

In vitro and in vivo Application of Eco-friendly Treatments to Control Postharvest Stem-end Rot of Naturally Infected Avocado (cv. Pollock)

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ABSTRACT

Purpose: Stem-end rot (SER) is an endophytic fungal infection of avocado causing significant postharvest losses, affecting its marketability. This study was conducted to identify effective concentrations of selected eco-friendly essential oils and chemicals to control SER pathogens by conducting in vitro bioassays and to develop treatments to control SER in naturally infected avocado (cv. Pollock) using less hazardous alternatives to synthetic fungicides.

Research Method: In vitro disc volatilization and poison food bioassays were conducted to identify inhibitory concentrations of some essential oils and chemicals against SER pathogens. Avocado fruits were subjected to eco-friendly fumigation and dip treatments and their pathological, physicochemical and sensory properties were assessed after 7 days of storage at 15 °C.

Findings: Disc volatilization bioassay revealed that 5 µL/plate clove oil was most effective against *Lasiodiplodia theobromae*, *Diaporthe nelumbonis* and *Fusarium oxysporum*. According to Poisoned food bioassay, 5% (w/v) sodium bicarbonate and 0.07% (v/v) acetic acid were highly effective against the test pathogens. SER incidence of avocado fruits has been successfully delayed for 7 days after subjecting to fumigation treatment with clove oil and dip treatments with sodium bicarbonate and acetic acid, followed by storage at 15 °C. None of the treatments adversely affected physicochemical and sensory properties of avocado.

Originality/Value: Treatments could be further improved by conducting a medium-scale in vivo trial to obtain good quality avocado with higher consumer acceptance.

Keywords: acetic acid, avocado, clove oil, eco-friendly, sodium bicarbonate, stem-end rot

INTRODUCTION

Avocado (*Persea americana* Mill.) belongs to the family Lauraceae and is one of the few commercially significant members of the genus *Persea* (Yahia, 2012). Avocado is a high fat fruit, containing rare sugars of high carbon number and is relatively rich in vitamins (A, B, C, E and K), dietary fibre, minerals (potassium, phosphorus, magnesium and iron and nitrogenous substances) (Yahia, 2012; Bill *et al.*, 2015). Avocado is one of the popular fruit crops grown and consumed in Sri Lanka. Pollock, Hass and Purple are some of the popular avocado cultivars grown in Sri Lanka and other sub-tropical regions. Although it is not grown in orchard scale in Sri Lanka, a

sizeable volume of harvested crop from backyard gardens come to the local market. Apart from the wet zone, avocado is becoming popular in the intermediate zone as a home garden crop in Sri Lanka (Sarananda *et al.*, 2004).

Higher susceptibility of avocado to qualitative and quantitative postharvest losses is one of the prime challenges faced by both growers and

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the consumers. Major postharvest losses are encountered throughout the supply chain, mostly due to anthracnose and stem end rot (SER) diseases (Bill *et al.*, 2015). SER affects the shelf-life, fruit quality and marketability of avocado. It is a disease reportedly caused by a group of fungal pathogens namely, *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Phomopsis* spp., *Fusarium* spp. and *Neofusicoccum* spp. (Hartill, 1991; Twizeyimana *et al.*, 2013). The first visible symptom of SER is shriveling around the stem base which is followed by decay, fruit discoloration, softening and lesions. Rot ultimately consumes the entire fruit and produces mycelia of the responsible fungi on the infected lesions (Hartill, 1991).

SER of avocado is a latent infection in which the symptoms are not produced until the fruits begin to ripen. The causative fungi which survive endophytically on branches progressively colonize and establish on stem-ends of young fruits without producing symptoms. When fruits initiate ripening, pathogenic fungi become activated due to the physiological and biochemical changes that are associated with a reduction in the concentration of fungal inhibitors such as diene present in avocado fruits (Hartill and Everett, 2002). Pathogenicity of the SER associated fungi of avocado (i.e. *L. theobromae*, *D. nelumbonis* and *F. oxysporum*) used in this study has been confirmed by Nilmini *et al.* (2020). According to previous findings, all three fungal species have significantly contributed to development of SER disease symptoms in avocado.

L. theobromae has been identified as a SER pathogen of avocado during a research conducted by Garibaldi *et al.* (2012). *F. oxysporum* has not been recognized as a pathogen causing SER of avocado in previous studies. However, Hartill (1991) has reported several *Fusarium* spp. (i.e. *F. crookwellense*, *F. pallidoroseum*, *F. equiseti* and *F. graminearum*) as SER pathogens of avocado. *D. nelumbonis* is found to be isolated from *Nelumbo nucifera* leaves by Chen and Kirschner (2018) and has not yet been reported as a postharvest pathogen of any fruit. Nevertheless, several other *Diaporthe* spp. including *D. foeniculina*, *D. sterili* and *D. rudis* have been previously identified as SER causing pathogens of avocado according to

Guarnaccia *et al.* (2016) and Torres *et al.* (2016).

To reduce postharvest losses of many fresh commodities, fungicides are applied immediately after harvest. However, depositing of fungicide residues on the fruits and their negative effects on the environment and consumer health have raised concerns regarding food safety (Muri *et al.*, 2009). These concerns have raised the interests of researchers towards the development of alternative approaches to combat SER in avocado.

Many non-hazardous organic and inorganic compounds have been found to be fungicidal and are effectively being used for postharvest disease control throughout the world. These compounds have shown broad spectrum antimicrobial activity with low mammalian toxicity (Olivier *et al.*, 1999) and considered by the United States Food and Drug Administration (FDA) as Generally Recognized as Safe (GRAS) compounds. Sodium salts have demonstrated inhibitory effect on the growth of fungal pathogens of fruits, field crops, vegetables and ornamentals (Oliver *et al.*, 1999; Mecteau *et al.*, 2002). Sodium bicarbonate has been used as an effective and safe fungicide against powdery mildew disease in tomato (Demir and Onogur, 1999).

Naturally occurring, biologically active plant essential oils are generally assumed to be more acceptable and less hazardous than synthetic fungicides. These volatile compounds are capable of inhibiting the growth of fungal pathogens and evaporating without leaving residues (Taghavi *et al.*, 2018). Essential oils represent a rich source of potential disease control agents due to the presence of many active compounds such as eugenol, camphor, camphene, caryophyllene, phyllandrene, etc. (Zaker, 2016). These antifungal components present in essential oils act synergistically to inhibit the growth of pathogenic fungi (Anthony *et al.*, 2004).

Therefore, the present research was carried out (i) to examine the *ex situ* activity of *Syzygium aromaticum* (clove) and *Cinnamomum zeylanicum* (cinnamon) essential oils, sodium bicarbonate, sodium metabisulfite and acetic acid in controlling SER of avocado and (ii) to develop a fumigation treatment with most effective

essential oil and dip treatment using highly efficacious test chemicals to control SER and to enhance the shelf life of avocado (cv. Pollock).

MATERIALS AND METHODS

Treatment material

S. aromaticum oil, *C. zeylanicum* leaf oil, sodium bicarbonate, sodium metabisulfite and glacial acetic acid were purchased from Glorechem Enterprises, Colombo, Sri Lanka, as eco-friendly treatment materials. Carbendazim 50WP fungicide was purchased from Hayleys Agriculture Holdings Pvt. Ltd., Colombo, Sri Lanka.

Test fungi

Lasiodiplodia theobromae (MK907912), *Diaporthe nelumbonis* (MK907914) and *Fusarium oxysporum* (MK907915) were isolated from avocado (cv. Pollock, Fuerte, Hass and some local cultivars) displaying symptoms of SER, as collected from the markets in Kandy, Matale, Nuwara-eliya, Matara, Rathnapura, Gampaha and Colombo Districts in Sri Lanka. The isolated fungi were identified based on their colony morphology, conidial structures and their identities were confirmed by molecular tools at Genetech Institute, Colombo, Sri Lanka and Macrogen Inc., Republic of Korea. Nucleotide sequences of identified fungi were deposited in the GenBank and accession numbers were obtained by Nilmini *et al.* (2020).

In vitro disc volatilization bioassay

This assay was carried out to determine the antifungal efficacy of selected essential oils. Petri plates (90 mm in diameter) containing PDA (15 mL per plate) were separately inoculated with 7 mm diameter mycelial discs cut from the periphery of a 7-day old pure culture of the respective test pathogen (Bill *et al.*, 2015). A sterilized filter paper disc with a diameter of 90 mm (Whatman No: 1) was placed inside the lid. Thereafter, an aliquot of selected essential oil (1

– 5 µL per plate) was added (at intervals of 1 µL) onto the filter paper discs using a micropipette. Sterilized distilled water in place of oil served as the negative control. Treatments were done in four replicates. Immediately after introducing essential oil, Petri plates were tightly sealed with para-film and incubated in an inverted position at room temperature (28 ± 2 °C) for 7 days (Feng *et al.*, 2011).

In vitro poisoned food bioassay

This was conducted to determine the antifungal efficacy of selected eco-friendly chemicals. Sodium bicarbonate (1 – 5% w/v), sodium metabisulfite (0.1 – 0.3% w/v), acetic acid (0.05 – 0.07% v/v) and carbendazim (0.06% w/v) were incorporated into sterilized PDA cooled to 45 °C to obtain the desired final concentrations. Then, medium was poured (15 mL per plate) into Petri plates (90 mm) under aseptic conditions. A 7 mm diameter mycelial plug cut from the periphery of a 7-day old pure culture of the respective test pathogen was placed in the center of each plate. Plates without test chemicals (i.e. containing PDA) were used as the negative control, while carbendazim (0.06% w/v) was used as the positive control. All treatments were replicated four times. Plates were tightly sealed with parafilm and incubated at room temperature (28 ± 2 °C) for 7 days (Ali-Shtayeh and Abu-Ghdeib, 1999; Kumar *et al.*, 2013).

Determination of the percentage inhibition

Radial mycelial growth of test fungi during *in vitro* bioassays were determined by measuring the colony diameter daily, along the two axes at right angles to each other using a ruler marked in centimeters. The antifungal efficacy was expressed as the percentage inhibition of radial mycelial growth (% IRMG) using the equation given by Abdollahi *et al.* (2011).

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

dc = mean radial mycelial growth in control plates and dt = mean radial mycelial growth in treatment plates.

Avocado fruits

Mature (98 – 105 days), unblemished avocado (cv. Pollock) fruits were obtained from the farm of the Fruit Research and Development Institute, Horana, Sri Lanka. Fruits showing light green colour at the stem-end and having finger feel firmness score at 2 (1 = hard, 2 = slightly soft, just starting to ripen, 3 = very soft) (Sellamuthu *et al.*, 2013) were selected as the fruits at the correct maturity stage.

Development of a disease severity index

A disease severity index was developed to monitor SER in avocado. Five avocado (cv. Pollock) fruits were washed in running tap water to remove dirt and debris followed by sterile distilled water. Cleaned fruits were allowed to drip dry on a laboratory bench for 30 minutes, then placed in a plastic tray at room temperature. One selected fruit showing a gradual development of SER was photographed daily. Diseased area of the fruit in each photograph was estimated by DIGIMIZER (Version 5.3.4) and the disease severity was determined as percentage SER with respect to the total area of the fruit. A disease severity index was prepared using the photographs along with their percentage SER values (Kodituwakku *et al.*, 2020a).

Preparation of avocado fruits

Avocado (cv. Pollock) fruits were washed in running tap water. Thereafter, surface of the fruits was sterilized by dipping each fruit in sodium hypochlorite (0.1% w/v) for 5 minutes followed by a dip in distilled water. Fruits were air-dried at room temperature (Bill *et al.*, 2015).

Treatment of avocado fruits

Clove oil – 2100 µL (based on the inhibitory concentrations determined from *in vitro* disc volatilization bioassay) was introduced into a Petri plate (65 mm in diameter) lid which was kept in the centre of a 12.5 L translucent plastic container at 90% RH. Four avocado fruits were

carefully placed in each container by avoiding contact between fruits and clove oil. The container was immediately sealed with a slip-on lid to start the fumigation process. Fruits were exposed to clove oil vapour for 24 hours at 20 °C and thereafter, placed on sterile paper towels on a bench top (Bill *et al.*, 2015).

Sodium bicarbonate (5% w/v), acetic acid (0.07% v/v) and carbendazim (0.06% w/v) concentrations were prepared based on the inhibitory concentrations determined from *in vitro* Poison food bioassay. Each compound was added to the 5 L of distilled water separately. Four drops of Tween 80 (Koch light) were added as a surfactant. Negative control was prepared in the same manner using only distilled water and Tween 80. Carbendazim was used as the positive control. Fruits were dipped in each solution for 30 minutes and the excess solutions were allowed to drain for 10 minutes (Tripathi and Shukla, 2009).

Each treatment (i.e. clove oil, sodium bicarbonate and acetic acid) and controls (i.e. carbendazim and distilled water) consisting of four replicate fruits were placed in plastic trays (35×25×8 cm³) and then in separate plastic boxes (65×35×18 cm³) with lids disinfected with ethanol (70% v/v). A piece of sterile cotton wool soaked in sterile distilled water was placed inside each box (Tripathi and Shukla, 2009). All boxes were stored at cold temperature (i.e. 15 °C) in an incubator (BOD-400, S/N Al 160 80 501-13, Israel) for 7 days. At the end of the storage period, fruits were subjected to induced-ripening, and pathological, physicochemical, sensory properties and peel colour were examined. The experiment was repeated once under identical conditions, immediately followed by the first.

Induced-ripening of avocado

At the end of treatment time fruits were removed from the boxes and subjected to induced-ripening at room temperature. Each set of fruits were placed in a plastic bucket of 5 L capacity. Ethepon (2-chloroethyl phosphonic acid) solution was prepared by dissolving 2 mL of Ethepon (480 g/L) (CIC Crop Guard Pvt. Ltd., Colombo, Sri Lanka) in 1 L of distilled water

(Sarananda *et al.*, 2004). One mL of diluted Ethepon solution (2 mL/L) was taken into a 50 mL beaker and placed in the bucket. Evolution of ethylene (C₂H₂) was facilitated by adding 5 drops of 0.1 M Sodium hydroxide solution to the beaker. The buckets were kept sealed for 24 hours at room temperature and then remained opened for another 24 hours until the avocado reached fully ripe stage. (Siriwardana, 2016).

Pathological properties

SER severity of each ripened avocado fruit was subjectively estimated as percentage SER by comparing with the developed SER disease severity index (Figure 03.).

Physicochemical properties

Physicochemical properties of treated avocado stored at 15 °C for 7 days were assessed after induced-ripening. Three randomly selected ripened avocado fruits from each treatment (i.e. six fruits per treatment from two trials) were analyzed for physicochemical properties. Total soluble solids (TSS) (°Brix) of the filtrates of fruit pulp were determined using a hand-held refractometer (PAL- α , Cat.No.3840, ATAGO Co. Ltd., Japan). pH of the filtrates was measured using a bench top pH meter (pH 7110, WTW, Germany). Firmness of the fruit pulp was measured using a portable fruit firmness tester (FT 011, QA Suppliers, Italy) (Siriwardana, 2016).

Peel colour

Peel colour of avocado stored at 15 °C for 7 days were assessed before and after induced ripening. Peel colour was visually assessed in comparison with the RHS colour chart (1 = dark green, 2 = green, 3 = light green, 4 = yellow) (Royal Horticultural Society, 2015).

Sensory properties

Sensory properties of avocado stored at 15 °C

for 7 days were assessed after induced-ripening. Eight ripened fruits from each treatment (from the two trials) were provided to a ten-member trained sensory panel along with a questionnaire to evaluate skin surface, flesh texture, flesh colour proximal to skin, flesh colour proximal to seed, sweetness of flesh and general taste (moderate = 1, good = 2, very good = 3) (Owusu, 2012).

Statistical analysis

Data with respect to *in vitro* bioassays (n=4 per concentration/treatment) were analyzed using Two-way ANOVA and mean separation was done using Tukey's pair-wise comparison test. Pooled results of pathological, physicochemical, sensory properties and peel colour obtained from two trials were subjected to the statistical analysis as follows. Results obtained for physicochemical properties (TSS, pH and firmness) (n=6 per treatment) were analyzed using One-way ANOVA and mean separation was done using Tukey's pair-wise comparison test. Kruskal Wallis non-parametric test was used to analyze data with respect to pathological properties (n=8 per treatment), sensory properties (n=6 per treatment) and peel colour (n=8 per treatment) (Siriwardana, 2016).

RESULTS

***In vitro* antifungal efficacy of selected essential oils**

According to the disc volatilization bioassay, clove oil at 4 μ L/plate concentration, was sufficient to inhibit radial mycelial growth of *D. nelumbonis* completely. Further, clove oil completely inhibited the radial mycelial growth of other two fungi at 5 μ L/plate concentration (Figure 01.A). However, cinnamon leaf oil at vapour phase did not contribute to 100% inhibition of radial mycelial growth of any test fungus. It was revealed that cinnamon leaf oil at its highest concentration (i.e. 5 μ L/plate) displayed only 66.07, 56.25 and 38.20% inhibition of *F. oxysporum*, *D. nelumbonis* and *L. theobromae*, respectively (Figure 01.B).

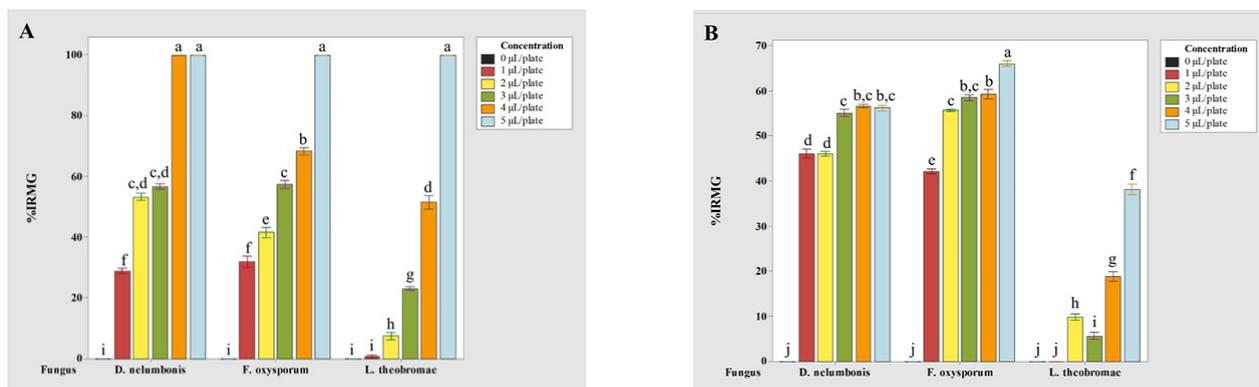


Figure 01: Percentage inhibition of radial mycelial growth (% IRMG) of avocado stem-end rot associated fungi determined by *in vitro* disc volatilization bioassay. (A) clove oil and (B) cinnamon leaf oil. Each data point represents the mean of four replicates \pm standard error. Means sharing a common letter(s) are not significantly different by Tukey's pairwise comparison test ($p < 0.05$).

In vitro antifungal efficacy of selected eco-friendly chemicals

Among the tested eco-friendly chemicals, sodium bicarbonate at 0.30% (w/v) achieved 100% inhibition of radial mycelial growth of *L. theobromae* and *D. nelumbonis* during the poison food bioassay. However, it was effective in controlling *F. oxysporum* completely at 0.50% (w/v) concentration (Figure 02.A). Sodium metabisulfite revealed the lowest effectiveness by indicating the least inhibitory effect against all test pathogens. Concentrations of sodium metabisulfite tested were unable to control test pathogens completely and the maximum % IRMG has been observed as 59.86% at 0.20% (w/v) concentration against *L. theobromae*. (Figure 02.B). Further, 0.07% (w/v) acetic acid was equally effective against all three pathogens and exhibited 100% IRMG. Other lower concentrations of acetic acid were less effective in inhibiting the pathogens completely (Figure 02.C).

Meanwhile, carbendazim fungicide which was used as the positive control achieved a 100% IRMG against all test fungi during two *in vitro* assays. When the test fungus, concentration of treatment and the interaction between the fungus and treatment concentration were considered as three separate factors, % IRMG values obtained

for all treatments during two bioassays were significantly different ($p < 0.05$) according to two-way ANOVA.

SER disease severity index for avocado

The subjective disease severity index prepared during this study (Figure 03) can be used to assess the approximate extent of damage caused to avocado (cv. Pollock) fruits due to SER. Stem-end rot disease severity of avocado exposed to different treatments during the present study was determined using this index.

Treatment strategies for avocado

Pathological properties: When considering the SER disease severity of naturally infected avocado, none of the fruits subjected to different treatments (i.e. clove oil, acetic acid and sodium bicarbonate) and positive control (i.e. carbendazim) displayed SER symptoms at 7 days of storage in the incubator (after induced ripening) (Figure 04.). 54.38% of SER severity was reported in the negative control which was significantly different from other treatments ($p < 0.05$) (Figure 05.).

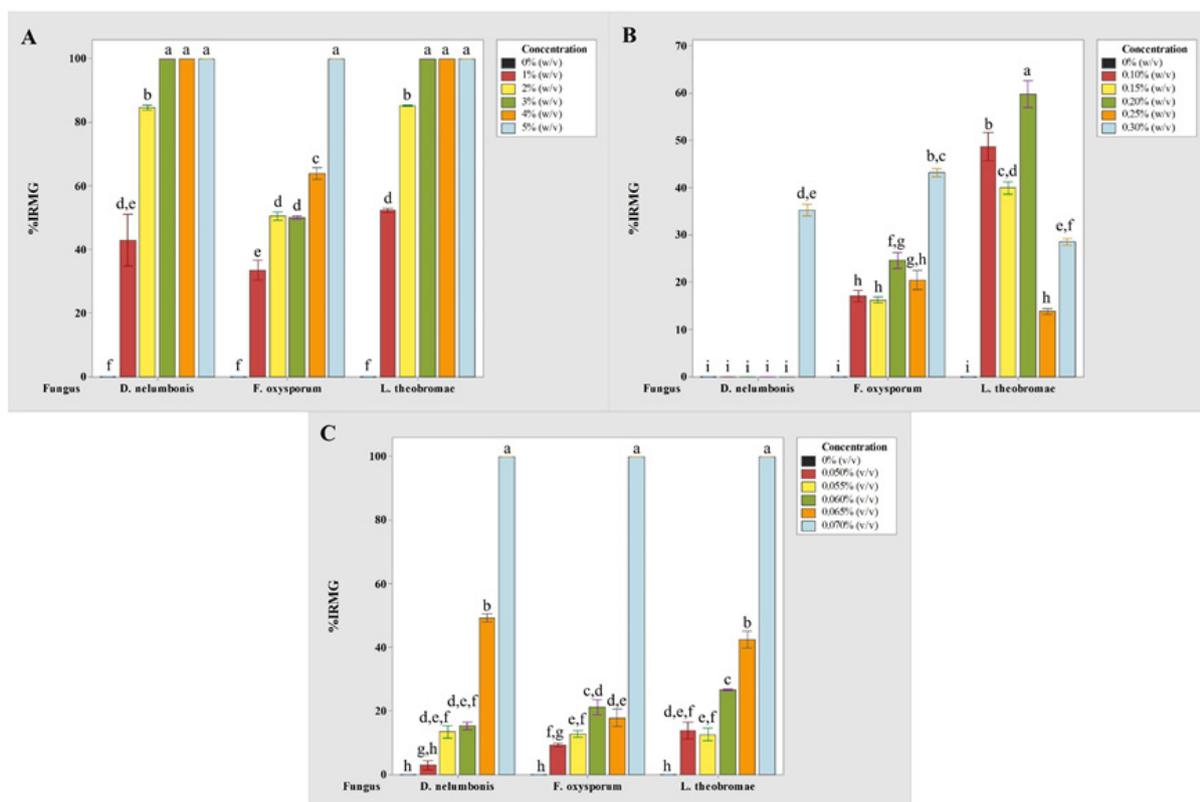


Figure 02: Percentage inhibition of radial mycelial growth (% IRMG) of avocado stem-end rot associated fungi determined by *in vitro* poisoned food bioassay. (A) sodium bicarbonate, (B) sodium metabisulfite and (C) acetic acid. Each data point represents the mean of four replicates \pm standard error. Means sharing a common letter(s) are not significantly different by Tukey's pair-wise comparison test ($p < 0.05$).

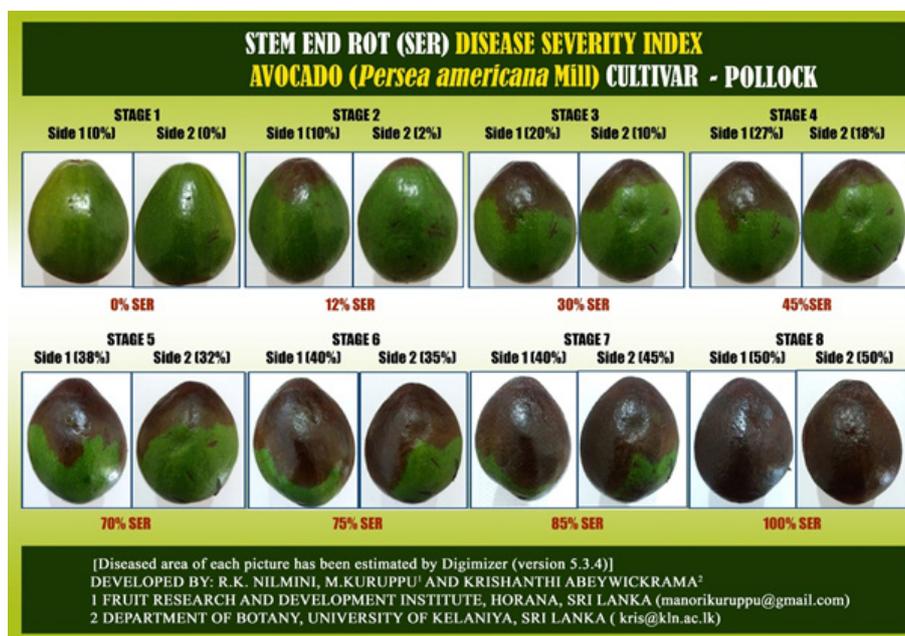


Figure 03: The subjective disease severity index prepared to monitor stem-end rot development in avocado (cv. Pollock).

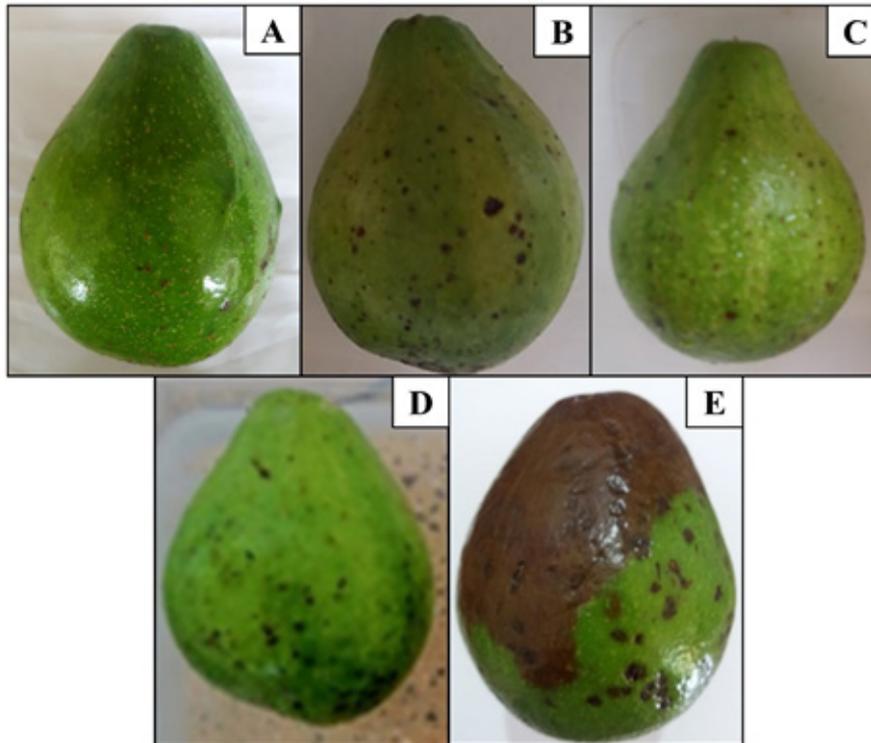


Figure 04: Appearance of avocado (cv. Pollock) fruits subjected to dip and fumigation treatments, stored at 15 °C for 7 days and subsequent induced-ripening. (A) 0.07% (v/v) acetic acid and (B) 5% (w/v) sodium bicarbonate treated fruits during dip treatment. (C) clove oil treated fruits during fumigation treatment. (D) positive control treated with 0.06% (w/v) carbendazim and (E) negative control treated with distilled water.

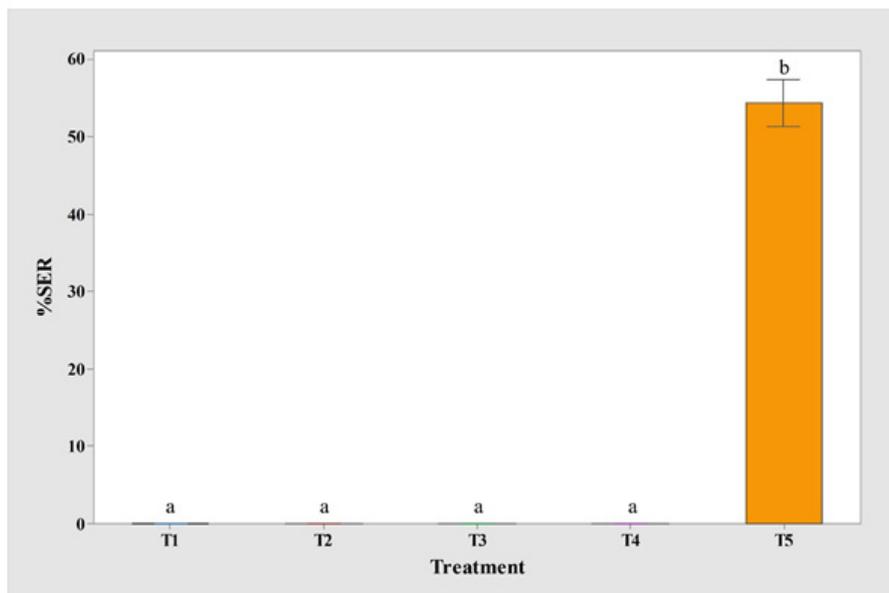


Figure 05: SER disease severity of avocado (cv. Pollock) fruits subjected to dip and fumigation treatments, stored at 15 °C for 7 days and subsequent induced ripening. T1: 0.07% (v/v) acetic acid, T2: 5% (w/v) sodium bicarbonate, T3: 0.5 $\mu\text{L mL}^{-1}$ clove oil, T4: 0.06% (w/v) carbendazim and T5: negative control. Each data point represents the mean of eight replicates \pm standard error. Means sharing a common letter(s) are not significantly different by Kruskal Wallis non-parametric test ($p < 0.05$).

Physicochemical properties: Avocado fruits treated with clove oil had the highest TSS value (i.e. 31.33 °Brix) after 7-day storage at 15 °C. The lowest TSS value was observed in carbendazim treated avocado fruits, which was 26.13 °Brix. Even though clove oil treated fruits were lightly more sweeter, there was no statistically significant difference in TSS among all treated and control samples ($p > 0.05$) (Table 01.). pH values of avocado subjected to acetic acid, sodium bicarbonate, clove oil treatments and negative control were found to be slightly lower than the pH of carbendazim treated avocado (i.e. 6.50). The lowest pH was observed in avocado treated with clove oil (i.e. 6.07). pH values of acetic acid and clove oil treated fruits were significantly different from the negative control, sodium bicarbonate and carbendazim treatments ($p < 0.05$) (Table 01.). However, firmness of all treated and control samples were almost similar. Firmness values of all samples were around 0.03 – 0.04 kg cm⁻² in which no statistically significant difference was observable ($p > 0.05$) (Table 01.).

Peel colour: At the end of 7-day storage period at cold temperature, avocado fruits were at the

‘dark green’ (index value = 1) or ‘green’ (index value = 2) stages. After induced ripening, peel colour of avocado exposed to each treatment and negative control was around 3 (i.e. light green). However, peel colour of avocado treated with carbendazim was around 1 or dark green and it was statistically significant from other treatments and the negative control ($p < 0.05$).

Sensory properties: When considering the sensory properties of avocado stored at 15 °C for 7 days, none of the treated and control samples displayed a statistically significant difference with respect to skin surface, flesh colour proximal to skin and sweetness of flesh ($p > 0.05$). Flesh texture and general taste of sodium bicarbonate and acetic acid treated avocado were significantly different from other treatments and the negative control ($p < 0.05$). Flesh colour proximal to seed of all treated samples was statistically significant when compared to the negative control ($p < 0.05$). In general, sensory attributes of avocado subjected to different treatments were at an acceptable level. However, sensory panelists had a slight preference towards the untreated control than other treatments (Table 02.).

Table 01: Physicochemical properties of avocado (cv. Pollock) treated with eco-safe compounds followed by storage at 15 °C for 7 days and subjected to induced-ripening.

Treatment	TSS* (°Brix)	pH	Firmness (kg cm ⁻²)
T1	30.13 ^a ± 1.39	6.17 ^b ± 0.08	0.03 ^a ± 0.00
T2	26.93 ^a ± 0.74	6.48 ^a ± 0.02	0.03 ^a ± 0.00
T3	31.33 ^a ± 2.19	6.07 ^b ± 0.03	0.03 ^a ± 0.00
T4	26.13 ^a ± 2.70	6.50 ^a ± 0.02	0.04 ^a ± 0.00
T5	27.87 ^a ± 1.88	6.44 ^a ± 0.01	0.03 ^a ± 0.00

T1: 0.07% (v/v) acetic acid, T2: 5% (w/v) sodium bicarbonate, T3: 0.5 µL mL⁻¹ clove oil, T4: 0.06% (w/v) carbendazim and T5: negative control.

*Total Soluble Solids

Each value represents the mean of six replicates ± standard error.

Means sharing a common letter(s) within the same column are not significantly different by Tukey's pair-wise comparison test.

Table 02: Sensory properties of avocado (cv. Pollock) treated with eco-safe compounds followed by storage at 15 °C for 7 days and subjected to induced-ripening.

Treatment	Skin surface	Flesh texture	Flesh colour proximal to skin	Flesh colour proximal to seed	Sweetness of flesh	General taste
T1	1.50 ^a ± 0.22	1.10 ^a ± 0.10	2.90 ^a ± 0.10	2.20 ^a ± 0.38	2.10 ^a ± 0.18	2.40 ^a ± 0.22
T2	1.50 ^a ± 0.16	1.50 ^a ± 0.22	2.70 ^a ± 0.15	1.70 ^a ± 0.21	2.00 ^a ± 0.14	2.00 ^a ± 0.00
T3	1.50 ^a ± 0.16	1.70 ^b ± 0.21	3.00 ^a ± 0.00	1.40 ^a ± 0.22	2.10 ^a ± 0.18	2.70 ^b ± 0.26
T4	1.50 ^a ± 0.16	2.50 ^b ± 0.22	3.00 ^a ± 0.00	1.40 ^a ± 0.16	2.30 ^a ± 0.15	2.80 ^b ± 0.20
T5	1.50 ^a ± 0.16	1.90 ^b ± 0.31	2.60 ^a ± 0.22	3.20 ^b ± 0.32	2.20 ^a ± 0.13	2.80 ^b ± 0.20

T1: 0.07% (v/v) acetic acid, T2: 5% (w/v) sodium bicarbonate, T3: 0.5 µL mL⁻¹ clove oil, T4: 0.06% (w/v) carbendazim and T5: negative control.

Each value represents the mean of eight replicates ± standard error.

Means sharing a common letter(s) within the same column are not significantly different by Kruskal Wallis non-parametric test.

DISCUSSION

Disc volatilization is a simple bioassay for antimicrobial evaluation of essential oils in vapour phase and therefore, could be used as a primary screening method for antifungal effects of essential oils (Juan, 2015). From the essential oils used during present study, only clove oil was highly effective against all test fungi when compared to cinnamon leaf oil. Therefore, only clove oil was tested to develop the fumigation treatment for avocado.

Even though, cinnamon leaf oil at a low concentration was unable to inhibit the growth of *L. theobromae* during the present research, Sukatta *et al.* (2008) reported that cinnamon leaf oil vapour at higher concentrations (i.e. 10 µL/plate) resulted in 100% inhibition of *L. theobromae* isolated from grape. Contrary to Sukatta *et al.* (2008), the present study showed that clove oil was successful in inhibiting growth of *L. theobromae* at concentrations lower than 10 µL/plate. According to a similar *in vitro* disc volatilization assay carried out by Kodituwakku *et al.* (2020b), clove and cinnamon oils inhibited the growth of SER pathogen *L. theobromae* isolated from mango at a concentration of 2 µL/plate, which is comparatively lower than the inhibitory concentrations reported in the present study. This might be due to differences

in composition of the same oil, depending on its origin. According to Barra (2009), chemical composition of an essential oil varies depending on factors such as light, precipitation, growing site and nature of soil of the environment where the plant species is growing.

Another fumigation bioassay conducted by Massoud *et al.* (2012) revealed that cinnamon leaf oil at 1 µL/plate concentration has successfully controlled *Fusarium moniliforme*, even though higher concentrations than 1 µL/plate could not achieve a complete inhibition of *F. oxysporum* during the present research. Present study is not in conformity with Massoud *et al.* (2012), as they have reported that clove oil has attained 100% inhibition of *F. moniliforme* at a higher concentration (i.e. 10 µL/plate). However, if higher concentrations (>5 µL/plate) of clove oil were tested in a future trial, it may reveal higher mycelial inhibition of *F. oxysporum*. According to Karunanayake *et al.* (2020), mango treated with a high concentration of basil oil showed low preference by taste panel when compared to the control. Therefore, present study was aimed to control test pathogens using minimum concentrations of oils.

GC-MS conducted by Kodituwakku *et al.* (2020b) revealed that eugenol (79.11%), β-caryophyllene (9.07%), caryophyllene oxide (0.40%) and

eugenyl acetate (0.20%) are the major bioactive components of clove oil. Eugenol, which is the dominant component of clove oil as reported in many studies, varies from 30 – 95% depending on the plant part used for extraction. The lowest content of eugenol (i.e. 28%) was found in the oil isolated from growing clove leaves (Nowak *et al.*, 2012). Similarly, some of the major components of cinnamon leaf oil such as eugenol (63.76%), cinnamaldehyde (1.37%) and linalool (1.27%) are found to be antifungal against SER causing fungal pathogens of mango (Kodituwakku *et al.*, 2020b). The two main antifungal components present in cinnamon oils are eugenol and cinnamaldehyde. According to the raw material (either cinnamon leaves or bark) from which the oil was obtained, the ratio of these two components may change (Vangalapati *et al.*, 2012). In the present study, inhibition of test fungi by cinnamon leaf oil vapour was not as high as with clove oil, because the chemical compositions of clove and cinnamon oils are different. Nevertheless, with the synergistic action of different bioactive constituents present in essential oils in different proportions they may inhibit the growth of pathogenic fungi (Anthony *et al.*, 2004).

Well characterized antimicrobial components present in essential oils display different modes of action against plant pathogens including their membrane disruption, distraction of adhesion to hosts, disruption of cell wall integrity, inactivation of enzymes and intercalation of DNA which results in the inhibition of protein synthesis (Zaker, 2016).

According to Bill *et al.* (2015), fumigation with thyme oil (i.e. 960 μ L per box) has significantly reduced SER in artificially inoculated avocado (cv. Hass) fruits. Further, Bill *et al.* (2016) reported a complete inhibition of SER in un-inoculated avocado (cv. Ryan) after fumigation with thyme oil. Potential has shown by many essential oils on their antifungal activity in the efficient control of SER of avocado at low concentrations. Therefore, it is possible to consider the results of the present study positively, which are in agreement with Bill *et al.* (2015) and Bill *et al.* (2016).

A significant increase of fruit firmness was observed in avocado fumigated with thyme oil by

Bill *et al.* (2015) and Bill *et al.* (2016). However, this result is not in agreement with the present findings with regard to clove oil fumigation, as it did not cause any significant firmness change in avocado. Having mature and firm fruits are important to minimize the mechanical damages that could occur during transport (Sarananda and Amarakoon, 1999). Furthermore, Bill *et al.* (2016) observed a significant increase of sensory properties in thyme oil treated avocado including taste and texture when compared to the control. Even though, clove oil did not have any significant improvement on sensory properties, all sensory properties tested received similar scores as the control and they were well within the acceptable range.

Poisoned food method is universally used to evaluate antifungal effects against moulds. From the compounds used, sodium bicarbonate and acetic acid showed a higher antifungal activity at lower concentrations against SER pathogens of avocado in comparison with sodium metabisulfite. Therefore, only sodium bicarbonate and acetic acid were used for the development of dip treatments for avocado.

Guimarães *et al.* (2019), revealed that even 1% (w/v) sodium bicarbonate could completely inhibit the growth of *L. theobromae* isolated from *Citrus* fruits in Poisoned food bioassay. However, this finding is not in accordance with the present study, since 52.36% inhibition of *L. theobromae* isolated from avocado was observed at the same concentration. This observation may probably be due to strain difference of the pathogens isolated from two different fruits. However, results of the present investigation are somewhat similar to the findings reported by Türkkan and Erper (2012), as both studies have revealed that 2% (w/v) sodium bicarbonate is capable of inhibiting *F. oxysporum* up to 50 – 55%.

The US FDA recommends that sodium bicarbonate could be applied on food substrates with no limitation other than Good Manufacturing Practice (GMP) specifications (eCFR, 2020). Since higher concentrations of sodium bicarbonate could impart an unacceptable flavour on avocado, greater than 5% (w/v) sodium bicarbonate levels were not tested. Nevertheless, Kalupahana *et al.* (2020) reported a slight increase in TSS of

Tom EJC mango treated with 8% (w/v) sodium bicarbonate, when compared to control.

Fischer *et al.* (2018) reported that avocado (cv. Hass) fruits treated with 2% w/v sodium bicarbonate have exhibited a significant reduction in postharvest disease of 'anthracnose'. Irrespective of the targeted disease, this finding could be allied with the present study because both studies revealed the potential of sodium bicarbonate to control postharvest pathogenic fungi of avocado. Germination of spores of many fungal species prefer acidic conditions. Since sodium bicarbonate solutions are more alkaline, that alkalinity could inhibit the germination of fungal spores (Chalker-Scott, 2009).

According to Fischer *et al.* (2018), soluble solids of avocado treated with sodium bicarbonate have been slightly reduced compared to the untreated control and this finding is in conformity with the results of the present research. These also agree with Fischer *et al.* (2018) who reported that flesh firmness and peel colour of sodium bicarbonate treated avocado did not show a significant difference when compared to the control.

Even though, Türkkan and Erper (2012) highlighted that 0.40% (w/v) sodium metabisulfite could result in 100% inhibition of *F. oxysporum*, the same compound at 0.30% (w/v) concentration only achieved a 43.22% inhibition of the respective fungus during the present study. However, Kalupahana *et al.* (2020) revealed that physicochemical properties of mango even treated with a low concentration (i.e. 0.15%) of sodium metabisulfite were the lowest among all treatments. By taking that point into consideration, use of sodium metabisulfite to control test pathogens was limited to low concentrations.

Antifungal action of sodium metabisulfite is quite different when compared to sodium bicarbonate. It reacts with water to release sulfur dioxide which causes toxic effects on many fungi by interfering with many cellular components such as membrane lipids, enzymes and their cofactors, nucleic acids, etc. (Arslan, 2015).

Although, the precise modes of action of inorganic salts in controlling postharvest pathogens have not been recognized, it is possible that interactions between salt residues and fruit tissues may cause hostile conditions against the target pathogens such as pH alterations, induction of plant defense mechanisms and accumulation of antimicrobial compounds (Palou *et al.*, 2016).

An *in vitro* liquid bioassay carried out by Hassan *et al.* (2015) discovered that 5% (v/v) acetic acid has inhibited the growth of *F. oxysporum* only up to 23.65%. The present results indicate that the respective pathogen could be completely inhibited by acetic acid at significantly low concentrations at 15 °C. As an organic acid, acetic acid affects the permeability of cellular membrane of most fungi by neutralizing its electrochemical potential. Due to this altered membrane permeability, leakage of electrolytes may disrupt the cellular functions of the target pathogen (Dalié *et al.*, 2010).

CONCLUSIONS

According to *in vitro* bioassays, vapour of clove oil, sodium bicarbonate and acetic acid were more effective in controlling SER pathogens isolated from avocado (i.e. *L. theobromae*, *D. nelumbonis* and *F. oxysporum*) than cinnamon leaf oil and sodium metabisulfite. All avocado fruits treated with acetic acid, sodium bicarbonate and clove oil and followed by storage at 15 °C did not exhibit SER at the end of the storage period. Therefore, storage at 15 °C could further enhance shelf-life of avocado that have been exposed to different eco-friendly SER controlling treatments up to one week. None of the treatments adversely affected physicochemical and sensory attributes of avocado. Therefore, these eco-friendly treatment strategies along with cold storage could be further improved for commercial application in order to provide avocado fruits to the market with a better and endure postharvest quality at higher consumer acceptance.

REFERENCES

- Abdollahi, A.L.I., Hassani, A., Ghosta, Y., Meshkatsadat, M.H., and Shabani, R. (2011). Screening of antifungal properties of essential oils extracted from sweet basil, fennel, summer savory and thyme against postharvest phytopathogenic fungi. *Journal of Food Safety*. 31 (3), 350-356. DOI: <https://doi.org/10.1111/j.1745-4565.2011.00306.x>
- Ali-Shtayeh, M.S., and Abu-Ghdeib, S.I. (1999). Antifungal activity of plant extracts against dermatophytes. *Mycoses*. 42, 665-672. DOI: <https://doi.org/10.1046/j.1439-0507.1999.00499.x>
- Anthony, S., Abeywickrama, K., Dayananda, R., Wijeratnam, S.W., and Arambewela, L. (2004). Fungal pathogens associated with banana fruit in Sri Lanka, and their treatment with essential oils. *Mycopathologia*. 157, 91-97. DOI: <https://doi.org/10.1023/B:MYCO.0000012226.95628.99>
- Arslan, U. (2015). Evaluation of antifungal activity of sulfur-containing salts against phytopathogenic fungi. *Fresenius Environmental Bulletin*. 24 (5a), 1879-1886. https://www.researchgate.net/publication/284633283_Evaluation_of_Antifungal_Activity_of_Sulfur-Containing_Salts_Against_Phytopathogenic_Fungi. 15.03.2020.
- Barra, A. (2009). Factors affecting chemical variability of essential oils: a review of recent developments. *Natural Product Communications*. 4 (8), 1147-1154. DOI: <https://doi.org/10.1177/1934578X0900400827>
- Bill, M., Obianom, P.C., Korsten, L., Mavuso, Z., van Rooyan, Z., and Sivakumar, D. (2016). Using plant volatiles in biodegradable sachets and exposure of volatiles under pallet covers to control postharvest decay in avocados. *South African Avocado Growers' Association Yearbook*. 39, 44-49. http://www.avocadosource.com/Journals/SAAGA/SAAGA_2016/SAAGA_2016_39_PG_044.pdf. 11.02.2020.
- Bill, M., Sivakumar, D., van Rooyan, Z., and Mavuso, Z.S. (2015). New methods of postharvest disease control: Using thyme oil fumigation. *South African Avocado Growers' Association Yearbook*. 38, 98-104. http://www.avocadosource.com/Journals/SAAGA/SAAGA_2015/SAAGA_2015_38_PG_98.pdf. 05.01.2020.
- Chalker-Scott, L. (2009). Baking soda will fungi fail and roses rejoice? Puyallup Research and Extension Center, Washington State University, Puyallup, Washington. <https://s3.wp.wsu.edu/uploads/sites/403/2015/03/baking-soda.pdf>. 15.03.2020.
- Chen, K.L., and Kirschner, R. (2018). Fungi from leaves of lotus (*Nelumbo nucifera*). *Mycological Progress*. 17 (1-2), 275-293. DOI: <https://doi.org/10.1007/s11557-017-1324-y>
- Dalié, D.K.D., Deschamps, A.M., and Richard-Forget, F. (2010). Lactic acid bacteria – Potential for control of mould growth and mycotoxins: A review. *Food Control*. 21 (4), 370-380. DOI: <https://doi.org/10.1016/j.foodcont.2009.07.011>
- Demir, S., Gül, A., and Onogur, E. (1999). The effect of sodium bicarbonate on powdery mildew in tomato. *Acta Horticulturae*. 491, 449-452. DOI: <https://doi.org/10.17660/ActaHortic.1999.491.71>
- eCFR (2020). Electronic Code of Federal Regulations. https://www.ecfr.gov/cgi-bin/text-idx?SID=ac854f13bffc9c5c9b64395995da734a&mc=true&node=pt21.3.184&rgn=div5#se21.3.184_11736. 06.02.2020.
- Feng, W., Chen, J., Zheng, X., and Liu, Q. (2011). Thyme oil to control *Alternaria alternata* in vitro and in vivo as fumigant and contact treatments. *Food Control*. 22 (1), 78-81. DOI: <https://doi.org/10.1016/j.foodcont.2010.05.010>
-

- Fischer, I.H., Moraes, M.F.D., Palharini, M.C.D.A., Fileti, M.D.S., Cruz, J.C.S., and Firmino, A.C. (2018). Effect of conventional and alternative products on postharvest disease control in avocados. *Revista Brasileira de Fruticultura*. 40 (1), e408. DOI: <https://doi.org/10.1590/0100-29452018408>
- Garibaldi, A., Bertetti, D., Amatulli, M.T., Cardinale, J., and Gullino, M.L. (2012). First report of postharvest fruit rot in avocado (*Persea americana*) caused by *Lasiodiplodia theobromae* in Italy. *Plant Disease*. 96 (3), 460-461. DOI: <https://doi.org/10.1094/PDIS-10-11-0886>
- Guarnaccia, V., Aiello, D., Cirvilleri, G., Polizzi, G., Susca, A., Epifani, F., and Perrone, G. (2016). Characterization of fungal pathogens associated with stem-end rot of avocado fruit in Italy. *Acta Horticulturae*. 1144, 133–140. DOI: <https://doi.org/10.17660/ActaHortic.2016.1144.19>
- Guimarães, J.E., de la Fuente, B., Pérez-Gago, M.B., Andradás, C., Carbó, R., Mattiuz, B.H., and Palou, L. (2019). Antifungal activity of GRAS salts against *Lasiodiplodia theobromae* in vitro and as ingredients of hydroxypropyl methylcellulose-lipid composite edible coatings to control *Diplodia* stem-end rot and maintain postharvest quality of citrus fruit. *International Journal of Food Microbiology*. 301, 9-18. DOI: <https://doi.org/10.1016/j.ijfoodmicro.2019.04.008>
- Hartill, W.F.T. (1991). Post-harvest diseases of avocado fruit in New Zealand. *New Zealand Journal of Crop and Horticultural Science*. 19 (3), 297-304. DOI: <https://doi.org/10.1080/01140671.1991.10421814>
- Hartill, W.F.T., and Everett, K.R. (2002). Inoculum sources and infection pathways of pathogens causing stem-end rots of ‘Hass’ avocado (*Persea americana*), *New Zealand Journal of Crop and Horticultural Science*. 30 (4), 249-260. DOI: <https://doi.org/10.1080/01140671.2002.9514221>
- Hassan, R., El-Kadi, S., and Sand, M. (2015). Effect of some organic acids on some fungal growth and their toxins production. *International Journal of Advances in Biology*. 2 (1), 1-11. <https://aircse.com/ijab/papers/2115ijab01.pdf>. 03.02.2020.
- Juan, B. (2015). Models of evaluation of antimicrobial activity of essential oils in vapour phase: a promising use in healthcare decontamination. *Natural Volatiles and Essential Oils*. 2 (2), 16-29. <https://www.semanticscholar.org/paper/Models-of-evaluation-of-antimicrobial-activity-of-a-Bueno/c1be240d23a16af761f8c8c0d59f3f532c67c1e7>. 30.01.2020.
- Kalupahana, K.I.M., Kuruppu, M. and Dissanayake, P.K. (2020). Effect of essential oils and GRAS compounds on postharvest disease control in mango (*Mangifera indica* L. cv Tom EJC). *The Journal of Agricultural Sciences-Sri Lanka*. 15 (2), 207-221. DOI: <http://doi.org/10.4038/jas.v15i2.8802>
- Karunanayake, K.O.L.C., Liyanage, K.C.M., Jayakody, L.K.R.R. and Somaratne, S. (2020). Basil oil incorporated beeswax coating to increase shelf life and reduce anthracnose development in mango cv. Willard. *Ceylon Journal of Science*. 49 (5), 355-361. DOI: <https://doi.org/10.4038/cjs.v49i5.7802>
- Kodituwakku, T.D., Abeywickrama, K., and Karunanayake, K.O.L.C. (2020a). Pathogenicity of stem-end rot associated fungi isolated from Karthakolomban mango and their control by spray and fumigation treatments with selected essential oils. *The Journal of Agricultural Sciences-Sri Lanka*. 15 (1), 19-36. DOI: <https://doi.org/10.4038/jas.v15i1.8669>

- Kodituwakku, T.D., Ekanayake, G.C.M., Abeywickrama, K., and Jayakody, R. (2020b). *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 characterization of antifungal components. *Journal of the National Science Foundation of Sri Lanka*. 48 (2), 101-111. DOI: <http://dx.doi.org/10.4038/jnsfsr.v48i2.8952>
- Kumar, S.N., Nambisan, B., Sundaresan, A., Mohandas, C., and Anto, R.J. (2013). Isolation and identification of antimicrobial secondary metabolites from *Bacillus cereus* associated with a rhabditid entomopathogenic nematode. *Annals of Microbiology*. 64, 209-218. DOI: <https://doi.org/10.1007/s13213-013-0653-6>
- Massoud, M.A., Saad, A.S.A., Soliman, E.A., and El-Moghazy, A.Y. (2012). Antifungal activity of some essential oils applied as fumigants against two stored grains fungi. *Journal of the Advances in Agricultural Researches*. 17 (2), 296-305. https://www.researchgate.net/publication/293817314_Antifungal_activity_of_some_essential_oils_applied_as_fumigants_against_two_stored_grains_fungi. 28.01.2020.
- Mecteau, M.R., Arul, J., and Tweddell, R.J. (2002). Effect of organic and inorganic salts on the growth and development of *Fusarium sambucinum*, a causal agent of potato dry rot. *Mycological Research*. 106 (6), 688–696. DOI: <https://doi.org/10.1017/s0953756202005944>
- Muri, S.D., van der Voet, H., Boon, P.E., van Klaveren, J.D., and Brüschweiler, B.J. (2009). Comparison of human health risks resulting from exposure to fungicides and mycotoxins via food. *Food and Chemical Toxicology*. 47 (12), 2963-2974. DOI: <https://doi.org/10.1016/j.fct.2009.03.035>
- Nilmini, R.K., Panapitiya, D., Abeywickrama, K. and Kuruppu, M. (2020). Morphological and molecular identification of fungal species associated with postharvest stem-end rot disease of avocado in Sri Lanka. *Sri Lanka Journal of Food and Agriculture*. 6 (1), 47-56. DOI: <http://doi.org/10.4038/sljfa.v6i1.81>
- Nowak, K., Ogonowski, J., Jaworska, M. and Grzesik, K. (2012). Clove oil-properties and applications. *Chemik*. 66 (2), 145-152. http://yadda.icm.edu.pl/yadda/element/bwmeta1.element.baztech-article-BPP2-0017-0048/c/Nowak_eng.pdf. 14.06.2020.
- Olivier, C., MacNeil, C.R., and Loria, R. (1999). Application of organic and inorganic salts to field grown potato tubers can suppress silver scurf during potato storage. *Plant Disease*. 83 (9), 814-818. DOI: <https://doi.org/10.1094/PDIS.1999.83.9.814>
- Owusu, A.A. (2012). Formulation and shelf life evaluation of avocado (*Persea americana*) fruit spread. M.Sc. thesis, Kwame Nkrumah University of Science and Technology, Ghana. <http://ir.knust.edu.gh/xmlui/handle/123456789/4727>. 17.01.2020.
- Palou, L., Ali, A., Fallik, E., and Romanazzi, G. (2016). GRAS, plant-and animal-derived compounds as alternatives to conventional fungicides for the control of postharvest diseases of fresh horticultural produce. *Postharvest Biology and Technology*. 122, 41-52. DOI: <https://doi.org/10.1016/j.postharvbio.2016.04.017>
- Royal Horticultural Society (2015). RHS colour chart. 6th edition. Royal Horticultural Society, London.

- Sarananda, K.H., and Amarakoon, R. (1999). Methods to minimize postharvest rot of three local cultivars of mango. In: Proceedings of the Annual Symposium of the Department of Agriculture, Sri Lanka. pp. 327-333. https://www.researchgate.net/publication/280387291_METHODS_TO_MINIMIZE_POSTHARVEST_ROT_OF_THREE_LOCAL_CULTIVARS_OF_MANGO. 07.02.2020.
- Sarananda, K.H., Kumari, U.N.G.C., Eeswara, J.P., and Rathnayaka, R.M.N.D. (2004). Artificial ripening to reduce postharvest losses of avocado. *Tropical Agricultural Research and Extension*. 7, 144-149. http://www.agri.ruh.ac.lk/tare/pdf/V_7/AG.7.16.pdf. 16.03.2020.
- Sellamuthu, P.S., Sivakumar, D., and Soundy, P. (2013). Antifungal activity and chemical composition of thyme, peppermint and citronella oils in vapour phase against avocado and peach postharvest pathogens. *Journal of Food Safety*. 33, 86-93. DOI: <https://doi.org/10.1111/jfs.12026>
- Siriwardana, S.S.D.H.L.S. (2016). Improvement of the postharvest storage life of banana (Embul – *Musa acuminata*, AAB) and (Cavendish – *Musa acuminata*, AAA) using novel crown rot disease control strategies. Ph.D. Thesis, University of Kelaniya, Sri Lanka.
- Sukatta, U., Haruthaithanasan, V., Chantarapanont, W., Dilokkunanant, U., and Suppakul, P. (2008). Antifungal activity of clove and cinnamon oil and their synergistic against postharvest decay fungi of grape *in vitro*. *Kasetsart Journal: Natural Science*. 42, 169-174. https://kukr.lib.ku.ac.th/journal/KJNS/search_detail/result/28709. 03.02.2020.
- Taghavi, T., Kim, C., and Rahemi, A. (2018). Role of natural volatiles and essential oils in extending shelf life and controlling postharvest microorganisms of small fruits. *Microorganisms*. 6 (4), 104. DOI: <https://doi.org/10.3390/microorganisms6040104>
- Torres, C., Camps, R., Aguirre, R., and Besoain, X.A. (2016). First report of *Diaporthe rudis* in Chile causing stem-end rot on ‘Hass’ avocado fruit imported from California, USA. *Plant Disease*. 100 (9), 1951. DOI: <https://doi.org/10.1094/PDIS-12-15-1495-PDN>
- Tripathi, P., and Shukla, A.K. (2009). Application of essential oils for postharvest control of stem end rot of mango fruits during storage. *International Journal of Postharvest Technology and Innovation*. 1 (4), 405-415. DOI: <https://doi.org/10.1504/IJPTI.2009.030689>
- Türkkan, M., and Erper, I. (2014). Evaluation of antifungal activity of sodium salts against onion basal rot caused by *Fusarium oxysporum* f. sp. *cepae*. *Plant Protection Science*. 50 (1), 19-25. DOI: <https://doi.org/10.17221/9/2013-PPS>
- Twizeyimana, M., Förster, H., McDonald, V., Wang, D.H., Adaskaveg, J.E., and Eskalen, A. (2013). Identification and pathogenicity of fungal pathogens associated with stem-end rot of avocado in California. *Plant Disease*. 97 (12), 1580-1584. DOI: <https://doi.org/10.1094/PDIS-03-13-0230-RE>
- Vangalapati, M., Satya, N.S., Prakash, D.S. and Avanigadda, S. (2012). A review on pharmacological activities and clinical effects of cinnamon species. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 3 (1), 653-663. <https://www.semanticscholar.org/paper/A-Review-on-Pharmacological-Activities-and-Clinical-Vangalapati-Prakash/3e85ede09a3f2f26c36a59df4f804af7131bbb4d>. 16.06.2020.
- Yahia, E.M. (2012). Avocado. In: Crop post-harvest: science and technology: Perishables. Volume 3. (Rees, D., Farrell, G. and Orchard., J. Eds.) Blackwell Publishing Ltd, UK. pp. 159-186.

Zaker, M. (2016). Natural plant products as eco-friendly fungicides for plant diseases control – A review. *The Agriculturists*. 14 (1), 134-141. DOI: <https://doi.org/10.3329/agric.v14i1.29111>