ANTIFUNGAL ACTIVITY OF TRUE CINNAMON (*Cinnamomum zeylanicum* Blume) ESSENTIAL OIL AGAINST POSTHARVEST PATHOGENS, ASPERGILLUS, PENICILLIUM AND FUSARIUM

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INTRODUCTION

Postharvest diseases in fruits and vegetables are affected by a wide variety of fungal and bacterial pathogens and cause considerable losses of fruits and vegetables. Among those, black mould rot caused by *Aspergillus* spp., dry rots caused by *Fusarium* spp., anthracnose caused by *Colletotrichum* spp. and green mould rots caused by *Penicillium* spp. are considered as some of the major diseases causing severe post-harvest losses in fruit and vegetable crops. Present practice is application of synthetic fungicides during storage. However, chemical fungicides on food crops have been a growing social concern. Therefore, alternatives for synthetic fungicides were investigated by exploring natural sources with antifungal activity. Essential oils from various plants have previously been reported to contain antifungal activity (Tabassum and Vidyasagar, 2013). Essential oils are concentrated hydrophobic liquids containing volatile chemical compounds from plants. They are also known as volatile oils, ethereal oils or as the oil of the plant from which they were extracted, Essential oils extracted from cinnamon have previously been reported to have significant inhibitory effects against several fungi, including *Aspergillus* spp, *Eurotium* spp., *Laetiporus sulphureus*, and *Penicillium* (Chipley and Uraih 1980; Cao 1993; Mastura et al. 1999; Guynot et al. 2003; Simić et al. 2004; Cheng et al. 2006). Cinnamon (*Cinnamomum zeylanicum)* oil is extracted through steam or hydro-distillation. Primary constituents in cinnamon oil differ according to the plant part from which oil has been extracted. For instance, cinnamaldehyde in bark oil, eugenol in leaf oil and camphor in root-bark oil (Wijesekera, 1978). Previously, it has been reported that trans- cinnamaldehyde from C. *zeylanicum* oil contains strong antifungal activity against 17 tested fungal pathogens (Simić et al. 2004). Therefore, the objective of the present study was to evaluate antifungal activity of essential oils extracted from leaves and bark of C. *zeylanicum* against common postharvest fungal pathogens.

METHODOLOGY

Isolation of fungal pathogens

Fungal pathogens were isolated from onion, potato, and citrus showing symptoms of black mold rot, Fusarium dry rot, and green mold rot respectively. Briefly, symptomatic plant parts were surface sterilized by 70% ethanol for 2 mins and washed with sterilized distilled water. Surface sterilized plant parts were transferred onto Potato Dextrose Agar (PDA) aseptically and then incubated at 25°C for 5 days (Jogee et al., 2017). Pure cultures were obtained by several passage onto fresh PDA. Fungal isolates were identified up to genus level by using colony morphology and microscopic characteristics of hyphae and reproductive structures (Guarro et al., 1999).

Essential oil from Cinnamon

Cinnamon bark oil and leaf oil were extracted from dried cinnamon bark and leaf by hydro distillation method. The GCMS analyses of the oils were carried out using Trace 1300/ISQ QD Gas Chromatography Mass Spectrometer and identification of the individual component in the oil was accomplished by the comparison of retention time with standard substances and by matching mass spectral data with the MS library (NIST MS search 2.0).

Determination of antifungal activity

Antifungal activity of cinnamon oil from the bark and leaves were evaluated on PDA using well diffusion method. Briefly, wells were made on PDA by using a cork borer (diameter 4 mm) and filled with 10 µl of undiluted cinnamon oil. Mycelial discs of actively growing selected fungi were placed at the center of the plate and incubated at 25 +/- 2 °C. For control, PDA (no cinnamon oil) was inoculated with mycelial discs of fungi at the center of the plate. Experiment was carried out in triplicates. When the fungus in the control were fully grown until the edge of the Petri plate, diameter of clear zone surrounding cinnamon oil filled wells were measured using a meter ruler at three directions and obtained average diameter of the clear zone.

Determination of Minimum Inhibitory Concentration (MIC) (Poison Food Technique)

Potato dextrose agar was prepared supplemented with cinnamon oil at concentrations of 100 ppm, 50 ppm, 25 ppm, 12.5 ppm, 5 ppm and for control only PDA was used. Each plate was inoculated at the center with a mycelial disc cut out from an actively growing culture using a cork borer. Inoculated Petri plates were incubated at 25 ±2 °C. Experiment was carried out in triplicates. Growth of fungal colonies were estimated by measuring colony diameter when the fungus in the control were fully grown until the edge of the Petri plate. MIC was evaluated by comparing relative growth of fungus in treatment with control. (Janssen et al., 1986; and Barrata et al., 1998).

Data analysis

Data obtained on diameter of inhibition zone were subjected to the analysis of variance (ANOVA) under two factor (type of oil and species of fungus) factorial design to find the statistical significance and means separation. Data processing was conducted by the SAS version 9.1 computer software package.

RESULTS AND DISCUSSION

Fungi isolated from onion, potato, and citrus were identified as *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. respectively. Undiluted cinnamon bark oil and leaf oil inhibited the growth of *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. in well diffusion method (figure 01). According to the diameter of inhibition zone, bark oil showed significantly higher inhibition than cinnamon leaf oil at 0.05 significance level. Diameter of inhibition zone was significantly different at 0.05 significance level among the tested fungal species. Fusarium sp. showed the highest inhibition against cinnamon bark oil.

According to the MIC assay, diluted cinnamon bark oil and leaf oil at 100 ppm (0.1 ml/ml), 50 ppm (0.5 ml/ml), 25 ppm (0.25 ml/ml), 12.5 ppm (0.0125ml/ml), 5 ppm (0.005 ml/ml) concentrations, completely inhibited the mycelial growth of *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. compared to control plates. Therefore, MIC of cinnamon bark oil and leaf oil against *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. should be below 5 ppm.

Table 1.0: Mean diameter of inhibition zone/cm where a, b and c are letters that showed the significance differences of mean values

Factor	**Levels**	**Mean diameter of inhibition zone**
Oil type	Cinnamon bark oil	**2.2^{a}**
	Cinnamon leaf oil	**1.7^{b}**
	CV value	**13.3**
	Probability	**0.0001**
Pathogenic fungi	*Aspergillus* sp.	**1.9^{b}**
	Fusarium sp.	**2.4^{a}**
	Penicillium sp.	**1.6^{c}**
	CV value	**13.3**
	Probability	**<0.0001**
Oil type vs pathogenic Fungi	Cinnamon bark oil vs *Aspergillus* sp.	**2.0 ^{b}**
	Cinnamon bark oil vs *Fusarium* sp.	**3.1^{a}**
	Cinnamon bark oil vs *Penicillium* sp.	**1.6^{c}**
	Cinnamon leaf oil vs *Aspergillus* sp.	**1.9b^{c}**
	Cinnamon leaf oil vs *Fusarium* sp.	**1.7b^{c}**
	Cinnamon leaf oil vs *Penicillium* sp.	**1.6^{c}**
	CV value	**13.3**
	Probability	**<.0001**

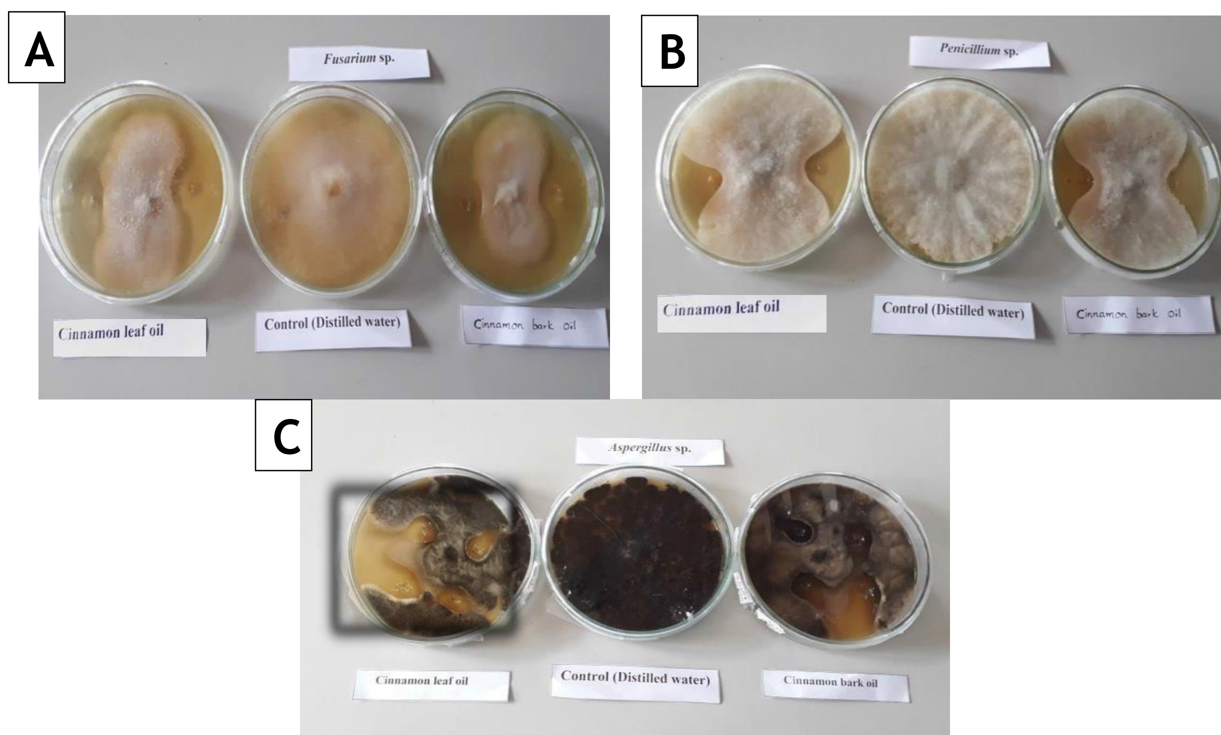

Figure 01: Antifungal activity as zone of inhibition A- *Fusarium* sp. zone of inhibition against cinnamon oil, B -*Penicillium* sp. zone of inhibition again cinnamon oil, C- *Aspergillus* sp. zone of inhibition against cinnamon oil.

CONCLUSIONS/RECOMMENDATIONS

Cinnamon bark oil and leaf oil exhibited strong antifungal activity against common postharvest fungal isolates, *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. The most efficient antifungal activity was shown by bark oil. The MIC against *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. found to be less than 5 ppm of either bark or leaf oil.

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