STUDIES ON THE INCIDENCE OF ANTHRACNOSE DISEASE IN DIFFERENT VARIETIES OF MANGO (MANGIFERA INDICA L.) AND IT'S IN VITRO BIOCONTROL MEASURES

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ABSTRACT

Anthracnose of mango caused by *Colletotrichum gloeosporioides*) is a very serious disease. Leaves, panicles, immature and mature fruits of mango are infected and damaged by this disease. The symptoms of this disease on infected parts were noted. The incidence of disease on leaves was studied on fourteen varieties of mango *viz.* Himsagar, Dashehari, Vanraj, Farnandin, Mulgoa, Bombai, Kishanbhog, Bangalora, Alphanso, Langra, Mallika, Zardalu, Chausa and Suvarnrekha. In all the varieties the incidence of anthracnose ranged from 1 - 15 per cent. The disease incidence was maximum (15.00 per cent) in Kishanbhog followed by Bombai (12.00 per cent), Himsagar (9.00 per cent) and Bangalora (8.00 per cent). In contrast, the disease incidence was recorded minimum in Alphanso (1.00 per cent). *Trichoderma viride* and *Beauveria bassiana* were applied against *Colletotrichum gloeosporioides* in vitro and their antagonistic activity were recorded.

Keywords: Anthracnose, mango, variety, *Trichoderma*, *Beauveria*

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INTRODUCTION

Mango (*Mangifera indica* L.) is universally considered one of the most important fruit crop in India. India produces near about 54.2% of World's mangos and exports Rs. 170.71 Crore of mangoes in 2008—09(APEDA, 2010).

Anthracnose, the most important mango disease, is caused by the fungus *Colletotrichum gloeosporioides*. Flower blight, fruit rot, and leaf spots are among the symptoms of this disease (Arauz, 2000). Fruits infected at mature stage carry the fungus into storage and cause considerable loss during storage, transit and marketing (Haggag, 2010). Generally to combat this disease, fungicides (e.g. bavistin, blitox -50) are applied but fungicides are environmental pollutant. The non-chemical or eco-friendly methods are now popularized in developed countries (America, U.K. etc.). Cultivation of disease resistant variety and bio control method are eco-friendly strategies to manage diseases.

Therefore, the main objectives of this work are to record mango varietal differences in susceptibility to anthracnose and antagonistic efficacy of biocontrol agents - *Trichoderma viride*, and *Beauveria bassiana* against *Colletotrichum gloeosporioides, causal* organism of anthracnose of mango *in vitro.*

MATERIAL AND METHODS

a) Study of symptoms of anthracnose: The infected leaves, petioles, twigs, panicles, flower and fruits were collected separately in sterilized biodegradable polythene bags and carried in laboratory and the symptoms caused by anthracnose were studied with the help of simple microscope.

b) Isolation and purification of pathogen from diseased parts : The infected leaves , petioles , twigs , panicles , flower and fruits were collected separately in sterilized biodegradable polythene sheets ,carried in laboratory ,and isolation of the pathogen was done in PDA medium in Petri dishes at 28^{o} C by the method presented by Dhingra & Sinclair(1994).

For purification of isolated pathogen, single hyphal tip method was taken.

c) Identification or Characterization of the pathogen: The identification of the pathogen was done by cultural and microscopical characteristics with the help of published fungal Key and books (Nagamani *et al*., 2006; Domsch *et al*.; Bailey and Jeger, 1992; Freeman *et al*, 1998). The identifications of isolates of *T. viride* were done by IARI, Delhi, India

d) Pathogenecity test of the pathogen: It was done by koch's postulates

e) Mango varietal susceptibility test : The anthracnose disease incidence on mango leaves was recorded during the period of flowering and before the fruit set in the years of 2009 & 2010 in the Gayaspur Farm , under BCKV , Mohanpure , Nadia , West Bengal ,India Fourteen mango varieties (Himsagar , Dasehari , Vanraj ,Farnandin, Mulgoa ,Mumbai, Krishnabhog, Bangalore, Alfanso , Langra, Zardalu, Chausa, Mallika and Suvarnrekha) were screened in randomized block for the study of incidence of anthracnose. The scale proposed by Khalid & Alam (2002) was taken to observe the incidence of anthracnose of mango.

Standard Scale for assessment of anthracnose of mango (Khalid &Alam 2002)

Disease grading	**Types of symptoms**
1—5 %	**Affected leaves per twig per plant -Mild**
6—10%	**Affected leaves per twig per plant –High**
11—15%	**Affected leaves per twig per plant -Severe**

f) Isolation and characterization of antagonistic fungi: Isolation of fungi from different soils from different geographical regions of west Bengal, were done in the laboratory by dilution Plate Method followed by Dhingra & Sinclair (1994).

Fungal colonies were isolated and sub-cultured repeatedly for getting pure colonies and then preserved in slant tubes for further identification. . The fungal strains were identified after staining them with cotton blue, by following the keys of Domsch et.al 1980**...**Nagamani *et al*, (2006) and www.mycobank.org

g) Antagonistic potentiality test or rating of mycoparasitism of isolated antagonistic fungi: Five mm diameter of mycelial colony from the margin of actively growing colony of *C. gloeosporioides* and that of antagonist were incubated simultaneously at opposite ends of a Petri dish containing 25 ml of sterilized PDA medium. The plates containing the paired culture were incubated at $28^0 \pm 1^0$ C for 9 days in a B.O.D. incubator and were subsequently scored for degrees of antagonism on a 1-5 scale (Bell *et al*., 1982).

An isolate of the mycoparasite was considered highly antagonistic to the pathogen when the mean score for a given comparison (when rounded to the nearest whole class number) was ≤ 2, but not highly antagonistic if the number was ≥ 3.

Among the Class—I antagonists, a comparative analysis was done on the basis of ability of antagonist to grow faster than other over *C. gloeosporioides.*

The selected cultures from pairing of mycoparasitized pathogen were observed under microscope (Leitz Laborlux K, Germany) to study of hyphal interactions between the antagonist and the pathogen and photographed when required.

RESULT AND DISCUSSION

Symptoms of the disease:

i) **Leaves**: On the leaves the symptom starts as circular or oval shaped irregular brownish to black color spots variable in sized(2-6 mm) .Under conducive condition (Humidity >90 and temperature 30^0C) the spot increases in size and disease tissues become rotted. During dry weather, the spot cannot enlarge and it becomes dried and drops off (Fig. 1). The disease spots are observed on the petiole also.

ii) **Twig**: On the young twig black spot appears on the young leaves. During rainy season, the disease rapidly covers the twig causing it to turn black and it becomes dry.

iii) **Inflorescence:** Spots appears as black, they enlarged and coalesced on the inflorescence or panicle axis, the infected axis becomes dry and withered before fruit set (Fig.2).

Fig .1 Symptom on leaves

Fig.2 Symptoms on panicle

Fig.3 Symptom on fruit

iv) **Fruit:** The symptoms are found both young as well as mature fruits. Black spots are formed on the fruits and their skin becomes discolored. The pulp beneath the infected skin becomes hard (Fig3).

Similar symptoms were recorded by many workers (Ploetz, 1994**;** Arauz, 2000; Singh 2005).

Identification of the pathogen: The isolated pathogen was *Colletotrichum* gloeosporioides (Penz) (Fig.4-7).The identification of the pathogen was done by cultural and microscopical characteristics with the help of published fungal Key and books (Nagamani *et al*., 2006; Domsch *et al*.; Bailey and Jeger, 1992; Freeman *et al*, 1998).

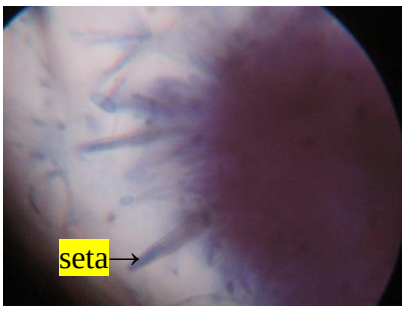

Fig. 4 Acervulae of *C. gloeosporioides*

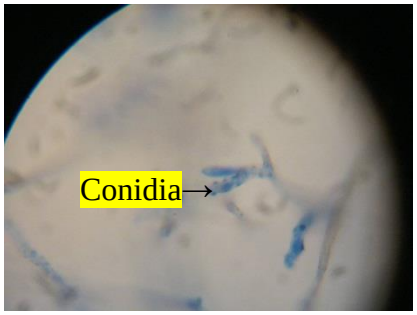

Fig. 5 Conidia & conidiophore

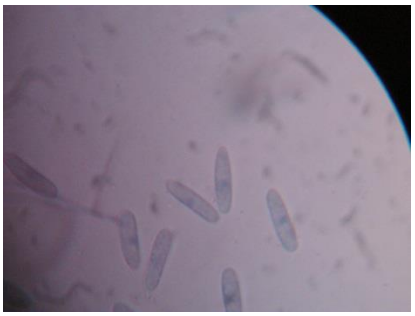

Fig. 6: Conidia of *C.gloeosporioides*

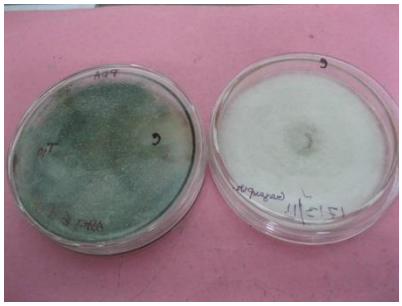

Fig. 7: (Left) Dual culture

Mango varietal susceptibility test: The presented in the table-1 exhibited that all varieties of mango trees tested were susceptible to anthracnose disease. The maximum (15) per cent of disease incidence (PDI) occurred in the Krishanbog followed by Bombai (12 per cent), Himsagar (9.00 per cent), Dashehari (8.50) and Bangalora (8.00 per cent).

Table 1 Study of mango varietal susceptible test against anthracnose

S. No.	Mango variety	Per cent of disease incidence			Type of disease
		2009	2010	Average	
1	Himsagar	08.50*	09.50	09.00	High
2	Dashehari	08.75	08.25	08.50	High
3	Vanraj	06.00	05.00	05.50	High
4	Farnandin	02.00	02.00	02.00	Mild
5	Mulgoa	05.00	08.00	06.50	High
6	Bombai	09.00	15.00	12.00	Severe

7	**Kishanbog**	**13.00**	**17.00**	**15.00**	**Severe**
8	**Bangalora**	**08.00**	**08.00**	**08.00**	**High**
9	**Alphanso**	**01.00**	**01.00**	**01.00**	**Mild**
10	**Langra**	**06.00**	**09.00**	**07.50**	**High**
11	**Mallika,**	**09.00**	**06.00**	**07.50**	**High**
12	**Zardalu**	**05.50**	**06.50**	**06.00**	**High**
13	**Chausa**	**07.00**	**09.00**	**08.00**	**High**
14	**Suvarnrekha.**	**05.50**	**07.50**	**06.50**	**High**

S.Em± **0.0012**
C.D. (P≤0.05) **0.3130**
*Average of three replicated plants

In contrast, the disease incidence was recorded minimum in Alphanso (1.00 per cent). Out of fourteen mango varieties, two (Farnandin and Alphanso) were categorized as mild susceptible while ten varieties (Himsagar, Deshahari , Bangolora Chausa,Langra, Mallika, Mulgoa,Subarnkeka, Zardalu andVanraj were under high. And only two (Krisanbag and Bombai) were severe susceptible. In the year 2010 per cent of disease incidence was higher in many varieties in comparison to 2009. It is due to prevailing weather during this period.

Varietal differences in susceptibility have been noted in Egypt. Maximum damage was observed on Alphanso, whereas variety Tommi and Fagr Kelan were recorded to be resistant (Haggag, 2010).

Antagonistic potentiality test or rating of mycoparasites of isolated antagonistic fungi: A total of seven fungal isolates were screened against *C. gloeosporioides* under *in vitro* condition by Bell's test (Fig.8) for determining mycoparasitic activity. The data presented in the Table -2 indicate that all seven isolates were rated as class—I mycoparasites /antagonists .Out of them, five isolates(T1.T2.T3,T10 &T12) identified by IARI as *Trichoderma viride* .Two isolates(BB& B2) were two strains of *Beauveria bassiana*.

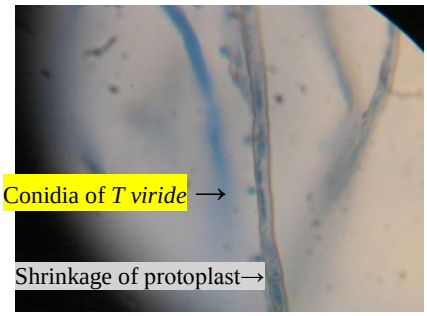

Fig. 8 T10 x pathogen

Table-2 Rating of mycoparasites in dual culture plate by Bell's method.

Serial No.	Organism or isolates	Mean scores [I II III]
1	T1	1.12 (1.00)
2	T2	1.50(1.00)
3	T3	2.00(2.00)
4	T10	1.06(1.00)
5	T12	1.40(1.00)
6	BB	1.80(1.00)
7	B2	2.00(2.00)

I=Mean of three replications.
II =Data are recorded 9 days after incubation.
III =Scale of classes described by Bell *et al*. (1982) was followed. ; The figures in parenthesis indicate the whole class.

Further, the results from Table –3 revealed that out of five strains of *T. viride* ,T10 showed maximum (3.10) mycoparasitism over C. gloeosporioides followed by T12(3.00),T1(1.40) ,T3(1.40) and T2 (1.00). Out of two strains of *B. bassiana*, BB was better than B2

Table 3: Comparative mycoparasitic activity of different isolates of hyperparasites showing class –I activities against *C. gloeosporioides*

Serial No.	**IARI Herb. No.**	**Organism or isolate**	**Scientific name of mycoparasite**	**Radial growth(cm) of mycoparasites over *C. gloeosporioides* at 24 hr. interval**		
				24	**48**	**72**
1	**108**	**T1**	***Trichoderma viride***	**0.70***	**1.09**	**1.40**
2	**109**	**T2**	***Trichoderma viride***	**0.45**	**0.86**	**1.00**
3	**110**	**T3**	***Trichoderma viride***	**0.61**	**1.00**	**1.30**
4	**112**	**T10**	***Trichoderma viride***	**1.00**	**2.25**	**3.10**
5	**115**	**T12**	***Trichoderma viride***	**0.80**	**2.02**	**3.00**
6	**--**	**BB**	***Beauveria bassiana***	**0.20**	**0.42**	**1.00**
7	**--**	**B2**	*Beauveria bassiana*	**0.15**	**0.32**	**0.60**

S.Em±	0.093
C.D. (P≤0.05)	0.452

*Each insertion is average of three replications

Kefialew and Ayalew(2008) reported that four isolates of bacteria, five yeasts and two filamentous fungi were evaluated in this study. Cell suspensions and culture filtrates of the isolates inhibited spore germination and hyphal growth of *C. gloeosporioides in vitro.*

Mango plants treated with Trichoderma's pellet and spraying with spore suspension of *Trichoderma harzianum* PC01 and *T. hamatum* PC02 (404 x 10^{10}) and some chemical fungicide treated ones. It was observed that the biological treatments gave better yield than the chemical fungicides (Carbendazim, Zinep, Manep and Copper oxychloride) treatment. (Noiaium and Soytong, 2010).

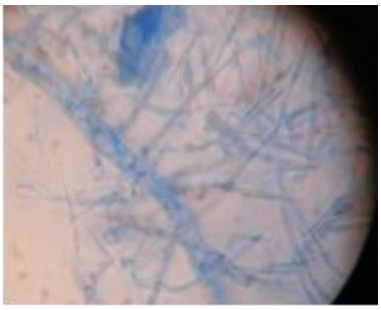

Fig. 9: T10 xPathogen

Interaction between *Trichoderma viride* (T10) and *C. gloeosporioides* under microscope showed that spores of T10 adhered on the wall of C. gloeosporioides and shrinkage of protoplast of the latter were recorded (Fig. 9).The Fig. - 9 depicted that the hyphae of C. gloeosporioides was surrounded by T 10 and the shrinkage of the protoplast of the pathogen.

Similar phenomenons were reported in the interaction between mycoparasites (*Trichoderma viride*) and plant pathogens by other workers (Chat *et al*., 1981; Pan and Ghosh, 1997).

In conclusion, all varieties of mangoes are susceptible to anthracnose from mild to severe. *Trichoderma viride* strains are good biocontrol agents as tested *in vitro* and this experiment encourages other to apply this biocontrol agent in the field of mango (in *vivo*).

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