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RESEARCH ARTICLE

Biological control of zonate leaf spot of sorghum caused by *Gloeocercospora sorghi*

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ABSTRACT

Zonate leaf spot caused by *Gloeocercospora sorghi* is one of the most destructive diseases of sorghum. The present investigation was carried out to test the efficacy of ten isolates of *T. harzianum* for their antagonistic potential against *G. sorghi* by dual culture technique *in vitro*. Th-32 isolate showed maximum inhibition of radial growth (86.1%) of the test pathogen. Volatile compounds of Th-43 isolate inhibited maximum mycelial growth of the pathogen (83.3%) followed by Th-38 (71.1%) and Th-32 (67.0%). In a seed bio-priming followed by two foliar sprays experiment, maximum increase in plant height (241.25 cm) and reduction in disease severity (37.48%) was recorded with Th-32 under field conditions.

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INTRODUCTION

Sorghum is the fifth most important cereal crop in the world after maize (*Zea mays* L.), wheat (*Triticum vulgare* L.), rice (*Oryza sativa* L.) and barley (*Hordeum vulgare* L.). Sorghum is attacked by a wide range of pathogens because of a range of environments in which it is cultivated. Zonate leaf spot caused by *Gloeocercospora sorghi* Bain and Edgerton is one of the most destructive diseases of sorghum crop in India. The disease causes damage upto 85 per cent of photosynthetic area under humid and cloudy weather conditions (Agnihotri and Pandey, 1977). The present investigation was carried out to investigate the efficacy of *T. harzianum* isolates against the pathogen *in vitro* and *in vivo.*

MATERIALS AND METHODS

Dual culture screening :

Ten *T. harzianum* isolates (Th-6, 10, 15, 25, 31, 32, 36, 38, 39, and 43) procured from Biocontrol Laboratory of Department of Plant Pathology, Pantnagar, were screened for their antagonistic potential against the pathogen following the dual culture technique (Morton and Stroube, 1955). Twenty ml of sterilized and melted Oat meal agar (OMA) was aseptically poured in a sterilized 90 mm diameter Petri plates and allowed to solidify. Five mm of 6 days old mycelial disc of pathogen (*G. sorghi*) and test biocontrol agents cut with the help of sterilized cork borer from the edge of 4 days old culture, were placed on solidified OMA in such a manner that they lie just opposite to each other (approximately 6 cm apart from each other). Inoculated petri plates were incubated for seven days at 28 ± 1^0C because the pathogen took a week to completely fill the Petri plate. OMA amended Petri plate and inoculated centrally with 5mm mycelial disc of pathogen served as control. Observation on inhibition of radial growth of the pathogen was recorded after 7 days. Experiments were conducted in Completely Randomized Design (CRD) and each treatment was replicated three times.

Effect of volatile compounds :

Five *T. harzianum* isolates *viz.,* Th-43, 32, 38, 36 and 31

that were found promising in dual culture test were further tested for antifungal volatiles. The bio-agents were grown on Petri plates containing OMA medium for 2, 4, 6 and 8 days. The lids of these Petri plates were replaced with the freshly inoculated test pathogen under aseptic condition. Petri plate pair, containing freshly inoculated test pathogen on upper side and amended with OMA medium without bio-agent on lower side served as control. The pair of each plate was sealed together with parafilm. The plates were incubated at 28±1°C and observations were recorded after 7 days. Experiments were conducted in completely randomized design (CRD) with three replications. The growth inhibition per cent was recorded by the following formula:

$$I = \frac{C - T}{C} x100$$

where,

I = Per cent inhibition

C = Radial growth in check in mm

T = Radial growth in treated plates in mm

Effect of seed bio-priming and foliar spray in glasshouse experiment :

***Seed bio-priming* :**

T. harzianum isolates-32, 25, 38, 39, and 43 were used for seed bio-priming @ 10 g spores (2×10^9 cfu/ml) / kg seeds of susceptible sorghum variety Pant Chari-4. Ten gram of spore powder of each isolate was mixed with 50 ml of 2% jaggery solution and then seeds were incubated at 28±1°C in this solution for 24 hours before sowing, to allow covering of seed surface with the mycelial growth of *T. harzianum* isolates. The jaggery solution acts as a sticking material here and keeps the bio-agents glued to the seed surface. Ten bio-primed seeds were, then shown in each pot with three replications. After 20 days, all the other seedlings were uprooted from each pot to keep only 5 seedlings per pot. Thirty days old seedlings were artificially inoculated by spraying the spore suspension of the pathogen containing 5×10^4 spores /ml. Two sets of experiment were conducted *viz.,* first set- seed bio-priming and one foliar spray; second set-seed bio-priming and two foliar sprays. In both the sets, first spray of *T. harzianum* isolates @ 10 g of spores / lit. of water was given after two days of inoculation with zonate leaf spot pathogen. In second set of experiment, second spray was given after 15 days of first spray. Control plants were sprayed with sterilized water only. In glasshouse, temperature was maintained about 30±1°C and relative humidity >85%. Experiments were conducted in completely randomized design (CRD) with three replications. The observations on disease severity were recorded in 1-5 scale {1 = No symptoms (Highly resistant), 2 = upto 10 % leaf area covered (Resistant), 3 = 11-25% leaf area covered (Moderately resistant), 4 = 26-50% leaf area covered (Susceptible), 5 = above 50% leaf area covered (Highly susceptible)}, 60 days after sowing (DAS). Following formula was used to calculate the per cent disease severity :

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

Field trials :

Field experiments were conducted during 2008 *Kharif* season at Pantnagar to evaluate the efficacy of selected bio-agents in controlling zonate leaf spot of susceptible sorghum variety, Pant Chari-4. The isolates, Th-43, Th-32 and Th-38 found effective in glass house experiments were further evaluated in field trials *viz.,* seed bio-priming, seed bio-priming and one foliar spray, seed bio-priming and two foliar sprays, one foliar spray and two foliar sprays, as per procedure described in glasshouse experiments. Observations on disease severity were recorded 45 and 60 DAS. Field trials were laid out in randomized block design (RBD) with three replications.

Statistical analysis :

Statistical analysis was done using STPR software available in the Department of Mathematics, Pantnagar.

RESULTS AND DISCUSSION

The experimental findings of the present study have been presented in the following sub heads:

***In vitro* antagonism between *T. harzianum* isolates and *G. sorghi* :**

Using dual culture method, antagonistic potential of 10 isolates of *Trichoderma harzianum* was evaluated against *G. sorghi* (Table1). In dual culture test, all the isolates reduced

Table 1 : Per cent inhibition of radial growth of *G. sorghi* by different isolates of *T.* harzianum after 7 days

Treatments	Radial growth (cm)	Inhibition of radial growth (%)
Th-6	5.99	33.4 (35.3)
Th-10	5.25	41.6 (40.1)
Th-15	3.40	62.2 (52.0)
Th-25	2.97	67.0 (55.2)
Th-31	2.62	70.8 (57.3)
Th-32	1.25	86.1 (68.1)
Th-36	2.25	75.0 (60.0)
Th-38	1.60	82.2 (65.0)
Th-39	4.42	50.8 (45.5)
Th-43	1.90	78.6 (62.4)
control	9.00	-
C.D. at 5 %	0.28	

*Figures in parentheses are angular transformed values

the colony growth of the pathogen. Th-32 performed best which gave 86.1 per cent inhibition of radial growth followed by Th-38 (82.2 %), Th-43(78.6 %), Th-36 (75.0 %) and Th-31(70.8%) whereas least inhibition was obtained with Th-6 (33.`4 %). The difference in per cent inhibition of radial growth indicates the difference in their antagonistic potential for the test pathogen. These observations are similar to the finding of Kucuk and Kivance (2004). In present results, a clear cut zone of inhibition was observed with all the 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 by several workers

Table 2: Effect of volatile compound of *T. harzianum* isolates on radial growth of *G. sorghi* after 7 days

Treatments	Age of culture (in days) of *T. harzianum* isolates							
	2		4		6		8	
	Radial growth (cm)	Inhibition (%)	Radial growth (cm)	Inhibition (%)	Radial growth (cm)	Inhibition (%)	Radial growth (cm)	Inhibition (%)
Th-43	1.82	79.7	1.50	83.3	2.92	67.5	3.02	66.4
Th -38	3.10	65.5	2.60	71.1	3.30	63.3	3.37	62.5
Th -36	4.00	55.5	3.32	63.1	3.02	66.4	3.30	63.3
Th -32	3.60	60.0	2.97	67.0	3.00	66.6	3.12	65.3
Th -31	3.15	65.0	3.17	64.7	3.70	58.8	3.87	57.0
Control	9.00	-	9.00		9.00		9.00	-
C.D. at 5%	0.28		0 .21		0.42		0.29	

Table 3 : Effect of seed bio-priming followed by two foliar sprays of *T. harzianum* on germination, plant height and disease severity of zonate leaf spot under glass house conditions

Treatments	Germination (%)	Plant height (cm)	Disease severity (%)	Decrease in disease severity (%)
Th -43	87.0(69.5)	64.2	26.4 (37.7)	44.3
Th 32	83.5(70.0)	63.2	23.0 (36.0)	50.8
Th -38	85.2(67.8)	61.3	24.3 (36.5)	48.7
Th -25	78.7(63.0)	55.7	29.5 (38.9)	37.7
Th -39	81.3(64.4)	55.7	31.9 (39.5)	32.8
Control	79.9(63.2)	54.0	47.5 (43.6)	
C.D. at 5%	1.43	2.67	2.90	

*Figures in parentheses are angular transformed values

Table 4 : Effect of seed bio-priming followed by two foliar sprays of *T. harzianum* isolates on plant height, stem diameter and disease severity of zonate leaf spot in field experiment

Treatments	Plant height (cm.)	Stem diameter (cm.)	Disease severity (%)			Decrease in disease severity (%)
			45 DAS	60 DAS	Mean	
Th-43	236.00	1.37	41.27	48.10	44.69	25.52
Th-32	241.25	1.42	31.30	43.48	37.39	37.48
Th-38	231.37	1.22	38.77	44.51	41.64	30.37
Control	215.50	1.07	58.23	61.40	59.81	-
C.D. at 5%	2.38	1.03				
Treatment (A)				0.17		
Interval (B)				0.24		
A × B				0.34		

(Sivan and Chet, 1989; Upadhyay and Mukhopadhyay, 1986; Sharma and Dohroo, 1991; Harman, 2000).

Effect of volatile compounds of *T. harzianum isolates* on the radial growth *of G. sorghi*

Biocontrol agents on OMA produced volatile compounds which significantly inhibited the radial growth of the test pathogen. The results presented in Table 2 suggest that Th-32, 38, 31, 36, 43 were capable of influencing the growth of pathogen through production of volatile inhibitors under controlled conditions. Maximum (83.33%) inhibition of the mycelial growth of the pathogen was recorded with Th-43 which was followed by Th-38 (71.1%), Th-32 (67.00%), Th-31 (64.7%) and Th-36 (63.1%) when 4 days old culture were used. As the culture of biocontrol agents got older, their effect on the growth of pathogen was significantly reduced. The effect was minimum on the pathogen from 8 days old culture of *T. harzianum* isolates. There was a linear relationship: fresher the *T. harzianum* isolates culture, greater the amount of volatile compounds and consequently less radial growth and more inhibition percentage. Dennis and Webster (1971a and b) have also reported the volatile action of *Trichoderma* spp.

Glasshouse experiment :

It is evident from the data presented in Table 3 that all the treatments significantly reduced the disease severity over control. Maximum reduction in disease severity was observed with Th-32(50.8%) followed by Th-38 (48.7%) and Th-43 (44.3%). Maximum plant height was observed with Th-43 (64.2 cm). However, Th-32 (63.2 cm) was statistically at par with Th-43 (64.2 cm).

Field experiment :

It is evident from the data presented in Table 4 that the seed bio-priming followed by two sprays of *T. harzianum* significantly increased plant height and reduced disease severity. Maximum reduction in disease severity was observed with Th-32 (37.48%) followed by Th-38 (30.37%) and Th-43 (25.52%). Maximum plant height was observed with Th-32 (241.2 cm) followed by Th-43 (236.00 cm). The present findings are in conformity with those reported by Sivan and Chet, 1989; Sharma, and Dohroo, 1991; Inbar *et al.*, 1996; Tiwari, 2006 and Indira *et al.,* 2006.

Therefore, seed bio-priming followed by two foliar sprays with Th-32 may be recommended for management of zonate leaf spot of sorghum.

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