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RESEARCH ARTICLE

Biological control of anthracnose of sorghum caused by *Colletotrichum graminicola*

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ABSTRACT

Forty isolates of *T. harzianum* and one isolate of *P. fluorescens* were tested for their antagonistic potential against *Colletotrichum graminicola*. Th-43 isolate brought maximum inhibition of radial growth (72.5%) of the test pathogen. In glass house seed biopriming experiment, maximum germination was obtained with Th-43 (84.0%), whereas maximum plant height (102.0 cm) with Th-39 and maximum reduction in disease severity (43.3 %) was observed with Th-39 and Th-43. In field trial of seed biopriming and BCA colonized compost amended soil, Th-39 recorded maximum height (234.7 cm) and stem diameter (1.8 cm) whereas maximum reduction in disease severity was obtained with Th-39 (28.1%). In seed biopriming and foliar spray trial under field conditions, maximum reduction in disease severity (45.2%) and highest green fodder yield (90 t/ha) was found in seed biopriming and three foliar spray treatment with Th-39. In foliar spray experiment carried out under field conditions, Th-39 showed maximum reduction in disease severity (34.0 %) as well as maximum green fodder yield (81.9 t/ha) where as Th-43 was also at par Th-39 in terms of green fodder yield.

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INTRODUCTION

Sorghum is attacked by a broad range of plant pathogens. Anthracnose caused by *Colletotrichum graminicola* is one of the most destructive diseases of sorghum. Reduction in grain and stover yield by 47 and 23 per cent, respectively have been reported (Pande *et al*., 2003). It occurs everywhere in the world where sorghum is grown and most of the cultivars presently grown in the country have varying degree of susceptibility to the disease. For management of this disease besides many cultural practices chemicals are also used. Chemicals are necessary at present, but are not a long term solution to crop health. Besides their non target effects and hazardous nature, many of them are now losing their effectiveness because of development of resistant strains. Moreover, application of chemicals to sorghum crop is to be avoided as the fodder is fed to the cattle. Therefore, the present investigation was carried out to evaluate the efficacy of biocontrol agents against the anthracnose pathogen.

MATERIALS AND METHODS

Source of biocontrol agents :

Forty isolates of *Trichoderma harzianum* and one of *Pseudomonas fluorescens* were used throughout the course of investigation. For *in vitro* studies and glasshouse experiments, the various *T. harzianum* isolates used were Th - 1to Th-23, Th-25 to Th-34, Th-36 to Th-40,Th-42 to Th-45 whereas *P. fluorescens* isolate was Psf 28. For field evaluation, the formulations of Th-43, 39 and PSF -28 were used. These bioagents were obtained from Biocontrol Laboratory of Department of Plant Pathology, G.B. Pant

University of Agriculture and Technology, Pantnagar.

***In vitro* testing of antagonism between *T. harzianum* isolates and *C. graminicola* by bangle method :**

A 5 mm disc was cut from the periphery of actively growing culture (3 to 5 days old) of the test fungus (on oat meal agar medium) with the help of sterilized cork borer and was centrally inoculated in Petri plates (9 cm diameter) seeded with oat meal agar (approx. 20 ml/ plate). A flame sterilized glass bangle (6cm diameter) was dipped in conidial suspension of different *Trichoderma harzianum* isolates in 2 per cent autoclaved sterilized gelatin solution and transferred carefully to Petri plates with the help of sterilized forceps. After dipping the bangle in spore suspension, it was held for some time to allow excess of suspension to trickle down before transferring to the Petri plates. In case of control bangles were dipped in 2 per cent gelatin solution only. The test pathogen being relatively slow growing was inoculated 48 hours prior to *Trichoderma harzianum*. Three replications were used for each treatment. All the plates were incubated at 28±1^{0}C. Radius of the fungal pathogen was recorded 48 hours after inoculation. Per cent inhibition of growth and time taken by *Trichoderma* to completely overgrow the fungal pathogen were taken into account as criteria to compare their antagonistic potential. The percent inhibition of radial growth was calculated with following formula :

$$\text{Per cent inhibition of radial growth} = \frac{\text{Radial growth in check} - \text{radial growth in treatmens}}{\text{Radial growth in check}} \text{x100}$$

Glass house experiment :

***Seed biopriming experiment* :**

Pots of 30 cm. size were filled with sterilized soil and then the bioprimed seeds were sown in these pots. Biocontrol agents were used @ 10 g spores/cell (2 ×10^9 cfu/ml) / kg of seeds for each treatment. Each isolate was mixed with 50 ml of 2 per cent gum arabic solution and then seeds were incubated at 28±1^{0}C for 24 hours before sowing to allow covering of seed surface with bioagents. The gum arabic solution acts as a sticking material and keeps the bioagents glued to the seed surface. The observations on germination percentage, height of plants and disease severity were recorded. Each treatment was replicated thrice. Congenial growth conditions were provided to the experimental plants. The inoculation of the plants with the pathogen was done when they were 1 month old. The observation on plant height, stem diameter and disease severity were recorded after 60 days of sowing while germination was recorded after 15 days of sowing.

Field trials :

Field experiments were conducted during the kharif season of 2007 at Livestock Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar to evaluate the efficacy of selected bioagents in controlling anthracnose of sorghum.

Seed biopriming :

Biopriming was done 24 hours before sowing by treating the seeds with talc based formulation of biocontrol agents (Th-39, Th-43 and Psf-28) @ 10g spores/cells (2×10^9 cfu/ml) / kg seeds in 2% gum arabic solution. Thirty days old plants were artificially inoculated by spraying the spore suspension (5×10^4 spores/ml) of test pathogen on the sorghum plants. Bioprimed seeds were used for sowing in two separate experiments; one in which soil was amended with BCA colonized compost while other was without any amendment. Observations on disease severity, plant height and stem diameter were recorded 60 days after sowing whereas green fodder yield was recorded after harvesting.

Seed biopriming and foliar spray :

Seeds were bioprimed in aforementioned manner with (Th-43 Th-39) and (Psf-28). Thirty days old plants in the field were artificially inoculated by spraying the spore suspension of the pathogen containing 5×10^4 spores/ml. The inoculum was sprayed between 6-7 pm as night temperature and humidity were conducive for infection. Three sets of experiment were conducted *viz.,* first set: bioprimed seed and one spray; second set-bioprimed and two spray; third set: bioprimed seed and three spray. In all the sets, first spray of the biocontrol agent @ 10 g spores/cells (2 ×10^9 cfu /ml) /lit. of water was given before three days of inoculum spray. Second and third sprays were given at 10 days interval.

Foliar spray :

Talc based formulations of Th-43, 39 and Psf-28 were sprayed as in seed biopriming and foliar spray experiment to test their efficacy against the pathogen. Trials were laid out in a Randomized Block Design (RBD) with three replications. Observations on disease severity were recorded in 1-5 scale proposed by All India Coordinated Sorghum Improvement Project after 60 days of sowing as follows: 1 = No symptoms (Highly resistant), 2= upto 10% intensity (Resistant), 3=11-25% intensity (Moderately resistant), 4=26-50% intensity (Susceptible), 5=above 50% intensity (Highly susceptible). Following formula was used to calculate the percent disease severity :

$$\text{Per cent disease severity (S)} = \frac{\text{Sum of numerical rating}}{\text{Total no. of samples x maximum rating grade}} \text{x100}$$

RESULTS AND DISCUSSION

The results of the present study as well as relevant discussions have been presented under following sub heads:

***In vitro* testing of antagonism between *Trichoderma* isolates and *C. graminicola* using bangle method :**

Antagonistic potential of 40 isolates of *T. harzianum* and 1 isolate of *P. fluorescens* (Psf-28) was evaluated against the pathogen, *C. graminicola* by bangle method (Table 1). Th-43 performed best which gave 72.5% inhibition of radial growth followed by Th-39 (72.3%), Th-36 (64.2%), Th-37 (63.4%), Th-4 (62.2%), Psf-28 (61.8%) and Th-22 (61.7%) while least inhibition (7.7%) was obtained with Th-44. All *T. harzianum* isolates tested for antagonism *in vitro* were found to be effective to varying extents. In fact the difference in per cent inhibition of radial growth indicates, the difference in their antagonistic potential for the test pathogen. In present results, a clear cut zone of inhibition was observed with Th-3, 4, 9, 13, 22, 32, 37, 39, 38, and 43 isolates tested against the pathogen. This may be due to mechanism of antibiosis by the antagonists. *Trichoderma* spp. inhibiting the growth of pathogens by the mechanism of antibiosis has been reported earlier. (Bangari and Singh, 2011).

Table 1: Per cent inhibition of radial growth of *C. graminicola* by different isolates of *T. harzianum* and *P. fluorescens* under *in vitro* condition by Bangle method after 7 days

Treatments	Average radial growth of fungus (cm)	Inhibition of radial growth (%)
Th-1	2.4	59.4 (50.4)
Th-2	2.8	51.8 (46.0)
Th-3	2.3	61.2 (51.0)
Th-4	2.2	62.2 (52.0)
Th-5	2.4	58.5 (49.9)
Th-6	2.5	56.9 (48.9)
Th-7	4.7	21.3 (27.1)
Th-8	2.6	55.7 (48.3)
Th-9	4.0	32.1 (34.5)
Th-11	4.5	24.0 (29.3)
Th-12	2.6	56.2 (48.6)
Th-13	4.8	18.4 (25.4)
Th-14	5.0	16.3 (23.8)
Th-15	4.6	22.1 (28.0)
Th-17	4.1	30.1 (33.2)
Th-18	2.7	54.7 (47.7)
Th-19	5.5	8.0 (16.5)
Th-20	5.4	9.3 (17.7)
Th-21	5.6	5.9 (14.1)
Th-22	2.2	61.6 (51.7)
Th-23	4.4	26.2 (30.8)
Th-25	2.3	60.6 (51.1)
Th-26	4.5	23.6 (29.0)
Th-27	2.6	55.9 (48.4)
Th-28	4.2	29.7 (33.0)
Th-29	4.4	25.7 (30.4)
Th-30	5.0	16.4 (23.8)
Th-31	2.4	59.5 (50.4)
Th-32	2.3	61.0 (51.3)
Th-33	5.3	11.1 (19.5)
Th-34	5.1	14.4 (22.3)
Th-36	2.1	64.2 (53.2)
Th-37	2.1	63.7 (52.9)
Th-38	2.3	61.0 (51.3)
Th-39	1.6	72.3 (58.2)
Th-40	5.4	9.7 (18.1)
Th-42	5.4	8.4 (16.8)
Th-43	1.6	72.4 (58.4)
Th-44	5.2	7.7 (16.1)
Th-45	4.8	19.5 (26.2)
Psf-28	3.5	61.8 (51.3)
Control	9.0	--
C.D. at 5 %	0.20	1.15

*Figures in parentheses are angular transformed values.

Glass house experiments :

***Seed biopriming experiment* :**

As bio-control agents are known to promote growth and induce the resistance in plants, biopriming of seeds was done with forty different *T. harzianum* isolates and one *P. fluorescens* isolates for 24 hours and observations on germination, height of the plant and disease severity were recorded (Table 2). Among all treatments Th-1, 2, 3, 4, 9, 21, 22, 27, 28, 29, 32, 34, 36, 38, 39, 40, 43 and Psf-28 showed significant increase in germination over control. Maximum germination was observed with Th-43 (84%) followed by Th-36 (83.7%) and Th-39 (83.3%). Plant height was observed maximum in Th-39 (102cm) treated seeds followed by Th-43 (100cm), Th-36 (98.7cm) and Psf-28 (97.7cm), while in control plant height was 89.0 cm. While studying the effects of different isolates of *Trichoderma* on host plants, Chet (1987) and Kleifeld and Chet (1992) obtained similar growth promotion results. Maximum reduction in disease severity was obtained with Th-39 and Th-43 (43.3%) followed by Th-32 (41.6%), Th-11 (39.1%), Th-36 (38.3%), Th-45 (37.5) and Psf-28 (33.8%). The present findings are in consonance with those reported by Nzojiyobiri *et al.* (2003); Chen *et al.* (2005) and, Vidhyasekaran and Muthamilan (1995). Mechanism of disease reduction is probably induced systemic resistance because the pathogen is not in direct contact with the bio-control agent; the resistance thus exerted by the plant seems to have originated systemically.

Field trials :

***Bioprimed seeds in plots amended with BCA colonized compost* :**

Seeds of susceptible sorghum variety PC-4 which were bioprimed for 24 hours with different biocontrol agents were

Table 2: Effect of seed biopriming on seed germination, plant height and disease severity under glass house condition

Treatment	Germination (%)	Plant height (cm)	Disease severity (%)	Decrease in disease severity (%)
Th-1	78.0 (62.0)	92.5	34.3 (35.9)	14.2
Th-2	80.3 (63.7)	92.6	29.0 (32.6)	27.5
Th-3	76.7 (61.1)	89.8	34.3 (35.9)	14.2
Th-4	75.7 (60.4)	95.5	34.7 (36.1)	13.4
Th-5	67.7 (55.4)	89.8	34.7 (36.1)	13.3
Th-6	69.3 (56.4)	91.5	27.0 (31.3)	32.5
Th-7	67.7 (55.4)	88.5	34.0 (35.6)	15.0
Th-8	66.7 (54.8)	90.4	31.3 (34.0)	21.7
Th-9	74.3 (59.6)	92.1	26.0 (30.6)	35.0
Th-11	63.3 (52.7)	89.5	24.3 (29.6)	39.2
Th-12	68.3 (55.8)	92.5	29.3 (32.8)	26.7
Th-13	63.7 (52.9)	93.5	31.3 (34.0)	21.7
Th-14	65.7 (54.1)	90.9	32.0 (34.4)	20.0
Th-15	67.0 (54.9)	91.4	29.0 (32.6)	27.5
Th-17	68.0 (55.6)	92.2	26.3 (30.9)	34.2
Th-18	65.6 (54.1)	93.9	26.0 (30.7)	35.0
Th-19	69.0 (56.2)	94.6	30.0 (33.2)	25.0
Th-20	70.0 (56.8)	94.3	34.7 (36.1)	13.4
Th-21	71.0 (57.4)	92.9	35.3 (36.7)	11.7
Th-22	70.7 (57.2)	96.6	35.3 (36.5)	11.7
Th-23	73.0 (58.7)	93.6	33.0 (35.1)	17.5
Th-25	68.0 (55.6)	90.7	32.0 (34.4)	20.0
Th-26	69.3 (56.4)	91.0	32.3 (34.7)	19.2
Th-27	71.0 (57.4)	92.3	31.7 (34.2)	20.9
Th-28	72.7 (58.5)	92.7	31.7 (34.2)	20.9
Th-29	72.7 (58.5)	92.6	32.7 (34.9)	18.4
Th-30	68.3 (55.8)	94.0	33.7 (35.5)	15.9
Th-31	68.7 (55.9)	91.0	32.0 (34.4)	20.0
Th-32	80.7 (63.9)	98.0	23.3 (28.9)	41.7
Th-33	70.0 (57.2)	91.5	33.3 (35.3)	16.7
Th-34	72.3 (58.3)	96.5	31.3 (34.0)	21.7
Th-36	83.7 (66.2)	98.7	24.7 (29.8)	38.4
Th-37	68.7 (55.9)	92.0	35.0 (36.3)	12.5
Th-38	82.7 (65.5)	95.2	24.7 (29.8)	38.4
Th-39	83.3 (66.0)	102.0	22.7 (28.4)	43.4
Th-40	74.7 (59.8)	93.5	31.7 (34.0)	30.9
Th-42	69.3 (56.4)	92.0	32.7 (34.9)	18.4
Th-43	84.0 (66.5)	100.0	22.7 (28.4)	43.4
Th-44	70.0 (56.8)	92.5	31.0 (33.8)	22.5
Th-45	70.0 (56.8)	92.2	25.0 (29.9)	37.5
Psf-28	76.3 (60.9)	97.7	26.5 (41.8)	33.8
Control	68.4 (55.8)	89.0	44.0 (41.6)	--
C.D. at 5 %	2.3	1.2	1.4	----

*Figures in parentheses are angular transformed values

used for sowing in this experiment. The observations on plant height, stem diameter and disease severity were recorded. Results presented in Table 3 indicate that all the treatments recorded significant increase in height over control. A maximum plant height of 234.7 cm was observed in Th-39 treated seeds followed by Th-43 (234.3 cm) and Psf-28 (230.8 cm). All the treatments increased the stem diameter significantly over control except Psf-28. Maximum stem diameter was observed in Th-39 (1.8 cm). All the treatments were found to be significantly superior in reducing disease severity over control. A maximum reduction in disease severity was observed in Th-39 (28.1%). Maximum increase in green fodder yield was observed with Th-39 (28.1%) followed by Th-43 (27.2%).

Seed biopriming and foliar spray :

Effect of seed biopriming and foliar spray with bio-control agents on plant growth and disease severity of anthracnose was evaluated. Results presented in Table 4 indicate a significant increase in plant height, stem diameter and reduction in disease severity in all treatments. Increase in plant height, stem diameter, green fodder yield and reduction in disease severity were observed as number of spray increased. Maximum reduction in disease severity (45.3%) and highest green fodder yield (90 t/ha) was found in seed biopriming and 3 foliar spray treatment with Th-39. Present findings are in consonance with those reported by Julien (2006) on effect of spray with biological control agent *P. fluorescens* in phylloplane upon plant growth. Similar findings have been reported by Singh and Singh (2008) while studying the effect of seed biopriming and spraying *T. harzianum* isolates in reducing disease severity and increasing plant growth and yield of sorghum. Th-39 was superior in increasing in plant height (31%) and green fodder yield (17%) over control in a foliar spray experiments. Th-43 resulted in reduced disease severity over control in treatments with seed biopriming, compost colonized by biocontrol agents and seed biopriming

Table 3: Effect of seed biopriming and BCA colonized compost on plant growth and disease severity

Treatments	Plant height (cm)	Stem diameter(cm)	Disease severity (%)	Decrease in disease severity (%)	Green fodder yield (q/ha)	Per cent increase in green fodder yield
Th-39	234.7	1.8	39.0 (38.6)	28.1	82.6	28.1
Th-43	234.3	1.7	39.8 (39.1)	26.5	82.0	27.2
Psf-28	230.8	1.6	42.1 (40.4)	22.4	74.7	15.9
Contaf	225.9	1.5	38.8 (38.5)	28.4	74.0	14.7
Control	220.2	1.5	54.2 (47.4)	--	64.5	--
C.D. at 5 %	0.8	1.0	0.2		0.7	

*Figures in parentheses are angular transformed values

Table 4: Effect of seed biopriming and foliar spray on plant growth and disease severity

Treatments	No. of spray	Plant height (cm)	Stem diameter (cm)	Disease severity (%)	Decrease in disease severity (%)	Green fodder yield (q/ha)	Per cent increase in green fodder yield
	1	232.3	1.7	36.5 (37.1)	32.5	77.7	20.5
Th-39	2	236.6	1.8	30.5 (33.5)	43.9	82.0	27.2
	3	237.6	1.9	29.7 (33.0)	45.3	90.0	39.6
	1	232.5	1.7	39.4 (38.9)	27.5	82.0	27.2
Th-43	2	235.2	1.8	35.7 (36.7)	37.3	83.7	29.8
	3	236.4	1.8	34.0 (35.7)	39.2	85.7	32.9
	1	229.9	1.5	41.1 (39.9)	24.2	81.3	26.7
Psf-28	2	233.5	1.6	37.8 (37.9)	30.3	86.3	33.9
	3	235.1	1.6	37.2 (37.6)	31.4	88.7	37.5
	1	226.1	1.5	35.9 (36.8)	33.9	74.0	14.7
Contaf	2	228.2	1.6	32.9 (35.0)	39.3	75.3	16.7
	3	232.8	1.6	30.9 (33.8)	42.9	76.0	17.8
Control		220.2	1.5	54.3 (47.4)	--	64.5	--
C.D. at 5 %		0.9	0.5	0.4		8.5	

*Figures in parentheses are angular transformed values

Table 5: Effect of foliar spray of biocontrol agents on plant growth and disease severity

Treatments	No. of spray	Plant height (cm)	Stem diameter (cm)	Disease severity (%)	Decrease in disease severity (%)	Green fodder yeld (q/ha)	Per cent increase in geen fodder yield
	1	230.3	1.6	40.0 (39.3)	26.2	80.7	25.1
Th-39	2	232.8	1.7	36.4 (37.1)	32.9	81.8	28.4
	3	233.1	1.7	35.6 (36.7)	34.3	81.9	27.1
	1	230.3	1.6	40.1 (39.3)	26.2	80.9	25.1
Th-43	2	232.4	1.7	36.3 (37.1)	33.0	81.5	26.4
	3	232.9	1.7	35.8 (36.7)	34.0	81.9	27.1
	1	225.7	1.5	43.8 (41.4)	19.3	73.6	14.2
Psf-28	2	227.3	1.6	40.8 (39.7)	24.8	74.7	15.8
	3	227.4	1.6	40.7 (39.7)	24.9	74.9	16.1
	1	225.1	1.5	40.0 (39.2)	26.3	74.0	14.7
Contaf	2	227.2	1.6	32.1 (34.5)	40.9	74.9	16.1
	3	237.8	1.6	31.6 (34.1)	41.8	75.9	17.7
Control		220.2	1.5	54.3 (47.4)	--	64.5	--
C.D. at 5 %		0.7	0.6	1.8		0.7	

*Figures in parentheses are angular transformed values

and foliar spray by 20, 22 and 21per cent, respectively.

Foliar spray :

Effect of foliar spray of Th-43, Th-39 and Psf-28 on plant growth and disease severity of anthracnose was determined. Results presented in Table 5 indicate a significant increase in plant height in all treatments over control. Stem diameter was observed to be significantly superior in all the treatments over control. A maximum (34.0%) reduction in disease severity was observed by 3 spraying with Th-39. However, Th-43 was at par with Th-39 in reducing disease severity. Maximum yield was obtained with Th-43 and Th-39 (81.9t/ha) with 3 spray treatment. Similar observations on reduction of disease severity were obtained by Sati (2005) while working with *T. harzianum* and *P. fluorescens* foliar spray against *C. graminicola,* the anthracnose pathogen of sorghum.

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