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EVALUATION OF *TRICHODERMA HARZIANUM* AND *PSEUDOMONAS FLUORESCENS* ISOLATES FOR THEIR ANTAGONISTIC POTENTIAL AGAINST *EXSEROHILUM TURCICUM* CAUSING LEAF BLIGHT OF SORGHUM

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ABSTRACT
Antagonist potential of seventeen isolates of *Trichoderma harzianum* and ten isolates of *Pseudomonas fluorescens* was determined against *Exserohilum turcicum in vitro* using dual culture technique. Th-39 and Psf- 82 gave maximum inhibition of mycelial growth of the pathogen by 77.11 and 56.00 percent respectively. Th-39 and Psf-82 isolates proved their worthiness in glasshouse as well as field conditions also reducing the maximum disease severity by 58.64% and 50.00% (glasshouse) and 35.59% and 30.30% (field) respectively using three sprays. Considering the biocontrol potential of these agents in disease suppression they can be recommended for disease management.

INTRODUCTION

Sorghum (*Sorghum bicolor*) is one of the most important drought tolerant food and fodder crop of India grown in many parts of the country. Leaf blight of sorghum caused by *Exserohilum turcicum* is a serious disease in India as well as other sorghum growing areas of the world. It affects the crop from seedling to adult stage leading to varying yield losses, depending on severity of infection. Leaf blight caused by *Exserohilum turcicum* has become a limiting factor in sorghum cultivation. Ahmed and Reddy (1993) have reported a severe yield loss in susceptible cultivars of sorghum as a result of seedling losses due to early infection and severe damage to foliage during favourable weather conditions. Bunker and Mathur (2006) have reported disease severity in sorghum due to *E. turcicum* in terms of percent disease index ranges from 43 to 74 percent resulting in 20-36 percent reduction in grain yield and 12 -27 percent reduction in fodder yield. Use of biocontrol agents in disease management is considered as ecofriendly, ruling out chances of environmental pollution and sustainable approach for disease management. Moreover, resistance development in pathogen against biocontrol agents has not been reported so far while frequent use of fungicides has led to resistance development in various pathogens. Biocontrol agents have been used in disease management for long time as seed treatment but their use as foliar spray is rarely followed approach and needs screening in different crops and under different environmental conditions. Kharayat and Singh (2012) found *Trichoderma* sp effective against zonate leaf spot pathogen of sorghum. *P. fluorescens* encompasses a group of common, Gram negative, rod shaped, nonpathogenic saprophytes that colonize soil, water and plant surface environments (Gade *et al.*, 2014). Keeping in view the importance of the disease and lack of information on effective isolates of *T. harzianum* and *P. fluorescens* for managing the leaf blight pathogen, the present investigation was undertaken.

MATERIALS AND METHODS

Seventeen isolates of *T. harzianum* and ten isolates of *P. fluorescens* used in the experiments were obtained from Biocontrol Laboratory of Department of Plant Pathology, G. B Pant University of Agriculture and Technology, Pantnagar. *T. harzianum* isolates included Th 1, 2, 3, 4, 5, 6, 9, 12, 13, 14, 19, 22, 31, 32, 37, 39, 43 whereas *P. fluorescens* isolates were Psf 2, 3, 4, 12, 18, 25, 27, 82, 101 and Psf Pant. Pathogen was isolated from diseased leaves on Potato Dextrose Agar (PDA) medium, incubated at 27 ± 1ºC and was regularly subcultured.

In vitro* efficacy of *T. harzianum* isolates against *E. turcicum

Isolates were screened for their antagonistic potential against

the pathogen using dual culture technique (Morton and Stroube, 1955). Five mm discs were cut from the periphery of actively growing ten days old culture of the test fungus with the help of sterilized cork borer, similarly *T. harzianum* discs were cut with borer and placed in such a manner that both the discs lie opposite to each other (approximately 4 cm apart from each other) in petri plates (9cm diameter) seeded with PDA (approx. 20 ml/ plate). The test pathogen being relatively slow growing was inoculated 48 hours prior to *T. harzianum*. Three replications were used for each treatment. All the plates were incubated at 27 ± 1^0C. Petri plates without *T. harzianum* served as control.

In vitro efficacy of *P. fluorescens* isolates against *E. turcicum*

Screening was done using dual culture technique as above. Paper discs were cut with the help of punch, sterilized in autoclave at 15 psi for 20 minutes. Five mm discs were cut from the periphery of actively growing ten days old culture of the test fungus, with the help of sterilized cork borer. Sterilized paper discs were dipped in *P. fluorescens* culture, then placed in such a manner that both the discs (pathogen and antagonist) lie opposite to each other (approximately 4 cm apart from each other) in petriplates (9cm diameter) seeded with PDA amended with King's medium B (in 50:50 ratio, approx. 20 ml/plate). Three replications were used for each treatment. All the plates were incubated at 27 ± 1°C. Petri plates without *P. fluorescens* served as control.

The percent inhibition of radial growth was calculated with following formula (Vincent, 1947):

$$\%\text{inhibition of radial growth} = \frac{\text{Radial growth in check - Radial growth in treatments}}{\text{Radial growth in check}} \times 100$$

Table 1: Percent inhibition of radial growth of *Exserohilum turcicum* by different isolates of *Trichoderma harzianum*

Treatment	Radial growth (cm)	Percent inhibition (%)
Th-1	2.60	71.11
Th-2	2.96	67.11
Th-3	2.83	68.55
Th-4	3.00	66.66
Th-5	3.16	64.88
Th-6	2.67	70.33
Th-9	2.73	69.66
Th-12	2.66	70.44
Th-13	2.56	71.55
Th-14	2.53	71.88
Th-19	2.90	67.77
Th-22	3.06	66.00
Th-31	2.66	70.44
Th-32	2.50	72.22
Th-37	2.20	75.55
Th-39	2.06	77.11
Th-43	2.33	74.11
Control	9.00	00.00
CD at 5%	0.45	-

Table 2: Percent inhibition of radial growth of *E. turcicum* by different isolates of *P. fluorescens*

Treatment	Radial growth (cm)	Percent inhibition (%)
Psf-2	6.33	29.66
Psf-3	6.40	28.88
Psf-4	5.46	39.33
Psf-12	6.13	31.88
Psf-18	6.16	31.55
Psf-25	6.10	32.22
Psf-27	5.93	34.11
Psf-82	3.96	56.00
Psf-101	6.40	28.88
Psf-Pant	5.53	38.55
Control	9.00	00.00
CD at 5%	0.96	-

Screening of *T. harzianum* and *P. fluorescens* isolates in glasshouse and field conditions

Five isolates of each *T. harzianum* and *P. fluorescens* found most effective *in vitro* were evaluated under glasshouse conditions. Three sets of experiment were conducted viz., first set: one foliar spray, second set: two foliar sprays and third set: three foliar sprays. Three replications were maintained for each set. TNS 603 susceptible cultivar was used for glasshouse and field experiments. Ten healthy seeds were sown in pots filled with sterilized soil. These pots were kept in glass house. Thirty days old plants were artificially inoculated by spraying 1×10^5 conidia ml^{-1} suspension of the pathogen, plants in all three sets were sprayed with *T. harzianum* and *P. fluorescens* isolates @ 10g/liter water, after two days of inoculation, in second set of experiment second spray was given after 10 days of first spraying and in third set of experiment third spray was given after 10 days of second spraying. Similarly three sets of experiment were conducted in field condition, where three isolates of each *T. harzianum* and *P. fluorescens* found most effective in glasshouse were further evaluated. The seeds were sown in two rows of 6 m length in the plots with spacing dimensions of 45 × 15 cm. Plants were inoculated by spraying pathogen and biocontrol agents as in glasshouse experiment. Observations on disease severity were recorded in 1-5 scale proposed by All India Coordinated Sorghum Improvement Project at 60 DAS where, 1 = Highly resistant (No symptom); 2 = Resistant (upto 10% intensity); 3 = Moderately resistant (11-25% intensity); 4= Susceptible (26-50% intensity) and 5 = Highly susceptible (above 50% intensity). Disease severity was calculated as per the formula given by Mckinney (1923):

$$\%\text{Disease severity (S)} = \frac{\text{Sum of numerical rating}}{\text{Total no. of samples} \times \text{Maximum rating grad}} \times 100$$

RESULTS AND DISCUSSION

In vitro screening of antagonists

Using dual culture method antagonistic potential of 17 isolates of *T. harzianum* was evaluated against the pathogen *E. turcicum* (Table 1). Among *T. harzianum* isolates Th-39 performed best which gave 77.11% inhibition of radial growth followed by Th-37 (75.55%), Th-43 (74.11%), Th-32 (72.22%) and Th-14 (71.88%), whereas least inhibition was obtained with Th-5 (64.88%). The difference in percent inhibition of radial growth indicates the difference in antagonistic potential of *T. harzianum* isolates for the test pathogen. Secondary metabolites play important role in inhibiting growth of other

Table 3: Effect of one, two and three foliar spray of *T. harzianum* isolates on disease severity of leaf blight under glasshouse condition

Treatment	Disease severity (%)			Reduction in Disease severity (%)		
	One spray	Two spray	Three spray	One spray	Two spray	Three spray
Th-14	40.46	34.00	35.33	31.02	42.03	39.77
Th-32	39.33	31.40	29.33	32.95	46.47	50.00
Th-37	37.40	30.66	26.00	36.24	47.73	55.67
Th-39	35.93	28.66	24.26	38.74	51.14	58.64
Th-43	38.26	30.93	27.00	34.77	47.27	53.97
Control	58.66	58.66	58.66	00.00	00.00	00.00
CD at 5%	2.00	2.75	2.50	-	-	-

Table 4: Effect of one, two and three foliar spray of *P. fluorescens* isolates on disease severity of leaf blight under glasshouse condition

Treatment	Disease severity (%)			Reduction in Disease severity (%)		
	One spray	Two spray	Three spray	One spray	Two spray	Three spray
Psf- 4	42.80	39.80	34.86	27.03	32.15	40.57
Psf- 25	44.00	41.06	35.73	24.99	30.00	39.08
Psf- 27	41.13	36.06	31.06	29.88	38.52	47.05
Psf- 82	40.00	35.80	29.33	31.81	38.97	50.00
Psf- Pant	42.66	38.40	33.73	27.27	34.53	42.49
Control	58.66	58.66	58.66	00.00	00.00	00.00
CD at 5%	2.95	2.07	2.27	-	-	-

Table 5: Effect of one, two and three foliar spray of *T. harzianum* isolates on disease severity of leaf blight under field condition

Treatment	Disease severity (%)			Reduction in Disease severity (%)		
	One spray	Two spray	Three spray	One spray	Two spray	Three spray
Th-37	52.06	48.00	41.46	12.06	18.91	29.96
Th-39	50.66	45.93	38.13	14.42	22.41	35.59
Th-43	53.20	48.93	43.20	10.13	17.34	27.02
Control	59.20	59.20	59.20	00.00	00.00	00.00
CD at 5%	1.32	1.74	1.60	-	-	-

microorganism. Kucuk and Kivanc, 2004 reported retardation of growth and sporulation of *G. graminis* by *T. harzianum* isolates. Harlapur *et al.*, 2007 evaluated in vitro efficacy of five *Trichoderma* species along with one species each of *Bacillus subtilis* and *Pseudomonas fluorescens* and found *T. harzianum* most effective in reducing growth of *Exserohilum turcicum* in maize. This may be due to mechanism of antibiosis, mycoparasitism and competition by the antagonists. *Trichoderma* spp. inhibiting the growth of pathogens by the mechanism of antibiosis has been reported by several workers (Sharma and Doharoo, 1991; Sivan and Chet, 1989). Sab *et al.* (2014) reported maximum 70% inhibition of mycelial growth of *Sclerotium rolfsii* by *T. harzianum*- 55 IIHR isolate *in vitro*.

Similarly dual culture method was used to evaluate antagonistic potential of 10 isolates of *P. fluorescens* against the pathogen (Table 2). All the isolates reduced the radial growth of pathogen however, isolate Psf-82 performed best which gave 56% inhibition of radial growth followed by Psf-4 (39.33%), Psf-Pant (38.55%), Psf-27 (34.11%) and Psf-25 (32.22%) whereas least inhibition was obtained with Psf-3 and Psf-101 (28.88%). These results are corroborated by the study conducted by Zegeye *et al.* (2011) in which *P. fluorescens* inhibited the radial growth of the late blight pathogen *P. infestans* by 88 percent. *Pseudomonas fluorescens* inhibited the mycelial growth of *Fusarium oxysporum* (61.85%), *Sclerotium rolfsii* (63.15%), *Rhizoctonia bataticola* (55.56%) and *R.solani* (53.15%) *in vitro* (Dewangan *et al.*, 2014).

Glasshouse screening of antagonists

Five out of seventeen *T. harzianum* isolates found effective *in vitro* were further tested in glasshouse conditions. In single spray, all the treatments were significantly superior over control. *T. harzianum* isolate Th-39 (38.74%) was found most effective in reducing the disease severity followed by Th-37 (36.92%), and Th-43 (34.77%), though statistically Th-39 and Th-37 were at par in reducing severity. In case of two foliar sprays, all the treatments reduced the disease over control Th-39 (51.14%) was most effective followed by Th-37 (47.73%) and Th-43 (47.27%). With three foliar sprays, reduction in disease severity was maximum with Th-39 (58.64%) followed by Th-37 (55.67%) and Th-43 (53.97%) (Table 3). *T. harzianum* has been successfully used as a biopesticide in greenhouse by Tondje *et al.* (2007). Also Prasad and kumar (2011) reported 29.70 percent and 23.01 percent reduction in sheath blight (*Rhizoctonia solani)* severity in paddy under glasshouse condition when sprayed with *T. harzianum*. Anthracnose and zonate leaf spot diseases were effectively managed by *Trichoderma harzianum* under glasshouse (Purohit *et al.*, 2013; kharayat and Singh, 2012 and Bangari and Singh, 2011).

P. fluorescens isolates found more effective *in vitro* were further evaluated under glasshouse condition. In single foliar spray, *P. fluorescens* isolates under glass house conditions resulted in significant reduction in disease severity of leaf blight over

Table 6: Effect of one, two and three foliar spray of *P. fluorescens* isolates on disease severity of leaf blight under field condition

Treatment	Disease severity (%)			Reduction in Disease severity (%)		
	One spray	Two spray	Three spray	One spray	Two spray	Three spray
Psf- 27	55.13	50.73	44.40	6.90	14.30	25.00
Psf- 82	53.20	47.66	41.26	10.13	19.50	30.30
Psf- Pant	55.26	53.60	46.66	6.67	9.46	21.18
Control	59.20	59.20	59.20	00.00	00.00	00.00
CD at 5%	2.94	2.62	2.74	-	-	-

control. Psf-82 was found most effective in reducing the disease severity over control (31.81%). However, Psf-27, Psf-Pant and Psf-4 were statistically at par in reducing disease severity. In two sprays all treatments reduced disease severity over control. Isolate Psf-82 gave maximum reduction (38.97%). However, Psf-27 (38.52%) was statistically at par Psf-82 in reducing disease severity. Disease severity was reduced considerably over control with three sprays. Psf-82 gave maximum reduction (50%) followed by Psf-27 (47.05%) and Psf-Pant (42.49%) (Table 4). Purohit *et al.* (2013) reported 45.05 percent reduction in disease severity of zonate leaf spot of sorghum by three sprays of *P. fluorescens* isolate Psf-28.

Field screening of antagonists

T. harzianum isolates found more effective under glasshouse were further evaluated in field condition for efficacy against the pathogen. Foliar spray with *T. harzianum* isolates reduced disease severity significantly over control. Maximum reduction in disease severity was obtained with Th-39 (14.42%) followed by Th-37 (12.06%) and Th-43 (10.13%) in case of one spray. With two sprays, Th-39 recorded maximum reduction in disease severity by 22.41 % followed by Th-37 (18.91%) and Th-43 (17.34%) Th-37 and Th-43 were statistically at par. Three sprays with Th-39 recorded maximum reduction in disease severity by 35.59 % followed by Th-37 (29.96%) (Table 5). Singh and Singh (2008) reported reduced disease severity and increased plant growth and yield, with Th-39 and Th-43 against anthracnose of sorghum. Nzojiyobiri *et al.* (2003) reported that peroxidase and phenylalanine aminotransferase activity was enhanced significantly in treated seeds than control which suggests that induced resistance may be one of the mechanisms of biocontrol strains. Kumar *et al.* (2011) reported highest â-1, 3-glucanase activities in *T. harzianum* in presence of *Rhizoctonia solani*. Purohit *et al.*, (2014) observed 36.62 percent reduction in disease severity of zonate leaf spot in sorghum with three sprays of *T. harzianum* isolate Th-39over control.

Numerous species of soil bacteria which flourish in the rhizosphere of plants, but which may grow in, on, or around plant tissues, stimulate plant growth by a plethora of mechanisms, *Pseudomonas sp* has been reviewed for the biofertilizer, phytostimulator and phytopathogen biocontrol activities (Sharma *et al.*, 2014). *P. fluorescens* isolates effectively suppressing disease intensity in glasshouse were selected for field evaluation. In our results one foliar spray with *P. fluorescens* isolates reduced disease severity significantly over control. However they were statistically at par in reducing disease severity. Two sprays further augmented *P. fluorescens* population on phylloplane, thereby increased reduction of disease was observed with Psf-82, which reduced the disease by 19.50 % followed by Psf-27 (14.30%). Three sprays with Psf-82 reduced maximum disease severity (30.30%), followed by Psf-27 (25.00%) whereas Psf-Pant (21.18%) was least effective (Table 6). Our findings are in consonance with those reported by Vidhyasekaran *et al.* (1997) in rice against blast (*Pyricularia oryzae*) pathogen in field, who reported that, maximum *P. fluorescens* multiplication on leaves took place from 3 to 15 days of spray and also effectiveness of spraying persisted up to 15 days. Jeyalakshmi *et al.* (2010) reported that two sprays at 15 days interval gave maximum reduction in disease severity of bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) of rice. Purohit *et al.* (2013) reported that three sprays of *P. fluorescens* isolate Psf-28 reduced *Gloeocercospora sorghi* severity by 26.19% over control.

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