



## Management of post flowering stalk rot of maize (*Zea mays*) caused by *Fusarium moniliforme* with native biocontrol agents

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### ABSTRACT

Nine isolates of *Trichoderma* species were screened *in vitro* against post flowering stalk rot of maize (*Zea mays* L.) caused by *Fusarium moniliforme* Sheld. by dual culture test. All the isolates significantly inhibited the mycelial growth of *F. moniliforme*. *Trichoderma harzianum* isolate Th-1 showed strong mycoparasitism followed by *T. viride* isolate Tv-3 which was effective in suppression of mycelial growth of test pathogen and these were further selected for pot house and field studies. Under pot experiments, Th-1 based seed treatment @ 20 g/kg seed and amending the soil with FYM supplemented with talc based formulation of Th-1 were found superior in reducing the stalk rot incidence and severity and increasing the seed germination, root and shoot length and biomass in maize. Under field experiments, maximum disease control and seed germination were obtained when soil was broadcasted with FYM supplemented with Th-1 @ 2 kg talc/q FYM and amendment of soil with FYM supplemented with Th-1 @ 100g/hill, respectively. Maximum increase in grain yield (40.5%) was obtained in FYM supplemented with Th-1 @ 100g/hill and further enhanced the growth of the plants indicating growth promoting ability of Th 1 isolate.

**Key words:** *Fusarium moniliforme*, Isolate, Maize, Seed treatment, Stalk rot, *Trichoderma harzianum*

Post flowering stalk rots (PFSR) play a vital role in affecting the productivity of maize (*Zea mays* L.) crop in Punjab. This complex is caused by *Fusarium moniliforme*, *Macrophomina phaseolina* and *Cephalosporium maydis*, out of which *F. moniliforme* Sheld. is of economic importance (Khehra *et al.* 1982). The disease has been recorded by different workers in different parts of the country (Desai and Hegde 1990, Kumar *et al.* 1998, Harlapur *et al.* 2002). Reduction in yield due to PFSR disease in susceptible maize germplasm has been estimated to the tune of 39.5 per cent (Payak and Sharma 1978). A reduction of 18.7 per cent in cob weight and 11.2 per cent in 1000-grain weight in infected plants has been reported by Cook (1978). Most of the commercially grown cultivars have shown a high level of disease incidence at the grain filling stage. Due to its soil borne nature, the major limitation in chemical control is the requirement of large amount of fungicide which is economically unviable and often leads to atmospheric pollution and development of fungicide resistant strains of the pathogen, thus upsetting the biological equilibrium in

soil. It was, therefore, necessary to develop an effective, economically viable and environment friendly method to manage this disease. Keeping in view the importance of crop and severity of disease in presently available cultivars, a need was felt to identify potent bio-control agents for eco-friendly management of this disease.

### MATERIALS AND METHODS

Isolations were done by using dilution plate technique from the rhizosphere soil of maize plant. In all, nine isolates of *Trichoderma* spp were obtained from rhizosphere. The most virulent isolate of *Fusarium moniliforme* isolated from the infected maize plants was selected and maintained on Potato Dextrose Agar (PDA) medium for further studies. The level of antagonism/hyperparasitism of selected isolates against *F. moniliforme* was studied *in vitro* by dual culture technique (Johnson and Curl 1972). Three replications were kept for each treatment. Mode of inhibition was observed as zone of inhibition and mycoparasitism after 7 days of incubation. The most promising isolate *T. harzianum* (Th1) exhibiting maximum mycoparasitism and the consortium of two best bioagents, *T. harzianum* (Th1) and *T. viride* (Tv3) were evaluated for their biocontrol potentiality against *F. moniliforme* under pot house and field conditions during spring crop seasons of 2010 and 2011. For *in vivo* evaluation,

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maize cultivar JH 3459 was selected.

Ten days old broth culture of *T. harzianum* (Th1)/*T. viride* (Tv3) grown on 100 ml potato dextrose broth was mixed with 1 kg talc under laminar air flow cabinet to get the final inocula with colony forming units of  $1 \times 10^6$ /g formulation. Carboxy methyl cellulose (CMC) @ 1.0 per cent was also added to the formulation. Similarly, the mixed formulation of Th1 and Tv3 was also prepared based on talc formulation. The culture of *F. moniliforme* was mass multiplied on sterilized peas mixed with sand (1:1) in conical flasks. The soil in each pot was inoculated with *F. moniliforme* at  $1 \times 10^3$  cfu/g of soil. The experiment was performed during spring crop season 2010 in pots containing 3.0 kg sterilized soil/pot. The total of eight replications were kept for each treatment. The seed treated with Bavistin 50 WP (carbendazim) @ 3g/kg seed as well as untreated seed grown in pots filled with infested soil were used as standard check in all the treatments. The pot house experiment was conducted with three delivery systems, viz;

**Seed treatment:** Seed priming was done with talc based formulation of Th1 as well as consortium (Th1 and Tv3) @ 10, 15 and 20 g/kg seed having inocula of  $1 \times 10^6$  cfu/g formulation. After the treatment, seed were incubated at  $25 \pm 2^\circ\text{C}$  for 24 hours. Initially 10 seeds were sown in one pot and after germination 5 seedlings were maintained in each pot.

**Soil treatment:** Talc based formulation of effective bioagent @ 10, 15 and 20g/kg soil was added separately two days after inoculation of the pathogen. The formulation was mixed in soil one day prior to sowing.

**Amendment with farmyard manure (FYM):** Well decomposed and sterilized FYM was supplemented with talc based formulation of Th1 in the ratio of 25:1 and was incubated for 72 hours at  $25 \pm 2^\circ\text{C}$ . After the treatment, it was added to the soil @ 25, 50 and 100 g/kg soil in pots.

Under field conditions, the most effective isolate of *T. harzianum* (Th1) was evaluated as seed treatment and soil treatment against *F. moniliforme* during spring crop season 2011. The plot size consisted of 4 rows of 2 m each with row to row and plant to plant spacing of 60 cm and 20 cm, respectively. Seed of maize hybrid JH 3459 were treated

with talc based formulation of Th1 @ 10, 15 and 20g/kg seed having  $1 \times 10^6$  cfu/g formulation. The mass culture of *F. moniliforme* was added @ 5g/hill in each plot. All the treatments were replicated thrice. Soil treatment was done by amending the soil with well decomposed FYM supplemented with talc based formulation of Th1 in the ratio of 25:1 following hill inoculation and broadcast method. In hill inoculation, FYM supplemented with Th1 was added to the soil @ 25, 50 and 100 g/hill two days prior to sowing. Non-amended soil served as control. As control, FYM alone (without supplementation with Th1) was added to the soil @ 25, 50 and 100 g/hill to assess the effect of FYM alone on plant growth. In broadcast method, FYM supplemented with talc based formulation of Th1 was broadcasted @ 2 kg, 1 kg and 0.5 kg talc/q FYM/acre in each plot. Inoculum of *F. moniliforme* was also broadcasted in the field so as to make inoculum concentration of  $1 \times 10^3$  cfu/g soil.

The seed germination was recorded after 15 days of sowing. Disease intensity was recorded by splitting the infected stalks longitudinally and scoring the individual plant on 1-9 scale at the time of harvesting (Payak and Sharma 1983). Growth parameters like root length, shoot length, fresh weight and dry weight of individual plants were recorded after 15 days of sowing in pot house experiments. In the field experiments, growth parameters of individual plant were recorded after 90 days of sowing and yield parameters were recorded at maturity.

## RESULTS AND DISCUSSION

Six isolates (Th1, Th 2, Th3, Th4, Th5, Th6) of *Trichoderma harzianum* and three isolates (Tv1, Tv2, Tv3) of *T. viride* were obtained from rhizosphere soil samples of maize plants. The data in Table 1 revealed that isolates of *T. viride* exhibited antibiosis and inhibited the growth of the pathogen in dual culture method. There was a clear cut zone of inhibition developed and it varied from 2.0 to 2.5 mm after 6 days of incubation at  $25 \pm 2^\circ\text{C}$ . However, the isolates of *T. harzianum* exhibited mycoparasitism over *F. moniliforme*. *Trichoderma harzianum* isolate, Th1 showed strong mycoparasitism by completely covering the mycelium of pathogen within 4 days of incubation.

Table 1 Interaction and mechanism of inhibition of *Fusarium moniliforme* with different antagonists in dual culture technique

Bioagent	Mode of inhibition	Isolate	Zone of inhibition/ Mycoparasitism
<i>Trichoderma harzianum</i>	Mycoparasitism	Th1	Antagonistic mycelium completely covered mycelium of pathogen after 4 days
		Th2	Antagonistic mycelium completely covered mycelium of pathogen after 5 days
		Th3	Antagonistic mycelium completely covered mycelium of pathogen after 5 days
		Th4	Antagonistic mycelium completely covered mycelium of pathogen after 6 days
		Th5	Antagonistic mycelium completely covered mycelium of pathogen after 5 days
		Th6	Antagonistic mycelium completely covered mycelium of pathogen after 6 days
<i>Trichoderma viride</i>	Antibiosis	Tv1	2 mm
		Tv2	2 mm
		Tv3	2.5 mm

Table 2 Effect of seed treatment with *Trichoderma harzianum* on disease severity of stalk rot and growth parameters<sup>1</sup> of maize under pot house conditions

Seed treatment	Doses of talc based formulation (g/kg seed)	Germination (%)	Disease severity (%)	Per cent disease control	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
<i>T. harzianum</i> (Th1)	10	80.8 <sup>c</sup>	40.5 <sup>b</sup>	41.5	11.9 <sup>ab</sup>	37.1 <sup>a</sup>	4.1 <sup>d</sup>	0.5 <sup>ab</sup>
	15	88.3 <sup>bc</sup>	30.1 <sup>ab</sup>	56.4	14.2 <sup>ab</sup>	42.3 <sup>ce</sup>	5.2 <sup>ce</sup>	0.5 <sup>ab</sup>
	20	95.0 <sup>b</sup>	23.1 <sup>a</sup>	66.6	17.4 <sup>b</sup>	49.4 <sup>b</sup>	5.8 <sup>be</sup>	0.6 <sup>b</sup>
Consortium of <i>T. harzianum</i> (Th1) and <i>T. viride</i> (Tv3)	10	82.4 <sup>c</sup>	31.4 <sup>ab</sup>	54.6	13.8 <sup>ab</sup>	41.4 <sup>aed</sup>	3.4 <sup>a</sup>	0.4 <sup>a</sup>
	15	84.0 <sup>c</sup>	28.1 <sup>ab</sup>	59.3	15.2 <sup>ab</sup>	45.1 <sup>bd</sup>	4.6 <sup>cd</sup>	0.5 <sup>ab</sup>
	20	86.5 <sup>c</sup>	26.6 <sup>a</sup>	61.6	16.3 <sup>ab</sup>	43.7 <sup>cde</sup>	4.9 <sup>c</sup>	0.6 <sup>ab</sup>
Bavistin 50 WP	3	81.8 <sup>c</sup>	22.2 <sup>a</sup>	67.9	14.5 <sup>ab</sup>	38.9 <sup>a</sup>	4.8 <sup>a</sup>	0.6 <sup>a</sup>
Control		60.2 <sup>a</sup>	69.2 <sup>c</sup>		10.9 <sup>a</sup>	34.9 <sup>ae</sup>	3.0 <sup>e</sup>	0.3 <sup>b</sup>

<sup>1</sup>Values within experiments indicated by the same letter are not significantly different at P = 0.05, 15 days after sowing

Variability in antagonistic potential among different species of *Trichoderma* against different pathogens has been reported (Harman *et al.* 2004, Pan and Bhagat 2007). Results of *in vitro* studies on the antagonistic activity of *Trichoderma* spp. were corroborated with the earlier findings that had also suggested that *T. viride* inhibited the mycelial growth and sporulation of *F. moniliforme* under *in vitro* conditions (El-Hasan *et al.* 2007, Calistru *et al.* 1997). Nayaka *et al.* (2010) revealed that *T. harzianum* isolate Th-8 showed maximum antifungal activity against *F. verticillioides*. In the present study, *Trichoderma* isolates- Th1 and Tv3 were highly efficient in their antagonistic potential against *F. moniliforme*.

*Effect of bioagent treatments on seed germination, Fusarium stalk rot development and growth parameters of maize in pot experiments.*

#### As seed treatment

All the treatments, viz Th1 alone, Th 1+ Tv 3 (1:1 ratio) and Bavistin 50 WP significantly improved germination of maize seedlings compared to untreated control. The maximum per cent germination (95.0%) was observed with seed treatment of Th1 @ 20 g/kg seed which was significantly higher than control (60.2%) treatment (Table 2). However, there were no significant differences between different doses

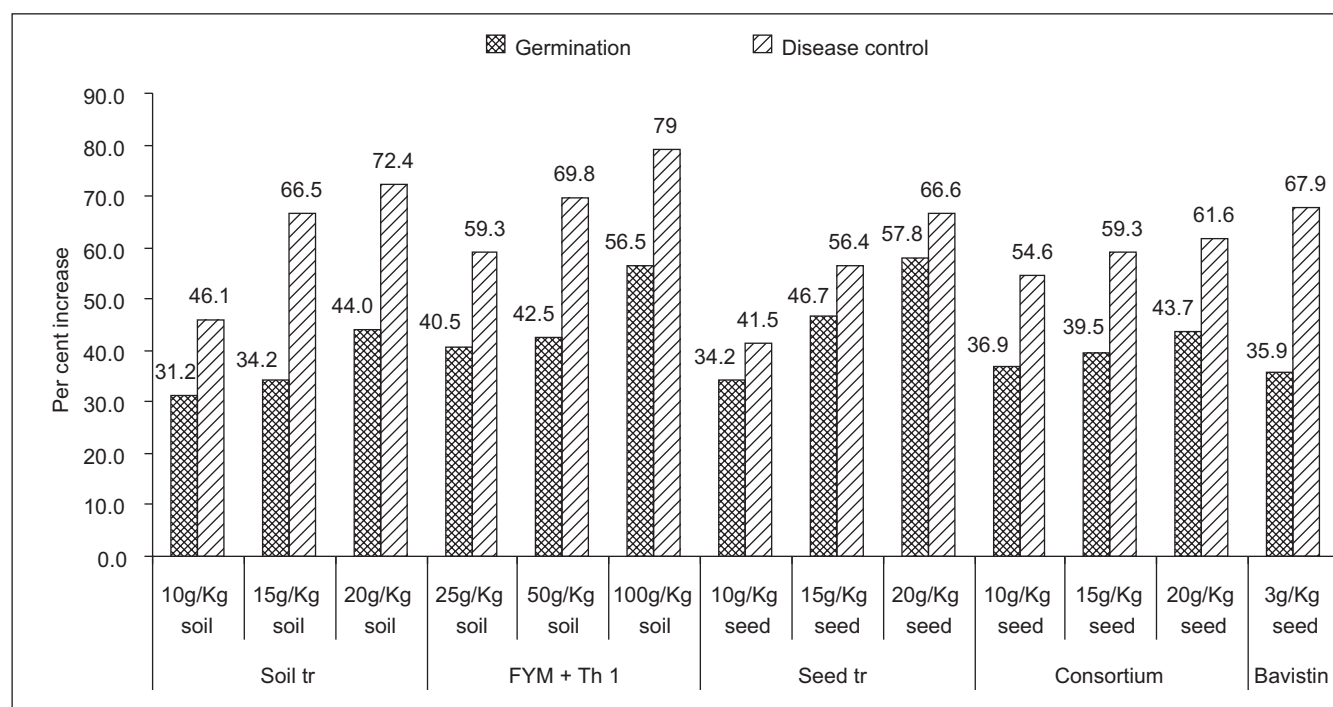


Fig 1 Effect of different treatments with *Trichoderma harzianum* on seed germination and Fusarium stalk rot of maize under pot house conditions

Table 3 Effect of soil treatment with *Trichoderma harzianum* on seed germination, severity of stalk rot and growth parameters<sup>1</sup> of maize under pot house conditions

Soil treatment	Doses of talc based formulation (g/kg soil)	Germination (%)	Disease severity (%)	Per cent disease control	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
Soil amendment with <i>T. harzianum</i> (Th1)	10	79.0 <sup>c</sup>	37.3 <sup>b</sup>	46.1	16.1 <sup>bc</sup>	38.0 <sup>c</sup>	4.6 <sup>d</sup>	0.5 <sup>ad</sup>
	15	80.8 <sup>c</sup>	23.2 <sup>d</sup>	66.5	17.1 <sup>bc</sup>	42.0 <sup>d</sup>	5.2 <sup>d</sup>	0.5 <sup>ad</sup>
	20	86.7 <sup>bc</sup>	19.1 <sup>cd</sup>	72.4	18.0 <sup>b</sup>	48.3 <sup>b</sup>	6.7 <sup>e</sup>	0.7 <sup>bc</sup>
Soil amend-ment with FYM supplemented with <i>T. harzianum</i> (Th1) in 25:1 ratio	25	84.6 <sup>c</sup>	28.2 <sup>bd</sup>	59.3	14.2 <sup>c</sup>	42.8 <sup>d</sup>	7.0 <sup>ce</sup>	0.6 <sup>dc</sup>
	50	85.8 <sup>c</sup>	20.9 <sup>cd</sup>	69.8	15.5 <sup>bc</sup>	47.9 <sup>b</sup>	7.7 <sup>bc</sup>	0.7 <sup>bc</sup>
	100	94.2 <sup>b</sup>	14.5 <sup>c</sup>	79.0	18.5 <sup>b</sup>	50.4 <sup>b</sup>	8.2 <sup>b</sup>	0.8 <sup>b</sup>
Bavistin 50WP	3	81.8 <sup>c</sup>	22.2 <sup>d</sup>	67.9	14.5 <sup>c</sup>	38.9 <sup>c</sup>	4.8 <sup>d</sup>	0.6 <sup>cd</sup>
Control		60.2 <sup>a</sup>	69.2 <sup>a</sup>		10.9 <sup>a</sup>	34.9 <sup>a</sup>	3.0 <sup>a</sup>	0.3 <sup>a</sup>

\*Values within experiments followed by the same letter are not significantly different at P = 0.05, <sup>1</sup>15 days after sowing

of consortium treatments. The per cent increase in seed germination was maximum (57.8%) and minimum (34.2 %) in seed treatment with Th-1 @ 20g/kg and 10g/kg seed, respectively (Fig 1). Disease severity in seed treatment with Th-1 @ 20g/kg seed was significantly less (23.1 %) as compared to control (69.2 %). Maximum disease control (67.9 %) was observed in Bavistin @ 3g/kg seed closely followed by Th 1 @ 20g/kg seed where disease control of 66.6 per cent was observed. In rest of the treatments, disease control ranged from 41.5 to 61.6 per cent. All the treatments with Th-1 gave quantitative increase in plant growth attributes per plant. Seed treatment with Th-1 @ 20g/kg seed gave maximum root length of 17.4 cm, shoot length of 49.4 cm, fresh weight of 5.8 g and dry weight of 0.6 g followed by 14.2 cm, 42.3 cm, 5.2 g and 0.5 g, respectively with seed treatment @ 15 g/kg seed. Similar trend was observed in seed treatment with consortium of Th-1 and Tv-3 at different doses.

#### As soil treatment

The data in Table 3 revealed that all treatments differed significantly from each other including untreated control. The maximum per cent germination (94.2%) was obtained with amendment of soil with FYM supplemented with Th1 @ 100g/kg soil followed by amendment of soil with talc formulation of Th1 @ 20g/kg soil (86.7%). Amendment of soil with FYM supplemented with Th1 @ 100g/kg soil recorded minimum disease severity of 14.5 per cent thereby significantly reducing the severity of stalk rot by 79.0 per cent (Fig 1). Bavistin also reduced stalk rot severity by 67.9 per cent which was significantly lower as compared to treatment with bioagents. Similarly, the amendment of soil with FYM supplemented with Th1 @ 100g/kg soil gave best results by yielding maximum root length of 18.5 cm, shoot length of 50.4 cm, fresh weight of 8.2 g and dry weight of

0.8 g which was significantly higher as compared to control and Bavistin treatment. More than two times increase in biomass production was recorded in treatment as compared to the control.

#### Effect of bioagent applications on seed germination and *Fusarium* stalk rot development under field conditions

##### As hill treatment

Data (Table 4) revealed that germination of maize seedlings was significantly enhanced when talc based formulation of Th-1 was applied as seed treatment as well as along with FYM as hill treatment. The maximum germination (97.7 %) was obtained in amending the soil with FYM supplemented with Th-1 @ 100g/hill which was significantly higher than the control treatment (74.1 %). Seed treatment with Th-1 @ 20g/kg seed also resulted in 96.9 per cent germination which was at par with amending the soil with FYM supplemented with Th-1. Disease incidence and severity of stalk rot was significantly reduced in both seed treatment as well as amending soil with supplemented FYM. The disease incidence and disease severity varied from 49.6 to 87.4 per cent and 33.4 to 77.4 per cent, respectively in different treatments. FYM supplemented with Th-1 @ 100 g/hill yielded least disease incidence (49.6 %) and disease severity (33.4 %) with maximum disease control (55.0 %) closely followed by 55.6 and 35.3 per cent disease incidence and severity, respectively with 52.3 per cent disease control in seed treatment @ 20g/kg seed (Fig 2).

##### As broadcast method

Maximum germination of 99.1 per cent was obtained when soil was amended with FYM supplemented with Th1 @ 2 kg talc/q FYM which was significantly different from

Table 4 Efficacy of different treatments with *Trichoderma harzianum* on incidence and severity of stalk rot of maize under field conditions (applied per hill)

Treatment	Dose	Germination (%)	Disease incidence (%)	Disease severity (%)	Per cent disease control
Seed treatment with <i>T.harzianum</i> (Th1)	10g/kg	81.1 <sup>ab</sup>	67.3 <sup>de</sup>	51.4 <sup>dc</sup>	30.7
	15g/kg	82.6 <sup>ab</sup>	60.2 <sup>ce</sup>	43.6 <sup>ce</sup>	41.2
	20g/kg	96.9 <sup>b</sup>	55.6 <sup>c</sup>	35.3 <sup>be</sup>	52.3
FYM supplemented with <i>T. harzianum</i> (Th1) in 25:1 ratio	25g/hill	90.9 <sup>b</sup>	71.3 <sup>d</sup>	55.8 <sup>d</sup>	24.8
	50g/hill	96.9 <sup>b</sup>	62.1 <sup>ce</sup>	42.1 <sup>ce</sup>	43.1
	100g/hill	97.7 <sup>b</sup>	49.6 <sup>b</sup>	33.4 <sup>b</sup>	55.0
FYM alone (control)	25g/hill	87.9 <sup>ab</sup>	83.5 <sup>a</sup>	70.1 <sup>a</sup>	
	50g/hill	90.1 <sup>b</sup>	85.1 <sup>a</sup>	73.0 <sup>a</sup>	
	100g/hill	91.7 <sup>b</sup>	87.1 <sup>a</sup>	77.4 <sup>a</sup>	
Control ( <i>F. moniliforme</i> only)		74.1 <sup>a</sup>	87.4 <sup>a</sup>	74.2 <sup>a</sup>	

\*Values within experiments indicated by the same letter are not significantly different at P = 0.05

Table 5 Effect of different treatments with *Trichoderma harzianum* on incidence and severity of stalk rot of maize under field conditions (applied as broadcast)

Treatment	Dose/acre	Germination (%)	Disease incidence (%)	Disease severity (%)	Per cent disease control
FYM supplemented with <i>T. harzianum</i> (Th1) in 25:1 ratio	2 kg talc/q FYM	99.1 <sup>b</sup>	46.3 <sup>b</sup>	30.2 <sup>a</sup>	58.6
	1 kg talc/q FYM	98.4 <sup>b</sup>	61.1 <sup>c</sup>	48.0 <sup>b</sup>	34.2
	0.5 kg talc/q FYM	96.8 <sup>b</sup>	70.1 <sup>d</sup>	51.4 <sup>b</sup>	29.6
FYM only	1q	98.9 <sup>b</sup>	88.1 <sup>a</sup>	75.7 <sup>c</sup>	
Control ( <i>F.moniliforme</i> only)		86.9 <sup>a</sup>	86.4 <sup>a</sup>	73.0 <sup>c</sup>	
Uninoculated control		91.6 <sup>c</sup>	65.1 <sup>cd</sup>	55.7 <sup>b</sup>	

\*Values within experiments indicated by the same letter are not significantly different at P = 0.05

inoculated control (86.9%) and uninoculated control (91.6%) treatments (Table 5). Minimum disease incidence of 46.3 per cent and disease severity of 30.2 per cent with maximum disease control of 58.6 per cent were obtained with FYM supplemented with 2 kg talc based formulation of *T. harzianum* per quintal FYM (Fig 2) as compared to maximum disease incidence of 88.1 % and disease severity of 75.7 per cent in control treatment. Significant inverse correlation was obtained between the stalk rot and the dosage of talc based formulation of *T. harzianum* used.

#### Effect of bioagent applications on growth parameters and yield of maize under field conditions

##### As hill treatment

Data (Table 6) indicated that plant height in seed treatment with Th-1 @ 20g/kg and FYM supplemented with Th-1 @ 50g/hill and 100 g/hill differed significantly from the control whereas all other treatments were at par with each other. However, there were no significant differences observed between the cob length in different treatments. In case of cob girth and mean cob-weight, two treatments, viz. seed treatment with Th-1 @ 20g/kg and FYM+ Th-1 @

100g/hill differed significantly from control. Similarly, 1000-grain weight in seed treatment @ 20g/kg and FYM supplemented with Th-1 @ 50g/hill and 100g/hill differed significantly from all other treatments. The 1000-grain weight (211.7 g) was found maximum in amending soil with FYM supplemented with Th-1 @ 100 g/hill, closely followed by seed treatment @ 20g/kg seed (205.3 g) and found minimum in control (178.7 g). All the treatments increased the 1000-grain weight significantly. In case of grain yield, all the treatments differed significantly from the control. The total grain yield varied from 38.4 to 54.0 q/ha being maximum (54.0 q/ha) in soil amended with FYM supplemented with Th-1 @ 100g/hill and minimum in control (38.4 q/ha). The increase in grain yield in all treatments compared to untreated control varied from 12.2 to 40.5 per cent, being maximum in FYM supplemented with Th-1 @100g/hill and minimum in seed treatment @ 10g/kg (Fig 2). Similar trend was observed in mean cob weight, which was found highest in FYM supplemented with Th-1 @ 100g/hill (105.0g) followed by seed treatment @ 20g/kg (98.3 g).

##### As broadcast method

The data in Table 7 indicated that plant height, cob length

Table 6 Effect of different treatments with *Trichoderma harzianum* on growth parameters and yield of maize under field conditions (applied per hill)

Treatment	Dose	Plant height (cm)	Cob length (cm)	Cob girth (cm)	Mean cob wt (g)	1000-grain wt (g)	Grain yield (q/ha)
Seed treatment with <i>T.harzianum</i> (Th1)	10g/kg	111.7 <sup>a</sup>	13.7 <sup>a</sup>	4.1 <sup>ab</sup>	85.7 <sup>ab</sup>	194.7 <sup>ab</sup>	45.7 <sup>a</sup>
	15g/kg	117.0 <sup>ab</sup>	14.0 <sup>a</sup>	4.1 <sup>ab</sup>	92.7 <sup>ab</sup>	200.3 <sup>ab</sup>	47.6 <sup>b</sup>
	20g/kg	121.3 <sup>b</sup>	14.5 <sup>a</sup>	4.3 <sup>b</sup>	98.3 <sup>b</sup>	205.3 <sup>ab</sup>	52.5 <sup>c</sup>
FYM supplemented with <i>T. harzianum</i> (Th1) in 25:1 ratio	25g/hill	115.0 <sup>ab</sup>	13.8 <sup>a</sup>	4.1 <sup>ab</sup>	94.3 <sup>ab</sup>	197.3 <sup>ab</sup>	48.6 <sup>b</sup>
	50g/hill	120.0 <sup>b</sup>	14.1 <sup>a</sup>	4.2 <sup>ab</sup>	96.5 <sup>ab</sup>	204.7 <sup>ab</sup>	52.8 <sup>b</sup>
	100g/hill	125.8 <sup>b</sup>	14.7 <sup>a</sup>	4.3 <sup>b</sup>	105.0 <sup>b</sup>	211.7 <sup>b</sup>	54.0 <sup>c</sup>
FYM alone (Control)	25g/hill	120.0 <sup>b</sup>	13.5 <sup>a</sup>	4.0 <sup>ab</sup>	85.3 <sup>ab</sup>	199.3 <sup>ab</sup>	43.1 <sup>b</sup>
	50g/hill	122.7 <sup>b</sup>	14.0 <sup>a</sup>	4.1 <sup>ab</sup>	87.0 <sup>ab</sup>	201.0 <sup>ab</sup>	45.8 <sup>b</sup>
	100g/hill	124.7 <sup>b</sup>	14.0 <sup>a</sup>	4.2 <sup>ab</sup>	91.7 <sup>ab</sup>	204.7 <sup>ab</sup>	49.8 <sup>b</sup>
Control ( <i>F.moniliforme</i> only)	-	103.1 <sup>a</sup>	12.5 <sup>a</sup>	3.8 <sup>a</sup>	70.0 <sup>a</sup>	178.7 <sup>a</sup>	38.4 <sup>a</sup>

\*Values within experiments indicated by the same letter are not significantly different at P = 0.05

and cob girth increased with increase in doses of FYM supplemented with Th-1. The maximum plant height (134.3 cm), cob length (15.0 cm) and cob girth (4.4 cm) was recorded in treatment of FYM and Th-1 (2 kg talc/q FYM). In case of cob length, there was no significant difference between the treatments. The 1000-grain weight in uninoculated control significantly differed from FYM + Th-1 @ 2 kg talc/q FYM and control treatment whereas it was non-significant with other treatments. Increase in 1000-grain weight was maximum (14.3%) in FYM + *T. harzianum* @ 2 kg talc/q FYM (Fig 2). Similar trend was observed in mean cob weight in different treatments. Maximum grain yield (58.2 q/ha) was recorded in

FYM + Th-1 @ 2 kg talc/q closely followed by 1 kg talc/q (55.5 q/ha). The per cent increase in yield varied from 18.9 to 36.3 per cent in different treatments as compared to control treatments (Fig. 2).

*Trichoderma* spp. have been investigated as potential biocontrol agents because of their ability to reduce the incidence of diseases caused by soil borne pathogens (Elad 2000, Freeman *et al.* 2004, Ashrafizadeh *et al.* 2005, Dubey *et al.* 2007). The pot house studies indicated that seed treatment as well as FYM supplemented with bioagent caused significant increase in germination of maize seedlings, however, under field conditions, addition of FYM

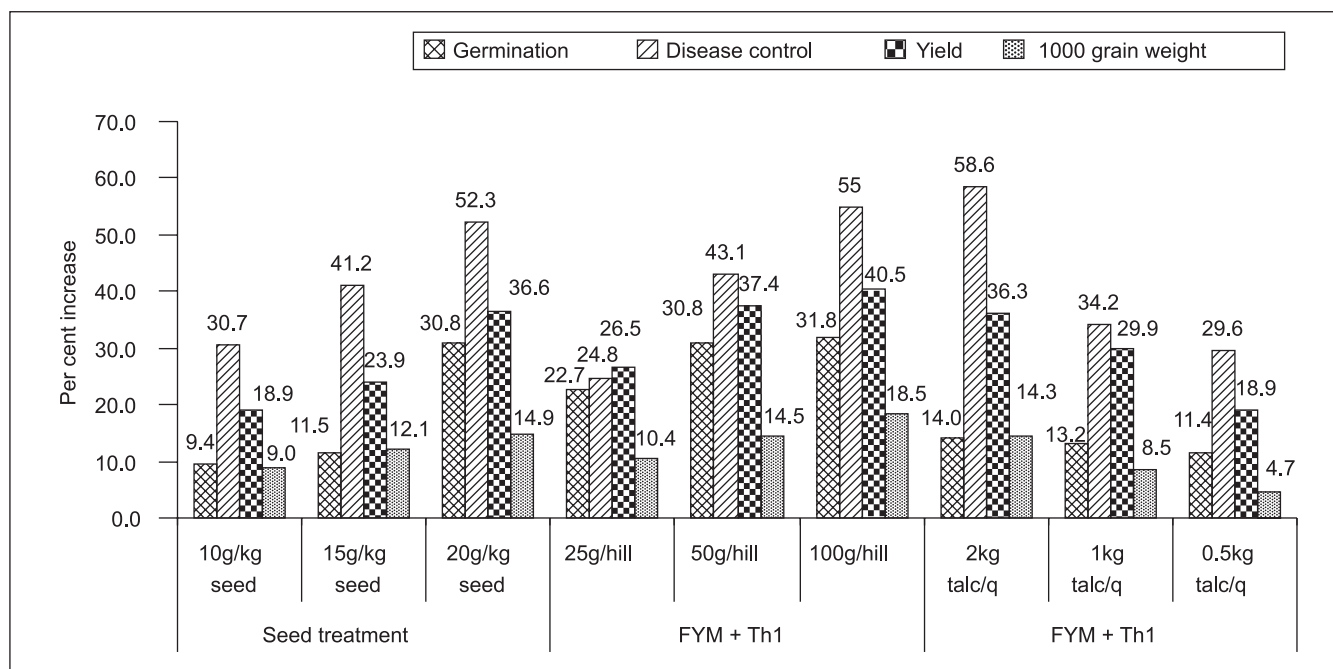


Fig 2 Effect of different treatments of *Trichoderma harzianum* on seed germination, disease control and yield parameters of maize plants under field conditions

Table 7 Effect of different treatments with *Trichoderma harzianum* on growth parameters and yield of maize under field conditions (applied as broadcast)

Treatment	Dose/acre	Plant height (cm)	Cob length (cm)	Cob girth (cm)	Mean cob wt (g)	1000-grain wt (g)	Grain yield (q/ha)
FYM supplemented with <i>T. harzianum</i> (Th1) in 25:1 ratio	2 kg talc/q FYM	134.3 <sup>b</sup>	15.0 <sup>a</sup>	4.4 <sup>b</sup>	107.7 <sup>b</sup>	210.7 <sup>b</sup>	58.2 <sup>b</sup>
	1 kg talc/q FYM	125.7 <sup>ab</sup>	14.8 <sup>a</sup>	4.3 <sup>ab</sup>	100.3 <sup>ab</sup>	200.0 <sup>ab</sup>	55.5 <sup>b</sup>
	0.5 kg talc /q FYM	125.3 <sup>ab</sup>	14.3 <sup>a</sup>	4.2 <sup>ab</sup>	96.3 <sup>a</sup>	193.0 <sup>ab</sup>	50.8 <sup>c</sup>
FYM only (control)	1 q	128.0 <sup>ab</sup>	13.5 <sup>a</sup>	4.0 <sup>ab</sup>	87.3 <sup>a</sup>	189.3 <sup>a</sup>	43.5 <sup>a</sup>
Control ( <i>F. moniliforme</i> only)		110.0 <sup>a</sup>	12.7 <sup>a</sup>	3.9 <sup>a</sup>	81.7 <sup>a</sup>	184.3 <sup>a</sup>	42.7 <sup>a</sup>
Uninoculated control		121.0 <sup>ab</sup>	13.7 <sup>a</sup>	4.1 <sup>ab</sup>	89.3 <sup>a</sup>	191.5 <sup>ab</sup>	45.1 <sup>a</sup>

\*Values within experiments indicated by the same letter are not significantly different at P = 0.05

supplemented with *T. harzianum* significantly increased seed germination, reduced stalk rot severity, promoted plant growth and increased grain yield of maize. These results are in conformity with the earlier findings (Raju *et al.* 1999, Celar and Valic 2005). Nayaka *et al.* (2010) reported that *T. harzianum* increased the seed germination in Kanchan, Pioneer and Sweet corn cultivars of maize. Cook and Baker (1993) applied the same strategy and successfully used *T. harzianum* as seed treatment to improve stand of maize, beans and other vegetable crops. Orole and Adejumo (2009) revealed *T. koningii* as the best endophyte which reduced wilting rate to 25 % in maize under *in vivo* conditions. Harman *et al.* (2004) have shown that plants grown from *T. harzianum* strain T-22 treated seed induced systemic resistance in maize. The soil amendment with organic matter is well known to activate bio-control capacity of various antagonists. There are several reports regarding amendment of organic matter in soil and management of soil borne diseases as Kaur (2000) reported that amendments with FYM along with non-pathogenic *Fusarium* enhanced the biocontrol potentiality of antagonists. Organic amendments make the soil suppressive against soil borne pathogens. Differences in growth parameters among the treatments is an important tool for measuring stimulating effect of Th1 on maize plants. In the present investigation, seed treatment with *T. harzianum* and FYM supplemented with Th1 had positive effect on growth parameters of maize plant. This has been demonstrated earlier by Va'squez *et al.* (2000) who reported that *T. harzianum* increased shoot length and dry weight in maize. Similarly, several other workers have also explained different mechanisms for plant growth enhancement by *Trichoderma* spp (Hoitink *et al.* 2006, Vinale *et al.* 2008, Harman *et al.* 2004). Increased growth of plants by timely application of *T. harzianum* might be due to the elimination of minor pathogens in the rhizosphere.

To sum up, the results revealed that application of FYM supplemented with *T. harzianum* isolate (Th 1) both in hill inoculation and broadcast method exhibited great antagonistic potential against *F. moniliforme* causing stalk rot of maize and can be further exploited to develop eco-friendly strategies

for disease management. The use of Th1 isolate of *Trichoderma harzianum* as a biocontrol is expected to have high potential for controlling *F. moniliforme* in maize crop. However, its ecological parameters must be determined before its use can be commercialized.

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