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EFFICACY OF FUNGICIDES AND *TRICHODERMA VIRIDE* AGAINST *FUSARIUM OXYSPORUM* F. SP. *CUBENSE IN- VITRO*

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ABSTRACT
Fusarium wilt of banana is one of the most destructive diseases of banana worldwide. Four fungicides Mancozeb, Saaf, Carbendazim and Cuprozin in three different concentrations (0.01%, 0.02% and 0.03%) and one biocontrol agent *Trichoderma viride* were evaluated against mycelial growth of the causal pathogen *Fusarium oxysporum* f. sp. *cubense in-vitro* by poison food and dual culture techniques respectively. Observations were taken at 24 hours intervals up to 192 hours for the assessment of their inhibitory effects. Among them, Carbendazim at its all concentrations was found to be the most effective against the pathogen followed by Saaf. Carbendazim at 0.01% and 0.02% inhibited fungus at 1.66mm and 1.16mm of mycelial growth respectively after 72 hours of treatment, while at 0.03% concentration it inhibited fungus growth at 0.99mm after 48 hours only. The biocontrol agent *Trichoderma viride* in dual culture with pathogen completely inhibited its mycelial growth. The pathogen fungus continued to increase up to 192 hours showing a radial growth of 38.99mm in control but in dual culture it showed growth of 17.50mm up to 120 hours only and after that there was no further growth. Thus, fungicide Carbendazim and biocontrol agent *Trichoderma viride* can be used for the amelioration and control of wilt disease in banana.

INTRODUCTION

Fusarium oxysporum is the soil born and omnipresent phytopathgenic fungi, causing various diseases, such as vascular wilt, root rot and damping off (Saremi, 1996). One group of these pathogenic fungi *Fusarium oxysporum* f. sp. *cubense* (FOC) causes fusarium wilt disease in banana. Many prominent cultivars of banana of the region such as Malbhog and Alpan are facing the threat of extinction due to wilt disease. Fungicides under *in-vitro* test system were found to be effective against FOC. However, chemical control of the disease in the field with the use of fungicides proved ineffective (Strover, 1962). Dar *et al.* (2013) evaluated systemic fungicide Carbendazim and non-systemic fungicides Mancozeb, to check the disease suppression in *Abie spindrow* (Himalayan fir). Evaluation of these chemical fungicides under *in-vitro* test system against pathogen FOC for the management of banana wilt disease will help the crop in the region.

Biological control agents manage the soil borne phytopathogens in an environment friendly approach compared to hazardous fungicides (Saleem *et al.*, 2000 and Anu Rajan *et al.*, 2013). These biological control agents either use the mechanism of antibiosis or mycoparasitism against the fungal pathogen. Biocontrol agents *Bacillus subtilis* and *Gliocladium virens* utilize antibiosis, whereas *Trichoderma* spp. utilize mycoparasitism (Baker and Paulitz, 1996). Several studies suggested that the *Trichoderma* spp. have antagonistic and biological control potential against a diverse group of soil borne pathogens including FOC (Hanson and Howell, 2004 and Afzal *et al.*, 2013). Seetharamulu *et al.* (2012) evaluated the efficacy of *Trichoderma viride* against *Fusarium solani* in *in-vitro* system while in *in-vivo* system it was effective against the disease in combination with fungicide Mancozeb. Comparisons of chemical control of *Fusarium oxysporum* f. sp. *cubense* fungus with fungicides and biological control with *Trichoderma viride* will help in selection of better prevention and management of the wilt disease. Thus, the paper deals with the evaluation of the effect of fungicides and biocontrol agent *Trichoderma viride* against banana wilt pathogen *Fusarium oxysporum* f. sp. *cubense*.

MATERIALS AND METHODS

Cultures

The strain of pathogen *Fusarium oxysporum* f. sp. *cubense* was isolated from the wilt infected banana vascular tissues from the farmers field at Pannapur, Dharampur in Vaishali and of *Trichoderma viride* from the experimental field of banana at Rajendra Agricultural University, Pusa, Samastipur, Bihar. They were identified macroscopically and microscopically (Lesile and Summerell, 2006). The pathogen was tested for pathogenicity(Venkatesh *et al.*, 2013).These cultures were grown and maintained on PDA medium for further experiments.

Test with Fungicides

Four fungicides *viz.*, Mancozeb, Saaf, Carbendazim and Cuprozin were evaluated against colony growth of *Fusarium oxysporum* f. sp. *cubense*. Fungicides were used @ 0.01%,

0.02% and 0.03% concentrations in autoclaved PDA medium by poisoned food technique (Dhingra and Sinclair, 1985). Twenty ml of such medium was poured in each sterilized Petri plate and solidified. After solidification, 5 mm disc of seven days old cultures of FOC were cut by using sterile cork borer and placed in the centre of Petri plates containing different concentrations of fungicides and incubated at 25 ±1ºC. Treatments were replicated thrice along with suitable control in which fungicide was omitted in the medium. The radial growth of *Fusarium oxysporum* f. sp. *cubense* was measured at intervals of 24 hours up to eight days (Table 1).

Antagonistic Test

The antagonistic activity of biocontrol agent *Trichoderma viride* against *Fusarium oxysporum* f. sp. *cubense* was determined by dual culture technique in *in-vitro* condition (Kumar and Honda, 2007). Mycelial discs measuring five mm diameter from seven days old cultures of fungi *Trichoderma viride* and the test pathogen were placed at equidistant on sterile Petri plates containing PDA medium. The Petri plates were then incubated at 28 ± 1ºC. Three replications of each treatment were maintained and observed for a period of eight days. Suitable controls were kept without antagonist. Growth of the pathogenic fungi was measured at 24 hours intervals up to eight days of inoculation of antagonist. Percentage inhibition of mycelial growth of test pathogen was calculated using the formula (Vincent, 1947).

$$I = \left(\frac{C - T}{C}\right) \times 100$$

Where,

I = Per cent radial mycelial growth inhibition

C = Radial growth of pathogen in check Petri-plate.

T = Radial growth of pathogen in dual culture.

The data were collected daily at an interval of 24 hours. All the data were analyzed by executing one factor analysis of variance (ANOVA) using OP Stat. The means were compared using Duncan's multiple range test (Duncan, 1955) to find the difference at 5% ($P<0.05$) level with fungicidal effect of the test fungi (Table 1).The results were expressed as a mean of three replications.

RESULTS AND DISCUSSION

Effect of Mancozeb, Saaf, Carbendazim and Cuprozinon on growth of *Fusarium oxysporum* f. sp. *cubense*.

Use of chemical fungicides for control of fusariumwilt is a general practice in many of the cultivated plants (Arunodhyam *et al.*, 2014). Such measures are largely ineffective in banana. The chemical fungicides showed their effect on pathogen fungus FOC under *in-vitro* test system but were ineffective in field (Stover, 1962). However, chemical fungicide carbendazim, when applied at 0.2% concentration for soil drenching or injected 2.0% in the rhizomes of banana plant, controlled the fusarium wilt of banana (Thangvelu *et al.*, 2001). Mancozeb, Saaf, Carbendazim and Cuprozin are the commonly used fungicides. Carbendazim is a systemic fungicide, approved by International Standardization Organization (ISO), which inhibits the synthesis of β-tubulin of the fungal pathogen (Pfeil and Dellarco, 2005). Mencozeb and Cuprozin are contact non-systemic fungicides that inhibit the formation of germ tube of fungal species. Whereas Saaf is the mixture of Carbendazim (12%) and Mencozeb (63%) and has a collective effect of systemic and contact fungicides. The effect of these fungicides on the mycelial growth of the FOC was observed *in-vitro*. There was a gradual increase of fungal mycelial growth with increasing periods of incubation in control as well as in presence of different doses of selected fungicides. The maximum radial growth of fungus was observed in control (38.99mm) followed by those treated with Cuprozin (32.37 - 34.76mm), Mancozeb (8.27 - 13.65), Saaf (3.60 - 5.90) and Carbendazim (0.99 - 1.66) respectively. The fungus continued its growth upto 192 hours of treatment with all the three concentrations of fungicides Cuprozin and Mancozeb. The growth was uninhibited in control, less inhibited when treated with Cuprozin and more inhibited when treated with Mancozeb. The rate of fungus growth decreased with longer duration of treatment representing higher inhibition. However, these two fungicides failed to

Table 1: Effect of Mancozeb, SAAF, Carbendazim and Cuprozinon radial growth (mm) of *Fusarium oxysporum* at 24 hours interval.

Treatments	Radial growth (mm) of *Fusarium oxysporum*							
	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs	192hrs
Control (untreated)	4.00	10.00	16.01	23.00	31.00	36.17	38.66	38.99
Cuprozin (0.01%)	3.80	9.29[a]	14.78 [a]	21.09	28.23 [a]	32.62	34.61	34.76
Cuprozin (0.02%)	3.75[a]	9.18 [a]	14.55 [a]	20.58	27.40 [a]	31.71	33.52	33.62
Cuprozin (0.03%)	3.69[a]	8.90	14.08	19.96	26.24	30.23	32.14	32.37
Mancozeb(0.01%)	2.93	4.79	6.22	8.67	11.19	12.93	13.63	13.65
Mancozeb(0.02%)	2.66	4.36	5.66	7.39	9.78	10.98	11.65	11.69
Mancozeb(0.03%)	2.33[b]	3.89[b]	4.63	5.62 [a]	6.98	7.83	8.25	8.27
Saaf(0.01%)	2.25[bc]	3.76 [b c]	5.18	5.61 [a]	5.78	5.90	5.90	5.90
Saaf(0.02%)	2.23 [bc]	3.63 [c]	3.96	4.33	4.46[b]	4.49	4.60	4.60
Saaf(0.03%)	2.13[c]	2.79	3.06	3.33	3.56[b]	3.60	3.60	3.60
Carbendazim (0.01%)	1.13[d]	1.33	1.66	1.66	1.66 [c]	1.66	1.66	1.66
Carbendazim (0.02%)	0.99 [de]	1.00 [d]	1.16 [b]	1.16 [b]	1.16 [c]	1.16 [a]	1.16 [a]	1.16 [a]
Carbendazim (0.03%)	0.86[e]	0.99 [d]	0.99 [b]	0.99 [b]	0.99 [c]	0.99 [a]	0.99 [a]	0.99 [a]
SE(m)	0.050	0.068	0.079	0.128	0.331	0.121	0.136	0.11
CD	0.145	0.199	0.232	0.375	0.968	0.355	0.398	0.324
CV	3.404	2.404	1.944	2.338	4.707	1.516	1.610	1.307

Data are mean of three replicates; [a] Note: values in the same column followed by a similar letter are not significantly different by applying DMRT, (Amadioha, 2000).

Table 2: Radial growth of *Fusarium oxysporum* as influenced by dual culture of *Trichoderma viride* at 24 hours interval and per cent inhibition

Time	Radial growth (mm) of *Fusarium oxysporum*		
	Control	Dual Culture	Per cent Inhibition
24 Hrs.	4.00	3.80	5.00
48 Hrs.	10.00	9.25	7.50
72 Hrs.	16.01	14.70	8.18
96 Hrs.	23.00	17.30	24.78
120 Hrs.	31.00	17.50	43.50
144 Hrs.	36.17	17.50	51.61
168 Hrs.	38.66	17.50	54.73
192 Hrs.	38.99	17.50	55.11
SE(m)	0.45	0.09	
CD	1.36	0.26	
CV	3.16	1.02	

inhibit fungal mycelial growth even at 192 hours of treatment (Table 1).

There was total inhibition of mycelial growth of FOC when treated with fungicide Saaf and Carbendazim. Carbendazim was more effective and elevated in inhibiting the growth of FOC than Saaf. The Saaf treatment completely inhibited fungus expansion at 3.60mm mycelial growth after 144 hours of treatment. Carbendazim at 0.01% and 0.02% inhibited fungus at 1.66mm and 1.16mm of mycelial growth respectively after 72 hours of treatment, while at 0.03% concentration it inhibited fungus growth at 0.99mm which was at par to the growth at 0.2% after 48 hours only (Table 1). Thus, the best fungicide for the control of soil born pathogenic fungus FOC as observed by the minimal *in-vitro* growth of fungus was Carbendazim followed by Saaf, Mancozeb and Cuprozin respectively.

Harender Raj *et al.* (2005) observed the efficacy of different fungicides against *Fusarium oxysporum* f. sp. *gladioli* under *in vitro* conditions and found that Carbendazim and Saaf were more efficient with former better than the latter. Singh and Jha (2003) also found Carbendazim the most effective fungicide against *Fusarium oxysporum* f.sp. *ciceris* under *in vitro* conditions. Similar observations were also found by many other workers on different formae specials of *Fusarium oxysporum* (Raju *et al.*, 2008 and Srivastava *et al.*, 2011).Thus, FOC the causal pathogen for wilt disease of banana can be effectively controlled by the use of fungicide Carbendazim in *in-vitro* test system and possibly in the fields also.

Growth of *Fusarium oxysporum* f. sp. *cubense* as influenced in dual culture with *Trichoderma viride*.

Trichoderma species are free living filamentous fungi, being used worldwide as one of the common biocontrol agents for suitable management of various foliar and soil borne plant pathogens including *Fusarium* species (Thangavelu *et al.*, 2004).The antagonistic activity of *Trichoderma viride* against the pathogenic fungus *Fusarium oxysporum* f. sp. *cubense* was determined by dual culture technique *in-vitro*.The growth of pathogen in control was more compared to that of in dual culture (Fig. 1A). The growth rate of *Trichoderma viride* was faster compared to FOC. It reached the pathogen mycelium within 96 hours and overgrew it, inhibiting further growth of pathogen (Fig. 1B). Thus, the presence and growth of *Trichoderma viride* in dual culture controlled the growth of pathogen *Fusarium oxysporum* f. sp. *cubense*.

The growth of pathogen fungus *Fusarium oxysporum* f. sp. *cubense* increased with the increasing duration of culture in control as well as in dual culture with biocontrol fungus *Trichoderma viride*. The pathogen fungus continued to increase upto 192 hours in control but in dual culture it showed growth of 17.50mm upto 120 hours only and after that there was no further growth. The percent inhibition of the mycelia growth of pathogen fungus in dual culture increased with increasing duration (Table 2).

Biocontrol agent *Trichoderma* species reduces the effect of fungal pathogen *Fusarium* through a mechanism consisting of mycoparasitism, a competition for space and nutrients, antibiosis involving enzymes and secondary metabolites and elicitation of plant defence system (Thangavelu and Mustaffa, 2012).*Trichoderma* species during mycoparasitism entered the hyphe of pathogen. These produced extracellular proteolytic, glucanolytic and chitinase enzymes, which were responsible for release of bioactive molecules namely proteins and cell wall fragments and lysis of pathogen cells (Weindling,

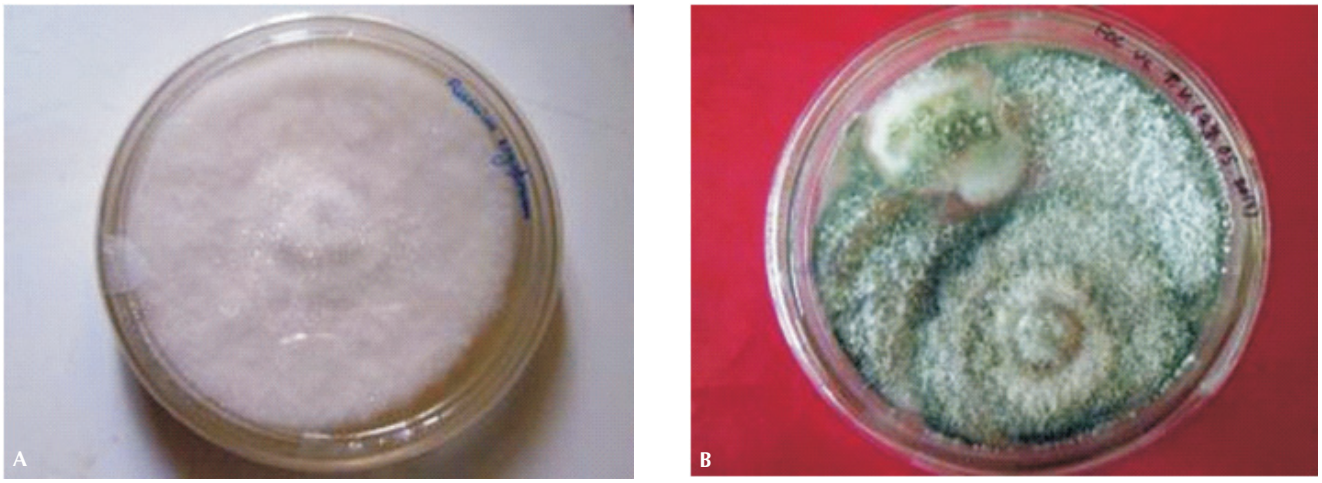

Figure 1: Pure culture of *Fusarium oxysporum*f.sp. *cubense* A, and antagonistic effect of *Trichoderma viride* against *Fusarium oxysporum* f.sp. *cubense* B.

1941).These released molecules and cell wall fragments were responsible for elicitation of induced systemic or localized resistance. The secondary metabolites produced by *Trichoderma* such as volatiles and antibiotics were responsible for antibiosis(Thangavelu and Mustaffa, 2012). Further, *Trichoderma* also helped in removing the mycotoxin produced by the fungal pathogen (Pates *et al.*, 1999)

The management of pathogen *Fusarium oxysporum* f. sp. *cubense* by biocontrol agent *Trichoderma viride* was superior compared to three of the chemical fungicides used namely Mancozeb, Cuprozin and Saaf, but inferior to all the three concentrations of Carbendazim. However, considering the ecofriendly nature, biocontrol agent *Trichoderma viride* is more desirable for the control of fusarium wilt in banana. Thus, the work illustrated the possibility of using biocontrol agent *Trichoderma viride* for the amelioration and control of wilt disease in banana caused by *Fusarium oxysporum* f. sp. *cubense*.

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