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Effects of cinnamon extract, chitosan coating, hot water treatment and their combinations on crown rot disease and quality of banana fruit

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Abstract

The antifungal activities of cinnamon extract (CE), piper extract (PE) and garlic extract (GE) were evaluated on banana crown rot fungi (*Colletotrichum musae*, *Fusarium* spp. and *Lasiodiplodia theobromae*) *in vitro*. The assay was conducted with extracts of CE, PE and GE with concentrations of 0, 0.1, 0.5, 1.0, 5.0, 10.0 and 0.75 g L^{-1} of carbendazim (CBZ) on potato dextrose agar at room temperature. CE completely inhibited conidial germination and mycelial growth of all fungi at 5.0 g L^{-1}. PE totally suppressed mycelial growth of all fungi at 5.0 g L^{-1} and conidial germination at 10.0 g L^{-1} except for *Fusarium* spp. GE had no significant effects but low concentrations (0.1 and 0.5 g L^{-1}) enhanced germ tube elongation of the three fungi. The ED_{50} values were higher for mycelial growth than for conidia except for *Fusarium* spp. Combined treatments were investigated on crown rot development in banana fruit (*Musa* AAA group 'Kluai Hom thong'). Treatments included 5.0 g L^{-1} CE, 1% (w/v) chitosan solution, hot water treatment (HWT, 45 °C for 20 min), CE plus chitosan, CE plus HWT and 0.75 g L^{-1} of CBZ, applied before and after inoculation of the fruit. Crown rot development was assessed during storage at 13 °C for 7 weeks. Disease development was least (25%) on CE treated fruit after inoculation compared to CBZ but was higher when CE was applied before inoculation. Chitosan significantly delayed ripening as in terms of peel color, firmness, soluble solids and disease severity. CE showed no negative effects on quality of fruit. CE plus HWT caused unacceptable peel browning.

Keywords: Banana; Cinnamon; Chitosan; Crown rot; Hot water treatment; *Musa* AAA group; Quality

1. Introduction

Banana (*Musa acuminate* L.) is a highly perishable fruit that has a short shelf-life and suffers severe postharvest losses (Basel et al., 2002; De Costa and Erabadupitiya, 2005). Fungal diseases including crown rot caused by a fungal complex, *Colletotrichum musae*, *Fusarium* spp. and *Lasiodiplodia theobromae*, are a major cause of postharvest disease (Paull et al., 1998), and disease severity is higher when combinations of virulent pathogens attack the fruit (Antony et al., 2004). Crown rot causes losses in banana-exporting countries and its incidence rises periodically in the rainy season (Krauss and Johanson, 2000).

Disease symptoms first appear at or near the cut surface of the crescent-shaped crown and the tissues become black and soft. White, gray or pink mold appears on the surface of the cut crown and the mold can penetrate the finger stalks and progress to fingers. Ultimately the finger stalks become weak and detach. Postharvest storage conditions may accelerate the development of the disease (Paull et al., 1998). Fungicides are the primary means of controlling postharvest diseases, the most commonly used ones being benzimidazoles such as benomyl and thiabendazole (TBZ) (Aked et al., 2001). However, strains resistant to these fungicides have emerged and residues may also be a problem (De Lapeyre de Bellarie and Maurichon, 1998; Mari et al., 2003). Consumer demand for non-chemically treated fruit is increasing (De Costa and Erabadupitiya, 2005).

Many plant extracts have been reported to have activity against a wide range of fungi (Kumar and Tripathi, 1991; Doubrava et al., 1998), and those with fungitoxic properties may include volatile constituents. When two or more substances are present in the extracts, the fungitoxic potential may be enhanced. Possible synergism among compounds could be beneficial for

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postharvest protection of banana because the pathogens would be less likely to develop resistance against mixed components (Tripathi and Dubey, 2004).

Cinnamon extracts have fungistatic and fungicidal activity against the anthracnose and crown rot pathogens and spraying them on Embul banana prior to storage, controlled crown rot and extended shelf-life (Ranasinghe et al., 2002; Ranasinghe et al., 2003). Chitosan has also been reported to maintain the quality of fruit and vegetables (El Ghaouth et al., 1991a, 1991b; Li and Yu, 2001; Zhao et al., 2004), and as a coating it is safe and shows antifungal activity against several fungi (Jiang et al., 2005). Chitosan (1%) coating of banana fruit prolonged storage life for up to 27 days at 13 °C with 90–95% RH (Wattanakorn, 2003). Hot water treatment is also an effective non-chemical method for controlling postharvest pests and diseases if suitable combinations of temperatures and exposure times are selected to prevent quality loss (Lurie, 1998). The use of a combination of such techniques in the postharvest handling of fresh products is increasing.

The objectives of this research were to investigate the antifungal activity of plant extracts on cultures of banana crown rotting fungi, *C. musae*, *Fusarium* spp. and *L. theobromae* and interactions between cinnamon extract, and a chitosan and hot water treatment on the incidence of crown rot and quality changes in banana during and after cool storage.

2. Materials and methods

2.1. Fungal isolation and inoculum preparation

The causal fungi, *C. musae*, *Fusarium* spp. and *L. theobromae*, were freshly isolated from infected banana hands. Pure cultures were maintained on PDA media. Inoculum was prepared by using the conidia of 14-day-old cultures of these fungi. The conidia were dislodged from the surface of the media by flooding with sterile distilled water and gentle rubbing with a sterile glass rod. The suspensions were filtered through three layers of muslin cloth to remove mycelial fragments and adjusted to 10^5 conidia/mL with a haemacytometer. Then, all three conidial suspensions were mixed and adjusted to 5×10^3 conidia/mL.

2.2. Preparation of plant extracts and chitosan

The plant materials, dry cinnamon bark, dry piper leaves and fresh garlic bulbs were collected from local sources. Samples (500 g) were chopped into small pieces and immersed in 1000 mL of 75% ethanol for 6 h (Bautista-Baños et al., 2003). The extract solutions were filtered and the crude extract was then concentrated by rotary evaporation at 40 °C and stored at 5 °C. The volumes of dry crude extracts harvested from cinnamon bark, piper leaves and garlic bulbs were 20, 146 g kg^{-1} of dry weight and 25 g kg^{-1} of fresh weight, respectively. Chitosan solution (1%, w/v) was prepared by dissolving 1 g of chitosan powder in 100 mL of 0.5% acetic acid in distilled water, with agitation overnight.

2.3. Fruit preparation

Bananas cv. Kluai Hom Thong (AAA group) were harvested at 75–80% maturity from a commercial plantation in Petchaburi province, Western Thailand. Hands, free of visual defects and of uniform weight and shape of banana were selected. The hands were surfaced-sterilized with 0.1 g L^{-1} of sodium hypochlorite solution for 3 min. The crown surface of each hand was re-cut using a sterilized knife to make a fresh wound surface for artificial inoculation and allowed to air dry for 2–3 h. The hands were then randomly allocated to treatment groups. A treatment unit consisting of seven hands was used to assess disease development. Five hands of banana were allocated for measurement of fruit quality. Treatments were applied within 24 h of harvest.

2.4. Antifungal assays

Antifungal activity of plant extracts was evaluated using three fungi cultured on agar plates at concentrations of 0, 0.1, 0.5, 1.0, 5.0 and 10.0 g L^{-1}. For comparison, 0.75 g L^{-1} of CBZ and unamended media were used as controls. The prepared extracts were added to conical flasks containing previously sterilized and cooled agar medium. After thorough mixing, 15 mL of media were poured into sterilized Petri dishes 9 cm in diameter (Thangavelu et al., 2004).

One millilitre of conidial suspension (10^5 conidia/mL) was evenly spread on the agar plates and incubated at room temperature. The number of germinated conidia were counted in 10 microscopic fields of 100 conidia in three replicate plates and presented as percent germination. Conidia were considered to have germinated if the germ tubes were equal to or longer than the length of the conidia itself (Khan et al., 2001).

Fungal plugs (0.5 mm in diameter) were removed with a cork borer from the growing margin of each fungus colony and placed at the center of the test plate. Five replications were made for each treatment and the cultures were incubated at room temperature. Colony diameter was measured in two directions daily until the fungus covered the whole of the agar in the control plate. Data were expressed as growth rate (mm/day) relative to control. Percentage inhibition (conidial germination and mycelial growth) was converted to probit values (Finney, 1978) and the ED_{50} was determined.

2.5. Efficacy of integrated methods for controlling crown rot development

The effects of 5.0 g L^{-1} cinnamon extract (CE), 1% (w/v) chitosan and hot water treatment (HWT, 45 °C for 20 min) and their combinations on crown rot development and quality of fruit were evaluated in three different experiments. For the first and second experiments, banana hands were artificially inoculated as before and after treatment. The spore suspension was sprayed evenly on the cut surface of each banana hand using an air brush and mini air compressor (Model 150-4-PK, Badger Air-Brush Co. Ltd.). For the third experiment, natural disease incidence on hands was evaluated.