International Journal of Agriculture and Environmental Research

ISSN: 2455-6939

Volume:02, Issue:05

EFFICACY OF BASIL OIL AND ALUM IN CONTROLLING CROWN ROT DISEASE OF CAVENDISH BANANA (*Musa acuminata*, AAA) DURING COLD STORAGE

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ABSTRACT

Effect of *Ocimum basilicum* (basil) oil spray treatment, alum in combination with modified atmosphere packaging (MAP) was investigated in extending the shelf life of Cavendish banana (*Musa acuminata*, AAA - Grand Naine cultivar) at 12-14 ^{0}C. Twelve week mature Cavendish banana fruits were treated with 1% (w/v) alum (Potassium aluminium sulphate), 1% (w/v) alum + 0.4% (v/v) basil oil, 0.5 g/L carbendazim and distilled water (control). Treated banana samples were packed in Low Density Polyethylene bags and stored at 12-14 ^{0}C. In-package gases were analysed every seven days up to 28 days of storage. Physicochemical properties (pH, firmness, TSS, TA), sensory properties (peel colour, flesh colour, aroma, flavour, taste, overall acceptability) and crown rot disease severity were determined in ripening induced fruits after each storage period. At the end of 28 days of storage O_2 in all packages remained between 5.0-5.4% while CO_2 varied from 5.1 to 5.6%. Further, treatment of 1% alum+0.4% basil oil effectively controlled crown rot disease of Cavendish banana completely up to 21 days. Most of physicochemical and sensory properties of treated banana were not adversely affected by the treatment.

Keywords: Crown rot disease, Basil oil, Cavendish banana, cold storage

1. INTRODUCTION

Banana (*Musa acuminata* L.) is one of the widely grown and consumed fruits all over the world. Among the various commercial subgroups of banana, Cavendish (genome AAA) is largely

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traded, particularly in the export market (Alvindia, 2013). The dominant commercial cultivars of Cavendish subgroup include, Lacatan, Poyo, Williams, Grand Naine, and Petite Naine (Aurore *et al.,* 2009).

Banana fruit has short storage life due to its highly perishable nature and suffers severe post harvest loss both in terms of quality and quantity due to post harvest diseases (Sangeetha *et al.,* 2010). Crown rot disease is the major postharvest and post packing disease of banana and all commercial cultivars of dessert bananas are susceptible to this disease (Abd-Alla *et al.,* 2014).

Crown rot disease is caused by different pathogenic fungi such as *Colletotrichum musae*, *Lasiodiplodia theobromae*, *Fusarium proliferatum* and *Verticillium theobromae* (Ploetz *et al.,* 1994). However, *L. theobromae* and *C. musae*, have been reported as the major pathogens associated with the Crown rot disease in majority of banana producing countries (Anthony *et al.,* 2003).

The above fungal pathogens infect the banana crown through wounds created in dehanding process and contaminated water used for washing. Mycelia are formed on the surface of the cut crown, turning infected tissue in to black colour. The rot may then advance into the finger stalks, causing fingers to detach from the crown when handled, reducing the consumer appeal to buy such fruits, or rejecting export consignments (Alvindia, 2013).

Crown rot disease of banana is often controlled commercially by post-harvest treatment of fungicides such as thiobendazole, imazalil or benomyl (Sangeetha *et al.,* 2010). However, due to hazardous effects of fungicides on humans and environment, investigations on use of nonchemical approaches are more promising. The current world trend is for eco-friendly treatments for banana which safeguard the health of consumers.

Use of plant essential oils is a safer alternative to synthetic fungicides. Essential oils obtained through steam distillation of aromatic plant materials have been used for centuries in medicine, perfumery, cosmetic, and food industries (Hyldgaard *et al.,* 2012).

Sweet basil (*Ocimum basilicum* L.) belonging to *Lamiaceae* family is a herb which is grown in several regions around the world. Basil is used to extract oil for perfumery, food and medical industries. This oil possesses a range of biological activities such as insect repellent, nematicidal, antibacterial, antifungal and antioxidant activities (Wogiatzi *et al.,* 2011).

Modified atmosphere packaging (MAP) refers to the technique of sealing actively respiring produce in a packaging film to modify the O_2 and CO_2 levels within the package atmosphere. Generating of an atmosphere low in O_2 and high in CO_2 influence on the reduction of respiration

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rate and ethylene production of the packaged product and thereby improve storability or the shelf life (Mir and Beaudry, 2002). A combination of essential oils and MAP may have positive impact in controlling Crown rot disease and extending the storage life of banana than using essential oils alone.

Previous investigations conducted in Sri Lanka revealed that spraying Embul variety banana with an emulsion of basil oil (*Ocimum basilicum*) controlled Crown rot disease and increased shelf life up to 28 days at MA conditions (14 ^{0}C with 90% RH) (Abeywickrama *et al.,* 2009). However, this method has not been tested on Cavendish banana, which is the predominant commercial cultivated variety worldwide. Further, to the best of our knowledge, reports on the effect of basil oil for the effective management of Crown rot disease in Cavendish banana are scarce.

Further, Banana meant for long distance export via sea shipment have to be treated with a desirable botanical/chemical strategy. Appearing of Crown rot disease during sea shipment may result in rejection of consignments of banana which may invariably result in loss of markets and income to a country. Therefore, it is important to develop an eco-friendly treatment system to control Crown rot disease completely.

This research was conducted to identify the efficacy of basil oil + alum in controlling Crown rot disease and extending shelf life of Cavendish banana which were subjected to MA packaging and stored at 12-14 ^{0}C for a month. Our objectives were to examine headspace respiratory gas composition and crown rot disease severity and to assess physicochemical and sensory properties of basil oil treated Cavendish banana which were subjected to packaging and storage as above.

2. MATERIALS AND METHODS

2.1 Preparation of Cavendish banana

Twelve week mature Cavendish banana (Grande Naine cultivar) bunches were harvested from CIC banana plantation in Pelwehera, Dambulla, Sri Lanka. Banana bunches were transported to the CIC banana pack house, in CIC Agri Business Centre, Dambulla, Sri Lanka. Bunches were dehanded and approximately 1 kg hands were selected as experimental units. All hands were washed in water to remove dirt and then washed with potassium aluminium sulphate (alum) (1% w/v) except control. Subsequently, banana were allowed to drip dry.

2.2 Preparation of treatments

Ocimum basilicum (sweet basil) oil was purchased from Aromatica laboratories (Pvt.) Ltd. Sri Lanka. Basil oil (400 uL) was added to 0.1 L of distilled water to prepare 0.40% (v/v) concentration with a drop of 'Tween'80 (Park Scientific Limited, Northampton, UK). The mixture was stirred using a magnetic stirrer for 10 min and transferred to a hand-sprayer and mixed well by shaking. Carbendazim (0.5 g L^{-1}) solution was also prepared as a standard fungicide. The control was prepared by adding one drop of 'Tween' 80 to 0.1 L of distilled water and stirring for 10 min (Abeywickrama *et al.,* 2009).

2.3 Application of treatments

Cut surfaces of crown and fingers of banana hands were sprayed with 0.40% (v/v) basil oil emulsion, fungicide or distilled water. Another set of banana hands were washed in 1% alum solution only. Subsequently, banana hands were allowed to drip dry and placed in low density polyethylene (LDPE) bags (150 gauge) of 31.5 × 32 cm surface area and Flora foam liners were placed on top of banana to provide protection to fruit. Mouths of bags were tied with rubber bands and packed in (40×29×19 cm) ventilated 3-ply fibre board cartons. Each treatment comprised of four replicate boxes, each with five hands (weighing 5.0-5.5 kg). All treatments were stored at 12-14 ^{0}C in a cold room at 85-90% relative humidity at CIC Agri Business Centre, Dambulla, Sri Lanka (Abeywickrama *et al.,* 2009). Observations were made after 7, 14, 21 and 28 days of storage. The experimental arrangement was a completely randomized design (CRD). This experiment was repeated once under identical conditions.

2.4 In-package gas analysis

In-package respiratory gas (O_2 and CO_2) variations within bags were measured on 7, 14, 21 and 28 days during cold storage using a Digital Oxygen and Carbon Dioxide Head Space analyzer (Model 902 D, Quantek Instruments, Grafton, MA). A needle was inserted in to each bag and a small sample of package headspace gas was pumped into the gas analyzer. The stable oxygen and carbon dioxide measurements were recorded (Kudachikar *et al*., 2011). Five replicate measurements were taken per treatment.

2.5 Ripening of banana

After each storage period, banana hands were subjected to induced ripening by exposure to ethylene (thrill, ethephon, 1 L of water) for 2-3 days at ambient temperature (Abeywickrama *et al.,* 2009).

Pathological, physicochemical and sensory properties of ripened Cavendish banana fruits were analysed after each storage period.

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2.6 Pathological properties

Crown rot in each hand was recorded using a standard index developed at the Department Botany, University of Kelaniya (CRD Severity) 0=No rot, 1=25% Crown rot, 2=50% Crown rot, 3=75% Crown rot, 4=100% Crown rot) (Abeywickrama *et al.,* 2009).

2.7 Physicochemical properties

Ten fingers selected at random from each treatment was subjected to physicochemical analysis. The firmness of the cross sections of ripe fruits (1 cm thickness) were measured using a fruit firmness tester (FT 011, QA Supplies, Italy). pH of the filtrates were measured using a digital pH meter (PC 510, EUTECH Instruments, Singapore). Total soluble solids (TSS) of filtrates were recorded using a hand-held Refractometer (ATC, ATAGO, Japan, Brix; 0-32%). Titratable acidity (TA) (% acid) was assessed by a titration of an extract of banana with 0.1 M NaOH using phenolphthalein as the indicater (Abeywickrama *et al.,* 2009; Siriwardana *et al.,* 2015).

2.8 Sensory Properties

Peel colour, flesh colour, flavour, taste, aroma, texture and overall acceptability of fruit were assessed by a trained ten member sensory panel. Each quality parameter was scored as follows: Excellent=9-10, Good=6-8, Fair=4-5, Poor=1-3 (Abeywickrama *et al.,* 2009).

2.9 Statistical Analysis

Data obtained for in-package gases and physicochemical properties were subjected to General Linear Model and mean separation was done using Tukey's Multiple Comparison test using Minitab. Data obtained for pathological and sensory properties were analysed using Kruskal-Wallis non-parametric statistical test.

3. RESULTS

3.1 In-package gas analysis

O_2 level in all treated and control samples gradually decreased throughout 28 day storage period and final O_2 levels were between 5.0-5.4% (Figure 1A). Further, CO_2 level in all treated and control samples increased gradually throughout 28 day storage period and the final values were between 5.1-5.6% (Figure 1B). According to statistical analysis, there was no significant difference in O_2 and CO_2 levels among the different treatments and control when treatment and interaction of treatment x time were considered. However, there was a significant difference in O_2 and CO_2 levels when only time was considered as a factor.

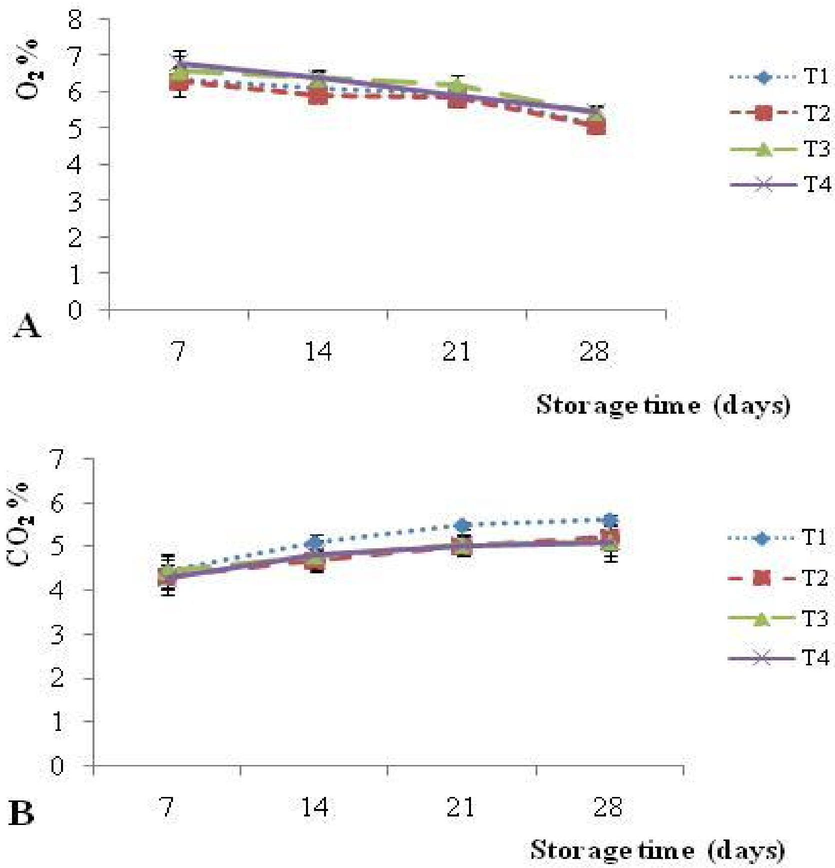

Figure 1. O_2% **(A)** and CO_2% **(B)** of MA packaged Cavendish banana treated with 1% alum (T1), 1% alum + 0.4% basil oil (T2), 0.5 g L^{-1}carbendazim (T3) and distilled water (T4) after each storage period at 12-14 ^{0}C. Each data point represents the mean of five replicates ± standard error.

3.2 Pathology

All treated and control samples showed no crown rot disease up to 7 days. Both alum treated and control banana showed similar crown rot disease severity on day 14, where it increased with time Control samples showing the highest crown rot disease severity on day 28. Alum + 0.4% basil oil showed no crown rot disease up to 21 days, however, showed mild crown rot disease on day 28. Fungicide treatment showed no crown rot disease in any of the analysis day (Figure 2). However, mean crown rot disease severity values were <1 in all treated and control samples.

According to statistical analysis, crown rot disease severity was not significantly different among treatments and control.

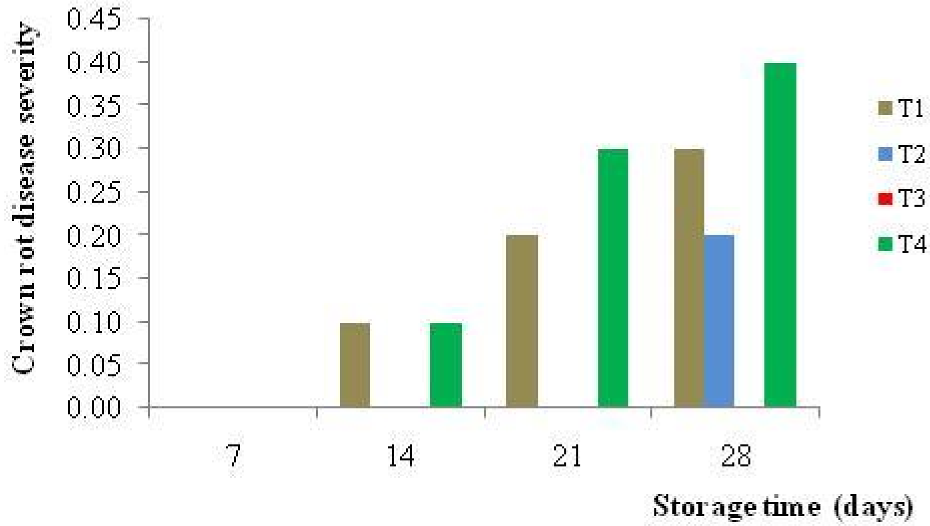

Figure 2. Crown rot disease severity of MA packaged Cavendish banana treated with 1% alum (T1), 1% alum + 0.4% basil oil (T2), 0.5 g L^{-1}carbendazim (T3) and distilled water (T4) after each storage period at 12-14 ^{0}C and subjected to induced ripening. Each data point represents the mean of ten replicates.

3.3 Physicochemical properties

There were slight variations of pH over time. The pH values of alum treated and alum+0.4% basil oil treated banana slightly increased up to 14 days and thereafter a decrease was observed. In fungicide treated samples pH increased up to 21 days and decreased thereafter. Further, pH of control samples dropped over time during the 28 day storage period. However, the values were within the range of 4.46-4.96 (Table 1). pH values were not significantly different when the effect of treatment was considered. However, there was a significant difference in pH when time and the interaction of treatment x time were considered (Table 2).

Table 1. pH and Titratable Acidity (TA) of MA packaged Cavendish banana

Treatment	Storage time			
	day 7	day 14	day 21	day 28
		pH		
T1	4.85 ± 0.02	4.86 ± 0.03	4.76 ± 0.02	4.72 ± 0.03
T2	4.86 ± 0.01	4.96 ± 0.01	4.82 ± 0.03	4.67 ± 0.03
T3	4.70 ± 0.01	4.89 ± 0.04	4.96 ± 0.02	4.66 ± 0.01
T4	4.46 ± 0.02	4.74 ± 0.04	4.74 ± 0.03	4.70 ± 0.04
		TA (% Malic Acid)		
T1	0.72 ± 0.02	0.68 ± 0.02	0.67 ± 0.02	0.76 ± 0.00
T2	0.67 ± 0.03	0.61 ± 0.03	0.69 ± 0.03	0.80 ± 0.01
T3	0.72 ± 0.02	0.66 ± 0.02	0.64 ± 0.03	0.76 ± 0.03
T4	0.73 ± 0.02	0.69 ± 0.02	0.67 ± 0.02	0.70 ± 0.03

T1 – 1% alum, T2- 1% alum+0.4% basil oil, T3- 0.5 g L^{-1}carbendazim, T4- control. Each data point represents the mean of ten replicates ± standard error.

Opposite trend of pH was observed for Titratable acidity (TA) of most samples. TA values of alum+0.4% basil oil treated banana slightly decreased up to 14 days and increased thereafter. In alum, fungicide treated and control samples, TA decreased up to 21 days and increased subsequently. Further, the values were within the range of 0.61-0.80 (Table 1). TA values were not significantly different when the effect of treatment and the interaction of treatment x time were considered. However, there was a significant difference in TA when only time was considered as a factor (Table 2).

Table 2. ANOVA results for effect of different treatments and time on physicochemical properties of MA packaged Cavendish banana

Factor	d.f.		F-value	P value
		pH		
A (treatment)	3		1.59	0.194
B (time)	3		11.73	0.000
A × B	9		3.70	0.000
		TSS		
A (treatment)	3		23.51	0.000
B (time)	3		24.10	0.000
A × B	9		5.07	0.000
		TA		

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A (treatment)	3	0.16	0.925
B (time)	3	9.74	0.000
A × B	9	1.73	0.086
	Firmness		
A (treatment)	3	2.65	0.051
B (time)	3	5.69	0.001
A × B	9	0.66	0.741

P<0.05 indicates a significant difference among variables and control.

During the current research, TSS values of all treatments decreased up to 21 days and then increased except in alum+0.4% basil oil treated banana which showed a slight decrease in TSS during first 14 days and thereafter an increase. Highest TSS values were observed for alum+0.4% basil oil treated banana while the lowest values of TSS were reported for fungicide treated banana in all analysis days (Figure 3A). Total Soluble Solid values ranged from 14.50 to 18.80 (^{0}Brix) during the 28 day storage period. Further, TSS values were significantly different when treatment, time and interaction of treatment x time were considered (Table 2).

The firmness of the samples from different treatments and control were within the range of 0.47 - 0.39 kg cm^{-2} during analysis period of 28 days. The lowest firmness of 0.39 kg cm^{-2} was recorded for control banana on day 28 while highest firmness of 0.47 kg cm^{-2} was recorded for the carbendazim treated banana on day 7. Decrease of firmness over time could be observed for all treatments (Figure 3B). According to statistical analysis, there was no significant difference (p<0.05) in firmness when treatment and interaction of treatment x time were considered. However, there was a significant difference in firmness when only time was considered as a factor (Table 2).

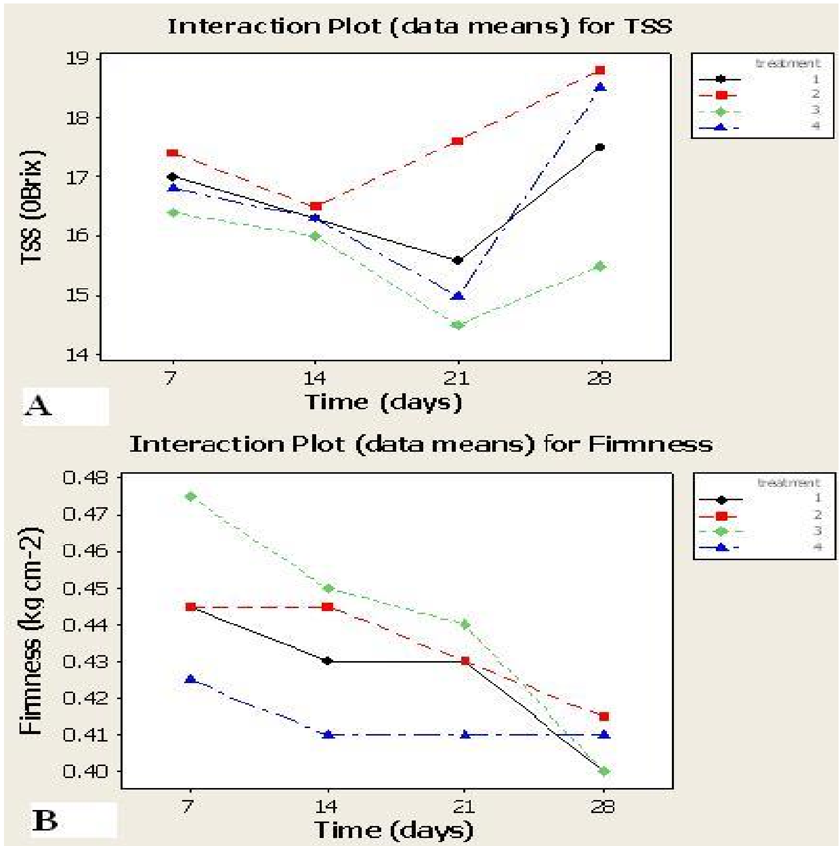

Figure 3. Interaction plot for TSS **(A)** and Firmness **(B)** of modified atmosphere packaged Cavendish banana (treatment 1 - 1% alum, 2 - 1% alum + 0.4% basil oil, 3 - 0.5 g L^{-1}carbendazim, 4 - distilled water)

3.4 Sensory Properties

When sensory properties were considered, it was noted that sensory panelists preferred the alum + 0.4% basil oil treated banana over other treatments. However, all score values obtained for sensory properties including aroma were 6 or better indicating all samples were of 'good' quality (Figure 4).

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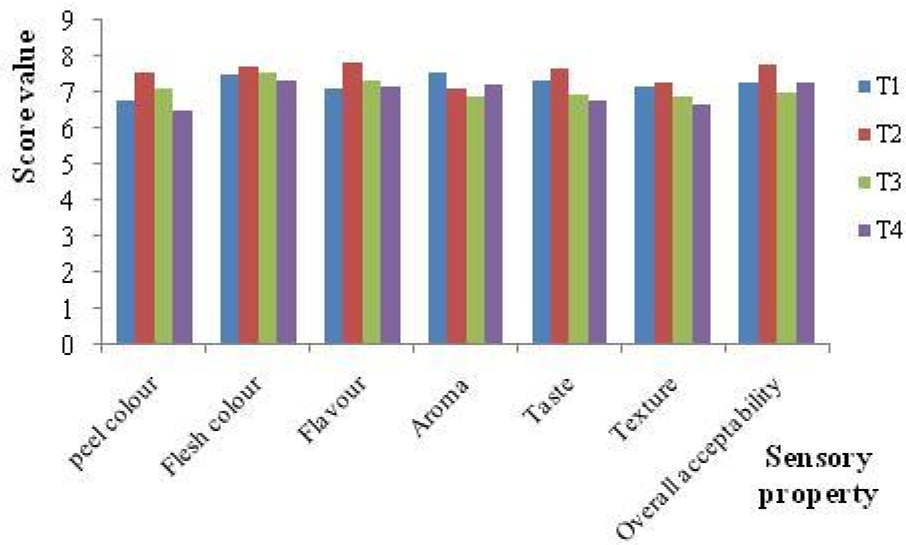

Figure 4. Sensory properties of MA packaged Cavendish banana treated with alum (T1), 1% alum + 0.4% basil oil (T2), 0.5 g L^{-1}carbendazim (T3) and distilled water (T4) after 28 day storage period as scored by the trained sensory panel. Each data point represents the mean of twenty replicates.

4. DISCUSSION

During the current study, O_2 levels in all treated and control samples were between 5.0-5.4% while CO_2 levels were between 5.1-5.6% at the end of storage period at 12- 14 ^{0}C. According to Kudachikar *et al*., (2011) banana cv. *Robusta* (AAA) stored under MAP at 12 ± 1°C and 85–90% maintained 8.2 - 8.6% CO_2 and 2.6 - 2.8% O_2 levels in packages, respectively, after 3 weeks of storage. Abdulla *et al.,* (1993) reported that banana cv. Berangan (AAA) packed in polyethylene bags with or without ethylene absorbent maintained the levels of CO_2 and O_2 around 3.4-6.7% and 1.6-6.1% from first to fourth week of storage at 14 ^{0}C. These findings are in accordance with the current research data. Slight variations in gas levels reported within packages could be attributed to cultivar differences & maturity, initial quality of commodity and film thickness.

According to Abdulla *et al.,* (1993) CO_2 levels below 10% and O_2 levels beyond 1% are needed for safe storage of banana. Fermentation and off-flavours/odours may develop if decreased O_2 levels cannot sustain aerobic respiration. Kader (1993) reported that O_2 levels below 1-1.5% cause grayish/brown peel discolouration, off flavour and failure to ripen properly and CO_2 levels greater than 6-8% cause pulp to soften while the peel is still green, and result in undesirable texture and flavour. Several researchers have recommended optimal MA conditions for fresh

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banana as 13-15 ^{0}C, 90- 95% relative humidity with 2-5% CO_2 and O_2 concentration (Lee *et al.,* 2010; Sen *et al.,* 2012). Therefore, it could be inferred that O_2% and CO_2% in MA packages during the present study remained at desired gas levels maintaining quality or freshness of banana.

Alum + 0.4% basil oil was more effective (than the alum treatment alone and control) and crown rot disease was completely controlled up to 21 days. However, slight crown rot disease severity was observed in oil treated samples on day 28. The results of the present study suggested that basil oil have the potential to control crown rot disease in Cavendish banana up to 21 days. Previously, basil oil have been reported to effectively control crown rot disease in Embul cultivar banana and extend the storage life up to one month (Abeywickrama *et al.,* 2009).

Senanayake *et al.,* (1997) reported, eugenol (35%), β-selanine (15.5%) and β-caryophyllene (10.7%) as the major constituents of *Ocimum basilicum* oil, when analyzed using gas chromatography. Anthony *et al.,* (2003) reported the presence of eugenol (10.76%) and β-caryophyllene (0.5%) in authentic basil oil. Control of crown rot disease due to basil oil could be attributed to eugenol, which inhibits conidial germination, appressoria formation and affect membrane permeability of fungal pathogens (Anthony *et al.,* 2003). Conidia of *C. musae* germinate after depositing on a plant surface and produce a melanised, thick walled appresoria which is essential to firmly adhere to the host surface. In a previous research, it was noted that, basil oil prevents melanisation of appressoria and cause leakage of cell contents in conidia of *C. musae* resulting in the death of microbial cells (Herath and Abeywickrama, 2008).

pH is an important measure of fruit palatability. It depends both on the total quantity and strength of acids present in fruit. According to Opara *et al.,* (2013) pH of Dwarf Cavendish banana followed an irregular pattern with ripening i.e. increase of pH followed by a decrease and pH ranging between 4.98-5.43. According to Marin *et al.,* (1996) after ripening pH of Grande Naine' (AAA) banana were within 4.94-4.95. Dadzie (1998) reported after ripening, pH of Grande Naine' banana attained a value of 4.93. It could be inferred that observations of present study are in accordance with previously published literature.

When ripening proceeds, TA increases causing a drop in the pH. Opara *et al*., (2013) reported that in Dwarf Cavendish banana TA increased until the banana fruits became fully ripe and declined at the overripe stage after 3 weeks of storage at 11-12 ^{0}C and values were within the range of 0.34-0.41% Malic acid. Dadzie (1998) reported, TA of Grande Naine' banana was about 0.30% MA after ripening. However, results reported in current study are somewhat higher compared to previous reports indicating higher acidity of samples.

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Total Soluble Solids (sugars) of banana is linked to consumer taste preference. Generally, fruits above 12% ^{0}Brix are considered more acceptable to consumers (McGlone and Kawano, 1998). In the current study, Highest TSS values were observed for alum+0.4% basil oil treated banana in all analysis days. Sweeter taste in Cavendish banana samples treated with alum+basil oil were relatively more preferred by the sensory panelists than other treatments. Further, TSS values increased with time and ranged between 14.50 and 18.80 (^{0}Brix) during the 28 day storage. Similarly, Opara *et al.,* (2013) reported that TSS of Dwarf Cavendish banana increased as fruit ripened and were within 19.6-21.2 ^{0}Brix. Further, Dadzie (1998) reported that in ripe Grande Naine banana TSS was in the range of 14.00 ^{0}Brix.

Firmness is an important parameter for determining the eating quality and ripeness of banana. As fruits ripen, flesh firmness decreases down to a relatively narrow optimal eating range of 0.7-0.4 kg cm^{-2} beyond which the fruit becomes senescent. Softening or loss of pulp firmness during ripening is due to solubilization of peptic substances in the cell wall and middle lamella (Dadzie, 1998). According to Opara *et al.,* (2013) fruit firmness of Dwarf Cavendish banana gradually decreased over 21 day storage period at 11-12 ^{0}C. Firmness values decreased over time during current study and are in accordance with the previously published literature.

Sensory score values obtained were 6 or better indicating all samples were of 'good' quality. At times, aroma of essential oil, could cause negative organoleptic effects exceeding the threshold acceptable to consumers. However, in present study, sensory panelists preferred the alum+basil oil treated banana over other treatments indicating that basil oil did not adversely affect the sensory properties of banana.

Basil oil control pathogenic fungi on banana and subsequently, volatilize leaving no residues on commodity. The United States Food and Drug Administration (FDA) classify basil oil as generally recognized as safe (GRAS) compounds (Hyldgaard *et al.,* 2012). A range of essential oil components have been accepted by the European Commission for their intended use as flavourings in food products and eugenol (bioactive component in basil oil) is one of the registered flavouring agents which is considered to present no risk to the health of the consumer (Hyldgaard *et al.,* 2012). Further, toxicological data of eugenol indicate that a dose of 2680 mg/kg is needed to observe LD_{50} (Lethal Dose, 50%) in rats (Goubran and Holmes, 1993). This means that a relatively high dose of basil oil is needed to observe lethal effect on mammals including humans.

It could be inferred that the eco-friendly treatment technology developed in this research could be adopted in organic banana industry where fungicides are not used to treat fresh Cavendish banana for local consumption and also for export to various destinations which require transit time up to three weeks.

5. CONCLUSION

Treatment of alum + 0.40% basil oil combined with MAP in cold storage at 12-14 ^{0}C controlled crown rot disease effectively compared to alum treatment or untreated control and extended the storage life of Cavendish banana up to 21 days. Most of the physicochemical and sensory properties of treated banana were not significantly different compared to the control.

ACKNOWLEDGEMENTS

Financial assistance provided by the National Research Council (NRC), Grant 12-003, Sri Lanka and support provided by staff of CIC Agri Business Centre, Dambulla, Sri Lanka are highly appreciated.

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