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Management of quiescent pathogens rots of mango with preharvest spraying of true fungicides and salicylic acid

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Abstract

The shelf life of mango 'the king of fruit' is found to be affected by several biotic and abiotic stresses as well as their various combinations. However, pathogenic infections in latent and quiescent state reduce commercial values by aggravating the dynamics of rotting and reduction in quality. Mango is succumbed to infection by several pathogens both before and after harvest. *Colletotrichum* state of *Glomerella cingulata* and *Lasiodyplodia theobromae* are important quiescent postharvest pathogens of mango and both of these survive within old foliage despite the prevailing high summer temperature above 35°C, chilling winter below 10 °C and heavy rains approx. 1100mm during monsoon months. A single preharvest foliar spray at 85 days before harvest with two fungicides namely Dithane M-45 (0.25%) and Bavistin (0.15%) followed by a single spray of Salicylic acid (0.5 – 2.0mM) before 30 days of harvest on young immature fruits was carried out to evaluate the efficacy of different treatments. The present finding suggests that both Dithane M-45 (0.25%) or Bavistin (0.15%) followed by Salicylic acid (2.0mM) are equally effective treatments with disease reduction of 66.67% over control for a storage period of 15 days at ambient room temperature.

Keywords: Postharvest, *Colletotrichum*, *Lasiodyplodia theobromae* quiescent, fungicide, salicylic acid

Introduction

Mango fruit has been infected by several postharvest pathogens which result in considerable loss in fruit quality. Postharvest disease development is a major constraint to the quality and shelf life of mango fruit thereby limiting its domestic and export marketing (Bally *et al.*, 2009)^[4] as well as resulting in heavy economic losses (Barkai-Golan, 2001; Narayanasami, 2006)^[5, 21]. Like other fresh commodities, mango has also been found prone to postharvest fruit decay due to rapid disease development during storage and ripening (Prusky *et al.*, 2009)^[22]. Anthracnose (*Colletotrichum gloeosporioides*) is regarded one of the major postharvest diseases of mango (Bally *et al.*, 2009)^[4]. Stem end rot (SER) and black spots (i.e., *Alternaria* rot) have also been reported to cause significant postharvest decay in mango (Prusky *et al.*, 2009)^[22]. Mango sea-freight trial shipments from Pakistan to Germany also indicated SER as the major concern for high postharvest losses (Malik *et al.*, 2010)^[18]. The role of *C. gloeosporioides*, *Cytosphaeria manqiferae*, *Lasiodyplodia theobromae* and *Pestalotiopsis* sp. has also been described by various researchers (Sangchote, 1987; Bagshaw, 1989; Johnson *et al.*, 1991b)^[26, 2, 14]. Most of these pathogens get invaded into the plant tissues; colonize the fruit peduncle during panicle emergence and flowering; penetrate into the fruit tissues during fruit development and maturation period by endophytic hyphal growth and cause SER during ripening (Johnson *et al.*, 1991a, 1992, 1993; Everett, 2001)^[12, 11, 13, 8]. In the past, various attempts have been made worldwide to ascertain the pathogen, mode and source of infection, stage of infection and development of SER (Johnson *et al.*, 1993)^[13]. Furthermore, various pre and postharvest disease management studies have been reported in literature including fungicidal treatments (Sanders *et al.*, 2000; Mortuza *et al.*, 2003)^[25, 20], hot water dips and vapor heat treatments (Esguerra *et al.*, 2004; Sopee & Sangchote, 2005)^[7, 28], emulsion coatings (Diaz-Sobac *et al.*, 2000)^[6]. Disease control by inducing host resistance and activating the defense mechanisms in plants (especially herbaceous plants) and harvested fresh produce has also been explored (Johnson & Hofman, 2009)^[10]. Plant hormones were tried and found to be effective in extending shelf-life and reducing post-harvest losses by delaying

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ripening and senescence in many fruit species (Lurie *et al.* 2010) [17]. Salicylic acid is a well known natural inducer of disease resistance in plants (Sticher *et al.*, 1997) [29] and its performance against mango anthracnose has been reported (Zainuri *et al.*, 2001; Zeng *et al.*, 2006) [33, 35]. SA at 200 ppm was found to be most effective in delaying the ripening cum senescence processes of mango (amrapalli) (Reddy and Sharma, 2016) [23]. Carbendazim has been used widely to control various pre and postharvest diseases in horticultural crops (Mortuza *et al.*, 2003; Stovold & Dirou, 2004; Khanzada *et al.*, 2005) [20, 30, 15]. However, its use has been restricted by some markets of the world due to its hazardous residual effects, acute (WHO, 1996) [32] and chronic (Mantovani, 1998) [19] on the health of consumers. The modern food safety concerns have led to withdraw the approval for the use of carbendazim on fruit crops (Anonymous, 2009) [1].

The natural resistance at host peel gradually reduced along with the onset of ripening and the phenomenon allows the quiescent pathogens to proliferate within the fruits and cause extensive damage to mesocarp. The induction of resistance in field crops by chemical method is gradually being utilized in LDC and Developing countries to combat the diseases yet commercial success in fruit crops is limited and Indian experience is still at infancy. Preharvest spraying just prior to harvest of several fungicides although effective but residual toxicity of pesticides also poses serious concern to human health and trigger reluctance due to visible residues to consumers. Therefore, an attempt has been made to integrate both direct fungicidal control at long prior to harvest and use of resistance inducers as a successful management strategy to get rid of the menace of these two serious pathogens.

Materials and Methods

The experiment was carried out during 2017 on the mango cv. *Amrapali* in the 15 years old orchard of Palli-Siksha Bhavana Agricultural Farm, Sriniketan, West Bengal, India. For pre-harvest fungicide application, 22 mango trees were selected at Agricultural farm of Palli Siksha Bhavana, Sriniketan, Birbhum, West Bengal. These 22 trees were selected randomly eliminating the border rows. The mango variety selected was amrapali and each tree was considered as a treatment. After 85 days of fungicide treatment and 30 days of salicylic acid treatments the fruits were harvested, graded, packed in corrugated cardboard boxes and brought to department of plant protection, PSB and kept at room temperature for observation and data collection. Harvested fruits were stored during May 19th to 8th June 2017 at room temperature approx. 32±3 °C.

Details of treatment: Selected mango trees were sprayed with Dithane M- 45 at the rate of 0.25% and Bavistin at the rate of 0.15% using foot sprayer during flowering stage. Control trees were also maintained simultaneously without any spray. Salicylic acid was sprayed in different concentrations at 30 days before harvest and the treatment were arranged in following manner.

T₁ = Trees sprayed with Dithane M 45 @ 0.25%

T₂ = Trees sprayed with Bavistin @ 0.15%

T₃ = Control without any spray

Salicylic acid (SA) were sprayed with following dose

S₁ = 0.5mM concentration solution

S₂ = 1.0mM concentration solution

S₃ = 1.5mM concentration solution

S₄ = 2.0mM concentration solution

Combination of treatments: T₁S₁ = Dithane M-45 @ 0.25% + SA @ 0.5 mM, T₁S₂ = Dithane M-45 @ 0.25% + SA @ 1.0 mM, T₁S₃ = Dithane M-45 @ 0.25% + SA @ 1.5 mM, T₁S₄ = Dithane M-45 @ 0.25% + SA @ 2.0 mM, T₂S₁ = Bavistin @ 0.15% + SA @ 0.5 mM, T₂S₂ = Bavistin @ 0.15% + SA @ 1.0 mM, T₂S₃ = Bavistin @ 0.15% + SA @ 1.5 mM, T₂S₄ = Bavistin @ 0.15% + SA @ 2.0 mM

PDI (Percent Disease Index) calculation:

10 point (0-9) scale of disease have been adopted for calculating the PDI. The scale was as follows

Disease Grade	Percent of fruit surface infection
0	: No symptom/infection
1	: < 2% infection
2	: 2-5% of infection
3	: 5-10% of infection
4	: 10-25% of infection
5	: 25-45% of infection
6	: 45-70% of infection
7	: 70-85% of infection
8	: 85-95% of infection
9	: > 95% of infection

$$PDI = \frac{\sum \text{All disease ratings}}{\text{No of observations} \times \text{Maximum disease grade}} \times 100$$

Percent reduction of postharvest rotting of Mango:

Percent reduction of rot over control was calculated by using the following formula

$$\text{Percent reduction of rot} = \frac{C-T}{C} \times 100$$

Where, C = rotting in control

T = rotting in the treatment

Statistical analysis: All experiments were conducted in a completely randomized design with ten replications, for each treatment. The statistical analysis of the results was conducted in MS excel sheet using analysis of variance (ANOVA) at p = 0.05 significance level.

Results and Discussion

Disease index of mango: The PDI of mango fruits were calculated and presented in table no.1 and 2 for different fungicides and salicylic acid treatments. Table 1 indicated that PDI of each treatment with salicylic acid differs significantly. Cent percent fruits were rotted under control in 20days after harvest where as fruits treated with Dithane M-45 and salicylic acid (SA) at 2.0mM rotted 68.89% which showed the best result. If we look at 15th day after harvest treatment 2.0mM SA and Dithane M-45 showed only 22.22% of rotting as compare to 66.67% rotting in control (Plate no.1). There was no rotting at 8th day after harvest in SA treatment with 1.5mM and 2.0mM. Similar observations were also noticed in treatments with Bavistin and SA. The results implied that fruits treated with Dithane M-45 and Bavistin and SA with 2.0mM has the significant effect on disease reduction over control.

Table 1: PDI of Mango fruit rot sprayed with Dithane M-45 @ 0.25% and different dose of salicylic acid before fruit harvest

Treatments	Dithane M-45 @0.25%			
	8th Day	10th Day	15th Day	20th Day
SA @0.5 mM	2.22	8.89	64.44	95.56
SA @1.0 mM	2.22	8.89	44.44	88.89
SA @1.5 mM	0.00	4.44	40.00	82.22
SA @2.0 mM	0.00	2.22	22.22	68.89
Control	7.99	17.78	66.67	100.00
SEm	0.00	1.62	3.35	3.49
CD (p=0.05)	0.01	5.11	10.54	11.00

Table 2: PDI of Mango fruit rot sprayed with Bavistin @ 0.15% and different dose of salicylic acid before fruit harvest

Treatments	Bavistin @0.15%			
	8th Day	10th Day	15th Day	20th Day
SA @0.5 mM	2.22	8.89	62.22	96.56
SA @1.0 mM	0.00	6.67	40.00	93.33
SA @1.5 mM	0.00	2.22	28.89	76.78
SA @2.0 mM	0.00	0.00	22.22	71.11
Control	7.99	17.78	66.67	100.00
SEm	0.00	1.28	3.76	3.23
CD (p=0.05)	0.01	4.04	11.85	10.16

Percent reduction over control: Table 3 also reflected that the maximum disease reduction was recorded in fruits treated with 2.0mM SA treatment as preharvest spray in both Dithane M-45 and Bavistin sprayed fruits. Maximum shelf life of fruits without any infection recorded upto 11 days after harvest in fruits treated with Bavistin and 2.0mM SA. However both the results showed both Dithane M-45 and Bavistin with SA (2.0mM) are equally effective in reducing the postharvest rot of mango. The trial was preliminary and it is necessary to take more such trial with more different combination for finalization of recommendation.

Table 3: Percent reduction of postharvest rot of mango due to quiescent pathogens by preharvest spraying of true fungicide and salicylic acid.

Treatments		Shelf life without Infection (days)	Percent reduction of rotting over control			
			8 th day	10 th day	15 th Day	20 th day
Dithane M-45 (0.25%)	SA 0.5mM	8	72.18	50	6.33	4.44
	SA 1.0mM	8	72.18	50	33.33	11.11
	SA 1.5mM	9	100	75.02	39.99	17.78
	SA 2.0mM	10	100	87.51	66.67	31.11
Bavistin (0.15%)	SA 0.5mM	8	72.18	20	6.66	4.44
	SA 1.0mM	9	100	62.49	39.99	6.67
	SA 1.5mM	10	100	87.51	56.67	22.22
	SA 2.0mM	11	100	100	66.67	28.89
Control		7	0	0	0	0

Discussion

Use of SA to delay in fruit ripening, softening, and inducing disease resistance and reducing disease incidence were discussed by Raskin, 1992^[24]. Reddy and Sharma, 2016^[23] reported that application of salicylic acid as a pre-harvest spray with 200 ppm, effectively modulate the ripening behavior of the Amrapali mango fruits during storage at ambient conditions. Liu *et al.*, 1998^[16] reported the disease index of mango fruits caused by *Colletotrichum gloeosporioides* decreased significantly when the inflorescences were treated with salicylic acid 100 mg⁻¹ L. They concluded that salicylic acid could induce mango fruits to have the effect in resistant to *Colletotrichum gloeosporioides*. Fruits inoculated with *Colletotrichum gloeosporioides* and treated with SA showed the disease incidence of 62.5% as that in control in the fourth day and concluded that SA could enhance resistance of mango fruits to anthracnose disease (Zeng and Jiang, 2005)^[34] SA at the concentration of 200 mg⁻¹ L reduce the Anthracnose disease and changes of fruit skin color and reduce firmness significantly (Zainuri *et al.*, 2001)^[33]. Application of SA with suitable dose enhanced the efficiency of antioxidant system in plants (Hayat *et al.*, 2010)^[9]. Tian *et al.*, 2007^[31] recorded that SA treatment at nontoxic concentrations reduces postharvest diseases in fruits by enhancing activities of different enzymes like PPO, PAL and β -1,3-glucanase. SA and KMnO₄ treatments, alone or in combination, is effective methods of increasing the shelf life of kiwifruits in storage

(Bal and Celik, 2010)^[3]. Shi, *et al.* (2006)^[27] reported preharvest treatment by a foliar spray of 1 mM SA might have a signaling function in induction of heat tolerance in cucumber seedling as indication by an increase in H₂O₂ concentration which act as signal molecules initiating several protective resistance mechanisms against pathogens, chilling and heat stress. Salicylic acid application as pre-harvest spray, one week prior to harvest could effectively change the ripening behavior of the Amrapali mango fruits and extend the fruit shelf life by 3 days (Reddy and Sharma, 2016)^[23] Our findings also corroborated the findings of all the researchers. Salicylic acid and fungicide at preharvest spray induce resistance in mango (Amrapali) fruits. The use of fungicides may not have the residual effect on the fruit as these were used at flowering state, although it has not been tested. However more works to be carried out to find the residual effect on the mango as pre flowering to fruit formation stage at fruit ripening.

Conclusion

Preharvest spraying of both Dithane M-45 (0.25%) and Bavistin (0.15%) followed by Salicylic acid (2.0mM) are equally effective treatments with disease reduction of 66.67% over control for a storage period of 15 days at ambient room temperature. The enhancement of further shelf life may be possible with a combination of low temperature storage and fungicidal treatments. Large scale trial, however, becomes essential before commercial recommendation.



A.

B.

Plate 1: Fruits showing disease severity at 15 days after harvest. **A:** Dithane M-45(0.25%) + Salicylic acid @2.0mM; **B:** Bavistin(0.15%) + Salicylic acid @2.0mM

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