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Article *in* International Journal of Food Science & Technology · April 2010

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Original article

Evaluation of plant oils for suppression of crown rot disease and improvement of shelf life of banana (*Musa* spp. AAA subgroup, cv. Robusta)

Ganesan Sangeetha,[1]* Raman Thangavelu[2] & Swaminathan Usha Rani[1]

1 Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Chidambaram 608002, Tamil Nadu, India
2 National Research Centre for Banana, Thiruchirapalli 620 102, Tamil Nadu, India

(Received 5 January 2010; Accepted in revised form 11 February 2010)

Summary Fourteen plant oils were evaluated to control the crown rot disease caused by *Lasiodiplodia theobromae* and *Colletotrichum musae*. Five of these, viz. *Ocimum sanctum*, *Cymbopogan citratus*, *C. martinii*, *C. nardus* and *Pelargonium graveolens* oils completely arrested the mycelial growth of both test pathogens at their lowest concentration compared to other oils. Besides, these plant oils have also inhibited the activity of cellulolytic and pectinolytic enzymes produced by these pathogens effectively under *in vitro* condition. The treatment of banana fruit var. Robusta (Cavendish-AAA) with oils of *O. sanctum*, *C. citratus*, *C. nardus* and *C. martinii* not only reduced the crown rot severity significantly, but also increased the shelf life of banana fruits. However, under low-temperature storage (14 °C) condition, *O. sanctum* oil increased the shelf life of banana fruits up to 48 days without affecting their organoleptic properties. Hence, *O. sanctum* oil could be used as an alternative to chemical fungicides for the management of crown rot disease.

Keywords Banana, crown rot, *O. sanctum* oil, plant oils, total shelf life.

Introduction

Banana (*Musa acuminate* L.) fruit is highly perishable and its storage life is often affected by number of post-harvest diseases. Among these, crown rot is the most serious post-harvest disease in commercial bananas worldwide, as the disease shortens the storage life and spoils the appearance of fruit (Ranasinghe *et al.*, 2003). Although the disease is reported to be caused by different pathogenic fungi such as *Colletotrichum musae*, *Lasiodiplodia theobromae*, *Fusarium proliferatum* and *Verticillium theobromae* (Finlay & Brown, 1993; Ploetz *et al.*, 1994), the fungi *L. theobromae* and *C. musae*, have been reported to be the major pathogens associated with the cause of the crown rot disease in majority of banana producing countries (Anthony *et al.*, 2003; Haque *et al.*, 2003). Our earlier study also showed that in India too, *L. theobromae* and *C. musae* are the major pathogens causing this disease (Thangavelu *et al.*, 2007). However, the research on the management of crown rot disease caused by these complex pathogens (*L. theobromae* and *C. musae*) is meagre. Moreover, crown rot disease is often controlled commercially by a post-harvest treatment, which involves submerging clusters of banana in solutions of thiobendazole (TBZ) or imazalil or benomyl fungicides (Krauss *et al.*, 1998). Due to this intensive practice, the disease could not be controlled effectively as the pathogens have developed resistance/tolerance to these fungicides (Johanson & Blazquez, 1992). In recent times, an increasing number of countries are also demanding fresh products not treated with agrochemicals, particularly those applied after harvest (Ranasinghe *et al.*, 2003). Under these circumstances, an alternative method of controlling crown rot disease without the use of chemical is urgently required.

Among the safer alternatives to synthetics, use of plant oils has attracted researchers for the management of several diseases of fruits (Smid *et al.*, 1994; Dixit *et al.*, 1995). The essential oils are complex volatile compounds, and their constituents have often been used as biological agents for their therapeutic activity and toxicity against insects and plant pathogenic fungi (Delespaul *et al.*, 2000). The presence of oils in plants belonging to the genera *Ocimum*, *Thymus*, *Origanum*, *Anethum*, *Eucalyptus*, *Foeniculum* and *Citrus* are well known. Essential oils of cinnamon and clove such as cinnamaldehyde and eugenol have been tested on fresh fruits of mandarin, kiwi and rambutan to control post-harvest diseases (Thanassoulopoulos & Yanna, 1997; Sivakumar *et al.*, 2002). In the case of banana, Ranasinghe *et al.* (2003)

*Correspondent: E-mail: sangeethaau@hotmail.com

doi:10.1111/j.1365-2621.2010.02232.x

reported that spraying Embul banana (*Musa accuminata* – AAB) with an emulsion of oil extract of cinnamon bark, prior to storage, controlled crown rot and increased shelf life up to 14 days at ambient temperature (28 ± 2 °C) and 21 days at modified atmospheric conditions (14 °C with 90% RH). However, oils, which can extend the shelf life up to 21 days, may not be useful for the countries (for example India) which require storage life of more than 21 days for the export trade. Moreover, reports on the effect of oils for the effective management of crown rot disease caused by complex of *L. theobromae* and *C. musae* pathogens particularly in Cavendish banana, which is the predominant commercial variety worldwide, are also lacking. Therefore, in this study, we made an attempt to bridge these research gaps, by formulating the objectives namely viz. (i) screening and identification of potential plant oils for the suppression of crown rot disease as well as to increase the shelf life of banana fruits, (ii) studying the inhibitory effect of oils on the activity of cell wall degrading enzymes produced by potential crown rot pathogens and (iii) effect of these oils on physico-chemical and organoleptic properties of banana fruits.

Materials and methods

Crown rot infected banana hands were collected from the market place in Thiruchirapalli, Tamil Nadu, India and brought to the laboratory and the fungi were isolated from infected fruits. These fungi were identified as *Lasiodiplodia theobromae*, *Colletotrichum musae*, *Fusarium* spp. and *Deightoniella torulosa* on the basis of colony characters on media, mycelial characters, types of conidiophore and conidial characters as per the descriptions by Goos *et al.* (1961); Punithalingam (1976) and Sutton & Waterson (1970). As the majority of the isolations (more than 80%) yielded *L. theobromae* and *C. musae* (data not shown), further studies on *in vitro* and *in vivo* evaluation of potential plant oils were carried out using these two pathogens. Hence, pathogenicity was proved for both *L. theobromae* and *C. musae* isolates in cv. Robusta.

Collection of plant oils

The four plant oils viz., *Ricinus communis, Sesamum indicum, Azadiracta indica* and *Pongamia glabra* were purchased from M/S Ayurvedic Research Laboratory, Sirkazhi, India. Remaining ten oils were purchased from the manufacturer M/S S.R. Biotech Pvt. Ltd, Attur, Tamil Nadu, India and confirmed that these oils were extracted by hydro distillation process (Table 1).

Determination of minimum inhibitory concentration of plant oils

Fourteen plant oils were screened for fungal growth inhibition in the concentration ranged from 0.02 to 0.1% (v/v) against *L. theobromae* and *C. musae* by following the poisoned food technique in liquid bioassay (Baratta *et al.*, 1998). Tween 80 at 0.01% was used as a

Table 1 The minimum inhibitory concentrations and minimum lethal concentrations of different plant oils against crown rot pathogens *L. theobromae* and *C. musae*

Name of the plant oil with its scientific name and family	*Minimum inhibitory concentration† % (v/v)		*Minimum lethal concentration‡ % (v/v)	
	L. theobromae	*C. musae*	*L. theobromae*	*C. musae*
Castor oil (*Ricinus communis* L. – Euphorbiaceae)	NI	NI	NI	NI
Citrodara oil *(Monarda citrodara* Cew. Ex Lag.)	0.6	1.0	1.0	1.0
Citronella oil (*Cymbopogan nardus* Rendl. – Graminae)	0.08	0.08	0.1	0.1
Curry leaf oil (*Murraya koenigii* Spr . – Rutaceae)	1.6	0.6	2	0.8
Eucalyptus oil (*Eucalyptus globulus* Labill. – Myrtaceae)	0.6	0.6	1.0	1.0
Geranium oil (*Pelargonium graveolens* L. Hert. – Geraniaceae)	0.06	0.06	0.8	0.6
Gingelly oil (*Sesamum indicum* L. – Pedaliaceae)	NI	NI	NI	NI
Illupai oil (*Madhuca longifolia* (L) Mac Bride. – Sapotaceae)	NI	NI	NI	NI
Lemongrass oil (*Cymbopogan citratus* Stapf. – Graminae)	0.04	0.04	0.08	0.06
Neem oil (*Azadiracta indica* A. Juss – Meliaceae)	1.6	1.6	2.0	1.6
Palmarosa oil – (*Cymbopogon martinii* (Roxb.) Wats – Graminae)	0.04	0.04	0.06	0.06
Pungam oil – (*Pongamia glabra* Vent. – Papilionaceae)	NI	NI	NI	NI
Tulsi oil (*Ocimum sanctum* L. – Labiatae)	0.04	0.04	0.08	0.06
Turmeric leaf oil (*Curcuma longa* L. – Zingiberaceae)	1.6	1.6	2.0	2.0
Benomyl 0.1%	0.08	0.08	0.08	0.1

*Mean of four replications.
†Zero mycelial growth at minimum inhibitory concentration (MIC).
‡Zero revival of fungi at minimum lethal concentration (MLC).
NI, no inhibition.

surfactant to disperse the oil. The % inhibition was determined as mentioned below as described by Baratta *et al.* (1998):

$$\%\text{Inhibition (minimum inhibitory concentration, MIC)} = \frac{(C_m - I_m) - (T_m - I_m)}{(C_m - I_m)} \times 100$$

where, T_m is the mean weight of mycelium in the treatments, C_m is mean weight of mycelium in control (without oils, but added with Tween-80 at 0.01% conc.) and I_m is mean weight of initial inoculum.

Determination of minimum lethal concentration of plant oils

Fungal discs, which did not show any growth in liquid bioassay, were transferred on to fresh potato dextrose agar (PDA) plates in order to test the survival of the fungus. After 1 week, fungal discs indicating no growth were flooded with a freshly prepared solution of 2,3,5 triphenyl tetrazolium chloride (1.0%) for 30 min to confirm the death of fungal cells and MLC required (to kill the test fungus completely) for each oil was calculated.

Influence of plant oils on the *in vitro* production of cell wall degrading enzymes of crown rot pathogens

Among fourteen plant oils, only seven oils *viz*., *C. martinii, C. nardus, O. sanctum*, *M. citrodara*, *P. graveolens, C. citratus* and *E. globulus* which showed effectiveness in its least concentration were only taken for this study. The other oils were not taken for further study since they needed higher concentration of more than 1.6% (v/v) to control both test pathogens.

Preparation of enzyme source

To prepare the enzyme source from *L. theobromae* and *C. musae*, flasks (250 mL) containing 100 mL of Czapek's dox broth devoid of sucrose but supplemented with 0.3% carboxy methyl cellulose (CMC) for cellulolytic enzymes and 3% pectin for pectinolytic enzymes were prepared and autoclaved. To these broths, plant oils at respective MIC against the test pathogens were added separately, so as to get the required final concentration. The flasks were inoculated with 8 mm culture discs of *L. theobromae and C. musae* separately and incubated at 28 ± 2 °C. Necessary controls were also maintained. Two weeks after incubation, the mycelial mat was harvested for both test pathogens by filtering through two layers of sterile cheesecloth. The filtrates collected were centrifuged at 3000 rpm for 30 min to remove the spores and the supernatant was used as enzyme source and used for further estimation.

Assay of cellulase activity

Cellulase activity was measured spectrophotometrically as described by Sadasivam & Manickam (2004) using dinitrosalicylic acid (DNS) reagent (Sigma, St Louis, MO, USA). The absorbance was measured at 540 nm, and the enzyme activity was expressed as mg glucose released min^{-1} mg^{-1} protein.

Assay of pectic enzymes

The activity of endo-polygalacturonase (Endo-PG) (Hancock *et al*., 1964), polygalacturonate *trans*-eliminase (PGTE) (Ayers *et al*., 1966) and pectin trans-eliminase (PTE) (Sadasivam & Manickam, 2004) was estimated by percent loss in viscosity by viscometric method. Viscosity measurements were made using an Ostwald-Fenske viscometer. The percent viscosity reduction was calculated as per the formula, $V = \{T_0 - T/T_0 - T_w\} \times 100$, where V is % loss in viscosity, T_0 is flow time in seconds at zero time, T is flow time of reaction mixture at time T, and T_w is flow time of distilled water. The activity of the enzyme was expressed as percent reduction in viscosity.

Preparation of the oil emulsion

The earlier study conducted by Anthony *et al.* (2003) indicated the concentration twice that of MLC would be most effective without affecting the organoleptic and physico-chemical properties of the fruit, in controlling the disease incited by complex pathogen. This was again confirmed in our preliminary tests (data not shown). Therefore, the MLC of each test oil (*C. martini, C. nardus, O. sanctum*, *Monarda citrodara*, *Pelargonium graveolens, C. citratus* and *Eucalyptus globulus*) was doubled and added accordingly into 100 mL of distilled water separately along with 0.01% Tween 80 to disperse the oil into water. The mixture was transferred to a hand-sprayer and mixed well by shaking. A control containing water with Tween 80 at 0.01% was also included. Although the oils such as *M. koenigii*, *C. longa* and *A. indica* were shown effective against both crown rot pathogens, these oils have affected the quality of fruit; hence these oils were not included for further study.

In vivo evaluation of plant oils on the crown rot severity of banana

Matured (90%) healthy banana bunches (collective name for the hands of fruit) var. Robusta with 12–15 uniform-sized fingers (individual fruit arise from each flower is called finger; cluster of tightly packed fruits are called hand) which were free from pesticide treatment were selected from a local farmer's field. The fruits were

then dehanded along with the crown portion from the bunch, washed in running water to remove any dust and allowed to air dry for 2 h. On the crown portion of the hand, a small cavity was made and 100 μL of spore suspension prepared from 10 days old cultures of *L. theobromae* and *C. musae* (10^6 spores/mL) were placed in this cavity. The individual oil emulsion of *C. martinii, C. nardus, O. sanctum, M. citrodara, P. graveolens, C. citratus* and *E. globulus*, benomyl (0.1% w/v) and Tween 80 (0.01% v/v as control) were then sprayed with a hand sprayer on to the cut surface of crowns and also onto the fingers separately and allowed to air dry for 12 h. Each polythene bag contained one hand, loosely packed and the mouth of the bag was tied to avoid cross contamination.

One 'set' of treatments was incubated at room temperature (28 ± 2 °C) and disease assessment was made on the twelfth day after treatment. Another 'set' of identical treatments was incubated under cold storage (14 °C, 90% RH) and disease assessment was made on twenty-fourth day after treatment. Each treatment was replicated four times. The banana hands were then assessed for disease severity at regular intervals based on crown rot severity (0–5 scale), crown colour (1–7 scale) and crown texture (0–4 scale) (Finlay & Brown, 1993). The shelf life (green and yellow life) periods were also examined and recorded.

Evaluation of plant oils for the organoleptic and physico-chemical property of banana fruits

To assess the quality of the fruits (peel colour, flavour, texture and overall acceptability) due to treatment with oils, the fruits were treated with oils, loosely packed in polythene bag (one hand/bag), and stored at 14 °C for 1 month. Then, the polythene bags were removed and kept at room temperature (28 ± 2 °C) for a week for natural ripening. All the quality parameters were assessed by an expert panel of ten people. Assessment of texture, flavour and overall acceptability was done by following the scorecard developed by Hewage (1996) for each parameters as excellent (scale 9–10), good (scale 6–8), fair (scale 4–5) and poor (scale1–3). The assessment of peel colour was done by standard colour index (SCI) as described by Anthony *et al.* (2003). The effect of oils on the total soluble solids in the pulp of banana fruits was assessed by hand held refractometer (Brix; 0–32%) (Somogyi, 1952).

Experimental design and data analysis

Four replicates (per treatment) for each storage regime were maintained in a completely randomised block design. All the data in the present study were subjected to an analysis of variance (ANOVA) and means were separated by Duncan's multiple range tests (DMRT).

Results

In vitro effect of plant oils against *L. theobromae* and *C. musae*

The *in vitro* evaluation of 14 plant oils showed the fungicidal and fungistatic properties against both the crown rot pathogens within the range of 0.04–2%. However, among all oils, *O. sanctum, C. citratus* and *C. martinii* were fungistatic at 0.04% (v/v) and fungicidal at 0.06–0.08% (v/v) against both *L. theobromae* and *C. musae* pathogens. The *P. graveolens* and *C. nardus* oils were fungistatic at 0.06 and 0.08% respectively. *A. indica, M. koenigii* and *C. longa* oils were also fungistatic and fungicidal to both the test pathogens but at their higher concentrations (0.6 to 2.0%). The oils of *R. communis, P. glabra, S. indicum* and *M. longifolia* did not exhibit any inhibitory effect to both the test pathogens (Table 1).

Effect of oils on the *in vitro* activity of cell wall degrading enzymes of crown rot fungi

Generally, incorporation of plant oils into medium significantly reduced all lytic enzymes of both pathogens (Tables 2 and 3). However, among the oils, *C. martinii* and *C. citratus* recorded maximum reduction of *L. theobromae* cellulase activity (84.61%) as compared to benomyl and other oils tested (Table 2). Although there was significant reduction in PG enzyme activity by *C. martinii, C. nardus, O. sanctum* and *C. citratus* oils, their effect was not significantly different from the chemical benomyl (0.1%). In case of PTE activity, maximum reduction of PTE activity (79.96%) was observed with *O. sanctum* oil and benomyl followed by *C. martinii* and *C. citratus* oils. With regard to PGTE enzyme activity, *C. nardus* oil recorded maximum reduction of 88.40% followed by *C. martinii* (88.21%), *O. sanctum* (88.01%) and benomyl (87.72%).

Of the plant oils tested on cell wall degrading enzymes of *C. musae*, maximum reduction of cellulase activity was observed by addition of oils such as *C. martinii* (79.31%), *C. citratus* (75.86%) and *O. sanctum* (72.41%) and also by benomyl (0.1%) (72.41%) and their effect was on par with each other (Table 3). In case of other enzymes viz., PG, PTE and PGTE maximum reduction in activity was recorded due to *C. martinii, O. sanctum, C. citratus* oils and with benomyl (0.1%), which were also on par in their activity.

In vivo evaluation of plant oils on the crown rot severity of banana

Among the seven oils tested for crown rot severity under high temperature storage conditions (28 ± 2 °C), *O. sanctum, C. citratus* and *C. martinii* oils were significantly effective compared to other oils and also

Table 2 Influence of plant oils on the cell wall degrading enzymes activity of crown rot pathogen *L. theobromae* under *in vitro* condition

Plant oils	MIC of oils (%)	Cellulase activity*	Per cent reduction over control	Percent viscosity reduction of substrate†		
				PG	PTE	PGTE
C. martinii	0.04	0.04ab	84.61	8.37a (87.51)	14.13b (78.41)	8.13a (88.21)
C. nardus	0.08	0.06c	76.92	8.77a (86.91)	14.18b (78.34)	8.00a (88.40)
O. sanctum	0.04	0.05bc	80.76	8.10a (87.91)	13.12a (79.96)	8.27abc (88.01)
M. citrodara	0.6	0.15e	42.30	18.33c (72.65)	20.07d (69.34)	16.23d (76.47)
P. graveolens	0.06	0.12d	53.84	15.16b (77.38)	22.40e (65.78)	17.20e (75.07)
C. citratus	0.04	0.04ab	84.61	8.28a (87.64)	14.97c (77.13)	8.53bc (87.63)
E. globulus	0.6	0.18f	30.76	19.50c (70.90)	27.28f (58.33)	20.43f (70.39)
Benomyl	0.08	0.05bc	80.76	8.32a (87.58)	13.33a (79.63)	8.47bc (87.72)
Control	-	0.26g	–	67.03d	65.47g	69.00g

*mg sugars released mg^{-1} enzyme protein min^{-1}.
†Mean of four replicates.
Readings were taken at zeroth and sixtieth minute after adding test compound. Data in parentheses are arcsine transformed values. In a column means followed by the same letter do not differ significantly by Duncan's multiple range test ($P \leq 0.05$).

Table 3 Influence of plant oils on the cell wall degrading enzymes activity of crown rot pathogen *C. musae* under *in vitro* condition

Plant oils	MIC of oils (%)	Cellulase activity*	Per cent reduction over control	Percent viscosity reduction of substrate†		
				PG	PTE	PGTE
C. martinii	0.04	0.06a	79.31	7.72a (89.06)	8.80a (87.91)	12.23a (81.02)
C. nardus	0.08	0.09b	68.96	10.63b (84.94)	12.67b (82.60)	16.73b (74.04)
O. sanctum	0.04	0.08a	72.41	7.47a (89.41)	8.63a (88.15)	12.27a (80.96)
M. citrodara	1.0	0.18e	37.93	14.57d (79.36)	13.43b (81.56)	18.21d (71.75)
P. graveolens	0.06	0.14c	51.72	13.23c (81.26)	14.96c (79.46)	17.50c (72.85)
C. citratus	0.04	0.07a	75.86	7.67a (89.13)	8.48a (88.35)	12.37a (80.81)
E. globulus	0.6	0.16d	44.82	15.55e (77.97)	18.30d (74.87)	19.73e (69.39)
Benomyl	0.08	0.08a	72.41	7.62a (89.20)	8.03a (88.97)	12.27a (80.96)
Control	–	0.29f	–	70.60f	72.84e	68.47f

*mg sugars released mg^{-1} enzyme protein min^{-1}
†Mean of four replicates.
Readings were taken at zeroth and sixtieth minute after adding test compound. Data in parentheses are arcsine transformed values. In a column means followed by the same letter do not differ significantly by Duncan's multiple range test ($P \leq 0.05$).

comparable with the effect of fungicide benomyl (0.1%) (Table 4). A similar effect was also reflected in the texture of crown, as the crown tissue was as hard as that of normal hand as compared to black, decayed fibrous rotten tissue in the pathogen alone inoculated hands. With regard to colour of the crown, it was yellow in *O. sanctum*, *C. martinii* and *C. citratus* oils treated hands compared to complete blackening in pathogen alone inoculated hands (Table 4). In addition, these oils also increased the total shelf life period of banana significantly (8 days more) as compared to pathogen alone inoculated fruits and the effect was on par with that of fungicide benomyl (0.1%). Among the oils, *E. globulus* and *M. citrodara* oils were found to be least effective in controlling the crown rot severity.

In the case of oils tested under cold storage condition (14 °C), the oils viz., *O. sanctum*, *C. martinii*, *C. citratus* and *C. nardus* significantly reduced the crown rot severity (score 1 to 1.3) as against the rot score of 4.5 in pathogens alone inoculated fruits and their effect was not significantly different with each other. The crown portion of banana fruits treated with these oils was hard with $<25\%$ decay (Table 5). With regard to shelf life period, the treatment with *O. sanctum* oil recorded the maximum of 48 days, which was similar to the effect of fungicide benomyl (0.1%). However, oils of *C. martinii*, *C. nardus* and *C. citratus* also increased the shelf period of banana fruits to 45 days as against 22 days in pathogen alone inoculated hands, under cold storage (14 °C) conditions.

Effect of plant oils on organoleptic and physico-chemical properties of banana

The fruits in *O. sanctum* and *C. martinii* treatment were still in colour index stage 1 (still green) after one month

Table 4 *In vivo* evaluations of certain plant oils for the suppression of crown rot disease and on the total shelf life of banana fruits in cv. Robusta under room storage (28 ± 2 °C)

Plant oils	Concentration (%)	Severity score*			Total shelf life (days)
		Rot (0–5)	Colour (1–7)	Texture (0–4)	
C. martini	0.12	1.5 ab	4.5 ab	1.3 ab	17.0a
C. nardus	0.20	1.8 bc	5.0 bc	1.5 b	17.3 a
O. sanctum	0.12	1.3 ab	4.3 a	1.0 a	17.3 a
M. citrodara	2.00	3.3 d	6.0 de	2.5 d	14.3 b
P. graveolens	0.12	2.3 c	6.3 ef	2.5 d	13.8 b
C. citratus	0.12	1.3 ab	4.5 ab	1.3 ab	17.8 a
E. globulus	0.20	3.3 d	6.5 ef	3.5 e	13.5 b
Benomyl	0.10	1.0 a	4.5 ab	1.0 a	17.3 a
L. theobromae + *C. musae*	–	4.5 e	6.8 f	4.0 f	9.3 c
Control (Tween 80 alone)	–	1.3a	5.5 cd	2.0 c	14.3 b

*Mean of four replicates. Severity score was taken after 12 days of treatment.
In a column means followed by the same letter do not differ significantly by Duncan's multiple range test ($P \leq 0.05$).

Table 5 *In vivo* evaluations of certain plant oils for the suppression of crown rot disease and on the total shelf life of banana fruits in cv. Robusta under cold storage (14 °C)

Plant oils	Concentration (%)	Severity score*			Total shelf life (days)
		Rot (0–5)	Colour (1–7)	Texture (0–4)	
C. martinii	0.12	1.3 ab	4.5 a	1.0a	45.3 b
C. nardus	0.20	1.3 ab	4.8 a	1.3 a	45.0 b
O. sanctum	0.12	1.0 a	4.5 a	1.0 a	48.3 a
M. citrodara	2.00	3.0 cd	6.0 b	3.5 d	32.5 e
P. graveolens	0.12	2.5 c	6.3 b	2.8 c	35.3 d
C. citratus	0.12	1.0 a	4.8 a	1.0 a	45.5 b
E. globulus	0.20	3.5 d	6.0 b	3.5 d	27.8 f
Benomyl	0.10	1.5 ab	4.3 a	1.3 a	48.3 a
L. theobromae + *C. musae*	–	4.5 e	6.8 b	3.8 d	22.3 g
Control (Tween 80 alone)	–	1.8 b	4.5 a	2.0 b	39.3 c

*Mean of four replicates. Severity score was taken after 24 days of treatment.
In a column means followed by the same letter do not differ significantly by Duncan's multiple range test ($P \leq 0.05$).

of cold storage. However, significant differences in peel colour was noticed (colour index stage 2 – colour break) in banana fruits treated with *C. nardus* and *C. citratus* oils. Similarly, in pathogen-inoculated control, the fruits were in colour index stage 2 (colour break) and this might occurred due to induced ripening by the crown rot pathogens. The overall acceptability was significantly higher ($P \leq 0.05$) in fruits treated with *C. martinii, C. nardus* and *O. sanctum* oils and fungicide benomyl. However, treatment with *C. citratus* oil resulted in significant difference in quality of fruits which affected peel colour, flavour, texture and thus the overall acceptability (Fig. 1).

The total soluble solids content has increased as ripening progressed. The total soluble solids (TSS) values of all treatments were in the range of 4.7–5.4 (^{0}Brix) after cold storage and before subjected to natural ripening. The total soluble solids contents were significantly lower in all treated fruit after cool stored and subsequently subjected to natural ripening. The TSS values of plant oils treated and untreated fruit were in the range of 20.4–22.0 (^{0}Brix) after ripening (Fig. 2).

Discussion

A wide range of secondary metabolites produced by plants, such as essential oils are endowed with antimicrobial, allelopathic, antioxidant and bioregulatory molecules (French, 1985; Elakovich, 1988). Many of the essential oils and their constituents are commonly used as culinary herbs and spices (Isman, 2000). In the past, several attempts have been made to study the effect of essential oils on different post-harvest pathogen in many crops. However, study on the plant oils against

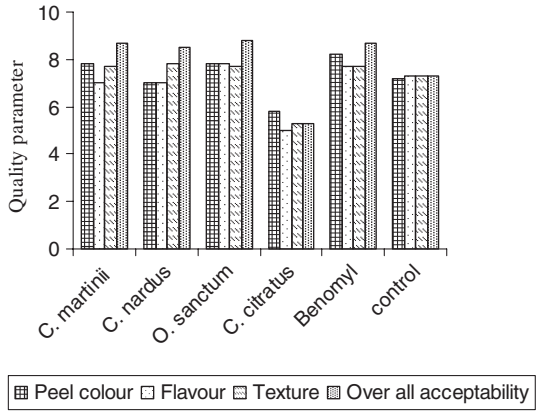

Figure 1 Organoleptic properties of banana cv. Robutsa treated with plant oils, kept for one month at cold storage (14 °C) and then subjected to natural ripening for 1 week after cold storage.

post-harvest pathogens of banana, particularly crown rot disease caused by *L. theobromae* and *C. musae* complex in a commercial variety Robusta (AAA) is lacking. Hence, an attempt has been made to find out the effect of locally available plant oils in controlling rotting of fruits as well as in extending the shelf life of bananas without affecting their organoleptic properties. In this study, the *in vitro* evaluation of fourteen different plant oils against the crown rot pathogens *L. theobromae* and *C. musae* indicated that *O. sanctum*, *C. citratus* and *C. martinii* oils, at a concentration of 0.04% (v/v) (400 mg L^{-1} conc), were effective in arresting the mycelial growth of both the test pathogens, while *P. graveolens* and *C. nardus* oils needed 0.06% and 0.08%, respectively, for similar effectiveness. Lambat *et al.* (2004) had also evaluated plant oils against seven plant pathogens and reported that *C. citratus* oil at 0.02–0.06% and eucalyptus oil at 1.5–3.0% possessed broad-spectrum antifungal activities. Similarly, Herath & Abeywickrama (2008) have observed the inhibition of condial germination and disruption of conidial activity of *C. musae* and *F. proliferatum* by the oils of *O. basilicum* and *C. citratus*.

In this study, the effect of oils on the *in vitro* activity of cell wall degrading enzymes of *L. theobromae* and *C. musae* were tested to understand the mode of action of oils on pathogenesis. The oils of *C. martinii, C. citratus* and *O. sanctum* strongly inhibited the cellulase as well as pectinolytic enzymes of both crown rot pathogens. Reduction in the production of pectinolytic and cellulolytic enzymes of *B. theobromae* was observed when mango fruits were dipped in *O. sanctum* extract (Patil *et al.*, 1995). Earlier Chitra *et al.* (2001) had also reported that the extracts of fruits and flowers of *Datura innoxia* were inhibitory to the cellulolytic enzymes of *C. capsici*.

With regard to studies of plant oils on crown rot severity under *in vivo* condition, out the seven different oils selected, four oils viz. *C. martinii*, *C. nardus*, *O. sanctum* and *C. citratus* were highly effective in reducing the crown rot severity under both storage conditions. The reduction of crown rot severity might be due to the reduction in cell wall degrading enzymes produced by the pathogen, which has been observed in this study. Treatment of oranges with the essential oils of *Mentha arvensis, O. canum* and *Zingiber officinale* has been found to control blue mold, thereby enhancing shelf life (Tripathi, 2001). In this study, the treatment of oils which were found effective in reducing the crown rot severity also increased the total shelf life period of fruits in cv. Robusta (AAA) for up to 48 days with *O. sanctum* oil. The oils of *C. martinii*, *C. nardus* and *C. citratus* oils increased the shelf life of banana fruits to 45 days; however, treatment with *C. citratus* oil affects the overall acceptability of the fruit. Whilst overall acceptability of banana fruit treated with *O. sanctum*, *C. nardus*, *C. martinii* oil and fungicide

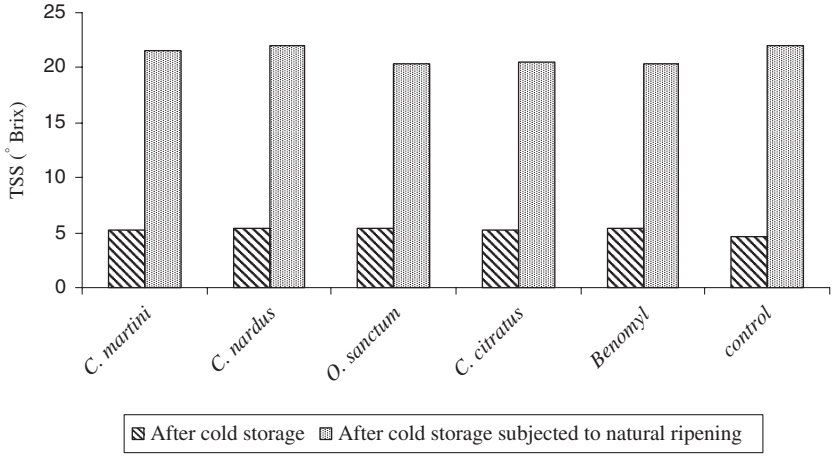

Figure 2 Changes in total soluble solids of banana cv. Robusta treated with plant oils and kept for 1 month at cold storage (14 °C) and then subjected to natural ripening for 1 week after cold storage. *C. martinii* – *Cymbopogon martini*; *C. nardus* – *Cymbopogan nardus*; *O. sanctum* – *Ocimum sanctum*; *C. citratus* – *Cymbopogan citratus*; TSS – total soluble solids.

benomyl was good. Similarly, Ranasinghe *et al.* (2003) have reported that cinnamon oil sprays in Embul bananas induced resistance to crown rot disease and increased the shelf life without any detrimental effect of the physico-chemical properties.

The toxicological studies conducted by Goubran & Holmes (1993) also indicated that the LD_{50} values of *O. sanctum* and *C. martinii* oils are greater than 2000 mg kg^{-1} body weight in experimental mammals. Hence, our study results suggest that these essential oils and their individual components could be safely used at low concentrations to treat banana fruits against possible fungal attack. To conclude, this study resulted in the identification of plant oils, which can (i) significantly reduce the crown rot complex disease caused by *L. theobromae* and *C. musae* pathogens in var. Robusta (AAA), (ii) considerably increases the shelf life of banana fruits for up to 48 days with *O. sanctum* oil treatment. These findings are important for countries which require long storage life of banana fruits especially for the cv. Robusta (which is the common variety in most of the export trade), which is often in transit for more than 21 days, and for which *L. theobromae* and *C. musae* are the major pathogens of crown rot disease complex. Further studies should be conducted on components of *O. sanctum* oil active against specific pathogens involved in crown rot disease and before commercial application, suitable method of application should also be formulated, so as to reach the banana traders, as an effective strategy of controlling the postharvest diseases of banana.

Acknowledgments

The authors are grateful to Dr A Anandan, Sr Lecturer, Department of Agricultural Botany, Annamalai University for his help rendered during statistical analysis.

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