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Evaluation of different botanicals, bioagents and fungicide against *Colletotrichum gloeosporioides* under *in vitro* condition

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Abstract
Anthracnose a post-harvest disease is important in *konkan* region because losses also reported in *Alphanso*. Hence by management aspect evaluation of different botanicals Garlic clove (*Allium satium*) at concentration 20% found to be significantly superior over rest of the botanicals by inhibiting the mycelial growth at (54.12%) followed by Tulsi (*Ocimum tenuiforum*) (46.88%). In case of biogents maximum mycelial growth inhibition resulted with *Trichoderma viride* (92.38%), followed by *T. harzianum* (89.33%), *B. subtilis* (88.41%) and *T. koningi* (75.30%). Finally using fungicides Azoxystrobin 23% SC at concentration 0.1 and 0.05% was found to be most effective which inhibited per cent growth of the fungus at both concentration followed by Tebuconazole 50% + Trifloxystrobin 25% WG at 0.1 and 0.05 per cent concentration.

Keywords: Bioagents, botanicals, fungicides, post-harvest disease and anthracnose

Introduction
Mango (*Mangifera indica* L.) is the most important fruit crop of India. Its delicious taste, exceedingly acceptable flavour, pleasing colour and exemplary nutritive value made it 'National fruit of India' and is known as 'King of fruits'. It is originated from South East Asia, the Indo-Burma region, in the foot hills of the Himalayas (Mukherjee, 1951) [13]. It is one of the most-relished fruits of tropics. Besides, it has an intimate association with cultural, religious, aesthetic, academic and economic life of the Indian since long time. Mango fruits is put to many uses right from raw fruit to mature green and ripe fruit. The availability, acceptability and multipurpose utilization have adorned King's crown on mango. Hence mango has been called as "The First Fruit of India". In Maharashtra, mango covered the area of 157.07 thousands ha. With production of 514.87 thousands MT and the productivity is 3.28 MT/ha. Konkan region is traditional belt for mango production in the country. The area under mango in Konkan is 111715 ha. And annual production is around 353066 MT with the productivity of 3.16 MT/ha. (Anonymous 2017d) [2]. The Konkan region of Maharashtra state comprises Palghar (4,990 ha.), Thane (1800 ha.), Raigad (12325 ha.), Ratnagiri (61000 ha.), Sindhudurg (31600 ha.) (Anonymous. 2016a) [1]. In the Konkan region 90 per cent of area occupied by mango, which is single cultivar 'Alphonso', and they locally called as "Hapus".
Colletotrichum is one of the most important genera of plant pathogenic fungi worldwide, in tropical and subtropical regions. It causes anthracnose, and post-harvest rots in many economically important crops such as cereals, pulses, vegetables, fruits, spices and cash crops. In *Konkan* region, mango is commonly infected by *Colletotrichum gloeosporioides* causing anthracnose disease during rainy season and at the post-harvest ripening stage. Post-harvest diseases of fresh fruits are traditionally being controlled by synthetic chemical fungicides (Eckert and Ogawa, 1985) [7]. However, when harvested fruits are treated with fungicide to manage post-harvest diseases, there is greater likelihood of direct human exposure to them. Development of resistance in pathogens due to application of same repeated fungicides applied for controlling the post-harvest diseases (Spotts and Cervantes, 1986) [18] underlines the necessity to develop new and effective methods of controlling post-harvest diseases that are perceived as safe by the public and pose negligible risk to human health and environment. Considering the status and occurrence of anthracnose, it was felt necessary to undertake studies on this disease.

Material and Methods

a) Evaluation of botanicals

The present investigation was aimed to study the antifungal activity of some plant extracts. The plant extracts mentioned in Table 1 which were locally available was evaluated against the test fungi as per the procedure given by Shinde and Patel (2004) [19]. One hundred gram fresh plant material was weighed and thoroughly washed with tap water followed by sterilized water. The plant material was then homogenized in sterile distilled water 1 ml/g of tissues with a pestle and mortar. The crude material was then expressed through double-layered muslin cloth and was centrifuged in a centrifuge machine at 5000 rpm for 20 minutes. After centrifugation, the supernatant was taken and pellets were discarded avoid bacterial contamination. This formed the standard plant extract solution (100 per cent).

The effect of plant extracts on mycelial growth was studied by Poisoned Food Technique (Nene and ThapliyaI, 1993) [15]. The principle involved in this technique is to poison the nutrient medium with fungi-toxicant and allowing the fungus to grow on it. The potato dextrose agar medium was used as a basal medium. The requisite quantity of plant extract was added to sterilized Potato Dextrose Agar medium by using sterile pipette under aseptic conditions so as to get desired Concentration. These were poured into sterilized petri plates. The mycelial discs of 8 mm in diameter of the test fungi were cut with the help of sterile cork borer and transferred aseptically at the centre of each sterilized plate already poured with poisoned medium separately. The inoculated Potato Dextrose Agar medium without incorporation of plant extract served as the control. The plates were incubated at room temperature, three replications per treatment were maintained. The efficacy of plant product or botanical was expressed in percentage of radial growth over the control which was calculated by the formula of Horsfall (1956) [8].

$$I = C - TC \times 100$$

Where, I - Percent inhibition
C – Radial growth in control
T - Radial growth in treatment

Further angular transformation were made for data and analysed statistically.

b) Evaluation of bioagents

In vitro study was carried out on mango fruits cv. Alphonso against post-harvest anthracnose caused by *Colletotrichum gloeosporioides* by imposing various bioagents (Table 2). The antagonistic activity of Bioagents against *Colletotrichum gloeosporioides* was determined by dual culture technique under *in vitro* condition (Bhuvaneswari and Rao, 2001) [3]. Mycelial discs measuring 8 mm diameter from one week old cultures of both fungal antagonist and the test pathogen were placed at equidistant on sterile Petri plates containing Potato Dextrose Agar medium. However for *Bacillus subtilis,* one day old culture of bacteria was streaked on opposite side of the pathogen on Nutrient Agar medium. The Petri plates were incubated at 28±2°C. Three replications were maintained in each treatment. Suitable controls were kept without antagonist. Zone of inhibition were measured simultaneously day after inoculation of antagonist. Percentage inhibition of mycelial growth of test pathogen was 34 calculated. The experiment was conducted completely randomized design with four replications and five treatments.

c) Evaluation of fungicides

The efficacy of six fungicides and their concentration tested against the mango anthracnose. The treatment details of the fungicides used are given Table 3. Required quantity of individual fungicide was added separately into molten and cool potato dextrose agar so as to get the desired concentration of fungicide. Later 20 ml of the poisoned medium was poured into sterile Petri plates. Mycelial discs of 8 mm size from actively growing one weeks old culture of the fungus were cut by sterile cork borer and one such disc was placed at the centre of each agar plate. Control was maintained without adding any fungicide to the medium. Each treatment was replicated thrice. The plates were incubated at room temperature for ten days and radial colony growth was measured. The efficacy of fungicides was expressed as percent inhibition of mycelia growth over control and was calculated by using the formula suggested by Horsfall (1956) [8].

$$X = Y - ZY \times 100$$

Where,
X = Per cent inhibition
Y = Growth of fungus in control (mm)
Z = Growth of fungus in treatment (mm)

Results and Discussion

a) Evaluation of botanicals

The data presented in the Table 4, revealed that the treatment T4 Garlic clove (*Allium satium*) at concentration 20% found to be significantly superior over rest of the botanicals used in the study by inhibiting the mycelial growth at (54.12%) followed by Tulsi (*Ocimum tenuiforum*) (T6-46.88%), Clove leaves (*Syzygium aromatic*) (T2-35.01), Garlic clove (*Allium satium*) (T3-33.20) at 10% concentration and Soapnut (*Sapindus mukorossi)* (T1-24.55%) for inhibiting the mycelia growth of the fungus *C. gloeosporioides.* From overall it indicates that effect of plant extracts on the fungal growth was significantly superior for *in vitro* management of *C. gloeosporioides.*

All the Phytoextracts were evaluated under *In-vitro* condition against *Colletotrichum gloeosporioides* to know the fungi toxic nature of their extracts. Though, complete inhibition of pathogen was not observed in any of the plant extracts tested but considerable amount inhibition was noticed in some of them (Khadar, 1999 and Nagesh, 2000) [9, 14]. The highest percent inhibition (54.12%) was observed in garlic extracts which was statistically different from all other treatments. The second highest inhibition (46.88%) was observed in case of tulsi extract which was also statistically different from the other treatments. Similar observation recorded by Mukherjee, *et al.* (2011) [12] and Kolase *et al.* (2014) [10].

b) Evaluation of bioagents

The results presented in Table 5, revealed that all the antagonists significantly reduced the growth of *C. gloeosporioides* either by over growing or by exhibiting inhibition zones. Most of the antagonist inhibited colony growth of *C. gloeosporioides* by fast and over growing nature as observed in antagonists. The result revealed that maximum mycelial growth inhibition resulted with *Trichoderma viride* (92.38%), followed by *T. harzianum* (89.33%), *B. subtilis* (88.41%) and *T. koningi* (75. 30%) and at par with each other. Earlier studies carried out by Rocha *et al.,* (1998) [17], Patel (2000), Bhuvaneshvari and Rao (2001) [3], Lakshmi *el al.,*

(2004) [11] showed the efficacy of *T. harzianum* against *Colletotrichum gloeosporioides.* The efficacy of *T. harzianum* against *C. gloeosporioides* was also studied by Dev *et al.* (2015) [6] up to 87 to 92% mycelium growth inhibited by the *Trichoderma* spp.

c) Evaluation of fungicides

It was revealed from the data depicted in Table 6 that, the effect of different fungicides on growth and sporulation of *C. gloeosporioides* was significant. Among different fungicides Azoxystrobin 23% SC at concentration 0.1 and 0.05% was found to be most effective which inhibited cent per cent growth of the fungus at both concentration followed by Tebuconazole 50% + Trifloxystrobin 25% WG at 0.1 and 0.05 per cent concentration and at par with each other. Similar results were reported by Diedhiou *et al.* (2014) [5] and Patil *et al.* (1992) [16]. Biradar S.V. (2002) [4].

Table 1: List of botanicals used to check their efficacy against *C. gloeosporioides*

Tr. No.	Common name	Botanical name	Plant part used for extract	Conc.%
1	Soapnut	*Sapindus mukorossi*	Rind of seed	10
2	Clove	*Syzygium aromatic*	Leaves	10
3	Garlic	*Allium satium*	Rhizome	10
4	Garlic	*Allium satium*	Rhizome	20
5	Tulsi	*Ocimum tenuiforum*	Leaves	10
6	Tulsi	*Ocimum tenuiforum*	Leaves	20
7	Control	-	-	-

Table 2: List of bioagents used to check their efficacy against *C. gloeosporioides*

Tr. No.	Biocontrol agent
1	*Trichoderma harzianum*
2	*Trichoderma viride*
3	*Trichoderma koningii*
4	*Bacillus subtilis*
5	Control

Table 3: List of fungicides used to check their efficacy against *C. gloeosporioides*

Tr. No.	Chemical name	Conc. (%)
1	Carbendazim 12% + Mancozeb 63% WP	0.1
2	Azoxystrobin 23% SC	0.05
3	Azoxystrobin 23% SC	0.1
4	Tebucanazole 50% + Trifloxystrobin 25% WG	0.05
5	Tebucanazole 50% + Trifloxystrobin 25% WG	0.1
6	Copper oxychloride 50% WP	0.1
7	Control	-

Table 4: *In vitro* efficacy of various plant extracts against mycelial growth of *C. gloeosporioides*

Tr. No	Plant Extracts	Botanical Name	Conc. (%)	Mean* (mm)	Per cent Inhibition* of mycelial growth
T1	Soapnut	*Sapindus mukorossi*	10	62.5	24.55 (29.70)
T2	Clove leaves	*Syzygium aromatic*	10	53.8	35.01 (36.28)
T3	Garlic clove	*Allium satium*	10	55.3	33.20 (35.18)
T4	Garlic clove	*Allium satium*	20	38.0	54.12 (47.36)
T5	Tulsi leaves	*Ocimum tenuiforum*	10	65.0	21.53 (27.65)
T6	Tulsi leaves	*Ocimum tenuiforum*	20	44.0	46.88 (43.21)
T7	Control	-	-	82.8	-
SE (m) ±		4.20			
CD @ 1%		17.69			

* Mean of three replications

Figures in parentheses are Arcsine values

Table 5: *In vitro* efficacy of various bio-agents against mycelial growth of *C. gloeosporioides*

Tr. No.	Bio-agents	Radial Growth (mm)*	Per cent Inhibition* of mycelial growth
T1	*T. harzianum*	8.75	89.33 (70.73)
T2	*T. viride*	6.25	92.38 (73.98)
T3	*T. koningii*	20.25	75.30 (60.20)
T4	*B. subtilis*	9.50	88.41 (70.10)
T5	Control	82.00	-
SE (m) ±		1.04	
CD@ 1%		4.35	

* Mean of three replications

Figures in parentheses are Arcsine values

Table 6: *In vitro* efficacy of various fungicides against mycelial growth of *C. gloeosporioides*

Tr. No.	Fungicides	Conc. (%)	Mycelial growth* (mm)	Per cent Inhibition of mycelial growth*
T1	Carbendazim12% + Mancozeb 63% WP	0.1	1.38	83.43 (65.98)
T2	Azoxystrobin 23% SC	0.05	1.00	88.02 (69.75)
T3	Azoxystrobin 23% SC	0.1	0.70	91.62 (73.17)
T4	Tebuconazole 50% + Trifloxystrobin 25% WG	0.05	1.08	87.03 (68.89)
T5	Tebuconazole 50% + Trifloxystrobin 25% WG	0.1	1.05	87.43 (69.23)
T6	Copper oxychloride 50% WP	0.1	1.92	77.05 (61.38)
T7	Control	-	8.35	0.00
SE (m) ±	1.78			
CD @ 1%	7.49			

* Mean of three replications
Figures in parentheses are Arcsine values

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