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Plant growth promoting activity of some indigenous *Trichoderma* isolates and their field performance against sheath blight of rice in old alluvial zone of North Bengal

Goutam Mondal

Regional Research Station (Old Alluvial Zone), Uttar Banga Krishi Viswavidyalaya, Majhian, Patiram-733133, Dakshin Dinajpur, West Bengal, India. E-mail: gm_rrsubkv@yahoo.co.in

A B S T R A C T

Five *Trichoderma* spp. were isolated from the old alluvial zone of North Bengal and their biocontrol activity were tested in dual culture and found effective against soil-borne plant pathogens, namely, *Fusarium oxysporum* f. sp. *ciceri* (Padw.) Matuo. and *Rhizoctonia solani* Kühn. Efficacy in plant growth promoting activity of all the isolates was also tested on cauliflower (*Brassica oleracea* var *botrytis* L.), chilli (*Capsicum frutescens* L.) and tomato (*Lycopersicon esculentum* Mill.) with culture filtrate of biocontrol agents and found both positive and negative effect on seed germination, plant growth and vigour. Maximum increase in germination by 13.64% (chilli), shoot length by 59.69% (tomato), root length by 84.51% (cauliflower) and biomass by 46.55% (cauliflower) were obtained by the isolate B16 followed by B14 and B13. However, the isolate, B18 showed negative result with maximum reduction in germination by 25.56% (cauliflower), shoot length by 67.06% (cauliflower), root length by 88.50% (cauliflower) and biomass by 34.91% (chilli). Whereas, in the field experiment with talc based bioformulation ($3x10^8$ cfu), all *Trichoderma* isolates, particularly the isolates, B18 and B16, were found effective to increase yield (12.50% to 23.14%) and reduce rice sheath blight disease incidence (46.32% to 70.54%) over check plot significantly. Again, the efficacy of the biocontrol agents was significantly greater in the block with green manured than the block without green manured. Application of hexaconazol 5% EC@ 2.0 ml $litre^{-1}$ and carbendazim 50% WP @ 1.0 ml $litre^{-1}$ also reduced the sheath blight disease incidence by 53.25% and 56.86% over the check plot respectively. It was interesting to observe that all biocontrol agents were found effective significantly than the fungicides. Therefore, the fungal biocontrol agents, particularly, B18 and B16 could be used as biofungicide as well as biofertilizer for the management of sheath blight of rice.

***Keywords*:** *Trichoderma* spp., *Rhizoctonia solani*, sheath blight of rice, plant growth promotion

Introduction

Sheath blight of rice caused by *Rhizoctonia solani* Kühn is one of the most important diseases in all the rice growing areas of the world (Tamilvanan & Kandhari 2009; Naeimi *et al.* 2010; Prasad & Kumar 2011) causing considerable yield loss, 25-50% from Philippines and Vietnam, 20-50% from Japan and 5.2-70% from India (Naidu 1992). Roy (1993) recorded loss in grain yield ranging from 10-36% in Assam. The disease has become one of the major constraints for rice cultivation in West Bengal, particularly in the northern part. The pathogen can survive for a long period in soil, rice stubbles and seeds (Roy 1993). The pathogen has a very wide host range, and strong sources of resistance in rice against this disease have yet to be found out (Singh *et al*. 2002; Anonymous 2006). The disease has been more alarming due to intensive cultivation of modern high yielding varieties with use of high dose of nitrogenous fertilizer. All popular varieties cultivated in this zone have been found susceptible to this pathogen.

Chemical pesticides have proved unsafe for humans, animals, fishes and other non-target beneficial organisms, with toxic residues and its by-products to contaminate the environment (Anon 2006; Tamilvanan & Kandhari 2009; Naeimi *et al.* 2010; Prasad & Kumar 2011). Thus, use of pesticides is posing a big threat to social as well as economic and environmental issues and thus will continue to be the target of public pressures worldwide. Resistance to conventional pesticides is one of the three most important challenges facing the future agriculture, besides pollution and soil erosion. At least 200 plant pathogens are known to exhibit fungicide resistance (Dover & Croft 1984; Carlton 1988). In fact, all the current popularly used fungicide group viz. benzimidazoles, phenylamides, morpholines, dicarboximides conilinopyrimidenes and pyrroles have a high inherent risk for resistance, and have lost substantial market share due to resistance, particularly benzimidazoles and dicarboximides (Urech 1997). The impetus for developing biocontrol agents has been the public perception of pesticide toxicity in the environment (Steyaert *et al.* 2003). In this context, biocontrol of plant pathogens using mycoparasite attracted wide spread attention in recent year with many commercial biofungicides available worldwide. Soil application of *neem* cake, foliar spray with leaf extract of *Ocimum sanctum,* cow dung etc are other methods to reduce incidence of the sheath blight of rice and its severity (Anonymous, 2006). Application of *Trichoderma* has also been found to effective and was reported to reduce disease incidence by 10-70% (Anonymous 2006; Tamilvanan & Kandhari 2009; Naeimi *et al.* 2010; Prasad & Kumar 2011). Biological control of plant pathogens using microorganisms has been investigated intensively because of lack of alternative management (Mishra *et al.* 2011). It has been found that the efficacy of biocontrol agent isolated from local or same agro climatic zone is found more effective than originated from other zone (Naeimi *et al.* 2010). Keeping this in view, a field experiment was conducted to find out the efficacy and field performance of some indigenous isolates of *Trichoderma* spp. against sheath blight of rice.

Materials and Methods

Several *Trichoderma* spp. were isolated from the agro ecological zone of Old alluvial Zone of the Northern part of West Bengal. Among them, five biocontrol agents (isolates No. B1, B13, B14, B16 and B18) were identified after screening through dual culture test against two destructive and widespread soil-borne pathogens*, F. oxysporum* f. sp. *ciceri* (Padw.) Matuo. and *R. solani*. For observation the interaction between *Trichoderma* isolate B1 and pathogen was observed under microscope using following technique. Sterilized molten potato dextrose agar (PDA) medium was poured in a sterilized Petri plate containing two glass microscope slides placed side by side in such a way that the depth of the medium on the slides was approximately 1.0 mm. *Trichoderma* sp. B1 and the pathogen were inoculated on either side of each slide and the plates were incubated at 28 ± 1^{0}C for 7 days. The slides were taken out and the me-

dium was discarded from both the sides keeping the interaction zone intact and then, lactophenol blue was poured on it for observation under microscope. The culture filtrate was prepared by culturing the five isolates separately in 250 ml conical flask containing 25 ml potato dextrose broth (PDB) and incubated at 28±1^{0}C for 21days. The broth was filtered by using Whatman filter paper (No.42) and the filtrate of each isolate was used for the further experiment. The efficacy of *Trichoderma* isolates on germination and growth of crop plant, viz., cauliflower (*Brassica oleracea* var *botrytis* L.), chilli (*Capsicum frutescens* L.) and tomato (*Lycopersicon esculentum* Mill.) was carried in Petriplate with following procedure. Two Whatman filter papers (No.42) were kept in each Petri plate, sterilized and wetted by pouring 2ml culture filtrate of *Trichoderma* isolates. Twenty five seeds were kept on the soaked filter papers in each Petri plate, replicated thrice for each isolate and incubated at 28±1^{0}C for 5 days.

For field study, a talc based bioformulation of *Trichoderma* spp. consisting 10% carboxy methyl cellulose (CMC) was prepared with 3x10^8 cfu to use in field. The field experiment was conducted in *kharif* season in the year 2009 at Regional Research Station (Old Alluvial Zone), Uttar Banga Krishi Viswavidyalaya, Majhian, Patiram, Dakshin Dinajpur, WB, India. The experimental field was made rice sheath blight disease sick by artificial inoculation consecutively over more than three year with *R. solani*. The field was designed as split plot with 3 replications where each replication is divided into two blocks. Out of two blocks in each replication, one block was green manured with dhaincha [*Sesbania aculeata* (Willd.) Pers.] *in situ* and the other block was kept fallow from beginning of the experiment. The plot size was 16m x 5m and transplanted rice seedling spacing was 20cm x 15cm. The germinated seed and seedling of rice (cv. Swarna) were treated with the bioformulation of *Trichoderma* spp. @ 5 g litre^{-1} of water by dipping and spraying, respectively. For soil application, bioformulation @ 7.5 kg ha^{-1} was mixed in soil at the time of final land preparation. Two commonly used fungicides, namely, hexaconazol 5% EC @ 2.0ml litre^{-1} and carbendazim 50% WP @ 1.0 mllitre^{-1} were taken as chemical control along with another check plot without any treatment. The fungicides were applied as seed treatment and also seedling treatment as spray in the nursery plot 7 days before transplanting. Two sprays with fungicides were given in the main field, first at 21 days after transplanting (DAT) and second at 42 DAT. All the plots were given uniform fertilizer dose @ 30:40:40 NPK kg ha^{-1} at the time of final land preparation and @ 30 N kg ha^{-1} after 42 DAT. Data for percentage of disease incidence were taken as number of infected tiller over healthy tiller. INDOSTAT software was used for statistical calculation.

Results and Discussion

During screening for biocontrol activity in dual culture tests, all the isolates showed hyper parasite over the pathogens (Fig 1& 2).

Under light microscope, it was noticed that at the interaction zone the hyphae of *Trichoderma* isolate B1 coiled around the hyphae of pathogen, *R. solani* (Fig 3a). Crystalline structures at the interaction zone were also noticed in dual culture plate of isolate B1 and *F. oxysporum* f. sp. *ciceri* and hyphae of the pathogen were vacuolated and coagulated (Fig 3b). The phenomenon of hyphal contact, coiling, penetration and lysis of hypha of host pathogen is a common phenomenon (Heydari & Pessarakli 2010) and reported by several workers in *Trichoderma* (Steyaert *et al.* 2003; Almeida *et al.* 2007). Coiling capacity is an important attribute for effective biocontrol (Almeida *et al.* 2007). Crystalline structures were observed at the interaction zone under scanning electron microscope (Mondal 1998). Patibanda (1995) reported larger irregular particles at the interaction zone of *A. niger*AN27 and *F. oxysporum* f. sp. *melonis* and assumed that the particles were produced by the former. Toxin has been noted in interactions involving *Trichoderma* spp. and causing vacuolization and coagulation in the host hyphae (Dennis &Webster 1971).

Plant vigour is an important factor for yield and resistance of crop plants (Corsini *et al.* 1989). Fungi and bacteria have been reported to increase crop vigour besides disease reduction (Ebenezar *et al.* 1996; Kumar & Bezbaruah 1996). In this experiment, all the isolates had both positive and negative effect on seed germination, plant growth and vigour tested with culture filtrate of the biocontrol isolates (Table 1, 2 & 3). In this context, positive results on germination and plant growth were showed by four biocontrol isolates, namely, B1, B13, B14 and B16. Among them, B16 isolate showed better results on germination, shoot length, root length and biomass followed by B14 and B13. Maximum increase in germination by 13.64% (chilli), shoot length by 59.69% (tomato), root length by 84.51% (cauliflower) and biomass by 46.55% (cauliflower) were obtained with the isolate B16. However, the isolate, B18 showed negative result with maximum reduction in germination by 25.56% (cauliflower), shoot length by 67.06% (cauliflower), root length by 88.50% (cauliflower) and biomass by 34.91% (chilli). In this regard, a general characteristic of any growth regulator that promotes plant growth inhibits or retards at higher concentration (Thimann 1972). So, it may be assumed that as result of containing higher concentration of secondary metabolite(s) in culture filtrate of B18 was the cause of inhibition of seed germination and plant growth.

In the field experiment, all the bioformulations of *Trichoderma* isolates were found effective to increase yield (12.50% to 23.14%) significantly over check plots and the isolate, *Trichoderma* sp. B18 was given the highest yield (Table 4). Again, the efficacy of the biocontrol agents was significantly greater in the block green manured with dhaincha than the block without green manured as green manure enhance the activity and multiplication rate of *Trichoderma*. The rice sheath blight disease incidence caused by *R. solani* was significantly reduced with 46.32% to 70.54% on ap-

plication of bioagent and two fungicides (hexaconazol 5% EC and carbendazim 50% WP) comparing with the check plot. In general, *Trichoderma* species are ubiquitous fungi in the soil and have an antagonistic activity against several soil-borne plant pathogens including *R. solani* (Naeimi *et al.* 2010). It was also observed that the all biocontrol agents were found significantly effective than the fungicides. The *Trichoderma* spp. B18 and B16 were performed better in disease reduction. This result is consistent with the observation of Tamilvanan & Kandhari (2009), Naeimi *et al.* (2010), Prasad & Kumar (2011) who all found that *Trichoderma* spp. was an effective biocontrol agent in controlling rice sheath blight. Therefore, in this context, it can be concluded that all the fungal biocontrol agents, particularly, *Trichoderma* isolates B18 and B16, were found significantly effective in field and could be used as biofungicides and biofertilizer for the management of sheath blight of rice and to increase yield.

Literature Cited

Almeida FBdR Cerqueira FM Silva RdN Ulhoa CJ Lima AL. 2007 Mycoparasitism studies of *Trichoderma harzianum* strains against *Rhizoctonia solani*: evaluation of coiling and hydrolytic enzyme production. *Biotechnology Letters* **29** (8): 1189-93.

Anonymous. 2006 Rice fact sheet, International Rice Research Institute, Rice Knowledge Bank, Rice Knowledge Management Portal (RKMP) http://rkmp.co.in/sites/default/files/ris/rice-state-wise/Status%20Paper%20on%20Rice%20in%20West%20Bengal.pdf

Carlton BC. 1988 Development of genetically improved strains of *Bacillus thuringiensis*- a biological insecticide. In *Biotechnology for Crop Protection,* American Chemical Society, pp 260-79.

Corsini DL Thompson C Pavek JJ. 1989 The effect of plant growth regulators on verticillium wilt of potato. *American Potato Journal* **66**: 125-36.

Dennis C Webster J. 1971 Antagonistic properties of species-groups of *Trichoderma*. III. Hyphal interaction. *Transactions of the British Mycological Society* **57**: 363-69.

Dover M Croft B. 1984 'Getting Tough' Public policy and the management of pesticide resistance.World Resource Institute Publication, No.1/Washington, DC (USA), 80p.

Ebenezar EG Alice D Sivaprakasam K. 1996 Biological control of damping off disease in mustard caused by *Pythium aphanidermatum*. *Journal of Ecobiology* **8**: 55-57.

Heydari A Pessarakli M. 2010 A Review on Biological Control of Fungal Plant pathogens using microbial antagonists. *Journal of Biological Sciences* **10**(4): 273-90.

Kumar BSD Bezbaruah B. 1996 Antibiosis and plant growth promotion by a *Pseudomonas* strain isolated from soil under tea cultivation. *Indian Journal of Microbiology* **36:** 45-48.

Mishra DS Gupta AK Prajapati CR Singh US. 2011 Combination of fungal and bacterial antagonists for management of root and stem rot disease of soybean. *Pakistan Journal of Botany* **43**(5): 2569-74.

Mondal G. 1998 *In vitro* evaluation of *Aspergillus niger* AN27 against soilborne fungal plant pathogens and field testing against *Macrophomina phaseolina* on potato. *Ph.D. Thesis*, IARI, New Delhi, 117p.

Naeimi S Okhovvat SM Javan-Nikkhah M Vagvolgyi C Khosravi V Kredics L. 2010 Biological control of *Rhizoctonia solani* AG1-1A, the causal agent of rice sheath blight with *Trichoderma* strains. *Phytopathological Mediterranea* **49**: 287-300.

Naidu VD. 1992 Influence of sheath blight of rice on grain and straw yield in some popular local varieties. *Journal of Research Publication* **10**: 78-80.

Patibanda AK. 1995 Interactions between *Fusarium oxysporum* f. sp. *melonis,* muskmelon wilt pathogen and *Aspergillus niger,* its potential biocontrol agent. *Ph.D. Thesis,* IARI, New Delhi, 81p.

Prasad BN Reddi M Kumar MR. 2011 Comparative efficacy of different isolates of *Trichoderma* spp. against *Rhizoctonia solani*, incitant of sheath blight of rice. *Indian Journal of Fundamental and Applied Life Science* **1**(3): 107-11.

Roy AK.1993 Sheath blight of rice in India. *Indian Phytopathology* **46**: 197-05.

Singh R Kumar A Jalali BL. 2002 Variability, predisposing factors and management of stem rot of rice caused by *Sclerotium oryzae* Catt. : An overview. *Annual Review of Plant Pathology* **1**: 275-89.

Steyaert JM Ridgway HJ Elad Y Stewart A. 2003 Genetic Basis of Mycoparasitism: a mechanism of biological control by species of *Trichoderma*. *New Zealand Journal of Crop and Horticulture Science* **31**: 281-91.

Tamilvanan M Kandhari J. 2009 Bio-control of rice sheath blight through antagonists in field. *Indian Phytopathology* **62**(2): 204-08

Thimann KV. 1972 The natural plant hormones. In *Plant Physiology, Vol. 6B: Physiologyof Development - The Hormones* (Ed Steward FC), Academic Press, New York, 365p.

Urech PA Staub T Voss G. 1997 Resistance on a concomitant of modern crop. *Pesticide Science* **51**: 227-34.

Table 1.

Efficacy of *Trichoderma* isolates on seed germination and plant growth of cauliflower

Treatment	Germination (%)	Change over control	Shoot length (cm)	Change over control	Root length (cm)	Change over control	Biomass (gm)	Change over control
Trichoderma sp. B1	98.89 (9.94)[a]	-1.11	1.27[e]	-25.29	1.28[e]	-43.36	12.46[e]	-11.44
Trichoderma sp. B13	92.22 (9.60)[b]	-7.78	1.56[d]	-8.24	2.59[c]	14.60	15.08[c]	7.18
Trichoderma sp. B14	100.00 (10.00)[a]	0.00	2.06[b]	21.18	3.33[b]	47.35	19.10[b]	35.75
Trichoderma sp. B16	98.89 (9.94)[a]	-1.11	2.19[a]	28.82	4.17[a]	84.51	20.62[a]	46.55
Trichoderma sp. B18	74.44 (8.63)[c]	-25.56	0.56[f]	-67.06	0.26[f]	-88.50	9.59[f]	-31.84
Control	100.00 (10.00)[a]	-	1.70[c]	-	2.26[d]	-	14.07[d]	-
CV (%)	0.65		1.92		0.62		1.55	
CD (95%)	0.11		0.05		0.03		0.43	
S.E.Diff.	0.05		0.02		0.01		0.19	

Data in the parenthesis are the square root transformation for statistical calculation. Data are the average of three replications. Values followed by the same letter do not differ significantly (P = 0.05).

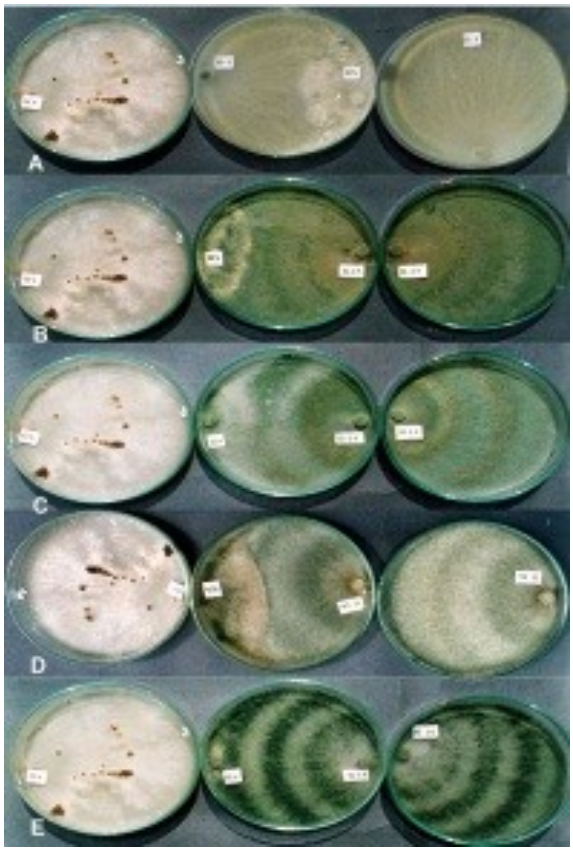

Fig 1

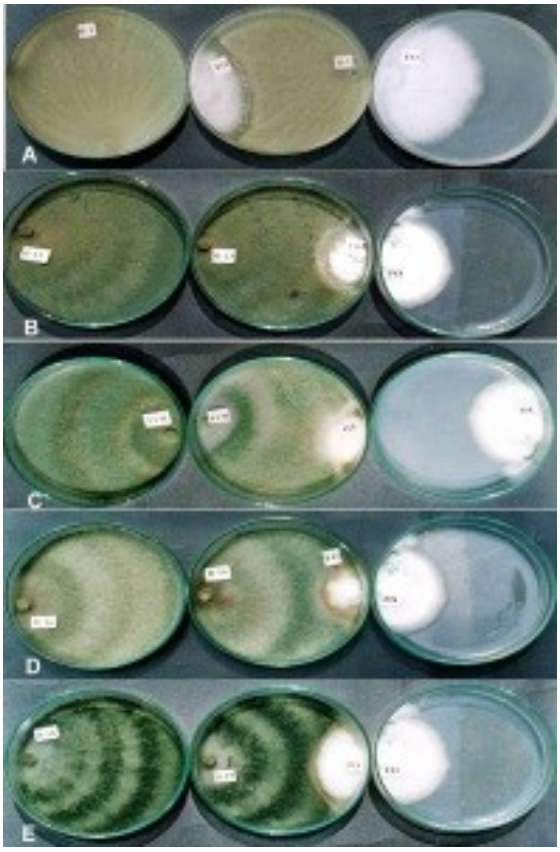

Fig 2

Fig 1. Interaction between biocontrol agent *Trichoderma* spp. and pathogen, *Rhizoctonia solani* (RS) in dual culture plate incubated at 28±1^{0}C for 7 days: (A) *Trichoderma* isolate B1 x RS, (B) *Trichoderma* isolate B13 x RS, (C) *Trichoderma* isolate B14 x RS, (D) *Trichoderma* isolate B16 x RS and (E) *Trichoderma* isolate B18 x RS, where dual culture plates are in the middle one and control plates of pathogen and biocontrol agent are at left and right side, respectively.

Fig 2. Interaction between biocontrol agent, *Trichoderma* spp. and pathogen, *Fusarium oxysporum ciceri* (FO) in dual culture plate incubated at 28±1^{0}C for 7 days: (A) *Trichoderma* isolate B1 x FO, (B) *Trichoderma* isolate B13 x FO, (C) *Trichoderma* isolate B14 x FO, (D) *Trichoderma* isolate B16 x FO and (E) *Trichoderma* isolate B18 x FO, where dual culture plates are in the middle one and control plates of pathogen and biocontrol agent are at right and left side, respectively.

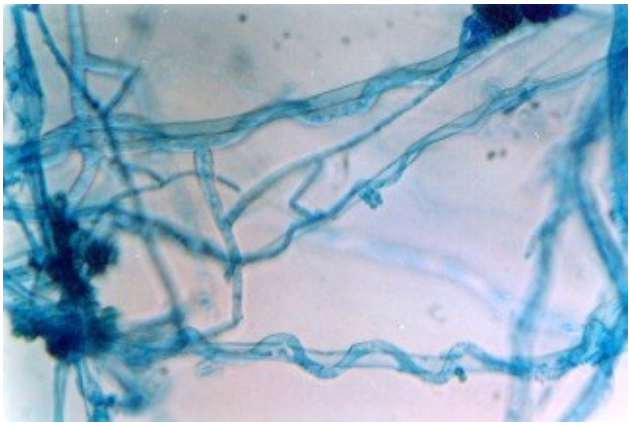

Fig. 3a

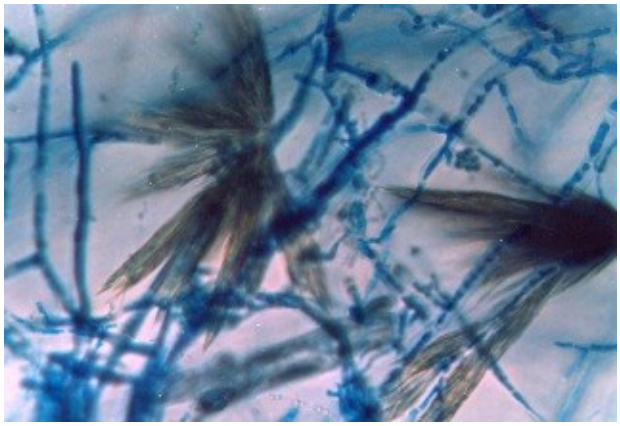

Fig. 3b

Fig 3. Observation of interaction between biocontrol agent, *Trichoderma* isolates B1 and pathogens in dual culture plate under light microscope: (Fig 3a) Hyphal coiling of *Trichoderma* isolates B1 on hyphae *Rhizoctonia solani* (RS). (Fig 3b) Formation of crystalline structures at interaction zone of isolate B1 and *Fusarium oxysporum ciceri* and hyphae of the pathogen were vacuolated and coagulated.

Table 2.

Efficacy of *Trichoderma* isolates on seed germination and plant growth of chilli

Treatment	Germination (%)	Change over control	Shoot length (cm)	Change over control	Root length (cm)	Change over control	Biomass (gm)	Change over control
Trichoderma sp. B1	92.38 (9.61)^{b}	10.23	1.90^{e}	-6.86	1.63^{c}	23.48	14.70^{e}	-0.54
Trichoderma sp. B13	86.67 (9.31)^{c}	3.41	2.38^{c}	16.67	1.49^{d}	12.88	15.33^{c}	3.72
Trichoderma sp. B14	93.33 (9.66)^{ab}	11.36	3.13^{a}	53.43	2.21^{a}	67.42	19.78^{a}	33.83
Trichoderma sp. B16	95.24 (9.76)^{a}	13.64	2.65^{b}	29.90	1.71^{b}	29.55	16.40^{b}	10.96
Trichoderma sp. B18	92.38^{b} (9.61)	10.23	1.59^{f}	-22.06	1.15^{f}	-12.88	9.62^{f}	-34.91
Control	83.81 (9.15)^{d}	-	2.04^{d}	-	1.32^{e}	-	14.78^{d}	-
CV (%)	0.75		2.82		1.61		0.22	
CD (95%)	0.13		0.12		0.05		0.06	
S.E.Diff.	0.06		0.05		0.02		0.03	

Data in the parenthesis are the square root transformation. Data are the average of three replications. Values followed by the same letter do not differ significantly (P = 0.05).

Table 3.
Efficacy of *Trichoderma* isolates on seed germination and plant growth of tomato

Treatment	Germination (%)	Change over control	Shoot length (cm)	Change over control	Root length (cm)	Change over control	Biomass (gm)	Change over control
Trichoderma sp. B1	100.00 (10.00)[a]	0.00	3.82[b]	-1.29	1.74[c]	-2.79	18.52[c]	1.20
Trichoderma sp. B13	100.00 (10.00)[a]	0.00	4.57[a]	18.09	1.84[a]	2.79	19.04[b]	4.04
Trichoderma sp. B14	98.10 (9.90)[b]	-1.90	2.54[d]	-34.37	0.51[e]	-71.51	16.48[e]	-9.95
Trichoderma sp. B16	100.00 (10.00)[a]	0.00	6.18[a]	59.69	1.84[a]	2.79	25.19[a]	37.65
Trichoderma sp. B18	100.00 (10.00)[a]	0.00	3.48[c]	-10.08	1.39[d]	-22.35	16.37[e]	-10.55
Control	100.00 (10.00)[a]	-	3.87[b]	-	1.79[b]	-	18.30[d]	-
CV (%)	0.33		0.80		1.66		0.54	
CD (95%)	0.06		0.06		0.05		0.19	
S.E.Diff.	0.03		0.03		0.02		0.08	

Data in the parenthesis are the square root transformation. Data are the average of three replications. Values followed by the same letter do not differ significantly (P = 0.05).

Table 4.
Effect of *Trichoderma* isolates on sheath blight disease and yield of rice (cv. Swarna)

Treatment	Block-I: without Dhaincha (B_1)		Block-II: with Dhaincha (B_2)		Block-I: without Dhaincha (B_1)		Block-II: with Dhaincha (B_2)	
	[1]Disease incidence (%)	[2]Disease reduction (%)	[1]Disease incidence (%)	[2]Disease reduction (%)	[1]Yield (kg ha^{-1})	[2]Increase (%)	[1]Yield (kgha^{-1})	[2]Yield increase (%)
Trichoderma sp. B1	11.17 (3.34)	60.30	6.03 (2.46)	70.54	5633.33	14.19	6683.33	14.57
Trichoderma sp. B13	13.43 (3.66)	52.25	7.33 (2.71)	64.19	5550.00	12.50	6783.33	16.29
Trichoderma sp. B14	12.37 (3.52)	56.04	6.77 (2.60)	66.93	5600.00	13.51	7116.67	22.00
Trichoderma sp. B16	8.97 (2.99)	68.12	4.53 (2.13)	77.87	5766.67	16.89	6850.00	17.43
Trichoderma sp. B18	8.03 (2.83)	71.44	3.33 (1.82)	83.73	6033.33	22.30	7183.33	23.14
Hexaconazol 5EC @ 2.0 ml litre^{-1}	14.33 (3.78)	49.05	9.57 (3.09)	53.25	5350.00	8.45	6016.67	3.14
Carbendazim 50WP @ 1.0 ml litre^{-1}	15.10 (3.88)	46.32	8.83 (2.97)	56.86	5533.33	12.16	6083.33	4.29
Check	28.13 (5.31)	-	20.47 (4.52)	-	4933.33	-	5833.33	-
	Main plot	Sub plot	Interaction		Main plot	Sub plot	Interaction	
			B x T	T x B			B x T	T x B
CD (95%)	0.18	0.17	0.24	0.27	310.13	456.33	645.35	660.06
SE Diff.	0.04	0.08	0.17	0.12	72.08	222.77	315.05	303.39

Data in the parenthesis are the square root transformation. [1]Data are the average of three replications. [2]Data in percentage were calculated over the data of check plot.