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Biological management of fusarial wilt of tomato by *Trichoderma* spp. in Andamans

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ABSTRACT

Twelve isolates of *Trichoderma* from Andaman and Nicobar Islands have been evaluated for their biocontrol potential under *in vitro* and field conditions during 2008-09, 2009-10 and 2010-11 against *Fusarium oxysporum* f. sp. *lycopersici* causing wilt of tomato. The isolates Th-CARI-50, Tv-CARI-73, Tv-CARI-85 and Th-CARI-61 were most efficient in the hyperparasitic action on the test pathogen in dual culture test. The biopriming of seeds with *Trichoderma* isolates and bacterial antagonist significantly improved the germination behaviour of tomato seeds as compared to control. Mycelial form of inocula was proved better than conidial inocula of *Trichoderma* in inducing germination (%) of tomato seeds. The isolates Th-CARI-61 was proved most effective in inducing per cent germination (90% - mycelial inoculums; 88% - conidial inocula), seedling vigour (945 – MI; 889 - CI), seedling biomass (395.5 mg - MI; 355.4 mg - CI) of tomato seedlings followed by Tv-CARI-85, Th-CARI-50, Tv-CARI-73, *P. fluorescens*, Tv-CARI-110, Th-CARI-72, whereas Th-CARI-130 was noted with least effective. The seed and soil application of *Trichoderma* spp. was most effective in reduction of disease incidence of fusarial wilt of tomato under both greenhouse and field conditions than that of either seed or soil application alone. Th-CARI-50 was most effective in inducing germination (92%), lowest disease (16.4%) and highest reduction of disease incidence (80%) followed by Th-CARI-61, Tv-CARI-85, Tv-CARI-73, Th-CARI-130 and the isolate Tv-CARI-100 was least effective (54.9% RDI). T_6 treatment (Th-CARI-50) was most effective in improving field emergence (90.2%) of tomato seedlings, reduction in fusarial wilt disease incidence (78.8%) and corresponding yield increase (138%) of tomato under field condition followed by T_{12} (Tv-CARI-73), T_{15} (Tv-CARI-85) and T_3 (Th-CARI-61).

Key words: Biocontrol, *Fusarium oxysporum* f. sp. *lycopersici*, tomato, *Trichoderma* spp.

INTRODUCTION

Tomato is most important vegetable crops in India and worldwide. Several biotic and abiotic factors are the main yield limiting constraints. Among the biotic constraints, bacterial wilt (*Ralstonia solani*), fusarial wilt (*F. oxysporum* f.sp. *lycopersici*), leaf curl (TYLCV), basal stem rot are the major diseases in Andaman and Nicobar Islands causing considerable yield loss. The wilt of tomato is a major yield limiting biotic constraints and it can cause heavy loss to the farmers (Bhagat, 7). This problem becomes more severe when it is affected by more than two or three diseases simultaneously. The use of conventional chemical pesticides are the most common practice to manage this disease but indiscriminate use of chemical pesticides not only cause pollution of soil and water ecosystem but also causes severe health hazards to human. Use of biocontrol agents are the suitable alternative to chemical pesticides with sustainable disease management without pesticide residues in food stuffs, development of resistance in plant pathogens and appearance of new races/strains of the pathogen.

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Trichoderma spp. are predominant and among the most frequently isolated soil fungi and present in plant root systems (Harman, 14; Vinale *et al.*, 22) and over wide geographic regions in all climatic zones. These fungi are opportunistic, avirulent plant symbionts and functions as parasites and antagonists of many phytopathogenic fungi, thus protecting plants from disease. They are characterized by rapid growth, an ability to assimilate a diverse array of substrates, and by their production of an array of antimicrobials. Strains have been exploited for production of enzymes and antibiotics, bioremediation of xenobiotic substances, and as biological control agents against plant pathogenic fungi and nematodes. Some species of *Trichoderma* can form intimate associations with plant roots, stimulating plant growth by producing soluble forms of mineral nutrients and growth-promoting metabolites (Altomare *et al.*, 1; Yedidia *et al.*, 23; Bhagat and Pan, 5 & 8). *Trichoderma*, a filamentous soil inhabiting mycoparasite, have been used in commercial preparation for biological control of many fungal induced plant diseases. For successful biological control with the antagonist, the biocontrol strain should be highly effective, it should be able to compete and ideally be able to colonize

and proliferate on existing and newly formed plant parts at times well after application and inexpensive in formulation of the biocontrol agent with excellent shelf life even under ordinary storage conditions as well as suitable method of delivery (Harman, 14; Bhagat and Pan, 5, 8 & 9). Therefore, present investigation was aimed at isolation and identification of native *Trichoderma* isolates from rhizosphere soil of tomato across the Bay Islands, physiochemical characterization, biopriming, evaluation of promising isolates of *Trichoderma* under greenhouse and field conditions against fusarial wilt of tomato.

MATERIALS AND METHODS

Twelve isolates of *Trichoderma* (6 *T. harzianum*, *T. viride* and 2 *T. hamatum*) were isolated from rhizospheres soil of tomato from various locations of Andaman and Nicobar Islands (Table 1) and were maintained on PDA (Potato dextrose agar) medium for subsequent use. *F.o.* f.sp. *lycopersici* strain was isolated from infected tomato seedlings by tissue segment method (Rangaswami, 20). The culture was purified by repeated subculturing and pure culture was used in present investigation.

The selected isolates were subjected to dual culture test against *F.o.* f.sp. *lycopersici*. The mycelial discs (6 mm dia) of *F.o.* f.sp. *lycopersici* were inoculated centrally on solidified PDA in the Petri plates one day advance to attain the point of contact at the middle of Petri plates. The inoculated plates were incubated at 28 ± 1°C for 10 days and were periodically observed for the mycelial suppression of pathogens. This set of experiment was replicated five times. Per cent inhibition of mycelia growth of pathogen was calculated as per formula:

IRG (%) = 100 $[(R_1-R_2)/R_1]$, where R_1 is the farthest radial distance grown by the pathogen in the direction of the antagonist (control), while R_2 represents the distance grown on a line between inoculation positions of the pathogen and the antagonist.

Mycelial plug (6 mm dia) from young growing region of 4-day-old culture of 12 isolates of *Trichoderma* was inoculated into Erlenmeyer flasks (250 ml) containing 100 ml potato dextrose broth medium (PDB) and incubated at 28 ± 1°C for 3-4 days into a BOD incubator. The mycelial mat was harvested individually from flasks by passing through the Whatman No. 42 filter paper and homogenized by a stirrer. The required concentration (1×10^8 cfu/ml) of each mycelial inoculum was prepared by adding sterilized distilled water and was used immediately. For conidial inocula of *Trichoderma*, same procedures was followed upto the inoculation of antagonist in the medium (PDB) but incubated for 9 days. The conidia of each *Trichoderma* isolates were separated from the mycelial mat by shaking the conical flasks clockwise and anti-clockwise. The conidial suspension was then collected individually into sterilized conical flask and centrifuged it at 6000 rpm for 10 min. The required concentration (1×10^8 cfu/ml) of conidial suspension was prepared by adding the sterilized distilled water and was used immediately. For *P. fluorescens* a talc-based formulation was prepared in a slurry form.

Table 1. Location, pH and organic matter of tomato field soils from which *Trichoderma* spp. were isolated from Andaman & Nicobar Islands.

Location	Soil type	Soil pH	Organic matter (%)	Nitrogen content (%)	C:N ratio	Isolation code	*Trichoderma* spp.
Diglipur	Loamy sand	6.3	0.37	0.021	15:1	Th-CARI-61	*T. harzianum*
Diglipur	Loamy sand	6.2	0.25	0.028	13:1	Th-CARI-72	*T. harzianum*
Manglutan	Sandy loam	7.2	0.56	0.050	16:1	Tv-CARI-73	*T. viride*
Calicut	Sandy loam	6.4	0.90	0.045	21:1	Th-CARI-50	*T. harzianum*
Sippighat	Loamy sand	5.5	0.77	0.037	23:1	Th-CARI-70	*T. harzianum*
Guptapara	Sandy loam	7.6	0.80	0.056	14:1	Th-CARI-64	*T. hamatum*
Nimbudera	Sandy loam	5.4	0.72	0.044	15:1	Tv-CARI-85	*T. viride*
Diglipur	Loamy sand	7.6	0.68	0.055	15:1	Tv-CARI-100	*T. viride*
Diglipur	Sandy loam	6.5	0.75	0.050	13:1	Tv-CARI-68	*T. viride*
Diglipur	Sandy loam	6.0	0.91	0.041	22:1	Th-CARI-130	*T. harzianum*
Hutbay	Sandy	6.9	0.84	0.038	22:1	Th-CARI-116	*T. harzianum*
Hutbay	Sandy	6.4	0.91	0.036	25:1	Th-CARI-110	*T. hamatum*

Tomato seeds were thoroughly washed with distilled water, air-dried and finally dipped into the suspension of bioagents for a few minutes and stirred thoroughly to ensure uniform coverage of seeds with suspension of bioagents. The treated seeds were spread on a clean blotter paper and allowed to shade dry. The treated seeds were seeded into Petri dishes lined with double-layered moist blotter on both sides and incubated for one week at 28 ± 1°C. The germination of seeds was observed periodically and the root and shoot lengths and root and shoot weights under wet and dry conditions were measured. The vigour index of seedlings in each crop was also calculated as:

Vigour index = [root (cm) + shoot (cm)] × germination (%).

Some isolates of *Trichoderma* were evaluated against fusarial wilt of tomato under greenhouse conditions. Five kg potting mixture of soil and farm yard 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 *F.o.* f.sp. *lycopersici* were also buried 2 - 4 cm deep in the potting mixture. MHC of the potting mixture was adjusted to 50% and filled into the aluminium tray ($50 \times 20 \times 10$ cm^3). Twenty five seeds were sown per tray. The experiment was replicated four times. The trays were supplied with irrigation water whenever required to maintain the MHC of potting mixture to 50%.

The details of treatments in the greenhouse test were as follows: T_1 : Seed treatment with *T. harzianum* (Th-CARI-61) @ 5 g wheat bran + mustard cake (1 x 10^8 cfu/g) /kg seed + *F.o.* f.sp. *lycopersici*; T_2 : Soil application of *T. harzianum* (Th-CARI-61) @ 25 g wheat bran + mustard cake (1×10^8 cfu/g)/pot; T_3 : $T_1 + T_2$; T_4 : Seed treatment with *T. harzianum* (Th-CARI-50) @ 5 g wheat bran + mustard cake (1×10^8 cfu/g)/kg seed; T_5 : Soil application of *T. harzianum* (Th-CARI-50) @ 25 g wheat bran + mustard cake (1×10^8 cfu/g)/pot; T_6 : $T_4 + T_5$; T_7 : Seed treatment with *T. harzianum* (Th-CARI-130) @ 5 g wheat bran + mustard cake (1×10^8 cfu/g) /kg seed; T_8 : Soil application of Th-CARI-130@ 25 g wheat bran + mustard cake (1×10^8 cfu/g) /pot and T_9 : $T_7 + T_8$; T_{10} : Seed treatment with *T. harzianum* (Tv-CARI-73) @ 5 g wheat bran + mustard cake (1×10^8 cfu/g) /kg seed; T_{11} : Soil application of Tv-CARI-73 @ 25 g wheat bran + mustard cake (1×10^8 cfu/g) /pot and T_{12} : $T_{10} + T_{11}$; T_{13} : Seed treatment with *T. harzianum* (Tv-CARI-85) @ 5 g wheat bran + mustard cake (1×10^8 cfu/g)/kg seed; T_{14} : Soil application of Tv-CARI-85 @ 25 g wheat bran + mustard cake (1×10^8 cfu/g)/pot and T_{15} : $T_{13} + T_{14}$; T_{16} : Seed treatment with *T. harzianum* (Tv-CARI-100) @ 5 g wheat bran + mustard cake (1×10^8 cfu/g) /kg seed; T_{17} : Soil application of Tv-CARI-100@ 25 g wheat bran + mustard cake (1×10^8 cfu/g) /pot and T_{18} : $T_{16} + T_{17}$

Field trial of some isolates of *Trichoderma* against fusarial wilt of tomato was conducted at the Sippighat Farm, Central Agricultural Research Institute, Port Blair under sick plot condition of *F.o.* f.sp. *lycopersici* (1×10^4 cfu/g soil) during 2008-09, 2009-10 and 2010-11. The cultivar "LE -3704" was sown at a spacing of 50×20 cm^2 in randomized block design with four replications. The detail of treatment schedules were same as greenhouse test except the dose of antagonists (200 g/plot wheat bran + mustard cake preparation).

RESULTS AND DISCUSSION

The results presented in Table 2 revealed that all isolates of *Trichoderma* spp. have significantly inhibited mycelial growth of *F.o.* f.sp. *lycopersici* as compared to control but the isolate Th-CARI-50, Tv-CARI-85,Th-CARI-61,Tv-CARI-100, Tv-CARI-73 were most efficient in per cent inhibition of test pathogen. Highest (65.9%) inhibition of pathogen was noted with Th-CARI-50, whereas, the isolate Th-CARI-110 was least effective to inhibit the growth of pathogen (44.9%). There was inhibition zone at the point of contact between pathogen and antagonist, the growth of test fungus was completely restricted at point of contact and antagonists overgrown and parasitized the *F.o.* f.sp. *lycopersici* completely.

Strong antagonism by *Trichoderma* spp. against a range of soil-borne plant pathogens has been reported

Table 2. Antagonistic potential of *Trichoderma* spp. against *F.o.* f.sp. *lycopersici* by dual culture.

Isolate	Radial mycelial growth (mm)*	Per cent inhibition
Th-CARI-61	33.3	63.0
Th-CARI-72	40.9	54.5
Tv-CARI-73	33.5	62.8
Th-CARI-50	30.7	65.9
Th-CARI-70	45.1	49.9
Th-CARI-64	42.0	53.3
Tv-CARI-85	33.5	64.8
Tv-CARI-100	32.0	59.9
Tv-CARI-68	43.5	51.7
Th-CARI-130	38.2	57.5
Th-CARI-116	43.5	51.7
Th-CARI-110	49.6	44.9
Control	90.0	0.0
$CD_{(0.05)}$	0.802	-

*Means of four replications

(Pan *et al.*, 18; Pan and Bhagat, 17; Bhagat and Pan, 6, 8 & 9). The fungal biocontrol agent, *Trichoderma* is known to antagonize numerous soil borne pathogenic fungi *in vitro* and under greenhouse/ field conditions (Papavizas, 19; Harman *et al.*, 14; Bhagat and Pan, 5). Though the results of *in vitro* studies for the antagonistic potential of the biocontrol agents are not always same degree under field conditions, yet such studies are of immense important for initial screening of the large number of antagonists against host fungi (Bell *et al.*, 3; Papavizas, 19; Vinale *et al.*, 22). In the present experiment strong selectivity of the isolates of *Trichoderma* in their antagonistic efficiency towards the pathogen has been observed. Higher growth rate and greater competitive ability of the selected strain are indicative of their better antagonistic potential. Variability in antagonistic potential among the different species of *Trichoderma* against different pathogens has been reported (Bell *et al.*, 3; Dubey *et al.*, 13).

The biopriming of tomato seeds with *Trichoderma* isolates and bacterial antagonist, *Pseudomonas fluorescens* significantly improved the germination (%), vigour index and seedling biomass as compared to untreated control (Table 3). The results also suggested that mycelial form of inoculum was more effective than conidial form of inoculum in improving germination behaviour of tomato seeds. Highest germination (92.8%), vigour index (1058.8) and seedling biomass (442.6 mg) was recorded with Th-CARI-61 by using mycelial form of inoculum followed by Tv-CARI-85, Th-CARI-50, Tv-CARI-73, whereas, Th-CARI-130 was least effective for the same. The lone bacterial antagonist *Pseudomonas fluorescens* was also improved the germination behaviour (896% germination, 878.1 vigour index and 397.8 mg of seedling biomass) of tomato and was superior than few isolates of *Trichoderma*. The significant increase in germination, vigour index and seedling biomass resulted from the inherent capacity of bioagents to protect the seeds from *F.o.* f. sp. *lycopersici* as well as providing necessary nutrients to germinating seeds by decomposition of organic substrates. 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 tomato seedlings when treated with bioagents.

All isolates of *Trichoderma* were significantly inhibited the *F.o.* f.sp. *lycopersici* under sick plot conditions and reduced fusarial disease incidence in tomato upto 60 days after planting (Table 4). The results also indicated that seed priming + soil application was proved better than either seed priming or soil application of *Trichoderma* spp. alone. The disease incidence at 60 days was ranged from 16.4% (Th-CARI-50) to 37.0% (Tv-CARI-100) in the treated plot whereas 82% disease incidence was recorded in the untreated plot. The seed priming +

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

Isolate	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.
Th-CARI-61	92.8	89.7	4.5	4.0	7.2	6.8	1085.8	968.8	442.6	405.8
Th-CARI-50	91.5	87.4	4.2	3.9	6.9	6.6	1015.6	917.7	418.1	399.3
Th-CARI-72	85.3	82.4	3.9	3.4	5.7	5.2	835.9	708.6	344.9	322.4
Th-CARI-130	79.8	77.5	3.6	3.4	5.5	4.9	726.2	643.2	323.2	306.6
Tv-CARI-73	90.6	88.2	4.2	3.7	6.8	6.4	996.6	890.8	414.2	398.8
Tv-CARI-85	91.9	89.2	4.3	3.9	6.9	6.6	1029.3	936.6	436.3	400.7
Tv-CARI-100	86.6	83.4	3.9	3.6	5.7	5.4	831.4	750.6	335.5	311.9
P. fluorescens	89.6 @	89.6	4.0	4.0	5.8	5.8	878.1	878.1	397.8	397.8
Control	70.0 (56.79)	70.0 (56.79)	2.4	2.4	3.5	3.5	413.0	413.0	252.0	252.0

	Germination			Root length			Shoot length		
	Isolate	Form of inocula	Isolate × Form of inocula	Isolate	Form of inocula	Isolate × Form of inocula	Isolate	Form of inocula	Isolate × Form of inocula
$CD_{(0.05)}$	0.77	0.45	1.34	0.56	0.45	NS	0.56	0.45	NS

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

Table 4. Evaluation of *Trichoderma* isolates against wilt (*F.o.* f.sp. *lycopersici*) of tomato under greenhouse and field conditions.

Treatment	Greenhouse trial (pooled data of two years)			Field trial (pooled data of three years)				
	Germination (%)†	Per cent disease incidence*	% RDI	Field emergence (%)*	Per cent disease incidence*	% RDI	Yield (q/ha)*	% increase in yield over control
		60 DAP			75 DAP			
T_1	88.5	28.1	65.7	86.4	28.5	66.1	192.0	92.0
T_2	87.0	26.9	67.2	84.8	27.6	67.1	196.0	96.0
T_3	91.4	17.0	79.3	89.0	18.3	78.2	235.0	135.0
T_4	89.7	27.6	66.3	87.5	28.9	65.6	187.0	87.0
T_5	86.8	25.0	69.5	84.9	27.0	67.8	196.0	96.0
T_6	92.0	16.4	80.0	90.2	18.2	78.3	238.0	138.0
T_7	76.0	36.2	55.8	74.5	36.9	56.1	157.0	57.0
T_8	72.3	34.0	58.5	70.1	34.7	58.7	165.0	65.0
T_9	82.5	23.0	71.9	80.8	24.0	71.4	205.0	105.0
T_{10}	86.5	29.2	62.2	84.1	30.5	63.7	180.0	80.0
T_{11}	82.7	25.6	68.8	79.9	26.8	68.1	195.0	95.0
T_{12}	91.0	17.5	78.6	90.0	18.4	78.1	236.0	136.0
T_{13}	88.0	28.5	65.2	88.0	28.9	65.5	191.0	91.0
T_{14}	82.8	24.2	70.5	80.0	25.1	70.1	199.0	99.0
T_{15}	91.5	17.2	79.0	89.6	18.0	78.6	235.0	135.0
T_{16}	82.0	37.0	54.9	81.0	37.5	55.3	151.0	51.0
T_{17}	77.7	32.9	59.9	75.8	33.1	60.6	170.0	70.0
T_{18}	84.0	25.8	68.5	81.9	26.0	69.0	197.0	97.0
Control	60.4	82.0	0.0	64.0	84.0	0.0	100.0	0.0
$CD_{0.05}$	1.404	0.212	-	3.168	1.338	-	0.648	-

†Means of 100 seedlings observed; *Means of four replications; DAP = Days after planting; RDI = Reduction in disease incidence

soil application of Th-CARI-50 was most effective in inducing germination (92.0%), lowest disease incidence (16.2%) and highest reduction of disease incidence (80.0%) as compared to untreated plot. The other promising isolates were Th-CARI-61, Tv-CARI-85, Tv-CARI-73, which were statistically at par in reducing the fusarial disease incidence when they were applied as seed + soil application.

The results presented in Table 4 revealed that all treatments significantly reduced the fusarial disease incidence in tomato as compared to control. The application of antagonistic fungus, *Trichoderma* as seed treatment and soil application alone have significant effect in reducing disease incidence at all the dates observed and per cent increase of yield over control but the combination of seed treatment + soil application of *Trichoderma* isolates had significantly edge over the seed or soil application alone. T_6 treatment (Th-CARI-50) was most effective in improving field emergence (90.2%), reduction in disease incidence (78.8%) and corresponding yield increase (138.0%) of tomato. The other treatments in the descending order of chronology in relation to per cent reduction in disease incidence and increase in yield, were T12 (Tv-CARI-73 – 78.1 & 136.0%), T15 (Tv-CARI-85 – 78.6 & 135%) and T3 (Th-CARI-61- 78.2 & 135.0%), which were statistically at par, whereas, the isolate Th-CARI-130 and Tv-CARI-100 were least effective with less than 55.0% RDI of fusarial wilt of tomato. The disease incidence was increased with crop growth stage and reached to 84.0% at 75 DAS in the control plot. Similar trend was also observed in treated plot but all treatments have significantly less disease incidence as compared to control.

Seed priming with bioagents for protection of seeds and control of seed borne diseases offers the growers/farmers an alternative means of chemical fungicides. 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.*, 11). Several researchers have reported the biological

seed treatments for protection of seed and control of pathogens causing seedling diseases (Bennett *et al.*, 4; Bhagat and Pan, 5 & 9). Likewise, there are several reports on biocontrol of pathogens *in vivo* and under field conditions (Dubey *et al*., 13; Bhagat and Pan, 5 & 6). The addition of microbial biocontrol agents during biopriming allows for colonization of the seed prior to planting and adds a new dimension to seed priming treatment. Pre-colonization provides the biocontrol agent with a competitive advantage over pathogens and often provides superior seed protection when compared to seed coating (Harman and Taylor, 15; Harman, 16). In present investigation there were significant increase in per cent germination of seeds, seedling vigour index and biomass of treated tomato seeds. The present results are in accordance with the findings of Chacon *et al*. (12), where they reported that increased plant fresh weight (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 Petri dishes inoculated with *T. harzianum* conidia. In our previous study, significant enhancement of germination (%) and seedling vigour index of solanaceous as well as leguminous vegetables have been reported (Bhagat and Pan, 8 & 9).

In the present investigation, the combined application of seed and soil application of *Trichoderma* spp. isolates gave better result than seed or soil application alone. The possible reason may be due to failure of *Trichoderma* isolates to proliferate readily in the soil and confined to seed coat/seed surface in case of seed treatment only, while the *Trichoderma* spp. well establish in the soil system (soil application) which can proliferate readily near root zone of plants, compete for root exudates, nutrient and/or space with other microorganisms. Infact, *Trichoderma* spp. occupy themselves in the vicinity of root hairs/or tips, which otherwise provide an ample chance of pathogen's attack. Simultaneous application of of *Trichoderma* as seed priming and soil incorporation results into a protective cover in the seed coat by rapid multiplication in the spermatosphere applied in seed as well as the *Trichoderma* population applied to soil, have enough strength to out compete the other microorganisms or directly parasitizing *in situ*.

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