Use of Selected Essential Oils for Controlling Stem-end Rot Disease in Mango (cv. Karuthacolomban)

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Abstract

In Sri Lanka, stem-end rot (SER) caused by *Lasiodiplodia* sp. is the most common and serious disease that affects the mango cv. 'Karuthacolomban'. At present, disease control is achieved with chemical fungicides which are hazardous to human health and environment. As such, this research looked into the possibility of using essential oils (EOs) as biological fungicides against *Lasiodiplodia* sp. in mango (cv. Karuthacolomban). Pure-grade Basil oil (*Ocimum basilicum*), Cardamom oil (*Elettaria cardamomum*), Citronella oil (*Cymbopogon nardus* L.), Lemon oil (*Citrus limon*), Mustard oil (*Brassica juncea*) and Orange oil (*Citrus sinensis)* were selected for the study. The most effective EOs against *Lasiodiplodia* sp. under *in vitro* conditions selected by a poisoned food bio-assay, were used in *in vivo* studies. The effect of the treatment on reducing disease development, organoleptic properties and the edibility of fruits after *in vivo* application was assessed. Twenty-four hours after artificial inoculation, fruits were dipped in the selected concentration of the EO solutions at room temperature or at 45°C for 3 minutes and then stored under ambient conditions. Disease

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development, percentage weight loss, titrable acidity and total soluble solids contents were measured and a sensory evaluation with a taste panel was also carried out for the treated fruits. Results revealed that basil, cardamom (700 µl l⁻¹) and citronella (400 µl l⁻¹) oils significantly reduced the growth of *Lasiodiplodia* sp. under *in vitro* conditions and cardamom oil (700 µl l⁻¹) in warm water effectively reduced the severity of SER under *in vivo* conditions with no effect on sensory properties or physico-chemical quality parameters. Thus, plant EOs can serve as safe, eco-friendly and effective alternatives to synthetic fungicides.

Key words: Mango, Essential Oil, Stem-End Rot, *Lasiodiplodia* sp., cardamom oil.

Introduction

In Sri Lanka, mango is a fruit that is in high demand in the domestic market and an important fruit for the export market (Jayasinghe and Fernando, 2009). However, commercial mango production is constrained by several diseases at all stages of its development. 'Karuthacolomban' (KC), a popular local mango cultivar, develops large stem–end rots caused predominantly by *Lasiodiplodia theobromae* and it is a major problem in the mango industry in Sri Lanka. (Karunanayake *et al.*, 2013). Stem-end rot of mango is mainly caused by *Lasiodiplodia theobromae* (Pat.) Griff et Maubl. in tropical Asia (Johnson *et al.*, 1993) and *Dothiorella* spp*., Phomopsis mangiferae*, *Pestalotiopsis* sp. and *Cytosphaera mangiferae* are also known as causal agents (Johnson *et al.,* 1992) in this process. At present, post-harvest diseases of Mango are controlled by using fungicides (Nascimento et al., 2000). Long term use of agrochemicals has caused serious health hazards to humans and environment in addition to inducing non-target pest and fungicide resistance (Fry, 1982). Thus, adoption of new disease preventive methodology which is safe for humans and environment is needed. Among the various alternatives, natural plant products, including essential oils (EOs) that are biodegradable and eco-friendly, are grabbing the attention of scientists worldwide (Macias *et al.,* 1997). Anthracnose of mango has been effectively controlled using EOs from basil (*Ocimum basillicum*), orange (*Citrus sinensis*), lemon (*Citrus medica*) and mustard (*Brassica juncea*) (Abd-alla and Haggag, 2013). In Sri Lanka, research

has been carried out on the use of EOs for the control of post-harvest diseases of banana fruit (Anthony *et al.*, 2003; 2004). Disease control is achieved by the antifungal properties shown by these EOs. Although much research has been carried out regarding controlling anthracnose of mango fruit with EOs, application of EOs to control stem-end rot disease of mango fruit has not been reported in Sri Lanka and limited work (Tripathy and Shukla, 2009) is available internationally. Therefore, in this research, the effects of natural plant products, which are essential oils (EOs) were evaluated as alternatives to fungicides in controlling SER of Mango fruit cultivar 'Karuthacolomban'.

Materials and Methods

Essential oils
Pure-grade Essential oils of basil (*Ocimum basilicum*), cardamom (*Elettaria cardamomum*), citronella (*Cymbopogon nardus*), lemon (*Citrus limon),* mustard (*Brassica juncea*) and orange (*Citrus sinensis*) were obtained from Herbal Exotics, Pugoda, Sri Lanka.

Fruits used in the study
Healthy unripe mango fruits at the harvesting maturity stage were obtained from 'Thabuththegama' (8.15˚ N, 80.30˚ E) within 24 h of harvest. The fruits were transported to the Botany Research Lab in The Open University of Sri Lanka, washed in running tap water and allowed to dry under ambient conditions. Healthy, unripe fruits of equal size and maturity were used in experiments.

Isolation of the SER pathogen
The SER pathogen was isolated on to Potato Dextrose Agar (PDA) (Meron Bacteriological Agar, superfine grade) from stem-end rot lesion on ripe K.C. mango fruit following surface sterilization with 0.5% NaOCl for 30 seconds. Sub-culturing was performed on the isolated fungi to obtain a pure culture. Sub-culturing was done on to PDA at two weeks intervals and pure cultures of the isolated fungus were maintained. Fungi were identified using colony and conidial morphology. Koch's postulates were performed to confirm the pathogenicity.

In vitro poisoned food bio-assay
The pure-grade essential oils in the selected concentrations (μl l^{-1}), were

dispersed individually as an emulsion using Tween 80 (0.05%) and added to flasks containing sterile PDA (at a temperature of 40-45 °C) just before pouring to Petri dishes (Pitarokili et al., 2003). Concentrations of EOs between 400 and 1000 µl l^{-1} were tested and the controls included the same quantity of sterile distilled water. The amount of EO added was adjusted according to the volume of PDA in the flask to obtain the required final concentration. A 5 mm diameter disk of the test fungal culture was placed in the middle of each plate prepared as mentioned above and incubated at room temperature (RT) (29±2°C) (Pitarokili *et al.*, 2003). Linear growth of the fungus was taken by measuring the diameter of the growing culture along two axes (at right angles to each other). Mean growth rates were calculated from three replicate plates of fungi every 24 h until fungal growth in the control filled the Petri dishes completely. The percentage mycelial inhibition was calculated as described by Tripathi *et al.* (2008).

In vivo application of essential oil treatment
A portion of one mm^2 mycelial plug from 14-day old *Lasiodiplodia* sp. culture was placed on top of the stem-end region of mango after making a slight cut on the peel of the fruit at the stem-end (Nisansala *et al.*, 2015). After 24 h incubation the mycelial plug was removed and fruits were dipped 2-3 min in the effective concentrations of EOs selected based on the *in vitro* study as a curative treatment. The treatments were: dipping mango fruits in the selected effective concentrations of EOs dissolved in distilled water at RT, or the same EOs and concentrations of EOs in warm water (45 °C) and the controls where mangoes were dipped in distilled water at RT or in warm water (45 °C).

Evaluation of disease development in EO treated fruits
The infection was identified on the basis of symptoms i.e., a brown, soft decay starting at the stem end and rapidly spreading to the whole fruit. The extent of stem end rot was determined daily up to seven days by tracing the diseased area on to graph paper as described by Karunanayake *et al.* (2013).

Effect of the *in vivo* essential oil treatment on fruit quality parameters

Physiological Weight Loss (%)

Weight loss was determined by daily weighing of fruits for 7 days on digital balance (Radwag, PS6000.R2). The average weights of all fruits in each treatment were taken and at the end of the experiment % of the weight loss was calculated by the formula of Gerefa *et al.* (2015).

Physiological weight loss = $\dfrac{\text{Initial weight of fruits- Final weight of fruits}}{\text{Initial weight of fruits}} \times 100$

Titrable Acidity (%)

To determine titrable acidity, ripe mango fruits (04) from each treatment were selected, peeled and juiced. Form each fruit 10 ml of juice sample was taken (three replicates) and 3 drops of phenolphthalein was added as a pH indicator. Titration was carried out against 0.1 N NaOH until the endpoint turns to pink. Titrable acidity percent as malic acid was calculated by the following formula (Gerefa *et al.*, 2015).

$$\text{Titrable acidity (TA\%) as malic acid} = \frac{0.0064 \text{ X ml of 0.1 N NaOH consumed}}{\text{Volume of juice}} \times 100$$

where 0.0064 is the malic acid conversion factor.

Total Soluble Solids (°Brix)

To determine total soluble solids (TSS) ripe mango fruits (04) from each treatment were selected and fruit pulp was made into juice without adding water. The Brix value of the pulp of each treatment was recorded using a portable refractometer (Brix/ATC 0 ~ 32%) of 0-30 Brix range at RT (25 ± 2 °C) and expresses as °Brix (Samane *et al.*, 2012). The readings were taken three times and analyzed.

Sensory evaluation of fruits treated with essential oils

A panel of untrained 26 adults (aged above 21 years), comprising both male and female, were randomly selected for sensory evaluation. A five point hedonic scale as described by Larmond (1977) was used, where 5 = very high, 4 = high, 3 = moderate, 2 = low and 1 = very low. The four

samples were served in identical plates. The organoleptic evaluation of interest conditions were: color (both peel and flesh color) and appearance, texture, odor and taste (Umuhozariho *et al.,* 2013).

Statistical Analysis
In-vitro experiments were run in triplicate, and data were reported as the mean ± standard deviation (SD). For the *in vivo* trials 8 fruits were used per treatment and the experiment was performed thrice. Data were analyzed using a statistical package, IBM SPSS version 20.0. One-way Analysis of variance (ANOVA) among means was performed using one way ANOVA. After applying the least significant Difference (LSD) test, differences of $P \leq 0.05$ were significant. For sensory evaluation data, Principal Component Analysis (PCA) and cluster analysis were used to identify the preferences of the panelists for the samples.

Results

Isolation of the SER pathogen
The mycelia grew uniformly in all directions and fully covered the surface of the media within 3 to 4 days. The colour of the colony changed gradually from light grey to dark gray after four to seven days of incubation and to greyish black after 2 weeks of incubation (Figure 1). Mature conidia were observed to have one septa, oval-shaped and brown in colour with the presence of irregular longitudinal striations (Figure 2). The identity of the fungus was confirmed as *Lasiodiplodia* sp. Based on colony and conidial morphology. Pathogenicity was confirmed by Koch's postulates.
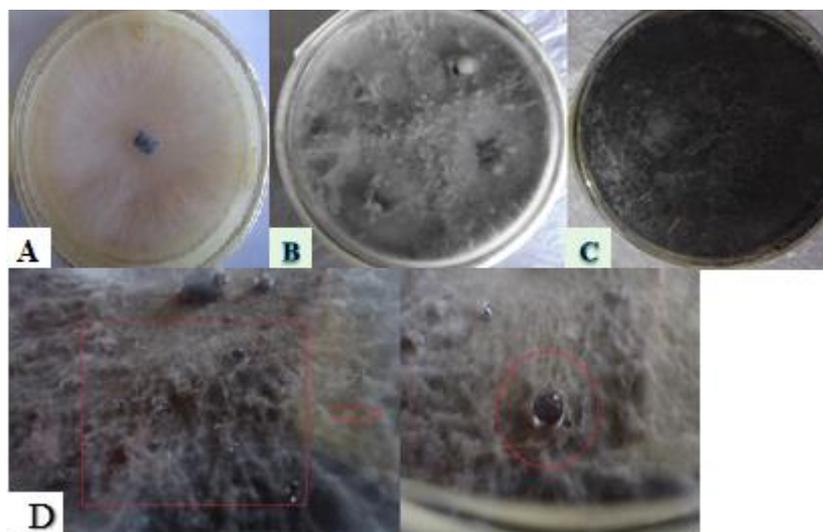
Figure 1: Colony characteristics of the fungus isolated from Mango (K.C.) fruit. (A). Top view (day 2); (B). Top view of colony (day 4); (C). Top view of colony (day 14); (D). Developing fruiting bodies (after 14 days).
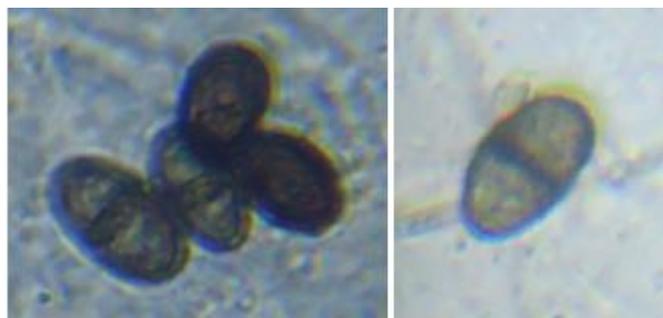
Figure 2. Conidial morphology of *Lasiodiplodia* sp. under light microscope (high power: 400x magnification).

In vitro poisoned food bio-assay

Of the six selected EOs Lemon, orange and mustard were not effective in inhibiting growth of *Lasiodiplodia* sp. under *in vitro* conditions (LSD p>0.05). Basil, cardamum and citronella oils showed a significant effect (LSD p= 0.00) on mycelial growth where cardamom oil was found to be the most effective (67.7% inhibition) at 700 µl l⁻¹ (Table 1).

Table 1. Percentage mycelial inhibition of *Lasiodiplodia* sp. in PDA media treated with different concentrations of essential oils by the time control cultures reached maximum growth (90mm colony diameter).

Essential oil Treatment	Concentration (μl l^{-1})	Inhibition (%)
Basil	600	46.50 ±14.9
	700	60.71±13.3
Cardamom	600	30.14±6.29
	700	67.69±13.9
Citronella	400	52.41±17.2
	500	57.31 ±14.53

Evaluation of disease development in fruits following in vivo application of Essential oils

In the *in vivo* treatment, the most effective is the cardamom in hot water treatment. Mean area of disease development was significantly lower in hot water dipped cardamom oil treated fruits, 72 hours after treatment (HAT), 96 HAT and 120 HAT when compared with the control. Disease development is high in basil-treated fruits than in control fruits at 72 HAT and 96 HAT (Figure 3).
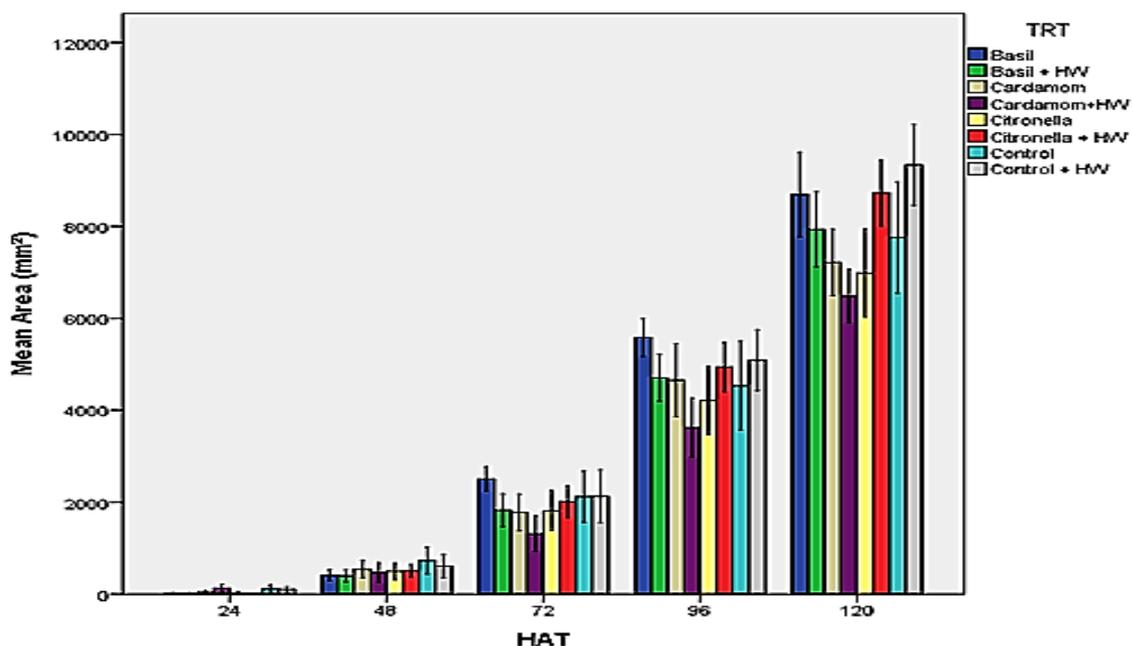
Figure 3. Mean disease area (mm^2) of 14 replicates of 'K.C.' fruits inoculated with *L. theobromae* and then treated with 3 selected EOs. Error bars represent the standard error of the means.

Mean area = diseased development in 14 replicates, TRT = Treatment, HAT = Hours after treatment, HW = EOs in hot water at (45 °C).

Effect of the in vivo essential oil treatment on physico-chemical parameters: physiological weight loss, Titrable acidity and TSS

The lowest %weight loss was seen in cardamom + HW treatment (Day 6: 1.52g, Day 7: 2.00g) while the highest was in the control + HW (Day 6: 2.25 and Day 7:2.7g). There was no significant difference in °Brix value among the treatments. However, the value was slightly higher in the control (13.25) compared to the effective cardamom treatments (cardamom 12.05, cardamom + HW 12.81) and the control + HW 12.55. There was no significant difference between the %Titrable acidity among treatments.

Sensory evaluation of fruits treated with essential oils

The Principal Component Analysis (PCA) extracted three components explaining a total variance of 51.541% of the total data set. The result of the PCA revealed that the treatment did not affect the taste of the fruits. However, treatment negatively affected the appearance, peel color, hardness and freshness of treated fruits (LSD p=0.00) (Table 2).

The most effective oil for reducing the growth of *Lasiodiplodia* sp. under *in vivo* condition was cardamom oil. As a treatment, cardamom oil in hot water was more inhibitory on the SER pathogen and rendered lower physiological weight loss in fruits compared to all the other treatments. According Olivero-Verbel [et al.] (2010) and Utta-U´r *et al.* (2000) inhibitory effect of cardamom essential oil on fungi maybe due to 1,8-cineol (29.7%) and α-terpineol acetate (26.1%) the main components found in the volatile oil. Hot water treatment is known to be an effective and environmental-friendly method for controlling postharvest diseases of mango (Kumah *et al.*, 2011; Duamkhanmanee, 2008). Therefore, in the present study the EO with fungicidal components and the hot water treatment which can also control post-harvest diseases would have in combination brought about the significant reduction in SER in treated mango fruits.

Table 2. Summary of the Principal Component Analysis (PCA) carried out on the sensory data of treated fruits

	Component		
	1	2	3
Eigen value	1.889	1.379	1.370
% variance explained	20.994	15.325	15.222
Total variance explained	20.994	36.319	51.541
Appearance	**0.697**	-0.116	-0.062
Peel Color	**0.652**	0.212	0.155
Hard	**0.620**	0.080	-0.074
Fresh	**0.537**	-0.065	0.466
Soft	0.040	**-0.685**	0.221
Flesh Color	-0.017	**0.664**	0.285
Sweet	0.141	**0.618**	0.100
Dry/Wrinkled	-0.324	0.074	**0.738**
Fruity	0.423	0.116	**0.660**

Discussion

The most effective essential oils for reducing the growth of the SER pathogen *Lasiodiplodia* sp. under *in vitro* conditions were cardamom oil, basil oil and citronella oil. Although citronella oil was effective in controlling pathogen, it was not used in the *in-vivo* experiment due to its strong and unpalatable aroma. The antimicrobial activity of basil against various microbes has been reported in previous studies (Bozin et al., 2006; Sokovic and Griensven, 2006) and many scientists have linked basil antimicrobial effects to the presence of high content of linalool which is the main component of the oil (Juliani and Simon, 2002).

In the present study, the highest Brix value was seen in the control

fruits. However, all treatments demonstrated TSS over 10% Brix which is an acceptable sugaur content for mango fruit (Harrill, 1998). Therefore, the treatment did not negatively affect the sugar content of the fruit. Though the taste of fruits under focus was not affected by the oil application, PCA data revealed that treatment did affect the appearance, peel color and hardness of the fruit all of which are important determinants of marketing the fruit. This is a negative aspect of EO treatment as the outer appearance is a major parameter for consumer acceptability.

Overall, these findings suggest that cardamom EO can be used to control the postharvest losses caused by *Lasiodiplodia* sp. in the mango cultivar *'Karuthacolomban'* fruit. However, the method of application needs slight modifications to overcome the negative effects of EO on the outer appearance of the fruit. Incorporation of EOs into edible waxes and other fruit coatings may be a suitable alternative to hot-water-dip treatments.

Conclusions

Essential oils of cardamom (*Elettaria cardamomum*) are an effective and environmentally safe alternative to synthetic fungicides in controlling the stem-end rot caused by *Lasiodiplodia* sp. in mango fruit cultivar 'Karuthacolomban' with no detrimental effects on sensory quality.

Acknowledgments

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