IN VITRO INTEGRATED CONTROL OF *COLLETOTRICHUM GLOEOSPORIOIDES* WITH BIOLOGICAL AND CHEMICAL AGENTS

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ABSTRACT

To study the effect of different biological and chemical agents on the growth of *Colletotrichum gloeosporioides*, *in vitro* experiments were conducted with *Trichoderma* spp. and two fungicides alone and in integration, at the Department of Plant Pathology, NWFP Agricultural University, Peshawar, in 2006. The results indicated that all the treatments had a significant inhibitory effect on the culture of *C. gloeosporioides* and reduced its colony diameter. Treatments with any of the species of *Trichoderma* integrated with 300 mg mancozeb/L PDA were effective and did not allow any growth of the pathogen. When mancozeb was used alone at 100 mg/L PDA or 300 mg/L PDA, it reduced the growth of the pathogen by 49.21% and 100%, respectively, as compared to the control. The cultures of *T. viride*, *T. harzianum* and *T. hamatum* reduced the growth of *C. gloeosporioides* by 28.00 %, 50.40 % and 58.41 %, respectively, when used alone. The dose of 300 mg captan/L PDA reduced the colony diameter by 31.74 %, while its integration with *T. hamatum* reduced the growth of the pathogen by 75.07 %. It is concluded that mancozeb is more effective than captan against the pathogen. *Trichoderma* spp. are also effective in controlling the growth of *C. gloeosporioides* whether used alone or in integration with the fungicides. However, further studies are needed to evaluate their potential under field conditions.

Keywords: *Colletotrichum gloeosporioides*, *Trichoderma* spp., Integrated Control, Mancozeb

INTRODUCTION

Colletotrichum gloeosporioides is a destructive pathogen of many crop species. It causes diseases in many annual, biennial and perennial plants. The fungus has got a large host range and infects a variety of economically important plants such as cucurbits, blue berries, citrus, guava, strawberry, turf grasses, cereals and some other fruit crops both in nursery and field. This pathogen exists in different forms in different hosts. It is found both in sexual (*Glomerella cingulata*) and asexual (*C. gloeosporioides*) forms (Agrios, 1997).

It is very difficult to control the pathogen. Chemical control may not be effective because the pathogen often forms hard overwintering structures (acervuli) which enable the pathogen to survive as dormant under a wide range of unfavorable environmental conditions. Moreover, problems like leaching, degradation, environmental pollution and killing the non-target organisms are also associated with chemical control. Host resistance is broken down easily by the frequent appearance of new virulent strains. Mainly due to these reasons, integrated control strategies have been adopted extensively.

Different species of *Trichoderma* have been used widely against many plant pathogens. *Trichoderma* is a filamentous fungus distributed in species like *Trichoderma viride*, *T. harzianum, T. hamatum* and *T. asperelum* (Khuls *et al*. 1999). *Trichoderma* has multiple mechanisms for control of pathogens (Benitez *et al*. 2004). *Trichoderma* spp. have a wide host range and so are strongly antagonistic to fungal pathogens like *Pythium, Rhizoctonia, Fusarium, Botrytis, Sclerotium, Colletotrichum, Alternaria,* nematodes and many other plant pathogens (Harman, 1996). Winidham *et al*. (1986) reported that *Trichoderma* increased plant growth by the production of a growth-stimulating factor. Leinhos and Buchenauer (1992) concluded that fungi release toxic metabolites and enzymes into the medium in which they grow. Benitez *et al.* (2004) isolated chemicals like harzianic acid, tricholin and viridine, which play a very important role in antagonistic behavior of *Trichoderma.*

The use of fungicides sometimes becomes unavoidable. However, their dose and frequency of application can be minimized by integration with other methods for effective control of plant pathogens. Solano and Arauz, (1995) applied mancozeb, captan, tricyclazole, chlorothalonil and prochloraz against papaya anthracnose (*Colletotrichum gloeosporioides*) and found that mancozeb and prochloraz resulted in lowest disease incidence. Freeman *et al.* (1997) assessed various fungicides like folpet, captan and propaconazole for their ability to control *C. gloeosporioides*. They found that captan was effective in 50 % and 70 % concentrations. Legard (2000) tested captan, thiram, Benlate, Topsin-M and mancozeb against *Colletotrichum* crown rot. He found Benlate, Topsin-M and mancozeb more effective in controlling the pathogen, as compared to captan and thiram. This study was aimed at finding out the effect of *Trichoderma* spp. alone and in integration with fungicides on the growth of *C. gloeosporioides*.

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MATERIALS AND METHODS

The culture of *C. gloeosporioides* was obtained from the Department of Plant Pathology, N.W.F.P. Agricultural University, Peshawar. The culture was then multiplied on Potato Dextrose Agar (PDA) medium. The cultures of *Trichoderma* spp. were also obtained from the Department of Plant Pathology, N.W.F.P, Agricultural University, Peshawar. These antagonists were grown on PDA medium at 25^{o}C for 1 week for mass culturing. The two fungicides, captan and mancozeb, were purchased from the local market.

Setting up of the Experiment

The experiment was carried out at the Department of Plant Pathology, NWFP Agricultural University, Peshawar to test the relative efficacy of the three species of *Trichoderma* (*T. viride*, *T. harzianum* and *T. hamatum*) against *C. gloeosporioides* alone and in integration with different doses of the two fungicides (captan and mancozeb). Two doses, 100 mg/L and 300 mg/L, of both the fungicides were used in PDA medium. The medium was prepared and autoclaved at 121^{o}C for 15 minutes. Both the fungicides were added after sterilization and thoroughly mixed before pouring into the Petri dishes. Petri dishes were inoculated in the centre with blocks of 5 mm diameter of a 15 days old culture of *C gloeosporioides*. Each of the *Trichoderma* spp. was applied individually as 5 mm diameter inoculum plugs at four equidistant sites around the pathogen. The Petri dishes were sealed with parafilm and incubated for 2 weeks at 25^{o}C. One treatment was also left untreated that served as a control. All these treatments were replicated seven times. The experiment was laid out in a randomized complete block design. Data were taken as the mean colony diameter of *C. gloeosporioides*, twice at weekly interval.

RESULTS AND DISCUSSION

Both the doses of the two fungicides and each of the three species of *Trichoderma* as well as their integration reduced the growth of *Colletotrichum gloeosporioides* significantly as compared to the control (Tables I and II). The high dose of 300 mg mancozeb/L PDA when used alone or in integration with any of the species of *Trichoderma* was the most effective and did not allow any growth of the pathogen. The low dose of 100 mg mancozeb/L PDA reduced the growth of the pathogen by 49.21% after 7 days, which remained the same after 14 days. Captan, on the other hand, reduced the colony diameter of the pathogen by 21.71% and 31.74% when used in the low and high doses of 100 and 300 mg/L PDA, respectively. When integrated with *T. hamatum*, the low dose of mancozeb reduced the growth of the pathogen by 85.77% during the first 7 days of incubation, but the growth inhibition was reduced to 76.98% after 14 days. *T. hamatum* also reduced the colony diameter of the pathogen by 80.95% in integration with 100 mg captan/L PDA in the first week, which was reduced to 76.18% after the second week. Among the *Trichoderma* spp., *T. hamatum* was also the most effective alone and reduced the colony diameter of the pathogen by 58.41%. *T. harzianum* and *T. viride* reduced the colony diameters by 50.40% and 28.00% after 7 days and 50.40% and 25.40 % after 14 days, respectively.

Several workers have used different species of *Trichoderma* against *Colletotrichum*, both in the field and laboratory (Freeman *et al*. 1997). The present study proved that among the fungicides, mancozeb as compared to captan, was more effective in controlling the growth of the pathogen whether used alone or in integration with the antagonists. This result compares favorably with the finding of Solano and Arauz (19995), Haddad *et al.* (2003) and Legard (2000). There may be several reasons for the greater efficacy of this fungicide. Mancozeb is a contact protective fungicide with a high molecular weight as compared to captan. It is a derivative of dithiocarbamic acid. These compounds are toxic to fungi because they are metabolized to isothiocynate radicals (N = S = C) inside pathogen cells, inactivating the –SH group of amino acids and enzymes showing their effect. On the other hand, captan is a low molecular weight contact fungicide used mostly against oomycetes (Haddad *et al.* 2003). Mancozeb also showed good results when it was integrated with the species of *Trichoderma*.

Among the species of *Trichoderma*, *T. viride* was not as efficient as *T. harzianum* and *T. hamatum*, which caused greater growth inhibition. These results are similar to the findings of Malathie *et al*. (2002), Marco (2003) and Verma *et al*. (2006). All the species of *Trichoderma* were inoculated to the Petri dishes at the same time but after 3 days of inoculating *C. gloeosporioides*. Still the hyphae of *T. hamatum* and *T. harzianum* grew vigorously over the culture of the pathogen (Fig. 1). The growth of *T. viride*, on the other hand, was much restricted. The culture was light green in colour right from the beginning, as compared to white colour of the other two species. Absence of direct parasitism and less competition may also be one of the reasons due to which *T. viride* was not as successful in controlling *C. gloeosporioides.*

CONCLUSION AND RECOMMENDATION

It is concluded that mancozeb is more effective than captan against the pathogen. *Trichoderma* spp. are also effective in controlling the growth of *C. gloeosporioides*, whether used alone or in integration with the fungicides. However, further studies are needed to evaluate their potential under field conditions.

Table I. ***Mean colony diameter (cm) of Colletotrichum gloeosporioides after 7 days of incubation at 25 °C as affected by fungicides and Trichoderma spp.***

Treatment No.	Treatments	Colony diameter	% reduction than control (T1).
T1	Control (*C. gloeosporioides* alone)	9.00 A	-
T2	*Trichoderma viride*	6.429 BC	28.00
T3	*T. harzianum*	4.457 EF	50.40
T4	*T. hamatum*	3.743 FG	58.41
T5	Captan (100 mg/L PDA)	7.029 BCD	21.71
T6	Captan (300 mg/L PDA)	5.857 B	31.74
T7	Mancozeb (100 mg/L PDA)	4.571 DEF	49.21
T8	Mancozeb (300 mg/L PDA)	0.0 K	100
T9	*T. viride* + Captan (100 mg/L PDA)	5.214 CDE	42.06
T10	*T. harzianum* + Captan (100 mg/L PDA)	3.429 FGH	62.0
T11	*T. hamatum* + Captan (100 mg/LPDA)	1.714 IJ	80.95
T12	*T. viride* + Captan (300 mg/L PDA)	4.386 EF	51.26
T13	*T. harzianum* + Captan (300 mg/L PDA)	3.014 GHI	66.51
T14	*T. hamatum* + Captan (300 mg/L PDA)	2.243 HIJ	75.07
T15	*T. viride* + Mancozeb (100 mg/L PDA)	3.429 FGH	61.90
T16	*T. harzianum* + Mancozeb (100 mg/L PDA)	2.571 GHIJ	71.43
T17	*T. hamatum* + Mancozeb (100 mg/L PDA)	1.286 JK	85.77
T18	*T. viride* + Mancozeb (300 mg/L PDA)	0.0 K	100
T19	*T. harzianum* + Mancozeb (300 mg/L PDA)	0.0 K	100
T20	*T. hamatum* + Mancozeb (300 mg/L PDA)	0.0 K	100
	$LSD_{(0.05)}$	1.319	
	CV (%)	36.00	

Means followed by different letters are significantly different from each other at 5 % level of significance.

Table II. ***Mean colony diameter (cm) of Colletotrichum gloeosporioides after 14 days of incubation at 25 ºC as affected by fungicides and Trichoderma spp.***

Treatment No.	Treatments	Colony diameter	% reduction than control (T1).
T1	Control (*C. gloeosporioides* alone)	9.00 A	-
T2	*Trichoderma viride*	6.714 B	25.40
T3	*T. harzianum*	4.457 DE	50.40
T4	*T. hamatum*	3.743 EF	58.41
T5	Captan (100 mg/L PDA)	7.029 BC	21.71
T6	Captan (300 mg/L PDA)	6.143 B	31.74
T7	Mancozeb (100 mg/L PDA)	4.571 DE	49.21
T8	Mancozeb (300 mg/L PDA)	0.0 H	100
T9	*T. viride* + Captan (100 mg/L PDA)	5.214	42.06
T10	*T. harzianum* + Captan (100 mg/L PDA)	3.857 EF	57.14
T11	*T. hamatum* + Captan (100 mg/LPDA)	2.143 G	76.18
T12	*T. viride* + Captan (300 mg/L PDA)	4.386 DE	51.26
T13	*T. harzianum* + Captan (300 mg/L PDA)	3.014 FG	66.51
T14	*T. hamatum* + Captan (300 mg/L PDA)	2.243 G	75.07
T15	*T. viride* + Mancozeb (100 mg/L PDA)	3.857 EF	57.14
T16	*T. harzianum* + Mancozeb (100 mg/L PDA)	2.786 FG	69.04
T17	*T. hamatum* + Mancozeb (100 mg/L PDA)	2.071 G	76.98
T18	*T. viride* + Mancozeb (300 mg/L PDA)	0.0 H	100
T19	*T. harzianum* + Mancozeb (300 mg/L PDA)	0.0 H	100
T20	*T. hamatum* + Mancozeb (300 mg/L PDA)	0.0 H	100
	$LSD_{(0.05)}$	1.279	
	CV (%)	33.94	

Means followed by different letters are significantly different from each other at 5 % level of significance.

Fig. 1: Growth of *Trichoderma harzianum* hyphae and spores over the colony of *Colletotrichum gloeosporioides.*

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