Pre-harvest and post-harvest application of benzothiadiazole for controlling anthracnose and extending shelf life of harvested banana

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Abstract: Anthracnose, caused by the fungus *Colletotrichum musae*, is a serious latent post-harvest disease of banana, which results in major economic losses during transportation and storage. Benzo-thiadiazole-7-carbothioic acid S-methyl ester (BTH), a functional analogue of the plant endogenous hormone-like compound salicylic acid (SA), has been known to possess resistant effects on some diseases caused by fungi. The aim of present study was to select an appropriate BTH concentration and an appropriate stage of banana ripening for its application in controlling anthracnose and extending shelf life of harvested banana fruit. Different concentrations of BTH (50, 100, 200 and 300 μg/mL) were applied at different stages of banana fruit ripening, including one week, two weeks and one month before harvest. The results suggest that while the concentrations of BTH ranging from 50 μg/mL to 200 μg/mL in both pre-harvest and post-harvest application, this could control anthracnose of harvested banana fruit, the appropriate concentration of BTH in both pre-harvest and post-harvest treatment was 100 μg/mL and the best time of BTH treatment was two weeks before harvest. Examination of quality parameters including peel color and firmness indicated that 100 μg/mL BTH treatment delayed banana fruit ripening at room temperature.

Keywords: banana fruit, shelf life, ripening, post-harvest preservation, BTH treatment, anthracnose, peel color, firmness

1   Introduction

Banana is the most popular and commercially important fruit crop grown in many tropical and subtropical countries for its utilization as dessert and staple food. However, diseases at the post-harvest stage cause significant loss in yield and quality of banana fruit.

Anthracnose, caused by *Colletotrichum musae* (*C. musae*), is a latent infection where fungal spores infect immature banana in the field but its symptoms occur as peel blemishes, as black or brown sunken spots of various sizes on fruit that may bear masses of salmon-colored acervuli with their associated conidia on the fruit peel after ripening[1]. Thus, any potential control measure which can effectively delay symptoms of anthracnose infection would play an important role in extending the shelf life of banana fruit during storage.

To date, several synthetic chemicals such as benomyl and thiabendazole (TBZ) have been used to effectively control these diseases in harvested fruits. However, these chemicals remain problematic, as far as contamination, pathogen resistance and environmental pollution are concerned. Therefore, there has been an increasing pressure on the banana industry to minimize the use of synthetic fungicides and to discover sustainable non-chemical alternative fungicides for controlling post-harvest diseases.

Benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH), a functional analogue of the plant endogenous hormone-like compound salicylic acid (SA), has been shown to be effective in inducing systemically-acquired resistance (SAR) in plants and in protecting different plant species against diseases caused by viral, bacterial or fungal pathogens[2,3]. The BTH has been used as an alternative, or complement to fungicide treatments to prevent fungal infection, because of its low toxicological risk and rapid degradation in plant tissues[4].

Recently, BTH was found to control several diseases caused by fungi in other harvested fruits such as mango, strawberry and papaya[5-7]. However, little information is available on whether BTH has effects on controlling anthracnose and extending post-harvest shelf life of banana fruit with pre-harvest and post-harvest applications. Finding an applicable concentration and a proper time for the application of BTH to effectively control post-harvest anthracnose and prolong the storage life of banana fruit is of agricultural significance. Therefore, the purpose of this study was to investigate the appropriate concentration and appropriate time in both pre-harvest and post-harvest periods for the application of BTH. In addition, several parameters of fruit quality including peel color and firmness were investigated after application of BTH in harvested banana fruit.

2   Materials and methods

2.1   Materials

Banana cultivar 'Brazil' (*Musa acuminata*, AAA Group) was collected at commercial maturity stage (green mature, at the 75% full stage) from Panyu of Guangzhou City in China. The BTH (50% of active ingredient, ACTIGARD™, Novartis Crop Protection Inc., United Kingdom) solution was prepared according to the product manual, with sterile deionized water plus 0.05% Tween 80. 500 μg/mL Sportak (25% prochloraz, Aventis Co., Ltd., Bayer, German) and 1000 μg/mL Carbendazim wettable powder (CWP, 50% active ingredient) were prepared as fungicides control in pre-harvest and post-harvest treatments, respectively.

2.2   *Colletotrichum musae* isolation and culture conditions

To isolate *C. musae* (Berk. Et Curt.) v. Arx. from infected banana, fruit was placed in a humid chamber at room temperature (25℃). Small portions of symptomatic tissues were placed on petri dishes containing potato dextrose agar (PDA) (Sangon, Shanghai, China) and incubated at 25℃. Once the mycelial growth was observed, the colonies were re-isolated on fresh PDA dishes to obtain pure cultures. The isolates developed were then identified based on their morphological and cultivar characteristics. Continuous re-isolations were carried out on PDA slants to maintain inoculum. Conidial suspensions of the pathogen were prepared by flooding the seven-day-old culture dishes incubated at 25℃ with sterile distilled water containing 0.05% Tween 80. The spore suspension was adjusted to 5.0×10^5 CFU per milliliter with sterile distilled water with a haemacytometer and prepared for the next inoculation experiment.

2.3   Pre-treatment of banana fruit

Banana fruits were cleaned and dried naturally. Defect-free fruits of uniform color, shape and size were selected to reduce variation in ripening. They were freshly cut and immediately dipped into chlorinated water

(10 mg/L of available chlorine) for 5 min, and then dried in air.

2.4   Post-harvest assay

2.4.1   Selection of the optimum BTH concentration

Banana fingers after pretreatment were soaked in 50, 100, 200 and 300 μg/mL, of BTH solutions for 5 min, and then air-dried.   Banana fingers soaked in 500 μg/mL Sportak solution for 5 min and then air-dried were used as fungicide control.   The control banana fruits were soaked in sterile deionized water plus 0.05% Tween 80 for 5 min and then air-dried.   After the treatments, five banana fingers per bag were put into a polyethylene bag (0.03 mm in thickness, unsealed) and ripened naturally at (23 ± 1) ℃ with 80% relative humidity (RH) in a constant temperature chamber (SANYAN, Japan).   After stored for three days, the fruits were dipped for one minute in 0.04% ethephon for ripening and then stored at (23 ± 1) ℃ with 80% RH for 7 d and 12 d. Maturation and disease index were investigated respectively.   The state of banana fruit coloring grade was classified according to the maximum intensity of yellow coloring on the peel using the maturation index of international standards[8].   Fruit color was rated on a scale from 0 to 6, where 0 = entirely green (green mature), 1 = break green, 2 = more green than yellow, 3 = more yellow than green, 4 = yellow with green tips, 5 = entirely yellow, and 6 = entirely yellow with brown freckles.   The maturation grade was defined and calculated as: maturation grade = ∑ (coloring grade × number of fruit) / total number of fruit, or maturation index = $100 \times$ ∑ (coloring grade × number of fruit) / (total number of fruit × maximum coloring grade).   Disease severity in terms of spot areas was classified into 5 grades (1-5), where 1 = 0% of fruit surface rotten; 2 = 1%-25%; 3 = 26%-50%; 4 = 51%-75% and 5 = 76%-100%[9].   These empirical scales made it possible to calculate disease index (DI) to show the average of the disease severity as actual percentage in terms of maximum disease severity. DI = [∑ (disease grade × number of inoculated spots) / (total number of inoculated spots × maximum disease grade)] × 100%.   Control effect (%) = [(DI of control − DI of treated fruit) / disease index of control] × 100%.   Each treatment was replicated four times with ten fruits per replicate and the entire experiment was repeated twice.

2.4.2   Effects of BTH on lesion diameter and disease incidence of fruit inoculated with *C. musae*

After pre-treated and BTH-treated, the ripening of banana fruits was accelerated, and then fruits were inoculated with suspensions of *C. musae* following as the method of Capdeville et al[10].   A 3 mm × 3 mm wound was made on each fruit and applied with a drop of 20 μL spore suspension.   Then the fruits were placed in plastic trays and stored at (23 ± 1) ℃ with 80% RH for 5 d and 7 d. Disease development was evaluated at these two days by measuring the diameter of the disease spots and calculating disease incidence.   Disease incidence = (number of disease occurred spots / total number of inoculated spots) × 100%.

2.4.3   Effects of post-harvest BTH treatment on fruit quality

Fruit quality was evaluated in terms of firmness and skin color in fruit of naturally occurred disease.   Fruit firmness was determined using a penetrometer (Model Instron 5542, INSTRON Co., USA) equipped with a cylindrical flat-surfaced plunger (8 mm diameter).   A small slice of fruit skin was removed and firmness was then recorded from three different types of fruit, with three different points per fruit, and finally mean values were expressed as newton (N).   Banana peel color was determined by measuring lightness (L) and hue angle with a reflectance colorimeter (Color Reader CR-10, Konica Minolta Sensing, Inc., Japan).   Color values of each fruit were computed as the mean value of three measurements taken from the blossom end, middle and stem end of the fruit peel.   The L value and hue value of each treatment were compared.

2.5   Pre-harvest assay

The field trial using BTH was conducted on banana trees grown in the horticultural field of South China Agricultural University.   'Brazil' banana was used in this experiment and two experiments were performed as below.   (1) To determine the best concentration of BTH application in pre-harvest banana, four concentrations of BTH solution at 50, 100, 200, 300 μg/mL were applied in banana plant in field two weeks before harvest.   (2) To determine the best period of application of BTH, the time

points, at the stages of one month before harvest, two weeks and one week before harvest, respectively, the trees were sprayed with BTH solutions, respectively. After harvested, the banana fruits of those two experiments were collected and treated with the method described in the sections of 2.3 and 2.4.1. The MI and DI were applied to describe the control effect. Three replicates were conducted for each treatment with ten trees per replicate. Trees sprayed with water or 1 000 μg/mL 50% CWP were used as control and fungicide control.

2.6   Statistical analysis

One-way analysis of variance (ANOVA) and Duncan's multiple range test ($P<0.05$) were used for data analysis, using SPSS 10.0 (SPSS Inc., Chicago, USA). Data were presented as means±standard errors (S.E.) and plotted using Sigmaplot (Jandel Scientific, San Rafael CA, USA)

3   Results and discussion

3.1   Post-harvest treatment of BTH on banana ripening and anthracnose

Banana fruits untreated with BTH solution were very susceptible to *C. musae* inoculation. However, when banana fruits were first treated with BTH and Sportak and then challenged by *C. musae*, the MI and DI were significantly ($P<0.05$) lower, compared to those treated with water (Table 1). The MI and DI were reduced gradually with the increase in the BTH concentrations from 50 μg/mL to 200 μg/mL. The fruit, treated with 200 μg/mL BTH, had the lowest DI and the best control effect on post-harvest anthracnose and the lowest MI. However, no significant differences in the MI and DI were observed between 100 μg/mL and 200 μg/mL BTH treatments. The results also showed that fruit treated with 100 or 200 μg/mL BTH had significantly ($P<0.05$) lower DI compared to that treated with the fungicide 500 μg/mL Sportak and 300 μg/mL BTH. The MI and DI of the control fruit all reached 100 on 15 d, but significantly ($P<0.05$) lower levels were still maintained in BTH treated fruit. Taking the various factors such as MI, DI and the cost of BTH into account, 100 μg/mL was selected as a better concentration of BTH to control

post-harvest anthracnose of banana fruit. These results showed that BTH could act as a plant defense elicitor and could control anthracnose of harvested banana fruit effectively, and the control effect of 100 μg/mL BTH was better than that of a systemic fungicide Sportak that had an effective inhibition on postharvest anthracnose of fruit. The results of the present study suggest that BTH had great effects against anthracnose (*C. musae*) in post-harvest treatment studies. However, when the concentration of BTH was higher than 300 μg/mL, a higher disease index and maturation index were seen in fruit and this might be related to phytotoxicity of BTH.

Table 1   Effects of post-harvest treatments with BTH on maturation index and disease index of banana fruit stored at (23±1) ℃

| Storage time	Treatments	Maturation index	Disease index
10 days	Control	83.3±0 a*	73.3±1.3 a
	50 μg/mL BTH	68.9±2.4 b	45.3±4.8 cd
	100 μg/mL BTH	59.4±4.7 c	34.7±7.4 de
	200 μg/mL BTH	56.1±4.3 c	28.7±3.5 e
	300 μg/mL BTH	82.2±0.6 a	55.3±1.8 bc
	500 μg/mL Sportak	78.9±1.1 a	51.3±4.4 bc
15 days	Control	100.0±0 a	100.0±0 a
	50 μg/mL BTH	96.7±2.9 b	61.3±2.4 d
	100 μg/mL BTH	96.1±1.9 b	62.0±4.2 d
	200 μg/mL BTH	97.2±1.0 b	56.0±2.3 d
	300 μg/mL BTH	100.0±0 a	81.3±5.2 bc
	500 μg/mL Sportak	100.0±0 a	77.3±4.7 c

Note: * Means data within a column followed by the same letter are not significantly different at the 0.05 level. Each data point represents the mean±S.E. (*n*=4).

3.2   Effects of post-harvest treatment of BTH on the naturally occurred anthracnose in banana

Post-harvest treatment of 100 μg/mL BTH significantly reduced disease incidence and lesion diameter in banana fruit inoculated with *C. musae* (Figure 1). The disease incidence in BTH-treated fruit was 0, 25.7% and 74.2%, respectively, of that in control fruit on day 7, 9 and 11 after stored at (23±1) ℃ (Figure 1a). Meanwhile, the lesion diameter on treated fruit was only 0, 20.4% and 50.1%, respectively, of that in control fruit on day 7, 9 and 11 after stored (Figure 1b). This result showed that BTH had powerful effects in controlling both inoculated and naturally occurred anthracnose in post-harvested banana fruit.
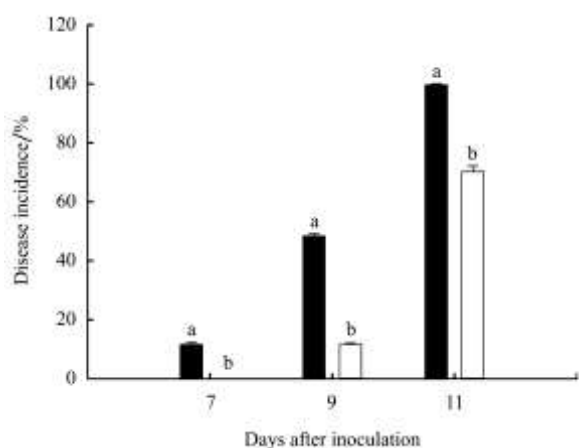
a. Disease incidence
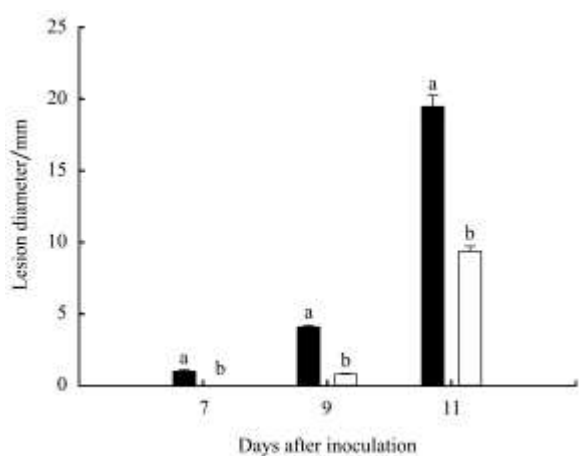
b. Lesion diameter

Figure 1   Anthracnose development in banana fruit after BTH treatment.   The fruit treated with water (■) and BTH (100 μg/mL) (□) are stored at 23 ℃ for 14 days.   Different letters indicate significant differences among means according to Duncan's multiple range test (P<0.05).

3.3   Effects of pre-harvest treatment of different concentrations of BTH on banana ripening and anthracnose

After being treated with 100 and 200 μg/mL BTH at the stage of two weeks before harvest and then stored at 25 ℃ for 6 d and 9 d, the fruit kept lower DI and MI (Table 2).   However, significant (P<0.05) difference was seen between 100 and 200 μg/mL BTH treatments. 50 μg/mL and 300 μg/mL BTH had significantly (P<0.05) higher MI and DI compared to those in 100 μg/mL and 200 μg/mL treatments.   DI was significantly (P<0.05) lowered after application of 1000 μg/mL CWP, but the MI had no significant (P<0.05) difference compared to that in the control.   These results suggested that 100-

200 μg/mL BTH could be used in the field to effectively control banana anthracnose; and the induction of resistance by BTH in banana to *C. musae* was dose-dependent, the most reliable and efficient induction were obtained with concentrations of 100-200 μg/mL, similar to the previous research that applied BTH in cauliflower to resistant downy mildew[11].

Table 2   Effects of different concentrations of BTH at the stage of two weeks before harvest on maturation grade and disease index of banana fruit stored at (23±1) ℃

Storage time	Treatments	Maturation grade	Disease index
6 days	Control	3.54±0.30 a*	4.90±0.65 a
	50 μg/mL BTH	2.89±0.21 b	2.05±0.43 c
	100 μg/mL BTH	2.08±0.16 c	1.19±0.54 d
	200 μg/mL BTH	2.05±0.21 c	1.14±0.08 d
	300 μg/mL BTH	2.80±0.12 b	3.51±0.73 b
	1000 μg/mL CWP	3.39±0.07ab	2.04±0.78 c
9 days	Control	5.79±0.26 a	53.58±0.82 a
	50 μg/mL BTH	4.67±0.37 b	45.66±1.05 b
	100 μg/mL BTH	4.98±0.45 c	38.74±0.41 c
	200 μg/mL BTH	4.84±0.43 c	38.33±0.59 c
	300 μg/mL BTH	5.36±0.77 b	49.27±1.26 ab
	1000 μg/mL CWP	5.61±0.08 ab	45.34±0.14 b

Note: * Means data within a column followed by the same letter are not significantly different at 0.05 level.   Each data point represents the mean±S.E. (n=4).

3.4   Effects of BTH application at different stages in the field on banana ripening and anthracnose

In comparison with the control group, the occurrence of anthracnose was decreased to different extents after the application of 100 μg/mL BTH at various stages of fruit development in the field (Table 3).   Among three stages tested, BTH application at two weeks before harvest showed the best effect with a maximum inhibition by 72.5% and was slightly more effective than that at the stages of one week and one month before harvest.   Fruit treated with BTH at the other two stages of fruit development also displayed lower occurrences of the disease than that treated with water.   However, no significant difference was found in the disease index between these two stages (P<0.05).

BTH has been developed as a novel crop protection agent, which does not have anti-microbial properties, but instead increases crop resistance to diseases[12].   It was shown to be a potent systemically acquired resistance

(SAR) inducer.   Li et al.[13] reported that the induced resistance against downy mildew in cucumber plants persisted for 15 days.   It is known that BTH-elicited resistance lasts longer in monocots than in dicots[14,15]. In the present study, time course resistance induction studies clearly showed that maximum induction was reached in post-harvest fruit after treatment at two weeks before harvest in the field, which may be related to the duration of induced resistance of BTH.   Therefore, it was concluded that in order to achieve an effective control on a postharvest disease, BTH should be applied at the appropriate time to give the best control of decay.

Table 3   Effects of different treatments at different stages before harvest on maturation grade and disease index of banana fruit stored at (23±1) ℃

Storage time	Treatment stage	Treatments	Maturation grade	Disease index
6 days	one week before harvest	Control	3.77±0.45 a*	7.43±0.64 a
		100 μg/mL BTH	2.62±0.11 b	4.36±0.32 b
		1000 μg/mL CWP	2.71±0.25 b	3.14±0.68 c
	two weeks before harvest	Control	3.85±0.30 a	7.37±0.65 a
		100 μg/mL BTH	1.64±0.20 c	2.03±0.41 c
		1000 μg/mL CWP	2.21±0.18b	3.35±0.28 c
	one month before harvest	Control	3.74±0.47 a	7.59±0.65 a
		100 μg/mL BTH	2.77±0.16 b	4.49±0.67 b
		1000 μg/mL CWP	2.81±0.36 b	4.09±0.08 b
9 days	one week before harvest	Control	5.63±0.42 a	52.31±0.12 a
		100 μg/mL BTH	5.11±0.94 b	46.56±0.31 b
		1000 μg/mL CWP	4.91±0.18 b	47.09±0.33 b
	two weeks before harvest	Control	5.59±0.32 a	53.58±0.20 a
		100 μg/mL BTH	4.18±0.10 c	35.48±0.61 c
		1000 μg/mL CWP	5.14±0.78 b	45.24±0.08 b
	one month before harvest	Control	5.69±0.06 a	54.38±0.31 a
		100 μg/mL BTH	4.83±0.15 b	48.65±0.01 b
		1000 μg/mL CWP	4.90±0.78 b	47.04±0.74 b

Note: * Means data within a column followed by the same letter are not significantly different at 0.05 level.   Each data point represents the mean±S.E. (*n*=4).

BTH has been largely employed to activate SAR for the control of plant disease caused by several pathogenic fungi, bacteria and viruses[3,6,16].   However, little is known about how it works and application time towards post-harvest anthracnose effectively.   Pre-harvest spray treatments at 1.43 mM and 2.86 mM BTH suppressed *B. cinerea* development on cut freesia flowerscvv, Dukaat and Cote d'Azur, respectively[17].   However, pre-harvest

spray applications of BTH had no effect on *B. cinerea* development on harvested wax flowercvv, Mullering Brook and My Sweet Sixteen Flowers[18].   Although many reporters have paid more attention to SAR occurred by BTH, our results indicated that the appropriate doses and application time before and post-harvest were also very important for the application of BTH on a large scale.

3.5   Effect of post-harvest treatment of BTH on fruit quality

BTH treatment reduced the decline of firmness of harvested of banana fruit stored at (23±1) ℃.   After being accelerated with ethylene, firmness of fruit declined sharply.   On day 5 and 7 after harvest, the firmness of BTH-treated fruit remained significantly higher than that of the control ($P<0.05$) (Figure 2).   Banana fruits are characterized by rapid softening once ripening is initiated[19].   In the present study, firmness changed from the initial value of 67.0 N to 1.7 N after 14 days of storage at (23±1) ℃, and BTH treatment significantly delayed this decrease (Figure 1a).
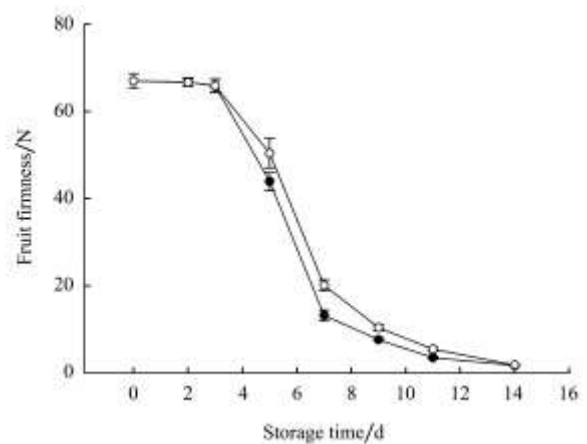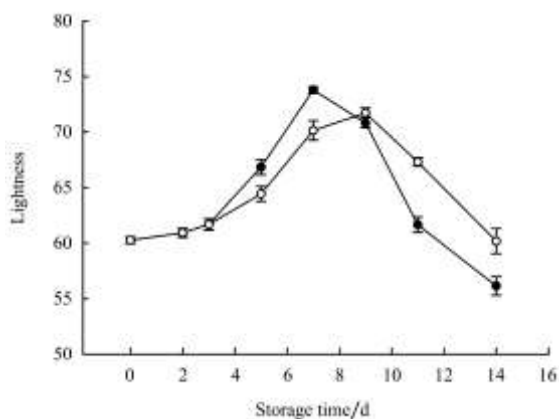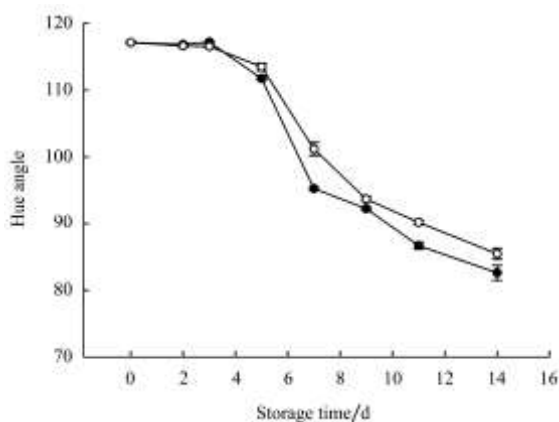

Figure 2   Effect of BTH on the firmness of harvested banana fruit. The fruit treated with water (●) and BTH (100 μg/mL) (○) are stored at 23 ℃ for 14 days.   Vertical bars represent the standard errors of the means (*n*=4)

As the banana fruit began to ripen, the peel lightness (L value) was similar in both BTH-treated and control fruits and they all showed an increased and then decreased trend, peaking on the 9th day and 7th day after treatment, respectively (Figure 3a).   On day 7, the symptoms of anthracnose appeared, and thereafter, the lightness of the control fruit declined sharply.   After nine days, the peel lightness of BTH-treated-fruit was

higher than that of the control fruit. Hue values decreased during ripening, indicating a change from green to yellow. After five days, BTH-treated-banana fruits showed higher hue values than those of control fruit, indicating a delay ripening of banana fruits in BTH treatment (Figure 3b). Post-harvest application of BTH to banana has reduced fruit softening and color development thereby extended the shelf life of fruit. The reduction in fruit color development with exogenous application of BTH is attributed to delay in the senescence process and lower chlorophyll degradation. During the normal fruit ripening process, rapid degradation of chlorophyll occurs in the peel with increased levels of carotenoids or other colored pigments. Similarly, it has been reported the application of SA, analogue of BTH, reduced fruit softning and color development in strawberry, banana and peach[20-22].

a. Lightness

b. Hue angle

Figure 3   Effect of BTH on lightness (A) and hue angle (B) of harvested banana fruit. The fruit treated with water (●) and BTH (100 μg/mL) (○) are stored at 23 ℃ for 14 days. Vertical bars represent the standard errors of the means (n=4)

4   Conclusions

Overall, this experiment showed the effectiveness of BTH on 'Brazil' banana anthracnose and fruit quality. The post-harvest and pre-harvest BTH treatment (i.e. 100-200 μg/mL) prevented fruit decay and ripening effectively and its effectiveness was better than that of fungicides Sportak or CWP. The post-harvest BTH treatment could also control the naturally occurred *C. musae* disease and reduce the lesion diameter and disease incidence of fruit. The field experiment showed that the appropriate stage of BTH application was two weeks before harvest. The present work showed that the application of the plant disease resistance elicitor BTH at pre-harvest and post-harvest periods could induce the defense/resistance system against post-harvest diseases in banana fruit. It could be a useful and promising measure for controlling post-harvest decays on a commercial scale.

Acknowledgments

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