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Potential of Fungicides on Spore Germination and Mycelial Growth of Fruit Rot/Anthracnose Disease of Banana Caused by *Colletotrichum gloeosporioides*

KEYWORDS: Banana, Fruit rot, Fungicides *Colletotrichum gloeosporiodes*

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ABSTRACT *The efficacy of fungicides was tested in vitro employing poison food technique against C. gloeosporioides. Efficacy of five fungicides viz., Bavistin 50 WP, Kavach 75 WP, Indofil M-45, Blitox-50 and Azadiractin at concentration of 100, 150, 200, 250, 500 and 750 µg/ml each were evaluated to study their effect on spore germination of C. gloeosporioides. All the treatments were found effective in suppression of germination of C. gloeosporioides at variable concentrations. Among fungicides Bavistin-50 was found most effective and 100 per cent inhibition was obtained at 150 ppm whereas, Kavach 75 WP gave the similar results at 500 ppm whereas Azadirachtin was found least effective in suppression of spore germination. Five different fungicides viz., Bavistin 50 WP, Kavach 75 WP, Indofil M-45, Blitox-50 and Azadirachtin at 200, 250, 300, 500, 750, 1000, 1250 and 1500 µg/ml each were evaluated to study their effect on mycelial growth of C. gloeosporioides by poison food technique on PDA. Among five fungicides, Bavistin-50 WP was found to be the best even at 250 µg/ml concentration inhibiting the growth of C. gloeosporioides whereas Azadirachtin was found less effective for inhibiting the radial growth of C. gloeosporioides as compared to the other four fungicides.*

Introduction

The banana which cultivated, are namely *Musa acuminata, Musa balbisiana.* The genus Musa is in the family *Musaceae*. Banana considered "queen of tropical fruit" and one of the oldest fruit known to mankind. Banana is the second largest fruit crop, is an important staple food commodity around the world. Banana is consumed as a staple food, fresh fruit or for processing. It also serves as a boost to farm income for millions of people in the tropical region. It is an important source of high-calorie energy and contributes about a quarter of the energy requirement of almost 70 million people in the West and Central African sub-region. It is also the fourth most important commodity at global level next to rice, wheat and dairy products (Hays, 1966).

In Bihar, the production areas are broadly grouped in two zones. They are Vaishali and Koshi. About 40% areas lies in Vaishali belt (Zone I) and the rest 60 per cent area is in Koshi belt (Zone II), Production has been seriously decreased and threatened by diseases and pests and soil fertility problems. The diseases are a major constrains of banana production both in field and also at post harvest. Several postharvest diseases of banana had been reported worldwide but fruit rot /anthracnose diseases had been reported as being the most prominent (Chadha, 2001)

Many fungicides and their changed formulas became available to market for disease management or post-harvest disease management. But time to time, potential of fungicides testing are very important in context with particular disease. Because of this fact we are emphasis to testing of fungicides for fruit rot/anthracnose of banana. Therefore, this experiment objective is paramount importance.

Materials and methods

Isolation and purification of the pathogen

The infected characteristic symptom of fruit rot showing water soaked grayish brown depressed spots were thoroughly washed in several changes of tap water to remove dirts if any present and were cut into small bits of 2-3 mm dimension and surface sterilized by dipping in mercuric chloride solution (0.1 %) for 30 seconds. The bits were washed in several changes of distilled water and transferred aseptically on sterilized potato agar medium slants and incubated at 28 $\pm$ 2^0C. After 4 days of incubation the culture was transferred to sterilized Petri plate and incubated in same manner. A bits of hyphal growth was transferred aseptically in potato dextrose agar slant with the help of inoculating needle and was incubated at 28 $\pm$ 2^0C for 10 days to obtain spore of fungus. The micro- organisms were brought into pure culture by dilution plate technique and single spore isolation (Riker and Riker, 1936).

Spore suspension of the fungus was prepared in sterilized water and diluted to the extent that one loopful contained 15-20 spores. Twenty ml of sterilized melted agar (2 %) at 40-45^{0}C was poured in sterilized Petri-plate and inoculated with one loopful spore suspension. The Petri-plate was rotated gently for uniform spread of spores. On solidification the spore was located on the reverse of the plate and a single spore was located and marked by glass marking pencil. After 12 hours of incubation the germinating spore was aseptically transferred to the potato dextrose agar slants and incubated. The culture obtained from a single spore was pure and maintained on potato dextrose agar slants by periodic transfers.

Pathogenicity

For estimating the pathogenicity, mature green fruits were used. They were washed in tap water, wiped with cotton then surface sterilized with 95 per cent ethyl alcohol and subsequently subjected to pin prick injury with sterilized needle. The fruit were inoculated with spore suspension made from 7 days old culture grown on PDA. Such inoculated fruits were kept in humid chamber and regularly observed for expression of characteristic symptoms of the fungus the fungus was then re-isolated do confirm its identity.

Morphology of the pathogen

Slides were prepared from monospori cultures. They were examined under research microscope. Calibration of Ocular was done with the help of stage micro meter. This calibrated ocular was used for measurement of conidia conidiophores, setae, acervulus and mycelium using the same microscope. The measurements were based on average of ten observations.

Measurement of fungal dimension

The structures of fungus were observed under microscope

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and measured with the help of micrometers. The unit of measurement was μm (1μ = 10-6 m)

Micrometers
The scale contained 100 divisions in grade 10, 20, 30, 40, 50……. up to 100. The value of the division of the scale varied from microscope to microscope according to their tube length. Therefore, calibration of ocular micrometer was made to record the value of one division with the help of stage micrometer.

Stage micrometer
The stage micrometer consisted of 100 divisions which was equal to 1mm

100 division = 1 mm

1 division = 0.01 mm- 10 μ (1 mm = 1000μ)

Calibration
During calibration both micro meters were used at the same time on the different segments. Ocular was placed inside the eyepiece of 10x and stage micrometer was brought into focus under the objective and ocular division eyepiece was concided and calculation was made.

Eyepiece = 10 x

Objective = 45 x

Since 100 division of stage micrometer = 1 mm

Therefore, 1 division of stage micrometer = 0.01 mm = 10 μm (1mm=1000μm)

Since 16 division of ocular micrometer concided with 10 division of stage micrometer, 1 division of ocular micrometer will concided with 10/16 division of stage micrometer (i.e. 0.62 division of stage micrometer)

Since 1 division of stage micrometer = 10μm

Therefore, 0.071 division of stage micrometer = 0.621 x 10 =6.2μm.

The details of the fungicides evaluated were given below:

Sl. No.	Trade Name	Common Name	Chemical Name
1.	Bavistin 50 WP	Carbendazim	Methyl Benzimidazole-2-Carbamate
2.	Kavach 75 WP	Chlorothalonil	Tetrachloroiso-phthalonitrite
3.	Indofil M-45	Mancozeb	Zinc, Iron and Manganese ethylene bisdithio carbamate
4.	Blitox-50	Copper oxychloride	Copper oxychloride
5.	Azadirachtin	Azadirachtin	Azadirachtin

Effect on spore germination of *C. gloeosporioides*
Five different fungicides viz., Bavistin 50 WP, Kavach 75 WP, Indofil M-45, Blitox-50 and Azadirachtin were tested for their efficacy against spore germination of C. gloeosporioides at 6 different concentrations i.e. 100, 150, 200, 500 and 750 mg/ml. Fifty ml of stock solution of 50,000 μm/ml (5%) strength of these fungicides were prepared in sterilized distilled water in 100 ml Erlenmeyer flasks. To obtain desired concentration of the fungicide, the stock solution was diluted with sterilized distilled water using following formula:

$C_1V_1 = C_2V_2$

Where, C1 = Concentration of stock solution μg/ml

C_2 = Concentration of the fungicides μg/ml

V_1 = Volume (ml) of the stock solution to be added

V_2 = Measured volume (ml) of distilled water in which fungicides is to be added.

One drop of each concentration of fungicide was placed on glass slide in which spores from 7 days old culture of C. gloeosporioides were mixed. The slides were kept in moist chamber and incubated at 28 $\pm$ 2^{0}C Same experiment was also conducted with sterilized distilled water. After 24 hours spore germination percentage was recorded under microscope.

Effect of fungicides on mycelial growth of C. gloeosporioides
In vitro effect of different fungicides on radial growth of C. gloeosporioides was studied at different concentration viz., 250, 300, 500, 750, 1000, 1250 and 1500 μg/ml by using poison food technique as described by Zentmeyer (1955). Fifty ml of stock solutions of 50,000 μg/ml strength of these fungicides were prepared in sterilized distilled water in 100 ml Erlenmeyer flasks. To obtain desired concentration of fungicides in the medium, amount of stock solution to be added in PDA was calculated by using the formula $C_1V_2 = C_2V_2$, as described earlier. Required amount of stock solution of all fungicides were poured into 60 ml sterilized molten PDA separately and mixed thoroughly so as to get final concentration. PDA poisoned with different fungicides was poured into sterilized petri-plate @ 20 ml/plate. These plates were allowed to solidify and then centrally inoculated with 5 mm disc of C. gloeosporioides obtained from 7 days old culture PDA plates not amended with fungicide but inoculated with test fungus served as control. These plates were inoculated at 28 $\pm$ 2^{0}C for 7 days. Three replications for each treatment were maintained. Observation on colony diameter of the test fungus was recorded after 7 days of inoculation.

Result and discussion
Effect on spore germination of *C. gloeosporioides*
Efficacy of five fungicides viz., Bavistin 50 WP, Kavach 75 WP, Indofil M-45, Blitox-50 and Azadiractin at concentration of 100, 150, 200, 250, 500 and 750 μg/ml each were evaluated to study their effect on spore germination of *C. gloeosporioides*. The results are presented in Table 1.

It is evident from data in table that all the treatments were found effective in suppression of germination of C. gloeosporioides at variable concentrations. Among fungicides Bavistin-50 was found most effective and 100 per cent inhibition was obtained at 150 ppm whereas, Kavach 75 WP gave the similar results at 500 ppm. Indofil M-45 and Blitox-50 showed somewhat similar magnitude of inhibition. Azadirachtin was found least effective in suppression of spore germination.

Effect of different fungicides on mycelial growth of *C. gloeosporioides*
Five different fungicides viz., Bavistin 50 WP, Kavach 75 WP, Indofil M-45, Blitox-50 and Azadirachtin at 200, 250, 300, 500, 750, 1000, 1250 and 1500 μg/ml each were evaluated to study their effect on mycelial growth of C. gloeosporioides by poison food technique on PDA. The results are presented in Table 2

Data presented in Table 2 revealed that all the fungicides at various concentrations under test were found highly effective in inhibiting the radial growth of the fungus as compared to control. Among five fungicides, Bavistin-50 WP was found to be the best even at 250 μg/ml concentration inhibiting the growth of *C. gloeosporioides*, Kavach 75 WP, Indofil M-45 and Blitox-50 inhibited the growth completely at 1000, 1250 and 1500 μg/ml, respectively. Azadirachtin was found less effective for inhibiting the radial growth of *C. gloeosporioides* as compared to the other four fungicides.

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The present findings were supported by a number of workers. Spraying of infected orchards with 0.1 per cent Bavistin or 0.2 per cent Daconil at 15 days intervals has been found effective to control the fruit rot of papaya (Rawal *et al*., 1983).

Field spraying of prochloroz (0.15%) and chlorothalonil (0.2%) at 15 days intervals have been found to be quite effective in controlling anthracnose of banana caused by C. gloeosporioides (Rawal and Ullasa, 1989).

Banik et al. (1998) in vitro studies showed complete inhibition of C. gloeosporioides at 400 ppm with carbendazim followed by captan (450 ppm) thiophanate methyl (450 ppm), ziram (550 ppm) and chlorothalonil (550 ppm).

Hua-Young Gang and Hua (2001) studied the effect of 10 fungicides on the conidial germination, germ tube elongation, appressorium formation and hyphal growth of C. gloeosporioides. The best control was obtained with chlorothalonil, thiram and carbendazim.

Lakshmi et al. (2003) reported that among fungicide benomyl treatment showed the minimum weight lose (5.01%) followed by thiophanate-methyl and carbendazim. Fruits from benomyl showed the lowest post harvest spoilage percentage (5.93%)

Table 1: Effect of different fungicides on spore germination of *Colletotrichum gloeosporioides* causal agent of fruit rot of banana

Fungicides	* Per cent of inhibition						Mean
	Concentration (ppm)						
	100	150	200	250	500	750	
Bavistin 50 WP	89.9 (71.5)**	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	98.3 (86.9)
Kavach 75 WP	53.3 (46.9)	61.1 (51.4)	69.2 (56.3)	76.6 (61.1)	100.0 (90.0)	100.0 (90.0)	76.7 (65.9)
Indofil M-45	33.3 (35.2)	42.2 (40.5)	50.5 (45.0)	68.8 (56.1)	86.6 (68.6)	100.0 (90.0)	63.5 (55.9)
Blitox-50	24.4 (29.6)	37.7 (37.9)	46.6 (43.0)	66.6 (54.7)	83.3 (65.9)	93.3 (75.1)	58.7 (51.0)
Azadirachtin	23.6 (29.0)	34.4 (51.9)	43.3 (41.1)	62.2 (52.0)	78.8 (62.6)	88.8 (70.5)	55.2 (51.2)
Mean	44.9 (42.4)	55.1 (54.3)	61.8 (55.1)	74.8 (62.8)	89.7 (75.4)	96.4 (83.1)	
	S. Em. ±	CD at 5%					
Treatment (fungicides) (A)	0.3	0.9					
Concentration (B)	0.3	0.9					
Treatment (A) x Concentration (B)	0.7	2.2					

*** Each value is an average of three replication**
**** Value given in parenthesis are after angular transformation control was 95.4 mm**

Table 2: Effect of different fungicides on mycelial growth of *Colletotrichum gloeosporioides* causal agent of fruit rot of banana

Fungicides	* Per cent of inhibition								Mean
	Concentration (ppm)								
	200	250	300	500	700	1000	1250	1500	
Bavistin 50 WP	90.6 (72.2)**	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	98.8 (87.7)
Kavach 75 WP	60.2 (50.6)	64.1 (53.2)	66.8 (54.8)	79.8 (54.6)	90.7 (72.3)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	82.7 (69.5)
Indofil M-45	44.4 (41.7)	46.0 (42.7)	46.3 (42.9)	66.0 (54.3)	77.6 (61.7)	88.8 (70.5)	100.0 (90.0)	100.0 (90.0)	71.1 (61.7)
Blitox-50	32.5 (34.7)	43.2 (41.12)	51.3 (45.7)	71.0 (57.4)	75.6 (60.4)	86.1 (68.1)	92.7 (74.3)	100.0 (90.0)	69.0 (59.0)
Azadirachtin	25.2 (30.1)	34.8 (36.1)	41.4 (40.0)	62.2 (52.0)	69.1 (56.2)	77.3 (61.5)	83.7 (66.2)	87.2 (69.1)	60.1 (51.4)
Mean	50.6 (45.9)	57.6 (52.6)	61.2 (54.7)	75.8 (61.7)	82.6 (68.1)	90.4 (76.0)	95.3 (82.1)	97.4 (85.8)	
	S. Em. ±	CD at 5%							
Treatment (fungicides) (A)	0.1	0.5							
Concentration (B)	0.2	0.6							
Treatment (A) x Concentration (B)	0.5	1.5							

*** Each value is an average of three replication**
**** Value given in parenthesis are after angular transformation control was 90.68 mm**

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