4 days into a biological oxygen demand (BOD) incubator. Top lid of each Petri-plates was replaced with bottom part of Petri-plate containing solidified PDA medium duly inoculated with mycelial plug (6 mm dia) of *R. solani* at a same time. The Petri-plates without antagonist at the lower lid but pathogen on the top lid were served as control. The pairs of each Petri-plate were sealed together with cellophane adhesive tape and incubated at 28±1°C for 3–4 days or until the pathogen cover the entire medium surface. This set of experiment was replicated 4 times. The radial mycelial growth of test pathogen was recorded daily and compared with control plates.

The test isolates of *Trichoderma* were grown in 100 ml sterilized potato dextrose broth (PDB) for 10 days in 250 ml Erlenmeyer flasks with periodical shaking to assess non-volatile inhibitors of antagonists on *R. solani*. Culture filtrate of *Trichoderma* was harvested by filtering it through Whatmann filter no. 42 filter paper into sterilized flasks. The culture filtrate was centrifuged at 6 000 rpm for 10 min, sterilized by passing it through Cellulose Millipore membrane filter paper (0.4 µm pore). The required volume of culture filtrate was added with known volume of molten PDA to obtain final concentration of 7.5 and 15% (v/v) culture filtrate. The amended media was poured into Petri-plates and inoculated with mycelial plug (6 mm dia) picked up from young culture of test pathogen and incubated at 28±1°C for 4 days. The PDA medium without addition of culture filtrate of antagonist was served as control. The per cent inhibition of mycelial growth of respective pathogen was calculated according to formula of Garcia (1991).

The rhizosphere competence of test isolates of *Trichoderma* was conducted with French bean *(P. vulgaris)* using natural, sundried and sterilized soils of both locations, ie Port Blair and Mohanpur. Two kg potting mixture of soil (natural, sundried and sterilized soil) and farmyard manure (2: 1 v/v) was mixed thoroughly with 20 g of wheat bran + mustard cake (20%) formulation of *Trichoderma* isolates (1×10^8 cfu/g of product), moisture-holding capacity of the potting mixture was adjusted to 50% and filled into the earthen pots (50 cm ×20 cm). Twenty five seeds of Frenchbean were sown per pot. The experiment was replicated 4 times. The pots were supplied with irrigation water whenever required to maintain the moisture-holding capacity of potting mixture to 50%. The rhizosphere soil was collected by gently uprooting the test crops and brushing the soil adhered to roots after 15 days of sowing. The observation was repeated at 15 days interval and the data were recorded up to 60 days after sowing. The rhizosphere soil collected from different plants was mixed thoroughly in each case and the rhizosphere population of *Trichoderma* spp was estimated by soil dilution technique (Dhingra and Sinclair 1995). The total number of cfu/g of rhizosphere soil was the rhizosphere population of *Trichoderma* spp.

The biopriming of Frenchbean seeds were done with 4 isolates of *T. harzianum* (including 1 isolate from West Bengal), 3 isolates of *T. viride* and 1 isolate of *P. fluorescens* with mycelial and conidial forms of inoculum of *Trichoderma* isolates and *P. fluorescens*. Seeds 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 seeded into Petri-plates lined with double layered moist blotter paper and covered with upper lid of petri plate 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 lengths, root and shoot weight under wet and dry condition was measured. The vigour index of seedlings were calculated as per Abdul Baki and Anderson (1973).

In vivo efficacy of test isolates of *Trichoderma* were

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evaluated against root rot of Frenchbean *(R. solani*) under greenhouse condition. Five kg potting mixture of soil and farmyard manure (2: 1 v/v) was mixed thoroughly with 25 g of wheat bran + mustard cake (20%) formulation of *Trichoderma* isolates (1×10^8 cfu/g of product) and 10 g of sand-maize meal culture of *R. solani* were also buried 2–4 cm deep in the potting mixture. Moisture-holding capacity of the potting mixture was adjusted to 50% and filled into the Aluminium tray (50 cm×20 cm×10 cm). Twentyfive seeds of Frenchbean were sown per tray. The experiment was replicated 4 times. The trays were supplied with irrigation water whenever required to maintain the moisture-holding capacity of potting mixture to 50%. 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 + 10 g sand-maize meal culture of *R. solani*]; T_2, [soil treatment with *T. harzianum* (ThrWB-1) @ 25 g wheat bran + mustard cake (1×10^8 cfu/g) /pot+ 10 g sand-maize meal culture of *R. solani*]; T_3, [{seed treatment with *T. harzianum* (ThrWB-1) @ 5 g wheat bran + mustard cake (1×10^8 cfu/g) /kg seed} + {soil treatment with *T. harzianum* (ThrWB-1) @ 25 g wheat bran + mustard cake (1×10^8 cfu/g) /pot+ 10 g sand-maize meal culture of *R. solani*}]; T_4, [seed treatment with *T. harzianum* (ThrAN-5) @ 5 g wheat bran + mustard cake (1×10^8 cfu/g) /kg seed+ 10 g sand-maize meal culture of *R. solani*]; T_5, [Soil treatment with *T. harzianum* (ThrAN-5) @ 25 g wheat bran + mustard cake (1×10^8 cfu/g) /pot+ 10 g sand-maize meal culture of *R. solani*]; T_6, [{seed treatment with *T. harzianum* (ThrAN-5) @ 5 g wheat bran + mustard cake (1×10^8 cfu/g) /kg seed} + {soil treatment with *T. harzianum* (ThrAN-5) @ 25 g wheat bran + mustard cake (1×10^8 cfu/g) /pot+ 10 g sand-maize meal culture of *R. solani*}]; T_7, {seed treatment with *T. harzianum* (ThrAN-13) @ 5 g wheat bran + mustard cake (1×10^8 cfu/g) /kg seed + 10 g sand-maize meal culture of *R. solani*}; T_8, {soil application of ThrAN-13 @ 25 g wheat bran + mustard cake (1×10^8 cfu/g) /pot+ 10 g sand-maize meal culture of *R. solani*}; T_9, [{seed treatment with *T. harzianum* (ThrAN-13) @ 5 g wheat bran + mustard cake (1×10^8 cfu/g) /kg seed} + [soil application of ThrAN-13 @ 25 g wheat bran + mustard cake (1×10^8 cfu/g) /pot+ 10 g sand-maize meal culture of *R. solani*}]; T_{10}, [seed treatment with TvAN-3 @ 5 g wheat bran + mustard cake (1×10^8 cfu/g) /kg seed+ 10 g sand-maize meal culture of *R. solani*]; T_{11}, [soil application of TvAN-3 @ 25 g wheat bran + mustard cake (1×10^8 cfu/g) /pot+ 10 g sand-maize meal culture of *R. solani*]; T_{12}, [(seed treatment with TvAN-3 @ 5 g @ 5 g wheat bran + mustard cake (1×10^8 cfu/g) /kg seed + {soil application of TvAN-3 @ 25 g wheat bran + mustard cake (1×10^8 cfu/g) /pot+ 10 g sand-maize meal culture of *R. solani*}]; T_{13}, [seed treatment with TvAN-5 @ 25 g wheat bran + mustard cake (1×10^8 cfu/g) /pot+ 10 g sand-maize meal culture of *R. solani*]; T_{14}, [soil application of TvAN-5 @ 25 g wheat bran + mustard cake (1×10^8 cfu/g) /pot+ 10 g sand-maize meal culture of *R. solani*]; T_{15}, [{seed treatment with TvAN-5 @ 25 g wheat bran + mustard cake (1×10^8 cfu/g)/pot + {soil application of TvAN-5 @ 25 g wheat bran + mustard cake (1×10^8 cfu/g)/pot+ 10 g sand-maize meal culture of *R. solani*}]; T_{16}, [seed treatment with TvAN-10 @ 5 g wheat bran + mustard cake (1×10^8 cfu/g) /kg seed+ 10 g sand-maize meal culture of *R. solani*]; T_{17}, [soil application of TvAN-10 @ 25 g wheat bran + mustard cake (1×10^8 cfu/g)/pot + 10 g sand-maize meal culture of *R. solani*]; T_{18}, [{seed treatment with TvAN-10 @ 5 g wheat bran + mustard cake (1×10^8 cfu/g)/kg seed} + {soil application of TvAN-10 @ 25 g wheat bran + mustard cake (1×10^8 cfu/g) /pot+ 10 g sand-maize meal culture of *R. solani*}] and T_{19}, (without *Trichoderma* isolates).

Field trial of some isolates of *Trichoderma* against root rot of Frenchbean was conducted at the Instructional Farm, Jaguli (BCKV, Mohanpur) under sick plot condition by artificial inoculation of *R. solani* [@ 50 g/plot (3×2 m^2) sand-maize meal-water medium (980 g: 20 g: 250 ml), was incorporated at the root zone of individual plant] during 2006–07 and 2007–08. 'Arka Komal' Frenchbean was sown at a spacing of 50 cm × 20 cm in randomized block design with 4 replications. The detail of treatment schedules were same as green house test except the dose of pathogen (50 g/plot sand-maze meal preparation of *R. solani*) and antagonists (200 g/plot wheat bran + mustard cake preparation).

RESULTS AND DISCUSSION

Table 1 reveals that the pre-tsunami isolates of antagonistic fungi performed better with respect to mycelial growth inhibition of pathogens than post-tsunami isolates. Highest percentage inhibition in growth of *R. solani* was noted with ThrAN-5, followed by TvAN-3, ThrAN-7. However, the isolates (ThrWB-1 and ThrWB-2) of *Trichoderma* from West Bengal were found with intermediate effect with respect to their hyperparasitic ability irrespective of chitin or non-chitin adaptation of *Trichoderma* isolates.

Table 2 shows that *R. solani*, suffered less inhibition (%) by the effect of volatile antibiotics than non-volatile antibiotics synthesized by *Trichoderma* spp (either chitin fed or devoid of chitin feeding, pre-or post-tsunami isolates). When the antagonists were pre-adapted to chitin, there was significant enhancement of antagonistic potential of *Trichoderma* spp by production of non-volatile antibiotics. Among the isolates of *Trichoderma,* ThrAN-5 was most effective in percentage inhibition of mycelial growth of *R. solani* by production of non-volatile substances at variable concentrations (7.5 and 15.0%, respectively), followed by ThrAN-7, ThrWB-1, TvAN-3, TvAN-10 and ThmAN-4 and the isolate ThrWB-2 (West Bengal) was least effective in inhibiting mycelial growth of *R. solani*.

The fungal biocontrol agent, *Trichoderma* is known to antagonize numerous soil borne pathogenic fungi *in vitro* and under greenhouse/field conditions (Harman *et al.* 2004,

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Table 1 Antagonistic potential of *Trichoderma* isolates from Andaman and Nicobar Island by dual culture test

Isolates of *Trichoderma*	Non-chitin adapted *Trichoderma* isolates		†Chitin adapted *Trichoderma* isolates	
	R. solani		*R. solani*	
	Radial mycelial growth (mm)*	Per cent inhibition	Radial mycelial growth (mm) *	Per cent inhibition
ThrWB-1	27.3	69.7	20.5	77.7
ThrWB-2	37.4	58.4	30.4	66.9
ThrAN-5	25.2	72.0	16.3	81.9
ThrAN-7	30.5	66.1	21.2	76.4
ThrAN-13	34.2	62.0	27.3	69.7
ThrAN-16	39.7	55.9	30.0	66.7
TvAN-3	29.0	67.8	18.5	79.4
TvAN-5	26.2	70.9	17.0	81.1
TvAN-8	32.9	63.4	22.5	75.0
TvAN-10	30.1	66.5	21.0	76.7
ThmAN-4	40.5	55.0	28.2	68.7
ThmAN-10	43.6	51.5	32.7	63.7
Control	90.0	0.0	90.0	0.0
SEm (±)	1.976		1.054	
CD (*P*=0.05)	4.0		2.140	

† Chitin supplementation up to 5th successive generations; *Mean of 5 replications

Table 2 Antagonistic potential of *Trichoderma* isolates against *R. solani* by production of volatile and non-volatile substances

Isolates of *Trichoderma*	Non-chitin adapted						†Chitin adapted					
	Volatile substances		Non-volatile substances				Volatile substances		Non-volatile substances			
	Radial mycelial growth (mm) *	Per cent inhibition	Radial mycelial growth (mm) *		per cent inhibition		Radial mycelial growth (mm) *	per cent inhibition	Radial mycelial growth (mm)*		Per cent inhibition	
			7.5%	15%	7.5%	15%			7.5%	15%	7.5%	15%
ThrWB-1	38.5	57.2	36.0	29.5	60.0	67.2	34.6	61.5	28.8	21.5	68.0	76.1
ThrWB-2	40.2	55.3	44.5	36.4	50.5	59.5	36.7	59.2	36.0	25.6	60.0	71.5
ThrAN-5	36.0	60.0	34.2	25.5	62.0	71.7	31.5	65.0	23.6	14.5	73.8	83.9
ThrAN-7	37.8	58.0	37.8	31.5	57.0	65.0	33.1	63.2	29.6	19.1	67.1	78.8
ThrAN-13	40.5	55.0	39.0	33.6	55.9	62.7	36.9	59.0	35.8	22.9	60.2	74.5
ThrAN-16	42.7	52.5	42.5	38.5	49.0	57.2	40.5	55.0	37.8	26.3	58.0	70.8
TvAN-3	34.6	61.5	35.0	26.0	61.1	71.1	31.0	65.5	24.0	15.8	73.3	82.4
TvAN-5	34.2	62.0	34.8	26.5	61.3	70.5	27.9	67.0	24.2	16.2	73.1	82.0
TvAN-8	42.8	52.4	40.0	32.1	54.5	64.3	37.4	58.4	29.9	21.4	66.8	76.2
TvAN-10	37.7	58.1	38.7	29.7	57.0	67.0	34.9	61.2	28.3	18.6	68.5	79.3
ThmAN-4	40.0	55.5	43.0	34.2	52.2	62.0	34.2	62.0	33.9	22.2	62.3	75.3
ThmAN-10	42.3	53.0	44.8	35.2	50.2	60.9	37.8	58.0	38.0	25.2	57.8	72.0
Control	90.0	0.0	90.0	90.0	0.0	0.0	90.0	0.0	90.0	90.0	0.0	0.0
SEm±	0.555		0.504	0.430			0.442		0.458	0.294		
CD (*P*=0.05)	1.142		1.037	0.883			0.911		0.941	0.604		

†Chitin supplementation up to 5th successive generations; *Mean of 5 replications

Joshi *et al.* 2007, Pan and Bhagat 2007). The isolates ThrAN-5, TvAN0-3 and TvAN-5 were most efficient in their hyperparasitic action against *R. solani* than other isolates of *Trichoderma*. Variability in antagonistic potential among the different species of *Trichoderma* against different pathogens have been reported (Joshi *et al.* 2007, Pan and Bhagat 2007). In present study, some of the *Trichoderma* isolates, like ThrAN-5, TvAN-5 and TvAN-3 were highly efficient and more or less equivalent to West Bengal isolate, ThrWB-1, in their antagonistic potential against test pathogens. The possible explanation of this result may be due to their inherent potential to adapt well to introduced conditions though it

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rarely occurs (Whips 2001).

The supplementation of chitin in the growth medium for successive generations seems to improve the antagonistic potential of *Trichoderma* spp (Vinale *et al.* 2008). This is due to enhanced expression of chitinase gene which is responsible for secretion of chitinase. Though there is no direct relationship between chitin feeding and secretion of other cell wall degrading enzymes, but definitely there is some synergistic action of cell wall degrading enzymes (CDE_s) and the plethora of antibiotics substances (both volatile and non-volatile substances) (Vinale *et al.* 2008) with biological activity, called as secondary metabolite. In general, the antagonistic activity of *Trichoderma* was noted less by volatile antibiotics than that of non-volatile inhibitors in the present findings. Similar observations were also noted by Dennis and Webster (1971 a, b). The present findings also suggested that the pre-tsunami isolates exhibited better antagonistic potential by dual culture technique and both volatile and non-volatile substances, against the test pathogen. This result may be due to sudden inundation with sea water after deadly tsunami wave on 26 December 2004, which lead to much salt deposition in the soil with great varieties of cations and anions. This temporary and high stress condition might have adversely affected the physiology of the *Trichoderma* isolates resulting in reduction of hyperparasitic activity in dual culture technique.

Fig 1 (A-F) indicates that all isolates of *Trichoderma* significantly colonized the rhizosphere of Frenchbean compared to control. The isolate TvAN-5 was most efficient in rhizosphere colonization of the Frenchbean in sterilized soil of both Mohanpur and Port Blair at 60 DAS, followed by ThrAN-5, TvAN-3, ThrWB-1, which were statistically at par with respect of rhizosphere competence in soils both Mohanpur, and Port Blair, Andaman soil. Rhizosphere competence of antagonistic fungi in root zone of many crops is a vital area of research that requires elaborated exploration for successful management of plant diseases by biocontrol agents. It is expected that the *Trichoderma* spp should come in contact and establish within the rhizosphere zone of plant earlier than any other micro-organisms, thus they will provide a protective cover for root tips and hairs which otherwise are vulnerable to attack by several plant pathogenic fungi. Interestingly, more aggregation of *Trichoderma* population have been observed near the stem base or collar region and root tip or root hairs than middle sections of root systems, which are the most vulnerable plant parts to be attacked by pathogens (McLean *et al.* 2005). Benitez *et al.* (2004) reported that *Trichoderma* has a strong capacity to mobilize and take up soil nutrients, thus making it more efficient and competitive than many other soil microbes. The present findings suggested that the Port Blair soil was most favourable for rhizosphere competence to Andaman isolates of *Trichoderma* than to West Bengal isolates. There was less rhizosphere population of *Trichoderma* spp when they were applied to crops which were grown in natural soil as compared to sun-dried and sterilized soil. The possible explanation of these findings may be due to ecological specificity of *Trichoderma* spp (Bae and Knudsen 2005) and existence of microbial competition which does not allow antagonist to flourish in that situation (Whips 2001). However, there was a reverse trend of increased rhizosphere population of antagonists in sterilized soils of both Port Blair (Andaman) and Mohanpur (WB) soil due to reduced or absence of microbial competition in such soil.

Table 3 shows that the mycelial form of inoculum was better than conidial inoculum of *Trichoderma* for priming the Frenchbean seeds. Highest per cent germination, vigour index and seedling biomass of Frenchbean seedlings were recorded with TvAN-5, ThrAN-5 and ThrWB-1, respectively. The isolates ThrAN-7 and *Ps. fluorescens* were observed with intermediate effect and ThrAN-13 was recorded to be least efficient in inducing germination behaviour of Frenchbean

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

Isolates of *Trichoderma*	Germination (%) †		Root length (cm) *		Shoot length (cm) *		Seedling vigour index		Biomass of seedlings (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)	90.0 (71.56)	5.5	5.3	8.3	7.9	1242.0	1188.0	752.5	709.0
ThrAN-5	90.0 (71.56)	88.0 (69.73)	5.7	5.5	8.6	8.0	1287.0	1190.0	766.2	728.5
ThrAN-7	85.0 (67.21)	81.0 (64.16)	5.4	5.0	7.7	7.5	1113.0	1021.0	689.5	658.2
ThrAN-13	80.0 (63.43)	77.0 (61.34)	5.0	4.8	7.7	7.2	1016.0	924.0	675.5	644.8
TvAN-3	90.0 (71.56)	89.0 (69.73)	5.5	5.3	8.4	8.0	1251.0	1184.0	759.2	716.7
TvAN-5	91.0 (72.54)	88.0 (69.73)	5.6	5.4	8.4	8.0	1274.0	1186.0	750.2	718.2
TvAN-10	86.0 (68.03)	83.0 (65.65)	4.9	4.5	7.4	6.7	1058.0	930.0	661.2	625.3
Ps. fluorescens	88.0 (69.73)@	88.0 (69.73)	5.5	5.5	8.4	8.4	1223.0	1223.0	744.8	744.8
Control	75.0 (60.00)	75.0 (60.00)	3.8	3.6	5.5	5.5	637.0	637.0	550.0	550.0
SEm±	0.769	0.839	0.183	0.154	0.188	0.172				
CD (*P*=0.05)	1.617	1.762	0.384	0.323	0.396	0.362				

M I, Mycelial inoculum; C I, conidial inoculum; @Culture filtrate; †Means of 100 seeds observed; *Means of 4 replications

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Fig 1 a–f. Rhizosphere colonization of *Trichoderma* spp in Frenchbean (a) natural soil of Port Blair; (b) natural soil of Mohanpur; (c) sun-dried soil of Port Blair; (d) sun-dried soil of Mohanpur (e) sterilized soil of Port Blair; (f) sterilized soil of Mohanpur

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Table 4 *In vivo* efficacy of *Trichoderma* isolates against root rot *(R. solani*) of Frenchbean

Isolates of *Trichoderma*	Seedling emergence† (%)	Per cent mortality of Frenchbean*				Pre cent reduction in disease incidence
		15 DAS	30 DAS	45 DAS	60 DAS	
ThrWB-1 (T_1)	87.0 (68.87)	8.5 (16.95)	12.7 (20.88)	17.2 (24.50)	23.4 (28.93)	70.0 CD
ThrWB-1 (T_2)	85.0 (67.21)	7.0 (15.34)	10.2 (18.63)	15.5 (23.18)	20.8 (27.13)	73.3 BC
ThrWB-1 (T_3)	89.0 (70.63)	4.6 (12.38)	7.7 (16.11)	11.1 (19.46)	16.2 (23.73)	79.0 A
ThrAN-5 (T_4)	88.0 (69.73)	8.2 (16.64)	13.0 (21.13)	18.0 (25.10)	23.8 (29.20)	69.5 CD
ThrAN-5 (T_5)	85.0 (67.21)	7.0 (15.34)	10.5 (18.91)	15.0 (22.79)	20.2 (26.71)	74.1 ABC
ThrAN-5 (T_6)	90.0 (71.56)	4.5 (12.25)	8.0 (16.43)	12.0 (20.27)	17.0 (24.35)	78.2 A
ThrAN-13 (T_7)	78.0 (62.03)	10.2 (18.63)	16.2 (23.73)	27.2 (31.44)	32.5 (34.76)	58.3 GH
ThrAN-13 (T_8)	76.0 (60.67)	8.6 (17.05)	15.1 (22.81)	24.7 (29.80)	27.6 (31.69)	64.6 EF
ThrAN-13 (T_9)	81.0 (64.16)	6.4 (14.65)	12.5 (20.70)	20.0 (26.57)	22.2 (28.11)	71.5 CD
TvAN-3 (T_{10})	89.0 (70.63)	8.4 (16.85)	12.5 (20.70)	17.0 (24.35)	24.0 (29.33)	69.2 CDE
TvAN-3 (T_{11})	87.0 (68.87)	6.8 (15.12)	9.8 (18.24)	15.2 (22.95)	21.0 (27.27)	73.1 BC
TvAN-3 (T_{12})	92.0 (73.57)	4.0 (11.54)	7.0 (15.34)	10.8 (19.91)	17.5 (24.73)	77.6 AB
TvAN-5 (T_{13})	89.0 (70.63)	8.5 (16.95)	12.9 (21.05)	18.0 (25.10)	23.6 (29.06)	69.7 CD
TvAN-5 (T_{14})	86.0 (68.03)	7.3 (15.68)	10.4 (18.81)	16.3 (23.81)	20.6 (26.99)	73.6 ABC
TvAN-5 (T_{15})	91.0 (72.54)	4.9 (12.79)	7.6 (16.00)	12.0 (20.27)	16.8 (24.20)	78.2 A
TvAN-10 (T_{16})	85.0 (67.21)	11.6 (19.91)	18.4 (25.40)	29.2 (32.71)	35.5 (36.57)	54.5 H
TvAN-10 (T_{17})	80.0 (63.43)	10.4 (18.81)	17.0 (24.35)	25.6 (30.40)	30.0 (33.21)	61.53 FG
TvAN-10 (T_{18})	87.0 (68.87)	8.0 (16.43)	14.0 (21.97)	24.1 (29.40)	25.4 (30.26)	67.4 DE
Control (T_{19})	70.0 (56.79)	23.0 (28.66)	44.5 9 (41.84)	63.0 (52.54)	78.0 (62.03)	0.0
SEm	0.848	0.327	0.323	0.323	0.302	
CD (*P*=0.05)	1. 72	0.663	0.655	0.655	0.612	

†Means of 100 seeds observed; *Means of four replications; DAS, days after sowing; RDI, reduction in disease incidence

seeds, exhibiting 80.0% germination, 1016.0 vigour index and 924.0 mg seedling biomass, when seeds of Frenchbean were primed with mycelial form of inocula *(Trichoderma* isolate) and bacterial cell inocula *(Ps. fluorescens*). There was significant increase in secondary and tertiary root systems with appreciable number of root hairs, which lead to increased vigour index and seedling biomass of Frenchbean seedlings when treated with bioagents.

Table 4 revealed that the root and collar rot of Frenchbean (c.o.-*R. solani*) was most effectively controlled by ThrWB-1, followed by TvAN-5, ThrAN-5 and TvAN-3 while the isolate TvAN-10 was being the least effective isolates of *Trichoderma* by seed and soil application of antagonist. The methods of application of antagonist were significantly differed with respect to their efficacy in controlling root and collar rot of Frenchbean. In general, combined application of biocontrol agents as seed + soil, proved best regardless of *Trichoderma* isolates tested, followed by soil and seed treatment alone.

Table 5 reveals that all isolates (with different treatments) significantly enhanced the field emergence (%), reduced the disease incidence at all the dates observed and corresponding increase in yield of green pod of Frenchbean as compared to untreated control. However, highest field emergence (%) in Frenchbean seeds was recorded with seed + soil application of antagonists even under the artificial infestation of field by *R. solani*. In untreated control, there was consistently high percentage of plant mortality at all the dates observed and it reached up to 85.0% at 60 days after sowing under sick plot condition. Lowest disease incidence and corresponding highest reduction in root and collar rot disease incidence of French-bean was recorded with simultaneous seed and soil application of ThrWB-1 (T_3), followed by T_{15}, T_6 and T_{12} which were statistically at par under the same condition. The highest yield of Frenchbean (green pod) was recorded with T_3 (3.72 tonnes/ha) the seed + soil application of ThrWB-1, followed by T_{15} (TvAN-5), T_6 (ThrAN-5), T_{12} (TvAN-3) and T_{18} (ThmAN-10) recorded with lowest yield (3.42 tonnes/ha) of green pod by combination of seed + soil application. The highest increase in yield was also recorded with ThrWB–1 over control followed by T_{15} (TvAN-5), T_6 (ThrAN-5), T_{12} (TvAN-3), while lowest increase in yield was noted with seed application of TvAN-10 (T_{16}).

Seed treatment with bioagents for protection of seeds and control of seed-borne diseases offer 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 micro-organisms. 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 (Challan *et al.* 1997). Effective biological control agents have been developed for control of seed and seedling pathogens

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Table 5 Field efficacy of *Trichoderma* spp against root rot *(R. solani*) of Frenchbean

Treatment	Field emergence (%)	Per cent mortality of Frenchbean				Pre cent reduction in disease incidence	Yield (tonnes /ha)	% increase in yield over control
		15 DAS	30 DAS	45 DAS	60 DAS			
T_1	82.2 (65.05)	10.2* (18.63)	15.1 (22.87)	21.4 (27.56)	24.4 (29.60)	71.3	3.28	295.2
T_2	88.4 (70.09)	8.8 (17.26)	12.2 (20.44)	18.2 (25.25)	20.2 (26.71)	76.2	3.45	315.7
T_3	95.0 (77.08)	6.8 (15.12)	8.0 (16.43)	11.5 (19.82)	14.3 (22.22)	83.2	3.72	348.2
T_4	80.0 (63.43)	10.5 (18.91)	15.5 (23.18)	20.0 (26.57)	25.0 (30.00)	70.6	3.25	291.6
T_5	88.0 (69.73)	9.0 (17.46)	13.0 (21.13)	17.9 (25.03)	21.2 (27.42)	75.0	3.42	312.0
T_6	94.4 (76.31)	7.0 (15.34)	8.5 (16.95)	11.7 (20.00)	15.0 (22.79)	82.3	3.68	343.4
T_7	74.4 (59.60)	12.5 (20.70)	24.4 (29.60)	29.5 (32.90)	32.4 (34.70)	61.9	2.93	253.0
T_8	78.0 (62.03)	10.2 (18.63)	18.2 (25.25)	24.6 (29.73)	27.2 (31.44)	68.0	3.15	279.5
T_9	85.0 (67.21)	7.9 (16.32)	11.4 (19.73)	17.2 (24.50)	19.9 (26.49)	76.6	3.47	318.1
T_{10}	83.0 (65.65)	11.0 (19.37)	14.8 (22.63)	20.7 (27.06)	25.5 (30.33)	70.0	3.23	289.1
T_{11}	90.0 (71.57)	8.8 (17.26)	12.0 (20.27)	18.4 (25.40)	21.8 (27.83)	74.3	3.38	307.2
T_{12}	95.0 (77.08)	6.6 (14.89)	7.8 (16.22)	12.0 (20.27)	15.6 (23.26)	81.6	3.65	339.7
T_{13}	81.0 (64.16)	10.4 (18.81)	15.7 (23.34)	22.0 (27.97)	25.0 (30.00)	70.6	3.25	291.6
T_{14}	88.8 (70.45)	8.5 (16.95)	12.2 (20.44)	17.5 (24.73)	20.0 (26.57)	76.5	3.47	318.1
T_{15}	95.0 (77.08)	6.5 (14.77)	9.0 (17.46)	11.5 (19.82)	14.5 (22.38)	82.9	3.70	345.8
T_{16}	69.2 (56.29)	16.0 (23.58)	28.2 (32.08)	32.2 (34.57)	35.0 (36.27)	58.8	2.82	239.7
T_{17}	73.4 (58.95)	13.6 (21.64)	21.4 (27.56)	26.4 (30.92)	28.6 (32.33)	66.3	3.10	273.5
T_{18}	79.0 (62.73)	10.2 (18.63)	15.0 (22.79)	18.8 (25.70)	21.2 (27.42)	75.0	3.42	312.0
Control	60.0 (50.77)	26.6 (31.05)	49.5 (44.71)	74.6 (59.74)	85.0 (67.21)	0.0	0.83	0.0
SEm (±)	1.097	0.236	0.356	0.420	0.459		0.023	
CD (0.05)	3.148	0.678	1.023	1.205	1.318		0.065	

*Means of 4 replications

such as *Pythium* spp, *R. solani, 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 (Bhagat and Pan 2007).

The root rot of Frenchbean is an important disease, causing considerable losses in crop yield particularly in early sown crop. Present investigation indicated that all isolates of *Trichoderma* (Andaman or West Bengal/pre-or post-tsunami isolates) significantly increased field emergence, reduced root rot incidence and increased yield of green pod of Frenchbean. The close observation of uprooted seedlings exhibited that there was increased number of secondary and tertiary roots, increased root length, plant height, number of branches, number of pods/plant when treated with *Trichoderma* isolates (seed, soil and seed + soil application). These results are in conformity with the earlier findings (Nath *et al.* 2004, Joshi *et al.* 2007). Joshi *et al.* (2007) observed that seed treatment of Frenchbean seed coupled with soil application resulted into increased germination, reduced root rot disease incidence, increased number of pods/plant and yield compared to untreated control. The recent findings showed that *Trichoderma* spp are opportunistic, avirulent plant symbionts, as well as being establish robust and long-lasting colonization of root surfaces and penetrate into the epidermis and a few cells below this level (Harman *et al*. 2004). They produce or release a variety of compounds that induced localized or systemic resistance responses and this explains their lack of pathogenicity to plants. Root colonization by *Trichoderma* spp often enhances root growth and development, crop productivity, resistance to abiotic stress and the uptake and use of nutrients.

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