Int.J.Curr.Microbiol.App.Sci (2018) *7*(3): 2322-2327

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 03 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.703.272

Bioefficacy of *Trichoderma* spp. against *Bipolaris sorokiniana* Causing Spot Blotch Disease of Wheat and Barley

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Keywords

Bipolaris sorokiniana, Biological control, *Trichoderma*, Mycelial Growth, Plant height and Disease severity

Article Info

Accepted:
20 February 2018
Available Online:
10 March 2018

A B S T R A C T

Spot blotch caused by *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem. is an important disease of Wheat and Barley. The biological control has been proved useful and economical in the control of spot blotch because of the harmful effect of fungicides to human and environment. The *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma virens* were used in dual culture method to see their effect on mycelial growth of *Bipolaris sorokiniana*. The inhibition varied among *Trichoderma* spp. was ranged from 60.76 to 73.07%. The bioagent *Trichoderma viride* exhibited maximum inhibition percentage (73.07%, 72.30%) on mycelial growth of *Bipolaris sorokiniana.* The seed and soil treatment with *Trichoderma viride* increased plant height, fresh and dry weight of shoot and root as compared to control. Soil treatment with *Trichoderma viride* (bio agent) was found best with minimum disease severity (21.51%) and minimum percent root infection (10.00%).

Introduction

The Wheat and Barley crop both have high nutritional value are cultivated for human consumption all over the world. In India wheat is second important staple food crop after rice whereas Barley was considered as poor man's crop because of its low input requirement and better adaptability to harsh environments, like drought, salinity, alkalinity and marginal lands. The most important uses of Barley in India are as grain feed to livestock and poultry, malt for manufacture of beer and other liquor like whisky, brandy etc. The Spot blotch caused by *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem. is a serious disease of Wheat and Barley. In last two decades spot blotch has emerged as serious concern for Wheat and Barley growers in developing world. *Bipolaris sorokiniana* causes several diseases in its hosts worldwide. It produces broad range of symptoms such as root rot, leaf blotch and black point of seed. The high temperature and high relative humidity

favours the outbreak of the disease particularly in South Asia s intensive irrigated rice – wheat production systems (Aggrawal *et al.,* 2000) and causes severe yield losses to Wheat crop in South East Asia (Saari, 1998) and marked reduction in grain yield and quality of Barley crop (Nutter *et al.*, 1985; Arabi and Jawahar, 2003). Annual yield loss of Wheat due to this disease in south Asia is estimated at 15-20% (Duvieller and Sharma, 2009). Yield losses of 25-45% in Barley crop have been reported in Kazakhstan and 41% in Russia (Iftikhar *et al.*, 2009). Spot blotch symptoms are characterized by formation of light brown to dark brown coloured elongated oval blotches surrounded by yellow halo. Wheat and Barley crop in eastern Uttar Pradesh, suffers from a number of fungal diseases such as loose smut, black rust, brown rust, yellow rust, covered smut, powdery mildew, stripe disease and net blotch etc. but spot blotch caused by *Bipolaris sorokiniana* is more severe than other diseases. Spot blotch can be controlled by a number of ways but integrated disease management is the best method for controlling the pathogen (Mehta, 1993; Dubin and Duvieller 2000). Biological control has also proved useful and economical in the control of spot blotch because of the harmful effect of fungicides to human and environment. *Trichoderma spp*. were used as a potential biological agent for control of foliar blight caused by *Bipolaris sorokiniana*. Jacobsen *et al.*, (2004) pointed out that biological control should be most effective when used in combination with disease resistance in an integrated disease management system. So, the present research work was carried to evaluate the potential of *Trichoderma* species for management of *Bipolaris sorokiniana*.

Materials and Methods

Isolation: In order to isolate the test pathogen (*Bipolaris sorokiniana*), infected leaves of barley and wheat showing characteristic blight symptoms were collected from Student Instructional Farm and Main Experimental Station, Narendra Deva University of Agriculture and Technology, Faizabad. The selected leaves were washed with fresh sterilized water in order to remove the dust particles and surface contaminants. The washed diseased leaves were cut into small bits. The cut leaf pieces were surface sterilized with 0.1 per cent Mercuric Chloride solution and washed thoroughly 3 to 4 times with sterilized water to remove the traces of Mercuric Chloride. The pieces were transferred in Petri dishes containing Potato Dextrose Agar and incubated at $25+2^{0}$C for 6 days.

Dual culture technique

To determine the effect of *Trichoderma spp*. on mycelial growth of *Bipolaris sorokiniana*, a dual culture method was used (Fokkema, 1973). The dual culture of three *Trichoderma spp*. and targeted pathogen were studied on Potato dextrose agar (PDA). 20 ml of PDA medium was poured in plates (9 cm) and was allowed to solidify.

The discs (5mm diameter) of mycelium cut from the margin of 6 days old culture of each *Trichoderma spp.* were placed at the edge of each plate, 10 mm from the periphery then disc of 5mm diameter of mycelium cut from the growing edge of 7 days old cultures of *Bipolaris sorokiniana* were placed on each plate, opposite to the mycelial discs of *Trichoderma spp.* where as in control plates, two mycelial disc of *Bipolaris sorokiniana* were placed at the edges of each plate, opposite to each other in complete aseptic condition. All the plates were incubated at 25+2˚C for about 7 days after inoculation and four replications were maintained for each treatment.

The radial growth of *Bipolaris sorokiniana* was recorded, compared with control and percentage of growth inhibition was calculated

by following the formula suggested by Sunder *et al.,* (1995).

$$\% \text{ Inhibition} = \frac{X-Y}{X} \times 100$$

Where X = Radial growth of pathogen alone (control) Y = Radial growth of pathogen along with antagonist.

Soil Treatment: The inoculum of *Bipolaris sorokiniana* and *Trichoderma viride* was grown on sterilized sorghum seeds as described by Joshi *et al.,* (1969). Sorghum seeds were soaked for 24 h and then transferred to conical flask of 250 ml capacity. The material was sterilized twice on subsequent days at 15 pound pressure for an hour. *Bipolaris sorokiniana* and *Trichoderma viride* were grown separately on Potato Dextrose Agar (PDA) and when they were grown bits of culture about 5x2 cm in size were added to each flask containing autoclaved sorghum seeds and incubated at 25^0C with periodical shaking to avoid cake formation. The infested seeds were mixed with soil @ 50g/ kg of soil and placed in pots in net house.

Seed Treatment: Barley seeds were treated with conidial suspension of *Trichoderma viride.* Conidia were harvested from the infested sorghum seeds and washed several time in sterile distilled water. Seed surface was disinfected by soaking in 0.5% sodium hypochlorite for 3 min then rinsed three times in sterile distilled water. The seeds were soaked for one hour in *Trichoderma* spore suspension and air dried. *Trichoderma* inoculated seed were sown in pots. Sowing was done on 12 December, 2015. Pots are watered at every three days of interval. Four plants were maintained in each pot. After 45 days of planting, disease severity, plant height, percent root infection, fresh and dry weight of shoot and root were recorded. All the treatments are arranged in CRD and data were statistically analyzed.

Results and Discussion

The effect of *Trichoderma* isolates i.e. *Trichoderma viride*, *Trichoderma virens* and *Trichoderma harzianum* were evaluated against mycelial growth of *Bipolaris sorokiniana* of Wheat and Barley by dual culture method (Table 1). The result showed that all isolates of *Trichoderma* inhibited the growth of *Bipolaris sorokiniana* and inhibition varied from 60.76- 73.07%. The maximum inhibition of mycelial growth of *Bipolaris sorokiniana* of Wheat and Barley was recorded with *Trichoderma viride* (73.07%, 72.30%) followed by *Trichoderma virens* (69.22%, 66.15%). Minimum inhibition of mycelial growth of *Bipolaris sorokiniana* of wheat and barley was recorded with *Trichoderma harzianum* (63.84%, 60.7%).

The *Trichoderma viride* exhibited maximum inhibition percentage to mycelial growth of *Bipolaris sorokiniana* was selected for treatment of seed and soil to evaluate its effect on survival of *Bipolaris sorokiniana* causing spot blotch disease in Barley and Wheat.

A perusal of data presented in table 2 indicated that all the treatments were significantly superior to control, minimum disease severity (21.51%) was recorded with untreated seed +*Trichoderma viride* treated soil followed by 26.38% with *Trichoderma viride* treated seed + infected soil and maximum disease severity (55.55%) was noted in control. The minimum percent root infection (10.00%) was recorded with untreated seed + *Trichoderma viride* treated soil followed by 12.90% with untreated seed + sterilized soil and 22.18% with *T. viride* treated seed+ infected soil. The maximum root infection (63.25%) was noted in control.

Table.1 Effect of *Trichoderma* spp. on mycelial growth of *Bipolaris sorokiniana*

S. No.	Treatment	Dual culture	
		Radial growth(cm)	Percent inhibition
T_1	*T. viride* x *B. sorokiniana* (wheat)	0.87	73.07 (58.81)
T_2	*T. harzianum* x *B. sorokiniana* (wheat)	1.07	63.84 (53.04)
T_3	*T. virens* x *B. sorokiniana* (wheat)	1.00	69.22 (56.33)
T_4	*T. viride* x *B. sorokiniana* (barley)	0.90	72.30 (58.33)
T_5	*T. harzianum* x *B. sorokiniana* (barley)	1.27	60.76 (51.23)
T_6	*T. virens* x *B. sorokiniana* (barley)	1.10	66.15 (54.43)
T_7	*B. sorokiniana* (wheat)	3.25	----
T_8	*B. sorokiniana* (barley)	3.25	----
	CD at5%	0.17	3.88

*Figures given in parenthesis are transformed value
*All the values are average of four replications

Table.2 Effect of *Trichoderma* seed and soil treatment on survival of *Bipolaris sorokiniana*

Treatments	Disease severity (%)	% Root infected	Plant height (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)
***T. viride* treated seed+ *B. sorokiniana* infected soil**	33.30 (35.07)	27.50 (31.60)	36.50	6.40	1.84	0.48	0.056
***T. viride* treated seed+ infected soil**	26.38 (30.82)	22.18 (28.07)	37.43	6.66	1.96	0.56	0.062
Untreated seed + *T. viride* treated soil	21.51 (27.54)	10.00 (18.21)	39.81	8.25	2.44	0.70	0.07
Untreated seed + sterilized soil	28.46 (31.98)	12.90 (20.62)	35.00	5.40	1.70	0.36	0.05
Untreated seed+ *B. sorokiniana* infected soil (control)	55.55 (48.19)	63.25 (52.63)	31.09	4.21	1.54	0.26	0.031
CD at 5%	6.88	4.52	4.3	1.37	0.49	0.16	0.02

*Wt. -Weight
*Figures given in parenthesis are transformed value
*All the values are average of four replications

Plant growth characters like plant height, fresh and dry weight of shoot and root were higher in all treatment compared to control. The maximum plant height (39.81 cm), fresh weight of shoot (8.25g)and root (0.70g)and dry weight of shoot (2.44g)and root (0.07g) were recorded with untreated seed + *Trichoderma viride* treated soil.

The minimum plant height (31.09cm) fresh weight of shoot (4.21g) and root (0.26g) and dry weight of shoot (1.54g) and root (0.031g) were recorded with control. The present findings are in agreement with the finding of Salehpour *et al.*, (2005) who reported that reduction in the growth of *Bipolaris sorokiniana* mycelia was highest with *Trichoderma viride* and also found that seed soaking and soil treatments with *Trichoderma viride* T112 and MO were the most effective in the reduction of infection caused by *Bipolaris sorokiniana* compared with the control ($p<0.01$), all isolates of *Trichoderma* increased plant height and fresh and dry weight of roots and shoots of wheat seedlings compared with the control. Kumar *et al.*, (2009) tested efficacy of *Trichoderma viride, Trichoderma harzianum* and *Trichoderma virens* against *Helminthosporium maydis*, in which *Trichoderma viride* inhibited the radial growth of *Helminthosporium maydis* to an extent of 60.7% followed by *Trichoderma harzianum* (55.1%) and *Trichoderma virens* (52.6%) and his studies on hyphal interaction between antagonists and test fungus revealed disorganization of protoplasmic content and lysis of host hyphae. The soil application of bioagents was more effective in protecting the crop than foliar spray. The seed and soil treatment with *Trichoderma spp.* alone increased plant height, fresh and dry weight of shoot and root compared with control. It is in accordance with result showing that some fungal isolates and bacterial strains are capable of promoting plant growth (El Abyad., 1992; Jones., 1996).

Among three *Trichoderma spp. Trichoderma viride* exhibited maximum inhibition percentage to mycelial growth of *Bipolaris sorokiniana* of Wheat and Barley. Soil treatment with *Trichoderma viride* (bio agent) effectively inhibits the growth of *Bipolaris sorokiniana* causing spot blotch of wheat and barley in turn protecting the losses of the crop. Wheat and Barley crops are cultivated for human consumption therefore manage the disease using bio agent instead of using chemical fungicide will protect the consumers from the ill effects of chemical fungicides.

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How to cite this article:

Divya Singh, S.K. Pande, Kavita, Jay Kumar Yadav and Sandeep Kumar. 2018. Bioefficacy of *Trichoderma* spp. against *Bipolaris sorokiniana* Causing Spot Blotch Disease of Wheat and Barley. *Int.J.Curr.Microbiol.App.Sci.* 7(03): 2322-2327.
doi: https://doi.org/10.20546/ijcmas.2018.703.272