POSTHARVEST APPLICATION OF DEFENCE ELICITORS TO MANAGAE ANTHRACNOSE IN MANGO FRUIT

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ABSTRACT

The use of defense elicitors; which are agents that stimulate or trigger disease resistance responses in plants have proved to be attractive alternatives to hazardous chemical fungicides in recent years. The present study investigated the possibility of using Salicylic acid and Bion® as postharvest elicitors on mango fruit. Three local mango cultivars were used in the study, ‘Karuthacolomban’, ‘Rata’ and ‘Willard’. Salicylic acid was applied as a postharvest spray at concentrations, 100, 500 or 1000 mg/L and Bion® (Acibenzolar-s-methyl, 500 WG, SYNGENTA) at 25, 50, 100 and 200 mg/L. Disease development following artificial inoculation (10^5 conidia / ml suspension) was assessed. Both elicitors were effective ($P < 0.05$) in controlling postharvest anthracnose. The most effective concentration of SA was 500 mg/L for ‘Rata’, 100 mg/L for ‘Willard’ and both 100 and 500 mg/L for ‘Karuthacolomban’. In Bion® the most effective concentration was 50 ppm for ‘Rata’ and ‘Willard’ and 25 ppm for ‘Karuthacolomban’. In SA treated fruits the percentage reduction in anthracnose ranged

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from 40- 77 % while values ranged from 67 – 99% for Bion® treated fruits. Results indicate that both SA and Bion® can be used to control postharvest mango fruit loss due to anthracnose.

Keywords: Mango, Anthracnose, Elicitors, Bion®, Salicylic acid

INTRODUCTION

Elicitors are defined as agents that stimulate or trigger disease resistance responses in plants. These agents may be physical, chemical, biotic or even genetic (Bell, 1981). Resistance may be induced as local acquired resistance (LAR), systemic acquired resistance (SAR) or induced systemic resistance (ISR) (Terry and Joyce, 2004). Use of elicitors to combat postharvest diseases of mango has been extensively studied. Zainuri *et al.*, (2001) reported a significant reduction in mango anthracnose when treated with 200 mg L⁻¹ of salicylic acid. A significant reduction in disease development, both under natural and artificial inoculation conditions, could be obtained by post-harvest dips in 2000 mg L⁻¹ of salicylic acid. In post-harvest vacuum infiltration, 1000 mg L⁻¹ of salicylic acid (SA) was sufficient to give a significant reduction in anthracnose (Zainuri *et al.*, 2001). The reduction in disease development was attributed to delay in fruit ripening brought about by an anti-ethylene effect of salicylic acid. In Thailand, mango inflorescences were treated with SA at a concentration of 100 mg L⁻¹ which significantly reduced anthracnose disease incidence (Leu, 1998). Postharvest dip treatments in SA increased polyphenol oxidase activity, phenyl alanine lyase activity, β -1,3-glucanase and chitinase activity in treated fruits of cultivar ‘*Matisu*’ (Zeng *et al.*, 2006). The level of hydrogen peroxide and superoxide radicals which can act as secondary messengers to activate plant defence were also enhanced by salicylic acid treatment (Zeng *et al.*, 2006). Zhu *et al.*, (2008) vacuum infiltrated mango fruits of the cultivar ‘*Tainong*’ with BTH (benzo-(1,2,3)-thiadiazole-7-

carbothioic acid S-methyl ester) (analogous to Bion®) at a concentration of 1.0 mM, incubated fruits at 13°C and 85–90% RH for 72 hr and then, inoculated fruits. Results showed that both disease severity and incidence were significantly reduced in fruits treated with BTH. Peroxidase, polyphenoloxidase, phenylalanine ammonia-lyase, chitinase and β -1,3-glucanase activities and total phenolic compound content in the fruits were enhanced by the BTH treatment during the incubation. Zhu *et al.*, (2008) suggested that these responses may be involved in the induced resistance against pathogen infection. In Sri Lanka, Karunanayake *et al.*, (2016) reported that pre-harvest field application of salicylic acid (SA) and Kasil (liquid potassium silicate) reduced postharvest anthracnose severity and incidence in mango cv ‘Karuthacolomban’. The present study investigates the effect of postharvest spray treatment with elicitors, SA and Bion® in reducing anthracnose development on three local mango cultivars with varying natural resistance to anthracnose.

METHODOLOGY

Sample selection

Healthy, unripe mango (*Mangifera indica*) fruits of three cultivars at harvesting maturity, showing no visible blemishes, were selected for experiments. Three cultivars used were; ‘Karuthacolomban’ highly resistant to anthracnose, ‘Willard’ highly susceptible to anthracnose and ‘Rata’ moderately resistant to anthracnose (Karunanayake *et al.*, 2014). Fruits used for experiments were obtained from a wholesale market at Kiribathkumbura, which had a regular supply of mango fruits from Dambulla and the fruits were transported to the laboratory within 24 hr of harvest.

Fruits were washed in running tap water, spread out on clean newspapers and allowed to air dry under ambient conditions.

Isolation and maintenance of the pathogen

Colletotrichum gloeosporioides was isolated from ripe mango fruits showing characteristic symptoms of anthracnose disease. Segments (1 cm³) were excised from the leading edge of characteristic anthracnose lesions of the fruit. Isolation and culturing of fungi were carried out under aseptic conditions. The excised tissue segments were placed in a sterile Petridish and surface-sterilized in a 1% solution of sodium hypochlorite (NaOCl) (commercial Clorox solution, diluted to 1/5) for one minute, then washed twice in sterile distilled water to remove all traces of NaOCl. The sterilized tissues were then trimmed to expose the causative organism within. Tissues were dried on sterile blotting paper and aseptically transferred to Petridishes containing potato dextrose agar (PDA). Sub-culturing was carried out once the fungal cultures were grown to obtain and maintain pure cultures. Koch's postulates were performed to confirm the pathogenicity of each isolate. Sub-culturing was carried out at 14 day intervals and conidia from 14 day-old pure cultures were used for inoculations.

Elicitor application

Surface sterilized fruits were sprayed with selected concentrations of elicitor solutions, salicylic acid (sodium salt) or Bion®, which were prepared approximately half an hour before the application. The concentrations of salicylic acid (sodium salt, SIGMA®) used were, 0 (control), 100, 500 and 1000 mg/L (1 mM= 160 mg/L) and the concentrations of Bion® (Acibenzolar-s-methyl, 500 WG, SYNGENTA) used were, 0 (control) 25, 50, 100 and 200 ppm. As Bion® (Syngenta) contained only 50 % active ingredients, to prepare 25 ppm, 2.5 mg Bion® was dissolved in 50 ml of sterile distilled water (1 ppm=1 mg/1000 ml). Each elicitor concentration was prepared to contain 50 ml of solution. Tween 20 (Sigma ®) was added to each elicitor solution (10 µl per 100 ml solution) to enable good wetting of the fruit. Sterile distilled water with Tween 20 served

as the control. The prepared solutions were evenly sprayed on to the entire surface area of each fruit, until finely misted, using an atomizer (KNF NEUBERG D-79112 Freiburg). Eight replicate fruits were used per elicitor concentration in one trial and three trials were performed. Sprayed fruits were maintained in moist chambers at 28 °C and under 100 % relative humidity.

Inoculation of fruits

After 72 hours of incubation, the fruits were removed from the moist chambers and the inoculated surface of each fruit was wiped with sterile cotton wool moistened with sterile distilled water. This was done to remove elicitor residues remaining on the fruit surface. The fruits were inoculated with a conidial suspension of *C. gloeosporioides* (10^5 conidia / mL). Three, 20 µl drops of the conidial suspension was placed along the long axis of each fruit and the position of each drop was outlined using a carbon pen. Inoculated fruits were returned to moist chambers for incubation.

Daily observations were made on salicylic acid treated, Bion® treated and distilled water treated (control) fruits. After the initiation of lesions, the diameter was measured on two axes at right angles to each other at each inoculated spot and the average was obtained. Diseased area was mathematically calculated using the average radius from the diameter measurement. The reduction in lesion area in treated fruits as an average value compared to the lesion area of control was mathematically calculated and a percentage value was calculated.

Statistical analysis

Three trials were performed and each trial was considered as a separate block and data pertaining to each day was analyzed for variance using ANOVA in a completely randomized block design with SAS computer software (version 6.12) at the 5 % probability level along with Duncan's MRT to compare the treatments.

The toxicity of elicitors against *C. gloeosporioides*

The direct fungi-toxicity of the effective concentrations of elicitors selected from the above experiment against *C. gloeosporioides* was determined using a spore germination assay. A double strength solution of the optimum concentration of elicitors were prepared and mixed in a 1:1 ratio with a suspension of conidia of *C. gloeosporioides* (also prepared at double strength). A 1:1 dilution of the conidial suspension with sterile distilled water was used as the control. Two 20 µl drops of the mixture were placed on either side of a clean glass slide. Two slides were prepared for each elicitor and two for the control. The slides were arranged in a slide rack and incubated in a moist chamber. Germination was terminated following 16 hours of incubation by adding a drop of lactophenol mixed with cotton blue. One hundred randomly selected conidia in each drop were counted (from several fields of vision under high power of a light microscope) for germination and the percentage germination was calculated. The experiment was performed twice.

RESULTS

Elicitor application

Salicylic acid

Different cultivars responded differently to the elicitor treatment. Anthracnose lesions were visible on cultivars 'Rata' and 'Willard' four days after inoculation whereas, lesions on cultivar 'Karuthacolomban' appeared 7 days after inoculation. In all three cultivars, anthracnose lesions were smaller in fruits which received salicylic acid treatments prior to inoculation compared to the control fruits. In cultivar 'Rata' the smallest anthracnose lesions were in the fruits treated with 500 mg/L salicylic acid and in fruits of cultivar 'Willard' upon inoculation, the smallest lesions were developed on fruits treated with 100 mg/L SA (Table 1). In cultivar 'Karuthacolomban' both 100 and 500 mg/L SA seemed to be effective. However, fruits treated with either higher or lower concentrations resulted in larger lesions (Table 1).

Statistical analysis showed that fruits treated with 500 mg/L salicylic acid in cultivar 'Rata' or 100 mg/L in cultivar 'Willard' developed significantly smaller lesions ($P < 0.05$) (Table 1).

Table 1. Mean lesion area (mm²) in inoculated fruits treated with varying concentrations of Salicylic acid as a postharvest treatment prior to inoculation.

Treatment	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Cultivar 'Rata'							
Control	8.16 ^a	13.65 ^a	17.5 ^a	38.33 ^a	70.51 ^a	136.12 ^a	187.7 ^a
100 mg/L	5.13 ^a	13.13 ^a	17.33 ^a	32.85 ^{ab}	70.37 ^a	115.41 ^{ab}	169.26 ^a
500 mg/L	4.24 ^a	9.7 ^a	9.97 ^b	17.9 ^b	38.85 ^b	82.43 ^b	99.7 ^b
1000mg/L	5.13 ^a	11.28 ^a	13.79 ^{ab}	31.94 ^{ab}	59.61 ^b	114.31 ^{ab}	155.7 ^a
Cultivar 'Willard'							
Control	46.57 ^a	149.7 ^a	284.63 ^a	441.2 ^a	622.3 ^a		
100 mg/L	16.20 ^b	40.29 ^b	91.02 ^b	172.4 ^b	224.0 ^b		
500 mg/L	20.15 ^b	70.69 ^a	161.42 ^a	329.2 ^a	407.2 ^a		
1000mg/L	16.20 ^b	33.84 ^b	106.44 ^b	230.6 ^a	323.8 ^a		
Cultivar 'Karuthacolomban'							
Control				2.9 ^a	4 ^a	14.5 ^a	23.6 ^a
100mg/L				1.6 ^a	2.5 ^a	7.5 ^a	8.9 ^b
500 mg/L				0.67 ^b	2.2 ^a	10.6 ^a	13.6 ^a
1000mg/L				1.8 ^a	4.4 ^a	8.6 ^a	12.6 ^a

Values followed by the same letter within each column of each cultivar do not differ significantly at the 5 % probability level (Duncan's Multiple Range Test).

Bion® (Acibenzolar-S-methyl)

Different cultivars responded differently to the elicitor treatment. Lesions were measured for ten consecutive days following inoculation and measurable lesions developed five days after inoculation in the cultivars ‘Rata’ and ‘Willard’ (Figure 1, 2). In cultivar ‘Karuthacolomban, lesions appeared 7 days after inoculation (Figure 3). The lesions which developed following inoculation in Bion® treated fruits were significantly smaller in size compared to the lesions in control fruits in all three cultivars (Figure 1, 2, 3). The smallest lesions were visible in the fruits treated with 50 ppm Bion® in the cultivars ‘Rata’ and ‘Willard’ (Figure 1, 2) and in fruits treated with 25 ppm Bion® in the cultivar ‘Karuthacolomban’ (Figure 3).

Figure 1: a) Anthracnose lesion development in fruits of cultivar ‘Rata’ after treatment with Bion® as a postharvest elicitor treatment followed by artificial inoculation.

Figure 2: Anthracnose lesion development in fruits of cultivar ‘Willard’ after treatment with Bion® as a postharvest elicitor treatment followed by artificial inoculation.

Figure 3: Anthracnose lesion development in fruits of cultivar ‘Karuthacolomban’ after treatment with Bion® as a postharvest elicitor treatment followed by artificial inoculation.

Treating fruits with 50 ppm Bion[®] reduced anthracnose development in cultivars ‘Rata’ and ‘Willard’ by a large percentage on days five to ten after inoculation. This reduction ranged from 67 to 95 % in cultivar ‘Rata’ and 80 to 99 % in cultivar ‘Willard’. In cultivar ‘Karuthacolomban’, treating fruits with 25 ppm Bion[®] reduced anthracnose development by a percentage ranging from 72 to 94 % (Table 2).

A high, significant percentage reduction in lesion area ranging from 67 % to 99 % was achieved by the Bion[®] treatment. The percentage reduction in anthracnose development in fruits treated with salicylic acid was less than that in fruits treated with Bion[®]. Treating fruits with 500 mg/l salicylic acid reduced anthracnose development by 40 % on day eight after inoculation and by 53 % on day 6 after inoculation which were the lowest and highest percentage reductions in lesion area (respectively) in the cultivar ‘Rata’. Similarly, the percentage reduction in anthracnose development by treating fruits of cultivar ‘Willard’ with 500 mg/L salicylic acid ranged from 60 to 73 %. Further, treating fruits of cultivar ‘Karuthacolomban’ with 100 mg/l salicylic acid reduced anthracnose development by a percentage ranging from 27 to 77 % (Table 2).

Table 2: Average reduction (%) in lesion area at the optimum elicitor concentration.

Treatment	Percentage reduction in lesion area (%)					
	Days after inoculation					
	5	6	7	8	9	10
Salicylic acid						
‘Rata’ (500 mg/l)	45	53.3*	45*	40*	46.8*	
‘Willard’ (100 mg/l)	65.2*	73.08*	68*	60.9*		
‘Karuthacolomban’ (500 mg/l)	‘ND’	‘ND’	76.9*	45	26.9	42.3
Bion						
‘Rata’ (50 ppm)	94.9*	88*	84.9*	66.9*	67*	69.2*
‘Willard’ (50 ppm)	99.2*	98*	95.4*	79.2*	87*	80.6*
‘Karuthacolomban’ (25 ppm)	‘ND’	‘ND’	94.3*	72.4*	81.8*	84.9*

‘ND’, measurable lesions had not developed.

* Denotes significant reduction in lesion area at the 5% probability level, opposed to the control

The toxicity of elicitors against *C. gloeosporioides*

Percentage conidia germination of *C. gloeosporioides* was not significantly different among the treatments at $P = 0.05$. Conidia treated with SA, Bion and untreated control reported 22.6, 22.4 and 22.3 % germination respectively, 16 hours after incubation in the moist chamber, indicating no significant reduction of conidia germination by the two elicitors.

DISCUSSION

Anthrachnose development commenced sooner in cultivars ‘Rata’ and ‘Willard’ which are more susceptible to anthracnose (Karunanayake *et al.*, 2014), compared to cultivar ‘Karuthacolomban’, which is more resistant. Further, the anthracnose lesions were also larger in cultivar ‘Willard’ known to be highly susceptible to anthracnose (Karunanayake *et al.*, 2014) while lesions were smaller in ‘Rata’ moderately susceptible to anthracnose and smallest lesions were seen in cultivar ‘Karuthacolomban’ which is reported to be highly resistant to anthracnose (Karunanayake *et al.*, 2014). These differences in anthracnose development between cultivars are due to innate cultivar effect and not due to elicitor treatments. In the postharvest spray treatments, within each cultivar, all concentrations of the elicitors, salicylic acid and Acibenzolar-S-methyl (Bion®), reduced anthracnose development compared to the untreated control. Of the two elicitors tested at postharvest stage, Acibenzolar-S-methyl (Bion®) was more efficient. The effective concentration of Bion®; 25 or 50 ppm was much lower than the effective concentrations of SA; 100 or 500 mg/L. However, the percentage reduction in lesion area was greater by treatment with Bion®. The

optimum concentration of salicylic acid was 500 mg/L (500 ppm) for cultivar 'Rata' but a lower concentration (100mg/l) was needed from salicylic acid to control anthracnose in the more susceptible cultivar 'Willard'. A lower concentration (25 ppm) of Bion® was sufficient to control anthracnose in the more resistant cultivar 'Karuthacolomban'. Wanigasekara *et al.*, (2014) however, reported that in Banana cv. 'Embul' pre-harvest spray treatment with SA at 500 mg/L was 22% more effective than 200 mg/L Bion® in controlling postharvest anthracnose.

In postharvest application, anthracnose severity was reduced to a greater extent in the more anthracnose susceptible cultivar 'Willard' as opposed to the other two cultivars. A similar incidence was reported by Dann *et al.*, (1998), where they could control white mold (*Sclerotinia sclerotiorum*) disease severity (20 to 60 %) in soybean with the application of Bion® as a soil drench.

Unlike in a fungicide where the fungus is killed and the most effective in doing so would be the highest concentration, elicitors activate the plant defences to protect against invading pathogens at low concentrations. The most effective dose of the two elicitors salicylic acid and its functional analogue Bion® was not the highest but an optimum. Yu and Zheng (2006) reported that salicylic acid could improve the efficacy of the bio-control agent *Cryptococcus laurentii* against blue mould disease in apple caused by *P. expansum*. The most effective concentration was 10 µg ml⁻¹ whereas higher or lower concentrations tested were less effective.

The optimum concentration of elicitors used in the present study was not directly toxic to *C. gloeosporioides*, as indicated by the conidia germination assay, although higher doses have been reported to have direct fungitoxic effects. Zainuri *et al.*, (2001) report that

1000, 2000 mg/l salicylic acid which effectively controlled mango anthracnose was directly fungitoxic. Similarly, Yu and Zheng (2006) have shown that salicylic acid significantly inhibited the spore germination of *P. expansum* *in vitro* when its concentration was increased to 1000 $\mu\text{g ml}^{-1}$. However, during this research, fruits sprayed with high concentrations (1000 mg/l) of salicylic acid showed some necrotic spots on the (lower) surface of the fruit where the run off of the spray solutions accumulated during the three day incubation. High concentrations of salicylic acid are reported to show phytotoxic effects (Lopez and Lucas, 2002). Phytotoxicity (causing injury to a plant) of a compound could be due to a number of reasons, in this case of salicylic acid it is not the chemical itself but the higher doses (overload) that appears to have this effect.

CONCLUSION

Postharvest spray application of Salicylic acid (sodium salt) and Bion® are significantly effective in controlling anthracnose caused by *Colletotrichum gloeosporioides* in mango fruit. Of the two Elicitors, Bion® at 25 or 50 ppm is more efficient as an elicitor than SA at 100 or 500 mg/L in controlling anthracnose in mango fruit.

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