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Screening mango germplasm against anthracnose and ecofriendly management of anthracnose in cultivar Neelum by pre and post-harvest treatments

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Abstract
Mango is one of the commercially important fruit crops cultivated in a large scale in Tamil Nadu. Though grown widely, there prevails a wide gap between the actual production and the quantity that reaches the consumers. There are many constraints which contribute to this gap among which is the post harvest incidence of anthracnose disease caused by *Colletotrichum gloeosporioides* (Penz. & Sacc.).

A preliminary screening of the mango germplasm at Horticultural College and Research Institute, Periyakulam against anthracnose caused by *C. gloeosporioides* (Penz. & Sacc.) revealed that among the 25 varieties, the choice varieties *viz*., Alphonso (12th day) and Sendhura (11th day) were found to have delayed initial infection of anthracnose and the time taken for more than 30 per cent infection on the fruit surface was 16th day in both the varieties. The least resistance to anthracnose was found in varieties *viz*., Neeleshan and Neelum which exhibited early initial infection on the fruit surface.

Three field trials were conducted at mango orchard of Horticultural College and Research Institute, Periyakulam during the period from March 2010 to April 2014 for the management of anthracnose in cv. Neelum being a susceptible variety. Ecofriendly and organic means *viz*., beneficial microorganisms, potassium bicarbonate were included in the treatment to formulate modules for the management. The result indicated that the treatment which comprised of two preharvest sprays of talc based formulation of *Pseudomonas fluorescens* (FP7) (0.5%) + *Burkholderia gladioli* strain TNAU 1 (0.5%) followed by post harvest hot water treatment (52ºC + 1ºC for 10 min) amended with potassium bicarbonate (1000 ppm) was very effective in the reduction of anthracnose to 19.78 PDI while in the untreated control, the incidence of post harvest anthracnose was found to be 98.00 PDI. However, this was next best to management using chemical fungicide for the mango anthracnose comprising two preharvest foliar sprays of carbendazim 50 WP (0.1%) starting from 30 days prior to harvest at 15 days interval followed by post harvest treatment of harvested fruits in hot water incorporated with carbendazim 50 WP (500 ppm). The widely adopted management practices in the pre and post harvest stages commonly involve chemical fungicides which lead to residual effect in the post harvest stage posing health risks to the consumers besides affecting the export market. Hence, as an alternative to chemical management, the next best treatment in this study using biocontrol agents can be recommended to the stakeholders.

Keywords: Anthracnose, *Colletotrichum gloeosporioides*, ecofriendly management, germplasm screening

Introduction
Mango occupies a distinguished place amongst the fruit crops grown in India and is being acknowledged as the king of fruits. Mango is adapted to wide range of soil, climate and altitude and is relatively easy to cultivate. Though 40% of total fruits grown in India is only mango (Pandey *et al*., 2012) [11], the productivity is low due to the wide range of climatic conditions and the diversity of the associated disease and disorder problems. A number of diseases affect the crop at all stages of its development right from the seedling in the nursery to the fruits in storage or transit. Among the diseases, anthracnose caused by *Colletotrichum gloeosporioides* Penz. is the most commonly prevalent fungal disease of mango (Prakash, 2003) [15]. The symptoms appear on all the aerial plant parts including leaves, stems, inflorescence and fruits. On the ripening fruits anthracnose is characterized by the development of black spots of varying forms which may be slightly sunken or may show cracks, eventually the entire fruit is covered by enlargement of the spots leading to fruit rot. This is more rapid after harvest as the fruits loose their natural resistance during ripening hastening the disease development. The infections leading to these losses may be initiated either in the field (pre harvest) or take place during post harvest phase (latent infection). Hence, to contain the post harvest infections control measures should be initiated in the field.

Prabakar *et al*. (2008) [12] had reported through *in vitro* studies that carbendazim (0.1%) inhibited the mycelial growth and conidial germination of *C. gloeosporioides*, as well when given as pre harvest spray in the field effectively checked the post harvest development of anthracnose in the fruits. The chemical fungicide though gives a thorough control of the disease, leads to residue problem especially when given as pre harvest sprays just before the harvest and post harvest treatment before transit. Also, the awareness among people on the ecofriendly measures has warranted the use of biological control which is an extensive area to be ventured. Vivekanathan *et al*. (2004) [25] recorded that the preharvest aerial spray with plant growth promoting rhizobacteria *Pseudomonas fluorescens* (FP7) amended with chitin at fortnightly intervals reduced latent expression of *C. gloeosporioides* in mango.

The application of bicarbonate salts in the biological management has exhibited the potential as an environment friendly measure in the control of wide range of fungi. Research on the use of bicarbonates and carbonates salts amended to water agar had been proved to be fungicidal to *Sclerotium rolfsii* by inhibiting the sclerotial germination (Punja and Grogan, 1982) [16]. Also application of bicarbonate salts was shown by Ziv and Zitter (1992) [28] to inhibit the fungal pathogens of cucurbits. Fallik *et al*., 1997 [2] have reported that preharvest foliar sprays with sodium and potassium bicarbonate solutions reduced the development of post harvest decay on pepper fruits. Therefore, management of the disease by combining chemicals and biocontrol agents will provide an amicable and ecofriendly strategy on which the study focuses. With this background, the study undertaken will promote the use of organic practices in the management of mango anthracnose through the usage of bicarbonate, biological agents and physical methods.

Materials and Methods

Screening the available mango germplasm

The mango germplasm at Horticultural College and Research Institute, Periyakulam was screened against anthracnose disease caused by *Colletotrichum gloeosporioides* (Penz. & Sacc.) for three consecutive mango seasons (2011-12, 2012-13 and 2013-14). The following varieties *viz*., Alphonso, Amrapali, Banganapalli, Himayuddin, Iswarya, Jehangir, Mulgoa, Mallika, Neeleshan, Neelum, Neeluddin, Panchatrakalasa, Pottalma, Ratna, Rumani, Sendura, Virudunagar local, PKM-1 and PKM-2 were assessed for the susceptibility to anthracnose during post harvest period. The experiment was conducted at ambient temperature and the disease rating scale (Dodd *et al*., 1991) [1] adopted for assessment is as follows.

Table 1: Rating scale for the assessment of mango fruit anthracnose

Disease rating	Symptoms
1	No fruit lesions
2	1 to 3 lesions on the fruit surface
3	4 to 6 lesions on the fruit surface
4	7 to 15 lesions on the fruit surface
5	More than 30% fruit surface covered with lesions

Management of anthracnose by pre and post harvest treatments: Field trials were conducted for three consecutive years (2011 – 12, 2012 – 13 and 2013 - 14) for the management of mango anthracnose with ten treatments and three replications in the cv. Neelum by pre and post harvest treatments. Two sprays starting from 30 days prior to harvest (@15 days interval) were given as pre harvest treatment. The harvested fruits were given hot water treatment at 52ºC + 1ºC for 10 min for treatments which included post harvest treatment. Water temperature was maintained at 52ºC + 1ºC for 10 min through an automatic thermostat installed water bath. The assessment of anthracnose incidence in the treatments was done under ambient condition using the above mentioned disease rating scale (0 - 5) on 25 fruits per replication. The treatment details are as follows

T_1: Preharvest spray of carbendazim 50WP @ 0.1 per cent concentration

T_2: Preharvest spray of potassium bicarbonate spray @ 0.5 per cent concentration

T_3: Preharvest spray of carbendazim 50WP @ 0.1 per cent followed by post harvest hot water treatment amended with carbendazim 50WP @ 500 ppm

T_4: Preharvest spray of potassium bicarbonate @ 0.5 per cent concentration followed by post harvest hot water treatment amended with potassium bicarbonate @ 1000 ppm

T_5: Preharvest spray of talc based formulation of *Pseudomonas fluorescens* (FP7) @ 0.5 per cent concentration

T_6: Spray of talc based formulation of *Burkholderia gladioli* strain TNAU-1 @ 0.5 per cent concentration

T_7: Preharvest spray of talc based formulations of *P. fluorescens* (FP7) @ 0.5 per cent + *B. gladioli* strain TNAU-1 @ 0.5 per cent concentration

T_8: Preharvest spray of talc based formulations of *P. fluorescens* (FP7) @ 0.5 per cent concentration + *B. gladioli* strain TNAU-1 @ 0.5 per cent followed by post harvest hot water treatment amended with potassium bicarbonate @ 1000 ppm

T_9: Post harvest hot water treatment alone at 52ºC + 1ºC for 10 minutes

T_{10}: Control (Untreated)

The PDI was assessed for the treatments when the infection in the control reached 100 per cent.

The Percent Disease Index (PDI) was calculated using Mckinney (1923) infection index

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{Total number of fruits/leaves observed}} X \frac{100}{\text{Maximum grade}}$$

The bacteria, *Pseudomonas fluorescens* (FP7) and *Burkholderia gladioli* strain TNAU-1 were reported to exhibit antagonism against *Colletotrichum gloeosporioides* when tested *in vitro* through dual plate technique (Vivekanathan *et al*., 2004 and Madhavan *et al*., 2011) [25, 6]. Hence, they were used in the experiment to evolve an ecofriendly measure alternative to the chemical methods used. The cultures of *Pseudomonas fluorescens* (FP7) and *Burkholderia gladioli* strain TNAU-1 was obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore and used in this study.

Results and Discussion

Screening the available mango germplasm

The initiation of anthracnose infection in the mango varieties screened at Horticultural College and Research Institute, Periyakulam and intensity of enlargement of the disease thereafter on the fruits for three consecutive years is given in the Table 2. The initiation of infection varied from 5 to 12 days and the expansion of the disease to cover more than 30 per cent of the fruit surface was 10 to 16 days across the varieties taken for observation. The varieties *viz*., Alphonso (12th day) and Sendura (11th day) had delayed initial infection of anthracnose and the time taken for more than 30 per cent infection on the fruit surface was 16th day in both the varieties which was comparatively longer than in other varieties. The least resistance to anthracnose was found in varieties *viz*., Neeleshan and Neelum which exhibited early initial infection on the fruit surface. Susceptibility of mango to number of diseases has been well documented through many studies. If left uncared, the nature and occurrence of the disease may completely killed or would debilitate the tree. Disease assessment technology is a useful tool to understand and minimise the damage caused by the disease. Horsfall & Cowling, 1978 [4] have stated that this technology is not only useful for research but is also essential for extension workers, administrator, environmental protection agencies, industries and the farmers to decide priorities and promotional activities. Sharma and Badiyala (1998) [18] observed that none of the cultivars of mango were resistant to anthracnose diseases in their studies. Further, recorded that under natural field condition, the cultivars *viz*., Amrapali, Toapuri, Safeda and Mallika were highly susceptible whereas Alphonso, Baramasi, Samer Bahisht, Rampur, Chausa and Sindhuri were moderately susceptible to anthracnose. The present investigation revealed that all varieties taken for the study were susceptible to the incidence of anthracnose. Alphonso and Sendura were able to resist the initial infection as well as the expansion of the disease to more than 30 per cent fruit surface as per the disease rating scale of Dodd *et al*., 1991 [1] when compared to other varieties. Hence, this study clearly indicates the variety which the farmers can choose for cultivation on a commercial scale especially aiming for the export market. Also, the knowledge on the variety which quickly expresses anthracnose infection posing a greater problem during the post harvest stage, can help in planning the management practices to be adopted in the pre and post harvest stage as the infection picks up in the field and remain latent without expression till the ripening starts.

Table 2: Screening of mango germplasm against anthracnose (2011 - 2014) (Pooled mean data of 2011-12, 2012-13 and 2013-14 mango seasons)

Sl. No.	Variety	Fruit Anthracnose incidence in the post harvest period	
		Appearance of initial infection or scale 2 (Days)	Time taken for attaining scale 5 (Days)
1.	Alphonso	**12**	**16**
2.	Amrapalli	9	13
3.	Banganapalli	9	13
4.	Himayuddin	8	12
5.	Iswarya	8	11
6.	Jahangir	6	10
7.	Malgoa	8	11
8.	Malika	9	12
9.	Neeleshan	5	9
10.	Neelum	5	9
11.	Neeludhin	6	10
12.	Panjatharakalasa	9	13
13.	Pottalma	10	14
14.	Ratna	9	12
15.	Rumani	7	12
16.	Senthura	11	16
17.	Virudunagar local	7	11
18.	PKM 1	8	13
19.	PKM 2	5	10
20.	Panjavarnam	9	13
21.	Peter	5	10
22.	Chinna swarnareka	9	14
23.	Kalapadi	5	10
24.	Banglora	8	13
25.	Neelum x Imam Pasand	9	13

Management of anthracnose by pre and post harvest treatments: In the experimental trail conducted from 2011-12 to 2013-14 period consecutively, the treatment with fungicides i.e., two pre harvest foliar sprays of carbendazim 50 WP @ 0.1 per cent starting from 30 days prior to harvest at 15 days interval followed by dip in hot water (52ºC + 1ºC for 10 min) incorporated with carbendazim 50 WP (500 ppm) was highly significant and the best in reducing the anthracnose incidence to 9.00 PDI. Also, the incidence of anthracnose on the fruits was 5% on the 5th day and 9.06% on the 10th day conversely the control recorded an incidence of 10.84% on the 5th day and 99% on the 10th day (Table 3). Singh *et al*., 2008 has reported that under *in vitro* condition, carbendazim was effective in completely inhibiting the growth of pathogen at 100 µg ml^{-1} concentration and besides provided remarkable control of anthracnose under field conditions. The complete inhibition of mycelial growth of *C. gloeosporioides in vitro* by carbendazim at all concentrations was reported by earlier research workers (Sharma and Verma, 2007; Tasival *et al.*, 2009 and Kolase *et al.*, 2014) [17, 21, 5].

However, the impetus given to the use of organic practices in the management of diseases warrants the research on the usage of biological methods. To make the concept viable, the treatments comprising of biological management was included in this trail and the result had shown that pre harvest foliar sprays with talc based formulation of *P. fluorescens* (FP7) (0.5%) + *Burkholderia gladioli* TNAU 1 (0.5%) twice starting from 30 days prior to harvest at 15 days interval followed by post harvest treatment of the harvested fruits in hot water (52ºC + 1ºC for 10 min) incorporated with potassium bicarbonate (1000 ppm) was effective in reducing the anthracnose disease to 19.78 PDI. In addition, the incidence per cent in this treatment was 34.33 when the control was 99 per cent (Table 3). Vidhyasekaran and Muthamilan, 1995 [22] have reported the use of talc based formulations of biocontrol agents for the management of several crop diseases. Studies conducted by Vivekanathan *et al*. (2004) [26] revealed that *P. fluorescens* (FP7) + chitin treatment reduced the anthracnose incidence to 60% and its efficacy was superior to standard fungicide carbendazim treatment when compared to untreated control. *In vitro* studies conducted showed higher inhibition of mycelial growth of the pathogen. In addition, preharvest application showed high levels of protection against latent infection development and reduced latent expression of anthracnose in stored condition.

Similar studies done by Viswanathan and Samiyappan (2001) [24] in sugarcane against red rot causing *Colletotrichum falcatum* revealed the enhanced biocontrol efficacy of flurorescent pseudomonads and the reason attributed for the efficacy was increased multiplication and enhanced chitinase activity of fluorescent pseudomonads. Madhavan *et al.* 2011 [6] reported that the foliar application of *Burkholderia gladioli* strain TNAU-1 was against *Colletotrichum gloeosporioides* and observed the induction of increased phenolics, three new peroxidase isozymes (PO-1, PO-2 and PO-3), polyphenol oxidase (PPO-1), chitinase and thaumatin-like proteins (TLP) in leaves of chilli (*Capsicum annuum* L.) in response to foliar application of *Burkholderia gladioli* strain TNAU-1.

The control of fungal diseases using chemicals which are safe to the environment and for the human directs the use of bicarbonates which exhibit potential for disease control in many crops and behaving as anion, several studies have registered it as the active portion in restricting the growth of the pathogen. The mode of action of bicarbonate salts is linked to the perturbation of pH, osmotic pressure and the bicarbonate/carbonate ion balance of sensitive fungi. Ziv and Zitter 1992 [28], found pronounced detrimental effects of bicarbonates on disease incidence and *in vitro* growth of several cucurbit foliar pathogens *viz*., *Alternaria cucumerina*, *Colletotrichum orbiculare*, *Didymella bryonlae* and *Ulocladium cucurbitae*. In the studies conducted by Palmer *et al*., 1997 [10] both potassium and sodium bicarbonates had similar effects in reducing radial growth of *Botrytis cinerea* and the colony diameter of *B. cinerea was* decreased by potassium bicarbonate at 50mM. In addition, recorded that bicarbonates may have several modes of action against fungi which includes buffering an elevated pH environment and increasing osmotic levels on leaf surfaces, both conditions were detrimental to fungal spores. Marku *et al*., 2014 [7] demonstrated that the severity of apple scab (*Venturia inequalis*) in leaves and fruit was significantly reduced by armicarb, a specially optimized formulation of potassium bicarbonate. The use of hot water in the post harvest treatment against anthracnose has been an old and successful technology known. Prakash *et al*., (2000) [14] observed that hot water treatment of 52ºC alone for 30 minutes was very effective in controlling anthracnose on mango cv. Dushehari.

Treating the affected fruits with hot water dips alone can significantly reduce anthracnose development, but the limitation in this technology is that the expression of heat damage by the fruit under some conditions of storage (Spalding and Reeder, 1986) [20]. Hence, to effectively use the technology, it is mandatory to optimise the temperature and duration of hot water treatment. One way to reduce the duration of exposure to hot water is by combining with chemicals which have fungicidal effects. McMillan and Mitchell, 1991 [9] have recorded that hot water treated fruits sprayed with preharvest fungicides had less anthracnose than the control or fruit treated with fungicide alone. Waskar and Gaikwad, 2005 [27] also observed that in mango, anthracnose and stem-end rot was effectively controlled by hot water treatment combined with bavistin (0.1%).

Table 3: Management of anthracnose in mango by pre and post harvest treatments – Pooled mean analysis (2011 - 14)

S. No.	Treatment	Incidence of Post harvest anthracnose (%)		Anthracnose (PDI)*	Per cent reduction over control *
		5th day	10th day		
1.	Carbendazim 50 WP (0.1%) spray	2.72	87.33	45.33^{e} (40.57)	53.76
2.	Potassium bicarbonate (0.5%) spray	4.94	95.00	75.11^{f} (51.01)	23.32
3.	Carbendazim 50 WP (0.1%) spray followed by hot water treatment amended with Carbendazim 50 WP (500 ppm)	0.00	9.06	9.00^{a} (24.59)	90.81
4.	Potassium bicarbonate (0.5%) spray followed by hot water treatment amended with potassium bicarbonate (1000 ppm)	0.00	36.56	24.11^{c} (32.80)	75.39
5.	*Pseudomonas* (FP7) (0.5%) spray	6.33	89.11	72.00^{f} (49.66)	26.54
6.	*Burkholderia gladioli* TNAU 1 (0.5%) spray	7.39	92.44	82.22^{g} (53.84)	16.12
7.	*Pseudomonas* (FP7) (0.5%) + *Burkholderia gladioli* TNAU 1 (0.5%) spray	4.44	82.50	73.78^{f} (50.33)	24.73
8.	*Pseudomonas* (FP7) (0.5%) + *Burkholderia gladioli* TNAU 1 (0.5%) spray followed by hot water treatment amended with potassium bicarbonate (1000 ppm)	0.00	34.33	19.78^{b} (30.89)	79.80
9.	Hot water treatment alone	0.00	49.56	29.00^{d} (34.80)	70.41
10.	Control (untreated)	10.94	99.44	98.00^{h} (65.85)	-
	SEd			0.72	
	CD(0.05)			1.45	
* pooled mean of 2011 – 12, 2012 – 13 and 2013 - 14					

Values in parentheses are arcsine – transformed values.

In a column, means followed by same letter do not differ significantly ($P< 0.05$) according to DMRT

Conclusion

Mango is one of the commercially important fruit crops cultivated in a large scale in Tamil Nadu. The quality of mango fruits affected by anthracnose results in poor market appeal and consumer acceptability. One of the important factors causing quality deterioration in mango is the postharvest incidence of anthracnose disease caused by *C. gloeosporioides* (Penz. & Sacc.). The incidence of anthracnose pathogen affects the tree in the pre harvest stage making the tree unproductive as it infects both immature and mature tree parts such as twigs, branches, panicle, flower and fruit stalks etc., providing inoculum for latent infection which expresses in the post harvest stage during ripening and storage of mango fruits. The widely adopted management practices in the pre and post harvest stages commonly involve chemical fungicides which lead to residual effect in the post harvest stage posing health risks to the consumers besides affecting the export market. The results of this study will help the stakeholders of mango industry especially the farmers and processing industries to choose a variety with minimum latent infection in order to minimise the post harvest loses. Secondly, it will promote the use of organic practices in the management of mango anthracnose through biological agents and physical methods.

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