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In-Vitro Evaluation of Fungicides and Antagonists against the Growth of *Colletotrichum musae*

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Abstract An experiment was conducted during 2013 to find out the effective measures against growth of *Clletotrichum musae.*The antifungal effects of six fungicides viz., Carbendazim, Mancozeb, Azoxystrobin Propiconazole, SAFF, Thiophanate methyl and three antagonists viz., *Trichoderma harzianum, Trichoderma viride* and *Pseudomonas fluorescens* were evaluated to control banana anthracnose disease caused by *Colletotrichum musae.* Among the chemicals tested, the fungal growth was totally inhibited in all the three concentrations (500 ppm, 750 ppm, 1,000 ppm) of carbendazim, propiconazole and carbendazim + mancozeb (SAAF), followed by azoxystrobin at 1,000 ppm (87.31%) and 750 ppm (80.88%). Thiophanate methyl at 1,000 ppm showed moderate inhibition of pathogen (78.02%) and mancozeb significantly inhibited the mycelial growth at 1,000 ppm (42.67%). Among three bio-agents *Trichoderma harzianum* and *Trichoderma viride* was significantly superior to *Pseudomonas fluorescens*. In case of *T. viride, T.harzianum* and *Pseudomonas fluorescens* the percent colony growth over control were 80.71, 70.54 and 44.82 respectively.

Keywords *In-vitro*, Chemicals, Antagonists, *Colletotrichum musae.*

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Introduction

Banana (*Musa* sp) is one of the important fruit crops of the world as well as India. Banana called as "Adam's fig" and "Apple of Paradise" belonging to the family Musaceae and the genus *Musa.* Bananas are widely grown in India with great socio-economic significance, interwoven in the cultural heritage of the country. It is known to be one of the earliest fruit crops grown by manking at the dawn of civilization considering its nutritive value. [1]. Anthracnose, caused by the fungus *Colletotrichum musae* (Berk. and M.A. Curtis) Arx, is the most important postharvest disease of banana that can results in 30 to 40% losses of marketable fruit [2]. Anthracnose is a latent infection where fungal spores infect immature banana in the field but symptoms occur as peel blemishes as black or brown sunken spots of various sizes on fruit that may bear masses of salmon-colored acervuli with their associated conidia on the fruit peel after ripening [3]. Thus, any potential control measure which can effectively delays the symptoms of anthracnose infection would have an important role in extending the shelf life of banana fruit during storage. Systemic fungicides eg. bayleton, benomyl, prochloraz and SAAF are successful in completely (100%) inhibiting the growth of *Colletotrichum gloeosporioides* [4].

In the light of present day constraints in plant disease management practices especially those on the use of pesticides, biological control is increasingly occupying the minds of scientists all over the world as they are eco-friendly and cost effective . In recent years, the use of Trichoderma has gained more importance. Hence, present studies were under taken

to evaluate the efficacy of chemicals and antagonists against the growth of the *Colletotrichum musae.*

Materials and Methods

An *in-vitro* experiment was conducted during 2013 at K. R. C. College of Horticulture, Arabhavi to find out suitable chemicals against growth of *Colletotrichum musae.*

The pathogen was grown on potato dextrose agar medium prior to the setting of the experiment. The fungicide suspension was made by adding required quantity of fungicides to the melted potato dextrose agar medium to obtain the desired concentration on the basis of active ingredient present in the chemical 30 ml of poisoned medium was poured into each sterilized petriplate and suitable checks were maintained without addition of fungicides. 5 mm of ten days old fungal disc was taken from the periphery of the culture and was placed in the center of the poisoned medium aseptically and incubated at 28 ^{0}C for seven days. Three replications were maintained for each treatment and the diameter of the colony was measured in 2 directions and the average was recorded after incubation for seven days.

$$I = \frac{C - T}{C} \times 100$$

Where, I = Per cent inhibition, C = Growth of the pathogen in control plate, T = Growth of the pathogen in dual culture plate.

Three antagonists such as *Trichoderma harzianum, Trichoderma viride* and *Pseudomonas fluorescens* were tested against *Colletotrichum musae.* Both biocontrol agents and test fungus were cultured on potato dextrose agar in order to get fresh and active growth of fungus. Five mm of fungal disc of the antagonist along with test fungus were kept on the potato dextrose agar medium in opposite direction. The plates were incubated for a week at 28 ^{0}C. The growth of antagonistic fungus and growth of pathogen was also recorded separately. The bacterial antagonist *Pseudomonas fluorescens* was streaked on one side of the potato dextrose agar medium and on the other side *Colletotrichum musae* disc was placed. The inhibition zone was measured. The observation on interaction zone or inhibition zone was recorded.

Table 1. *In-vitro* evaluation of chemicals against *Colletotrichum musae.* Figures in the parentheses are the square root transformation values.

Treatments	Concentration (ppm)	Colony diameter (mm)	Per cent inhibition of colony growth over control
T_1-Carbendazim	500	0.00 (1.00)	100.00
	750	0.00 (1.00)	100.00
	1000	0.00 (1.00)	100.00
T_2-Mancozeb	500	63.13 (8.00)	9.81
	750	58.25 (7.69)	16.78
	1000	40.13 (6.41)	42.67
T_3-Azoxystrobin	500	15.13 (4.01)	78.38
	750	13.38 (3.79)	89.88
	1000	8.88 (3.14)	87.31
T_4-Propiconazole	500	0.00 (1.00)	100.00
	750	0.00 (1.00)	100.00
	1000	0.00 (1.00)	100.00
T_5-SAAF	500	0.00 (1.00)	100.00
	750	0.00 (1.00)	100.00
	1000	0.00 (1.00)	100.00
T_6- Thiophanate methyl	500	27.88 (5.37)	60.17
	750	22.75 (4.87)	67.50
	1000	15.38 (4.04)	78.02
T_7- Control		70.00 (8.42)	
SEm ±			
CD at 1%			

After the period of incubation, the growth of the *Colletotrichum* colony was recorded and the per cent inhibition of the colony over control was calculated.

Results and Discussion

In vitro evaluation of fungicides against *Colletotrichum musae*

In vitro evaluation of different chemicals against *Colletotrichum musae* was done as described in material and methods using by following poisoned food technique. The fungicides were tested at 500, 750 and 1,000 ppm concentrations each and the observations on colony diameter and per cent inhibition of colony growth over control are presented in Table 1, Fig. 1 and Fig. 2.

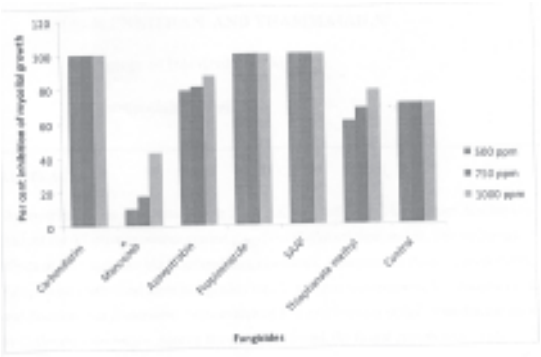

Fig. 1. *In vitro* evaluation of fungicides against *Colletotrichum musae*.

Carbendazim, propiconazole and SAAF (carbendazim + mancozeb) completely inhibited the mycelial growth of the fungus (100%) at 500 ppm followed by azoxystrobin (78.38%) and thiophanate methyl (60.17%). While mancozeb recorded the least inhibition of the fungus (9.81%). At 750 ppm, concentration, carbendazim, propiconazole and SAAF showed highest inhibition of 100% on mycelial growth of fungus followed by azoxystrobin (80.88%) and thiophanate methyl (67.50%). Mancozeb showed least effectiveness at 750 ppm by inhibiting 16.78% of fungus growth compared to 500 ppm. The results obtained at 1,000 ppm concentration of different fungicides clearly showed that carbendazim, propiconazole and SAAF were quite effective in inhibiting the mycelial growth of the fungus. A zoxystrobin and thiophanate methyl also shows good results in inhibiting pathogen growth (87.31 mm and 78.02 mm, respectively). Whereas, Mancozeb showed least effectiveness at 1,000 ppm by inhibiting 42.67% of mycelial growth compared to 500 and 750 ppm. The treatment thiophanate methyl even at very low concentration (100 ppm) carbendazim (250 ppm) or carbendazim + mancozeb mixture 2000 ppm) inhibits the growth of *Colletotrichum* spp.in vanilla [5].

Seven systemic and four non- systemic fungicides were evaluated at three concentrations against *Colletotrichum gloeosporioides.* They indicated that systemic fungicides bayleton, benomyl, prochloraz and SAAF gave complete control (100%) followed by non-systemic fungicide mancozeb (77.65%) [4].

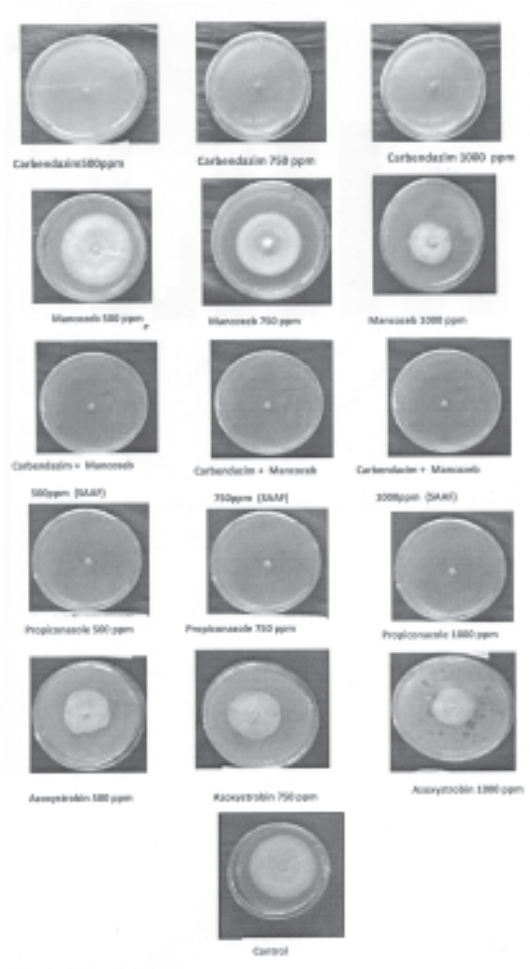

Fig. 2. *In vitro* evaluation of fungicides against *Colletotrichum musae*.

In vitro evaluation of antagonist against *Colletotrichum musae*

Among the antagonists *Trichoderma harzianum* showed strong antagonistic activity by inhibiting 80.71% of *Colletotrichum* colony as compared to control followed by *Trichoderma viride* (70.54%). Whereas, the least parasitic activity was noticed in case of *Pseudomonas fluorescens* which inhibited 44.82% of *Colletotrichum musae* colony (Table 2).

Trichoderma harzianum and *T. viride* of RAU, Pusa isolate were inhibiting the mycelial growth of

Table 2. *In vitro* evaluation of bioagents against *Colletotrichum musae.*

Sl. No.		Bioagents	Growth of the pathogen (mm)	Growth of the antagonistic (mm)	Per cent inhibition of colony growth over control
1	T_1	*Trichoderma harzianum*	13.50	69.00	80.71
2	T_2	*Trichoderma viride*	20.62	23.50	70.54
3	T_3	*Pseudomonas fluorescens*	38.62	36.12	44.82
4	T_4	Control	70		
		SEm ±	0.60	1.55	
		CD @ 1%	2.61	6.72	

Colletotrichum musae to the extent of 78.4 and 78% followed by *Trichoderma virens* (RAU, Pusa isolate) and *Trichoderma virens* (New Delhi isolate) to the extent of 76.8 and 75.1% respectively [6].

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