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EFFICACY OF FUNGICIDES TO CONTROL THREE VIRULENT ISOLATES OF *COLLETOTRICHUM GLOEOSPORIOIDES* ON COCOA

R. CHANDRA MOHANAN and K.M. KAVERIAPPA

ABSTRACT

The widespread occurrence of Colletotrichum disease of cocoa in India warrants the study on the control of the disease. Symptomatology of this disease revealed that besides pod rot, there are three kinds of foliar symptoms namely, blight, shot hole and irregular spot caused by *Colletotrichum gloeosporioides* Penz. The relative efficacy of nine fungicides on the growth of three virulent isolates of *C. gloeosporioides* causing blight, shot hole and irregular spot symptoms on cocoa leaves was studied in the laboratory. Out of the nine fungicides tested *in vitro* Carbendazim (Bavistin WP=0.05-0.10 per cent) mancozeb (Dithane M-45=0.20 and 0.30 per cent) and ziram (Liquid Cuman=0.3 per cent) inhibited the growth of all the three isolates completely. There was considerable variation in growth among the three isolates at different concentrations of the various fungicides tested.

The effect of the above three fungicides in controlling the foliar infection caused by the three isolates, when applied as pre- and post-inoculation sprays was studied. In both pre- and post-inoculation sprays, the leaf blight and irregular spots caused by the respective isolates were controlled effectively by Bavistin WP (0.05 per cent) followed by Dithane M-45 (0.2 per cent) whereas the shot hole symptom was effectively controlled by Dithane M-45 followed by liquid cuman (0.3 per cent). Thus Bavistin WP and Dithane M-45 were found to be promising fungicides to control *C. gloeosporioides* disease of cocoa.

INTRODUCTION

Until recently Colletotrichum disease was not a major problem on cocoa (*Theobroma cacao* L.). However, with the recent expansion in the cultivation of cocoa the disease has become widespread in the cocoa growing areas in India. It has also been observed in greater intensities in certain regions. In India, *Colletotrichum gloeosporioides* Penz. has been reported to have caused rotting of immature pods and three kinds of foliar symptoms namely, blight, shot hole and irregular spot (Chandra Mohanan and Kaveriappa, 1983). Studies on the pathogenicity of the isolates of *C. gloeosporioides* collected from the cocoa gardens of Kerala, Karnataka and Tamil Nadu states showed a great variation in pathogenicity among the isolates. Thus it has become necessary to find suitable control measures for the disease. The present investigation was undertaken to find out the relative efficacy of certain fungicides on three virulent isolates of *C. gloeosporioides*.

MATERIALS AND METHODS

A total of 299 pathogenie isolates of *C. gloeosporioides* were collected from different cocoa growing areas of Kerala, Karnataka and Tamil Nadu states. The most virulent isolates TNI/139, KeII/55 and KaIII/9 (Isolate No.) among the isolates causing blight, shot hole and irregular spot symptom on cocoa leaves respectively, were selected for the present studies.

In vitro studies

The relative efficacy of nine fungicides on the growth of the three isolates TNI/139, KeII/55 and KaIII/9, was studied in the laboratory by poisoned food technique (Nene, 1971). The fungicides tested were:

Bavistin WP (Carbendazim) (Methyl-1H-benzemedazol-2-ylcarbamate)
Kitazin (S-Benzyl-O, O-diisopropyl-phosphorothioate)
Calixin (Tridemorph) (N-Tridecyl-2, 6-dimethylmorpholine)
Dithane M-45 (Mancozeb) (Manganese ethylenebisdithiocarbamate plus zinc ion)
Dithane Z-78 (Zineb) (zinc ethylenebisdithiocarbamate)
Foltaf (Captafol) (cis-N-(1, 1, 2, 2-Tetrachloroethylthio)-4-cyc-

lohexene-I, 2-dicarboximide)
Liquid cuman (Ziram) (zinc dimethyldithiocarbamate)
Daconil 2787 (Chlorothalonil) (Tetrachloroisophthalonitrile)

Of the above Bavistin, Kitazin and Calixin are systemic fungicides. The test concentrations of fungicides are given in Table 1. The fungicides were mixed thoroughly in sterile distilled water and added to a known quantity of PDA medium cooled at 45°C to get the required concentration. The medium was gently shaken to ensure proper and uniform distribution of the fungicide. Fifteen ml of the medium was then poured into each of the sterile plates. Mycelial discs of 6 mm diameter were cut from the periphery of 10-day-old cultures of each isolate growing on PDA and placed in the centre of the plates containing the poisoned medium at the rate of one disc per plate. The plates were incubated at room temperature (28 ± 2°C) for 10 days. Three replications were maintained for each treatment and the plates without fungicide served as control. Radial growth of the fungus in each plate was recorded on the 10th day by measuring the diameter of the colony in two directions at right angles to each other. The average colony diameter was recorded and the per cent inhibition of growth was calculated by using the equation given by Vincent (1927). The data were statistically analysed.

In vivo **studies**

Based on the results of *in vitro* tests, three fungicides namely Bavistin WP, Dithane M-45 and liquid Cuman were selected for *in vivo* studies. They were tested at concentrations of 0.05 per cent, 0.2 per cent and 0.3 per cent respectively. These concentrations were selected on the basis of *in vitro* studies, as well as the earlier reports of their effect in controlling *Colletotrichun* disease of other crops. Cocoa seedlings were raised and eighteen day old healthy seedlings of uniform size were selected for the trial. Bavistin WP, Dithane M-45 and Liquid Cuman were separately mixed with distilled water to make 0.05 per cent, 0.2 per cent and 0.3 per cent concentrations respectively. They were applied as pre- and post-inoculation sprays. The inoculum was obtained from five day old cultures of the isolates, TNI/139, KeII/55 and KaIII/9 on oat meal agar medium. The seedlings were inoculated with a suspension of conidia and mycelia of each isolate separately in tap water.

Table 1. Effect of different concentrations of fungicides on the growth of the three selected isolates of C. gloeosporioides

Fungicides	Conc. (%)	*Percentage inhibition of growth			
		TNI/139	KeII/55	KaIII/9	Mean of three isolates
(1)	(2)	(3)	(4)	(5)	(6)
Bavistin WP	0.025	90.00 (100)	90.00 (100)	90.00 (100)	90.00 (100)
	0.05	90.00 (100)	90.00 (100)	90.00 (100)	90.00 (100)
	0.10	90.00 (100)	90.00 (100)	90.00 (100)	90.00 (100)
Kitazin	0.05	58.37 (72.50)	61.00 (76.50)	62.38 (78.50)	60.00 (75.88)
	0.1	65.65 (83.00)	66.04 (83.50)	67.62 (85.50)	66.44 (84.02)
	0.2	68.45 (86.50)	72.54 (90.99)	71.59 (90.02)	70.86 (89.25)
Calixin	0.05	56.48 (69.50)	47.00 (53.50)	53.43 (64.50)	52.30 (62.61)
	0.1	59.02 (73.50)	49.89 (58.50)	54.94 (67.00)	54.62 (66.47)
	0.2	64.16 (80.99)	54.63 (66.50)	60.67 (75.99)	59.82 (74.73)

Dithane M-45	0.10	90.00 (100)	90.00 (100)	41.55 (43.99)	73.85 (92.26)
	0.20	90.00 (100)	90.00 (100)	90.00 (100)	90.00 (100)
	0.30	90.00 (100)	90.00 (100)	90.00 (100)	90.00 (100)
Dithane Z-78	0.10	34.45 (31.99)	19.82 (11.49)	12.23 (4.49)	22.17 (14.23)
	0.20	35.67 (33.99)	23.58 (15.99)	22.38 (14.49)	27.21 (20.91)
	0.30	90.00 (100)	27.62 (21.49)	22.79 (14.99)	46.80 (53.15)
Foltaf	0.05	49.60 (57.99)	62.03 (77.99)	62.38 (78.50)	58.00 (71.92)
	0.10	54.94 (66.99)	63.79 (80.50)	63.79 (80.50)	60.84 (76.26)
	0.20	56.79 (69.99)	65.65 (83.00)	64.89 (81.99)	62.44 (78.59)
Captaf	0.10	51.35 (60.99)	58.05 (71.99)	58.69 (72.99)	56.03 (68.78)
	0.20	58.05 (71.99)	62.03 (77.99)	66.82 (84.50)	62.29 (78.39)
	0.30	63.43 (79.99)	65.65 (83.00)	90.00 (100)	73.03 (91.48)

(Contd.)

Table 1 (*Contd.*)

(1)	(2)	(3)	(4)	(5)	(6)
Liquid Cuman	0.1	65.24 (67.50)	90.00 (100)	66.82 (84.50)	70.69 (89.06)
	0.2	67.22 (85.01)	90.00 (100)	90.00 (100)	82.41 (98.25)
	0.3	90.00 (100)	90.00 (100)	90.00 (100)	90.00 (100)
Daconi	0.1	37.76 (37.49)	35.37 (33.49)	21.97 (13.99)	31.69 (27.61)
	0.2	45.86 (51.50)	49.31 (57.50)	31.94 (27.99)	42.37 (45.42)
	0.3	48.16 (55.50)	51.35 (60.99)	40.68 (42.49)	46.73 (53.02)
Mean		64.84 (81.92)	64.64 (81.66)	61.76 (77.61)	—

CD (P=0.05) for isolates 0.16
for treatments 0.48
for isolates × treatments 0.83

*After arcsine transformation.

Values in parentheses are original mean.

In the pre-inoculation fungicidal application, seedlings were sprayed with the respective fungicides separately and inoculated with the three isolates separately on the same day. In the post-inoculation application, the seedlings were inoculated with the three isolates separately and two days later the fungicides were sprayed. Twenty-five seedlings were used for each treatment. Seedlings inoculated separately with each isolate and maintained without fungicidal sprays served as control. In all the cases the seedlings were covered immediately after inoculation with polythene bags containing wet cotton pads for 72 hours to provide high humidity. The seedlings were kept under observation for eight days and the disease intensity was recorded on the eighth day. The disease intensity caused by each isolate was assessed by using grades of severity based on the leaf area affected. A quantitative index of severity was calculated based on these ratings (McKinney, 1923).

RESULTS AND DISCUSSION

The per cent inhibition of growth of each isolate in different concentrations of fungicides is given in Table 1. Out of the nine fungicides tested Bavistin WP (0.025-0.10 per cent), Dithane M-45 (0.20 and 0.30 per cent) and Liquid Cuman (0.3 per cent) inhibited the growth of all the three isolates completely. Dithane M-45 at 0.1 per cent concentration completely inhibited the growth of TNI/139 and KeII/55, whereas only 44 per cent inhibition of growth was found in isolate KaIII/9 at this concentration. Liquid Cuman at 0.2 per cent concentration inhibited the growth of the isolate KeII/55 and KaIII/9 completely, and there was only 85 per cent inhibition of TNI/139. The growth of KeII/55 was completely inhibited even at 0.1 per cent concentration of Liquid Cuman. But the other two isolates showed tolerance at this concentration. In the presence of Dithane Z-78 (0.3 per cent) only one isolate, (TNI/139) showed complete inhibition of growth. All the three isolates showed growth at 0.1 and 0.2 per cent concentrations of this fungicide. Dithane Z-78 at 0.4 per cent concentration has been reported to be very effective in reducing the incidence of inflorescence die-back of arecanut and is being recommended for commercial application (Reddy et al.,1978). In the presence of Captaf (0.3 per cent) also the growth of only one isolate KaIII/9 was completely inhibited. The growth of *C.*

gloeosporioides causing inflorescence die-back of arecanut has been reported to be completely inhibited in the presence of Thiram (0.25 per cent), Phenyl mercury Urea (0.1 per cent) and Captan (0.25 per cent) (Saraswathy et al., 1975). There was no complete inhibition of growth of any of the isolates at any of the concentrations of Foltaf, Kitazin, Calixin and Daconil tried. Among these, Daconil gave the least inhibition of growth of all the three isolates in all the concentrations tested. Thus there was considerable variation in growth among the three isolates at different concentrations of the various fungicides tested.

Since Bavistin (0.025-0.1 per cent), Dithane M-45 (0.2 and 0.3 per cent) and Liquid Cuman (0.3 per cent) were found to completely inhibit the growth of all the three isolates, these fungicides were selected for *in vivo* studies. The results of the effect of Bavistin (0.05 per cent), Dithane M-45 (0.2 per cent) and Liquid Cuman (0.3 per cent) in controlling the foliar infection caused by the three isolates when applied as pre- and post-inoculation sprays are presented in Table 2.

In both pre- and post-inoculation sprays leaf blight and irregular spot symptoms caused by TNI/139 and KaIII/9 respectively were controlled effectively by Bavistin followed by Dithane M-45. Spraying cocoa trees with maneb 30 per cent WP, Zinc ion-maneb complex 80 per cent WP, thiram 80 per cent WP, Zineb, Trifuncit 75 per cent WP and perenox was reported to be effective in reducing the leaf blight incidence in Ghana (Dakwa and Danquah, 1978). But comparative studies on the effect of these fungicides in controlling the leaf blight incidence are not available. In the pre-inoculation spray shot hole symptoms caused by KeII/55 were controlled effectively by Dithane M-45 followed by Liquid Cuman and Bavistin. In post-inoculation spray also the control of shot hole symptom was significantly high in Dithane M-45 treated seedlings. It was followed by Liquid Cuman. The fungicidal trial on cocoa seedlings in Costa Rica revealed that Difoltan and Dithane M-45 were quite effective in controlling *Colletotrichum* leaf spot (Newhall and Diaz, 1967). Dithane Z-78, copper oxide, fermate and DNC were reported to be effective in controlling anthracnose on young cocoa plants caused by *C. theobromicolum* in Colombia and Dithane Z-78 was found to be superior to other fungicides tested (Mazorra, 1954). Dithane Z-78 has also been reported to be effective in controlling the in-

Table 2. Effect of fungicidal sprays on cocoa seedlings inoculated with the three selected isolates of C. gloeosporioides

Treatments	Disease index							
	Pre-inoculation spray				Post-inoculation spray			
	TNI/139	KeII/55	KaIII/9	Mean	TNI/139	KeII/55	KaIII/9	Mean
Bavistin WP (0.05%)	5.06	4.99	1.48	3.84	6.25	28.33	5.56	13.38
Dithane M-45 (0.2%)	7.50	2.50	4.45	4.81	10.42	12.22	12.22	11.62
Liquid cuman (0.3%)	13.75	4.44	8.52	8.90	34.58	18.88	27.77	27.08
Control	46.25	64.55	47.33	52.71	41.67	60.55	47.78	50.00
Mean	18.14	19.12	15.45	—	23.23	29.99	23.33	—
CD (P = 0.05) for isolates			2.01				3.46	
for treatments			2.32				3.99	
for isolates × treatments			4.02				6.92	

florescence die-back of arecanut (Saraswathy et al., 1975). But in the present investigation, in *in vitro* tests Dithane Z-78 at 0.3 per cent concentration (the highest concentration tested) showed complete inhibition of growth of only leaf blight isolate (TNI/139) and hence was not included for the *in vivo* evaluation. Bavistin was thus found to be superior in controlling leaf blight and irregular spot caused by TNI/139 and KaIII/9 respectively, whereas Dithane M-45 was most effective in controlling shot hole symptoms caused by KeII/55. The infection was controlled more effectively when fungicides were applied as pre-inoculation spray.

From both *in vitro* and *in vivo* studies, Bavistin and Dithane M-45 were found to be the promising fungicides to control *C. gloeosporioides* infection on cocoa seedlings. Therefore these fungicides may be used for large scale field trials to control *C. gloeosporioides* disease of cocoa.

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DISCUSSION

Q : Will it be economical to carry out fungicide application using costly fungicides in view of the prevailing low price for cocoa?

Ans: The economics of the treatment has not been worked out.

Q : Have you investigated the possibility of the three different isolates of *C. gleosporioides* being three different strains of the fungus?

Ans: No.