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Effect of *Croton aromaticus* in controlling Crown Rot disease of Embul banana in combination with modified atmosphere and cold storage

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Abstract

Antifungal activity of aqueous, hot water and ethanolic leaf extracts of *Croton aromaticus* in controlling crown rot (CR) disease of Embul banana was investigated *in vivo* during the study. Embul banana hands were sprayed with *C. aromaticus* aqueous, hot water and ethanolic leaf extracts alone and in combination with alum and were stored in modified atmosphere packaging at 12-14 0 C for 14 days. In-package gases were analysed up to 14 days. Physicochemical properties (pH, firmness, TSS, TA), sensory properties (peel colour, flesh colour, aroma, flavour, taste, overall acceptability) and CR disease severity were determined in ripening induced fruits after 7 and 14 days of storage period. Oxygen and CO₂ in all packages were maintained at desired gas levels for safe storage of banana. Ethanolic leaf extract was the most effective in controlling CR disease compared to aqueous and hot water leaf extracts. Some of the physicochemical properties of Embul banana treated with *C. aromaticus* leaf extract alone and in combination with alum were affected significantly compared to control. *C. aromaticus* ethanolic leaf extract + alum in combination modified atmosphere packaging and cold storage could be used as a potential safe way of controlling CR disease of Embul banana.

Keywords: crown rot disease, in-package gases, physicochemical, sensory, banana.

Introduction

Crown Rot (CR) is the most common and serious postharvest disease causing severe postharvest losses in banana industry. The infection takes place at the exposed surface of the cut crown. Usually the rot is confined to the crown, but at advanced stages it spreads into the pedicels of the fingers. Whitish fungal mycelia and reproductive bodies appear on the rotted crown, finger stalks and on the fingers in severe infection. *Colletotrichum musae*, *Lasiodiplodia theobromae*,*Fusarium* spp. and *Verticillium* spp. and several other plant pathogenic species are reported to cause crown rot disease in banana (Indrakeerthi and Adikaram 2011). Synthetic fungicides are the primary means of controlling postharvest diseases. The excessive use of these chemicals for controlling crown rot fungi especially at postharvest level on fruit has been counterproductive, causing damage to the environment and humans which increase demand to reduce the use of these chemicals that accumulate in fruits (Talibi *et al.*, 2014).

Plant extracts of higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties. *Croton* genus belonging to Euphorbiaceae family is widespread in tropical regions. Some species are aromatic due to the possession of volatile oils and bioactive compounds such as diterpenes and alkaloids (Salatino *et al.*, 2007).

During a previous research by Bandara et al. (1987), leaf extracts of Croton aromaticus was found to have profound antifungal activity against *Cladosporium* cladosporioides. Further, ethanolic leaf extract of C. aromaticus significantly inhibited the mycelial growthand spore germination of postharvest fungal pathogens of Colletotrichum musae, Alternaria alternata. *Pestaloptiopsis* mangiferae and Colletotrichum gleosporioides isolated from a selected group of tropical fruits (Wijesundara et al., 2016). Modified atmosphere packaging (MAP) refers to the technique of sealing actively respiring produce in a packaging film to modify the O₂ and CO₂ levels within the package atmosphere. Generating of an atmosphere low in O_2 and high in CO_2 influence on the reduction of respiration rate and ethylene production of the packaged product and thereby improve storability or the shelf life of commodity of concern (Mir and Beaudry, 2002).

Therefore, the objectives of the present study were (1) to investigate *in vivo* antifungal activity of aqueous, hot water and ethanolic leaf extracts of *C. aromaticus* alone or in combination with alum on Embul banana under modified atmosphere and cold temperature to control crown rot disease and (2) to evaluate physicochemical and sensory properties of Embul banana after treating with fresh, hot water and ethanolic leaf extracts of *C. aromaticus* in combination with alum.

Materials and Methods

Preparation of Embul banana

Approximately 85-day mature Embul banana bunches were harvested from a plantation in Wewalduwa,

Sri Lanka. Bunches were dehanded at the field and transported to the laboratory at the Department of Botany, University of Kelaniya, Sri Lanka. Approximately 1 kg hands were washed in water to remove dirt. Each hand was washed in sodium aluminium sulphate (alum) (1% w/v) except control hands. Banana were allowed to drip dry on a laboratory bench (Abeywickrama *et al.*, 2009).

Preparation of leaf extract

Branches containing leaves of *Croton aromaticus* were collected separately from Malabe and Kaduwela areas in Colombo district in Sri Lanka.

Aqueous extract

Fresh leaf samples of *C. aromaticus* were thoroughly washed with tap water and then rinsed with sterile distilled water. Fresh leaves (1 g) were then crushed using a sterilized mortar and pestle with 10 mL of sterile distilled water. Resulting crude extract was filtered through sterilized Whatmann No. 1 filter paper, and the extract was subsequently filter sterilized (Pawar, 2011). The mixture was transferred to a hand-sprayer and mixed well by shaking.

Hot water extract

Fresh leaf samples were crushed as above and sample extract was boiled in a water bath for 6-7 hours at 45 ^oC. Extract was filtered after 18 hours and filtrate was taken as the hot water extract. Crude extract was transferred to a hand-sprayer and mixed well by shaking.

Solvent extract

Fresh leaf samples were thoroughly washed using tap water, air -dried and powdered using a grinder. The crude ethanolic extract was prepared from dried powdered leaf material (100 g), macerated with absolute alcohol (ethanol) (500 mL) for 72 hours at room temperature (28 ± 2 ⁰C). Extract was then filtered through Whatmann No.1 filter paper. Filtrate was concentrated and evaporated to dryness under vacuum at 40 ^oC using a rotary evaporator (Cole-Parmer Rotary Evaporator System; Diagonal, 230 VAC, U.S.A.).The crude extract was then stored at 4 ^oC until further use (Britto *et al.*, 2012). Solution of 400 mg ml⁻¹was prepared using crude ethanolic extract.

Application of treatments

Cut surfaces of banana crown and fingers of four banana hands were sprayed with C. aromaticus leaf extracts (aqueous, hot water and ethanolic leaf extract) or distilled water. Another set of banana hands were washed in 1% alum solution only. Crude extract was sprayed on another set of banana washed with 1% alum solution. Excess solutions of treated hands were allowed to drain for 10 minutes. All treated and control (only washed in water) banana samples were placed separately in low density polyethylene (LDPE) bags (150 gauge) of 31.5×32 cm surface area and the mouths of bags were tied with rubber bands. All banana hands were placed in ventilated telescopic type, 3-ply cardboard cartons (60cm x 35cm x 18cm) lined with perforated Manila paper (60 μ) and stored between 12-14 ^oC in a Walk-in Cold room (Iceman Technologies (Pvt.) Ltd.Wattala) providing a relative humidity (RH) of 85-90%. Samples were removed from the cold room after 7 and 14 days of storage for observation and evaluation. The experimental arrangement was a completely randomized design (CRD). This experiment was repeated once under identical conditions.

In-package gas analysis

In-package respiratory gas (O_2 and CO_2) variations within bags were measured on day 0 up to 14th day 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. Stable oxygen and carbon dioxide measurements were recorded (Kudachikar *et al.*, 2011). Four replicate measurements were taken per treatment.

Ripening of banana

After removing the samples from the cold room, all hands were taken out from the polyethylene bags and 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 Embul banana fruits were analysed after each storage period. This experiment was repeated once (Abeywickrama *et al.*, 2009).

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).

Physicochemical properties

Ten fingers selected at random from each treatment were 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 hand-held а Refractometer (ATC, ATAGO, Japan, Brix; 0-32%). Titratable acidity (TA)(% acid) was assessed by a titration of an extract with 0.1 M NaOH using phenolphthalein as the indicator (Abeywickrama et al., 2009).

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

Statistical analysis

All data parameters were analysed as Completely Randomized Design. Data obtained for in-package gases and physicochemical properties were subjected to ANOVA 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.

Results and Discussion

Oxygen level detected in Embul banana samples treated with aqueous extract of *C. aromaticus* were within 2.2-2.9 % while CO₂ were within 5.5-5.9% after 14 days of storage. Hot water leaf extract treated banana recorded 2.9-3.6% of O₂ and 7.6-8.4% of CO₂. Further, O₂ levels were in the range of 3.6-4.4% (Figure 1) while CO₂ levels were within 5.6-6.0% (Figure 2) in ethanolic leaf extract treated banana. Siriwardana *et al.*, (2016) reported that basil oil in combination with alum treated Cavendish banana stored under MAP at 12-14 ^oC maintained 5.3-5.9% of O₂ and 5.3-5.7% of CO₂ levels in packages,

respectively after 2 weeks of storage. Data reported during current study are in accordance with results reported by Siriwardana *et al.*, (2016). According to Abdulla *et al.*, (1993), for safe storage of banana, the level of CO_2 should not exceed 10% while O_2 should remain above 1%. Fermentation and off-flavours and off-odours may develop if decreased O_2 levels cannot sustain aerobic respiration. Therefore, O_2 % and CO_2 % in all packages during this study were maintained at desired gas levels for the safe storage of banana.

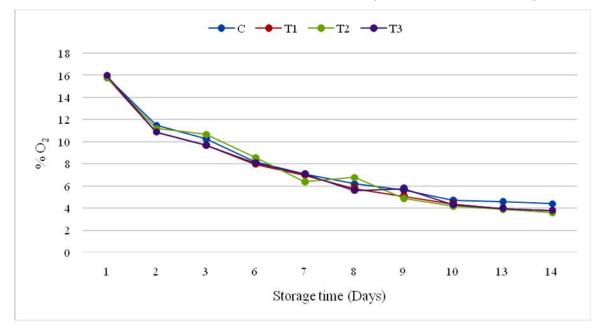
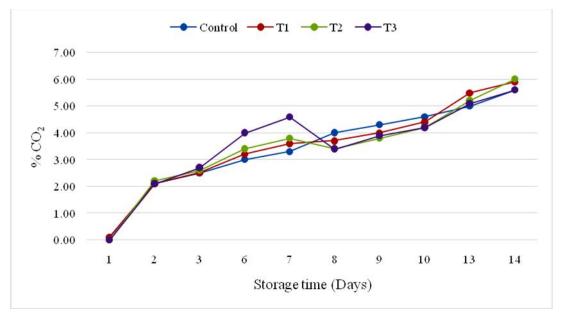
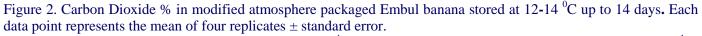


Figure 1.Oxygen % in modified atmosphere packaged Embul banana stored at 12-14 0 C up to 14 days. Each data point represents the mean of four replicates ± standard error.

C- control, T1- 1% alum, T2-1% alum+400 mg ml⁻¹C. *aromaticus* ethanolic leaf extract, T3- 400 mg ml⁻¹ C. *aromaticus* ethanolic leaf extract





C- control, T1- 1% alum, T2-1% alum+400 mg ml⁻¹C. *aromaticus* ethanolic leaf extract, T3- 400 mg ml⁻¹C. *aromaticus* ethanolic leaf extract

The banana samples packed in LDPE did not ripen during the storage period of 14 day. Storage of banana in polythene bags and the establishment of modified atmosphere conditions have been reported to extend the storage life of banana fruits as well as decline in respiration and delay in ethylene production (Sarananda et al., 1996). The conversion of 1aminocyclopropane-1-carboxylic acid (ACC) to ethylene is an oxygen dependent reaction by ACC oxidase during ethylene production process. Sarananda et al., (1996) reported that decline in ACC oxidase activity in modified atmosphere stored banana is the most important factor that lead to inhibition of ripening. This could lead to lengthening the storage life of Embul banana.

Pathogenicity of CR disease of *C. aromaticus* aqueous leaf extract treated Embul banana was lower at 7 day of storage, however, higher CR disease on evident on day 14. A significant reduction in CR was found in *C. aromaticus* 100% (v/v) aqueous leaf extract + 1% alum treated banana samples on both 7th and 14th day. Crown rot disease could be controlled to a higher extent when treated with *C. aromaticus* aqueous, hot water and ethanolic leaf extract in combination with 1% alum when compared to the single treatments without alum. Further, among all three extracts, ethanolic leaf extract was the most effective in controlling CR disease (Figure 3). Similar results were obtained during *in vitro* analysis of this study.

According to Dilhani et al., (2016) conidial germination of C. musae was 100% inhibited at the concentration of 600 (%w/v) of ethanolic extract and at the same concentration F. proliferatum was inhibited by 90.4 % in 96 well plate assay. Ethanolic leaf extract totally inhibited the mycelial growth of test pathogens at the concentration of 800 (% w/v)during liquid bioassay. Among three tested extracts, ethanolic extract was most effective in inhibiting both spore germination and mycelial growth of crown rot causing fungal pathogens. These results were in accordance with Wijesundara et al., (2016) who reported that ethanolic leaf extract of C. aromaticus significantly inhibited the mycelial growth and spore germination of C. musae isolated from a selected group of tropical fruits. Abeywickrama et al., (2009) reported that CR disease severity of 85-day mature and vacuum packed 1% alum (w/v) washed Embul banana was lower and comparable to carbendazim treatment. According to Siriwardana et al., (2016) alum + basil oil in combination with modified atmosphere packaging at 12-14 ^oC controlled CR disease of Cavendish banana significantly compared to alum alone and control. Abeywickrama et al., (2012) reported that, papaya washed in 1% (w/v) alum and subsequently sprayed with an emulsion solution of 0.16% (v/v) basil oil and enclosed in styrofoam sleeves could be stored for 14 days at 12-14 °C and fruits showed zero disease severity. These findings are in accordance with the current research data.

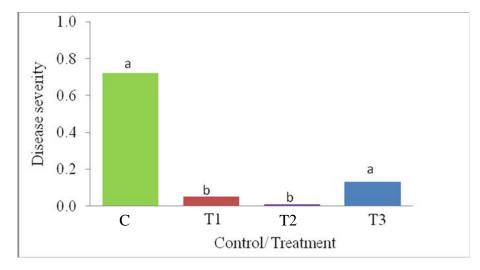


Figure 3. Effect of *C. aromaticus* ethanolic leaf extract alone and in combination with 1% alum with MAP on crown rot disease severity after 14 days of storage at 12-14 $^{\circ}$ C and induced ripening.

C- Control, T1- 1% alum, T2-400 mg ml⁻¹ C. *aromaticus* ethanolic leaf extract + 1% alum, T3- 400 mg ml⁻¹ C. *aromaticus* ethanolic leaf extract

(0=No rot, 1=25% Crown rot, 2=50% Crown rot, 3=75% Crown rot, 4=100% Crown rot, extended up to the finger stalk).

Each data value represents the mean of 4 replicates. Mean values sharing common letters are not significantly different by Kruscal-Wallis nonparametric test.

pH values of Embul banana treated with three different extracts indicated values in the same range (3.82-4.26). pH is dependent on the total quantity of acids as well as the strength of acid present. Malic, citric and oxalic acids are mainly responsible in providing an acidic taste to ripening banana (Hassan and Pantastico, 1990). According to Abeywicrama *et al.*, (2009), pH values of 85-day mature Embul banana treatments were not significantly different except in 1% alum wash treatment which had slightly low value (5.51±0.08).

In the current study, aqueous leaf extract treated banana showed TSS values in the range of 8.8-21.1 (⁰Brix), whereas hot water leaf extract treated samples,

indicated values of 17.7-19.7(⁰Brix). Further, ethanolic leaf extract treated samples showed TSS ranging from 14.9-22.0 from day 7 to day 14. Increment of TSS could be observed on day 14 as expected when compared to 7th day treatments. However, TSS values among treatments were not significantly different during tested storage periods. Abeywickrama et al., (2009) reported that, there was no significant difference among TSS contents of control and treatments except 1% alum + 0.16% basil oil treated 80-day mature Embul banana, which showed relatively low value. According to Anthony et al., (2003) Embul banana treated with Cymbopogon nardus, Cymbopogon flexuosus and Ocimum basilicum stored at cool or ambient temperature at 94% relative humidity and ripened induced had TSS values in the range of 21.0-22.0 (⁰Brix) (Table 1). Findings of previous research are in accordance with current research data, even though slight variations were observed.

Table 1. Physicochemical properties of Embul banana samples treated with <i>C. aromaticus</i> ethanolic leaf extract alone
and in combination with alum, alum alone and control (untreated banana).

Treatment	Day 7	Day 14			
	Firmness (kg cm ⁻²)				
С	0.33 ^a ±0.01	$\begin{array}{c} 0.32^{a}\pm 0.01\\ 0.32^{a}\pm 0.01\\ 0.34^{a}\pm 0.01\end{array}$			
T1	$0.35^{a}\pm0.02$				
T2	0.37 ^a ±0.01				
Т3	$0.36^{a}\pm0.03$	$0.32^{a}\pm0.01$			
	p	Н			
С	3.98 ^{a,b} ±0.01	4.01 ^{a,b} ±0.02			
T1	$3.93^{b} \pm 0.01$	4.00 ^{a,b} ±0.01			
T2	$3.93^{b}\pm0.03$	3.95 ^b ±0.01			
Т3	$4.02^{a}\pm0.00$	$4.04^{a}\pm0.02$			
	TSS (SS (⁰ Brix)			
С	$17.6^{a,b} \pm 0.70$	$\begin{array}{c} 19.2^{b}\pm 0.64\\ 22.0^{a}\pm 0.82\\ 22.0^{a}\pm 0.45\end{array}$			
T1	$21.2^{a}\pm 1.38$				
T2	$20.9^{a}\pm0.29$				
Т3	$14.9^{b} \pm 1.54$	19.4 ^b ±0.19			
	Titratable acidity (% Malic acid)				
С	$0.046^{a} \pm 0.001$	$0.044^{a}\pm0.002$			
T1	$0.049^{a}\pm0.002$	0.041 ^{a,b} ±0.003			
T2	$0.050^{a} \pm 0.001$	$0.047^{a} \pm 0.004$			
Т3	$0.049^{a} \pm 0.003$	$0.032^{b}\pm0.002$			

¹Each data point represents the mean of five replicates \pm standard error.

C - control, T1 - 1% alum, T2 - 1% alum + 400 mg ml⁻¹ *C.aromaticus* ethanolic leaf extract, T3 – 400 mg ml⁻¹*C. aromaticus* ethanolic leaf extract

When ripening proceeds, titratable acidity increases causing a drop in the pH. There was also an increase in titratable (malic acid) acidity in fruits of all treatments of aqueous leaf extract series on day 14 when compared to day 7. This is in accordance with (Siriwardana *et al.*, 2015) who reported titratable acidity increase causing a drop in the pH. In contrast, TA values decreased in hot water leaf extract and ethanolic leaf extract treated banana with storage time. TA values were in range of 0.025-0.045% aqueous leaf extract treated and ripening induced banana and the values were within the range of 0.032-0.05% for ethanolic leaf extract treated banana.

The Firmness of aqueous leaf extract treated samples ranged from 0.41 - 0.51 kgcm⁻² on day 7. A slight decrease of firmness could be observed in almost all treated samples of Embul banana during this research with storage time including the control. According to the work done by Siriwardana *et al.*, (2016) firmness of all Cavendish banana treated with basil oil (and control) stored at MAP ranged from 0.41-0.42 (Kgcm⁻²). According to the results obtained from hot water leaf extract and ethanolic leaf extract treatments, firmness values decreased gradually over time. This is due to the sequential degradation process of pectic, hemicellulossic polysaccharides and starch being converted to sugar during ripening of fruits.

When considering the sensory properties, sensory panelists preferred C. aromaticus 100% (v/v) aqueous leaf extract treated banana fruits when compared to the control on both 7th and 14th day. The Overall acceptability of C. aromaticus 100% (v/v) aqueous and hot water leaf extract sprayed samples were higher compared to the untreated banana. All sensory properties namely, peel colour, flesh colour, aroma, flavour, taste, texture obtained higher values for C. aromaticus 100% (v/v) hot water leaf extract treated banana on day 7. Further, sensory panel preferred C. aromaticus 400 mg ml⁻¹ ethanolic leaf extract treated banana on day 7. However, the highest overall acceptability of 9.1 was obtained for 1% alum + C. aromaticus 400 mg ml⁻¹ ethanolic leaf extract in combination with 1% alum treated banana on 14th day (Table 2). Similarly, Siriwardana et al., (2016) reported that sweeter taste in Cavendish banana samples treated with alum + basil oil were relatively more preferred by the sensory panelists than other treatments.

Table 2.Sensory properties of Embul banana samples treated with *C. aromaticus* ethanolic leaf extract alone and in combination with alum, alum alone and control (untreated banana).

day 7						day 14								
Treatment	Peel colour	Flesh colour	Flavour	Aroma	Taste	Texture	Overall acceptability	Peel colour	Flesh colour	Flavour	Aroma	Taste	Texture	Overall acceptability
T1	8.3 ^b	7.9 ^b	7.4 ^a	7.3 ^a	7.5 ^a	7.8 ^b	7.5 ^a	7.6 ^b	7.5 ^b	6.3 ^b	7.0 ^b	6.1 ^b	7.8 ^b	7.0^{b}
T2	7.5 ^a	8.2 ^b	7.6 ^a	7.7 ^b	7.9 ^b	7.9 ^a	7.5 ^a	9.1 ^b	8.3 ^b	7.8^{b}	7.4 ^a	8.0^{b}	7.4 ^a	9.1 ^b
T3	7.2 ^b	8.2 ^a	7.4 ^a	7.1^{a}	7.2^{a}	7.4 ^a	7.9 ^a	8.5 ^a	8.1 ^a	7.0^{a}	7.1 ^a	7.4 ^a	7.3 ^a	7.4 ^a
С	6.7 ^b	7.6 ^a	7.5 ^b	7.4 ^b	7.6 ^b	7.1 ^b	7.2 ^b	5.6 ^a	6.5 ^a	6.2 ^a	6.3 ^a	6.0^{a}	7.1 ^b	6.9 ^b

C - Control, T1 - 1% alum, T2 - 1% alum + 400 mg ml⁻¹C. *aromaticus* ethanolic leaf extract, T3- 400 mg ml⁻¹ C. *aromaticus* ethanolic leaf extract

¹Each data value represents the mean of ten replicates.

(9-10 – Excellent, 6-8 - Good, 4-5 – Fair, 1-3 - Poor)

Further, the *C. aromaticus* leaf extract had no adverse effect on peel colour of Embul banana. Peels showed the highest preferable peel colour after induced ripening. The coloration of the ripe edible flesh is a good indicator of carotenoid content (Englberger et al., 2006). According to Englberger et al. (2006) the major provitamin A carotenoids detected in all tested banana cultivars were *trans* -carotene and -carotene with cis -carotene at a much lower concentration. In present study, the flesh colour obtained higher score of above 6 for samples treated with aqueous, hot water and ethanolic leaf extracts indicating that the treated Embul banana had increased levels of carotenoids. Ripening may increase in simple sugars to provide sweetness, decrease in organic acids and phenolics to minimize the astringency and increase in volatiles to give the characteristic flavour of the fruits. The characteristic aroma of the banana fruit is due to the production of complex mixture of volatiles (Hassan and Pantastico, 1990).

According to the current study, it could be inferred that, aqueous leaf extract and hot water leaf extract of *C. aromaticus* have a potential in extending the storage life of Embul banana up to two weeks. Further, ethanolic leaf extract was the most effective extract in controlling CR disease. Combining ethanolic leaf extract with alum gave more promising outcome in CR disease control of Embul banana. Storage life of Embul banana can be extended for two weeks by treating with ecofriendly *C. aromaticus* 400 mg ml⁻¹ ethanolic leaf extract + 1% alum with improved sensory properties under modified atmosphere and cold storage.

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