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The Efficacy of Bioagents against Post Flowering Stalk Rot (PFSR) of Specialty Corn Caused by *Fusarium verticillioides* (sheldon)

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Abstract

The efficacy of three bio-agents *viz.* Pratap *Trichoderma viride*-1, *Trichoderma viride* and *Trichoderma harzianum* were assessed *in vitro* against four isolates (Fv SC-01, Fv SC- 02, Fv SC-03 and Fv SC- 04) of *Fusarium verticillioides*, cause Post flowering stalk rot (PFSR) of specialty corn. All used bio- agents were antagonistic in nature and significantly inhibited the growth of *Fusarium verticillioides.* Among the bio-agents tested maximum inhibition of mycelium growth was observed by Pratap *Trichoderma viride*-1 @ 1500 ppm on Fv SC-04. Out of these antagonists, the resident isolate Pratap *Trichoderma viride* -1(78.45%) provided the highest inhibitory effect followed by *Trichoderma viride* (78.22%) and *Trichoderma harzianum* (56.60%) was the lowest suppressive antagonist. Among all isolates of *Fusarium verticillioides* Fv SC-04 was maximum inhibited by all tested bio- agents.

Keywords: *Fusarium verticillioides*, Trichoderma, *in vitro* and Specialty Corn

1. Introduction

Maize (*Zea mays* L.) is monocotyledonous plant that belongs to grass family (Poaceae). The specialty corn viz. Pop corn, Baby corn, Sweet corn and QPM are increasing its hectares because of high economic returns. QPM and sweet corn having opaque-2 and Shrunken-2 gene respectively (Jat *et al*., 2009). *Trichoderma* spp. are fungi that are present in nearly all soils, they frequently are the most prevalent cultivable fungi (Ubalua and Oti, 2007). They are common inhitants of the rhizosphere and are well recognized as biological agents of soil borne plant pathogens. For controlling the disease, farmers usually applied synthetic fungicides to the plants. However, these treatments were ineffective because it led to the appearance of environmental contaminations (Jacobsen and Backman, 1993). Hence, biological control using microbial agents is an alternative way to manage plant pathogens because it's eco-friendly and cost-effective. Nowadays, many microbes have been used as biocontrol agent of plant disease including *Trichoderma* spp. The use of *Trichoderma* spp. as a biocontrol agent against *Fusarium* spp. showed a high efficacy when tested under *in vitro* and *in vivo* conditions (Thangavelu et al., 2004). Therefore, the purpose of this study was to screen the pathogens of PFSR and antagonistic ability of *Trichoderma* spp. to suppress the growth of PFSR pathogen under *in vitro* conditions.

2. Materials and Methods

The experiment was conducted at Department of Plant Pathology, Rajasthan College of Agriculture (RCA) MPUAT, Udaipur during *kharif,* 2015–16.

2.1. Isolation of biocontrol agents

Isolation of biocontrol agent (*Trichoderma* spp.) was attempted from air dried rhizosphere soil in the viscinity of healthy roots of specialty corn. Specific technique was employed for isolation of resident (native) biocontrol agent. The details of these techniques are given below.

2.2. Isolation by baiting

A loop full of the dried rhizosphere soil collected from specialty corn field was placed in the bottom of the empty sterilized Petri-plates and to this a drop of sterile water was added, thoroughly mixed and allowed to dry on the laminar flow bench for 3 minutes, over this, 20 ml of lukewarm media 0.1 per cent malt extract agar (MEA) was poured carefully without disturbing the soil at the bottom. The medium in these plates was allowed to solidify and then 2 mm bit of growing cultures of isolated pathogen was aseptically inoculated in each plate. The plates were incubated at 25±2 °C and examined after 2 days for growth of the organism under stereo binocular microscope and hyphae of antagonists which appeared piercing through the hyphae of the pathogens or found

growing over the pathogens or causing lysis thereof, were aseptically picked up with the help of sterilized needle and transferred on to fresh MEA plates and purified with hyphal tip culture technique and maintained in MEA slants.

3. Results and Discussion

Efficacy of bioagents of local isolates and procured cultures of *Trichoderma* spp. were studied *in vitro* as described in Material and Methods. Data revealed that *Trichoderma* spp. were potential antagonists against *F. verticillioides.* The resident isolate which was found fast growing and effective against pathogen *F. verticillioides* was identified as *Trichoderma viride* based on morphological characters and was designated as Pratap *Trichoderma viride*-1 (PTv-1) this isolates has to be registered with ITCC for accession number. All the tested biocontrol agents significantly (p=0.05) inhibited the mycelial growth at all concentrations from 500, 1000 and 1500 ppm.

Pratap *Trichoderma viride* -1 caused 55.42, 56.05, 56.45 and 56.77% inhibition of mycelium growth at 500 ppm concentration, at 1000 ppm it caused 64.15, 65.37, 66.45 and 66.77% inhibition of mycelium growth and 77.55, 77.87, 78.10 and 78.45% inhibition of mycelium growth at 1500 ppm in (Fv SC-01, 02, 03 and 04) all isolates respectively. The maximum per cent inhibition of mycelium growth *i.e.* 78.45% of *F. verticillioides* SC-04 isolate was followed by Fv SC-03 that caused 78.10% inhibition of mycelial growth.

Trichoderma viride caused 47.65, 49.87, 49.55 and 50.85% inhibition of mycelium growth at 500 ppm concentration, at 1000 ppm it caused 61.95, 63.11, 63.33 and 63.77% inhibition of mycelium growth and 75.44, 76.22, 76.52 and 78.22% inhibition of mycelium growth at 1500 ppm of (Fv SC-01, 02, 03 and 04) all isolates respectively. The maximum per cent inhibition of mycelium growth *i.e.* 78.22% of *F. verticillioides* SC-04 isolate was followed by Fv SC-03 that caused 76.52% inhibition of mycelial growth.

Trichoderma harzianum was slightly less effective, as it caused 26.85, 27.72, 27.85 and 28.72% inhibition of mycelial growth at 500 ppm concentration, at 1000 ppm it caused 39.72, 41.95, 43.22 and 45.55% inhibition of growth and 54.37, 54.95, 55.66 and 56.60% inhibition of mycelium growth at 1500 ppm in (Fv SC-01, 02, 03 and 04) all isolates respectively. The maximum per cent inhibition of mycelium growth *i.e.* 56.60 per cent of *F. verticillioides* SC-04 isolate was followed by Fv SC-03 that caused 55.66% inhibition of mycelial growth. (Table 1, 2, 3, 4).

Suhaida and Izzati, 2013 based on virulence assay, *F. proliferatum* B202c was the most pathogenic isolate among other species including *F. verticillioides*. This pathogen was challenged in dual culture assays with 72 isolates of *Trichoderma* spp., which were isolated from soil samples. *T. harzianum* T73s showed highest per centage inhibition of 73.10% was further tested for its efficacy to suppress FER under glasshouse conditions. Ferrigo *et al.*, 2014 reported that the ability of the biocontrol fungus *Trichoderma harzianum* to

Table 1: Antagonistic activity (Per cent inhibition) of two isolates *Trichoderma viride* and one isolate of *T. harzianum* against *Fusarium verticillioides* SC-01 isolate *in vitro*

Sl. No.	Con	Isolates of *Trichoderma* spp. Growth* Mycelial growth (mm)			Per cent growth inhibition*		
		PT	TV	*TH*	PT	TV	TH
1.	500	40.07	47.05	65.80	55.42 (48.09)	47.65 (43.63)	26.85 (31.19)
2.	1000	32.25	34.20	54.20	64.15 (53.19)	61.95 51.89)	39.72 (39.05)
3.	1500	20.15	22.10	41.02	77.55 (61.69)	75.44 60.24)	54.37 (47.49)
4.	Control	90.00	90.00	90.00	00.00 (0.0)	0.0 (0.0)	0.0 (0.0)
		SEm±	CD (p=0.05)		SEm+	CD (p=0.05)	
Biocontrol		0.04	0.119		0.06	0.133	
Concentration		0.04	0.119		0.05	0.133	
B×C		0.07	0.207		0.08	0.230	

Con: Concentration (ppm); PT: Pratap *T. viride* -1; TV: T. *viride; TH:* T. *harzianum;* *Mean of four replications; Figures in parentheses are arcsine *√* per cent angular transformed values

Table 2: Antagonistic activity (Per cent inhibition) of two isolates *Trichoderma viride* and one isolate of *T. harzianum* against *Fusarium verticillioides* SC-02 isolate *in vitro*

Sl. No.	Con	Isolates of *Trichoderma* spp. Growth* Mycelial growth (mm)			Per cent growth inhibition*		
		PT	TV	*TH*	PT	TV	TH
1.	500	39.5	45.10	65.00	56.05 (48.45)	49.87 (44.47)	27.72 (31.75)
2.	1000	31.12	33.20	52.20	65.37 (53.93)	63.11 (52.57)	41.95 (40.85)
3.	1500	19.87	21.40	40.00	77.87 (61.91)	76.22 (60.77)	54.95 (47.42)
4.	Control	90.00	90.00	90.00	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
		SEm±	CD (p=0.05)		SEm+	CD (p=0.05)	
Biocontrol		0.03	0.111		0.04	0.127	
Concentration		0.03	0.111		0.04	0.127	
B×C		0.07	0.193		0.08	0.220	

*Mean of four replications; Figures in parentheses are arcsine *√* per cent angular transformed values

Table 3: Antagonistic activity (Per cent inhibition) of two isolates *Trichoderma viride* and one isolate of *T. harzianum* against *Fusarium verticillioides* SC-03 isolate *in vitro*

Sl. No.	Con	Isolates of *Trichoderma* spp. Growth* Mycelial growth (mm)			Per cent growth inhibition*		
		PT	TV	*TH*	PT	TV	TH
1.	500	39.15	45.20	64.90	56.45 (48.68)	49.55 (44.72)	27.85 (31.83)
2.	1000	30.15	33.00	51.10	66.45 (54.58)	63.33 (52.69)	43.22 (41.07)
3.	1500	19.67	21.10	39.90	78.10 (62.07)	76.52 (60.99)	55.66 (48.19)
4.	Control	90.00	90.00	90.00	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
		SEm±	CD (*p*=0.05)		SEm+	CD (*p*=0.05)	
Biocontrol		0.04	0.124		0.05	0.131	
Concentration		0.04	0.124		0.05	0.131	
B×C		0.07	0.216		0.08	0.228	

*Mean of four replications; Figures in parentheses are arcsine √ per cent angular transformed values

Table 4: Antagonistic activity (Per cent inhibition) of two isolates *Trichoderma viride* and one isolate of *T. harzianum* against *Fusarium verticillioides* SC-04 isolate *in vitro*

Sl. No.	Con	Isolates of *Trichoderma* spp. Growth* Mycelial growth (mm)			Per cent growth inhibition*		
		PT	TV	*TH*	PT	TV	TH
1.	500	38.85	44.20	64.10	56.77 (48.87)	50.85 (45.46)	28.72 (32.39)
2.	1000	29.85	32.57	49.00	66.77 (54.77)	63.77 (52.92)	45.55 (42.40)
3.	1500	19.15	19.60	39.10	78.45 (62.31)	78.22 (62.14)	56.60 (48.71)
4.	Control	90.00	90.00	90.00	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
		SEm±	CD (*p*=0.05)		SEm+	CD (*p*=0.05)	
Biocontrol		0.03	0.103		0.04	0.122	
Concentration		0.03	0.103		0.04	0.122	
B×C		0.06	0.179		0.07	0.212	

*Mean of four replications; Figures in parentheses are arcsine √ per cent angular transformed values

induce systemic resistance in maize against *F. verticillioides* is still lacking. Shekhar and Kumar, 2010 tested efficacy of six isolates of *T. harzianum* using dual culture plate technique and inhibition through volatile substances against charcoal rot pathogen of maize and found that Hyderabad isolate of *T. harzianum* caused maximum inhibition (62.3%) of radial growth of *Macrophomina phaseolina* and regarded as potential bio-control agent for minimizing.

4. Conclusion

To develop effective management strategies for PFSR three bio-control agents were evaluated against *F. verticillioides* (Fv SC-01 to Fv SC-04) *in vitro* condition. Pratap *Trichoderma viride*-1 @ 500, 1000 and 1500 ppm was found most effective in inhibiting the mycelial growth of pathogen. Among the bioagents tested maximum inhibition of mycelium growth was observed by Pratap *Trichoderma viride*-1 @ 1500 ppm on Fv SC-04.

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6. References

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