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Research Article

Cinnamomum zeylanicum essential oil in the management of Anthracnose of Banana Fruits

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Abstract

Essential oils of 24 angiospermic taxa were screened for their antifungal activity against fruit rotting fungus *Colletotrichum musae* causing anthracnose of banana. The essential oil of three plants viz. *C. zeylanicum, A. indica* and *Mentha arvensis* showed 100 % activity against the test pathogen. The essential oil of *C. zeylanicum* oil was selected for further investigation. The minimum inhibitory concentration of *C. zeylanicum* oil was found to be 100ppm. The nature of toxicity of oil was fingistatic at its MIC but turned cidal at hypertoxic concentration of 200ppm.The fungitoxicity of the oil was thermostabal up to 80˚C.The shelf life of oil was found to be 24 months. The *C. zeylanicum* oil was found to exhibit a broad fungitoxic spectrum by inhibiting the mycelia growth of 10 common fruit rotting fungi at its MIC. During *in vivo* trial the C*. zeylanicum* oil treated banana fruits showed enhancement of storage life up to 4 days. Therefore, the essential oil of C*. zeylanicum* could be recommended as potential botanical fungicide.

Key words: *Cinnamomum zeylanicum,* essential oil, Anthracnose.

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1. Introduction

Among postharvest diseases, anthracnose caused by *Colletotrichum musae*, is the most important disease causing massive economic losses in bananas [1]. Being a latent infection, the fungus infects immature bananas in the field but the symptoms appear only after ripening [2]. Bananas are generally treated with the fungicides like prochloraz and imazalil to control postharvest pathogens [3]. Recently, increased concerns about the use of fungicides by many countries have demanded a fresh produce without treatment with any chemicals, particularly fungicides applied after harvest. Additionally, due to continuous use of these fungicides *C. musae* has developed resistance and reduced the effectiveness of these synthetic chemicals [4]. All these issues have stimulated the need to search

some alternative sources for controlling post harvest rots of perishables.

Application of essential oil is a very attractive method for controlling postharvest diseases. The potential of plant essential oil is of considerable importance in inhibiting the growth of phytopathogenic fungi. Essential oils are classified as GRAS (generally regarded as safe) and would therefore be more acceptable to consumers. The advantage of essential oils is their bioactivity in the vapor phase, a characteristic that makes them attractive as possible fumigants for stored product protection. The multi-component nature of essential oils makes it more difficult for pathogens to build up resistance. Sometimes the chemicals in the oil, as well as the oil itself, are registered as pesticide active ingredients. It is also fairly common for two or more oils to be used in the same commercial product.

The antimicrobial effects of essential oils or their constituents on post harvest pathogens have been quite extensively studied [5].The essential oils are reported to have some fungicidal properties against certain postharvest diseases of tropical fruits and vegetables [6, 7, 8]. Ranasinghe *et al.* [1] assayed the effectiveness of essential oil on quality parameters of banana fruits and found that cinnamon and clove oils had no effect on TSS, pH and TA of oil-treated fruits. The essential oils of *Cymbopogon nardus*, and *Ocimum basilicum* [9] have also been found to have fungicidal activity against the pathogens associated with banana. Therefore considerable emphasis have been given on studies involving plant essential oils and their constituents for inhibiting the growth of post harvest fungal pathogens under *in vitro* and *in vivo* conditions [10, 11].

Present research work is an aim to evaluate the efficacy of *Cinnamomum zeylanicum* essential oil in the management of anthracnose on banana caused by *Colletotrichum musae* .

2. Material and methods

Fungal culture

The fungal culture of *Colletotrichum musae* was maintained by isolating it from infected banana fruits. The Cultures of *Aspergillus niger* Van Tiegh and *Rhizopus stolonifer* (Ehren. *ex* FR.) Lind was isolated from the infected mango fruits in laboratory. The cultures of *C. musae, A.niger* and *R. stolonifer* were identified with the help of manuals [12, 13]. The cultures of *Botryodiplodia theobromae* Pat, *Botrytis cinerea* Pers *ex* Fr., *Ceratocystis paradoxa* (Dade) C. Moreau, *Colletotrichum gloeosporioids* Penz., *Monilinia fructicola* (Wint.) Honey, *Penicillium digitatum* (Pers) Sacc., *P. expansum* Link *ex* S.F Gray, *P. italicum* Wehmer and *Phomopsis citri* Fawe. were obtained from IARI New Delhi.. All the cultures were maintained on PDA medium. The Czepeks agar medium ($NaNO_3$,3.0g; K_2HPO_4, 1.0 g; $MgSo_4.7H_2O$,0.5g; KCl,0.5g; $FeSO_47H_2O$, 0.1; Sucrose 30 g; Agar 15 g and distilled water 1L) was used throughout the investigation. Five mg streptopenicillin (a mixture of streptomycin and penicillin) was added to the medium to prevent bacterial contamination.

Isolation of active constituents

Some locally available aromatic angiospermic taxa viz., *Aegle marmelos, Ageratum conyzoides, Azadirachta indica, Carum carvi, Cestrum nocturnum, Chenopodium album, Curcuma longa, Cymbopogon flexuosus C.martinii, Eucalyptus citriodora, Hyptis saveolens, Lavendula stachyas, Lawsonia inermis, Lippia alba, Melaleuca leucodendron, Mentha arvensis, Monarda citriodora, Maurraya koenigii, Ocimum basilicum, O.canum, O.gratissimum, Rosa damascena*

and *Z. officinalnale* belonging to different families were collected for the extraction of essential oils. A 250 g fresh leaf of each plant was used for extraction of the essential oils. In the case of *Zingiber officinale* 250 g rhizomes were used for essential oil extraction. The essential oils were isolated by hydro distillation through Clevenger's apparatus. Fresh plant parts (leaves or rhizome) were cut into small pieces and then thoroughly washed with sterilized water. The plant material was then placed in round bottom flask of the Clevenger's apparatus. The ratio between the plant material and water in the flask was maintained as 1:3. Water was heated to produce steam that carried the most volatile fractions of the aromatic material with it. The steam was then chilled (in a condenser) and the resulting distillate was collected. The essential oil was found to float on the top of the hydrosol (the distilled water component) and was separated off. The extracted oils were dehydrated by the addition of anhydrous sodium sulphate, followed by thorough shaking and standing for 6-8 hours and filtration. The essential oil of *C. zeylanicum* was procured from the manufacturing company HERBINS, Begumpur, New Delhi.

Screening of essential oils against Colletotrichum musae

Fungitoxic activity of the oils was tested by the poisoned food technique [14] using potato dextrose agar (PDA) medium against the test fungus *C. musae* at 500 ppm (µ g/l). The concentration of the essential oils was prepared by dissolving the requisite amounts in 0.5 ml of 0.1% Tween 80 and then mixing with 9.5 ml of PDA medium. The control sets were prepared similarly using equal amounts of sterilized distilled water in place of the oil. The prepared plates were inoculated aseptically with assay discs of the test fungus and incubated for 6 days. The observations were recorded on the seventh day and the percentage mycelial inhibition was calculated by the following formula:

$$\text{Percentage of mycelial inhibition} = \frac{dc-dt}{dc} \times 100$$

Where dc is mean colony diameter of control sets and dt is mean colony diameter of treatment sets. The essential oils of *Cinnamomum zeylanicum* was selected for further investigation due to high efficiency as compared to other fungitoxic oils.

Minimum inhibitory concentration (MIC) of C. zeylanicum essential oil

Minimum inhibitory concentration of the essential oil was tested by the poisoned food technique [15] using Czepeks agar medium. The concentration of the essential oil was prepared by dissolving the requisite amounts in 0.5ml of 0.1% Tween-80 and then mixing with 9.5 ml of Czepeks agar medium to produce 500ppm, 400ppm, 300ppm, 200ppm, 100ppm concentrations. The control sets were prepared similarly using equal amounts of sterilized distilled water in place of oil.

Nature of toxicity of C. zeylanicum essential oil

The nature of toxicity (fungitoxic/ fungicidal) of the essential oil was tested against the test fungus following Thompson [16]. The inhibited fungal discs of the oil treated sets were re-inoculated in to fresh medium and revival of their growth was observed.

Physicochemical properties of the oil

The oil was standardized through physicochemical properties viz. specific gravity, specific rotation, refractive index, solubility in different organic solvents,

acid number, saponification value, ester value, phenolic content and carbonyl content following [17].

Fungitoxic properties and effect of storage on fungitoxicity of** C. zeylanicum **essential oil

The effect of increased inoculums density of the test fungus on fungi toxicity of the oil was studied following [18]. The effect of storage and temperature on the fungi toxicity of the oil was determined at its MIC by poisoned food technique.

Range of fungi toxicity of** C. zeylanicum **essential oil

The range of fungitoxicity of *C. zeylanicum* essential oil was tested against 10 most common fruit rotting fungi viz. *Aspergillus niger, Botrytis cinerea*, *Ceratocystis paradoxa*, *Colletotrichum gloeosporioids*, *Monilinia fructicola*, *Penicillium digitatum, P.expensum*, *P.italicum*, *Phomopsis citri* and *Rhizopus stolonifer* by poison food technique.

Comparative study of** C. zeylanicum **essential oil fungi toxicity with some synthetic fungicides

The efficacy of the oils was compared with some fungicides, viz. benzimidazole (benomyl), diphenylamine, phenyl mercuric acetate (ceresan) and zinc dimethyl dithiocarbamete (zirum) by the usual poisoned food technique.

In vivo testing of** C. zeylanicum **essential oil in control of post harvest anthracnose of banana

The banana was treated with the essential oils by the standard techniques [19, 20] in order to find out the efficacy of the oils against anthracnose of banaana caused by *C. musae*. Mature and healthy fruits were used for the experiment. The fruit of control as well as of treatment sets were washed in running water and were surface sterilized with 0.1 % sodium hypochlorite solution and were then washed with distilled water. The pathogenicity of the fungus was tested, following Garcha & Singh [21]. The fruits were inoculated by 1ml of the standard spore suspension of *C. musae*. For fruit inoculation spores from a 7 day old culture were suspended in sterile distilled water and 0.03% Tween 80. Fruits were wounded by puncturing them with a pin on different sides of the fruits. Each wound site was then inoculated by spraying with 40 µl of spore suspension (10^5 spores/ ml) of *C. musae*. The inoculated fruits were kept in desiccators (four fruits per desiccators). In treatment sets the requisite amount of oils were introduced separately into the desiccators by soaking in a piece of cotton so as to give concentrations of *C. zeylanicum* oils at 100ppm. The initiations of rotting of the fruits were observed. Six replicates were kept for treatment and control sets.

3. Results

Selection of active essential oils against C. musae

Antifungal efficiency of different essential oils is given in Table1. Among the evaluated oils three essential oils viz. *Azadirachta indica*, *Cinnamomum zeylanicum* and *Mentha arvensis* were found to exhibit absolute toxicity by showing 100% inhibition of the test pathogen *C. musae.* The essential oil of *Carum carvi, Cymbopogon martini* and *Lawsonia inermis* showed 95 % inhibition while the antifungal activity of *Aegle marmelos, Ageratum conyzoides, Cestrum nocturnum, Chenopodium album, Curcuma longa, Rosa damascene* and *Zingiber officinale* oils were recorded to be between35 to 65 %. *Eukalyptus citriodora, Lippia alba, Monarda citriodora, Murraya koenigii, O. canum, O. gratisimum and O. basilicum* essential oils showed the

activity between 75 to 85 %. As the essential oil of *C. zeylanicum* showed 100 % activity and due to its various medicinal and biological properties it has been selected for further investigation. Though the essential oil of *A. indica* and *Mentha arvensis* have also shown the same 100% activity and were not selected for present investigation because a lot of work has already been done with these oils.

The MIC and Nature of toxicity of* C. *zeylanicum

The minimum inhibitory concentration of *C. zeylanicum* oil was found to be 100ppm.The MIC of *Azadirachta indica* and *Mentha arvensis* oil was found to be 100 ppm and 200ppm respectively. The oil was further standardized by fungitoxic properties. The nature of toxicity of the *C. zeylanicum* was found to be fungi static in nature at 100ppm as in inhibited fungal discs were revived on placing the fresh medium. While, essential oil was found fungicidal at 200ppm.

Physicochemical properties of essential oils of* C. *zeylanicum

The essential oil of *C. zeylanicum* was standardized by physicochemical properties. The yield of oil was 1.6-1.8 %. The oil was pale yellow in colour with musky odder. It was found to be soluble in methanol, absolute alcohol and Acetone. The specific gravity of the oil was found to be at 20˚C, 1.035-1.052. The specific rotation was recorded as +23.0. The refractive index at 20˚C was found to be 1.527-1.537.The optical rotation was 1˚36 to 0˚ 40.The density was found to be 1.03-1.05 (Table 2).

Fungitoxic properties of* C. *zeylanicum essential oil

It has been observed that the oils inhibited the fungal growth of the treatment sets containing even 64 discs of the test fungus indicating the potency of the essential oils to withstand high inoculum density of test fungus (Figure 1). This is the important potential of the oils to be exploited as botanical fumigant. It was found that these oils have long shelf lives. The oil of C*. zeylanicum* remained active for 24 months. The oils remained fungi toxic at different temperatures between 10 and 80 ˚C showing the thermo stable nature of their fungitoxicity. The oils were found to exhibit a broad fungitoxic spectrum by inhibiting the mycelia growth of 10 common fruit rotting fungi at viz. *Aspergillus niger, Botrytis cinerea*, *Ceratocystis paradoxa*, *Colletotrichum gloeosporioids*, *Monilinia fructicola*, *Penicillium digitatum, P.expensum*, *P.italicum*, *Phomopsis citri* and *Rhizopus stolonifer* at their respective toxic and hypertoxic concentrations.

Comparative efficacy of the essential oils with some prevalent synthetic fungicides

The MIC of synthetic fungicides viz benzimidazole (benomyl), diphenyl amine, phenyl mercuric acetate (ceresan) and zinc dimethyldithiocarbamate (ziram) was found to be at 3000, 1000, 1000, and 5000 ppm respectively which were higher than the essential oils tested in present study. Thus the oils have been found to be more efficacious than the synthetic fungicides.

***In vivo* applicability of C. *zeylanicum* oil**

The C*. zeylanicum* oil treated banana fruits showed enhancement of storage life up to 4 days, (Table 3). Thus the oil showed their antifungal efficacy in control of storage rotting of banana during *in vivo* trials.

Table 1. Screening of essential oils of angiospermic plants against *C. musae*

Angiospermic plants	Family	Percent mycelia inhibition
Aegle marmelos	Rutaceae	42
Ageratum conyzoides	Asteraceae	50
Azadirachta indica	Milliaceae	100
Carum carvi	Apiaceae	95
Cestrum nocturnum	Solanaceae	45
Chenopodium album	Chenopodiaceae	55
Curcuma longa	Zingiberaceae	65
Cymbopogon flexuosus	Poaceae	90
Cymbopogon martinii	Poaceae	95
Cinnamomum zeylanicum	Lauraceae	100
Eukalyptus citriodora	Mirtaceae	85
Hyptis saveolence	Labiateae	60
Lavendula stochyas	Lamiaceae	90
Lawsonia inermis	Lythraceae	95
Lippia alba	Labiateae	78
Melaleuca alternifolia	Myrtaceae	92
Mentha arvensis	Labiateae	100
Monarda citriodora	Lamiaceae	85
Murraya koenigii	Rutaceae	75
O.canum	Labiateae	80
O.gratisimum	Labiateae	75
Ocimum basilicum	Labiateae	80
Rosa damascena	Rosaceae	35
Zingiber officinale	Zingiberaceae	65

4. Discussion

The essential oils have been evaluated for antifungal activity by a number of workers and are known to play a role in plant defense mechanism against phyto-pathogens [22]. Most of the essential oils have been reported to inhibit post harvest fungi in *in vitro* conditions (23, 24, 25, 26]. *In vitro* antifungal activity of the essential oils from *Monarda citridora* and *Melaleuca alternifolia* was evaluated against various post harvest pathogens. Both the oils exhibited a high level of antifungal activity [23]. Recent findings on the success of essential oils as biodegradable and ecofriendly fungitoxicants have shown the possibilities for their exploitation as natural fungicides (27,28]. In the present investigation the essential oils of C*. zeylanicum* selected for further study due to their lower MIC as compared to other fungitoxic oils and were subsequently standardized through physicochemical properties, fungitoxic properties and practical applicability in controlling the anthracnose caused by *C. musae*. Such investigations are essential with most of the fungitoxic plant products and are also required to recommend them to agrochemical firms for their formulation.

Table 2. Physicochemical properties of the *C. zeylanicum* oil

Parameters	***Observations***
Yield of oil	*1.6-1.8 %*
Colour	*Pale yellow*
Odour	*musky*
Specific gravity at 20ºC	*1.035-1.052*
Specific rotation	*+23.0º*
Refractive index at 20˚C	*1.527-1.537*
Optical Rotation	*1˚36'to 0˚40'*
Density	*1.03-1.05*
Acid number	*49.54*
Saponification value	*203.33*
Ester value	*156.01*
Phenolic content	*1826GAEmg/L*
Carbonyl percentage	*74*
Solubility in organic solvents	
Acetone	*Soluble (1:1 Conc)*
Absolute alcohol	*Soluble (1:1Conc)*
90 % alcohol	*Soluble(1:1 Conc)*
Ethyl acetate	*Soluble (1:1Conc)*
Benzene	*Soluble (1:1Conc)*
Chloroform	*Soluble (1:1Conc)*
Hexane	*Soluble 1:1 Conc)*
Methanol	*Soluble (1:1Conc)*

Table 3. *In vivo* applicability of C. *zeylanicum* oil on Banana fruits

Essential oil	Initiation of rotting (in days)	*Enhancement (in days)*
Control	6	*0*
Fumigated at 100ppm	*10*	*4*

The quality of essential oils depend on a number of physical parameters such as specific gravity, optical rotation, refractive index, solubility in different organic solvents, acid number, saponification value, ester value and phenolic contents. A number of papers on the biological activity of essential oils have been published. Their data however show much variation between the same essences. The reason for this variability can be understood if we take in to account all the factors influencing the chemical composition of the oils such as climatic, seasonal and geographical conditions, harvest period and distillation techniques [29].

The oil has fungistatic nature at their respective MIC, which is a positive indication that there would not have any cidal effect on host tissues. As the fungicidal nature of the oil appeared at higher concentrations therefore the oils must be applied at their respective MIC to prevent the host tissues from the negative effect of excess essential oils.

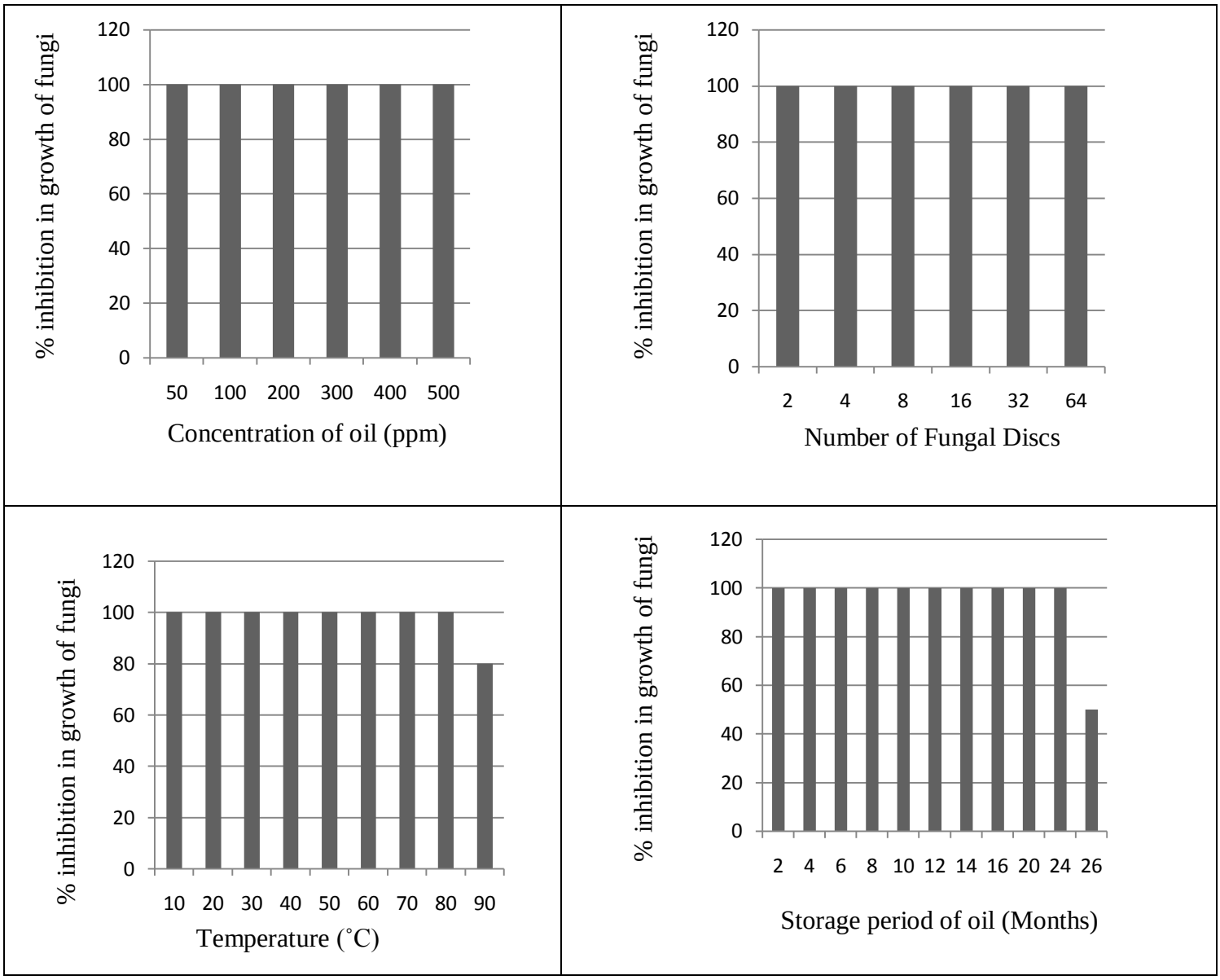

Figure 1. Essential oils effect: (a) minimum inhibitory concentrations (b) Fungi toxicity of oil on increasing fungal inoculums density (c) thermo stability of oil and fungi toxicity on keeping at different temperature for one hour (d) Effect of Storage on fungi toxicity of oil.

On comparing the MIC of the oils with some synthetic fungicides the oils were found to be more active than the synthetic pesticides as the MIC of the oils were found to lower as compared to synthetic fungicides (Figure 1).

Generally fungitoxicants of plant origin have been found to be non injurious to the treated food commodities and in some cases they have shown enhancement in the shelf life of the commodities. The essential oils of C*. zeylanicum* have shown significant fungitoxic activity and enhanced the shelf life of banana during storage by protecting them against anthracnose. The fruits were fumigated by the essential oils at their respective MIC. The fumigated fruits with C*. zeylanicum* oil treated sets showed enhancement of shelf

life up to 4 days. The oils did not show any adverse symptom on the fruit peel.

Therefore, the use of essential oils as antimicrobial agents can be an interesting field of investigation as the toxicity to mammals is mostly quite low, and their degree of volatility allows their use for fumigation in cold storage or for active packing. The essential oils of C*. zeylanicum* with strong fungitoxicity, low MIC, thermostable nature, long shelf life, fungistatic/fungicidal nature against the test fungus as well as against other common fruit rotting fungi, lower MIC in comparison to synthetic pesticides and the efficacy to withstand high inoculums density have all the desired characters of an ideal fungicide and could be recommended as botanical fungitoxicant. However, the potential use of essential oils to control postharvest diseases requires a detailed examination of their biological activity and dispersion in fruit tissues and the development of a formulation which inhibits the growth of pathogens at non phytotoxic concentrations.

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