RESEARCH ARTICLE

In vitro antifungal efficacy of selected essential oils in controlling fungi associated with the stem-end rot disease of mango (cv. Karutha Colomban) fruits and characterisation of antifungal components

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Abstract: Karutha Colomban is one of the most delightful mango varieties popular among Sri Lankan consumers. A significant postharvest loss of mango takes place every season due to diseases including stem-end rot (SER), which is caused by a group of endophytic fungal pathogens. In this research, *in vitro* antifungal efficacy of different concentrations of essential oils of basil, clove, and cinnamon were evaluated for their ability to control SER causing fungal pathogens of mango (cv. Karutha Colomban) as bio-safe alternatives to conventional fungicides, by conducting liquid and disc volatilisation bioassays. Major bioactive compounds of the selected essential oils were identified by gas chromatography-mass spectroscopy (GC-MS). Basil and cinnamon bark oils (0.20 – 0.30 μL/mL) in liquid bioassay showed high efficacy against *Lasiodiplodia theobromae*, while basil and cinnamon leaf oils (0.40 – 0.60 μL/mL) successfully inhibited *Pestalotiopsis* sp. Cinnamon bark oil (0.60 μL/mL) was identified as the most effective oil against *Phomopsis* sp. According to disc volatilisation bioassay, vapour of cinnamon oils (0.20 – 0.40 μL/mL) was the most effective in controlling *L. theobromae*. *Pestalotiopsis* sp. was efficiently controlled by clove and cinnamon bark oil (0.20 – 0.60 μL/mL) vapour. In vapour phase, clove and cinnamon oils (0.40 μL/mL) were the most effective against *Phomopsis* sp. According to GC-MS characterisation, methyl chavicol was the most abundant antifungal component in basil oil while it was (E)-cinnamaldehyde in cinnamon bark oil. Moreover, eugenol displayed the highest abundance in clove and cinnamon leaf oils. Based on *in vitro* studies, it could be concluded that cinnamon bark oil in liquid and vapour phases demonstrated a higher antifungal efficacy among the tested essential oils in controlling fungal pathogens causing SER of mango.

INTRODUCTION

Mango (*Mangifera indica* L.) is a popular fruit worldwide and it has become one of the most desirable fruits in international trade because of its delightful taste and high caloric value (Diedhiou *et al*., 2007). It bears important nutrients such as vitamins, minerals, polyphenolic and flavonoid antioxidant compounds as well as prebiotic dietary fiber (Kothalawala & Jayasinghe, 2017). Asia is recognised as the highest mango producing region, with a record 74.4 % of global production. Present extent under mango cultivation in Sri Lanka is about 3.1 million hectares (FAOSTAT, 2016). 'Karutha Colomban' is one of the most popular cultivars of mango among growers and consumers in Sri Lanka due to its orange flesh and delightful taste (Kothalawala & Jayasinghe, 2017).

However, the availability of high quality mango for the local and international consumers has been limited by its highly perishable nature and susceptibility to postharvest diseases, which lead to severe postharvest lossess (Bally *et al.,* 2009). Stem-end rot (SER) is considered as one of the most serious and frequently occurring postharvest and post-packaging diseases of mango (Tripathi & Shukla, 2009). SER is caused by a complex of fungi

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namely, *Lasiodiplodia theobromae* (syn. *Botryodiplodia theobromae*), *Neofusicoccum parvum* (syn. *Dothiorella dominicana*), *N. mangiferae* (syn *D. mangiferae*), *Phomopsis mangiferae*, *Pestalotiopsis mangiferae* and *Colletotrichum gloeosporioides* (Abeywickrama, 2006; Jabeen, 2016; Ekanayake, 2017).

At present, the disease control strategies include prophylactic use of synthetic fungicides to treat fruit crops including mango. Although fungicide usage is a successful chemical control strategy for postharvest diseases of many fruits, it has given rise to many controversial issues owing to their carcinogenicity, acute toxicity, long degradation periods, environmental pollution and contribution to the development of resistant pathogens. Due to these environmentally unsound effects, researchers are focusing on finding effective alternatives to synthetic fungicides in controlling postharvest diseases of fruits (Tripathi & Shukla, 2009).

Essential oils extracted from plants are generally assumed to be more acceptable and less hazardous than synthetic compounds as they possess bioactive properties, due to the presence of many chemical components such as eugenol, camphor, camphene, caryophyllene, phellandrene, *etc.* (Bakkali *et al.*, 2008; Zaker, 2016). In several previous studies, it has been recorded that essential oils of cinnamon (*Cinnamomum zeylanicum*) leaf and bark, clove (*Syzygium aromaticum*), and basil (*Ocimum basilicum*) as very effective sources of antifungal compounds (Ranasinghe, 2003; Sukatta *et al.*, 2008; Abeywickrama *et al.*, 2009; Costa *et al.*, 2015).

An *in vitro* liquid bioassay conducted by Anthony *et al.* (2004) showed that the essential oils of *Cymbopogon nardus* and *O. basilicum* have inhibited *L. theobromae* isolated from banana with crown rot disease at the minimum concentrations of 1.0 and 0.8 µL/mL, respectively. According to Abeywickrama *et al.* (2003), vapour of basil oil at 0.05 % – 0.2 % has found to be fungicidal against banana crown rot pathogens including *L. theobromae*. Dip treatment with cardamom (*Elettaria cardamomum*) oil at 0.70 µL/mL has significantly reduced SER of Karutha Colomban mango (Karunanayake *et al.*, 2018). Furthermore, fumigation with the essential oil of *Amomum subulatum* leaf has successfully controlled SER of mango (cv. Dasheri) (Dubey *et al.*, 2008).

The objectives of the present study were (i) to investigate *in vitro* bioactive efficacy of essential oils of cinnamon (*i.e.* leaf and bark), basil, and clove as eco-friendly antifungal agents against SER-associated fungi (*L. theobromae*, *Pestalotiopsis* sp., and *Phomopsis* sp.) isolated from Karutha Colomban mango, and (ii) to characterise these oils to identify effective antifungal components using gas chromatography-mass spectroscopy (GC-MS).

METHODOLOGY

Essential oils

Ocimum basilicum (basil) oil was purchased from Aromatica Laboratories (Pvt.) Ltd., Colombo 11. *Cinnamomum zeylanicum* (cinnamon) leaf oil, cinnamon bark oil and *Syzygium aromaticum* (clove) oil were purchased from Citro Essential Oils (Pvt.) Ltd., Mt. Lavinia. Extra pure eugenol as an authentic standard was obtained from Loba Chemie Indoaustranal Co., India. Carbendazim fungicide as a positive control was purchased from Hayleys Agriculture Holdings Ltd., Colombo 10.

Test fungi

SER-associated fungi, isolated and characterised in a previous study (Ekanayake, 2017) through morphology and molecular tools and designated as *Lasiodiplodia theobromae* (MH005085.1), *Pestalotiopsis* sp. (MH005090.1) and *Phomopsis* sp. (MH005086.1), were used for this study.

In vitro bioassays to determine the antifungal effects of essential oils

Liquid bioassay

According to the method described by Anthony *et al.* (2004), conical flasks (100 mL) containing 50 mL of SMKY semi synthetic liquid medium (7.0 g yeast extract, 1.5 g KNO_3, 20.0 g sucrose and 0.50 g $MgSO_4.7H_2O$ dissolved in 1 L of distilled water) was autoclaved for 20 min at 1.03 kg cm^{-2} and 121 ºC. A concentration series of oils; 0.10 – 1.00 µL/mL was prepared aseptically adding appropriate volumes of test oils to the flasks, and Tween 80 (Koch-Light Laboratories, England) was used as a surfactant to disperse oil. 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. Contents were mixed thoroughly by placing the flasks on reciprocal water bath shaker (New Brunswick Scientific, Model: R76) at room temperature (28 ± 2 ºC). A control (sterilised

distilled water and Tween 80 without oil) was included for comparison. Eugenol (2.00 µL/mL) prepared in a similar manner was used as an authentic standard to observe the antifungal efficacy. Carbendazim fungicide (0.1 % w/v) was used as the positive control. Six replicates of each treatment and controls were arranged according to completely randomised design (CRD) in the laboratory. After 7 d 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 (Memmert, UM 600) at 105 ºC overnight until a constant weight was reached to determine the mycelial dry weight (Anthony *et al*., 2004). The inhibition percentage was determined using the following equation.

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

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

Disc volatilisation bioassay

Petri plates (90 × 15 mm) containing potato dextrose agar (PDA) medium (15 mL per plate) were inoculated with a 5 mm diameter mycelial plug from the periphery of a 7-day old pure culture of the respective test pathogen (Perumal *et al*., 2016). A sterilised filter paper disc with a diameter of 90 mm (Whatman No: 1) was placed on the inner surface of the Petri dish lid. Aliquots of the essential oil ranging from 2.0 – 20.0 µL were added onto the filter paper discs placed in separate Petri dishes. A filter paper soaked with sterile distilled water served as the control. Similar treatments with eugenol (20.0 µL) and ethanol (100.0 µL) were used as authentic standard and positive control, respectively. The Petri plates were sealed with para-film immediately after adding essential oil, placed in an inverted position and incubated at room temperature for 7 d (Feng *et al*., 2011). The experimental design was a CRD with 6 replicates per treatment and controls. The radial mycelial growth of each test pathogen was evaluated after 7 d of incubation by measuring the colony diameter along two axes at right angles to each other. These values were compared with those of untreated controls. The antifungal efficacy was expressed as the percentage inhibition of radial mycelial growth (% IRMG) using the following formula (Abdollahi *et al*., 2011).

$$\text{\% IRMG} = [(dc - dt) / dc] \times 100 \qquad ...(2)$$

dc = mean radial mycelial growth in control Petri plates
dt = mean radial mycelial growth in treatment Petri plates

Determination of minimum inhibitory and minimum lethal values of test oils

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 d at room temperature (28 ± 2 ºC) to observe the recovery of the mycelial growth, in order to differentiate between the fungicidal and fungistatic activity of the selected essential oil treatments. Any revival of radial mycelial growth was categorised as the fungistatic effect and the corresponding concentration of the test oil was considered as the minimum inhibitory concentration (MIC). No growth of fungal mycelia (no revival) was considered as fungicidal effect and the corresponding concentration of the test oil was considered as minimum lethal concentration (MLC) (Sellamuthu *et al*., 2013; Bill *et al*., 2015; Perumal *et al*., 2016).

Gas chromatography-mass spectroscopy analysis of test oils

Basil, clove, cinnamon leaf, and bark oils were subjected to GC-MS analysis at the Industrial Technology Institute, Malabe. The analysis was carried out using a GC apparatus (Thermoscientific Trace 1300 model) equipped with an auto-injector AI 1310 (Thermoscientific) and a fused silica capillary column (Rtx-wax) of 30 m × 0.25 mm i.d., 0.25 µm film (Thermoscientific), using helium as the carrier gas with a flow rate of 1.0 mL min⁻¹. The temperature was programmed to start at 60 ºC, followed by an increase at a rate of 5 ºC min⁻¹ until 220 ºC was reached, then maintaining at 220 ºC for 10 min. The temperatures of the injector and the ion source were 240 ºC and 250 ºC, respectively. A volume of 0.3 µL essential oil diluted with 1 mL of hexane was injected at a partitioning rate of injected volume of 1:50 and a column pressure of 64.20 kPa. The MS was carried out with an ion capture detector operating in electronic impact mode with impact energy of 70 eV, a scan interval of 0.50 fragments, and fragments detected in the range of 50 – 450 Da. The essential oil components were identified by comparing the mass spectrum with spectra from the equipment database (NIST11). Additionally, the retention rates were compared with those in the literature (Adams, 2007).

Data analysis

Data with respect to *in vitro* bioassays were analysed using two-way analysis of variance (ANOVA), using Minitab 17 statistical software. The mean separation was done using Tukey's multiple comparison test (Anthony *et al*., 2004).

RESULTS AND DISCUSSION

Antifungal efficacy of essential oils in liquid phase

In vitro liquid bioassay technique allows determination of the antifungal activity of essential oils which are in direct contact with the mycelia of fungal pathogens growing in an aqueous medium (Anthony *et al.*, 2004). Out of the tested oils, basil oil was the most effective against *L. theobromae* which displayed 100 % mycelial inhibition at 0.20 µL/mL. Cinnamon bark oil was equally effective and achieved complete inhibition of mycelia of *L. theobromae* at 0.25 µL/mL. Clove and cinnamon leaf oils were slightly less effective against *L. theobromae* and displayed 100 % mycelial inhibition only at 0.40 µL/mL (Figure 1). Accordingly, each of these concentrations were considered as MIC values for basil, cinnamon bark, clove and cinnamon leaf oils, respectively (Table 1). With respect to the fungicidal effect, basil oil was more effective against *L. theobromae* than other oils, with an MLC of 0.20 µL/mL. According to two-way ANOVA, percentage inhibition values obtained for different oils were significantly different from the control when the

test oil type, concentration and the interaction between test oil type × concentration were considered (p < 0.05). According to a similar liquid bioassay carried out by Anthony *et al.* (2004), basil oil was fungistatic as well as fungicidal at a minimum concentration of 0.80 µL/mL against *L. theobromae* isolated from banana, which is comparatively greater than MIC and MLC values determined by the present study. MLC values of cinnamon bark and clove oils against *L. theobromae* were slightly higher being 0.30 and 1.00 µL/mL, respectively (Table 1). According to a previous record (Ranasinghe *et al.*, 2002) on a liquid bioassay using the same liquid medium, cinnamon bark oil was found to be fungicidal against *L. theobromae* isolated from banana at 0.45 µL/mL, which is slightly greater than the present value. MLC of clove oil against *L. theobromae* determined by Ranasinghe *et al.* (2002) was 0.60 µL/mL. However, present MLC value of clove oil against the same pathogen was somewhat lower compared to the previous result. The present MLC of cinnamon leaf oil against *L. theobromae* was 1.00 µL/mL and it is slightly greater than the value reported by Ranasinghe *et al.* (2002) (*i.e.* 0.80 µL/mL).
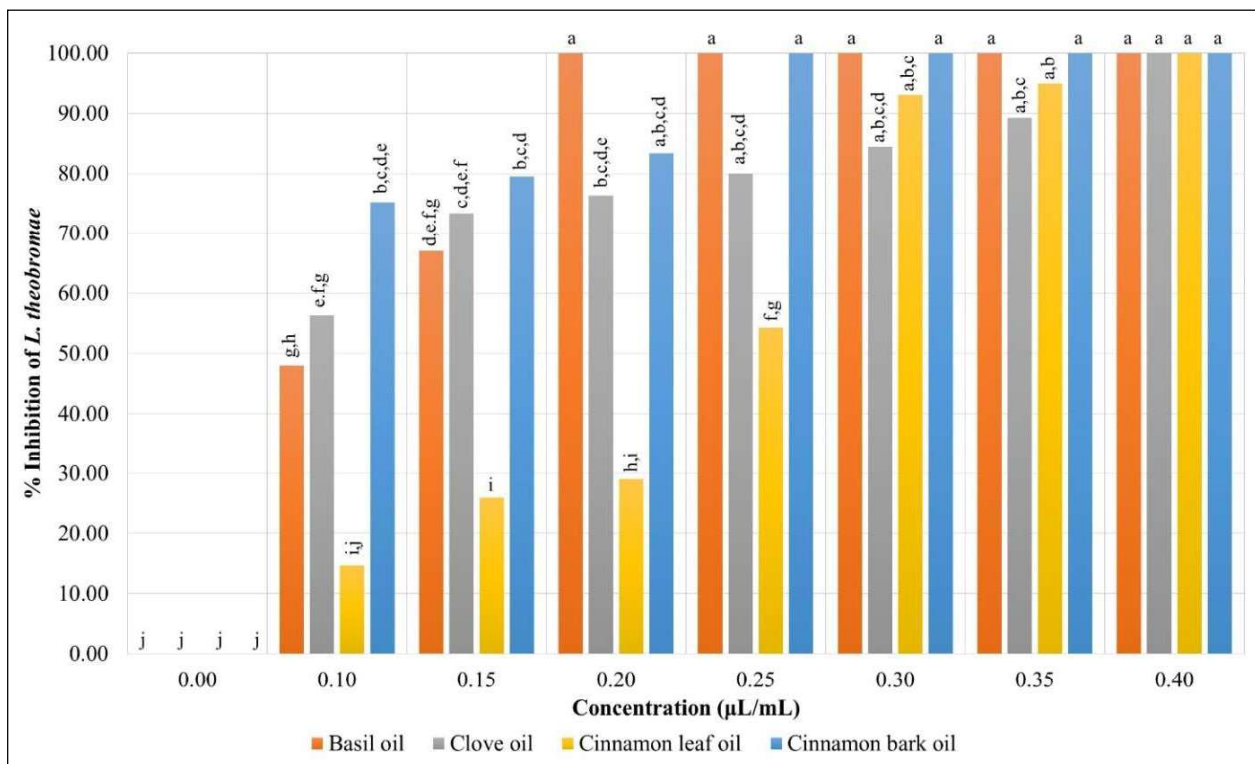

Figure 1: Inhibitory effect of test oils on the growth of *L. theobromae* in liquid bioassay. Each data point represents the mean of six replicates. Means sharing a common letter(s) are not significantly different by Tukey's multiple comparison test at p < 0.05.

Table 1: Minimum inhibitory and minimum lethal concentrations (MICs and MLCs) of test oils against SER-associated fungi of mango as determined by the liquid bioassay.

Test oil	*L. theobromae*		*Pestalotiopsis* sp.		*Phomopsis* sp.	
	MIC [a] (μL/mL)	MLC [b] (μL/mL)	MIC [a] (μL/mL)	MLC [b] (μL/mL)	MIC [a] (μL/mL)	MLC [b] (μL/mL)
Basil	0.20	0.20	0.60	0.60	0.80	0.80
Clove	0.40	1.00	0.60	1.00	0.60	0.80
Cinnamon leaf	0.40	1.00	0.40	0.60	0.60	0.80
Cinnamon bark	0.25	0.30	0.40	0.80	0.60	0.60

[a] Zero mycelial growth at minimum inhibitory concentration; [b] Zero revival of fungus at minimum lethal concentration. Each data point represents the mean of six replicates.

Growth of *Pestalotiopsis* sp. was completely inhibited by most of the test oils at very low concentrations (*i.e.* 0.40 – 0.60 μL/mL; Figure 2). Cinnamon leaf and bark oils were effective against *Pestalotiopsis* sp., as they displayed 100 % growth inhibition (MIC) at a lower concentration (*i.e.* 0.40 μL/mL; Figure 3) and fungicidal effect (MLC) between 0.60 – 0.80 μL/mL (Table 1). Basil and clove oils exhibited fungistatic effect against *Pestalotiopsis* sp. at a slightly higher concentration (*i.e.* 0.60 μL/mL). Further, MLC of basil oil was also recorded

Figure 2: Antifungal effect of test oils, eugenol and carbendazim against *Pestalotiopsis* sp. in liquid bioassay after 7 days of incubation at 28 ± 2 ºC [Negative control (A); 0.60 μL/mL basil oil (B); 0.60 μL/mL clove oil (C); 0.40 μL/mL cinnamon leaf oil (D); 0.40 μL/mL cinnamon bark oil (E); 2.00 μL/mL eugenol (F); and 0.1 % (w/v) carbendazim (G)].

at 0.60 μL/mL indicating its fungicidal effect. The two-way ANOVA revealed that, results obtained for test oils were significantly different from the control when the test oil type, concentration and the interaction between test oil type × concentration were considered as factors ($p < 0.05$).

Further, clove, cinnamon leaf and cinnamon bark oils were more effective than basil oil in terms of fungistatic activity against *Phomopsis* sp. (Table 1; Figure 3). MIC of basil oil was 0.80 μL/mL, which was slightly greater than the MICs of clove and two cinnamon oils (*i.e.* 0.60 μL/mL). However, when considering the fungicidal activity, cinnamon bark oil was the most effective against *Phomopsis* sp. than other oils. Percentage inhibition values were significantly different when concentration and interaction of test oil type × concentration were considered as factors ($p < 0.05$). However, there was no significant difference in percentage inhibition when only test oil type was considered as a factor ($p > 0.05$). Carbendazim and eugenol at fixed concentrations (0.1 % w/v and 2.00 μL/mL, respectively) completely inhibited the growth of all test fungi revealing high antifungal efficacy (data not presented). Eugenol is a bioactive compound found in the essential oils of many higher plants and its antifungal action has been confirmed by many studies (Herath & Abeywickrama, 2008; Wang *et al*., 2010).
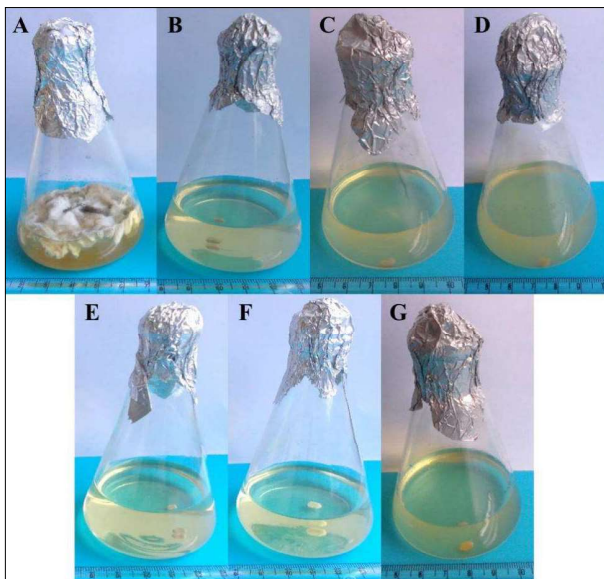
Antifungal activity of essential oils in vapour phase

In vitro disc volatilisation bioassay was carried out to identify the vapour phase effect of each essential oil against the growth of test fungi on a solid medium. The volatile nature of the bioactive compounds present in essential oils allows them to act against many fungal pathogens (Bill *et al*., 2015). Vapour of clove, cinnamon
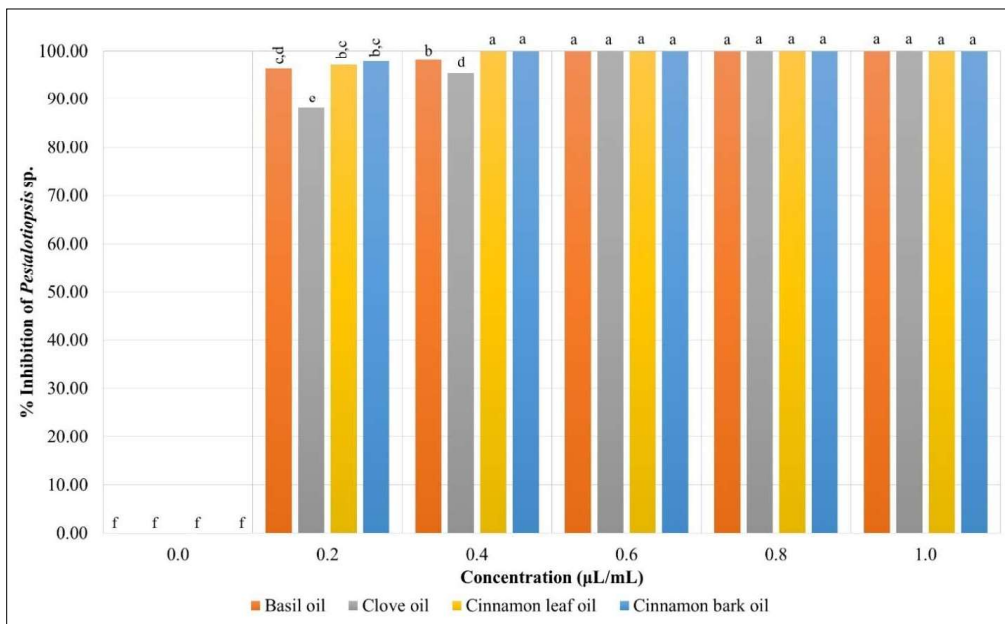
Figure 3: Inhibitory effect of test oils on the growth of *Pestalotiopsis* sp. in liquid bioassay. Each data point represents the mean of six replicates. Means sharing a common letter(s) are not significantly different by Tukey's multiple comparison test at p < 0.05.

Table 2: Minimum inhibitory and minimum lethal concentrations (MICs and MLCs) of test oils against SER-associated fungi of mango as determined by the disc volatilisation bioassay.

Test oil	*L. theobromae*		*Pestalotiopsis* sp.		*Phomopsis* sp.	
	MIC [a] (μL/plate)	MLC [b] (μL/plate)	MIC [a] (μL/plate)	MLC [b] (μL/plate)	MIC [a] (μL/plate)	MLC [b] (μL/plate)
Basil	10.0	Not achieved	16.0	16.0	12.0	12.0
Clove	2.0	Not achieved	2.0	6.0	4.0	4.0
Cinnamon leaf	2.0	4.0	4.0	8.0	4.0	4.0
Cinnamon bark	2.0	2.0	2.0	4.0	4.0	4.0

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

leaf, and cinnamon bark oils showed a complete inhibition of radial mycelial growth at 2.0 μL/plate concentration against *L. theobromae* (Table 2). However, according to Sukatta *et al.* (2008), higher concentration of cinnamon bark oil (*i.e.* 10 μL/plate) was needed for 100 % inhibition of mycelial growth of *L. theobromae*, while clove oil at the same concentration controlled the growth of *L. theobromae* only up to 74.07 %. Further, in the present study, cinnamon leaf and cinnamon bark oils showed a fungicidal effect against *L. theobromae* at concentrations of 2.0 μL/plate and 4.0 μL/plate

respectively, whereas, clove oil failed to inhibit the pathogen growth completely. Basil oil vapour achieved a 100 % IRMG at a concentration of 10.0 μL/plate and was the least effective oil against *L. theobromae* compared to other test oils, which could not display fungicidal effect either (Table 2). Abeywickrama *et al.* (2003) reported that basil oil showed a high antifungal effect against banana fruit pathogens, *Colletotrichum musae*, *Fusarium proliferatum* and *L. theobromae* at very low concentrations (*i.e.* MLC = 0.05 – 0.20 %) comparable with the results of the present research.

According to Table 2, clove and cinnamon bark oils achieved a 100 % IRMG of *Pestalotiopsis* sp. at a MIC of 2.0 µL/plate. Therefore, effectiveness of those two oils in the vapour form was high, when compared to basil and cinnamon leaf oils, having fungistatic effects at 16.0 and 4.0 µL/plate concentrations, respectively. Fungicidal activity of clove and cinnamon bark oils (*i.e.* MLC = 6.0 and 4.0 µL/plate, respectively) was more pronounced against *Pestalotiopsis* sp. than that of basil and cinnamon leaf oils (*i.e.* MLC = 16.0 and 8.0 µL/plate, respectively). Since clove oil demonstrated relatively lower MIC and MLC values than basil oil, it could be considered as a moderately effective fumigant against *Pestalotiopsis* sp. Clove, cinnamon leaf, and cinnamon bark oils displayed fungistatic as well as fungicidal effects against *Phomopsis* sp. at a concentration of 4.0 µL/plate. This value was notably less than the MIC and MLC of basil oil (*i.e.* 12.0 µL/plate) against *Phomopsis* sp. Therefore, efficacy of basil oil vapour against *Phomopsis* sp. was relatively less than that of clove and cinnamon oils, which could be recognised as the best oils in controlling the target pathogen (Table 2). According to Sukatta *et al.* (2008), a different strain of *Phomopsis* (*P. viticola*) was completely inhibited by cinnamon bark oil at 10 µL/plate concentration, while clove oil at the same concentration controlled the growth of *P. viticola* up to 91 %. However, the present MIC and MLC values of clove and cinnamon bark oils against mango SER-associated *Phomopsis* sp. were notably lower when compared to the results of the former study. According to the present study eugenol (20 µL/plate) at vapour phase was capable of inhibiting all test pathogens, completely. Although ethanol (100 µL/plate) was used as the positive control in this fumigation assay, it was unable to inhibit the growth of test fungi, completely. However, % IRMG values for ethanol were 21.60 and 50.62 % for *Pestalotiopsis* sp. and *Phomopsis* sp., respectively. According to Bacílková (2006), vapour of ethanol at 30 – 90 % (v/v) has totally controlled *Aspergillus niger* and *Penicillium notatum*, which are commonly considered as saprophytes. Since absolute ethanol was used against the two pathogenic fungi tested in the present study, a complete inhibition could not be achieved.

GC-MS characterisation of test oils

GC-MS analysis of the selected essential oils revealed the major chemical constituents of each tested essential oil along with their relative abundance. In basil oil, methyl chavicol was the major compound with a relative abundance of 74.44 % with relatively low amounts of eugenol and (E)-cinnamaldehyde (Table 3). However, eugenol was the most abundant constituent in both clove and cinnamon leaf oils. (E)-cinnamaldehyde in cinnamon leaf was relatively low. The composition of cinnamon bark oil consisted of 72.18 % (E)-cinnamaldehyde with a low abundance of eugenol. Linalool, which is one of the antifungal constituents present in many essential oils, was detected in basil, cinnamon leaf, and cinnamon bark oils with percentages of 15.01, 1.27 and 3.69, respectively (Table 3). All these chemical constituents have previously been reported as bioactive antifungal compounds against many postharvest pathogenic fungi. Methyl chavicol, which is the most abundant component present in basil oil was found to be antifungal against two postharvest pathogenic fungi *viz*, *C. gloeosporioides* and *Alternaria alternata* (Costa *et al.*, 2015). Eugenol, which was recognised as one of the most effective bioactive components present in the test oils has previously been reported for its strong antifungal activity against *C. musae*, *F. proliferatum*, and *Botrytis cinerea* (Herath & Abeywickrama, 2008; Wang *et al.*, 2010). According to Hong *et al.* (2015) and Marei and Abdelgalei (2018), cinnamaldehyde was found to be antifungal against *C. gloeosporioides*, *B. cinerea*, *Aspergillus niger*, and *Penicillium digitatum*. Further, Soković and van Griensven (2006) have identified linalool as an antifungal agent of controlling *Verticillium fungicola* and *Trichoderma harzianum*. Although several antifungal components are present in a single oil in different proportions, they appear to act synergistically to inhibit the growth of target fungi (Anthony *et al.*, 2004).

Antimicrobial constituents of essential oils affect cell structure by causing disruption of cell membrane, alteration of cell membrane integrity, and inhibition of cell wall formation. Integrity and functionality of cell membranes are governed by ergosterols, a structural component of cell membranes. Bioactive compounds of essential oils can bind with ergosterols or act as specific inhibitors that inhibit the biosynthesis of ergosterols, causing cell membrane destruction. Essential oils can also inhibit the cell wall formation by blocking the formation of β-glucans in fungi, which is one of the important constituents of the cell wall (Nazzaro *et al.*, 2017). Damage to cell wall and cell membrane by essential oils can cause leakage of macromolecules and electrolytes and cell lysis of pathogens (Bakkali *et al.*, 2008). Leakage of contents of *C. musae* and *F. proliferatum* conidia treated with essential oils of *Ocimum basilicum*, *Cymbopogon citratus*, eugenol and citral was reported by Herath and Abeywickrama (2008). Essential oils are also known to cause the dysfunction of mitochondria and inhibition of efflux pumps causing serious damages to pathogens. Mitochondrial electron transport chain can be inhibited by the antimicrobial constituents of essential oils, which reduce the mitochondrial membrane potential of fungal pathogens. Activity of proton pumping ATPases can be

Table 3: Chemical components of tested essential oils, their retention time (RT) and relative abundance (RA) as determined by GC-MS

Compound	Basil oil		Clove oil		Cinnamon leaf oil		Cinnamon bark oil	
	RT (min)	RA (%)	RT (min)	RA (%)	RT (min)	RA (%)	RT (min)	RA (%)
Anethole	18.21	0.27	-	-	-	-	-	-
Benzaldehyde	23.89	0.35	-	-	-	-	12.69	0.06
Benzyl benzoate	-	-	-	-	34.23	3.61	34.25	1.51
δ-Cadinene	-	-	17.69	0.30	-	-	-	-
Calamenene	-	-	19.37	0.14	-	-	-	-
Camphene	-	-	-	-	3.63	0.25	3.66	0.04
α-Caryophyllene	-	-	15.79	1.04	15.79	0.39	-	-
β-Caryophyllene	-	-	14.15	9.07	14.15	2.48	14.17	3.95
Caryophyllene oxide	-	-	22.53	0.40	22.53	0.30	22.55	0.30
Cinnamaldehyde, (E)-	24.19	1.63	-	-	23.82	1.37	23.85	72.18
Cinnamaldehyde, (Z)-	-	-	-	-	-	-	20.88	0.16
Cinnamyl acetate	-	-	-	-	25.83	1.08	25.47	5.16
Copaene	-	-	11.69	0.34	11.69	0.42	11.71	0.21
ρ-Cymene	-	-	-	-	6.89	0.82	6.91	0.35
D-Limonene	-	-	5.53	0.03	5.52	0.25	5.54	0.09
Epoxylinalool	11.65	1.57	-	-	-	-	-	-
Eugenol	26.41	0.71	26.15	79.11	26.14	63.76	26.17	4.82
Eugenol acetate	-	-	27.76	0.20	27.76	2.27	-	-
Geranyl vinyl ether	17.71	0.19	-	-	-	-	-	-
Humulene	-	-	-	-	-	-	15.81	0.45
Iso-β-terpineol	6.07	0.13	-	-	-	-	-	-
Linalool	13.35	15.01	-	-	13.03	1.27	13.05	3.69
Methyl chavicol	16.23	74.44	-	-	-	-	-	-
1-methyl-4-(1-methylethyl)-cyclohexanol	15.51	0.24	-	-	-	-	-	-
Methyl salicylate	-	-	18.32	0.11	-	-	-	-
1,2-Oxolinalool	10.99	1.60	-	-	-	-	-	-
α-Phellandrene	-	-	-	-	4.96	0.68	4.98	0.12
β-Phellandrene	-	-	5.72	0.02	5.71	0.40	5.73	0.32
α-Pinene	3.52	0.02	3.17	0.09	3.16	0.91	3.18	0.17
β-Pinene	-	-	-	-	4.14	0.21	4.16	0.04
Safrole	-	-	-	-	20.35	0.96	20.37	0.11
Terpinen-4-ol	-	-	-	-	14.35	0.07	-	-
α-Terpinene			5.21	0.07	5.20	0.12	5.22	0.04
γ-Terpinene	-	-	6.38	0.08	-	-	-	-
α-Terpineol	16.83	0.10	-	-	16.49	0.15	16.51	0.06
Trans-4-methoxycinnamaldehyde	33.40	1.28	-	-	-	-	-	-
Trans-α-bergamotene	14.23	0.43	-	-	-	-	-	-

inhibited by the essential oils, which diminishes ATP production in microorganisms. Inhibition of efflux pumps by essential oil constituents will disturb the removal of toxic substances out of the cell (Nazzaro *et al*., 2017). Essential oils are also capable of damaging cytoplasmic lipids and proteins leading to coagulation of cytoplasm (Bakkali *et al*., 2008). Many other mechanisms of action for various oil components have been proposed and tested against several fungal pathogens. As essential oils tested in this study contain many bioactive components in different proportions, it could be assumed that they act synergistically in inhibiting the mango SER pathogens with the aid of one or more mechanisms of action as highlighted in the literature.
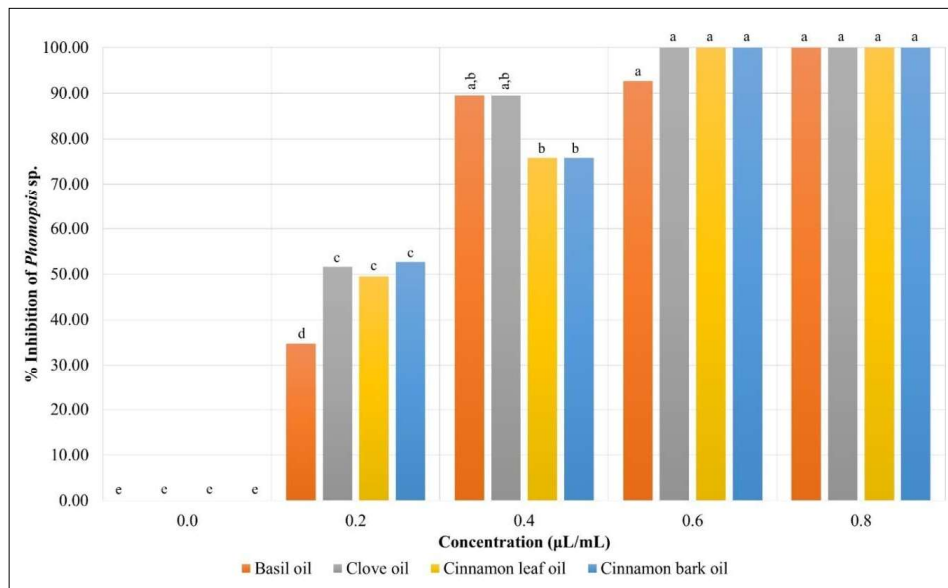
Figure 4: Inhibitory effect of test oils on the growth of *Phomopsis* sp. in liquid bioassay. Each data point represents the mean of six replicates. Means sharing a common letter(s) are not significantly different by Tukey's multiple comparison test at p < 0.05.
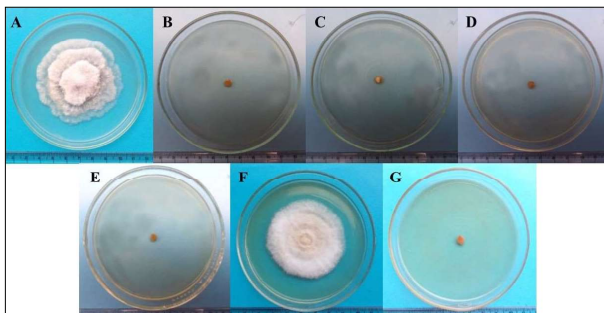

Figure 5: Antifungal effect of test oils, eugenol and carbendazim against *Pestalotiopsis* sp. in disc volatilisation bioassay after 7 days of incubation at 28 ± 2 ºC [Negative control (A); 16.0 μL/plate basil oil (B); 2.0 μL/plate clove oil (C); 4.0 μL/plate cinnamon leaf oil (D); 2.0 μL/plate cinnamon bark oil (E); 100 μL/plate ethanol (F); and 20 μL/plate eugenol (G)]

CONCLUSION

According to the *in vitro* bioassays carried out during the present study, liquid and vapour forms of basil, clove, and cinnamon oils successfully controlled SER-associated fungi of mango, *viz. L. theobromae*, *Pestalotiopsis* sp., and *Phomopsis* sp. However, their effectiveness depends on the type of oil and the fungal species tested. In general, cinnamon bark oil at liquid and vapour phases displayed a higher *in vitro* antifungal efficacy against the above SER associated fungi isolated from Karutha Colomban mango. The results highlight the importance of formulating suitable spray or fumigation treatments using these essential oils as eco-friendly alternatives to conventional fungicides, to control SER of mango in order to lengthen the postharvest storage life. Since, *L. theobromae* was the main pathogen frequently isolated from mango with SER, novel essential oil treatments could be focused towards controlling *L. theobromae* along with other SER-associated pathogens. Liquid spray and fumigation treatment systems are being currently developed *in vivo* to control SER in Karutha Colomban mango in combination with passive modified atmosphere packaging and cold storage.

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