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Biocontrol potential of some isolates of *Trichoderma viride* from Garo hills of Meghalaya

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Soils from different crop rhizosphere of East Garo hills, Meghalaya were collected from different crops and plants for evaluation and isolation of *Trichoderma* spp. and only *T. viride* isolates were obtained from the soil samples. The population in terms of c.f.u. of this isolates of antagonists ranged between 1 x 10^3 to 7 x 10^3 depending upon the location of soil. The antagonistic potential of all the ten isolates of *T. viride* was assesed by dual culture plate using PDA medium against four plant pathogens viz *Sclerotium rolfsii Rhizoctonia solani, Macrophomina phaseolina* and *Fusarium oxysporum* f.sp. *lycopersici.* The result showed that GH 2(3) and GH 3(1) obtained S_1 rating in 5 days. In case of *R. solani* six isolates viz. GH 1(1), GH 1(2), GH 2(3), GH 3(1), GH 4(1), and GH 4(2) attained S_1 rating within 4 days. The highest antagonistic potential against *M. phaseolina* was obtained from isolates GH 1(1), GH 2(2), GH 2(3), and GH 3(1) which attained S_1 rating within 4 days. In case of *F.oxysporum* f.sp. *lycopersici* six isolates GH 1(1), GH 2(1), GH 2(2), GH 2(3), GH 3(1), GH 4(1) attained S_1 rating within 5 days.

For production of volatile antibiotic isolates GH 3(1) and GH 4(1) appeared most superior irrespective of the plant pathogen screened resulting in 60% inhibition in mycelia growth of the test pathogen. In case of non-volatile inhibitors isolate GH 3(1) again appeared superior with respect to its antagonistic potential and production of non-volatile inhibitors which caused 65.1 % to 72.8% reduction in mycelial growth at 20% concentration.

In vitro seed treatment was carried out with chopped mycelial-conidial mat of *T. viride* isolate for the following seeds viz. pea (*Pisum sativum* L.), green gram [*Vigna radiata* (L.) Wilczek], and bengal gram (*Cicer arietinum* L.). Highest per cent germination of seed was obtained in mung bean 96% for the isolates GH 4(1) and GH 3(1) while the highest vigour index was recorded for isolate GH 1(1) followed by pea for isolate GH 3(1) and the lowest vigour index was recorded with bengal gram for GH2(3) the isolate.

Field experiment with wheat bran-mustard cake formulation of *T. viride* isolate against collar rot of mung bean (*Macrophomina phaseolina*) [T_6 treatment (seed treatment+soil application of *T. viride* + well decomposed FYM)] caused lowest disease incidence (12.6%) and highest yield (2650 kg/ha) of mung bean.

Key words: Biocontrol, Garo hills, soil borne pathogens, *Trichoderma*

INTRODUCTION

Biological control an alternative to the various chemical pesticides has attained importance in modern agricultural to curtail the hazards of intensive use of toxic chemicals. This approach is eco-sustaining, efficient, ecofriendly with long-term action, renewable, and may be socially acceptable. The importance on biological control of plant pathogens has gained momentum since 1965 and the potential of *Trichoderma* has been established as a biocontrol against a number of soil borne plant pathogens through continual experiments and developments for over two decades. The main, purpose of biocontrol of plant diseases is to suppress the inoculum load of the target pathogen below the level that caused economically significant outbreak of disease (Kumar and Dubey, 2001; Mukherjee and Tripathi, 2000). Several antagonistic microbes have recently been identified as effective

agents several soil borne plant pathogens (Walsh *et al*, 2000; Howell, 2002; Xue, 2003). Among various antagonists, *Trichoderma* spp. have gained wide attention due to their ability to control many fungal pathogens on a variety of crop plants under green house (Harman, 2000) and field conditions (Whipps, 1996; Harman, 1991) as well as their growth promotion affects on host plants.

The genus *Trichoderma* is widely distributed all over the world and is found in all kinds of soil and other natural habits, especially those containing or consisting of organic matter. *T. harzianum* Rifai a filamentous soil borne mycoparasitic fungus has been shown effective against many soil borne plant pathogens (Papavizas, 1985; Pan *et al.*; 2001; Jash and Pan, 2004).

MATERIALS AND METHODS

Soils were collected from different locations of Garo Hills, Meghalaya from various crop fields and from different topography such as maize field, cabbage field, orange orchard and forest region. The spots from where the samples collected were at least 10 kms apart from one another so as to represent an ecodistribution pattern of *Trichoderma* habitation.

Trichoderma spp. were isolated from the air dried, powdered and sieved rhizosphere soil using dilution plate method (Harris and Sommers, 1968) on *Trichoderma* specific medium (TSM) using a dilution of 10^{-3} at 28±1°C. The green colonies of the antagonists usually appeared at 4^{th} to 5^{th} day of incubation. Each colony was studied carefully under microscope. The genus *Trichoderma* was identified up to species level according the monograph of *Trichoderma* (Rifai, 1969).

The pathogens used for different investigations were taken from the culture collection of biocontrol laboratory, Department of Plant Pathology, B.C.K.V. and the pathogens used were *Sclerotium rolfsii* Sacc. (*Athelia rolfsii* Curzi.), *Rhizoctonia solani* Kuhn (*Thanatephorus cucumeris*.), *Macrophomina phaseolina* Tassi (Goid) and *Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) Synder and Hansen.

Ten isolates of *T. viride* were screened against the test pathogens and rated for their antagonistic potential as per Bell's scale (Bell *et al.*, 1982) by dual culture plate method.

To study the production of volatile antibiotics experiments were carried out by inoculating the antagonists centrally on the sterilized Potato Dextrose Agar (PDA) plates and incubating the plates at 28±1° C for 4 days. Similarly, some PDA plates were also inoculated centrally with culture discs of *R. solani, S. rolfsii, M. phaseolina* and *F.oxysporum*. f.sp. *lycopersici* and on the same day lids of the plates with 4 days old antagonists were replaced by the bottom part of the immediately inoculated pathogen plates. The control plates were maintained with PDA medium without antagonists at the lower part but inoculated with the pathogen on the upper part. The pairs of each plate were then sealed together with cellophane adhesive tape and incubated at 28±1°C. Colony diameter was recorded after 4 days and compared with the control.

To assay the non-volatile antibiotic production different isolates of *Trichoderma* were cultured in sterilized Potato Dextrose Broth (PDB). After 10-15 days mycelial mat was harvested using Whatman no. 1 filter paper. The culture filtrates which were collected and were sterilized by passing through pre-sterilized set of sintered glass filter (G-5) under vaccum and 10 ml of filtrates of different isolates of antagonists were mixed separately with 90 ml of molten PDA and 20 ml of culture filtrate with 80 ml of molten PDA to get 10% and 20% concentration respectively at 50 C in a thermostatic water bath. These media were subsequently poured in to sterilized Petriplates and inoculated with culture discs of *R. solani, S. rolfsii, M. phaseolina* and *F. oxysporum* f.sp. *lycopersici*. The Petriplates were incubated at 28±1°C for 4 days and the radial colony growth was recorded in each treatment.

In vitro seed treatment was carried out with the following seeds viz. pea (*Pisum sativum* L.), green gram (*Vigna radiata* (L.) Wilczek), and bengal gram (*Cicer arietinum* L.). The mycelial mat was stirred adding a little distilled water and mixed with 1% of jaggery on to which a pinch of Carboxy Methly Cellulose (CMC) was added. The seeds were then mixed thoroughly with the above mixture and dried under shade. After drying, the seeds were placed on plastic Petriplates on double layered moist filter paper and incubated at 28±1° C in a seed germinator. In control, the seeds were taken without treatments. After 4-5 days the root and shoot length of the seeds were recorded and its vigour index was determined.

A field trial was conducted using wheat bran + mustard cake (20%) preparation of *T. viride* against collar rot (*M. phaseolina*) of mung bean at the Instructional Farm, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, during *pre-kharif* season of 2006 and 2007. It was sown in randomized block design with a plot size of 6 m^2 at 45 cm x 10 cm spacing. A total of 6 treatments with three replications were as follows : T_1 - (control) without pathogen; T_2-soil application @ 100 g of wheat bran + mustard cake preparation of *T. viride* (1 x 10^8 cfu/g) + pathogen @ 100 g (10 sclerotia/g)/plot ; T_3 - seed treatment @ 4 g of *T viride* (1x10^8 cfu/g) formulation (wheat bran mustard cake preparation was mixed with 1 liter of water and seeds were dipped in to it for 30 min and shade dried before sowing) + pathogen @ 100 g/plot; T_4- soil application @ 200 g of wheat bran + mustard cake preparation of *T viride* (1 x 10^8 cfu/g) + pathogen @ 100 g (10 sclerotia/g)/plot; T_5 - T_3 + T_4 and T_6- T_5 + farmyard manure @ 5 kg/plot (500 g of well decomposed farmyard manure incubated for 20-25 days at room temperature and incorporated just before sowing of seed. The observations on the incidence of collar rot disease in mung bean were recorded starting from seedling emergence up to 45 days after sowing. The population density of *T. viride* (cfu/g of soil) in the rhizosphere soil of mung bean was also recorded up to 45 days after sowing at 15 days interval.

RESULTS AND DISCUSSION

Among the 10 isolates obtained from four different crops rhizosphere soil namely maize (2), forest (3), cabbage (3) and orchard (2) yielded *Trichoderma viride* (Table 1). The result also envisaged that the population of *Trichoderma* irrespective of species in different soils of Garo Hills varied from 1x10^3 to 7 x 10^3, highest population being 7 x 10^3 in Samanda (maize field) soil and the lowest being 1 x 10^3 in Rongap (orange orchard).

In vitro antagonistic potential of *Trichoderma* isolates revealed that they attained S_1 stage at 5-8 days for *S. rolfsii*, 4-5 days for *R. solani* and 4-6 days for *M. phaseolina* and *F.o. f.sp. lycopersici.* The results of Table 2 showed that none of the isolates of *T. viride* had very high antagonistic potential against the test pathogens. The dual culture plate clearly indicated that the antagonistic isolates exhibited variation among themselves for their antagonistic potential depending upon the fungal pathogen against which they were evaluated. Based on this result the isolates GH 1(1), GH 2(1), GH 2(3), GH 3(1) and GH 4(1) which appeared antagonistically more potent against four pathogens were selected for further studies.

From Table 3 it was found that in case of *S. rolfsii*, a strong inhibition in mycelial growth was obtained

Table 1: Isolates of *Trichoderma* from East Garo Hills, Meghalaya

Location	Species confirmed	Number of *Trichoderma* (cfu/g of soil)	Isolate code	Crop rhizosphere
Samanda	*T. viride*	6 x 10^3	GH 1(1)	Maize
Samanda	*T. viride*	7 x 10^3	GH I(2)	Maize
Rongrenggiri Reserve Forest	*T. viride*	2 x 10^3	GH 2(1)	Forest
Rongrenggiri Reserve Forest	*T. viride*	2 x 10^3	GH 2(2)	Forest
Rongrenggiri Reserve Forest	*T. viride*	3 x 10^3	GH 2(3)	Forest
Baija	*T. viride*	3 x 10^3	GH 3(1)	Cabbage
Baija	*T. viride*	4 x 10^3	GH 3(2)	Cabbage
Baija	*T. viride*	3 x 10^3	GH 3(3)	Cabbage
Rongap	*T. viride*	2 x 10^3	GH 4(1)	Orchard
Rongap	*T. viride*	1 x 10^s	GH 4(2)	Orchard

* cfu/g of soil = colony forming units/g of soil

GH = Garo Hills

Table 2: Antagonistic potential of *Trichoderma viride* isolates by dual plate method against four soil borne plant pathogens

Isolates	Antagonistic potential on modified Bell's scale			
	S. rolfsii	*R. solani*	*M. phaseolina*	*F.o.* f.sp. *lycopersici*
GH1(1)	7 S_1	4 S_1	4S_1	5 S_1
GH 1(2)	6 S_1	4 S_1	6 S_1	6 S_1
GH 2(1)	8 S_1	5 S_1	5 S_1	5 S_1
GH 2(2)	8 S_1	5 S_1	4 S_1	5 S_1
GH 2(3)	5 S_1	4 S_1	4 S_1	5 S_1
GH 3(1)	5 S_1	4 S_1	4 S_1	5 S_1
GH 3(2)	7 S_1	5 S_1	6 S_1	6 S_1
GH 3(3)	6 S_1	6 S_1	6 S_1	6 S_1
GH 4(1)	7 S_1	4 S_1	5 S_1	5 S_1
GH 4(2)	8 S_1	4 S_1	5 S_1	6 S_1

* Each insertion is based on five separate observations

with isolates GH4(1) resulting in 64.4% inhibition followed by GH3(1) 62.7%, GH 2(3) 58.3% and GH1(1) 48.3%. The lowest inhibition in mycelial growth with respect to volatile antibiotics was obtained with GH 2(1) 39.4%o. On the other hand, antibiotic produced by the same antagonist isolates and their inhibiting ability on *R. solani* by isolates GH 4(1) and GH 3(1) were equally effective 66.7% followed by GH 2(1) and GH 2(3) having no significant difference among themselves (57.2% and 56.6 % respectively). In case of the pathogen *M. phaseolina*, antibiotic produced by these isolates of *Trichoderma*, the present inhibition in growth of both isolates was GH4(1) (62.2%) and GH3(1) (63.3%) respectively and there was no significant differences among themselves as in case of *R. solani.* Highest inhibition was obtained in isolate GH 3(1) resulting in 65.0% inhibition on mycelial growth of *F.o.* f.sp. *lycopersici* followed by isolate GH4(1),(60.5%) and GH 2(3) (56.1%).

As regards to non-volatile inhibitors in general,

Table 3: Effect of volatile antibiotics produced by isolates of *Trichoderma viride* on growth of four soil borne plant pathogens

Isolates	inhibition over control (%)			
	S. rolfsii	*R. solani*	*M. phaseolina*	*F.o.* f.sp. *lycopersici*
GH1(1)	48.3*	56.6	58-9	45.5
GH2(1)	39.4	57.2	44.4	52.2
GH2(3)	58.3	56.6	51.1	56.1
GH3(1)	62.7	66.7	63.3	65.0
GH4(1)	64.4	66.7	62.2	60.5
Mean	54.6	59.8	56.0	55.9
SEm (±)	1.42	1.06	1.40	1.08
CD (0.05)	2.18	1.64	2.15	1.67
CD (0.01)	3.06	2.30	3.01	2.33

* Means of five replications

per cent inhibition in mycelial growth was more than that of volatile inhibitors when 20% cultural filtrate of the antagonist isolates were added with assay medium for evaluation. It was observed from the result that isolate GH 3(1) was most effective among the five antagonistic isolates in inhibiting the mycelial growth of the assay pathogens (Table 4). It could be further envisaged from the data that as high as 65.5% inhibition in the mycelial growth of *S. rolfsii* was recorded with antagonist isolate GH3(1) at 20% concentration of the non volatile- antibiotics followed by 58.9% inhibition of the same isolate GH3(1) against the same pathogen at 10% concentration. Other isolates showed over 50% inhibition in mycelial growth at 20% concentration of the non-volatile antibiotics. On the other hand in case of *R. solani* the same isolate GH 3(1) brought about 72.8% reduction in mycelial growth followed by 66.1% in case of both the isolate GH 1(1) and GH 2(3) at 20% concentration of the non-volatile antibiotics while around 60% reduction in the mycelial growth was noted at 10% concentration of the non-volatile antibiotics with the isolate GH 3(1).

While with *M. phaseolina* almost a repetition of the result was noted like that of *R. solani* where the higher inhibition (nearly 71.1%) was noted with antagonistic isolates GH 3(1) followed by GH 2(3) (66.7%) at 20% concentration of non-volatile inhibitors, while at 10% concentretion 65.0% inhibition in mycelial growth was obtained with the same isolate GH 3(1) and the least with GH 2(1) (44.4%).

The result further revealed that as high as 72.8% inhibition in the mycelial growth of *F.o.* f.sp. *lycopersici* was recorded with antagonist isolate GH 3(1) at 20% concentration of the non-volatile inhibitors followed by 64.4% inhibition of the same isolate against the same pathogen at 10% concentration.

Strong antagonism by different species of *Trichoderma* against wide range of soil borne plant pathogens has been reported earlier from this laboratory (Pan *et al*, 2001; Jash and Pan, 2004 a, b). Although the result of the present *in vitro* screening on the antagonistic property of isolate of *Trichoderma viride* against four soil borne plant pathogens viz. *R. solani, S. rolfsii, M. phaseolina* and *F.o.* f.sp. *lycopersici* reflected that the antagonistic potential of some of the antagonist microorganisms were not always similar against a particular plant pathogen. From the present result of antagonistic potential, it was clear that a particular antagonistic strain was not always equally effective against all kinds of the pathogens irrespective of their nature and form. Variability in antagonistic potential among the different species aggregates of *Trichoderma* against different pathogens had been

Table 4: Effect of non-volatile antibiotics produced by isolates of *Trichoderma viride* on growth of four soil borne plant pathogens at variable concentration of cultural filtrate

Isolates	inhibition in growth (%)							
	S. rolfsii		*R. solani*		*M. phaseolina*		*F.o.* f.sp. *lycopersici*	
	10% a	20% b	10%	20%	10%	20%	10%	20%
GH1(1)	51.1*	60.6	53.9	66.1	53.3	61.1	52.2	62.2
GH2(1)	51.1	57.8	41.7	66.1	44.4	55.6	48.9	56.1
GH2(3)	43.9	63.9	50.6	66.1	62.8	66.7	54.4	65.0
GH3(1)	58.9	65.5	61.1	72.8	65.0	71.1	64.4	72.8
GH4(1)	54.4	57.8	48.3	58.9	51.1	62.2	43.9	57.8
Mean	51.9	61.1	*51.1	65.0	55.3	63.3	52.8	62.8
SEm (±)	1.08	1.08	0.98	1.08	0.90	1.00	0.99	0.91
CD (0.05)	1.66	1.66	1.51	1.66	1.39	1.54	1.52	1.40
CD (0.01)	2.33	2.33	2.12	2.33	1.95	2.16	2.13	1.97

a, b concentration of cultural filtrate

* Means of five replications

reported earlier (Saha and Pan, 1996). Studies conducted by Mukherjee and Sen (l992) had similarly showed that while some isolates of *Trichoderma* were highly antagonistic to some pathogen yet there was clear isolate-to-isolate variability in degree of antagonism. In the present result, it had been clearly observed that variability in antagonistic potential also existed with specific antagonist pathogen interaction. Thus, the results of the present investigation for production of volatile and non-volatile antibiotics agreed with the many of the earlier experiments of this nature.

In vitro seed treatment was carried out with macerated mycelial mat of *T. viride* isolates for seeds of three crop plants pea, bengal gram and mung bean. The data in Table 5 revealed that the isolates of *T. viride* differed in their responses when used for seed treatment irrespective of the crop seed used. Highest per cent germination (96%) of seed was obtained in mung bean for the isolates GH 4(1) and GH 3(1), while the highest vigour index was recorded for isolate GH 1(1) followed by pea for isolate GH 3(1) and the lowest vigour index was recorded with bengal gram for the isolate GH 2(3).

In some of the observed seeds the entire root system were fully covered with whitish green mycelium growth of *Trichoderma*. In all the treated seeds there was significant increase in the development of secondary and tertiary root system with appreciable number of root hairs. There was appreciable rigidity and firmness of root and shoot system in case of treated seed, where as in case of untreated seeds majority of seeds after germination were found with lanky growth of root and shoot system and did not develop further. The root tips showing water soaked lesions with brown to dark brown dead ends and lysed root and shoot system at places were also observed in treated control.

Effective biological control agent had been developed for control of seed and seedling pathogens such as *Pythium* spp., *R. solani*, *S. rolfsii*, *M. phaseolina* and *Fusarium* spp. Several scientists had reported the biological seed treatment for protection of seed and control of pathogens causing seedling diseases (Harman, 1991; Bennett *et al*, 1992; Chet and Inbar, 1994). Likewise, there were several reports of biocontrol of pathogens in vitro and under field conditions (Gangwar *et al*, 2004; Jash and Pan, 2004a; Bhagat and Pan, 2007; Dubey *et al*, 2007).

In the present investigation, there were significant increase in per cent germination of seed and vigour index of treated seeds (pea, bengal gram and mung

Table 5 : Effect of seed treatment on pea, bengal gram and mung bean with mycelial mat of *Trichoderma viride* isolates

Isolates	Pea				Bengal gram				Mung bean			
	Germination of seed (%)	Root length (cm)	Shoot length (cm)	Vigour index	Germination of seed (%)	Root length (cm)	Shoot length (cm)	Vigour index	Germination of seed (%)	Root length (cm)	Shoot length (cm)	Vigour index
GH1(1)	76	4.68	1.84	495.5	76	5.25	2.36	578.4	92	5.28	2.66	730.5
GH2(1)	64	5.02	2.1,	455.7	70	4.36	2.56	484.4	92	4.38	2.22	607.2
GH2(3)	65	4.96	1.86	443.3	68	3.48	2.08	378.1	87	4.74	1.86	574.2
GH3(1)	80	5.78	2.64	673.6	78	5.36	2.46	609.9	96	5.32	2.22	723.8
GH4(1)	65	4.74	1.54	408.2	66	4.06	1.90	393.4	96	4.02	2.04	581.8
Untreated (control)	60	4.04	1.48	331.2	64	3.3	1.88	331.5	80	3.9	1.88	462.4
SEm (±)	1.18	0.06	0.09	-	0.82	0.04	0.03	-	1.24	0.09	0.04	-
CD (0.05)	1.82	0.09	0.15	-	1.26	0.06	0.05	-	1.92	0.15	0.07	-
CD (0.01)	2.55	0.13	0.21	-	1.77	0.09	0.07	-	2.69	0.20	0.10	-

* Means of 50 randomly observed seed

bean). These results were in consistent with the findings of Gurjar *et al* (2004), where they concluded that the treatments of seeds with fungal antagonist was superior over the untreated control and exhibited increased germination, seedling vigour and reduced seedling mortality in okra seeds treated with *T. harzianum* and *T. viride* against pathogenic fungi of okra. The increased secondary roots and root hairs were observed in the present study in all treated seeds. However, the increased root and shoot growth varied with test seedling with higher root and shoot length and corresponding vigour index was recorded with mung bean followed by pea and bengal gram. Similarly Harman *et al*, (2004) reported that the roots and shoots were lohger (roots were nearly twice as long) as maize seed treated with T-22 strain of *T. harzianum* than in its absence. They also reported that both main and secondary roots were increased in size and area and the root hairs were more in number with treated seeds. Prasad *et al.* (1999) reported that biopriming with *Trichoderma* resulted in to increased germination percentage and root and shoot length of red gram under field condition.

The result of the field experiment showed that all the treatments significantly reduced the mortality of mung bean plant and increased the seed yield over control (Table 6). Seed treatment and soil application of *T. viride* with well decomposed, sundried and remoistened farmyard manure (T_6) caused lowest disease incidence (12.6%) and highest yield (2650 kg/ha) followed by T_5 (22.6% and 2552 kg/ha). Very low disease control was achieved by seed treatment @ 4 g of wheat bran + mustard cake application of *T. viride* kg/seed (T_3). The yield was significantly higher in T6 treatment compared to seed treatment (T_3) and soil application which were statistically not significant.

Application of wheat bran mustard cake formulatio of *T. viride* individually as seed of soil treatment reduced the disease incidence and increased the seed yield of mung bean. The combination of soil application of antagonist with farmyard manure was more effective than seed or soil application alone. Soil Application of wheat bran mustard cake formulation of *T. viride* individually as seed or soil application of *T. viride* had an edge over the seed

Table 6: Field evaluation of wheat bran- mustard cake formulation of *T. viride* against collar rot (*M. phaseolina*) of mung bean.

	Treatments	Mortality of mung bean (%)			Yield kg/ha		
		Year 2006	Year 2007	Pooled	Year 2006	Year 2007	Pooled
TI	Control (without pathogen)	34.40 (37.1)	44.7 (41.95)	40.55	2080	2050	2065
T2	Soil application @ 100g (wheat bran+ mustard cake)+ pathogen @ IOOg/plot	45.5 (42.4)	52.4 (46.37)	48.95	1200	1725	1462.5
T3	Seed treatment @ 4g+ pathogen @ IOOg/plot	33.5 (35.36)	40.0 (39.2)	36.75	1970	2040	2005
T4	Soil application @100g (wheat bran+ mustard cake)+ pathogen@ IOOg/plot	26.2 (30.78)	32.6 (34.81)	29.4	2410	2500	2455
T5	T3 +T4	18.4 (25.4)	26.8 (31.1)	22.6	2536	2568	2552
T6	T5 + FYM @ 5kg/plot	10.2 (18.62)	15.0 (22.78)	12.6	2640	2660	2650
	SEm (±)	1.23	1.01	-	7.3030	4.4721	-
	CD(0.05)	2.74	2.26	-	16.27	9.96	-
	CD(0.01)	3.90	3.20	-	23.15	14.17	-

treatment, as this antagonist being a natural soil inhabitants easily established itself and proliferated very fast in soil.

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