Summary

Title: Investigation of biochemical and pathological changes following post harvest treatment of banana with essential oils

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Scientific Background and Scope/Objective of Project

Banana is an important starchy food consumed all over the world. In Sri Lanka banana are grown in large scale due to its nutritional value and export potential. About 20% of the banana is lost annually due to improper storage, post harvest disease, mechanical damages and unfavorable enzymatic reactions accompanied by mechanical damages.

Benlate (Benomyl) has been used on banana for effective control of post harvest fungal diseases. Processing of banana is being restricted due to the browning reactions. Polyphenol Oxidase is the enzyme responsible for the browning reaction.

Use of synthetic compounds as fungicides, antioxidants, enzyme inhibitors are fast becoming unpopular due to adverse health and environmental effects caused by these compounds. Hence information on safer alternatives using locally available resource such as essential oils would be very valuable.

The objectives of this study were to determine the effect of essential oils of cinnamon (*Cinnamomum zeylanicum* Blume) and clove [*Syzygium aromaticum* (L.) Merr et L.M.Perry]

in controlling post harvest fungal pathogens of banana and browning reactions of banana. Attempts were also made to develop a post harvest treatment system to prolong the storage life of banana in modified atmosphere by inhibiting the growth of post harvest pathogenic fungi.

Experimental methods

1. Fungal pathogens, which cause anthracnose and crown rot, were isolated from rotting 'embul' banana. Identification of isolated fungi was based on their reproductive and morphological characteristic with compared to the cited literature. International Mycological Institute (IMI) confirmed the identifications.
2. Semi synthetic (SMYK) liquid medium bioassay was carried out to determine the minimum inhibitory concentrations (MICs) and minimum lethal concentrations (MLCs) of cinnamon bark, cinnamon leaf and clove oils against *Fusarium proliferatum*, *Colletotrichum musae* and *Lasiodiplodia theobromae*.
3. The antifungal constituents of cinnamon bark, leaf and clove oils were identified using Thin Layer Chromatography (TLC) and Gas Chromatography (GC).
4. Banana were sprayed with different concentrations of cinnamon bark, cinnamon leaf and clove oils and stored in a modified atmosphere (MA) at 14 ^{0} C, 90% r.h. and ambient temperature (28 ± 2 ^{0} C) separately. Benomyl was sprayed as standard fungicide. After termination of storage periods banana were exposed to ethylene. Ripe banana were subjected to the analysis of pathological (anthracnose & crown rot), physico-chemical properties (weight loss, fruit firmness, pH, titratable acidity, total soluble solids,

carbohydrates, reducing sugars, total phenols & activity of polyphenol oxidase) and for evaluation of organoleptic properties (taste, odor, flavor & overall acceptability).

5. Polyphenol Oxidase (PPO) from banana pulp was isolated and the ID_{50} values for each essential oil were calculated.

Results and discussion

1. Three pathogens were identified as crown rot causing pathogens namely *Lasiodiplodia theobromae* (IMI # 384870), *Fusarium proliferatum* (IMI # 385824) and *Colletotrichum musae* while sole agent that causes anthracnose was identified as *Colletotrichum musae.*
2. The MICs and MLCs values are given in Table 1. The concentrations of the oils that required killing the fungi varied between 0.40 mg/ml – 1.17 mg/ml.
3. Major antifungal constituents of test oils, fungicidal on the growth of *Colletotrichum musae* and *Fusarium proliferatum* are shown in Table 2.
4. The storage life of banana was lengthened up to 14 days stored at ambient temperature, whereas banana stored at 14 ^{0} C was lengthened up to 21 days. The physico-chemical properties and the disease severity of anthracnose of the oil treated banana, stored at two different temperatures showed no significant difference compared to the control & benomyl. The sensory evaluation showed that there was no significant difference of the sensory properties of oil treated banana with compared to the control and benomyl treatment after 21 days at 14 ^{0}C. The sensory evaluation of banana after 14 days at ambient temperature and the diseases severity of crown rot are shown in Table 3 and 4. Cinnamon leaf and bark oil treated banana showed significantly higher taste and overall acceptability than that of the control & benomyl treatment. The disease severity of crown

rot was significantly low in cinnamon bark and leaf oil treated banana in comparison to the control at both temperatures.

5. The inhibitory effects of essential oils on the activity of banana PPO are shown in Figure 1. The ID_{50} values were 0.085 mg/ml (cinnamon leaf), 0.115 mg/ml (cinnamon bark) and 0.078 mg/ml (clove) (Figure 2).

Table 1. The Minimum Inhibitory Concentration & Minimum Lethal Concentration of test essential oils against anthracnose and crown rot pathogens.

Test oil	Test organism	MIC [a] / (mg/ml)	MLC [b] / (mg/ml)
Clove oil	*C. musae*	0.41	0.67
	L. theobromae	0.46	0.62
	F. proliferatum	0.51	1.02
Cinnamon bark oil	*C. musae*	0.30	0.40
	L. theobromae	0.35	0.45
	proliferatum	0.50	0.80
Cinnamon leaf oil	*C. musae*	0.53	0.75
	L. theobromae	0.64	0.85
	F. proliferatum	0.53	1.17
Benomyl	*C. musae*	0.004	0.005
	L. theobromae	0.005	0.006
	F. proliferatum	0.006	0.007

[a, b] Each data point represents the mean of 6 replicates

Table 2. Major antifungal constituents of test oils, fungicidal on the growth of *Colletotrichum musae* (1) and *Fusarium proliferatum* (2).

Antifungal constituents	*Cinnamomum zeylanicum* (leaf)		*Cinnamomum zeylanicum* (bark)		*Syzygium aromaticum*	
	(1)	(2)	(1)	(2)	(1)	(2)
Eugenol	+	+	+	+	+	+
Eugenyl acetate	*	*	*	*	+	+
Cinnamaldehyde	+	+	+	+	-	-
α - terpineole	+	-	-	-	-	-
Camphene	+	-	-	+	*	*
1,8 cineole	-	-	+	+	*	*
Cinnamyl acetate	-	-	+	+	*	*
β - caryophyllene	+	-	+	-	-	-
Terpinene - 4 – ol	-	-	+	-	*	*
Cinnamyl alcohol	-	-	+	-	*	*
Acetyl eugenol	+	+	*	*	*	*
Myrcene	-	-	+	-	*	*
α-Humulene	-	+	-	+	-	-

+ antifungal, - not antifungal, * not available

Table 3. Sensory evaluation of ripe embul banana after 14 days of treatments with different essential oils stored in MA at (28 ± 2 ^{0} C)

Treatment.	Concentration	Observations / %			
	(mg/ml)	Flavor	Taste	Odor	O.A.
Clove	1.03	86.8 ± 0.9 ^{abc}	87.8 ± 0.9 ^{abc}	87.5 ± 1.1	87.2 ± 1.0 ^{abc}
	2.06	88.2 ± 0.9 ^{ac}	90.0 ± 1.2 ^{ac}	87.7 ± 2.2	90.3 ± 1.0 ^{ac}
Cinnamon bark	0.80	87.8 ± 1.0 ^{ac}	88.5 ± 1.0 ^{abc}	87.0 ± 1.4	88.7 ± 1.2 ^{abc}
	1.60	89.3 ± 0.8 ^{a}	92.3 ± 1.6 ^{a}	87.8 ± 2.3	91.8 ± 1.1 ^{a}
Cinnamon leaf	1.17	85.2 ± 0.8 ^{abc}	87.5 ± 1.0 ^{abc}	85.2 ± 1.3	87.7 ± 0.9 ^{abc}
	2.34	86.3 ± 1.1 ^{abc}	90.8 ± 0.6 ^{a}	86.3 ± 1.9	90.8 ± 0.5 ^{a}
Benomyl	0.007	84.3 ± 0.8 ^{bc}	85.8 ± 1.1 ^{bc}	84.5 ± 0.7	86.2 ± 0.9 ^{bc}
Control	-	85.7 ± 1.4 ^{c}	87.3 ± 1.1 ^{c}	89.0 ± 0.7	87.2 ± 1.1 ^{c}
Significance ^{C}		**	**	NS	**

Each data point represents the mean of six replicates ± S.E. ^{a,b,c} Different letters in a same column denote significant difference ($p \leq 0.05$) by Mann-Whitney U Test. ^{C} **, mean values significant at the 5% level; NS, not significant. O.A; overall acceptability.

Table 4. The development of disease severity of crown rot after treatments with different essential oils stored in MA at two different temperatures

Treatment.	Concentration (mg/ml)	Crown rot	
		At (28 ± 2 ^{0}C) (after 14 days)	At 14 ^{0} C (after 21 days)
Clove	1.03	0.85 ± 0.04 ^{ac}	0.92 ± 0.03 ^{ac}
	2.06	0.76 ± 0.05 ^{ac}	0.89 ± 0.02 ^{ac}
Cinnamon bark	0.80	0.80 ± 0.03 ^{a}	0.87 ± 0.04 ^{ac}
	1.60	0.74 ± 0.04 ^{a}	0.83 ± 0.03 ^{a}
Cinnamon leaf	1.17	0.87 ± 0.03 ^{ac}	0.87 ± 0.04 ^{ac}
	2.34	0.76 ± 0.04 ^{a}	0.81 ± 0.02 ^{a}
Benomyl	0.007	0.16 ± 0.06 ^{b}	0.16 ± 0.05 ^{b}
Control	-	0.96 ± 0.06 ^{c}	0.99 ± 0.04 ^{c}
Significance ^{C}		**	**

Each data point represents the mean of five replicates ± S.E. ^{a,b,c} Different letters in a same column denote significant difference ($p \leq 0.05$) by Mann-Whitney U Test. ^{C} **, mean values significant at the 5% level.

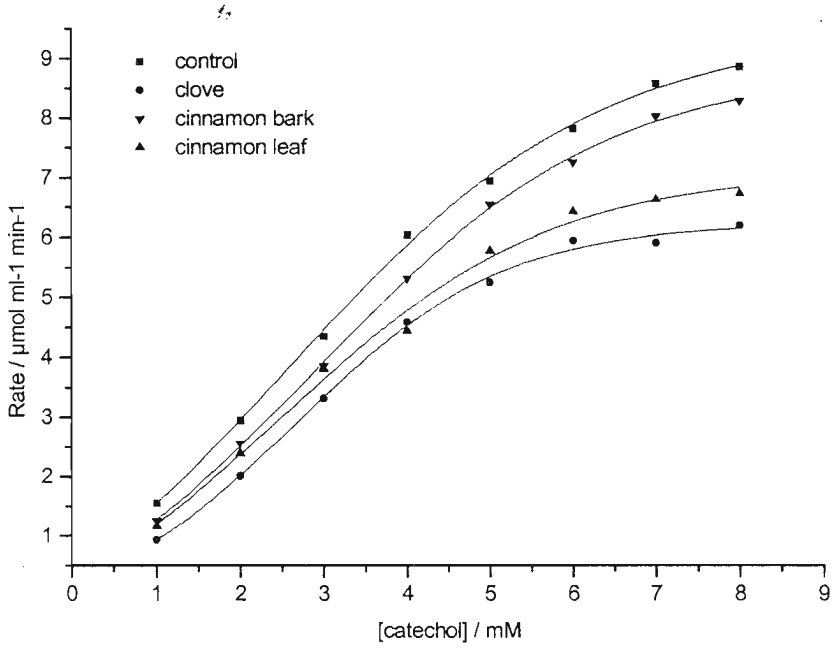

Figure 1. The inhibitory effect of cinnamon bark, leaf and clove oils on banana PPO activity.

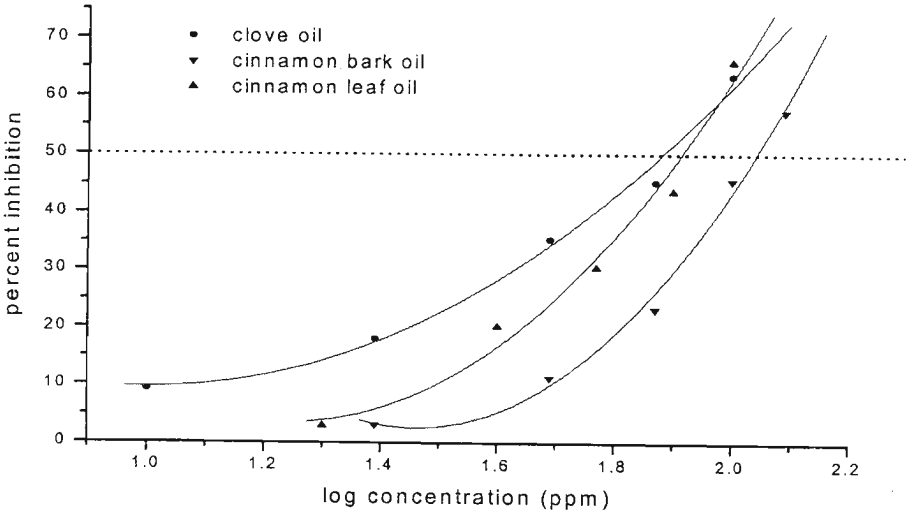

Figure 2. The relationship between log concentration and percent inhibition on banana PPO react of cinnamon bark, leaf and clove oils.

Conclusions

Since the growth of fungal pathogens, which causes anthracnose and crown rot were inhibited by the essential oils of cinnamon leaf & bark, these oils could be developed as naturally occurring post harvest treatment in modified atmosphere storage at ambient temperature (28 ± 2 ^{0}C) & 14 ^{0}C; 90% r.h. to lengthen the storage life of "embul" banana upto 14 and 21 days respectively.

As activity of banana polyphenol oxidase was inhibited by the essential oils of cinnamon leaf, bark and clove oils, these oils could be used as safer alternatives for the antibrowning agents.

Papers published on work done under the contract

Fungicidal Activity of Essential Oils of *Cinnamomum zeylanicum* (L.) and *Syzygium aromaticum* (L.) Merr et L.M. Perry against crown rot and anthracnose pathogens isolated from banana. *Letters in Applied Microbiology*. 2002 *35*: 208-211

"Effect of essential oils from *Cinnamomum zeylanicum* Blume and *Syzygium aromaticum* (L) Meer et L.M. Perry on storage life of banana and identification of antifungal constituents in these essential oils." Proceeding of the 58th Annual Session 2002, Sri Lanka Association for the Advancement of Science.

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