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Management of post-harvest anthracnose disease in mango using promising biocontrol agents

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Abstract
Mango (*Mangifera indica* L.) is universally considered as one of the leading fruit crop worldwide grown throughout the tropics and subtropics. Mango deserves to be the national fruit of India and because of its popularity and high nutritional value it has been acclaimed as "King of fruits". Post-harvest anthracnose disease incited by *Colletotrichum gloeosporioides* (Penz.) Penz., and Sacc., is a major yield and market constraint as it causes economical loss to a tune of 17–36% affecting the marketability of fruit. Use of chemical fungicides has been employed as primary method in postharvest disease management. However, due to development of resistance to fungicides and residual toxicity biological control has emerged as an alternative strategy and receiving a special attention to combat major postharvest decays of fruits. The promising bio-control agents for controlling of postharvest fruit diseases include antagonist fungi *Trichoderma*, bacteria with PGPR activity and yeasts as bio-protectant. Fruit bioassay was carried with promising antagonistic microflora isolated from fructoplane of mango for bio-management of anthracnose of mango. The percent disease index PDI (%) of anthracnose disease in Dasheri variety was recorded. Fruits treated with bioagents *Trichoderma*, *Bacillus subtilis*, *Pseudomonas fluorescens* and yeast *Meyerozyma caribbica* did not exhibit symptoms up to 2 days. Among the bioagents treated fruits, bacterial bioagents *P. fluorescens* and *B. subtilis* were most effective in reducing the anthracnose symptoms on fruits and recorded the minimum percent disease index PDI (%) of 37.70% and 33.81% at 10^{th} day of inoculation. Whereas, the PDI in *Trichoderma harzianum*, *T. viride*, *T. asperellum* treated fruits was 43.75%, 41.82% and 46.33% respectively and Yeast, *Meyerozyma caribbica* recorded 40.00% PDI while in control (Untreated fruits) the PDI was 85.50% on 10th day of incubation. The bacterial bioagents with their PGPR activity were effective in reducing the anthracnose disease symptoms by 45% over control. Use of such bioagents not only minimizes the losses to farmers but also help in reducing the fungicidal residue in fruits used for human consumption.

Keywords: Anthracnose, *Bacillus subtillis*, bioagents, *Colletotrichum gleosporidies*, *Pseudomonas fluorescens*, *Trichoderma* harzianum, mango, *Meyerozyma caribbica,* yeast

Introduction
Mango (*Mangifera indica* L.) is universally considered as 'King of Fruits' and deserves to be the national fruit of India, besides delicious taste, excellent flavour and attractive fragrance, it is rich in vitamin A and C, belonging to the family *Anacardiaceae* and native to India and South East Asia. Mango is grown throughout tropics and subtropics worldwide and is being promoted as cash crop for small holders (Arauz, 2000; Subramanyam *et al*., 1975) [1, 16]. The area under mango cultivation is 2262.8 thousand MT/ha with 34.92 percent of fruit area, production of 19686.9 thousand MT/ha with 21.20 fruit production and productivity of 8.7 MT. The major mango growing states are Andhra Pradesh, Uttar Pradesh, Karnataka, Bihar, Gujarat and Tamil Nadu. Uttar Pradesh ranks first in mango production with a share of 23.47% and highest productivity. India is also a prominent exporter of fresh mangoes to the world (Horticulture Statistics at a Glance, 2018) [3].
Anthracnose caused by *Colletotrichum gloeosporioides* is the most important disease widely distributed in all mango growing regions of the world and major constraint in the export trade. The disease was first identified in India by Mc Rae (1924) [6] and causes infection on leaves (black spots), blossoms (blight) and fruits (black spots and fruit drop) and subsequently tree becomes cent percent unproductive (Arauz, 2000) [1]. Anthracnose disease incited by *Colletotrichum gloeosporioides* is a major yield and market constraint. Because of their high moisture and the nutrient reserve, mango fruit is highly susceptible to the pathogen during the period between harvest and consumption. Being highly perishable, mango fruits have to be marketed immediately after harvest.

The post-harvest anthracnose on fruits was the most damaging as it caused economical loss to a tune of 17– 36% affecting the marketability of fruit rendering it worthless (Haggag, 2010) [4].

Most of storage management is restricted to small pockets of cultivation and not available to all the mango producers due to several socio-economic constraints in India. Use of chemical fungicides has been employed as primary method in postharvest disease management. However, due to development of resistance to fungicides and residual toxicity, use of many fungicides has been discontinued. Though physical or cultural methods, *viz*., hot water treatment, use of irradiation, careful handling of fruits to prevent wound infection can be applied, they have limitations in large scale application. Hence, new alternative non-chemical method using bio-control agents supersede these fungicides solely because of their high-specificity towards the target pathogen and the consequential eco-friendliness.

Biological control has emerged as an alternative strategy to combat major postharvest decays of fruits. The use of microbial antagonists for control of postharvest diseases received a special attention and has been extensively investigated. Biological control of postharvest has great potential for postharvest environmental parameters such as temperature and humidity could be rigidly controlled to suit the needs of the bio-control agent. Also, harvested commodities offer a concentrated target for the application of bio-control agents. The promising bio-control agents for controlling of several postharvest fruit diseases include antagonist fungi *Trichoderma*, bacteria with PGPR activity and yeasts as bio-protectant. The antagonists attack the pathogens by various modes of action *viz*., competition for nutrients, space, antibiotic production, siderophores and also triggering plant originated resistance mechanism called Induced Systemic Resistance (Ramamoorthy and Samiyappan, 2001) [10]. *Trichoderma* was used in strawberry grey mould management (Tronsmo and Dennis, 1977) [18]; *Bacillus subtilis* against brown rot management in strawberry (Pusey, 1989) [9]; yeasts against *Botrytis mold* infection in apple (Tian *et al*., 2002) [17].

Management of anthracnose disease has become one of the major concerns. Management of postharvest diseases by employing microbial agents has been demonstrated to be most suitable strategy to replace the chemical usage. The main strategies for the management of postharvest diseases outlined by Eckert and Ogawa (1988) [2] are inoculum reduction, prevention of field infection, inactivation of wound infection and suppression of disease development and spread. Use of biocontrol agents gives an alternate option for chemical free management of postharvest diseases. The advantages of using biocontrol agents are possibility of controlling environmental conditions during storage suitable for the bioagents as well as the fruits. The fungal antagonists *T. harzianum* and *P. fluorescens* were effective in checking the spread of pathogen *Colletotrichum gloeosporioides* on fruits compared with the pathogen-inoculated control (Prabakar *et. al*., 2008) [8]. Application of the yeast by immersion the mango fruits in yeast suspension could significantly reduce postharvest development of anthracnose lesions (Sriram and Poornachandra, 2013) [15].

In Karnataka mango is the major fruit crop which is widely distributed and the post-harvest spoilage is estimated about 30-35% due to anthracnose disease in mango caused by *Colletotrichum gloeosporioides* which is the most serious and destructive disease and a major post-harvest problem which degrades the quality and marketability of the fruit (Saxena, 2011) [12]. The main emphasis is to develop eco-friendly management of anthracnose of mango using bioagents. This will be useful not only in minimizing the loss to farmers but also in reducing the fungicidal residue in fruits used for human consumption. In this context, the present investigation was undertaken with an objective of management of anthracnose disease of mango using promising bio-control agents

Material and Methods

The laboratory studies and fruit bioassay experiments of this research were carried out from 2013 to 2015 at the Department of Plant Pathology laboratory and GKVK, Bengaluru

Management of post-harvest anthracnose disease of mango using promising bioagents

The effective bioagents *viz*., *Trichoderma*, *Pseudomonas*, *Bacillus* and Yeast screened under *in vitro* conditions were used for fruit dip and challenge inoculation method to evaluate their effectiveness against anthracnose disease of mango caused by *Colletotrichum gloeosporioides*.

Fruit bioassay by fruit dip method

Bioagents *Trichoderma*, yeast and bacterial cultures *Pseudomonas* and *Bacillus* isolated from fructoplane of mango were selected based on their inhibition studies carried out under *In-vitro* conditions against the postharvest pathogen *Colletotrichum gloeosporioides*. The selected pure cultures of the bioagents were grown on PDB, Kings B broth, Nutrient broth and Yeast Extract broth and the viability was checked and adjusted to 10^6 cfu/ml. Unripe fruits collected from the orchard were treated with the cell suspension of selected bioagent cultures. Three replications each with 5 fruits were maintained. Fresh fruits were dipped in sterile water containing *Trichoderma*, yeast and bacteria at 2×10^6 cfu/ml for five min and shade dried. Fruits were stored in cartons with holes and paddy straw lining inside. Prior to use, paddy straw was sterilized and treated with 0.2% Mancozeb solution for 2 min. Percent of fruits infected by *Colletotrichum gloeosporioides* and area of fruit surface damaged were recorded. Percent area of fruit surface covered by rotting due to the pathogen was recorded based on visual observation with 5 grade scale. Number of fruits that had been damaged in each category was recorded. Fruits without any treatment were maintained as control. From this average fruit surface area damaged was calculated.

Fruit bioassay by challenge inoculation

Healthy fruits were washed in tap water, followed by surface sterilized by using sodium hypochlorite for 30 seconds and rinsed thrice in sterile distilled water. Wound was made on the fruits with the help of a sterile needle/toothprick. The mycelium of the fungus *C. gloeosporioides* was inserted into the wounded surface of the fruit and kept in a humid chamber. The humid chamber was prepared by keeping water in the tray which was placed below and the perforated tray kept with inoculated fruits was placed above. The inoculated fruits were kept along with cotton plug dipped in water and the tray was covered with polythene sheet to maintain the relative humidity of over 90 percent and then incubated at 27 ± 1 °C. The symptom expression was recorded two days after inoculation and the following scale was used to record the spread of the infection on fruits.

Table 1: Disease scale (0-5) for scoring per cent fruit infection of anthracnose disease on mango fruits

Disease grade	Description
0	zero percent fruit area infected
1	1-10 percent fruit area infected
2	11-25 percent fruit area infected
3	26-50 percent fruit area infected
4	51-75 percent fruit area infected
5	>75 percent fruit area infected

The percent disease index was recorded with the following formula as given by Rose (1974) [11].

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of all individual rating}}{\text{Total number of fruits grade x Maximum disease grade}} \times 100$$

Statistical analysis

Statistical analysis was carried out as per the procedures given by Panse and Sukhatme (1985) [7]. Actual data in percentage were converted to angular transformed values, before analysis.

Results and Discussion

Management of anthracnose disease of mango using promising bio-control agents

Fruit dip method

Anthracnose diseases of mango cause serious losses worldwide. The observable external symptoms often become apparent after ripening, by which it is usually equated with the edibility of the fruit and causing serious losses during storage. Synthetic fungicides are primarily used to control postharvest decay loss. However, the recent trend is shifting toward safer and more eco-friendly alternatives for the control of postharvest decays. Search for microbial antagonists to control postharvest decay of fruits has been actively pursued and appears to be a promising strategy for managing disease.

Bioagents *Trichoderma*, yeast and bacteria isolated from fructoplane of mango were selected based on their inhibition percentage to control the postharvest pathogen *C. gloeosporioides*. The selected cultures were grown on Potato Dextrose Broth (PDB), Nutrient Agar (NB) and Yeast Extract Malt Glucose broth (YEMG) and the viability was checked and adjusted to 10^6 $cfuml^{-1}$. Unripe fruits Dasheri variety collected from the market were treated with the cell suspension of selected bioagent cultures. Three replications each with 5 fruits were maintained. Fresh fruits were dipped in sterile water containing bioagents at 2 x 10^6 $cfuml^{-1}$ for five min and shade dried. Fruits were stored in cartons with holes and paddy straw lining inside.

Fruits treated with bioagents *Trichoderma* spp., *B. subtilis*, *P. fluorescens* and yeast *Meyerozyma caribbica* and exhibited no symptoms for up to 2 days, but the symptom expression was observed in untreated fruits with 14.91 percent disease index on 2^{nd} day of incubation. Anthracnose symptoms started on fourth day in Dasheri variety and the percent disease index PDI (%) was recorded as 2, 4, 6, 8 and 10 days intervals according to the disease scale 0-5. Among the bioagents treated fruits bacterial bioagents *P. fluorescens* and *B. subtilis* recorded the minimum percent disease index of 37.70% and 33.81% on 10^{th} day of inoculation and were most effective as compared with untreated fruits the PDI on 10^{th} day of inoculation respectively. Whereas, the PDI in *Trichoderma asperellum* and *T. harzianum* treated fruits was 43.75% and 46.33%. Yeast *Meyerozyma caribbica* and *Torulaspora delbrueckii* recorded 41.82% on 10^{th} day of inoculation while in control (untreated fruits) the PDI was 85.50 percent on 10th day of incubation.

Challenge inoculation method

Fruits were treated with bioagents *Trichoderma*, *B. subtilis*, *P. fluorescens* and yeast *Meyerozyma caribbica* then challenge inoculated with the pathogen *Colletotrichum gloeosporioides*. The anthracnose symptoms expression stared from 2 days of incoluation (DAI) on Alphonso variety and the percent disease index PDI (%) was recorded as 2, 4, 6, 8 and 10 days intervals.

Among the bioagents treated fruits and challenge inoculated with pathogen *C. gloeosporioides*, the bacterial bioagents *P. fluorescens* and *B. subtilis* recorded the minimum percent disease index of 38.00% and 35.15% at 10^{th} day of inoculation as compared with untreated fruits the PDI was 86.33%. Whereas, the PDI in *Trichoderma asperellum* and *T. harzianum* treated fruits was 45.22% and 46.33%. Yeast *Meyerozyma caribbica* recorded PDI of 42.23% which was on par with yeast *Torulaspora delbrueckii* treated fruits while in control the percent disease index was 86.50% on 10th day of incubation.

The results were in accordance with Vivekananthan *et al*. (2004) [19], reported that the bioagents *viz.*, *Saccharomyces cerevisiae*, *P. fluorescens* and *B. subtilis* treated fruits exhibited no symptoms of anthracnose disease up to 2 days but the PDI (%) at 15th day was 40% in bioagents treated fruits and in untreated fruits the percent disease index reached up to 81 percent. Prabakar *et al*. (2008) [8], reported that *Trichoderma harzianum* and *P. fluorescens* reduced the disease spread significantly in the inoculated fruits when compared with uninoculated control. The PDI was 40.00% in the fruits inoculated with *Colletotrichum gloeosporoides* challenged with *T. harzianum*, 41.67 in the fruits inoculated and challenged with *P. fluorescens*, 98.33 in uninoculated control fruits on 12 th day after treatment. Sriram and Poornachandra (2013) [15] screened one yeast isolate and one bacterial isolate to be efficient in reducing the surface area affected by fruit rot caused by *C. gloeosporioides* and *D. natalensis* among five isolates tested by postharvest fruit dipping method. These isolates were identified as *Candida tropicalis* and *Alcaligenes* sp. using molecular tools. The rotting was 50.0 to 56.82% in treated fruits while in control it reached 93.64% on 12th day of incubation. Kefialew and Ayalew (2008) [5] reported that different isolates of bacteria and yeast antagonists significantly reduced anthracnose severity on fruit that had been artificially inoculated with *C. gloeosporioides.* The bioagent *Bacillus subtilis* significantly reduced anthracnose incidence in ripening fruits to much lower levels than those obtained by using a conventional single post-harvest treatment through prevention of early fruit infection (Senghor *et al*., 2007) [13]. The promising biocontrol agents for controlling of several postharvest fruit diseases includes fungi, bacteria and yeasts. Yeasts deserved particular attention as their modes of action tend to be competition for space and nutrients, rather than production of antibiotics or other toxic secondary metabolites. Substances secreted by yeasts mostly are enzymes, e.g. polygalacturonase, β-glucosidase (Sharma *et al*., 2009) [14].

Fruit dip with bacterial bioagents *Bacillus subtilis* and

Pseudomonas fluorescens were found to be very effective in reducing post-harvest development of anthracnose diseases on mango fruits as they compete with pathogen for nutrients and space, trigger antibiotic and siderophore production. The routine application of chemical fungicides leads to toxicity, residual effect and resistance developed by the pathogen. Hence, the management of anthracnose pathogen using biocontrol agents like *Trichoderma*, PGPR *Bacillus* and *Pseudomonas* and Yeast is ecofriendly.

Table 2: Evaluation of promising bioagents against anthracnose of mango by fruit dip method

Treatments	Treatment description	Percent disease index PDI (%)					
		2nd Day	4th Day	6th Day	8th Day	10th Day	% Disease reduction over control at 10th day
T_1	*Trichoderma asperellum*	0.00	20.56 (26.91)*	30.33 (33.40)*	40.19 (39.3)*	43.75 (41.45)*	34.33
T_2	*T. harzianum*	0.00	21.22 (27.42)	32.51 (34.70)	40.69 (39.70)	46.33 (42.93)	31.31
T_3	*Pseudomonas fluorescens*	0.00	17.55 (24.65)	25.60 (30.30)	30.65 (33.69)	37.70 (37.40)	41.40
T_4	*Bacillus subtilis*	0.00	18.52 (25.50)	22.30 (28.20)	25.57 (30.63)	33.81 (35.51)	45.95
T_5	*Meyerozyma caribbica*	0.00	21.80 (27.82)	30.54 (33.50)	32.80 (34.93)	40.00 (39.20)	38.71
T_6	*Torulaspora delbruekii*	0.00	18.30 (25.30)	26.62 (31.60)	36.56 (37.53)	41.82 (40.30)	36.58
T_7	Control	14.91	30.33 (33.41)	45.41 (42.40)	60.33 (51.30)	85.50 (66.63)	-
S.Em±		0.20	1.05	1.31	1.13	1.04	
CD @1%		0.59	3.09	3.86	3.32	3.07	
CV (%)		12.33	7.70	8.09	5.95	4.82	

* Figures in parentheses indicate angular transformed values

Table 3: Evaluation of promising bioagents against anthracnose of mango by challenge inoculation method

Treatments	Treatment description	Percent disease index PDI (%)					
		2nd Day	4th Day	6th Day	8th Day	10th Day	% Disease reduction over control at 10th day
T_1	*Trichoderma asperellum*	15.66 (23.15)*	25.33 (30.26)*	32.75 (34.90)*	42.80 (40.93)*	45.22 (42.20)*	33.94
T_2	*T. harzianum*	15.65 (23.22)	25.36 (30.23)	34.15 (35.70)	42.00 (40.45)	46.33 (42.90)	32.66
T_3	*Pseudomonas fluorescens*	10.45 (18.90)	21.55 (27.63)	26.55 (31.70)	32.43 (34.79)	38.00 (38.00)	42.31
T_4	*Bacillus subtilis*	11.33 (19.60)	18.10 (25.10)	24.45 (29.60)	31.72 (34.36)	35.15 (36.30)	45.61
T_5	*Meyerozyma caribbica*	15.66 (23.33)	25.36 (30.20)	32.45 (34.79)	35.63 (36.65)	42.36 (42.30)	37.26
T_6	*Torulaspora delbruekii*	12.82 (20.98)	20.41 (26.80)	28.40 (30.44)	38.33 (38.20)	42.20 (40.50)	37.44
T_7	Control	32.75 (34.90)	43.45 (41.20)	54.25 (47.45)	66.55 (54.71)	86.33 (60.36)	-
S.Em±		1.02	1.13	0.80	0.80	0.69	
CD @ 1%		3.01	3.32	2.36	2.34	2.04	
CV (%)		8.76	7.49	4.57	3.98	3.15	

* Figures in parentheses indicate angular transformed values

Conclusion

Management of anthracnose of mango was done using promising bioagents by fruit dip and challenge inoculation methods. Fruits treated with bioagents *Trichoderma harzianum*, *T. asperellum*, *B. subtilis*, *P. fluorescens* and yeast *Meyerozyma caribbica* and *Torulaspora delbrueckii* exhibited no symptoms up to 2 days on incubation on Alphonso variety, but after that the symptom expression on fruits gradually increased. Among the bioagents tested, bacterial bioagents *P. fluorescens* and *B. subtilis* recorded the minimum disease incidence of 37.70% and 33.81%. Whereas, the PDI in *Trichoderma asperellum* and *T. harzianum* treated fruits was 43.75% and 46.33% respectively. Yeast, *Meyerozyma caribbica* and *Torulaspora delbrueckii* recorded PDI of 40.00 and 41.82 percent whereas in control (untreated fruits) it reached control 85.50% on 10th day of incubation. In challenge inoculation method on Alphonso variety the similar results were obtained.

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