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Integrating plant defense inducing chemical, inorganic salt and hot water treatments for the management of postharvest mango anthracnose

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ABSTRACT

Preharvest dips of mango fruit in plant defense inducing chemicals (PDIC) integrated with postharvest treatments with inorganic salts and hot water were evaluated for the management of anthracnose on artificially inoculated mango fruit. Either of the PDICs salicylic acid or potassium phosphonate at 1000 mg L^{-1} combined with a fruit dip for 3 min in 3% aqueous sodium bicarbonate at 51.5 °C significantly reduced disease development as compared to other treatments and the control. This combination kept anthracnose severity (lesion development) below 5% during much of the 12 days experimental period and had the maximum proportion of marketable fruit (93.3%). The mean disease severity on untreated control fruit exceeded 30%, disease incidence reached 100% and marketability dropped to 0%. The treatments also maintained quality of mango; pH, TSS, TA, firmness and color of treated mango fruit significantly ($P < 0.001$) differed from those of the control. Heating calcium chloride (3%) to 51.5 °C did not significantly improve its effect on severity of mango anthracnose even when combined with preharvest PDICs. The integrated measures involving sodium bicarbonate offer effective options for the management of mango fruit rot due to anthracnose.

1. Introduction

Mango (*Mangifera indica* L.) is one of the world's most important fruit. The fruit suffer from several diseases, of which anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc., is the major postharvest disease in all mango producing areas of the world (Dodd et al., 1997).

Anthracnose is usually controlled by a combination of preharvest and postharvest fungicide treatments (Tuzun and Kloepper, 1995). Increasing public concern over the indiscriminate use of pesticides and associated health risks and environmental hazards, as well as occurrence of fungicide resistant pathogen strains, has stimulated research on alternative methods to control postharvest diseases (Wilson et al., 1994; Yao and Tian, 2005). Moreover, fungicides are unaffordable for many mango growers in developing countries. Postharvest control of mango anthracnose could also be accomplished by treatment of fruit in hot water, alone or in combination with chemicals (Dodd et al., 1997). However, precise control of temperature and time is critical, as fruit can be easily damaged by overexposure to heat (Arauz, 2000).

Several inorganic salts have shown antimicrobial activity (Deliopoulos et al., 2010). Some of these salts such as sodium bicarbonate have been widely used in the food industry and they are among compounds that are generally recognized as safe (GRAS). The potential of salts for the control of postharvest pathogens has been demonstrated in various pathosystems (Punja and Gaye, 1993; Youssef et al., 2012). However, Deliopoulos et al. (2010) concluded that inorganic salts are generally less effective than synthetic fungicides and could not replace them. Compounds that promote plant resistance may provide rational choices to complement postharvest treatments of inorganic salts and improve effectiveness. Local and systemic induced resistance can be activated in plants by chemical activators, such as salicylic acid and its analogs, or sodium and potassium salts (Reuveni et al., 2000).

Since mango anthracnose comes as quiescent infection and develops quickly after harvest with ripening of the harvested fruit and decline in resistance (Dodd et al., 1997), it is prudent to evaluate promotion of plant defense through preharvest treatments. Moreover, the potential of integrating plant defense inducing chemicals and postharvest salt treatments with hot water merits evaluation. If applied as part of complementary measures, hot water treatment could be effectively used with less exacting requirements for temperature and treatment duration.

Integrated disease management is a preferred strategy because of increased understanding of residual effects of chemical control on non-target organisms and the environment as well as the limitation of a single alternative management option to achieve the same level of control and reliability as that of chemicals. The present

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study reports on the effect of integrated use of plant defense inducing chemical (PDIC), inorganic salt and hot water treatments on mango anthracnose, fruit quality and marketability.

2. Materials and methods

2.1. Pathogen inoculum preparation

C. gloeosporioides was isolated from mango fruit showing anthracnose lesions. Potato dextrose agar (PDA) was used for initial isolation of the pathogen. An isolate of the pathogen grown in pure culture was maintained in PDA culture tubes at 4 °C and used as stock culture of the target organism throughout the study.

2.2. Preharvest PDIC treatment of mango fruit

Sample fruit of the local mango cultivar 'Amba Kurfa' at the orchards of the Bisidimo Leprosy Relief Center, near Harar, Ethiopia were marked using tags color-coded according to the treatment. Four PDIC treatments (i.e. salicylic acid and potassium phosphonate each at 500 and 1000 mg L^{-1}) were selected among 10 treatments based on disease reaction in a detached leaf test (data not shown). The four treatments were tested together with untreated control by applying them to nearly mature green fruit (95 days after fruit set) still attached to the mother tree a week before harvest. Treatments were applied by dipping individual fruit in PDIC solutions or sterile distilled water (for the control) for 30 s. Treated fruit were harvested at the physiological mature green stage and transported to the Plant Pathology Laboratory at Haramaya University for further test in combination with postharvest inorganic salt and hot water treatments.

2.3. Postharvest treatments

Harvested fruit were surface sterilized twice by dipping in 1% sodium hypochlorite solution containing 0.1% Tween 20 for 10 min. After rinsing in sterile distilled water and air-drying, the fruit were inoculated by dipping in conidial suspension of *C. gloeosporioides* previously adjusted to 10^7 conidia mL^{-1} using a haemocytometer. The fruit were covered with a plastic sheet and incubated at 25 °C for 15 h until the conidia germinated as reported by Koomen and Jeffries (1993).

The fruit were then treated separately with two types of inorganic salt solutions (sodium bicarbonate and calcium chloride) each at 3% (w/v) and a control without inorganic salt. These treatments were applied at two water temperatures (20 and 51.5 °C). The fruit were treated by dipping in the salt solutions or the controls (sterile distilled water) for 3 min. Fruit were then transferred into plastic trays (10 fruit per treatment per tray) and maintained at room temperature (ca. 20 °C) for 12 days. The experiment was laid out in completely randomized design (CRD) with factorial combination of five levels of preharvest PDIC, three levels of inorganic salt, and two water temperature levels in five replications (10 fruit for each treatment) and repeated.

2.4. Data collection

Fruit were assessed for disease incidence and disease severity at 48 h interval from the time of symptom appearance to 100% unmarketability of control fruit. Disease incidence was calculated as the percentage of fruit showing symptoms of anthracnose. Disease severity ratings were recorded by visual assessment of the percentage fruit area covered by lesions according to the diagrammatic scale developed by Corkidi et al. (2006).

Marketability of fruit was assessed according to the procedure employed by Kefialew and Ayalew (2008). Descriptive quality attributes were determined subjectively by observing the level of visible mold growth, decay, shriveling, smoothness, and shine of fruit. Marketability was expressed as percentage.

Data on quality of mango were recorded at full ripeness, i.e. color index 5 (Miller and McDonald, 1991). Samples of mango fruit were randomly taken from each treatment for analyses of total soluble solids (TSS), pH, titrable acidity (TA), firmness and color.

The TSS was determined using an aliquot of juice extracted with a juice extractor (60001 X, 31Je35 6X-00777, Hesteller). A bench top refractometer (60/70 ABBE, A90067, Bellingham & Stanley Ltd., England) with a range of 0–32 °Brix and refaction index range of 1.3–1.7 °Brix, with a precision of ±0.0003 °Brix was used to determine TSS by placing 1–2 drops of clear juice on the prism.

For pH and TA, mango juice was extracted according to Nunes and Emond (1999) with a juice extractor and clear juice was used for the analysis. The pH value of the mango juice was measured with a pH meter and the TA was measured according to the method described by Maul et al. (2000). Titrable acidity, expressed as percent citric acid, was determined by titrating 10 mL of mango juice to pH 8.2 with 0.1 NaOH.

Firmness was measured after peeling the fruit at three different points using a penetrometer (Model FT327, QA supplies, Norfolk, Virginia) and recorded as newtons (N). Subjective visual skin color ratings were recorded on a 1 to 5 scale where, 1 = 100% green; 2 = 25% yellow; 3 = 50% yellow; 4 = 75% yellow; and 5 = 100% yellow (Miller and McDonald, 1991).

2.5. Statistical analysis

Percentage data on disease incidence and severity and marketability were arc sine transformed before statistical analysis. The data were subjected to analysis of variance using SAS version 9.2 software, and least significant difference (LSD) was used for mean comparison.

3. Results and discussion

3.1. Effect of pre-harvest PDIC and post-harvest treatments on mango anthracnose

In general, preharvest treatments did not significantly affect incidence of anthracnose (Table 1). The lowest disease incidence of 44.4% was recorded on fruit treated with 3% sodium bicarbonate at 51.5 °C without preharvest PDIC. Since fruit showing any symptoms of anthracnose, even small inconspicuous spots that did not affect market appeal, are counted as diseased, incidence gives an indication of disease progress but not a good measure of the rotting caused by the disease.

Anthracnose severity was significantly lower in fruit subjected to preharvest application of PDIC, i.e., salicylic acid or potassium phosphonate, than the control (Table 2). However, no significant variation ($P \geq 0.05$) was observed between different concentrations of the PDICs in terms of disease severity. The postharvest treatments with inorganic salt (3% sodium bicarbonate or 3% calcium chloride) or hot water at 51.5 °C alone also reduced disease severity over the control (Table 2). The average disease severity in the control fruit reached 4.34 (i.e. nearly 30% fruit area affected). Individual treatments generally kept disease severity rating below four (less than 10% fruit area affected). The effects of salicylic acid and potassium phosphonate were statistically comparable. Among the inorganic salts, sodium bicarbonate markedly reduced disease severity as compared to calcium chloride. Nevertheless, none of the treatments applied alone were as effective as the integrated use of PDIC, inorganic salt and hot water treatments. Preharvest treatment with either salicylic acid or potassium phosphonate at