

## Control of Anthracnose in Banana Cultivar Kolikuttu (*Musa* sp.) with Essential Oils and Bio-safe Fruit Coatings

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### Abstract

Anthracnose caused by the fungus *Colletotrichum musae* is a widespread disease found among banana resulting in high postharvest losses in Sri Lanka. The use of essential oils (EO) to control postharvest diseases has become an ecologically friendly alternative to synthetic chemicals due to their antimicrobial properties while fruit coating is an effective method to increase shelf life. This study focused on controlling anthracnose and extending the shelf life of banana by incorporating selected EOs into a fruit coating. The gel matrix was extracted from the cortex of *Aloe vera* and mixed with a gelatin agent to serve as the fruit coating material. EOs was screened against *C. musae* which was isolated from banana cultivar *Kolikuttu* under *in vitro* bio-assay.

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Four different treatments were carried out under *in vivo* conditions. Two concentrations of gelatin (G) in 200 ml of *Aloe vera* (AV) coating material (2% GAV and 4% GAV) were independently mixed with the most effective EO at optimal concentration and one treatment with AV coating without EO. Untreated fruits with no EO or coating served as the control. Post-harvest quality parameters, % weight loss, total soluble solids (TSS), titratable acidity (TA), and flesh color were monitored and showed no significant undesirable effects when compared with the control. Disease severity was significantly low in coated fruits with Basil oil at 1000  $\mu\text{LL}^{-1}$  in 2% or 4% GAV. The Most effective EO for controlling banana anthracnose was basil oil (70.28%) at 1000  $\mu\text{LL}^{-1}$  followed by cardamom oil (53.06%). A sensory evaluation with untrained panelists showed that quality parameters were not affected by the treatments. Results indicated basil oil incorporated *A. vera* as a suitable bio-safe fruit coating against anthracnose in banana.

**Keywords:** Essential oil, Basil oil, *Aloe vera*, Fruit coating, Anthracnose, Banana

## Introduction

Banana (*Musa* sp.) which belongs to the family Musaceae, is one of the most important food crops next to rice, wheat, and maize (Perrier et al., 2011). It is one of the most desirable fruits in the international market because of its delicious taste and high caloric value (Sidhu & Zafar, 2018). Banana evolved in the humid tropical regions of Southeast Asia with India as one of its centers of origin. The total annual world production of banana is estimated at 86 million tons of fruits (FAO, 2010). In Sri Lanka banana is one of the most extensively cultivated fruits accounting for around 54% of total fruit-cultivating land (Ranathilaka et al., 2019). From the total production in the country, about 35-45% is lost at the post-harvest stage (Department of Census and Statistics, 2014).

Field diseases result in crop loss while postharvest diseases are directly linked with losses in the export and domestic market (Essien et al., 2005). One of the limiting factors that influences the economic value of the banana fruit is its short shelf life due to post-

harvest fungal attacks (Kuyu & Tola, 2018). Because of high nutrient reserve along with its low pH, banana fruits are highly susceptible to fungal decay (Singh & Sharma, 2007). Anthracnose, caused by *Colletotrichum* sp. is one of the most serious postharvest diseases of ripe bananas (Zakaria et al., 2009). It is the most common post-harvest banana disease in Sri Lanka (Imthiyas et al., 2021) affecting a wide range of commercial banana cultivars and resulting in post-harvest losses (Adikaram, 1986). It is reported that Anthracnose reduces the annual yield by 20%, along with crown rot (Anthony et al., 2004).

The most widely used approach for managing postharvest infections is the application of synthetic fungicides such as benomyl and thiabendazole (Khan et al., 2001). Even though synthetic fungicides have proven to be helpful in controlling postharvest infections, continuous use of synthetic fungicides may result in the pathogen developing fungicide-resistant strains, and the presence of fungicidal residues on the fruit surface causes serious health hazards to consumers and the environment (Maqbool et al., 2011).

Thus, there is a need for new disease-preventive technology which is safe for humans and environment friendly. Among the various alternatives, natural plant products, including essential oils that are safe, economic, and environmentally friendly are ideal candidates for use as alternatives to agrochemicals (Maicas et al., 1997).

It has been reported that essential oils have some fungicidal properties against postharvest diseases of tropical fruits and vegetables (Wilson et al., 1997; Meepagala et al., 2002; Imelouane et al., 2009). It has been found that cinnamon oil and lemon grass oil have antifungal activity against Anthracnose of banana caused by *C. musae* (Maqbool et al., 2010a, b). EO s from cinnamon, thyme, bitter and sweet almond were effective in controlling crown rot disease of banana (Abd-Alla et al., 2014). Study by Idris et al. (2015) confirmed the antifungal effect of basil, cinnamon, and rosemary essential oils on anthracnose of banana fruits.

In Sri Lanka, extensive work on fungal pathogens associated with banana and the use of essential oils for the control of postharvest diseases of banana fruit has been carried out by Abeywickrama and co-workers (Anthony et al., 2004; Anthony, Abeywickrama & Wijerathnam, 2003). EO s of *Cymbopogon nardus* and *Ocimum basilicum* were found to be directly fungitoxic to common postharvest disease-causing pathogens such as *Colletotrichum musae*, *Lasiodiplodia theobromae*, and *Fusarium proliferatum* (Anthony et al., 2004) while a study by Siriwardena et al. (2019) reported the effectiveness of basil oil and modified atmosphere packaging on Cavendish banana.

However, due to the highly volatile nature of essential oils, these oils need to be incorporated into coating material to exert a long-term effect on the fruit. The oils are mixed with a coating material and then applied to the fruit peel, so that essential oils and coating material have a beneficial effect on the quality of fruits by reducing water loss, rate of respiration and therefore delaying ripening and extending shelf life. Recently, the use of edible coatings has been widely studied for the preservation of fruits and vegetables (Senna et al., 2014).

Currently, scientific research on *Aloe vera* (AV) gel as a bio-safe fruit coating has gained much attention due to its antioxidant and antimicrobial properties (Lapena et al., 2020). AV gel has been used for postharvest application on various fruits as an edible coating. Sicari et al. (2020) reported reducing total soluble solids (TSS), increasing concentrations of total phenolics compounds and ascorbic acid and improving antioxidant activity of strawberry fruits in comparison to non-coated fruits. Similarly, another research revealed AV coating to be an effective, eco-friendly and non-chemical substitute treatment for maintaining the postharvest quality of guava fruit (Rehman et al., 2020). Mendy et al. (2019) indicated that coating fruits with AV can effectively extend the shelf life of papaya fruit.

The aim of the present study was to investigate the most effective combination of essential oil with *A. vera* coating in controlling anthracnose caused by *C. musae* of banana cv. *Kolikuttu* fruit and

to evaluate its effect on shelf life, physicochemical, and organoleptic properties of the fruit.

## **Methodology**

### **Plant Essential oils**

Pure grade essential oils, basil (*Ocimum basilicum*), cardamom (*Elettaria cardamomum*), mustard (*Brassica juncea* L.) and orange (*Citrus sinensis*) were obtained from 'Herbal Exotics', Pugoda, Sri Lanka. The composition of the EO s was analyzed as described by Herath et al. (2017) using a Trace 1300 Gas Chromatograph coupled with a single MS (Model: ISQ QD, Thermo Scientific, USA) at the Industrial Technology Institute, Colombo to confirm the constituents of the oils.

### **Fruits**

Healthy unripe banana cv. *Kolikuttu* fruits at harvesting maturity were obtained from an orchard in Galle (6.0535° N, 80.2210° E) within 24 hours of harvest. Fruits of approximately uniform size, shape and maturity (eight fruits per treatment) were used in experiments. Diseased banana cv. *Kolikuttu* were obtained from the Narahenpita (6.5332 °N, 79.5237 ° E) market to isolate the causal agent of banana Anthracnose.

### **Fungus isolation and Culture conditions**

The pathogen-causing banana anthracnose was isolated from the diseased banana peel onto Potato Dextrose Agar (PDA) following the standard protocol described by Wijesundara et al. (2015). Fungal species were identified based on mycelial/conidial morphology (CMI descriptions).

Pathogenicity of the causal agent was confirmed by performing Koch's Postulates. Conidia were obtained from the isolated pure culture of *C. musae* and a suspension was prepared (10<sup>5</sup> conidia/ml) to inoculate onto healthy fruits and reisolate the causal agent to confirm pathogenicity as described by Wijesundara et al. (2015). The pure cultures of *C. musae* causing Anthracnose

were maintained in the Research laboratory of the Open University of Sri Lanka under ambient conditions at  $28 \pm 2$  °C during the experiment.

### ***In vitro* screening of essential oils against anthracnose causing *C. musae* of banana cultivar Kolikuttu**

Poisoned food bioassay was carried out to examine the inhibitory effect of selected essential oils (basil, cardamom, mustard, and orange) against growth of *C. musae* of banana anthracnose under laboratory conditions. EO s were incorporated into molten PDA at concentrations 400, 500, 600, 750, 1000  $\mu\text{L}^{-1}$  and the PDA was allowed to solidify. Mycelial plugs (1cm x 1cm) of the isolated fungus were obtained from the leading edge of a pure culture and introduced onto the center of each PDA plate. This procedure was repeated for each concentration of the essential oil. The increase in diameter of the fungal colony was noted daily. An equal volume of sterile distilled water was incorporated into molten PDA, which served as the negative control and Daconil a commercial fungicide was used as the positive control at the recommended concentration. The experiment was repeated to confirm the most effective concentration of EO in controlling *C. musae*.

The percentage of mycelial growth inhibition was calculated by the formula given below (Tripathi et al., 2008).

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

dc= mean colony diameter of the negative control sets

dt=mean colony diameter of treatment sets

### **Composition of essential oils**

The composition of the EOs was analyzed as described by Herath et al. (2017) using a Trace 1300 Gas Chromatograph coupled with a single MS (Model: ISQ QD, Thermo Scientific, USA) at ITI, Colombo.

### **Preparation of *Aloe vera* coating material**

Mature leaves of *A. vera* plants were harvested, washed, and kept under ambient conditions for some time to get rid of moisture. Then the gel matrix was extracted from the cortex of the leaves, by scraping the inside of the fleshy leaves. This matrix was then blended and filtered to remove fiber. The resulting mixture was then pasteurized at standard pasteurizing temperatures (72 °C for 15 seconds) using a water bath (Sabarien et al., 2013). Gelatin agent (2% or 4%) was added to increase the gelling potential (Adetunji et al., 2018). This coating was stored (4 °C) in amber bottles and used when necessary.

### **Preparation of the essential oil mixed *Aloe vera* coating**

*Aloe vera* (AV) coating material was mixed with the optimal concentration of the most effective essential oil determined by *in vitro* trials. About 200 mL of AV coating material was applied to fruits in the following four treatments: (1) AV with 2% gelatin without essential oil, (2) AV with 2% gelatin with the most effective essential oil at the optimal concentration, (3) AV with 4% gelatin without essential oil (4) AV with 4% gelatin with the most effective essential oil at the optimal concentration and (5) Untreated control that was coated with water alone. The four coating material were applied separately on the fruit peel of individual fruits of the respective four treatments using a paint brush.

### ***In vivo* effect of essential oil mixed *Aloe vera* coating on postharvest quality**

Mature healthy banana fruits of cultivar *Kolikuttu* at harvesting maturity were obtained directly from a cultivation orchard in Galle (6.0535° N, 80.2210° E), which does not practice the use of chemical fungicides. Eight fruits were used per treatment as replicates. The fruits, both treated and controlled were arranged as a Completely Randomized Design (CRD) and kept on laboratory tables in clean plastic trays under ambient conditions for 5 days. Readings were taken on the weight of fruits in each treatment and recorded daily. This was used to calculate physiological weight loss.

### **Physiological weight loss (%)**

Weight of fruits was measured daily using a digital balance (Radwag, PS6000.R2). Percentage (%) weight loss was calculated using the formula given below (Gerefa et al., 2015).

$$\text{Physiological loss in weight \%} = \frac{\text{Initial weight of banana fruits} - \text{Final weight of banana fruits}}{\text{Initial weight of banana fruits}} \times 100$$

### **Effect of the treatments on physico-chemical and sensory properties**

#### **Total Soluble Solids (TSS) (°Brix)**

Ripe banana fruits 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 by using a portable refractometer (Brix/ATC 0 ~ 32%) of 0-30 Brix range at room temperature (28± 2 °C) and expresses as °Brix (Samane et al., 2012). The readings were taken three times for each treatment and averaged.

#### **Titrateable Acidity (TA)**

Ripe banana fruits from each treatment were selected, peeled, and juiced. From each fruit, 10 mL of juice sample was taken and three (3) drops of phenolphthalein were added as a pH indicator. Titration was done against 0.1 M NaOH until the solution starts to turn pink, this was taken as the end point of the titration. The titrateable acidity percentage for malic acid was calculated by the following formula given by Dadzie et al. (1997).

$$\% \text{ TA} = \frac{\text{Average titre} \times \text{multiplication factor}}{10\text{mL juice}} \times 100$$

% TA = Number of grams of malic acid per 100mL of juice



Average titre = Average number of ml of NaOH

Multiplication factor (acid Factor) = 0.0067

### **Flesh color**

At the ripe stage of the fruit, the pulp was taken, and the flesh color was noted with the colorimeter (CR-400-CHROMA METER, Japan).

### **Sensory evaluation**

A panel of 20 untrained adults (aged above 21 years old), comprising males and females, were randomly selected for the sensory evaluation. Out of the 20 panelists, 50% were males and 50% were females. 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. Five samples (five-day-old, treated fruits) were served on identical plates and the organoleptic properties of interest were color (both peel and flesh color), appearance, texture, odor, and taste (Umuhozariho et al., 2013).

### **Effect of the treatments on postharvest disease development**

Eight replicate fruits were used per treatment. Both curative and preventive methods were used. Fruits were coated with different essential oil mixed with AV coatings either one day prior to inoculation with the causal organism for banana Anthracnose (*C. musae*) (preventive) or 1 day following inoculation (curative). Preparation of the conidial suspension was done as described (Wijesundara et al., 2015) and the fruit surface was inoculated with 20 µL drops of the conidial suspension (4 drops per fruit) and maintained in a moist chamber ( $28 \pm 2$  °C, 100% RH). Daily observations were made and once symptoms developed, disease lesions were measured on two axes at right angles to each other for five consecutive days.

### **Statistical analysis**

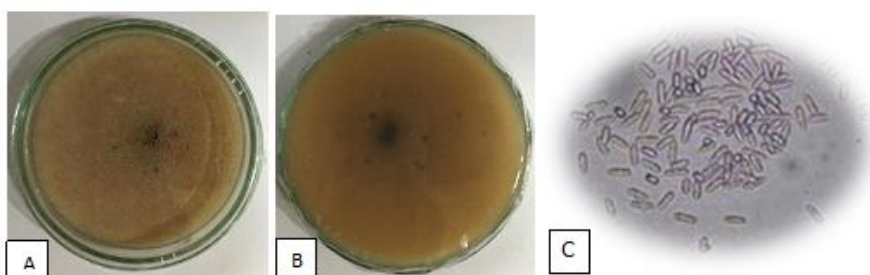
The experiments were run in triplicate and data are reported as the mean  $\pm$  standard deviation (SD). Data were analyzed using the statistical package, SPSS version 20.0. Analysis of variance

(ANOVA) among means was performed using one-way ANOVA. After applying the least significant (LSD) test, differences of  $P \leq 0.05$  were significant. For sensory evaluation data, Principal Component Analysis (PCA) & cluster analysis were used to identify the preferences of the panelists for the samples

## Findings and Discussion

### Isolation and maintenance of the causal agent

The fungi isolated from banana anthracnose showed typical morphological characteristics of *Colletotrichum* spp. The colony of the isolated fungus was initially greyish white, it became salmon pink colored, and production of conidia was observed by the sixth day. By the sixth day, Petri plates were fully covered with the fungus (Figure 1-A, B). Mature conidia of the fungus were typical of *C. musae* being hyaline, aseptate, one-celled, mostly ellipsoid to cylindrical with an obtuse apex and a truncate base (Figure 1-C). The causal agent was tentatively identified as *Colletotrichum musae* based on colony characters and morphology of the conidia as previously described by (Prusky & Plumbly 1992, CMI descriptions).



**Figure 1.** Colony characteristics of the fungus isolated from Banana cultivar *Kolikuttu*. (A). Top view of colony in Petri plate (day 6); (B). Reverse view of colony in Petri plate (day 6); (C). Conidial morphology of *C. musae* under light microscope (high power: 10 x 40 x 1 magnification).

### ***In vitro* bio-assay of plant essential oils against *C. musae***

*In vitro* studies revealed that the EO s basil and cardamom significantly inhibited the growth of *C. musae* compared to other EO s tested (Table 01). After 96 hours (day 4), basil oil caused the highest significant reduction (70.30%) of fungal colony growth at 1000  $\mu\text{LL}^{-1}$  whereas the reduction was low (38.11%) at 400  $\mu\text{LL}^{-1}$ . However, basil and cardamom showed a moderate effect on reducing the fungal growth at 500, 600, 750  $\mu\text{LL}^{-1}$ . Similarly, studies by Anthony et al. (2004) and Abeywickrama et al. (2009) also report the activity of basil oil against banana fruit pathogens. Idris et al. (2015) showed that *in vitro* test of basil oil completely (100%) inhibited the growth of the pathogen *C. musae* of banana at a concentration of 0.15-0.2% (v/v). Several researchers have also previously reported the antimicrobial activity of basil oil against various microbes (Bozin et al., 2006; Sokovic & Griensven, 2006). *In vitro* studies by Abeywikrama et al. (2012) showed that basil oil can be used as an alternative to fungicidal treatment against postharvest pathogens of papaya fruit. A similar study by Abeywickrama et al. (2010) reported an integrated treatment of basil oil (*Ocimum basilicum*) and Alum with modified atmosphere to control crown rot disease in embul banana.

**Table 1.**

*Percentage mycelial inhibition of *C. musae* in Petri plates enriched with different essential oils at the time when control reached its maximum growth (90 mm) at 96 h (Day 4) after inoculation*

Concentration ( $\mu\text{LL}^{-1}$ )	Inhibition (%) by different EOs			
	Basil	Cardamom	Mustard	Orange
<b>400</b>	38.11(7.67) <sup>ab</sup>	20.28(10.06) <sup>a</sup>	16.57 (11.20) <sup>a</sup>	21.67 (8.60) <sup>a</sup>
<b>500</b>	46.39 (6.73) <sup>b</sup>	38.22 (8.94) <sup>ab</sup>	17.31 (11.55) <sup>a</sup>	22.60 (6.35) <sup>a</sup>
<b>600</b>	48.89 (5.00) <sup>b</sup>	49.35 (7.30) <sup>b</sup>	18.43 (11.53) <sup>a</sup>	31.00 (18.74) <sup>ab</sup>
<b>750</b>	54.26 (8.47) <sup>b</sup>	51.85 (7.05) <sup>b</sup>	22.78 (13.35) <sup>a</sup>	32.96 (13.31) <sup>ab</sup>
<b>1000</b>	70.28 (7.72) <sup>c</sup>	53.06 (5.30) <sup>b</sup>	28.55 (11.34) <sup>a</sup>	43.00 (20.06) <sup>ab</sup>

*Note.* Values followed by the same letters in a column are not significantly different at ( $p \leq 0.05$ ), Standard deviation is given in parenthesis, Inhibition % = Mean inhibition of six replicates as a percentage.

### **Composition of essential oils**

According to the GCMS results, the highest percentage of constituents were estragole and linalool (Table 2) and results are comparable with constituents of basil oil reported in the literature. Many scientists have linked the antimicrobial effects of basil with the higher level of linalool which is the main component of the oil (Juliani & Simon, 2002). In the present study also the basil oil, had a high percentage of linalool (23%) and hence would have been one of the factors responsible for the observed antifungal effect. According to Kocic-Tanackova et al. (2012) estragol is the main constituent in the basil extract (86.72%). The present study also showed estragol (70%), as the main constituent of basil oil and estragol is also known to exhibit antifungal effects (Hussain et al., 2008). Both linalool and estragol are known to have antifungal and insecticidal properties (Chalchat & Ozcan, 2008; Rodrigues et al., 2016; Hussain et al., 2008). Similarly, Koba et al. (2008) also reported the antimicrobial activity of estragole and linalool along with other constituents of basil oil.

**Table 2.**

*Principal constituents of basil oil and their relative percentages of total chromatogram area (CTS 1813269)*

Compound	Retention time	Area %
$\alpha$ -Pinene	3.16	0.05
B- Pinene	4.13	0.04
D- Limonene	5.50	0.04
B-Phellandrene	5.69	0.25
Trans- $\beta$ -Ocimene	6.43	0.10
Sulcatone	8.62	0.09
Linalool	13.00	22.99
Trans- $\alpha$ -bergamotene	13.8	0.43
Caryophyllene	14.12	0.35
Levomenthol	15.13	0.38
Estragole	15.9	70.83
$\alpha$ - Citral	16.17	0.61
Humulene	18.01	1.29

### ***In vivo* effect of essential oil-mixed *Aloe vera* coating on postharvest quality**

#### **Physiological weight loss**

Banana fruits treated with *A. vera* coating showed a lower physiological weight loss (%) compared to untreated control fruits, however, it was not significant. Weight loss in fruits and vegetables is a sign of water loss and increases due to desiccation and metabolic activities (Zhu et al., 2008). Low weight loss is critical for preserving the quality of the fruits over time. A lower (%) weight loss was observed in fruits treated with 400  $\mu\text{LL}^{-1}$  basil oil in 2% GAV compared to the other treated fruits (table 3). Karunanayake et al. (2020) also report that basil oil in beeswax significantly reduced physiological weight loss in treated mango fruits compared to untreated control fruits. However, there are reports where weight losses were not significantly different ( $P < 0.05$ ) among treatments with oils from *O. basilicum*, *Cymbopogon nardus* and *Cymbopogon flexuosus* on banana fruits (Anthony et al., 2003).

Coating of banana fruits with *A. vera* creates a barrier to moisture loss and therefore, reduces weight loss (Mahmoud & Savello, 1992; Avena-Bustillos et al., 1997). *A. Vera* extract is composed of many complex ingredients including polysaccharides, glycoproteins, phenolic compounds, salicylic acid, lignins, hormones, amino acids, vitamins, saponins and enzymes (Larotonda et al., 2005). Maan et al. (2018) reported that the polysaccharides in *Aloe vera* act as a natural barrier to moisture and oxygen which can speed up food deterioration. Loss of water from guava fruit has been effectively reduced by *A. vera* gel coating (Rehman et al., 2020), reduced weight loss and increased shelf life have been reported in sapodilla (Khaliq et al., 2019), and cherry fruits (Ozturk et al., 2019).

**Table 3.**

*Physiological weight loss (%) in control banana cv. Kolikuttu fruits and fruits coated with A. vera, with or without basil oil treatment from day 1 to 5 after treatment*

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
<b>Control</b>	0.00(0.00) <sup>a</sup>	1.87(0.74) <sup>a</sup>	2.07(0.23) <sup>a</sup>	2.65(0.35) <sup>a</sup>	3.00(0.34) <sup>a</sup>
<b>2% GAV</b>	0.00(0.00) <sup>a</sup>	1.17(0.52) <sup>a</sup>	1.73(0.21) <sup>a</sup>	1.99(0.45) <sup>a</sup>	2.65(0.42) <sup>a</sup>
<b>4% GAV</b>	0.00(0.00) <sup>a</sup>	1.35(0.57) <sup>a</sup>	1.68(0.17) <sup>a</sup>	1.87(0.64) <sup>a</sup>	2.50(0.45) <sup>a</sup>
<b>Basiloil400+2%GAV</b>	0.00(0.00) <sup>a</sup>	1.06(0.65) <sup>a</sup>	1.19(0.22) <sup>b</sup>	2.05(0.65) <sup>a</sup>	2.47(0.24) <sup>a</sup>
<b>Basiloil400+4%GAV</b>	0.00(0.00) <sup>a</sup>	1.08(0.43) <sup>a</sup>	1.89(0.22) <sup>a</sup>	2.54(0.57) <sup>a</sup>	2.60(0.34) <sup>a</sup>
<b>Basiloil500+2%GAV</b>	0.00(0.00) <sup>a</sup>	1.37(0.57) <sup>a</sup>	1.93(0.32) <sup>a</sup>	2.58(0.45) <sup>a</sup>	2.65(0.47) <sup>a</sup>
<b>Basiloil500+4%GAV</b>	0.00(0.00) <sup>a</sup>	1.47(0.39) <sup>a</sup>	1.88(0.48) <sup>a</sup>	2.63(0.47) <sup>a</sup>	2.87(0.47) <sup>a</sup>
<b>Basiloil600+2%GAV</b>	0.00(0.00) <sup>a</sup>	1.56(0.69) <sup>a</sup>	1.65(0.57) <sup>a</sup>	2.65(0.58) <sup>a</sup>	2.98(0.47) <sup>a</sup>
<b>Basiloil 600+4%GAV</b>	0.00(0.00) <sup>a</sup>	1.78(0.41) <sup>a</sup>	1.89(0.37) <sup>a</sup>	2.35(0.57) <sup>a</sup>	2.87(0.58) <sup>a</sup>
<b>Basiloil750+2%GAV</b>	0.00(0.00) <sup>a</sup>	1.60(1.63) <sup>a</sup>	1.86(0.48) <sup>a</sup>	2.30(0.64) <sup>a</sup>	2.58(0.47) <sup>a</sup>
<b>Basiloil750+4%GAV</b>	0.00(0.00) <sup>a</sup>	1.70(0.41) <sup>a</sup>	1.90(0.37) <sup>a</sup>	2.25(0.57) <sup>a</sup>	2.57(0.47) <sup>a</sup>
<b>Basiloil1000+2%GA V</b>	0.00(0.00) <sup>a</sup>	1.76(0.85) <sup>a</sup>	1.98(0.45) <sup>a</sup>	2.72(0.96) <sup>a</sup>	2.97(0.48) <sup>a</sup>
<b>Basiloil1000+4%GA V</b>	0.00(0.00) <sup>a</sup>	1.85(1.42) <sup>a</sup>	1.98(0.56) <sup>a</sup>	2.73(0.78) <sup>a</sup>	2.98(0.57) <sup>a</sup>

*Note.* Values followed by the same letters within the column are not significantly different at ( $p \leq 0.05$ ), Standard deviation is given

in parenthesis. 2% GAV – 2% gelatin + *Aloe vera* 4% GAV – 4% gelatin + *Aloe vera*

### **Titratable acidity% (TA %), total soluble solids (Brix) and flesh color**

There was no significant difference in TA for all treated and control fruits except basil oil at 500  $\mu\text{LL}^{-1}$  in 4% GAV and basil oil at 750  $\mu\text{LL}^{-1}$  in 2% or 4% GAV where significantly higher TA was seen (Table 4). Abeywickrama et al. (2009) also reported application of basil oil on banana fruits to control crown rot disease did not show a significant difference in physico-chemical properties (titratable acidity, total soluble solids, pH, fruit firmness) when compared with the control.

**Table 4.**

*Physicochemical parameters in the control fruits and fruits coated with Aloe vera, with or without basil oil treatment at table ripe stage of maturity*

<b>Treatment</b>	<b>Titratable acidity%</b>	<b>Total Soluble Solids (%)</b>	<b>Flesh Color</b>
Control	0.06(0.00) <sup>a</sup>	13.34(0.22) <sup>a</sup>	Yellow
2% GAV	0.06(0.00) <sup>a</sup>	14.31(0.17) <sup>b</sup>	Yellow
4% GAV	0.06(0.00) <sup>a</sup>	14.43(0.15) <sup>b</sup>	Yellow
Basiloil400+2%GAV	0.06(0.00) <sup>a</sup>	14.00(0.13) <sup>b</sup>	Yellow
Basiloil400+2%GAV	0.06(0.00) <sup>a</sup>	14.07(0.15) <sup>b</sup>	Yellow
Basiloil500+4%GAV	0.07(0.00) <sup>b</sup>	14.50(0.15) <sup>b</sup>	Yellow
Basiloil500+2%GAV	0.06(0.00) <sup>a</sup>	14.38(0.15) <sup>b</sup>	Yellow
Basiloil600+2%GAV	0.06(0.00) <sup>a</sup>	14.24(0.08) <sup>b</sup>	Yellow
Basioil 600+4%GAV	0.06(0.00) <sup>a</sup>	15.02(0.12) <sup>c</sup>	Yellow
Basiloil750+2%GAV	0.08(0.01) <sup>c</sup>	14.51(0.03) <sup>b</sup>	Yellow
Basiloil750+4%GAV	0.07(0.00) <sup>b</sup>	14.14(0.11) <sup>b</sup>	Yellow
Basiloil1000+2%GAV	0.06(0.00) <sup>a</sup>	14.26(0.11) <sup>b</sup>	Yellow
Basiloil1000+4%GAV	0.06(0.00) <sup>a</sup>	14.27(0.21) <sup>b</sup>	Yellow

*Note.* Values followed by the same letters within the column are not significantly different at ( $p \leq 0.05$ ), Standard deviation is given in

parenthesis. 2% GAV – 2% gelatin + *Aloe vera* 4% GAV – 4% gelatin + *Aloe vera*

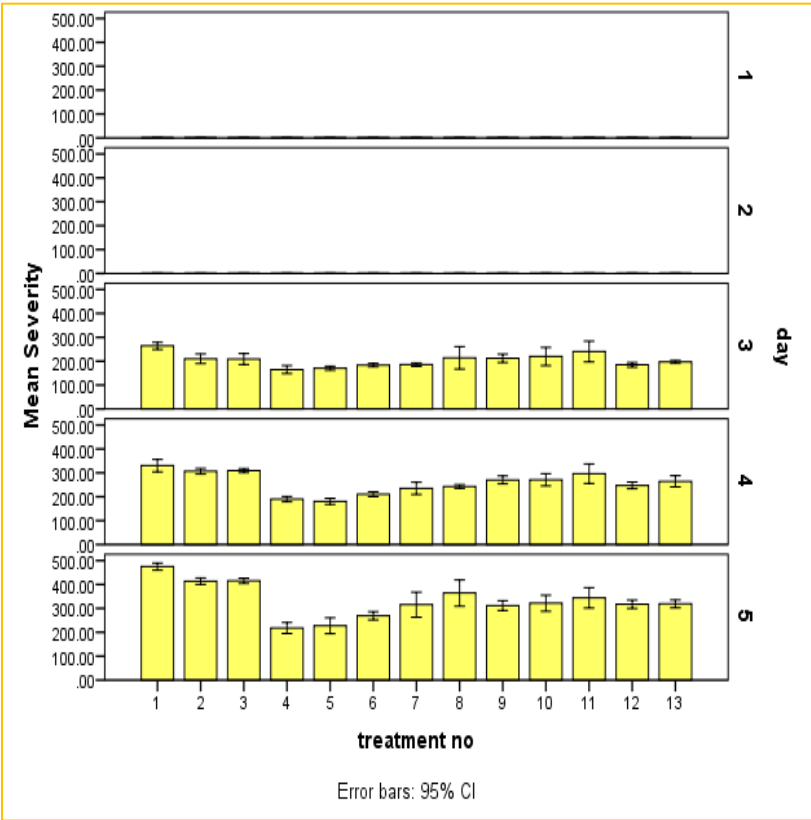
Fruits were tested for total soluble solid content at the table ripe stage of maturity (in five days after treatment. The highest Brix value (15.02) was observed in Basil oil at 600  $\mu\text{LL}^{-1}$  in 4% GAV coating. The lowest Brix value 13.34 was observed in the control fruits (Table 4). According to Harrill (1998), Brix value for sugar content was given as 4% for poor, 6% for average, 10% for good and 14% for excellent in banana fruits. In the present study, all samples showed over 10% Brix value which is considered as a good sugar content, and it suggested that the treatment had not negatively affected the sugar content of treated fruits. However, TSS represents the carbohydrate content, and increased TSS could indicate cell wall deterioration (Rehman et al., 2020). Kaushik et al. (2021) showed breakdown of organic acids and accumulation of soluble solids were slower in bananas protected with edible coatings of Aloe gel and Lemon peel extract. Mendy et al. (2018) showed *A. vera*-coated papaya fruits were able to reduce loss in weight and firmness and maintained higher soluble solid concentration, pH, and titratable acidity.

Flesh color was yellow in each treatment and there was no significant difference in flesh color between the treatments and the control (Table 4).

### **Effect of the treatments on postharvest disease development**

Lesion development was observed in banana fruits following 3 days of inoculation. The highest disease severity was observed in untreated control fruits and disease severity was significantly low in basil oil at 1000  $\mu\text{LL}^{-1}$  2% GAV and 4% GAV coated fruits at day 5 compared to other treatments (Figure 2). Abeywickrama et al. (2009) reported controlling of crown rot of banana with integrated treatment of alum and basil oil (at 0.16% or 0.2%). Linalool and Estragol are known to have antifungal and insecticidal properties (Chalchat & Ozcan, 2008; Rodrigues et al., 2016; Hussain et al., 2008; Kumar et al., 2015) which could be the reason for the reduced disease severity.



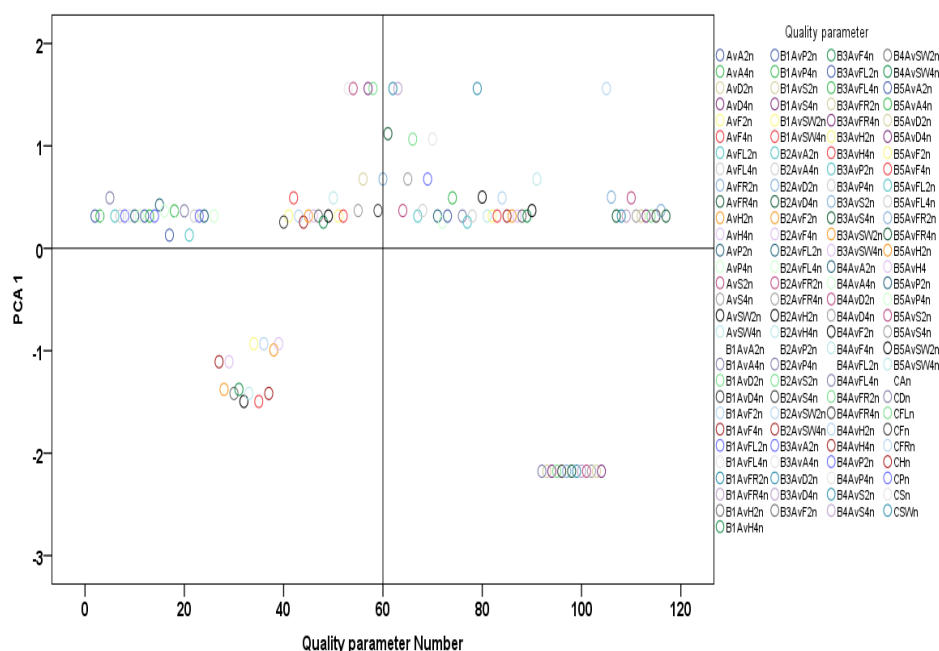


**Figure 2.** Mean disease area (Severity) at different days (5 days) of storage of cultivar *Kolikuttu* banana fruits after treating with different concentrations of basil oil mixed *Aloe vera* (AV) coating using two methods. **1**-Control, **2**- 2% AV, **3**- 4% AV, **4**- Basil oil 1000  $\mu\text{LL}^{-1}$  +2%AV, **5**- Basil oil 1000  $\mu\text{LL}^{-1}$  +4%AV, **6**- Basil oil 750  $\mu\text{LL}^{-1}$  +2%AV, **7**- Basil oil 750  $\mu\text{LL}^{-1}$  +4%AV, **8**- Basil oil 600  $\mu\text{LL}^{-1}$  +2%AV, **9**- Basil oil 600  $\mu\text{LL}^{-1}$  +4%AV, **10**- Basil oil 500  $\mu\text{LL}^{-1}$  +2%AV, **11**- Basil oil 500  $\mu\text{LL}^{-1}$  +4%AV, **12**- Basil oil 400  $\mu\text{LL}^{-1}$  +2%AV, **13**- Basil oil 400  $\mu\text{LL}^{-1}$  +4%AV

### Sensory evaluation effect of the treatments on sensory properties

The Principal Component Analysis (PCA) extracted a total variance of 91.83% of total data set. According to the results, the quality parameters such as appearance, peel color, hardness, freshness, softness, flesh Color, sweetness, dry/wrinkled, fruity were not

significantly affected by the treatments compared to untreated fruits (Figure 3). Similarly, Mohammadi et al. (2021) also showed that fruit coating with basil oil-incorporated *A. vera* could be a treatment for maintaining the quality of strawberry fruit during cold storage. But according to Abeywickrama et al. (2009) taste, flavor and odor of untreated 72-day mature embul banana with carbendazim treatment was preferred by the taste panelists over banana treated with basil oil and the differences were statistically significant.



**Figure 3.** Quality parameters of sensory evaluation depicted in one plot. B1- Basil oil 1000  $\mu\text{LL}^{-1}$ , B 2- Basil oil 750  $\mu\text{LL}^{-1}$ , B 3- Basil oil 600  $\mu\text{LL}^{-1}$ , B 4- Basil oil 500 ppm, B 5- Basil oil 400  $\mu\text{LL}^{-1}$ , CAN - Control Appearance, AvA2n - 2%AV Appearance, AvA4n - 4%AV Appearance, CPn- Control Peel color, AvP2n- 2% AV Peel color, AvP4n- 4%AV Peel color, CHn- Control Hard, AvH2n -2%AV Hard, AvH4n- 4%AV Hard, CFn- Control Fresh, AvF2n-2%AV Fresh , AvF4n-4%AV Fresh , CSn- Control Soft, AvS2n-2%AVSoft, AvS4n-4%AVSoft, CFIn-Control Flesh Color, AvFI2n-2% Flesh Color, AvFI4n-4% Flesh Color, CSWn-Control Sweet, AvSW2n-2%AV Sweet, AvSW4n- 4%AV Sweet, CDn-Control Dry, AvD2n-

2%AVDry, AvD4n-4% AV Dry, CFRn- Control Fruity, AvFR2n-2% AVFruity, AvD4n-4%AVFruity

## Conclusions

The results of the present study showed the possibility of using basil oil as a herbal fungicide and *Aloe vera* as a good coating material and carrier material for the essential oils. Basil oil at 1000  $\mu\text{L}^{-1}$  incorporated in *A. vera* was the most effective combination to control anthracnose in banana var. *Kolikuttu* fruit. The *A. vera* coating or the EO had no detrimental effects on any of the quality parameters tested and also had no undesirable effects on sensory properties either. Therefore, basil oil incorporated *A. vera* can be effectively used by organic farmers to reduce postharvest disease development while increasing the safety for consumers and increasing the quantity of Banana available for the local and export market.

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