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In vitro antifungal effect of *Aloe vera* and cinnamon essential oil incorporated *Aloe vera* on stem-end rot pathogens of mango (cv. Karthakolomban)

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ABSTRACT

Purpose: This research was conducted to determine the in vitro antifungal effect of *Aloe vera* and cinnamon oil incorporated *Aloe vera* on stem-end rot pathogens isolated from Karthakolomban mango. **Research method:** Effect of *Aloe vera* gel was determined using a liquid bioassay against the selected pathogens. Same bioassay was conducted to determine the effect of combinations of *Aloe vera* gel + cinnamon leaf and bark oils. A minimum inhibitory (MIC) and minimum lethal (MLC) concentration for each pathogen was determined. **Findings:** *Aloe vera* at 10% v/v showed the best effect against *Pestalotiopsis* sp. and *Phomopsis* sp. whereas *Lasiodiplodia theobromae* was controlled at the highest percentage at 12.5% v/v concentration. The MLC of *Aloe vera* + cinnamon leaf oil and *Aloe vera* + cinnamon bark oil against *Pestalotiopsis* were identified as 10% + 0.50 µl/ mL and 10% + 0.60 µl/ mL respectively. The MLC against *Phomopsis* for *Aloe vera* + cinnamon leaf and *Aloe vera* + cinnamon bark oils were identified as 10% + 0.80 µl/ mL, and 10% + 0.50 µl/ mL, respectively. When considering *L. theobromae,* concentrations of 12.5% + 0.60 µl/ mL and 12.5% + 0.15 µl/ mL were identified as the MLC for *Aloe vera* + cinnamon leaf oil and *Aloe vera* + cinnamon bark oil respectively. **Limitations:** *Aloe vera* gel without cinnamon oils was unable to inhibit fungal growth completely. **Originality/Value:** This study reveals the potential of using cinnamon oil in combination with *Aloe vera* as a preservative treatment against stem-end rot pathogens of mango.

INTRODUCTION

Among numerous tropical fruits 'mango' plays a noteworthy role as a horticultural commodity. Based on the records of the Food and Agriculture Organization, in 2019, mango accounts for almost quarter of total tropical fruit exports, whereas, the demand shows a gradual increment over the past ten years (FAO, 2020). Despite the increasing demand for mango, supply chain management becomes a challenge due to post-harvest diseases caused by pathogens. Therefore, attention has been drawn towards designing strategies to mitigate or eliminate post-harvest diseases related to mango fruit.

Stem-end rot (SER) is the most common postharvest disease of mango (*Mangifera indica* L.), resulting in periodic losses of up to 20% in the world (Johnson et al., 1992). According to certain researchers, SER disease in mango is caused by *Lasiodiplodia theobromae, Botryodiplodia theobromae, Dothiorella* spp., *Colletotrichum gloeosporioides, Phomopsis mangiferae*, and *Pestalotiopsis mangiferae* (Adikaram et al., 2010; Ekanayake et al., 2019).

The use of chemical fungicides in controlling postharvest diseases of fruit commodities is a popular practice in many production processes. Despite seemingly beneficial outcomes brought out by the use of chemical fungicides, many detrimental effects have been identified by researchers. Several studies have noted the fact that the use of agrochemicals had made alterations in metabolites associated with glycolysis in animals (Yang et al., 2021). On the other hand, it is reported in recent studies that, agrochemicals act as toxicants to hinder biological processes which cause acute and delayed effects in humans (Saquib et al., 2021). Apart from that, the ecosystems are being disturbed heavily, with the extensive use of agrochemicals (Singh et al., 2016). Therefore, there is an urgent necessity to replace these chemical fungicides with a sustainable and biologically safe solution.

Aloe vera has been in use among the traditional practices and it is well known for its therapeutic value. Specifically, *Aloe vera* leaf extract had shown the ability to be used in lengthening the shelf life of fruits and vegetables depending on the properties such as antimicrobial activity, film-forming properties, and biodegradability while maintaining the quality attributes of each commodity (Ajeethan & Mikunthan, 2016; Fardsadegh & Jafarizadeh-Malmiri, 2019). Further, *Aloe vera* causes the extension of the shelf-life of fruits and vegetables when applied as a coating by minimizing the rate of respiration and maturation development, prevents loss of moisture, delay oxidative browning and maintain quality attributes (Ajeethan & Mikunthan, 2016) of the commodity. Apart from many nutritional compounds *Aloe* consists of bioactive compounds such as aloesin, aloe-emodin, aloin, acemannan, aloe rid, ethyl chromones, flavonoids, naphthoquinones, sterols, anthraquinones, amino acids, salicylic acid, and phenolic compounds (Danish et al., 2020) which show a wide array of antifungal or antibacterial properties.

Essential oils have proven to be showing antibacterial, antiviral, antifungal, and insecticidal properties that have a commercial significance. Studies on oils of *Cinnamomum zeylanicum* (Ceylon cinnamon) have revealed that it has fungistatic and fungicidal properties against various pathogenic fungi (Ranasinghe et al., 2002; Kodituwakku et al., 2020). According to Kodituwakku et al. (2020), cinnamon leaf oil constitutes mainly Eugenol (63.76%), (E)-cinnamaldehyde (1.37%), and cinnamon bark oil constitutes (E) - cinnamaldehyde (72.18%), Eugenol (4.82%) according to GC-MS analysis, which accounts for antifungal and antibacterial properties.

The present study was carried out to (i) investigate the antifungal effect of *Aloe vera* gel and cinnamon oil incorporated *Aloe vera* gel against stem-end rot pathogens (*Lasiodiplodia theobromae*, *Phomopsis* sp. and *Pestalotiopsis* sp.) isolated from Karthakolomban mango and

(ii) determine the minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) against each mango pathogen using the combinations of *Aloe vera* gel + cinnamon (*Cinnamomum zeylanicum*) leaf oil and cinnamon bark oil.

MATERIALS AND METHODS

Preparation of *Aloe vera* gel

Aloe vera leaves were freshly harvested from the botanical garden at the University of Kelaniya. Extraction of *Aloe vera* gel was done based on the method described by Mendy et al. (2019). The extraction was done from fresh leaves of *Aloe vera* plant which were washed in tap water, disinfected by immersing in 70% ethanol for 1 min, then 10% Clorox solution for 2 min, and rinsed three times with sterilized distilled water. The gel was obtained from the soft, colorless, inner gel parenchyma which was separated from the outer cortex of the stems. The parenchyma was ground using an autoclaved mortar and pestle. *Aloe vera* gel was filtered with a muslin cloth. *Aloe vera* gel series (5 – 15% v/v) were prepared to determine the inhibitory percentage at each concentration against test pathogens.

Cinnamon oils

Cinnamomum zeylanicum (cinnamon) leaf oil (>99.9% v/v) and cinnamon bark oil (>99.9% v/v) were purchased from Citro Essential Oils (Pvt.) Ltd., Mt. Lavinia, Sri Lanka.

Test Fungi

SER – associated fungi previously isolated and characterized by Ekanayake et al., (2019) designated as *Lasiodiplodia theobromae* (MH005085.1), *Pestalotiopsis* sp. (MH005090.1) and *Phomopsis* sp. (MH005086.1) were used for the present research.

Liquid bioassay for *Aloe vera* gel only

The conical flasks (100 mL) containing 50 mL of SMKY semi-synthetic liquid medium (20.0 g sucrose, 7.0 g yeast extract, 1.5 g KNO_3, and 0.50 g $MgSO_4.7H_2O$ dissolved in 1 L of distilled water) were prepared (Anthony et al., 2004). Thereafter, the flasks were autoclaved for 20 min at 1.03 kg cm^{-2} and 121 ºC.

Concentration series of *Aloe vera* gel (5 – 15% v/v) were prepared aseptically along with three replicate flasks for each concentration. Each flask was separately inoculated with a 5 mm diameter fungal disc which was cut from 7-day old pure cultures of respective pathogens. The prepared flasks were incubated at room temperature (28 ± 2 °C) along with positive (Carbendazim fungicide – 0.1 % w/v) and negative control (sterilized distilled water). After incubation, the mycelia (if any) were recovered on pre-weighed filter paper (Whatman No: 1, 5.5 cm diameter), washed three times with sterile distilled water, and placed in a hot air oven (Memmert, UM 600) at 105 ºC overnight until a constant weight was reached. Subsequently, mycelial dry weight (Anthony et al., 2004) was determined. Percentage inhibition of mycelium was calculated for *Phomopsis* sp., *Pestalotiopsis* sp., *L. theobromae*, separately using the following equation (1) (Anthony et al., 2004; Kodituwakku et al., 2020).

$$\text{Inhibition } \% = [(C - T) / C] \times 100 \quad (1)$$

Where, C = mean dry weight of mycelium in control flasks
T = mean dry weight of mycelium in test flasks

In vitro liquid bioassay for the combinations of *Aloe vera* gel + Cinnamon leaf oil and *Aloe vera* gel + Cinnamon barks oil

Based on the results obtained for percentage inhibition for each pathogen, the least *Aloe vera* gel concentration which had given a significant growth inhibition was considered to carry out liquid bioassays, to further determine percentage inhibition under the combinations *Aloe vera* gel + Cinnamon leaf oil and *Aloe vera* gel + Cinnamon bark oil.

The treatments were prepared aseptically adding appropriate volumes of solution to the flasks. [*Pestalotiopsis* sp. – *Aloe* (10% v/v) + cinnamon bark and cinnamon leaf oil (0.20 – 0.60 µL/mL); *Phomopsis* sp. – *Aloe* (10% v/v) + cinnamon bark and cinnamon leaf oil (0.40 – 0.80 µL/mL); *L. theobromae* – *Aloe* (12.5% v/v) + cinnamon bark oil (0.05 – 0.45 µL/mL), *Aloe* (12.5% v/v) + cinnamon leaf oil (0.20 – 0.60 µL/mL)]. To disperse oil, Tween 80 (Koch-Light Laboratories, England) was used. Each assay flask was then separately inoculated with a 5 mm diameter fungal disc cut from the periphery of a 7-day old pure culture of the respective test pathogen. Flasks were placed on a reciprocal water bath shaker and were mixed thoroughly (New Brunswick Scientific, Model: R76) at room temperature (28 ± 2 ºC). A control (sterilized distilled water and Tween 80) was included for comparison. Carbendazim fungicide (0.1% w/v) was used as the positive control. Three replicates of each treatment and controls were arranged according to a completely randomized design (CRD) in the laboratory. After 7 days of incubation, the mycelia (if any) were recovered on pre-weighed filter paper (Whatman No: 1, 5.5 cm diameter), washed three times with sterile distilled water, and placed in a hot air oven as described previously and mycelial inhibition percentage was determined for each pathogen.

Determination of minimum inhibitory and minimum lethal concentrations for *Aloe vera* gel + Cinnamon leaf oil and *Aloe vera* gel + Cinnamon bark oil

The mycelial plugs that did not show any growth in each in vitro bioassay were transferred to freshly prepared PDA plates and incubated for 7 days at room temperature (28 ± 2 ºC) to observe the recovery of the mycelial growth. Hence, the fungicidal and fungistatic activities of the selected treatments were determined. Any revival of radial mycelial growth was categorized as the fungistatic effect and the corresponding concentration of the treatment was considered as the minimum inhibitory concentration (MIC). No growth of fungal mycelia (no revival) was considered as a fungicidal effect and the corresponding concentration of the treatment was considered as minimum lethal concentration (MLC) (Anthony et al., 2004; Kodituwakku et al., 2020).

Data analysis

Data with respect to in vitro bioassays were analyzed using one-way analysis of variance (ANOVA), using Minitab 19 statistical software. The means comparison was done using Tukey's multiple comparison test (Kodituwakku et al., 2020).

RESULTS AND DISCUSSION

Antifungal efficacy of *Aloe vera* gel in liquid phase

The present study tested the antifungal activity of five different concentrations of *Aloe vera* gel which were in direct contact with the mycelia of fungal pathogens growing in an aqueous medium. All these five concentrations were not able to control fungal mycelial growth completely. However, out of the five concentrations of *Aloe vera* gel the highest percentage inhibition of mycelia of *Pestalotiopsis* sp. and *Phomopsis* sp. were achieved at (10% v/v).

Percentage values for inhibition of mycelia were 80.15% and 81.67% respectively. The lowest *Aloe vera* gel concentration where *L. theobromae* displayed maximum percentage inhibition of mycelia (85.27%) was at 12.5% v/v. Positive control (Carbendazim) showed 100% inhibition of mycelial growth, while negative control (distilled water) showed 0% inhibition of mycelial growth. According to one-way ANOVA, percentage inhibition values obtained for different concentrations of *Aloe vera* gel were significantly different from the control ($p < 0.05$) (Fig. 1).

A recent study conducted by Mendy et al. (2019), had shown that the filtered fresh *Aloe vera* gel caused 100% inhibition of tested fungi, i.e. *Fusarium* sp., *L. theobromae*, *Aspergillus niger*, and *Colletotrichum gloeosporioides*. According to this previous research, 100% inhibition was shown by *L. theobromae* at 15% concentration at the end of the incubation period. In vitro investigations to observe the antifungal activity of *Aloe vera* gel against the pathogenic fungi, *A. niger*, *Aspergillus flavus*, *Alternaria alternata*, *Drechslera hawaiensis* and *Penicillium digitatum* had shown significant reduction in mycelial growth based on an agar diffusion plate method. According to this study *Aloe vera* gel at 0.35% concentration was significantly effective towards all tested fungi. At this concentration, the inhibition in mycelial growth was 24.29% against *A. niger*, 9.26% for *A. flavus* and only 6.24% for *P. digitatum* (Sitara et al., 2011). Similar to the present study, *Aloe vera* gel had effectively shown a certain antifungal activity against fungal pathogens in an in vitro assay.

In another study, the sensitivity of fungal spores of *Botrytis cinerea*, *A. alternata* and *P. digitatum* had been determined. The results of this study had shown that there was a 67 – 69% reduction in radial mycelial growth at 1 µL/L *Aloe vera* gel concentration for *P. digitatum*, *A. altemata*, and *B. cinerea* (Saks & Barkai-Golan, 1995). Similarly, the study conducted by Castillo et al. (2010), highlights the fact that, *Aloe vera* gel had brought inhibitory effect on mycelial growth on both *P. digitatum* and *B. cinerea* isolated from table grapes, in which the inhibitory effect had been comparatively higher on *P. digitatum* (99%) than *B. cinerea* (87%) at a 100 mL L^{-1} *Aloe vera* gel concentration. The mycelial growth inhibition against the several SER pathogens, by *Aloe vera* gel was observed clearly in the present study as well.

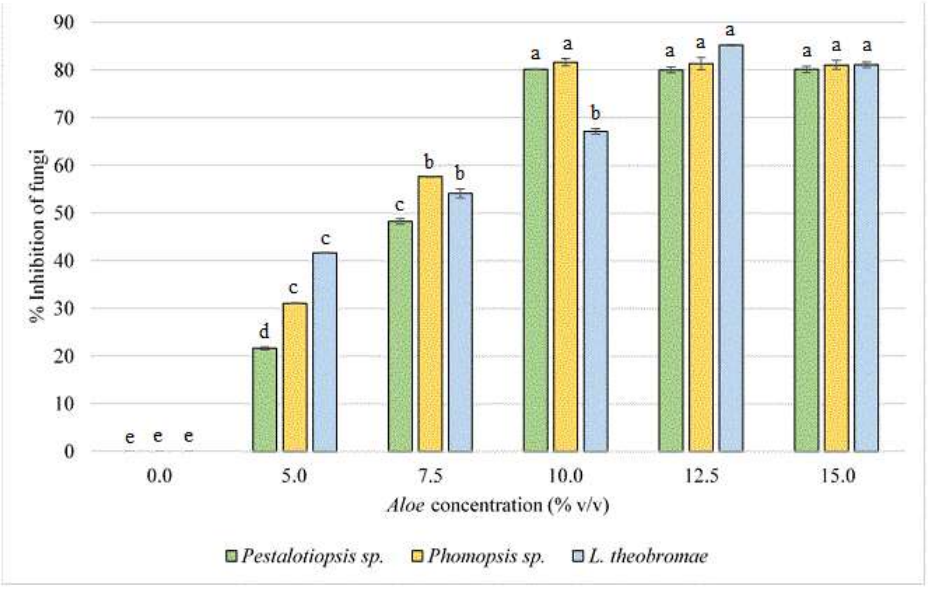

Fig. 1. Inhibitory effect of *Aloe vera* gel on the fungal growth of *Pestalotiopsis* sp., *Phomopsis* sp. and *L. theobromae* in liquid bioassay. Each data point represents the mean of three replicates. Means sharing a common letter(s) are not significantly different by Tukey's multiple comparison test at $\alpha = 0.05$.

Antifungal efficacy of *Aloe vera* gel + cinnamon oils in liquid phase

Best concentrations of *Aloe vera* gel for each pathogenic fungus were selected from the first experiment and two separate liquid bioassays were carried out in combination with cinnamon leaf or bark oil. Concentrations of cinnamon leaf and bark oils were selected based on the results of a previous study conducted by Kodituwakku et al. (2020) at University of Kelaniya.

According to a liquid bioassay carried out by Kodituwakku et al. (2020), cinnamon leaf oil alone was fungistatic and fungicidal at concentrations of 0.40 µL/mL and 1.00 µL/mL respectively against *L. theobromae* isolated from mango (cv. Karthakolomban). MIC and MLC values of cinnamon bark oil against *L. theobromae* were 0.25 µL/mL and 0.30 µL/mL respectively. The MIC value for both cinnamon leaf and bark oil against *Pestalotiopsis* sp. was 0.40 µL/mL. The MLC values obtained for cinnamon leaf and bark oil against *Pestalotiopsis* sp. were 0.60 µL/mL and 0.80 µL/mL, respectively. Further, 0.60 µL/mL was recorded as fungistatic (MIC) whereas 0.80 µL/mL was recorded as fungicidal (MLC) to *Phomopsis* sp.

The present study reported 100% inhibition of mycelial growth of *Pestalotiopsis* sp. by cinnamon leaf oil (0.30 µL/mL – 0.60 µL/mL) incorporated *Aloe vera* gel at a concentration of 10% (v/v) and cinnamon bark oil (0.40 µL/mL – 0.60 µL/mL) incorporated *Aloe vera* at a concentration of 10% (v/v) (Fig. 2). Cinnamon leaf oil (0.50 µL/mL – 0.80 µL/mL) incorporated in *Aloe vera* at 10% (v/v) and cinnamon bark oil (0.60 µL/mL – 0.80 µL/mL) incorporated *Aloe vera* at 10% (v/v) displayed 100% inhibition of mycelia of *Phomopsis* sp. (Fig. 3). Mycelial growth of *L. theobromae* was inhibited completely at 12.5% (v/v) *Aloe vera* gel + cinnamon leaf oil (0.30 µL/mL – 0.60 µL/mL) (Fig. 4) and 12.5% (v/v) *Aloe vera* gel + cinnamon bark oil (0.15 µL/mL – 0.45 µL/mL) (Fig. 5) in liquid medium. According to the one-way ANOVA and Tukey's multiple comparison tests, percentage inhibition values for all three tested fungi were significantly different from the control ($p < 0.05$). Between two above essential oils, cinnamon bark oil had shown a better performance in inhibiting *L. theobromae* (Fig. 6).

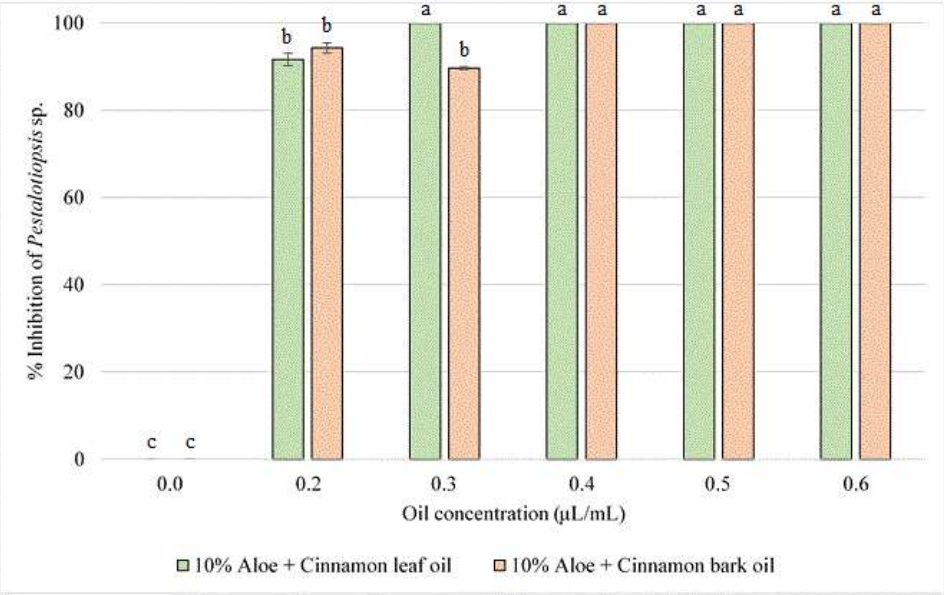

Fig. 2. Inhibitory effect of cinnamon oil in combination with 10% (v/v) *Aloe vera* gel on the growth of *Pestalotiopsis* sp. in liquid bioassay. Each data point represents the mean of three replicates. Means sharing a common letter(s) are not significantly different by Tukey's multiple comparison test at $\alpha = 0.05$.

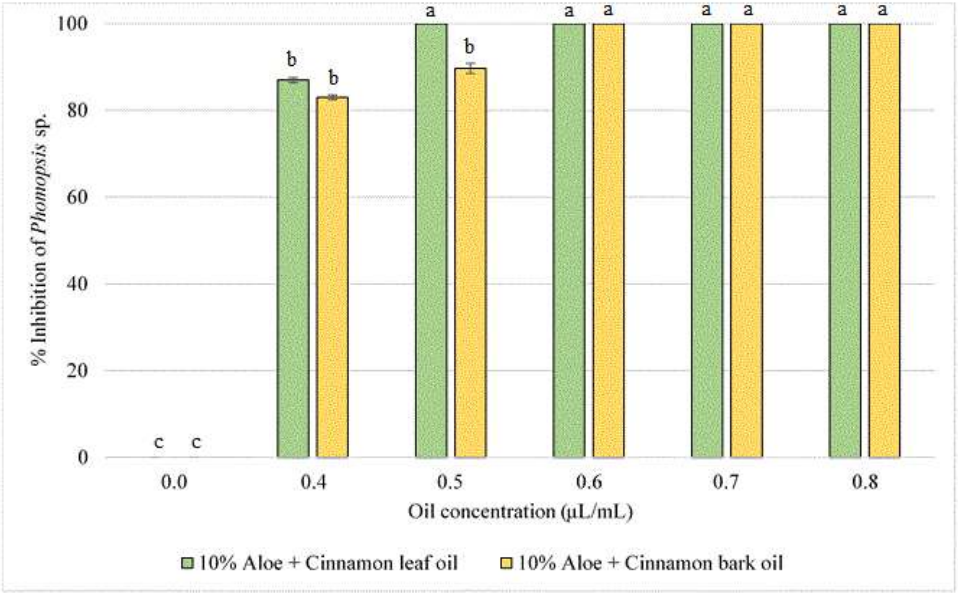

Fig. 3. Inhibitory effect of cinnamon oil in combination with 10% (v/v) *Aloe vera* gel on the growth of *Phomopsis* sp. in liquid bioassay. Each data point represents the mean of three replicates. Means sharing a common letter(s) are not significantly different by Tukey's multiple comparison test at $\alpha = 0.05$.

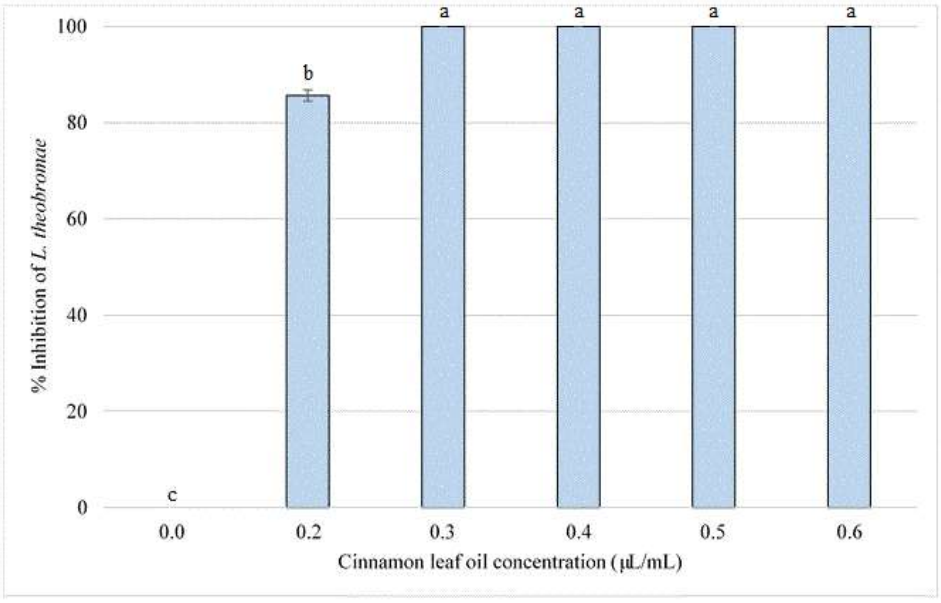

Fig. 4. Inhibitory effect of cinnamon leaf oil in combination with 12.5% (v/v) *Aloe vera* gel on the growth of *L. theobromae* in liquid bioassay. Each data point represents the mean of three replicates. Means sharing a common letter(s) are not significantly different by Tukey's multiple comparison test at $\alpha = 0.05$.

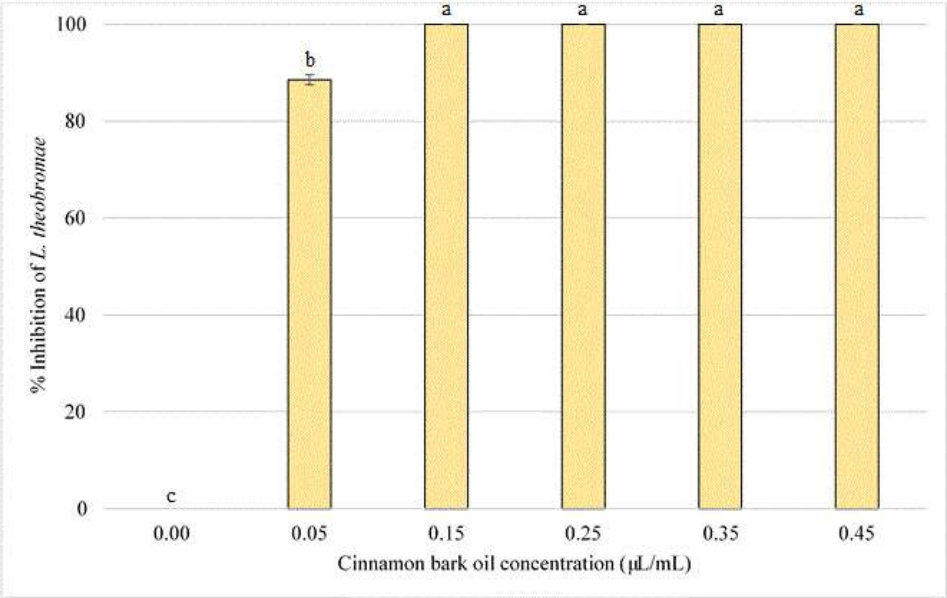

Fig. 5. Inhibitory effect of cinnamon bark oil in combination with 12.5% (v/v) *Aloe vera* gel on the growth of *L. theobromae* in liquid bioassay. Each data point represents the mean of three replicates. Means sharing a common letter(s) are not significantly different by Tukey's multiple comparison test at $\alpha = 0.05$.

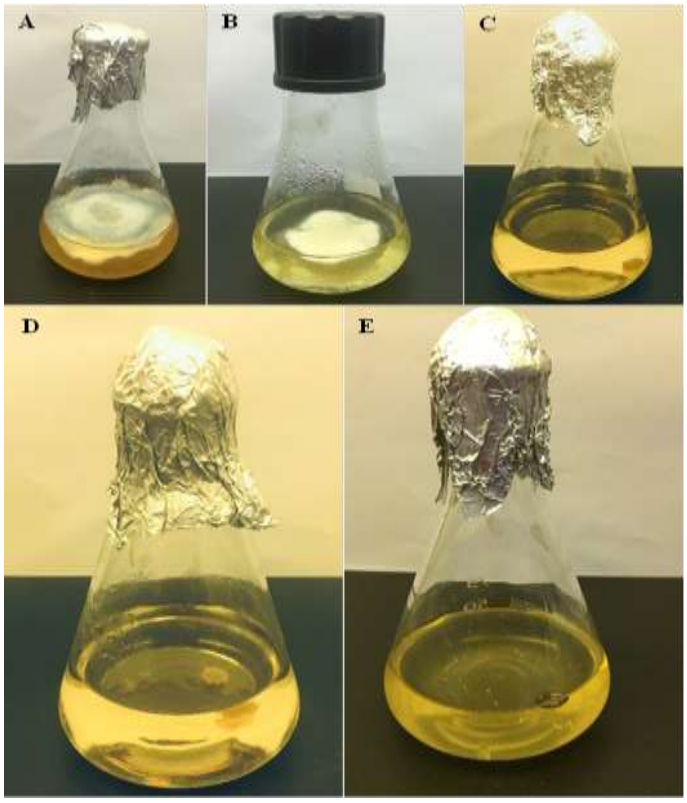

Fig. 6. Antifungal effect of *Aloe vera* gel, Cinnamon oils incorporated *Aloe vera* and carbendazim against *L. theobromae* in liquid bioassay after 7 days of incubation at 28 ± 2 ºC [Negative control (A); 12.5% *Aloe vera* gel (B); 12.5% *Aloe vera* gel + 0.30 µL/mL cinnamon leaf oil (C); 12.5% *Aloe vera* gel + 0.15 µL/mL cinnamon bark oil (D); 0.1% (w/v) Carbendazim (E)].

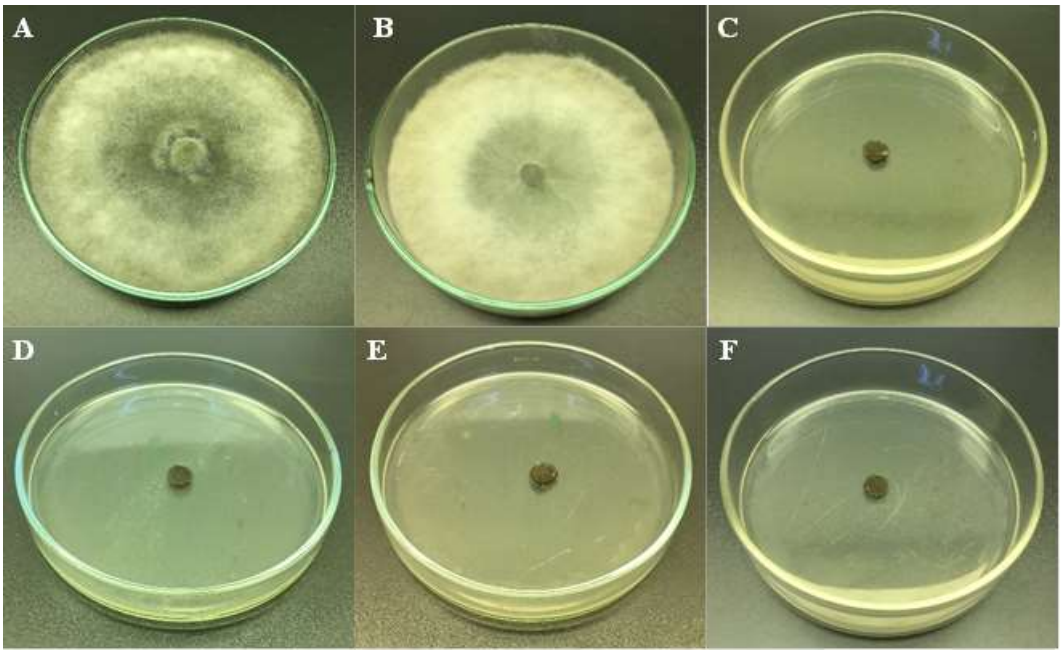

Fig. 7. Recovery of mycelial growth of *L. theobromae* on PDA after liquid bioassay. [Negative control (A); 12.5% *Aloe vera* gel + 0.05 µL/mL cinnamon bark oil (B); 12.5% *Aloe vera* gel + 0.15 µL/mL cinnamon bark oil (C); 12.5% *Aloe vera* gel + 0.25 µL/mL cinnamon bark oil (D); 12.5% *Aloe vera* gel + 0.35 µL/mL cinnamon bark oil (E); 12.5% *Aloe vera* gel + 0.45 µL/mL cinnamon bark oil (F)].

MIC and MLC values of cinnamon leaf oil incorporated *Aloe vera* gel against *Pestalotiopsis* sp. were 10% + 0.30 µL/mL and 10% + 0.50 µL/mL respectively. Cinnamon bark oil incorporated *Aloe vera* was fungistatic against *Pestalotiopsis* sp. at 10% + 0.40 µL/mL and fungicidal at 10% + 0.60 µL/mL. MIC and MLC values of cinnamon leaf oil incorporated *Aloe vera* against *Phomopsis* sp. were 10% + 0.50 µL/mL and 10% + 0.80 µL/mL respectively. Further, both MIC and MLC of cinnamon bark oil incorporated *Aloe vera* against *Phomopsis* sp. were 10% + 0.50 µL/mL. MIC and MLC values of cinnamon leaf oil incorporated *Aloe vera* against *L. theobromae* were 12.5% + 0.30 µL/mL and 12.5% + 0.60 µL/mL respectively. Cinnamon bark oil incorporated *Aloe vera* gel was effective against *L. theobromae*, as it displayed 100% growth inhibition (MIC) at a lower concentration (i.e. 12.5% + 0.05 µL/mL) and fungicidal effect (MLC) at just 12.5% + 0.15 µL/mL (Fig. 7) (Table 1). Present results revealed that *Aloe vera* gel in combination with cinnamon essential oil displayed a slightly higher antifungal activity against test fungi in liquid bioassay than using *Aloe vera* gel alone.

The present MLC of cinnamon oil with the combination of *Aloe vera* gel against *L. theobromae*, *Pestalotiopsis* sp. and *Phomopsis* sp. was somewhat lower than previous results identified by Kodituwakku et al. (2020) in which the essential oil had been tested against SER pathogens lonely. Ranasinghe et al. (2002) indicated that the fungicidal concentration of cinnamon bark oil for *L. theobromae* was 0.45 µL/mL which is a higher concentration compared to MLC identified by the present study. However, above research had been conducted using *L. theobromae* isolated from crown rot disease of banana. Same pathogen isolated from the different diseases/commodities may respond differently to botanical treatments.

Table 1. Minimum inhibitory and minimum lethal concentrations (MICs and MLCs) of cinnamon oil incorporated *Aloe vera* against SER-associated fungi of mango as determined by the liquid bioassay

Treatments	*Pestalotiopsis* sp.		*Phomopsis* sp.		*L. theobromae*	
	MIC[a]	MLC[b]	MIC[a]	MLC[b]	MIC[a]	MLC[b]
Aloe vera gel + cinnamon leaf oil	10% + 0.30 µL/mL	10% + 0.50 µL/mL	10% + 0.50 µL/mL	10% + 0.80 µL/mL	12.5% + 0.30 µL/mL	12.5% + 0.60 µL/mL
Aloe vera gel + cinnamon bark oil	10% + 0.40 µL/mL	10% + 0.60 µL/mL	10% + 0.50 µL/mL	10% + 0.50 µL/mL	12.5% + 0.05 µL/mL	12.5% + 0.15 µL/mL

[a] Zero mycelial growth at minimum inhibitory concentration; [b] Zero revival of fungus at minimum lethal concentration.

CONCLUSION

In vitro antifungal effect of cinnamon essential oils incorporated *Aloe vera* gel successfully controlled *L. theobromae*, *Pestalotiopsis* sp. *and Phomopsis* sp. which are the SER pathogens of mango (cv. Karthakolomban). Also, *L. theobromae* which is a prominent SER pathogen of mango was successfully controlled with a lower concentration to the values interpreted in contemporary studies when applied in a combination with the *Aloe vera* gel. *Aloe vera* gel coating acts as a protective layer which reduces fruit damage. Additionally, it plays the role of a successful antifungal agent alone and in combination with cinnamon leaf oil and cinnamon bark oil. Therefore, the use of *Aloe vera* gel in combination with cinnamon leaf oil and cinnamon bark oil in respective lethal concentrations could be suggested as successful treatments to extend the storage life of mango fruits since these treatments are eco-friendly and have economic advantages as smaller quantities are needed.

Conflict of interest

The authors declare no conflict of interest to report.

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