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Full Length Article

Effect of Cinnamon Oil on Incidence of Anthracnose Disease and Postharvest Quality of Bananas during Storage

MEHDI MAQBOOL, ASGAR ALI[1] AND PETER G. ALDERSON
School of Biosciences, Faculty of Science, The University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia
[1]Corresponding author's e-mail: Asgar.Ali@nottingham.edu.my; mehdimaqbool@yahoo.com

ABSTRACT

Colletotrichum musae is the causal organism of anthracnose in bananas during storage. A study using four concentrations of cinnamon oil (0.1, 0.2, 0.3 & 0.4%) along with untreated control was designed to investigate the antifungal effects of this plant product against anthracnose of bananas. The effects of cinnamon oil on quality of bananas during storage at (13±1°C, 80-90 relative humidity) for 28 days were also determined. A significant ($P < 0.05$) inhibition of mycelial growth and conidial germination of *C. musae* was observed in all treatments of cinnamon oil as compared to the control after 7 days of incubation at room temperature. However the Potato Dextrose Agar (PDA) plates amended with 0.4% cinnamon oil showed the most promising results among all the treatments in suppressing the mycelial growth and conidial germination inhibition (83.2%), while there was no effective conidial germination inhibition found in the control. The cinnamon oil treatments also delayed the disease incidence and maintained quality during storage however, some phytotoxic effects were observed on fruits treated with higher concentration. It can thus be concluded that the concentration up to 0.3% can be used for extending the storage life of bananas for up to 28 days without affecting the physico-chemical properties.

Key Words: Anthracnose; Banana; Cinnamon oil; Disease incidence; Postharvest quality

INTRODUCTION

Banana (*Musa acuminate* L.) being a climacteric fruit has a very short storage life. It is highly perishable, therefore susceptible to several diseases resulting in extensive postharvest losses (Basel *et al*., 2002). Among postharvest diseases, anthracnose caused by *Colletotrichum musae*, is the most important disease causing massive economic losses in bananas (Ranasinghe *et al*., 2003). Being a latent infection the fungus infects immature bananas in the field but the symptoms appear only after ripening (Gonzále-Aguilar *et al*., 2003). Thus for extending the shelf-life any potential control measure, which can be effectively used to delay symptoms expression would have a significant part.

Bananas are generally treated with the fungicides like prochloraz and imazalil to control postharvest pathogens (Aked *et al*., 2001). Recently, increased concerns about the fungicides by many countries have demanded a fresh produce without treatment with any chemicals, particularly fungicides applied after harvest. Additionally, due to continuous use of these fungicides *C. musae* has developed resistance and reduced the effectiveness of these synthetic chemicals (Mari *et al*., 2003).

The industry is under pressure to minimize the use of these synthetic fungicides employed postharvest to control *C. musae* of bananas. To avoid the use of these fungicides, some alternative and efficient methods for the control of this disease are required.

The essential oils are reported to have some fungicidal properties against certain postharvest diseases of tropical fruits and vegetables (Wilson *et al*., 1997; Meepagala *et al*., 2002; Imelouane *et al*., 2009) and are also safer for the environment than synthetics. The essential oil of cinnamon has been reported previously as a source of antifungal agent (Baratta *et al*., 1998; Delespaul *et al*., 2000). A previous study by Ranainghe *et al*. (2002) to control the crown rot disease of bananas also confirmed the effectiveness of cinnamon oil. This oil contains compound such as cinnamaldehyde, which has been tested on many fresh fruits such as mandarin, kiwi and rambutan to control postharvest diseases (Arras, 1988; Sivakumar *et al*., 2002). The cinnamon oil is commonly used in cooking as a flavoring agent and is also a safe preservative (Ranasinghe *et al*., 2003).

There are many studies concerning the screening and efficacy of essential oils but most of them are confined to *in vitro* studies only. Therefore the objectives of the present study were to determine the efficacy of cinnamon oil *in vitro* as well as *in vivo* to control postharvest anthracnose of bananas and also to study the effects on postharvest quality characteristics.

MATERIALS AND METHODS

Isolation and identification of *C. musae* from banana

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fruit samples: The *C. musae* hyphae were isolated from anthracnose lesions on banana fruit. Diseased areas were superficially disinfected, cut into small pieces (3-5 mm in diameter) and each piece was sterilized in 10% Clorox for 3-5 min and subsequently washed 3 times in distilled water. The washed tissues were then placed separately on Potato Dextrose Agar (PDA) (Difco Brand, USA) plates and incubated at room temperature (25-30°C). After incubation, hyphal tip isolation technique was used to transfer mycelium to fresh PDA plates to obtain pure cultures. Identification of the fungal isolate was carried out by microscopic observation according to appropriate taxonomic key and description (Sutton, 1980). To maintain pathogenicity of the inoculums, continuous re-isolations were carried out on PDA slants.

Preparation of cinnamon oil solution: Cinnamon oil was obtained from Tropical Bioessence Marketing, Melaka, Malaysia. PDA amended with different concentrations of cinnamon oil (0.1, 0.2, 0.3 & 0.4% v/v) using Tween 80 (0.01%) as a surfactant was prepared. These concentrations of cinnamon oil were selected based on the preliminary experiments done in the laboratory. The pH 5.6 of solution was adjusted by adding 1 N NaOH, using a digital pH meter (Model: Knick 646). The media were autoclaved for 15 min at 121°C.

***In vitro* antifungal assay of cinnamon oil:** The antifungal assay of cinnamon oil was carried out based on the inhibition in radial mycelial growth and conidial germination of *C. musae* on PDA using poison food technique. An agar disk (5 mm diameter) from a pure culture of *C. musae* was placed in the center of a PDA plate containing cinnamon oil (0.1, 0.2, 0.3 & 0.4% v/v). Control plates contained only PDA. Petri plates were incubated at 25°C for seven days. Daily radial growth measurements were taken until the fungus reached the edge of the control plate. *In vitro* conidial germination inhibition test was performed by the cavity slide technique and the results were expressed in percentage (Cronin *et al*., 1996).

***In vivo* antifungal assay of cinnamon oil:** Mature, green bananas (*Musa* AAA Pisang Berangan) without any visible defects were obtained from a commercial orchard located at Beranang, Semenyih, Selangor State of Malaysia. Hands were cut into individual fingers and washed with sodium hypochlorite (0.5%), rinsed with distilled water and air-dried at ambient temperature (25-28°C). Bananas were then dipped for 2-3 min in spore suspension of *C. musae* (1×10^5 $spores^{-1}$ mL) and kept at ambient temperature for drying. Air-dried fruits were dipped for 2-3 min in different concentrations of cinnamon oil (0.1, 0.2, 0.3 & 0.4% v/v) solutions and again kept at ambient for drying. After application of treatments bananas were packed in cardboard boxes and stored (13±1°C, 80±5% RH) for 28 days. The effect of cinnamon oil on disease incidence (DI) and disease severity (DS) was evaluated weekly for 28 days during cold storage. DI data was expressed as the percentage of fruits showing anthracnose out of the total number of fruits in each treatment, while DS was scored following the scale (1=0% of fruit surface rotten; 2=1-25%; 3=26-50%; 4=51-75% & 5=76-100%) (Sivakumar *et al*., 2002) and quality was evaluated as percentage weight loss, firmness, soluble solids concentration (SSC) and titratable acidity.

Experimental design and statistical analysis: The experiments were designed in a completely randomized fashion with four replications and data were subjected to analysis of variance (ANOVA) using MSTAT-C software (Dynamic Corporation, Texas, USA). The means were separated using Duncan's Multiple Range (DMR) test at ($P < 0.05$). Disease incidence data was transformed (arcsine of the square root of the proportion of affected fruit) before analysis. Four replicates of 20 plates were used for *in vitro* experiment, while for *in vivo* experiment each treatment was replicated four times with 20 fruits per replicate and the entire experiment was repeated twice.

RESULTS

Mycelial growth of *C. musae* was significantly ($P < 0.05$) affected by all the cinnamon oil treatments as compared to the control during the incubation period of 7 d. *In vitro* inhibition was directly related to the concentrations of cinnamon oil. However a maximum inhibition in mycelial growth was observed with 0.4% cinnamon oil concentration (Fig. 1). The cinnamon oil concentrations, 0.1, 0.2 and 0.3% also significantly ($P < 0.05$) suppressed the mycelial growth of *C. musae* as compared to the control but not as effective as 0.4%. Growth in the control plates was almost five times greater than in the 0.4% at the end of the incubation period.

The test regarding conidial germination inhibition was also carried out to confirm the efficacy of cinnamon oil treatments. The cinnamon oil treatments significantly ($P < 0.05$) inhibited the conidial germination as compared to the control after keeping in dark for 7 h (Fig. 2). The cinnamon oil concentration of 0.4% proved the best among all other treatments and inhibited the conidial germination up to (83.2%) followed by 0.3, 0.2 and 0.1% (80.1, 72.3 & 69.6%), respectively. However 0% inhibition in germination was found in the control treatment.

There were significant ($P < 0.05$) differences among treatments in bananas inoculated with spore suspension of *C. musae* and after treating with different concentrations of cinnamon oil during 28 days of storage (13°C, 80% RH) (Figs. 3 & 4). The DI and DS on bananas increased with the passage of time and reached up to 100% (5 score), respectively after 28 days of storage in control fruits. The anthracnose symptoms appeared on the control fruits after one week of storage and after 28 days most of the bananas were spoiled due to severe disease infection. Different concentrations of cinnamon oil not only delayed the onset of anthracnose disease but also maintained the freshness of bananas during first two weeks of storage and later on showed minimal symptoms. The highest fungicidal effect

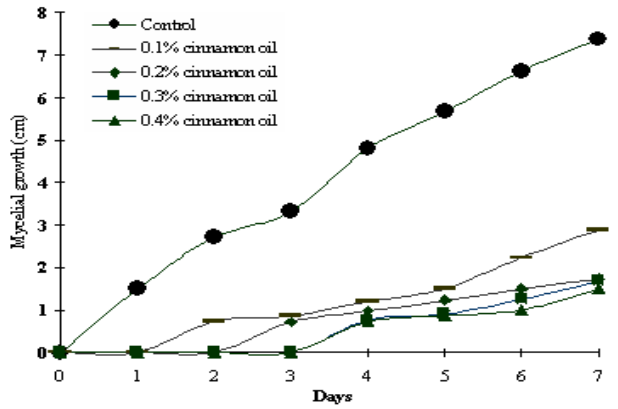
Table I: Effect of different concentrations of cinnamon oil on postharvest quality of banana fruits before and after storage (†)

Treatments	Weight loss (%)	Firmness (N)[a]	SSC (%)[b]	Titratable acidity (%)[c]
Control	16.62 a	46.11 a	8.3 a	0.19 a
0.1% cinnamon oil	14.20 a	50.42 a	7.4 a	0.20 a
0.2% cinnamon oil	14.10 a	52.23 a	7.2 a	0.22 a
0.3% cinnamon oil	14.98 a	56.20 a	6.9 a	0.20 a
0.4% cinnamon oil	14.46 a	52.71 a	7.0 a	0.21 a

†Where the letters are same, there is no significant difference between the means of different treatments. The data were recorded after 28 days at 13°C

[a,b,c] Firmness, SSC and titratable acidity value before storage = 75.21 N, 4.1% and 0.28%, respectively

Fig. 1: Effect of different concentrations of cinnamon oil on mycelial growth of *C. musae* during a 7 d incubation period



was observed in those bananas treated with 0.4% cinnamon oil (disease incidence of 8.0%) and disease severity (DS) score of 1.2 indicating fruit surface infection close to 1.0.

In present study, the cinnamon oil concentrations tested had no effects on weight loss, fruit firmness, SSC and titratable acidity values of bananas after 28 days storage at 13±1°C (Table I). The fruits treated with concentrations up to 0.3% showed no phytotoxic effects, while some phytotoxic effects appeared on bananas treated with 0.4% cinnamon oil after 28 days of storage at 13±1°C.

DISCUSSION

The results of the present study suggested that cinnamon oil treatments have the potential to control anthracnose disease caused by *C. musae* in bananas. Mycelial growth and conidial germination were clearly affected by cinnamon oil treatments indicating that these concentrations affected various stages of the development of *C. musae.* Among all cinnamon oil treatments, 0.4% was found by far the best in controlling the incidence of anthracnose disease both *in vitro* as well as *in vivo*. Cinnamon oil at lower concentrations also showed some fungistatic effects *in vitro* and also controlled growth of *C. musae* on bananas.

Fig. 2: Effect of different concentrations of cinnamon oil on conidial germination inhibition (%) of *C. musae*. ±SE

[A: Control; B: 0.1% cinnamon oil; C: 0.2% cinnamon oil; D: 0.3% cinnamon oil; E: 0.4% cinnamon oil]

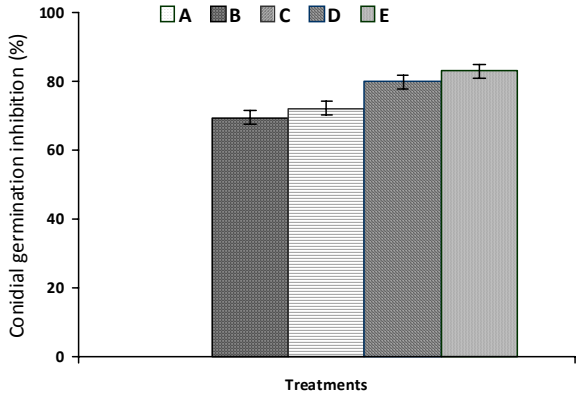


Fig. 3: Effect of different cinnamon oil on anthracnose disease incidence (%) on inoculated banana fruits during storage (13°C, 80% RH) for 28 days ±SE

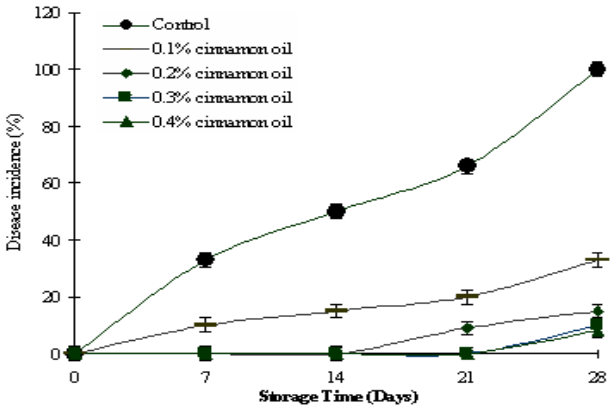
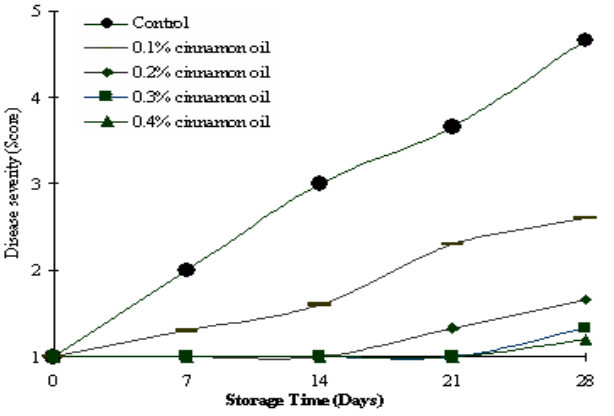


Fig. 4: Effect of different concentrations cinnamon oil on anthracnose disease severity (score) on inoculated banana fruits during storage (13°C, 80% RH) for 28 days. ±SE

[Disease severity: 1 = 0% of fruit surface rotten; 2 = 1-25%; 3 = 26-50%; 4 = 51-75% and 5 = 76-100%]



Cinnamon oil with fungitoxic or fungistatic activity could be considered as a suitable alternative to synthetic fungicides for managing anthracnose in bananas. *In vitro* inhibition was directly related to the cinnamon oil concentrations. In agreement with the findings, Thangavelu *et al.* (2004) found that the extracts of *Solanum torvum*, *Jatropha glandulifera* and *Emblica officinalis* were highly inhibitory to mycelial growth of *C. musae* in banana and the inhibitory effect was directly related to the quantity of extract added to the medium. In another study by Palhano *et al*. (2004) also confirmed that the inhibitory effects of citral on spore germination of *C. gloeosporioides* were higher with an increase in the concentration of essential oil.

Cinnamon oil has been reported by many researchers as a promising source of antifungal compounds (Baratta *et al*., 1998; Montes & Carvajal, 1998; Delespaul *et al*., 2000). Therefore it has been previously proposed as an alternative postharvest treatment for banana crown rot disease caused by *C. musae*, *L. theobromae* and *F. proliferatum* due to its fungistatic and fungicidal properties to these pathogens within a range of 0.03-0.11% (v/v) (Ranasinghe *et al*., 2002).

The major component of cinnamon oil is cinnamaldehyde, which is generally regarded as safe compound, since its toxicity to the mammalian was very low (ID_{50}, 2200 mg kg^{-1}) (Goubran & Holmes, 1993; Pattnaik *et al*., 2010). In addition, volatiles from plants can inhibit the growth of fungal pathogens before evaporating whilst leaving no residues. Some of them are constituents of the human diet and safe to use without causing any health risks (Hamilton-Kemp *et al*., 2000).

The cinnamon oil treatments of 0.3 and 0.4% delayed the onset of disease incidence up to 21 days in bananas during storage, while in control fruits the anthracnose symptoms appeared even after 7 days of storage. The actual mechanism of action of cinnamon oil needs to be further investigated. However this may be because anthracnose is a latent infection and starts from the germination of conidia, which produced appressoria and cinnamon oil penetrated into the appressoria of *C. musae* and therefore showed significant fungicidal effects (Ranasinghe *et al*., 2005). Singh *et al.* (1993) reported that leaf extracts of medicinal plants, such as *Calotropis procera*, *Vitex negundo* and *Azadirachta indica* may delay the appearance of initial disease symptoms of infected bananas.

Treatments with cinnamon oil up to 0.3% also maintained the quality of bananas during storage without adversely affecting the parameters tested. At 0.4% cinnamon oil, the fruits presented some phytotoxic effects. Earlier it was found that a concentration of 0.2 mg mL^{-1} of cinnamon oil obtained from waste water was effective in controlling crown rot disease without affecting the physico-chemical properties of bananas (Ranasinghe *et al*., 2003).

It is concluded from the study that cinnamon oil at the concentration up to 0.3% can be used commercially as a safe method for treating bananas to control anthracnose disease. Such control methods for postharvest diseases are more imperative due to the development of new resistant pathogens to conventional fungicides, whilst increasing concerns of the consumers on the use of permissible fungicides on fruits.

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