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Evaluation of fungicides, botanicals and biocontrol agents against banana antharcnose disease under *in vitro* condition

■ V.N. RAMANI, D.K. DAVARA, V.P. ANADANI* AND A.M. DETROJA

Department of Plant Pathology, Junagadh Agricultural University, JUNAGADH (GUJARAT) INDIA

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***Corresponding author:**
Email: dfrsvlb@jau.in

ABSTRACT

Banana (*Musa paradisiaca* L.) is the most popular tropical fruit crops grown in India. It is affected by several post-harvest diseases among them anthracnose (*Colletotrichum gloeosporioides* (P. Henn), caused huge loss in quality and quantity of fruits. Therefore affords were made to screen the different systemic, contact and combination of six fungicides, botaniclas and biocontrol agents *in vitro* condition against test fungus. Among systemic fungicides, carbendazim 50 per cent WP at 50 ppm, contact fungicides copper oxychloride 50 per cent WP at 500 ppm and combination of fungicides carbendazim 12 per cent WP + mancozeb 63 per cent WP at 100 ppm concentrations were found the most effective and gave cent per cent growth inhibition of *C. gloeosporioides*. Lantana leaves extract at 10 per cent solvent was also effective and resulted 66.41 per cent growth inhibition of *C. gloeosporioides*. Among the biocontrol agents, *Trichoderma harzianum* gave 62.43 per cent growth inhibition of *C. gloeosporioides* (62.43%).

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INTRODUCTION

Banana (*Musa paradisiaca* L.) is the most popular tropical fruit crop grown in India. It is a large herbaceous, perennial, monocotyledonous and monocarpic fruit crop. Banana belongs to family *Musaceae* in order Scitamineae. Banana is known as 'Apple of Paradise', origin in the tropical region of South–East Asia from *Musa accuminata* and *Musa balbisiana.* India is considered to be one of the centers of origin of banana *Musa balbisiana.* It can be grown round the year and it is widely adopted in India. A part from this it is considered as potential 'Dollar Earning Crop'. It is known since the dawn of ancient history as one of the delicious fruit in the world. Geographical, climatic and genetic studies of bananas indicate that all edible bananas are indigenous to the warm moist regions of the tropical and subtropical over a wide range from 20'N to 20'S of the equator (Shanmugavelu *et al.*, 1992). Major banana producing countries are India, China, Philippines, Brazil, Ecuador, Indonesia, Costa Rica, Mexico, Thailand and Colombia. It is cultivated on an area of 48,01,991 ha. With an average production of 9,99,96,519 MT in the world, India

produced 29 per cent of total banana production of the world during 2011 (Anonymous, 2011). Among the fruits, banana holds first position in production and productivity in India. In India, annual production of banana is 297.80 lacs MT from an area of 8.30 lacs ha spread all over the country (Anonymous, 2011). Banana covers 13 per cent of the total area under fruits, contributing 39.8 per cent of total fruit production in the country. In Gujarat state, areas under total fruit crops are 3,49,900 ha with production 72.45 lacs MT out of this, the banana crop occupies 64,700 ha area with annual production of 39,78,000 MT (Anonymous, 2011). In India, Tamil Nadu, Maharashtra, Kerala, Gujarat and Karnataka are the leading banana producing states. The highest productivity of banana has been reported to be (65.8 MT/ha) in Tamil Nadu followed by Gujarat (61.5 MT/ha) in the year 2010-11 (Anonymous, 2011). In Gujarat, the banana is grown in Anand, Bharuch, Junagadh, Kheda, Narmada, Navsari, Surat and Valsad districts. The ripe fruits are edible, delicious and very nutritious. The content of carbohydrates is very high with a calorific value of 67-137 mg/100 g fruit. It is good source of vitamin A (190 IU/100 g of edible portion) and vitamin C (100 mg/100 g pulp) and fair source of vitamin B and B_2.

Banana fruits are highly perishable in nature with estimated 25-30 per cent post harvest losses and it is very difficult to store for longer period, therefore, it needs immediate marketing and utilization. Cultivated banana is susceptible to many diseases, mostly fungal pathogen which attacks various part of the plant from root to fruit. Banana fruit suffers from many serious diseases such as anthracnose (*Colletotrichum* sp.), crown rot (*Fusarium* sp.), finger rot (*Botryodiplodia theobromae*) and cigar–end rot (*Verticillium theobromae*) disease. The current postharvest problems for bananas are mainly concerned with storage and marketing. Apparently, strategic, basic, applied and adapted researches on post-harvest diseases of banana fruits have not received the required attention. The fungus *Colletotrichum* has been the most notorious fungal pathogen, which causes severe rots deteriorating the fruit quality rapidly and rendering the fruit completely to a rotten with sticky mass tickling from the infected pulpy banana. The anthracnose disease is geographically widespread but is most common in the tropics and subtropics regions (Punithalingam, 1980). Therefore the study to be initiated to focus on understands the process of post harvest diseases and management of anthracnose disease.

MATERIAL AND METHODS

The diseased fruit sample of banana variety **'**Grand Nain' showing typical circular to angular, light to dark brown spots with a dark red or blackish margin on the fruit spots collected from fruit market, Junagadh and Lal Baugh, Junagadh Agricultural University, Junagadh during the year 2012-13. The infected fruit were brought in to the laboratory, placed in blotting papers under pressure with herbarium press and preserved for further investigations. The fresh infected fruits were subjected to microscopic examination and tissue isolation for the causal agent. Three to four surface-sterilized diseased fruit bits of 3 to 5 mm size were aseptically transferred to Potato Dextrose Agar (PDA) medium and plates incubated at room temperature (28 ± 2^0C) for seven days.

In vitro evaluaton of various fungicides to check the colony growth of the fungus *Colletotrichum gloeosporioides* were done through poisoned food technique described by Sinclair and Dhingra (1985) on PDA edium. The experiment conducted in Completely Rendomized Design (CRD) with tree replication of each treatment. Six systemic fungicides namely: Triadimefon 25 per cent WP, Propiconazole 25 per cent EC, Carbendazim 50 per cent WP, Hexaconazole 5 per cent SC, Thiophanate methyl 70 per cent WP, Benomyl 50 per cent WP were tested each at four concentration 50, 100, 250 and 500 ppm and five non-systemic fungicides *viz.*, Copper oxychloride 50 per cent WP, Thiram 75 per cent WP, Mancozeb 75 per cent WP, Chlorothalonil 75 per cent WP, Zineb 75 per cent WP were tested each at 4 concentration 500, 100, 1500 and 2000 ppm and Five combination of fungicides Cymoxanil 8 per cent WP + Mancozeb 64 per cent WP, Carbendazim 12 per cent WP + Mancozeb 63 per cent WP, Azoxystrobin 18.2 per cent WP + Difenoconazole 11.4 per cent WP, Pyraclostrobin 13.3 per cent WP + Epoxiconazole 5 per cent WP, Zineb 60 per cent WP + Hexaconazole 4 per cent WP were tested each at 100, 250,500 and 1000 ppm. After autoclaving, 25 ml PDA medium amended with fungicides in four different concentrations in separate 100 ml flasks was poured in sterilized 90 mm Petri dish. The PDA medium without fungicides was kept as control. 5 mm fungal disc of *Colletotrichum gloeosporioides* was picked from purified culture with the help of a sterilized cork borer and inoculated in the

center of each Petri dishs. Three replicate plates were inoculated for each fungicidal concentration. The inoculated plates were inoculated at 28 ± 2ºC and mean colony diameter was measured after one week of inoculation. The per cent inhibition in growth due to various fungicidal treatments at different concentrations was computed as follow:

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

where,

PGI = Per cent growth inhibition index

C = Area of test fungus in control (mm^2)

T = Area of test fungus in respective treatment (mm^2).

***In vitro* evaluation of plant extract :**

The antifungal properties of various plant species, botanicals were evaluated against *C. gloeosporioides in vitro*, applying poisoned food technique (Nene and Thapliyal, 1993). A total of 9 different plant species (Lantana Jatropha, Turmeric, Onion, Ardusi, Neem, Garlic, Ginger and Tulsi) free from any disease were selected on the basis of their antifungal properties reported earlier and easy availability round the year in the yield. Fresh and healthy leaves of selected plant species were collected from the field and campus of Junagadh Agricultural University, Junagadh and brought to the laboratory for further studies. The leaves were first washed under running tap water to remove dirt material adhering to the surface. One hundred gram (100 ml) in a mixture grinder. After through grinding, the extract was first filtered through muslin cloth and then through whatman filter paper number 1. Later, the extract was passed through zeitz filter to free them from bacterial contamination. The extract was then used as standard plant extract solution of 100 per cent concentration or 1:1 ratio.

The inhibitory effects of aqueous extract of the botanicals were assessed against the pathogens by poisoned food technique. 2, 5, 10 ml of plant extract was mixed in 98, 95, 90 ml of PDA medium, to prepare 2, 5 and 10 per cent of plant extract containing medium.

A mycelial disc of fungus, 5 mm in diameter was cut from periphery of 7 to 10 days old culture of fungus and aseptically inoculated into the medium; each set was replicated three times. The control was run side by using only sterilized PDA medium. The control was run side by side using only sterilized PDA medium. The Petri dishs were inoculated at 28 ± 2ºC temperature in BOD incubator and observation on mycelia growth were recorded and per cent growth inhibition over control was calculated as per formula of Vincent (1927).

Evaluation of bioagents agianst *C. gloeosporioides in vitro* :

The antagonistic potential of bioagents was assessed against *C. gloeosporioides* by dual culture technique on PDA medium as per procedure describes by (Martyn and Stack, 1990). Six antagonist's *viz.*, *Trichoderma harzianum*, *T. hamatum*, *T. viride*, *T. virens*, *Pseudomonas fluorescens* and *Bacillus subtilis* were collected from Department of Plant Pathology, JAU, Junagadh, Gujarat, India. For this, 20 ml of strilized PDA was poured in each Petri dish and allowed to solidify. A 5 mm disc of the respective bioagent was used. Control without inoculation of the bioagent was maintained, simultaneously. Observation regarding colony diameter of *C. gloeosporioides* and bioagent was recorded 10 days after inoculation by incubating at 28 ± 2ºC temperature.

RESULTS AND DISCUSSION

The growth inhibition of *C. gloeosporioides* causing anthracnose in banana has been tested at various concentration of systemic, non-systemic, combination of fungicides, plantextracts and bioagents *in vitro* recoded in Table 1, 4. The perusal of results showed that (Table 1) all the systemic fungicides were effective and more than 56.28 per cent inhibition growth of test fungus at 50 ppm concentration as compared to control. Carbendazim gave cent per cent growth inhibition at all concentration. This results is agreement with finding of Patel (2004), they found good mycelial growth inhibition of *C. gloeosporioides* of chilli at all its concentrations *i.e.*, 250, 500 and 1000 ppm by carbendazim. Patel and Joshi (2001), observed that carbendazim at 500 and 1000 ppm observed cent per cent inhibition of *C. gloeosporioides* causing leaf spot of turmeric under *in vitro* condition.

Similarly, non systemic fungicides copper oxychloride completely inhibited the mycelial growth inhibition at all concentrations over control and found significantly superior over rest of the treatments (Table 2). The next best fungicide was zineb, which exhibited

mean inhibition of 86.94 per cent within the treatments. These results are in accordance to the findings of Hegde *et al*. (1991), Thakare and Patil (1995) and Kumar and Yadav (2007) founded that copper oxychloride suppressed the growth of *C. gloeosporioides*, incidence of areca nut, leaf blight of chrysanthemum and betel vine, respectively.

The results of combination of fungicides presented in (Table 3) revealed that carbendazim 12 per cent WP + mancozeb 64 per cent WP were proved most effective and gave cent per cent growth inhibition at all concentration. Effectiveness of Carbendazim 12 per cent

Table 1 : Growth inhibition of *C. gloeosporioides* at different concentrations of various systemic fungicides after seven days of incubation at 28 ± 2ºC

Fungicide	Concentration (ppm)/per cent inhibition*				Mean	Toxicity Index#
	50	100	250	500		
Triadimefon	56.28	61.11	72.39	83.86	68.41	273.64
Propiconazole	70.25	77.22	88.26	100	83.93	335.73
Carbendazim	100	100	100	100	100	400
Hexaconazole	80.69	84.41	100	100	91.28	365.10
Thiophanate methyl	68.89	76.52	83.11	85.39	78.47	313.91
Benomyl	74.28	76.63	83.28	86.88	80.27	321.07
Control	0.00	0.00	0.00	0.00	0.00	0.00
	Fungicide (F)		Concentration (C)			F x C
S.E.±	0.26		0.22			0.54
C.D. (P=0.05)	0.76		0.62			1.51

* Mean of four replications # Maximum toxicity index = 400.00

Table 2 : Growth inhibition of *C. gloeosporioides* at different concentrations of various non-systemic fungicides after seven days of incubation at 28 ± 2^{0}C

Fungicide	Concentration (ppm)/per cent inhibition*				Mean	Toxicity Index#
	500	1000	1500	2000		
Chlorothalonil	60.39	66.11	71.82	74.13	68.11	272.45
Mancozeb	61.31	71.50	75.27	86.44	75.63	294.52
Zineb	82.83	84.80	88.80	91.33	86.94	347.76
Copper oxychloride	100	100	100	100	100	400
Thiram	52.36	54.30	56.30	60.75	55.93	223.70
Control	0.00	0.00	0.00	0.00	0.00	0.00
	Fungicide (F)		Concentration (C)			F x C
S.E..±	0.39		0.35			0.78
C.D. (P=0.05)	1.09		0.98			2.19

* Mean of four replications # Maximum toxicity index = 400.00

Table 3 : Growth inhibition of *C. gloeosporioides* at different concentrations of various combinations of fungicides after seven days of incubation at 28 ± 2^{0}C

Fungicide	Concentration (ppm)/per cent inhibition*				Mean	Toxicity Index#
	100	250	500	1000		
Cymoxanil + Mancozeb	46.06	52.52	62.33	73.80	58.68	234.71
Carbendazim + Mancozeb	100	100	100	100	100	400
Pyraclostrobin + Epoxiconazole	75.62	84.91	93.16	100	88.42	353.69
Zineb + Hexaconazole	72.44	79.08	88.03	91.16	82.68	330.71
Azoxystrobin + Difenoconazole	81.76	83.64	100	100	91.35	365.40
	Fungicide (F)		Concentration (C)			F x C
S.E.±	0.22		0.19			0.44
C.D. (P=0.05)	0.62		0.55			1.24

* Mean of four replications # Maximum toxicity index = 400.00

Table 4 : Growth inhibition of *C. gloeosporioides* at different concentrations of various phytoextracts after seven days of incubation at 28 ± 2°C

Phytoextract	Concentration (%)/per cent inhibition*			Mean	Toxicity Index#
	2	5	10		
Lantana	57.44	60.50	66.41	61.45	184.35
Jatropha	26.39	65.14	66.17	52.57	157.70
Turmeric	18.25	63.47	65.17	48.96	146.89
Onion	16.26	32.33	64.25	37.61	112.84
Ardusi	16.22	44.34	53.72	38.09	114.28
Neem	16.02	35.55	53.71	35.09	105.28
Garlic	15.33	16.66	24.45	18.81	56.44
Ginger	14.42	17.19	20.15	17.25	51.76
Tulsi	14.30	16.54	16.65	15.83	47.49
Control	0.00	0.00	0.00	0.00	0.00
	Phytoextract (P)		Concentration (C)		P x C
S.E.±	0.28		0.16		0.49
C.D. (P=0.05)	0.80		0.46		1.38

* Mean of four replications # Maximum toxicity index = 300.00

Table 5 : Growth inhibition of *C. gloeosporioides* by different biocontrol agents after seven days of incubation at 28 ± 2°C

Biocontrol agent	* Growth reduction (%)
Trichoderma harzianum	62.43
T. hamatum	52.02
T. viride	60.28
T. virens	55.94
Pseudomonas fluorescens	42.53
Bacillus subtilis	39.94
Control	0.00
S.E.±	0.54
C.D. (P=0.05)	1.61

* Average of four replications

WP + Mancozeb 64 per cent WP against *C. gloeosporioides* have been reported by Ekbote *et al*. (1997) and Prashanth *et al*. (2008).

The antifungal properties of nine phytoextrects were evaluated *in vitro* against *C. gloeosporioides*. Results revealed that all nine leaf extract tested at 2, 5 and 10 per cent concentrations were found to be significantly effective in checking the mycelial growth of *C. gloeosporioides* as compare the control.

Table 4 revealed that maximum per cent growth inhibition (PGI) was obtained in Lantana leaf extract (61.45%) which followed by Jatropha leaves extract (52.57%) and turmeric rhizomes extract (48.96%). The findings of present investigation were in favour of work done by Patel and Joshi (2001) and Win *et al.* (2007).

Six antagonists were studied for their antagonism against *C. gloeosporioides* by dual culture method. All the antagonists significantly helped in inhibiting the mycelial growth of *C. gloeosporioides* over control (Table 5). The highest mycelial growth inhibition was observed in *T. harzianum* (62.43%) followed by *T. viride* (60.28%), *T. virens* (55.94%) and *T. hamatum* (52.02%) after 7 days of incubation. The bacterial antagonist *viz.*, *Pseudomonas fluorescens* and *Bacillus subtilis* were observed moderate mycelial growth inhibition (42.53 and 39.94%), respectively. These findings are in conformity of earlier findings of Bhuvaneshwari and Rao (2001) and Patel (2004).

Conclusion:

This is helpful to the farmer to manage banana anthracnose disease and avoid fungal resistance against

fungicides.

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