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IN VITRO CHEMICAL CONTROL OF *COLLETOTRICHUM GLEOSPORIOIDES*

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ABSTRACT

Colletotrichum gleosporioides Penz. was isolated from the leaves of citrus suffering from anthracnose disease. *In vitro* efficacy of four fungicides viz. Acrobat MZ, Dithan M-45, Aliette and Ridomil Gold was evaluated against the isolated fungal species in Basal Broth medium. Different doses of the test fungicides viz. recommended (R), 0.50R and 0.25R were employed in the experiment. All the employed doses of the Dithane significantly reduced the biomass of the test fungal species. Similarly 0.5R and 0.25R Ridomil Gold significantly reduced the test fungal biomass. In contrast to that Aliette and Acrobat MZ found ineffective against the test fungal species.

Key words: *Colletotrichum gleosporioides*, anthracnose, citrus, fungicides.

INTRODUCTION

Colletotrichum gloeosporioides (Penz.) Penz. & Sacc. (teleomorph: *Glomerella cingulata*) a phytopathogenic fungus belonging to the order Melanconiales, is an important post harvest fungal pathogen attacking a wide variety of tropical and subtropical fruits (Coates *et. al.*, 1993, Prusky and Plumbley, 1992). This fungus also causes anthracnose disease in cassava, one of the major economic diseases of cassava. It occurs frequently in the cassava-growing regions of Africa (Theberge, 1985), South America (Lozano *et al.*, 1981) and Asia (Chadrasekharan-Nair *et al.*, 1979). Anthracnose caused by *C. gloeosporioides* is a most damaging disease of *Dioscorea alata* worldwide (Abang *et al.*, 2002). *C. gloeosporioides* spores germinate on fruits in the orchard by growing a germ tube that develops an appressorium (Binyamini and Schiffmann-Nadel, 1972). The appressorium produces infection hyphae that remain quiescent in the cells of the fruit's epidermal layer until the fruit ripens and softens during storage (Prusky, 1996). The present study was carried out to isolate the *C. gloeosporioides* from citrus leaves and to evaluate the *in vitro* control potential of some fungicides against this pathogen.

MATERIALS AND METHODS

Isolation of the pathogen

The diseased leaf specimens were cut into small pieces and surface disinfected by immersing in 1% sodium hypochlorite solution for one minute and then rinsed thrice in sterilized water. The surface sterilized pieces were placed on to the malt extract agar (MEA) and potato dextrose agar (PDA) in petriplates and incubated at 25°C. After 8 days the fungal isolates appearing on the leaf pieces were identified and transferred to PDA slants for purification.

In vitro bioassay

Recommended (R) as well as lower doses viz. 0.50R and 0.25R of four fungicides namely Acrobat MZ, Dithan M-45, Aliette and Ridomil Gold were employed in the experiment (Table 1). The weighed quantity of each fungicide was added to 100 ml of autoclaved basal broth medium in a 250 ml conical flask. Control treatment was without any fungicide. Inoculum discs of 6 mm diameter, obtained from the margins of 7-days old healthy growing culture of *C. gleosporioides*, isolated from leaves of infected citrus tree, were transferred to flasks aseptically. Each treatment was replicated thrice. The flasks were incubated at 25 °C. After 10 days fungal biomass in each flask was filtered, dried at 60 °C and weighed. All the data were analyzed by applying two way analysis of variance (ANOVA) followed by Duncan's Multiple Range (DMR) Test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

There was a significant difference in fungicidal potential among the four test fungicides against the test fungal species *C. gleosporioides*. Effect of different doses of fungicides as well as the interactive effect of the doses and fungicides on the fungal biomass production was also significant (Table 1).

Table 1. Doses of four fungicides used in the experiment.

Fungicides	Dose	Concentration (g/100ml)
Acrobat MZ 75/667WP	R	0.300
	0.50R	0.150
	0.25R	0.075
Dithane M-45 WP 80	R	0.800
	0.50R	0.400
	0.25R	0.200
Aliette 80% WP (w/w)	R	0.200
	0.50R	0.100
	0.25R	0.050
Ridomil Gold 72%WP(w/w)	R	0.500
	0.50R	0.250
	0.25R	0.012

R: Recommended dose of fungicides.

Table 2. ANOVA for the effect of different concentrations of four fungicides on *in vitro* growth of *C. gloeosporioides.*

Sources of variation	df	SS	MS	F values
Treatments	15	259929	17329	14.5*
Fungicides (F)	3	140889	46963	39.41*
Doses (D)	3	35289	11763	9.87*
F × D	9	83751	9306	7.81*
Error	32	38126	1191	
Total	48	2365586		

*, significant difference at P≤0.001

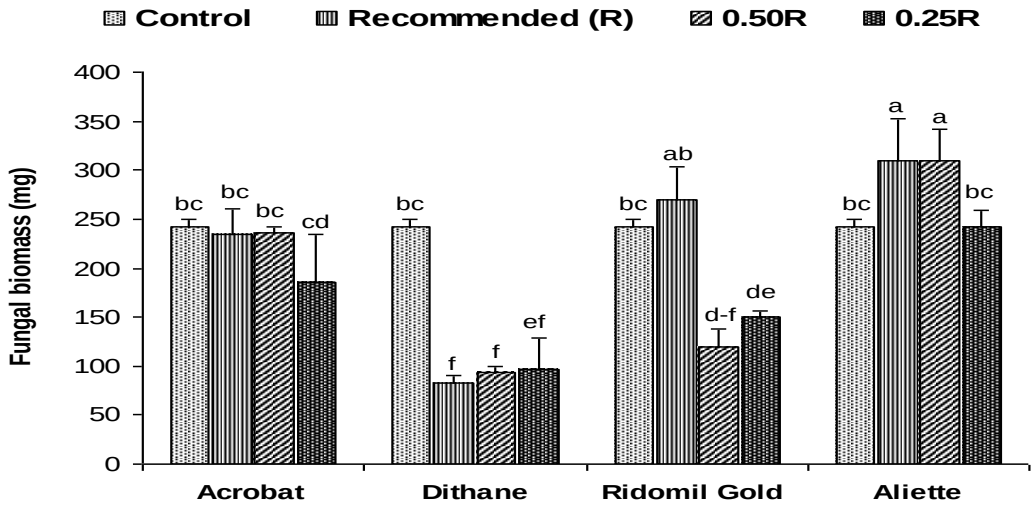

Fig. 1. Effect of different concentrations of the four fungicides on *in vitro* biomass production of *Colletotrichum gleosporioides.*Vertical bars show standard error of means of three replicates. Values with different letters show significant difference (P≤0.05) as determined by Duncan's Multiple Range Test.

Among the four fungicides, Dithane was found to be the most effective where all the employed doses significantly suppressed the fungal biomass. There was 60–66% reduction in fungal biomass due to application of recommended and various lower doses of this fungicide (Fig. 1). Dithane has also been found very effective against *Botriodiplodia theobromae* Pat., the cause of dying back mango (Javaid *et al.,* 2007). Bradley *et al.* (2007) reported that seed treatment with Dithane significantly reduced the occurrence of root rot fungi on flax (*Linum usitatissimum*L.) cultivars. Sudisha *et al*. (2006) reported a significant reduction in disease incidence in muskmelon by fungal pathogen *Didymella bryoniae* when seeds were treated with 0.2% Dithan suspension. Dithane is also effective in controlling yam (*Discorea rotundata*) rot fungi namely species of *Aspergillus, Botryodiplodia, Fusarium, Penicillium* and *Rhizopus* **(**Efiuvwevwere and Nwachukwu, 1998**).**

Ridomil Gold was found to be the second most effective fungicide against the target fungal species. The recommended dose of this fungicide resulted in an insignificant increase in fungal biomass. By contrast, lower doses of 0.50R and 0.25R significantly reduced fungal biomass –and --%as compared to control (Fig. 1).Ridomil Gold is also known to be very effective against *Phytophthora infestans, the cause of late of potato* (Evenhuis *et al.,* 2006). Similarly Fravel *et al.* (2005) found that Ridomil Gold was very effective against *Fusarium oxysporum.*

Effect of all the employed doses of Acrobat was insignificant. Similarly lowest dose of 0.25R of Alliette was found ineffective in suppressing the fungal biomass. Conversely, recommended and 0.50R doses of this fungicide significantly enhanced fungal biomass as compared to control (Fig. 1).

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