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Field Demonstration of *Trichoderma harzianum* as a Plant Growth Promoter in Wheat (*Triticum aestivum* L)

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Abstract

A three year study (2008-2011) on the validation of the *Trichoderma* technology for growth promoting ability of *Trichoderma harzianum* (Th3) was evaluated in the popular wheat (*Triticum aestivum* L.) variety Raj 3765 at farmer's field through TIFAC-DST project entitled "On Farm Demonstration and Commercial Production of *Trichoderma* as Biopesticide and Growth Promoter". The biological formulation was developed at Biological Control Laboratory, Division of Plant Pathology, IARI, New Delhi, and was successfully demonstrated in two districts of Rajasthan viz., Jaipur and Kota belonging to different agro climatic zones. Rhizospheric Competence Index along with its growth promotion effect on rootlets, tillers, weight of grains and grain yield were evaluated by using it at three stages of crop viz., seed, flowering and preharvesting @ 4g/kg and @ 4ml/L along with soil treatment with a mixture of farm yard manure and formulation @ 50:1 before sowing. Compared to the first year where the farmers were unaware of *Trichoderma* in 2008-09, a significant increase in yield of wheat from 36.25 to 46.73Q/ha (29% in Jaipur) and from 36.88 to 50.12Q/ha (36% in Kota) has been observed after continuous application for three years (2008-2011) The total income and the benefit cost ratio of farmers increased both at Jaipur (Rs 56242/ha, 1:1.8) and Kota (Rs 60332/ha, 1:1.9).

Keywords: *Trichoderma harzianum*, plant growth promotion, wheat, rhizosphere competence, yield

1. Introduction

Trichoderma spp. are most popular research tools as microbial inoculants which have been largely used against several plant pathogenic fungi causing soil borne, air borne and post harvest diseases of plant through their high antagonistic and mycoparasitic potential in lab conditions. In recent years, they have become popular as plant growth promoter (Hermosa et al., 2012). Some *Trichoderma* rhizosphere-competent strains have been shown to have direct effects on plants, increasing their growth potential and nutrient uptake, fertilizer use efficiency, percentage and rate of seed germination, and stimulation of plant defences against biotic and abiotic damage (Shoresh et al., 2010). Studies have demonstrated that *Trichoderma* increases root development, crop yield, proliferation of secondary roots, seedling fresh weight and foliar area. *Trichoderma* spp. was found to colonize the root epidermis and outer cortical layers and release bioactive molecules that cause walling off of the *Trichoderma* thallus. In addition to induction of pathways for resistance in plants, increased plant growth and nutrient uptake also occurs (Harman, 2006; Sharma et al., 2011).

The success of fungal biocontrol agent depends on its colonizing ability in rhizospheric region of any crop and also on the soil structure of different agro climatic zones for which field evaluation is required. The present study was based on the field demonstration of bioformulation of *Trichoderma harzianum* strain Th3 in wheat crop (variety Raj 3765) at two different agro climatic zones of Rajasthan (viz., Jaipur- "Semi- Arid Eastern Plains" and Kota- "Humid South-Eastern Plains"). Rhizospheric competence (R.C.) index and colony forming unit (C.F.U) of *Trichoderma harzianum* was observed at three stages of crops viz., seedling, flowering and pre-harvesting to study the colonization ability of Th3 on the rhizosphere of wheat crop in two different soil types at two different locations of Rajasthan. The effect of Th3 application on the yield and growth promoting parameters were studied.

2. Materials and Methods

2.1 Study Sites and Plant Species

The assays of plant growth promotion were carried out in the experimental field of Jaipur ($27^0$00'N Latitude and $75^0$82'E Longitude) and Kota ($25^0$10'N Latitude and $75^0$52'E Longitude). Jaipur comes under "Semi- Arid Eastern Plains" zone covering 11,152 km^2 areas (Av. elevation- 431m), having clay and sandy soil and Kota comes under "Humid South-Eastern Plains" zone covering 12,436km^2 areas (Av. elevation- 271m), having black soil. The annual rain fall in Jaipur and Kota are 650 mm and 885 mm respectively. Temperature varies from 25^0-45^0C in summers and from 5^0-22^0C in winters at Jaipur and from 30^0- 43^0C in summers and from 11^0- 25^0C in winters at Kota. The agronomic plant used was rust resistant and heat tolerant variety of Wheat viz. Raj-3765 which was sown during first week of October with a spacing of 15x18cm.

2.2 Use of Lab Bioformulation of Trichoderma harzianum Th3

The bioformulation used as inoculants in different seed, seedling and foliar treatments were prepared by using *Trichoderma harzianum* (Th3) strain in powdered ($2x10^8$ CFU/g) and liquid ($2x10^8$CFU/g) forms developed by Biological control Laboratory, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi.

2.3 Experimental Design

The field experiments were carried out to evaluate the growth promotion in wheat crops treated with bioformulation (Th3). Eight fields (2500 sq. m) were selected at both Dodhsar village (Jaipur) and Digodh village (Kota) for the study. Out of eight, four fields of each village were considered for Th3 bioformulation application to the farmers practice (Table 1) represented as '+Th3' and remaining four fields were considered without application represented as '-Th3'. For measurement of root colonization and plant growth promotion, 125-130 kg/ha seeds of the wheat variety Raj- 3765 were sown at both sites. At sowing time seeds were treated with bioformulation @ 4g/kg. As per the recommended practices for the fertilizers viz., farm yard manure (FYM), diammonium phosphate (DAP), Urea and Super Phosphate were used @ 6-7 Tons/ ha, 1.5-2.0 Q/ha,1.5-2.0 Q/ha, and 3.5-4.0 Q/ha respectively and other pesticides viz., Mancozeb (1.0-2.0 l/ha), Carbendazim (1.0-2.0 l/ha) and Endosulphan (1.5-2.5 l/ha) were used. Crop irrigation was at regular interval of 20, 40, 60 and 80 days after sowing. Weeds were manually removed from the crop.

Table 1. Application module of *Trichoderma harzianum* (Th3) bioformulation (2008-11)

Crop Stages	Mode of Treatment	Dose
Soil	Powder bioformulation + FYM application (1:50)	4g/kg soil
Seed/ Seedling	Powder bioformulation	4g/kg seed
Flowering/ Foliage	Powder and liquid bioformulation	Powdered bioformulation+ FYM (1:50), Liquid 4ml/L
Before harvesting	Liquid bioformulation	4ml/L

2.4 Rhizospheric Competence Index of Trichoderma harzianum (Th3)

Rhizospheric soil samples were collected from four +Th3 and -Th3 fields each, at a depth of 1cm, 3cm and 5cm during the seedling, flowering and pre-harvesting stages respectively. Serial dilutions of the soil attached to roots were plated on *Trichoderma* Selective Medium (TSM). After 5-8 days incubation the colonies produced characteristic green spores which were characterized as *Trichoderma harzianum* by microscopic observation. Total number of colonies produced (CFU) at different dilutions were recorded by colony counter. Along with the colony forming unit the depth of root and length of root and shoot were also recorded. The Rhizospheric Competence Index (RC Index) of the introduced bioformulation at both +Th3 and -Th3 fields were calculated for the study of colonization, verification and survivability. The R.C. Index was calculated as per the following formula:

$$\text{R.C. Index} = \sum_{N}^{i=1} [\log (Pi+1) \ln (Di+1)]/n$$

Where *P* is the population density per mg of rhizosphere/rhizoplane/bulk soil, *D* is the root depth, and *n* is the total root length.

2.5 Measurement of Different Growth Parameters in Wheat Crop

Different growth parameters viz., number of rootlets, tillers per 500 plants were recorded at the interval of 15, 30, 45, and 60 days after germination of seed. The grain yield, weight of thousand seeds and number of grain per spike were also recorded for wheat crop grown at both '+Th3' and '-Th3' fields in Jaipur and Kota during three years.

2.6 Statistical Analysis

Data were statistically analyzed using analysis of variance (ANOVA) at a significance level ($p < 0.0001$) using statistical software PRISM version 3.0.

2.7 Cost of Production

Cost of production was calculated by taking into consideration the expenditure incurred on cost for field preparation, fertilizer application, hoeing and weeding, pesticide application, material cost like seed, pesticides, biocontrol agents (Th3 bioformulation), IPM inputs, fertilizers, and irrigation. The data on grain yield (kg/ha), cost of production (Rs/ha) including all inputs and cost of plant protection (Rs/ha) and net return (Rs/ha) were used to determine the cost benefit ratio.

3. Results

3.1 Rhizospheric Competence Index of Trichoderma harzianum in Wheat Crop

The bioformulation of *Trichoderma harzianum* (Th3) was applied to wheat (Raj 3765) as both powder and liquid forms @ 4g/kg or 4g/L and 4ml/L respectively as per Table 1. A significant increase (at $p<0.0001$) in colony forming unit (CFU) count of *Trichoderma harzianum* was observed in +Th3 field at both Jaipur and Kota districts during the three stages of sample collection viz., seedling, flowering and preharvesting. Whereas, -Th3 fields showed negligible CFU count of *Trichoderma harzianum*. Increase in CFU count of Th3 was seen during 2010-11 than the previous year's viz., 2009-10 and 2008-09 in the +Th3 fields. CFU count was found maximum at the preharvesting stage in +Th3 field at Jaipur (50.75) and Kota (50.50) during 2010-11. The CFU count during the study ranged in seedling stage of wheat from 0 to 6.75 (Jaipur) and 0.75 to 8.75 (Kota), in flowering stage from 0.25 to 42.5 (Jaipur) and 0.75 to 44.25 (Kota). At preharvesting stage the CFU ranged from 0.25 to 50.75 (Jaipur) and 0.75 to 50.50 (Kota).

As per the CFU count (Table 2), root depth and root length, the RC Index was calculated which showed a significant difference at $p<0.0001$ level (Figure 1). The RC index values significantly increased in +Th3 fields as compared to the farmer's field with -Th3. The RC index values was 0.02 (Jaipur) and 0.04 (Kota) at the preharvesting stage in first year which gradually increased in the second year at Jaipur (0.31) and Kota (0.33) and in third year the RC index values were 0.39 (Jaipur) and 0.35 (Kota). In the -Th3 fields RC index values were negligible.

Table 2. Colony forming unit (CFU) count in the wheat field with Th3 technology at two districts of Rajasthan

Effect of Th3 Bioformulation on C.F.U. count x10^6 of Rhizospheric soil of Wheat Raj-3765*						
	Jaipur			Kota		
Treatments	Seedling	Flowering	Preharvesting	Seedling	Flowering	Preharvesting
2008-09: -Th3	0.00	0.25	0.25	0.75	0.75	0.75
2008-09: +Th3	4.50	20.50	27.00	5.75	23.00	27.50
2009-10: -Th3	0.75	0.75	0.75	1.00	4.50	4.75
2009-10: +Th3	5.75	40.25	44.75	7.50	41.25	47.50
2010-11: -Th3	0.75	5.50	0.75	1.00	6.75	5.50
2010-11: +Th3	6.75	42.50	50.75	8.75	44.25	50.50
SEm±		10.92			10.07	
CD (P<0.0001)		43.68			40.27	

*Mean of four replications

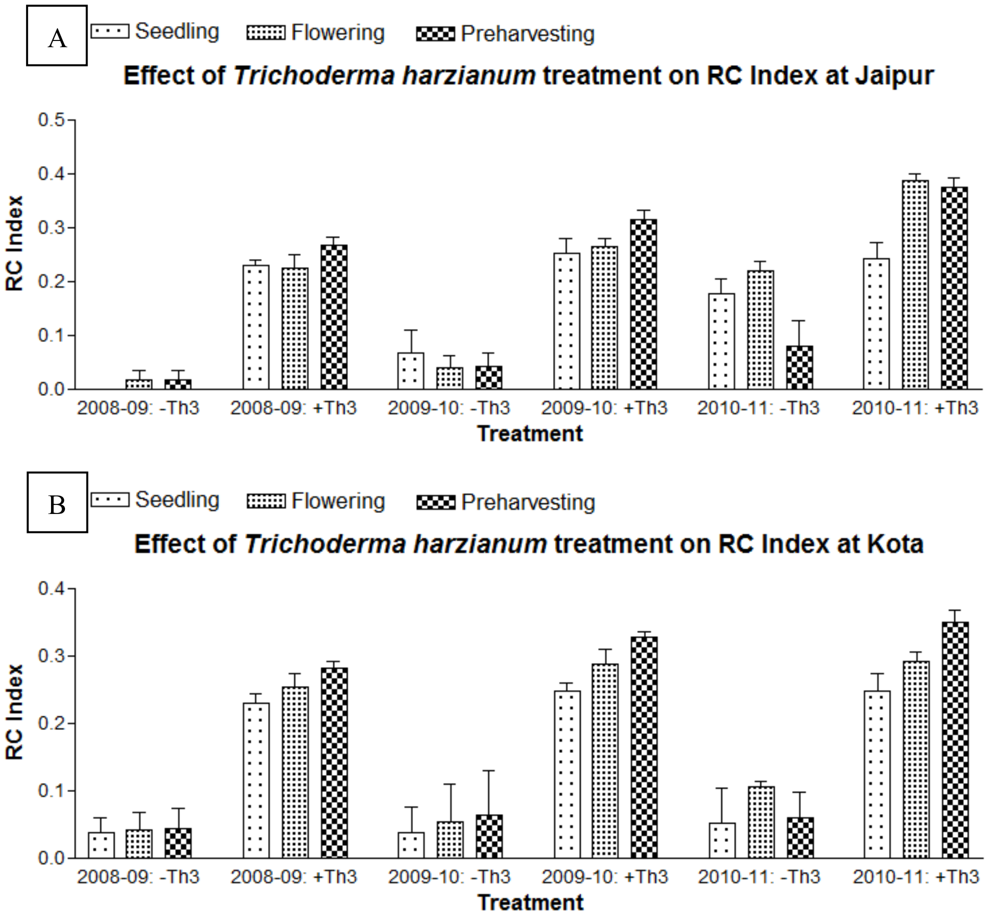


Figure 1. Effect of Th3 Bioformulation application on R.C. Index of *Trichoderma harzianum* in Wheat Raj-3765 at three different stages viz. Seedling, Flowering and Pre-harvesting in A) Jaipur and B) Kota districts of Rajasthan

3.2 Effect of Trichoderma harzianum on Growth of Wheat

3.2.1 Tillers

The number of tillers produced per 500 plants in the wheat variety (Raj 3765) increased from 1.8 to 7 (Jaipur) and 1.8 to 9.5 (Kota). Increase was found maximum at 60 days of sampling. Significant difference was observed among the number of tillers per plant produced in both +Th3 and –Th3 fields with similar results at Jaipur and Kota (Table 3).

Table 3. Effect of *Trichoderma harzianum* bioformulation application on number of tillers/500 plants of Wheat (Raj 3765) at 15, 30, 45 and 60 days of sampling

Effect of *Trichoderma harzianum* on the number of Tillers produced/500 plants of Wheat (Raj3765)								
	Jaipur				Kota			
Treatments	15 days	30 days	45 days	60 days	15 days	30 days	45 days	60 days
2008-09: -Th3	1.8	2.3	2.8	4.0	1.8	2.5	3.8	5.8
2008-09: +Th3	3.0	4.5	5.8	6.3	3.3	4.8	6.5	8.8
2009-10: -Th3	2.0	3.0	3.0	4.3	2.3	3.3	4.3	6.3
2009-10: +Th3	3.3	4.8	6.0	6.8	3.5	5.5	6.5	8.8
2010-11: -Th3	2.3	3.3	3.1	4.5	1.7	2.5	3.0	4.8
2010-11: +Th3	3.5	5.0	6.3	7.0	4.3	6.8	7.5	9.5
SEm±	0.4				0.5			
CD (p<0.0001)	1.7				1.9			

*Mean of four replications

3.2.2 Rootlets

The number of rootlets increased significantly in third year as compared to first and second year of applications (Table 4). The increase was observed after application of Th3. Highest number of rootlet produced (27) was in 60 days of root samples during the year 2010-11 both at Jaipur and Kota.

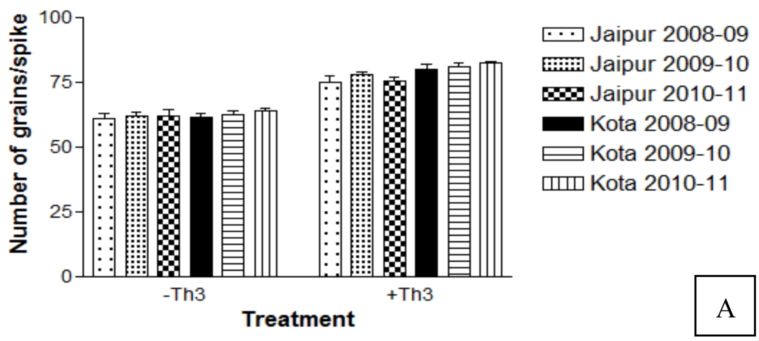
Table 4. Effect of *Trichoderma harzianum* bioformulation application on number of rootlets per 500 plants of Wheat (Raj 3765) at 15, 30, 45 and 60 days of sampling

Effect of *Trichoderma harzianum* on the number of Rootlets /500 plants produced in Wheat (Raj3765)								
	Jaipur				Kota			
Treatments	15 days	30 days	45 days	60 days	15 days	30 days	45 days	60 days
2008-09: -Th3	1.5	4.8	7.3	11.0	2.0	6.5	7.5	13.0
2008-09: +Th3	2.5	7.5	12.0	17.5	4.0	8.8	12.8	19.3
2009-10: -Th3	1.8	5.0	7.8	10.5	2.5	6.8	8.0	12.3
2009-10: +Th3	2.8	8.0	12.0	18.3	4.5	9.0	12.8	19.3
2010-11: -Th3	4.0	8.5	14.0	24.5	3.5	6.5	9.0	14.5
2010-11: +Th3	5.8	13.0	19.5	27.0	7.0	11.8	18.0	27.0
SEm±	2.3				1.8			
CD ($p<0.0001$)	9.4				7.2			

*Mean of four replications

3.2.3 Yield of Wheat (Variety: Raj 3765)

Number of grains produced per spike of wheat was calculated manually from representative four fields with +Th3 and –Th3 treatment along with farmer's practice at both the Jaipur and Kota site. Significant difference in the number of grains produced per spike was found among the +Th3 and –Th3 wheat crop. The minimum number of grains produced per spike of wheat was about 61 (Jaipur) and 61.5 (Kota) during 2008-09 whereas it was maximum viz., 75.5 (Jaipur) and 82.25 (Kota) during 2010-11. Similarly when weight of one thousand seeds were determined, it was found to increase in the +Th3 crop in all the three years of treatment. Significant difference was observed in weight of thousand seeds among the Th3 treated and untreated wheat crop. During three years the average weight of thousand seeds was 36.17g (Jaipur) and 38.42g (Kota) in –Th3 crop whereas it was 43.5 (Jaipur) and 46.42 (Kota) in +Th3 crop. This result showed a marked increase in the production in both number of grains/spike of wheat and weight of 1000 seeds (Figure 2) in the +Th3 treated wheat crop.



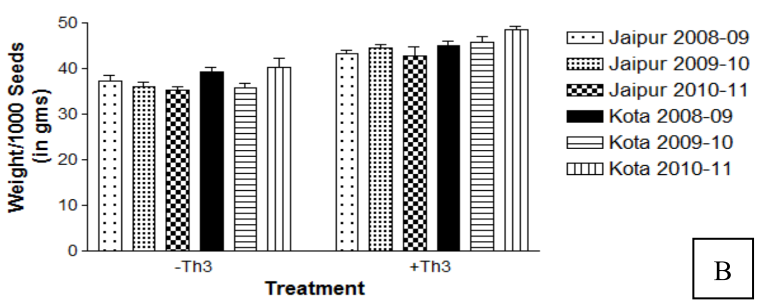


Figure 2. Effect of *Trichoderma harzianum* treatment on production of Wheat as A) Number of grains/spike, B) weight in gm of 1000 seeds in Jaipur and Kota of Rajasthan during 2008-2011

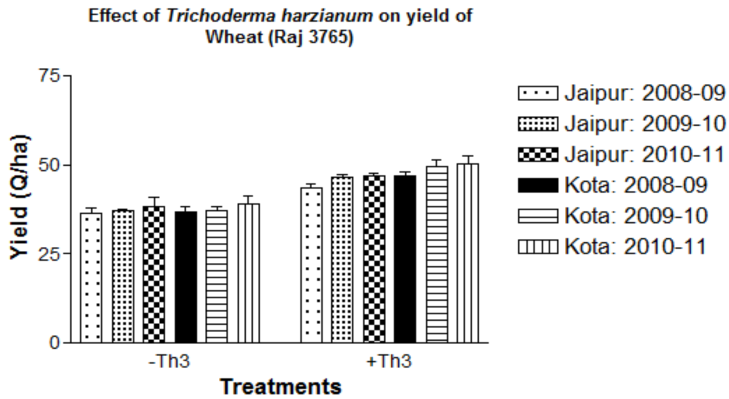


Figure 3. Effect of *Trichoderma harzianum* treatment on yield of wheat (Q/ha) in Jaipur and Kota of Rajasthan during 2008-2011

Average yield of the wheat crop were recorded during all the three years viz., 2008-09 to 2010-11 at both the places in Jaipur and Kota. A significant increase in yield was observed in the +Th3 wheat crop as compared to the –Th3 crop (Figure 3). During 2008-09 average yield increased from 36.25Q/ha to 43.38Q/ha (at Jaipur) and 36.88Q/ha to 46.75Q/ha (at Kota). During 2008-10 the average yield increased from 37.13Q/ha to 46.38Q/ha (at Jaipur) and 37.28Q/ha to 49.59Q/ha (at Kota). Similarly, during 2010-11, the average yield increased from 38.41Q/ha to 46.73Q/ha (at Jaipur) and 39.03Q/ha to 50.12Q/ha (at Kota). A significant increase in yield of wheat of 29% (Jaipur) and 36% (Kota) has been observed from the zero stage where the farmers were unaware of the treatment of *Trichoderma* in 2008-09 to 2010-11 where the continuous application of the three years has significantly increased the yield from 36.25Q/ha to 46.73Q/ha (Jaipur) and 36.88Q/ha to 50.12Q/ha (Kota).

3.3 Economic Growth of the Farmers

Economic analysis of the data of Jaipur Wheat crop (Table 5) showed higher economic returns and benefit-cost ratios in +Th3 practice (Rs. 56242/ha, 1:1.8) as compared to farmers practice (-Th3) (Rs. 45983 /ha, 1:1.5). Similar observations were seen for the Kota Wheat production (Table 6) where economic returns and benefit-cost ratios in +Th3 practice was (Rs. 60332/ha, 1:1.9) as compared to farmers practice (-Th3) (Rs. 46532 /ha, 1: 1.5). Similar, benefit of farmers was observed for both Jaipur and Kota. *Trichoderma harzianum* (Th3) treatment helped in increasing the production along with the yield thereby increased the annual income of the wheat farmers.

Table 5. Economic growth of farmers cultivating Wheat (Raj 3765) using Th3 technology in Jaipur district of Rajasthan during 2008-2011

Parameters	2008-09		2009-10		2010-11		Pooled Mean (2008-2011)	
	+Th3	-Th3	+Th3	-Th3	+Th3	-Th3	+Th3	-Th3
Total cost of production (Rs/ha) All inputs	29300	28300	31500	30500	34700	33700	31833	30833
Mean Yield (Q/ha)	43.38	36.25	46.38	37.13	46.73	38.41	45.50	37.26
Rate of selling (Rs/Q)	1100	1100	1400	1400	1200	1200	1233	1233
Total Returns (Income)	47718	39875	64932	51982	56076	46092	56242	45983
Net returns (Rs/ha)	18418	11575	33432	21482	21376	12392	24409	15150
Cost benefit Ratio	1:1.6	1:1.4	1:2.1	1:1.7	1:1.6	1:1.4	1:1.8	1:1.5

Table 6. Economic growth of farmers cultivating Wheat (Raj 3765) using Th3 technology in Kota district of Rajasthan during 2008-2011

Parameters	2008-09		2009-10		2010-11		Pooled Mean (2008-11)	
	+Th3	-Th3	+Th3	-Th3	+Th3	-Th3	+Th3	-Th3
Total cost of production (Rs/ha) (All inputs)	30100	29100	32050	31050	33620	32620	31923	30923
Mean Yield (Q/ha)	46.75	36.88	49.59	37.28	50.12	39.03	48.82	37.73
Rate of selling (Rs/Q)	1100	1100	1400	1400	1200	1200	1233	1233
Total Returns (Income)	51425	40568	69426	52192	60144	46836	60332	46532
Net returns (Rs/ha)	21325	11468	37376	21142	26524	14216	28408	15609
Cost benefit Ratio	1:1.7	1:1.4	1:2.2	1:1.7	1:1.8	1:1.4	1:1.9	1:1.5

4. Discussion

Trichoderma spp. is widely used in agriculture as biopesticides, bioprotectants, biostimulants, and biofertilizers on a wide variety of plants (Harman et al., 2004; Sharma & Sain 2005; Sharma et al., 2003, 2004, 2005) against soil borne, foliar and postharvest phytopathogenic fungal pathogens. Hermosa et al. (2012) and Sharma et al. (2011) have reviewed the plant-beneficial effects of *Trichoderma* and its genes.

The success of biocontrol agent depends on the colonization in the rhizospheric region which makes them survive during the cropping period and work through its different biocontrol mechanisms against the soil borne pathogens. In this process these microbes are also reported to release secondary metabolites which besides inhibiting the pathogen population also help in growth enhancement of the crop. This study is based on these parameters and therefore, CFU and Rhizospheric Competence (RC) index was evaluated.

In the present study the soil application of *Trichoderma harzianum* with FYM allowed the increase in the colonization which is evident through the RC index values (Figure 1). The seed, seedling and preharvesting treatment allowed optimum amount of *Trichoderma* colonies which may be responsible for growth promotion. Th3 strain was able to promote plant growth, colonize and adhere plant roots in both sandy and black cotton soil which clearly indicates the ability of the strain to acclimatize in two different agroclimatic zones. This study can be supported by findings of other workers reporting the role of *Trichoderma* as growth promoter. Several studies have documented that *Trichoderma harzianum sensu lato*, *T. asperellum* and *T. asperelloides* are highly rhizosphere competent and are able to stimulate growth and immune defense of plants (Harman et al., 2004). Mc Lean et al. (2005) reported colonization and proliferation of *Trichoderma atroviride* (C52) when applied as bioformulation in onions against *Sclerotium cepivorum* providing nutrient to the biocontrol agent as compared to a very low colony count when applied only on seed. *Trichoderma harzianum* was shown to solubilize phosphate and micronutrients that could be made available to plant (Singh, 2010). Similarly, *Trichoderma koningi* was found to colonize roots of *Lotus japonicus* which produced isoflavonoid phytoalexin vestitol and increased plant dry weight (Masunaka et al., 2011). The colonization of *Trichoderma harzianum* in the root resulted in increase in growth of root thus providing enough strength for more nutrient uptake by the roots in fields with limited irrigation facility. Bae et al. (2009) reported similar results in *Theobroma cacao* by using *Trichoderma hamatum* thus enhancing crop growth in drought prone area.

The *Trichoderma harzianum* (Th3) application (+Th3) on wheat crop significantly increased the growth promotion in terms of number of tiller, rootlets, number of grains per spike and weight of 1000 seeds in comparison to the –Th3 crop without Th3 treatment. El-Gizawy (2009) reported significant increase in the number of tillers after the application of bacterial consortia of *Azospirillum lipoferum*, *Bacillus polymxa* and *B. megatherium* in wheat which also explains that the microbial inoculants are responsible for the increased growth of the crop. Fungal colonization stimulated plant growth by factors including increased root size and root depth, which aid in nutrient uptake (Harman et al., 2004). The auxin dependant mechanism has been explained for increase in lateral roots by Contreras-Cornejo et al. (2009) in Arabidopsis by *Trichoderma virens*.

The increase in yield of wheat was found similar to the findings of Sallam et al. (2008) where the formulation of *Trichoderma* spp. treatments enhanced green yield of bean plants compared to infected control. In this experiment the increase in yield can also be attributed to the application of *Trichoderma harzianum* (Th3) bioformulation along with the Farm yard manure which helped increasing the colonies by providing nutrient to *Trichoderma* thereby increasing the plant growth and yield of wheat.

Trichoderma from rhizosphere, promoted growth of wheat and soybean under greenhouse conditions (Rojan et al., 2010). Some early work revealed that *Trichoderma* also promotes growth responses in some other crops viz., bean (*Phaseoulus vulgaris* L.), cucumber (*Cucumis sativus* L.), pepper (*Capsicum annum* L.), wheat (*Triticum aestivum* L.), radish (*Raphanus sativus*), bitter guard (*Momordica charantia*), loofah (*Luffa Acutangula* Roxb) and tomato (*Solanum lycopersicum*). Thus, the applications of *T. harzianum* in plant production, therefore, can reduce the use of fungicides, growth regulators and labor which eventually will lower the production costs and environmental impact. Plant growth promotion measured as root and shoot lengths significantly higher in case of treated seeds of Chilli with *T.harzianum* (Joshi et al., 2010). This somewhere highlights *Trichoderma harzianum* strain Th3 used as bioformulation in wheat crop at Rajasthan can colonize the soil and root of the plant, occupying a physical space and avoiding the multiplication of the pathogens and help in promoting the plant development inducing the defensive mechanisms of the plant.

This technology can be well utilized by the resource poor and marginal farmers who in absence of high inputs can still depend on a quality bioformulation and FYM (field resource) to increase the crop production and plant health with good income.

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