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Full Length Research Paper

Effect of hot water dip treatment on postharvest anthracnose of banana var. Berangan

Amin Mirshekari[1,4], Phebe Ding[1]*, Jugah Kadir[2] and Hasanah Mohd Ghazali[3]

[1]Department of Crop Science, Universiti Putra Malaysia, Malaysia.
[2]Department of Plant Protection, Universiti Putra Malaysia, Malaysia.
[3]Department of Food Science, Universiti Putra Malaysia, Malaysia.
[4]Department of Agronomy and Plant Breeding, Yasouj University, Iran.

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Heat treatment becomes a feasible method for controlling postharvest decay in many freshly harvested commodities. Anthracnose is the main disease affecting the quality of banana fruits during export and marketing. In this study, inoculated Berangan banana with *Colletotrichum musae* was dipped in hot water at 50°C for 0, 10 and 20 min with or without fungicide, respectively. The disease development was determined by measuring the anthracnose infected areas after 10 days of treatment. There was a significant difference in size of lesion on Berangan banana as affected by different dipping time (0, 10 and 20 min) of hot water alone at 50°C. Anthracnose infection rating was reduced with increase dipping time. Dipping fruit in hot water at 50°C for 20 min was more effective in suppressing disease development as compared to hot water with fungicide as control (0 min). Conidia germination of *C. musae* was also assessed with hot water treatment at the same time-temperature combination with or without fungicide as previous. In general, conidia germination rate increases with increasing incubation time, while increasing the hot water dipping time with or without fungicide suppressed conidia germination with varying incubation times. There was a significant difference between hot water treatment alone and with fungicide for 0 min as control. Hot water dip for 10 and 20 min at 50°C inhibited conidia germination (100 %) of *C. musae* better than application of fungicide alone (55.92 %). It is suggested hot water dip treatment at 50°C for 20 min could be used to control anthracnose in Berangan banana instead of using fungicide as practically used in commercial now.

Key words: Hot water dip, anthracnose, banana, *Colletotrichum musae.*

INTRODUCTION

Banana (*Musa* spp.) with world production of 95.6 million tonnes per year (FAO, 2010) is a widely grown fruit crop in tropical and subtropical countries. In Malaysia, banana is the second largest cultivated fruit crop. It has a high consumer demand and fetches a good price all over the world. The susceptibility of fresh harvested produce to postharvest diseases increases during prolonged storage, as a result of physiological changes and senescence which enable pathogens to develop in the fruit (Prusky and Kobiler, 2007). Banana being a highly perishable fruit suffers severe postharvest losses both in terms of quality and quantity (De Costa and Erabadupitiya, 2005).

Anthracnose is the main disease affecting the quality of banana fruits during export and marketing. The causal pathogenic fungus of this postharvest disease is *Colletotrichum musae*. In plantations, *C. musae* conidia contaminate banana fruits after flowering (De Lapeyre de Bellaire, 1997).

Anthracnose infects young banana. The fungal spores quickly germinate and form appressoria and then infect immature banana in the field. The symptoms appear at the ripening stage when appressoria germinate and form infected hyphae, leading to the development of quiescent anthracnose. Sometimes wound anthracnose can

*Corresponding author. E-mail: phebe@agri.upm.edu.my. Tel: +60389466942. Fax: +60389435973.

seriously damages fruit when it develops during container transport, and markedly diminishing the quality of banana before they are located in ripening rooms (Chillet et al., 2007).

Most postharvest diseases are control by fungicides immediately after harvest as a spray or dip application. This is to minimize the development of pathogens on the fruits during postharvest phase and remain on the fruits as chemical residues. Because of efficacy and feasibility use of this chemicals although are widespread, they are becoming increasingly unpopular as a result of increasing awareness among consumers about fungicide residues. So there is an urgent need to develop effective, non-damaging physical treatments disease control in fresh horticultural products (Lurie, 1998). Heat treatments as a beneficial method for control of postharvest problems especially diseases on various horticultural produce have been reported (Afek et al., 1999; Fallik, 2004). Hot water treatments of fruit have been demonstrated to protect against postharvest decay. On the other hand, efficacy of hot water treatment depends on product and is restricted to a narrow range of temperatures and exposure time. Moreover, variety of crop, preharvest agronomic practices in field and climactic regions of crop grown could vary with hot water treatment efficiency (De Costa and Erabadupitiya, 2005). Universally effective temperatures and exposure times to all postharvest pathogens are not available (Barkai-Golan, 1991). Another way to minimize the fungicide to control postharvest decay of horticultural produces has been supplied by using combination of heat treatment and agrochemicals (Schirra et al., 2000). Therefore, optimum temperature, exposure time and their adverse effects on anthracnose development when treated with hot water alone or with fungicide need to be determined for Berangan dessert banana variety.

MATERIALS AND METHODS

Plant material

Mature green banana (*Musa* AAA var. Berangan) was bought from local market. The fruits were immediately transferred to laboratory and treated within 24 h. After dehanding, fruit hands were selected for similar size, colour and maturity. Banana fingers were washed and surface sterilized in 1% sodium hypochlorite solution for two min, then used for disease development assessment.

Isolation and identification of the causal pathogen

Colletotrichum musae was isolated from diseased Berangan banana fruits collected from local market, Serdang, Malaysia. Diseased fruits were washed and surface sterilized with 1% sodium hypochlorite solution for two min, washed twice with sterilized distilled water, then left to air-dry under laminar air flow. Some pieces of lesion from diseased fruits were cut and transferred to sterilized Petri dishes containing potato dextrose agar (PDA) medium. They were incubated at 26°C and observed daily. The isolated fungus was purified using single spore technique and kept in a refrigerator on slant PDA culture. The isolated pure colony was identified according to morphological characteristics with reference report of Sutton and Waterston (1970) and confirmed at the Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, Malaysia.

Preparation of inoculums

Fourteen-days old PDA cultures of *C. musae* were used for inoculation. Conidia suspension was obtained by pouring sterilized distilled water into the culture plate and gently dislodging the conidia by scrubbing the surface of medium with glass rod. The conidia were filtered through a sterilized double layer of muslin cloth and collected in a sterile beaker. The concentration of conidia in the suspension was adjusted to 10^5 spores/ml using a heamacytometer.

Pathogenicity test

The isolated fungus *C. musae* was investigated for its pathogenicity on healthy mature green banana fruits. Surface sterilized fruits were wounded at three points of peel region with a sterilized cork borer (5 mm diameter) into 1 to 2 mm depth. The wounded and unwounded fruits were inoculated with a disc of mycelia or 50 µl of spore suspension. The inoculated fruits were put in sterilized fiberboard carton and kept at 26°C and 90 to 95% RH. The diameter of lesions (mm) was measured for evaluation of anthracnose rot for 10 days.

Preliminary experiment

This experiment was carried out to determine the best combination of temperature and dipping time in hot water. Healthy fruits were washed, drained and randomized for treatment. The fruits were dipped in hot water at 45, 50 and 55°C for 0 (control), 10, 20 and 30 min, respectively. Peel blackening was recorded for treatments after one week by scaling from no blackening until severe injury.

Inoculation, hot water dip treatment and assessment of anthracnose development

Banana fruits were wounded as explained before, and inoculated with 50 µl of spore suspension (10^5 spores/ml) of 14-days old *C. musae*. These inoculated fruits were kept at 26±1°C and 90 to 95% RH for 12 h to induce appressorial development. Hot water dip treatments were performed by dipping inoculated fruits in water bath (BioCote – SBS40 – UK). The banana fruits were divided into two lots and treated in hot water (HW) at 50°C alone or with fungicide (HW+F) (Benomyl 500 mg/l) with dipping time of 0, 10 and 20 min, respectively. The control (0 min) fruits dipped in distilled water with or without fungicide. After treatment, fruits were cooled in distilled water (22 to 23°C) and then allowed to dry. The fruit were placed in air tight container and treated with 100 µl/l ethylene for 24 h in 25°C to initiate uniform ripening. All fruits were then kept in room of 80-85% RH at 25 ± 1°C. The anthracnose severity was determined with diameter of lesions (mm) every two days during 10 days after treatment.

Conidia germination experiment

Conidia suspension that obtained from 14-day old *C. musae* as explained before was used for conidia germination test. This suspension was dipped in hot water by falcon tube at the same time-temperature combination with or without fungicide. Two drops

Table 1. Effect of hot water dip treatment on peel blackening of Berangan banana.

Temperature (°C)	45				50				55			
Dipping time* (min)	0	10	20	30	0	10	20	30	0	10	20	30
Peel blackening**	1	1	1	1	1	1	1	2	1	1	1	4

*0 min as control, **1- no blackening; 2- negligible blackening (<10% damage); 3- slight blackening (10 to 25% damage); 4- moderate blackening (25 to 50% damage); 5- severe injury (>50%).

Table 2. Effect of hot water treatment at 50°C alone (HW) or with fungicide ((HW+F) in three dipping time (0 (control), 10 and 20 min) on conidia germination (%) of *C. musae.*

	Treatment						
Incubation		HW			HW+F		
time (h)	0	10	20	0	10	20	Mean*
5	40.75	0.00	0.00	11.34	0.00	0.00	8.68 a
6	88.83	0.00	0.00	47.92	0.00	0.00	22.79 b
7	94.25	0.00	0.00	55.92	0.00	0.00	25.03 b
Mean*	74.61 a	0.00 c	0.00 c	38.39 b	0.00 c	0.00 c	

*Mean separation within column and row followed by the same letter are not significantly different at p≤ 0.05 by Duncan's new multiple range test.

of conidia suspension of each treatment was spread by glass rod over the surface of PDA thin layer culture plate aseptically and incubated at 26°C. After 5, 6 and 7 h of incubation, 5 mm diameter discs of the agar plate were removed aseptically, placed on glass microscope slides and stained with lactophenol trypan blue. The germination of conidia were observed and quantified microscopically by counting the germinated conidia, which was considered to have taken place if the germ tube was equal to or longer than the spore diameter (Khan et al., 2001). There were three replicates (agar plates) of each treatment and three randomly selected objective fields from each replicate were examined at 40x magnification for one hundred spores per field. Percentage of conidia germination could then be calculated.

Statistical analysis

The data were statistically analyzed using the completely randomized design in factorial arrangement and the data were analyzed using ANOVA (SAS version 9.1). The means were compared with Duncan's multiple range tests where significant differences occurred. Each experiment was performed in triplicate.

RESULTS

Pathogenicity test of causal pathogen

To verify the pathogenicity of *C. musae*, the fruits of mature green banana were used. Anthracnose symptoms appear on wounded fruit inoculated with conidia or mycelia plug. No symptom was observed on unwounded fruits although they were inoculated with the fungus. Wounded fruit inoculated with conidia had the most effect among the inoculation methods, so it was used for fruit infection in lesion development assay. Inoculate wounded fruits showed dark-brown necrotic and sunken lesions on wound area after 5 days in 26°C.

Effects of hot water dip treatment on peel blackening

Table 1 indicates that banana fruit peel blackening occurred in hot water at 50 and 55°C while the other treatments didn't show blackening. The most serious of peel blackening was observed in 50°C for 30 min, and negligible blackening at 55°C for 30 min and 55 °C for 20 min.

Effect of hot water dips treatment alone or with fungicide on conidia germination

The present study found that hot water dip inhibited conidia germination of *C. musae.* Germination rates of *C. musae* conidia, as indicated by the formation of germ tubes, varied with the dipping time and incubation time (Table 2). In general, increased germination rate in control with increasing incubation time, while increasing the dipping time with or without fungicide suppressed conidia germination with varying incubation times. There was a significant difference between control and control with fungicide. Hot water at 55 °C for 10 and 20 min without fungicide significantly inhibited conidia germination as compared to fungicide alone.

Effects of hot water dip treatment alone or with fungicide on disease severity

There was a significant difference in disease severity development of wound anthracnose on Berangan banana as affected by different dipping time of hot water treatment (Table 3). Diameter of lesion was reduced with

Figure 1. Effect of hot water treatment at 50°C alone (HW) or with fungicide (HWF) in three dipping time (0, 10 and 20 min) on anthracnose lesion development of Berangan banana in day 4 (left) and 8 (right) after inoculation.

Table 3. Mean effects of hot water treatment at 50 °C alone (HW) or with fungicide (HW+F) in three dipping time (0 (control), 10 and 20 min) on anthracnose lesion development 10 days after inoculation.

Treatment	Dipping time (min)	Diameter of lesion (mm)
HW	0	19.98[a]
	10	11.12[b]
	20	0.38[c]
HW+F	0	2.57[c]
	10	0.06[c]
	20	0.00[c]

Mean separation followed by the same letter are not significantly different at p≤ 0.05 by Duncan's new multiple range test.

increasing dipping time. Anthracnose symptoms did not develop in the fingers dipping in hot water at 50°C for 20 min either with or without fungicide amendment (Figure 1). There was also significant difference between hot water for 10 min and control (0 min) without fungicide (Table 3 and Figure 1).

DISCUSSION

Control of postharvest diseases is necessary when long transit time is needed for exporting banana. *C. musae* is the main fungus causing anthracnose rot in banana. Demands for the non-chemically treated horticultural crops are increasing day by day worldwide (De Costa and Erabadupitiya, 2005).

The results from this study clearly demonstrated that hot water treatment at 50°C for 20 min alone or with fungicide of inoculated Berangan banana reduced anthracnose severity caused by *C. musae.* Control of anthracnose by hot water dip with fungicide at 50°C for 10 min was better than 0 min. These results are concurrence with the work of De Costa and Erabadupitiya (2005). Lopez-Cabrera and Marrero-Dominguez (1998) showed hot water dip treatment were much effective in controlling postharvest pathogen of banana. Anthracnose and finger rot of 'Latundan' and 'Saba' bananas inhibited by hot water dip at 47 to 52°C for 10 to 20 min (Acedo Jr. et al., 2001). Similary, heat treatment reduced disease incidence in plantain banana (Aborisade and Akomolafe, 2007), mango (Acedo Jr. et al., 2001; Mansour et al., 2006), lychee and longan (Follett and Sanxter, 2001).

In vitro experiment indicated that hot water dip treatment at 50°C for 10 and 20 min alone or with fungicide completely suppressed spore germination of *C. musae*. Heat treatment directly affect on spors by retarding germ tube growth, reducing activity or completely killing germinating spore, thus reducing the rate of infection effective inoculums (Schirra et al., 2000).

A longer dipping time of hot water were required to achieve a reduction in fungal growth in the fruit tissues than in agar. This suggests that the effective hot water

temperature or dipping time to which the fungus is exposed is much lower than the dipping time applied to the fruit surface and highlights the penetration of pathogen in first few layer of living fruit peel tissues. The effect of hot water on anthracnose is basically due to reduction in the viability of fungi spores. Heat may also reduce pathogen growth by inducing resistance mechanisms in the outer layers of epicarp (Fallik, 2004). Anthracnose lesions by *C. musae* may not appear on mature green bananas before fruit ripening (Jinyoung et al., 2002). It may be due to the mechanism of fruit resistance. The association of preformed fungitoxic polyphenolic compounds in the pathogen defence system of bananas has already been suggested (Chillet et al., 2007).They could affect the development of hyphae during wound anthracnose infection.

In conclusion, it is suggested that hot water dip treatment at 50°C for 20 min could be used to control postharvest anthracnose of Berangan banana instead of using fungicide. The combination of hot water dip at 50°C for 10 min with fungicide is recommended for minimizing agrochemical treatment in banana.

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