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STUDIES ON MORPHOLOGY OF MANGO ANTHRACNOSE DISEASE CAUSING FUNGUS *COLLETOTRICHUM GLOEOSPORIOIDES* (PENZ.) PENZ. AND SACC. AND EFFICACY OF AZOXYSTROBIN AGAINST THE FUNGUS UNDER *IN VITRO* AND *IN VIVO* CONDITION

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ABSTRACT
Anthracnose disease caused by *Colletotrichum gloeosporioides* is one of the most serious diseases causing yield losses in mango. For controlling this disease much of attention and efforts has concentrated on the application of fungicides. The indiscriminate use of different fungicides possesses potential threat to human health, phytotoxicity, environmental hazards and development of pathogen resistance. Keeping this in view, the efficacy of azoxystrobin, one of the strobilurin class fungicides, which has broad-spectrum activity against large number of plant diseases, was evaluated both under *in vitro* and *in vivo* conditions. In *in vitro* tests, azoxystrobin significantly reduced both mycelial growth and conidial germination of *C. gloeosporioides* in PDA media. Although the mycelial growth and conidial germination of the fungus was declined continuously in 100, 200, 300 and 400ppm, the optimum rate was obtained at 100ppm where it responded continuously to climb with increasing rates. In field experiment, azoxystrobin treated trees showed lesser leaf anthracnose than control. The reduction of anthracnose intensity and yield increased curve obtain, shows flattening between the range 100 and 400ppm concentrations, hence the optimum rate of azoxystrobin was fixed to be at 100ppm for the control of anthracnose disease.

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the world's most important and esteemed fruits and described by some as the "king of all fruits". India is the world's largest producer (52%) in global mango production. Because of diverse production conditions and the vast area grown, mango suffers from a number of diseases, some of them taking heavy toll on the crop and limiting production and productivity. Among the diseases, mango anthracnose caused by *Colletotrichum gloeosporioides* is the most serious disease (Ploetz, 1999). Initial infection starts from leaves and spreads to reproductive structure (flower) causing blossom blight, which destroys inflorescence leading to considerable reduction in fruit yield. Much attention and efforts on anthracnose control has concentrated mostly on the use of fungicides. The indiscriminate use of different fungicides causes potential threat to human health, increase in pathogen resistance, mutation and causes environmental hazards. Using of organic sulphur (Dithio-carbamates) fungicides like zineb, maneb and heterocyclic nitrogen compounds like captan gave adequate control against anthracnose. However these fungicides have shown phytotoxic effect to flowers (McMillan, 1972). The fungus developed resistance to benomyl (0.1%), a systemic fungicide used for controlling of anthracnose disease of mango (Akthar *et al.,* 1998; Dodd *et al.,* 1991). Assessment of these reports, focus on the evaluation of new fungicides for controlling mango anthracnose and increasing mango production.

Strobilurins are the leading systemic fungicide, developed from naturally occurring antifungal compounds found in wood-decaying mushroom fungus like *Oudemansiella mucida and Strobilurus tenacellus*. It has broad-spectrum activity against large number of plant diseases such as leaf spot (*Cercospora beticola*), black spot (*Guignardia citricarpa*) of citrus, gray mold (*Botrytis cinerea*) of fruits and vegetables, powdery mildew (*Erysiphe betae*) of sugar beet, post-harvest rot (*Colletotrichum gloeosporioides*) of avocado (Slawecki *et al*., 2002; Anesiadis *et al*., 2003; Miles *et al.,* 2004)

Hence the present study was carried out to evaluate the efficacy of azoxystrobin on mycelial growth and conidial germination of *C. gloeosporioides* under *in vitro* condition and in controlling, mango leaf anthracnose disease under *in vivo* condition. Its effect on the fruit yield was also evaluated.

MATERIALS AND METHODS

The pathogen *C. gloeosporioides* was isolated from infected mango leaves. Isolation was made by cutting a small section of anthracnose infected portion along with healthy areas, which was surface sterilized with 1% NaOCl solution (Iqbal *et al.,* 2010), and rinsed in sterilized distilled water. It was then placed into the sterile petri plates containing solidified potato dextrose agar (PDA) medium, and incubated at 28 ± 2^{o}C. The pure culture was maintained in PDA slants.

Morphological studies

A small amount of culture of *Colletotrichum gloeosporioides* obtained from twelve days old culture was placed on the slide and teased thoroughly with lactophenol to obtain uniform spread. A cover slip was placed over it. Length and breadth of 100 conidia, acervuli and setae were measured under compound microscope and the average sizes were calculated with the help of ocular and stage micrometer. Conidia, acervuli and setae produced in the infected leaves were also measured in same manner.

Studies on *in vitro* efficacy of azoxystrobin in solid media

In vitro efficacy of azoxystrobin against radial growth of *C. gloeosporioides* was tested by poisoned food technique (Nene and Thapliyal, 1993). Mycelial discs of half centimetre diameter were cut using cork borer from the fungal colony. The discs were transferred at the centre of the petri plates containing PDA media amended with azoxystrobin at 25, 50, 100, 200, 300 and 400 ppm concentrations. A control was maintained without fungicide. Three replications were maintained for each treatment. These plates were incubated at 28 ± 2ºC for ten days. After incubation the length and breadth of circle to ellipse shaped growth of *C. gloeosporioides* on poisoned medium were measured and total area covered by mycelial growth was calculated by ellipse area formula which was also used by Anco *et al*. (2009) and Miclea and Puia (2012):

$$A = (l \times b \times \frac{\pi}{4}) - a$$

Where,

A = Area of radial growth (cm^2)

l = Length of radial growth (cm)

b = Breadth of radial growth (cm)

a = Area of the mycelial disc of half centimetre diameter which was transferred in media = 0.20 cm^2.

Per cent reduction in radial growth (area) over control of each treatment was calculated by using the following formula suggested by Vincent (1947).

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

Where,

I = Per cent growth inhibition (area) over control (%)

C = Area of radial growth (cm^2) in control

T = Area of radial growth (cm^2) in treatment.

Few discs of filter paper of half centimetre diameter were cut and soaked into sterile distilled water for few seconds with the help of forceps. Wet disc were then swept into colony of sporulated *C. gloeosporioides* and placed into sterile petri plates containing solidified water agar media. After sometimes the discs were pulled out of the media. Petri plates were then incubated at 28 ± 2ºC. After 24h, those were observed under microscope and germinated conidia as well as total number of conidia were counted. Per cent conidial germination was calculated for each treatment with three replications. Per cent inhibition of conidial germination over control was also calculated by the same formula suggested by Vincent (1947) with the data of per cent conidial germination in control and in treatment.

Experiment design and treatments in field condition

Field experiment was conducted at Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India in a mango orchard with 10-15 years old trees during 2011 and 2012. 21 trees with uniform flowering were chosen and treatments were assigned (each with three replications) employing completely randomized block design. Efficacy of azoxystrobin was tested with six different doses *viz*. 25, 50, 100, 200, 300 and 400 ppm of the fungicide.

Disease and yield assessment

Disease severity assessments were made regularly at 15 days intervals starting from first appearance of disease. The assessment carried out using the 0 – 9 scale given by Jamadar and Desai (1997):

Rating	Description
0	No infection observed
1	1 – 10%
3	10.1 – 15.0%
5	15.1 – 25.0%
7	25.1 – 50.0%
9	More than 50%

The percent disease intensity (PDI) was calculated using the formula developed by McKinney (1923)

$$PDI = \frac{\text{Sum of all Numerical ratings}}{\text{Total no. of leaves observed} \times \text{Maximum Ratings}} \times 100$$

Disease reduction over control was also calculated. Mango fruit yield was calculated as kg /tree.

RESULTS AND DISCUSSION

Studies on morphological characteristics of *Colletotrichum gloeosporioides*

The morphology of the fungus both on infected host tissue and PDA culture media depicted in Table 1. Microscopic examination of infected tissue revealed that acervuli were saucer shaped, measuring 141-381× 33-90 μ with an average of 170 × 42 μ. The acervulus was covered with a mucilaginous mass and it contains numerous conidia measuring 8-20 ×4-7 μ. Dark brown to black setae were arising through this mass; they were erect in habit, measuring 32-76 × 1-4 μ. Conidia were hyaline, single celled and smooth walled. Similar type of trend was also found in the results observed by Palo (1932) and Sattar and Malik (1939).

The fungal colony from 10 days old culture on PDA media was white with smooth margins. The mycelium was hyaline, superficial, septate and branched. The aerial mycelium was white. Sporulation was abundant with maximum fruiting bodies at the centre of the plate and profuse mycelium growth was found towards the periphery. The acervuli and conidia

Table 1: Morphological characteristics of *C. gloeosporioides*

Characters	On host		On PDA media	
	Range (μ)	Average (μ)	Range (μ)	Average (μ)
Acervuli	141-381 × 33-90	170 × 42	130-252 × 28-76	158 × 37
Setae	32-76 × 1-4	45 × 2	-	-
Conidia	8-20 × 4-7	13 × 5	7-11 × 2-5	9 × 3

from the culture measured 130-252 × 28-76μ and 7-11 × 2-5μ respectively. More or less same trend was also observed by Sutton (1992) in case of *C. gloeosporioides* on PDA media.

In vitro* efficacy of Azoxystrobin against *C. gloeosporioides

The efficacy of azoxystrobin against mango anthracnose pathogen *C. gloeosporioides* was tested under *in vitro* conditions. Azoxystrobin significantly reduced both mycelial growth and conidial germination on PDA media. Azoxystrobin at 25, 50 and 100 ppm slightly inhibited the mycelial growth and conidial germination whereas 200 and above ppm completely inhibited the mycelial growth and conidial germination of *C. gloeosporioides*. In control, 63.82 cm^2 surface areas were covered with mycelial growth and 94.38% conidia were germinated (Table 2).

Similar type of trends in mycelial growth inhibition over control was also observed by Sundravadana *et al*. (2006) where five concentrations of azoxystrobin was studied against the pathogen.

This experiment was useful to determine the optimum rate of azoxystrobin for inhibition of *C. gloeosporioides* under *in vitro* condition. The response of azoxystrobin at different concentrations against mycelial growth and conidial germination of *C. gloeosporioides* was illustrated graphically (Fig. 1). Although the mycelial growth was declined continuously, in 100, 200, 300 and 400ppm, the optimum rate was arrived at 100ppm by considering the flattening of the curve between concentrations. Similarly optimum inhibition of conidial germination was also found at 100 ppm where it responded continuously to climb with increasing rates (Fig. 1).

***In vivo* efficacy of Azoxystrobin against mango anthracnose disease**

Bioefficacy of azoxystrobin against mango anthracnose was tested under field conditions. Leaf anthracnose was first observed at flowering stage just before the spraying and after spraying, observations were taken upto final harvest at 15 days intervals. Azoxystrobin treated trees showed lesser leaf anthracnose than control. The reduction of leaf anthracnose varied between the doses of azoxystrobin. At the time of final observation (*i.e.* 120 days after spraying) azoxystrobin at 25 and 50ppm concentrations slightly reduced the leaf anthracnose *i.e.* 50.60% and 54.91% disease reduction over control respectively whereas other high doses 100, 200, 300 and 400 ppm significantly suppressed the leaf anthracnose

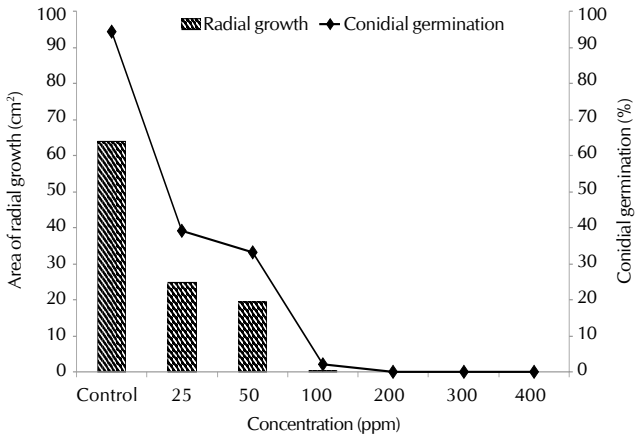

Figure 1: *In vivo* efficacy of azoxystrobin at different concentrations on per cent disease index of anthracnose and mango yield

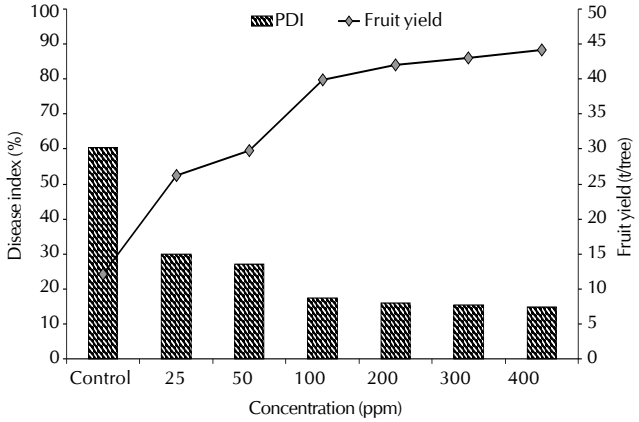

Figure 2: *In vivo* efficacy of azoxystrobin at different concentrations on per cent disease index of anthracnose and mango yield

i.e. 71.26%, 73.45%, 74.64% and 75.29% disease reduction over control respectively. So, the efficacy of azoxystrobin increased with increase in the concentrations (Table 3). Due to higher leaf anthracnose, controlled trees produced less fruit yield (12.08 kg/tree), whereas azoxystrobin treated trees produced maximum yield (44.10 kg/tree from 400 ppm treated trees) which was found to be statistically significant (Table 3).

Similar type of trends in both disease intensity and fruit yield was also observed by Sundravadana *et al*. (2007) where five

Table 2: Mycelial growth and conidial germination of *C. gloeosporioides* in different concentrations of Azoxystrobin treated PDA media

Concentration (ppm)	Area of radial growth(cm^2)	Per cent growth inhibition (area) over control(%)	Per cent conidial germination(%)	Per cent inhibition of conidial germination over control(%)
25	24.77* ^{b}(30.18)**	61.18	39.25^{b}(39.08)	58.41
50	19.46^{c}(26.53)	69.51	33.16^{c}(35.46)	64.87
100	0.20^{d}(4.76)	99.69	2.05^{d}(9.19)	97.83
200	0.00^{d}(4.05)	100.00	0.00^{e}(4.05)	100.00
300	0.00^{d}(4.05)	100.00	0.00^{e}(4.05)	100.00
400	0.00^{d}(4.05)	100.00	0.00^{e}(4.05)	100.00
Control	63.82^{a}(53.33)	-	94.38^{a}(76.94)	-
SEm(±)	0.59		0.27	
CD (5%)	0.20		0.09	

* Values are mean of four replications; ** Values in parentheses are arcsine-transformed values

In a column, means followed by a common letter are not significantly different at the 5 % level by DMRT.

Table 3: Per cent disease index of anthracnose disease and yield in different concentrations of azoxystrobin treated mango orchard

Concentration (ppm)	Per cent Disease Index (PDI) (%)								Disease reduction over control (%)	Fruit Yield (kg/tree)
	Before spray	15 DAS[1]	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	105 DAS	120 DAS		
25	4.20* ^{a}(12.52)**	6.73^{b}(15.60)	9.61^{b}(18.54)	12.98^{b}(21.54)	19.35^{b}(26.46)	22.49^{b}(28.65)	25.95^{b}(30.95)	28.26^{b}(32.43)	29.79^{b}(33.39)	50.60	26.16^{f}
50	4.26^{a}(12.60)	6.48^{b}(15.32)	9.03^{c}(17.98)	12.13^{c}(20.82)	17.96^{c}(25.45)	20.78^{c}(27.47)	23.94^{c}(29.63)	25.95^{c}(30.95)	27.19^{c}(31.75)	54.91	29.80^{e}
100	4.13^{a}(12.43)	5.40^{c}(14.06)	6.85^{d}(15.73)	8.34^{d}(17.30)	10.86^{d}(19.70)	12.45^{d}(21.09)	14.11^{d}(22.47)	14.73^{d}(22.97)	17.33^{d}(24.98)	71.26	39.88^{d}
200	4.18^{a}(12.49)	5.31^{c}(13.95)	6.60^{de}(15.45)	7.93^{de}(16.88)	10.08^{e}(18.98)	11.58^{e}(20.34)	13.09^{e}(21.63)	13.56^{e}(22.02)	16.01^{e}(23.97)	73.45	41.96^{c}
300	4.15^{a}(12.45)	5.21^{c}(13.82)	6.40^{de}(15.23)	7.64^{e}(16.58)	9.66^{ef}(18.59)	11.08^{f}(19.89)	12.51^{f}(21.14)	13.91^{e}(22.31)	15.29^{ef}(23.41)	74.64	43.08^{b}
400	4.10^{a}(12.38)	5.14^{c}(13.74)	6.29^{e}(15.10)	7.49^{e}(16.42)	9.44^{f}(18.38)	10.80^{f}(19.64)	12.19^{f}(20.87)	13.56^{e}(22.02)	14.90^{f}(23.11)	75.29	44.10^{a}
Control	4.39^{a}(12.78)	11.25^{a}(20.05)	18.29^{a}(25.69)	26.34^{a}(31.20)	36.73^{a}(37.60)	41.49^{a}(40.39)	48.25^{a}(44.28)	54.48^{a}(47.86)	60.30^{a}(51.24)	-	12.08^{g}
SEm(+)	-	0.25	0.16	0.16	0.14	0.12	0.11	0.10	0.21		0.11
CD (5%)	NS	0.75	0.48	0.49	0.42	0.35	0.32	0.31	0.64		0.33

[1]DAS- Days After Spray;* Values are mean of four replications;** Values in parentheses are arcsine-transformed values.
In a column, means followed by a common letter are not significantly different at the 5 % level by DMRT.

doses of azoxystrobin was studied against mango anthracnose.

This experiment was useful to determine the optimum rate of azoxystrobin for anthracnose disease control. The lower concentrations 25 and 50ppm had shown higher infecting rates, more disease than the higher concentrations of 100, 200, 300 and 400ppm. The response of azoxystrobin at different concentrations applied to PDI of anthracnose was illustrated graphically (Fig. 2). Although the leaf anthracnose incidence continued to decline in 100, 200, 300 and 400ppm the optimum rate was obtained at 100 ppm by considering the flattening of the disease curve between rates. Similarly optimum yield was also achieved at 100ppm where yield responded continuously to climb with increasing rates (Fig. 2).

From this experiment we can conclude that azoxystrobin may be very useful and effective fungicide against mango anthracnose disease with the optimum dose of 100 ppm.

REFERENCES

Akthar, K. P., Khan, I., Khar, I. A. and Khan, S. M. 1998. Studies on the incident and pathogenesis of *Colletotrichum gloeosporioides* Penz. causing anthracnose of mango and chemical control. *Pak. J. Phytopathol*. **10:** 42-44.

Anco, D. J., Kim, S., Mitchell, T. K., Madden, L. V. and Ellis, M. A. 2009. Transformation of *Phomopsis viticola* with the green fluorescent protein. *Mycologia*. **101(6):** 853-858.

Anesiadis, T., Karaoglanidis, R. and Klonari, K. T. 2003. Protective, curative and eradicant activity of the strobilurin fungicide azoxystrobin against *Cercospora beticola* and *Erysiphae betae*. *J. Phytopathol*. **151:** 647-651.

Dodd, J. C., Bugante, R., Koomen, I., Jeferies, P. and Jeger, M. J. 1991. Pre and post harvest control of mango anthracnose in the Philippines. *Plant Pathol*. **40:** 576-583.

Iqbal, Z., Pervez, M. A., Saleem, B. A., Ahmad, S., Dasti, A. A. and Saleem, A. 2010. Potential of *Fusarium mangiferae* as an etiological agent of mango malformation. *Pak. J. Bot*. **42:** 409-415.

Jamadar, M. and Desai, S. A. 1997. Bioefficacy of dimethomorph against downy mildew of grapevine. *Adv. Agric. Res. India*. **4:** 81-85.

McKinney, H. H. 1923. Influence of soil temperature and moisture on infection of wheat seedling by *Helminthosporium sativum*. *J. Agric. Res*. **26:** 195-217.

McMillan, R.T. 1972. Enhancement of anthracnose control of mangoes by combining copper with Nu-film-17. *Fla. State Hort. Soc*. **85:** 268-271.

Miclea, R. V. and Puia, C. E. 2012. *In Vitro* Studies Regarding the Biology and the Biological Control of some *Botrytis cinerea* Pers. Isolates. *Bull. UASVM Agric*. **69(1):** 300-301.

Miles, A. K., Willingham, S. L. and Cooke, A. W. 2004. Field evaluation of strobilurins and a plant activator for the control of citrus black spot. *Aust. Plant Pathol*. **33:** 371-378.

Nene, Y. L. and Thapliyal, P. N. 1993. Fungicides in plant disease control, 3rd edition. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi. pp. 526-531.

Palo, M. A. 1932. Sclerotium seed rot and seedling stem rot of mango. *The Philipp. J. Sci*. **52**(3): 237-261.

Ploetz, R. 1999. Anthracnose: The most important disease in much of the mango producing world. *The Newsl. Plant Pathol*. **3:** 1-6.

Sattar, A. and Malik, S. A. 1939. Some studies on anthracnose of mango caused by *Glomerella cingulata* (Stonem.) Spauld. Sch. (*Colletotrichum gloeosporioide*s Penz.). *Indian J. Agric. Sci*. **1**: 511-521.

Slawecki, R. A., Ryan, E. P. and Young, D. H. 2002. Novel

fungitoxicity assays for inhibition of germination associated adhesion of *Botrytis cinerea* and *Puccinia recondita* spores. *Appl. Environ. Microbiol*. **68:** 597-601.

Sundravadana, S., Alice, D., Kuttalam, S. and Samiyappan, R. 2006. Control of Mango Anthracnose by Azoxystrobin. *Tunis. J. Plant Prot*. **1(2):** 109-114.

Sundravadana, S., Alice, D., Kuttalam, S. and Samiyappan, R. 2007. Efficacy of azoxystrobin on *Colletotrichum gloeosporiodes* penz. growth and on controlling mango anthracnose. *J. Agric. Biol. Sci*. **2(3):** 10-15.

Sutton, B. 1992. The genus *Glomerella* and its anamorph *Colletotrichum*. In: *Colletotrichum*: biology, pathology and control, J.A. Bailey and M. J. Jeger (Eds). CAB International, Wallingford. pp. 1-26.

Vincent, J. M. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*. **159:** 850.