World J Microbiol Biotechnol (2008) 24:39–46
DOI 10.1007/s11274-007-9435-2

ORIGINAL PAPER

Use of some essential oils as post-harvest botanical fungicides in the management of grey mould of grapes caused by *Botrytis cinerea*

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Received: 13 February 2007 / Accepted: 14 May 2007 / Published online: 8 June 2007

Abstract During screening of twenty six essential oils against *Botrytis cinerea*, the essential oils of the ten plants viz. *Chenopodium ambrosioides*, *Eucalyptus citriodora*, *Eupatorium cannabinum*, *Lawsonia inermis*, *Ocimum canum*, *O. gratissimum*, *O. sanctum*, *Prunus persica*, *Zingiber cassumunar* and *Z. officinale* were found to exhibit absolute fungitoxic activity (100% growth inhibition). The essential oils of *O. sanctum*, *P. persica* and *Z. officinale* were selected for further investigation because these oils showed lower Minimum Inhibitory Concentration (MIC) as compared to the other fungitoxic oils. The selected oils were subsequently standardized through physico-chemical and fungitoxic properties. The MIC values of *O. sanctum*, *P. persica* and *Z. officinale* were found to be 200, 100 and 100 ppm (mg/l) respectively. The oils showed fungistatic nature at their respective MIC. The oils were thermostable, and exhibited a wide range of fungitoxicity against 15 other post-harvest fungal pathogens. The oils had the potency to withstand high inoculum density. The antifungal potency of oils was found to be greater in comparison to some prevalent synthetic fungicides. Practical applicability of the essential oils was observed in control of grey mould of grapes caused by *B. cinerea* during storage. The *O. sanctum*- and *P. persica*-oil-treated grapes showed enhancement of storage life up to 5 and 4 days respectively. The storage life of *Z. officinale*-oil-treated grapes was found to be enhanced up to 6 days. The oils did not exhibit any phytotoxic effect on the fruit peel. Therefore, the oils could be recommended as a potential source of ecofriendly botanical fungicide, after long term and wide ranging trials.

Keywords Essential oils · Grey mould · Fungitoxicity · *Botrytis cinerea* · Shelf life

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Introduction

Post-harvest diseases render heavy losses to perishables during transit and storage. Higher water contents, nutrient composition and pH of most of the perishables make them capable of supporting the growth of a number of microorganisms. Fruits due to their low pH are spoiled primarily by fungi which in addition to causing rot, may also contaminate the fruits by producing mycotoxins (Phillips 1984; Moss 2002). Worldwide post-harvest loss of perishables due to fungi is between 10% and 50%.

Among post-harvest fungal pathogens, *Botrytis cinerea* Pers.: Fr. is one of the common causal agent of grey mould of grapes. The grapevine industry is particularly affected by this fungus that attacks grapes at pre- and post-harvest stage under a wide array of environmental conditions and over a large geographical area. Infection, caused during post-harvest conditions lowers the shelf life and adversely affect the market value of the fruits.

Although the use of synthetic pesticides in plant protection had made a great contribution to plant protection, many are no longer used because of economic,

environmental or health concerns, or due to development of resistant strains. Fungicides that are primarily used for controlling post-harvest diseases have recently come under special scrutiny as posing a potential oncogenic risk. Therefore, the scientific community at international level is looking for safer alternative products from plants for effective control of pests during storage. Naturally occurring biologically active compounds from plants are generally assumed to be more acceptable and less hazardous than synthetic compounds and represent a rich source of potential disease-control agents.

Biologically active essential oils represent a rich potential source of an alternative and perhaps environmentally more acceptable disease management compounds. With a broad range of natural fungicidal plant volatiles, numerous opportunities exist to explore their usefulness in controlling post-harvest diseases. The general antifungal activity of essential oils is well documented (Reuveni et al. 1984; Deans and Ritchie 1987; Alankararao et al. 1991; Baruah et al. 1996; Gogoi et al. 1997; Pitarokili et al. 1999; Meepagala et al. 2002) and there have been some studies on the effects of essential oils on post-harvest pathogens (Bishop and Thornton 1997). The advantage of essential oils is their bioactivity in the vapour phase, a characteristic which makes them attractive as possible fumigants for stored product protection.

Ocimum sanctum L. (holi basil) called Tulsi in India is ubiquitous in Indian tradition. It is the most common and most revered of all household in India. Perhaps its role as a healing herb was instrumental in its ''sacred'' implication. The antifungal properties have been reported from the essential oil of *O. sanctum* (Tewari and Nayak 1991; Mohamed et al. 1996). *Prunus persica* (L.) Stackes (peach) is also treated as medicinal plant in African countries and the plant has been reported to exhibit strong antifungal activity (Caccioni et al. 2002). *Zingiber officinale* Rosc. has been recommended by Chinese medicine for over 2,500 years. It was called the universal medicine. The antifungal activity of its oil is well documented (Ficker et al. 2003).

Keeping all these points in consideration, an attempt has been made in the present piece of work to find out the practical applicability of essential oils of *Ocimum sanctum*, *Prunus persica* and *Zingiber officinale* in enhancing the shelf-life of grapes by protecting them from fungal rotting caused by *Botrytis cinerea*.

Materials and methods

Isolation of active constituents

Some locally available aromatic angiospermic taxa viz *Aegle marmelos, Ageratum conyzoides, Ammomum subulatum, Azadirachta indica, Caesulia axillaries, Callistemon lanceolatus, Chenopodium ambrosioides, Citrus medica, C. reticulata, Cymbopogon citrates, Elettaria cardemomum, Eucalyptus citriodora, Eupatorium cannabinum, Hyptis suaveolens, Lawsonia inermis, Lippia alba, Melaleuca leucodendron, Murraya koenigii, Nepeta hindostana, Ocimum basilicum, O. canum, O. gratissimum, O. sanctum, Prunus persica, Zingiber cassumunar* and *Z. officinale* belonging to different families were collected for the extraction of essential oils. 250 g fresh leaves 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 hydrodistillation through Clevengers 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 the round-bottom flask of the Clavengers 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 h and filtration.

Maintenance of fungal cultures

The common fruit rotting pathogens viz., *Botryodiplodia theobromae*, *Botrytis cinerea*, *Colletotrichum gloeosporioides*, *Fusarium roseum*, *Monilinia fructicola*, *Phomopsis citri*, *Alternaria citri*, *Geotrichum candidum* and *Ceratocystis paradoxa* were obtained from IARI New Delhi. *Aspergillus niger*, *A. flavus*, *Penicillium italicum*, *P. expansum*, *P. digitatum*, *Rhizopus stolonifer* and *Mucor pyriformis* were isolated from infected fruits. These fungi were identified on the basis of morphological characters, viz. colony characters on media, mycelial characters, types of conidiophore and conidial characters with the help of manuals of Barnett and Hunter (1972) and Domsch et al. (1980). The pathogens were maintained on potato dextrose agar medium (200 g, scrubbed and diced potato in 1000 ml distilled water, 15 g agar, 20 g dextrose, pH ± 5.6). A seven-day-old culture of each fungus was used during further investigation.

Screening of essential oils against *B. cinerea*

Fungitoxic activity of the oils was tested by the poisoned food technique of Perrucci et al. (1994) using potato

dextrose agar (PDA) medium against the test fungus *B. cinerea* at 500 ppm (mg/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:

$$\text{Percentage of mycelial inhibition} = \frac{d_c - d_t}{d_c} \times 100$$

where d_c is mean colony diameter of control sets and d_t is mean colony diameter of treatment sets.

The essential oils of *O. sanctum*, *P. persica* and *Z. officinale* were selected for further investigation due to their lower minimum inhibitory concentration (MIC) as compared to other fungitoxic oils.

Physicochemical properties of essential oils of *O. sanctum*, *P. persica* and *Z. officinale*

The oils were standardized through GLC and physicochemical properties viz. specific gravity, refractive index, acid number, saponification value, ester value and phenolic content were estimated following Chowdhury and Kapoor (2000).

Minimum inhibitory concentration and nature of toxicity of essential oils of *O. sanctum*, *P. persica* and *Z. officinale*

To find out the minimum inhibitory concentration at which the oils showed absolute fungitoxicity, experiments were carried out by the above-mentioned poisoned food technique using graded concentration of essential oils below 500 ppm. The nature of the toxicity (fungistatic/fungicidal) of the oils against the test fungus was determined following Thompson (1989). The inhibited fungal discs of the oil treated sets were reinoculated into fresh medium and revival of their growth was observed.

Fungitoxic properties of the essential oil of *O. sanctum*, *P. persica* and *Z. officinale*

The effect of increased inoculum density of the test fungus on fungitoxicity of the oils was studied following Molyar and Pattisapu (1987) and Shahi et al. (1999) at their respective MIC values. The effect of storage and temperature on the fungitoxicity of the essential oils was evaluated according to Tripathi et al. (1978) and Shahi et al. (1999). The range of fungitoxicity of the oils was determined at their respective toxic and hypertoxic concentrations by the poisoned food technique.

Comparison of the fungitoxicity of the essential oils with some prevalent synthetic fungicides

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

In vivo applicability of the oils of *O. sanctum*, *P. persica* and *Z. officinale*

The grapes were treated with the essential oils by the standard techniques followed by Chandra (1984) and Sharma and Yadav (1996) in order to find out the efficacy of the oils against grey mould of grapes caused by *B. cinerea*. Mature and healthy bunches of fruits were used for the experiment. The fruit bunches 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 and Singh (1980). The fruits were inoculated by 1 ml of the standard spore suspension of *B. cinerea*. 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 bunch. Each wound site was then inoculated by spraying with 40 µl of spore suspension (10^5 spores/ml) of *B. cinerea*. The inoculated fruits were kept in desiccators (four bunch per desiccator). 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 *O. sanctum*, *P. persica* and *Z. officinale* oils at 200,100 and 100 ppm respectively (at their respective MIC values). The initiations of rotting of the fruits were observed. Six replicates were kept for treatment and control sets.

Results

Selection of active essential oils against *B. cinerea*

It is evident from the Table 1 that out of twenty six essential oils tested against *B. cinerea*, ten oils viz. *Chenopodium ambrosioides, Eucalyptus citriodora, Eupatorium cannabinum, Lawsonia inermis, Ocimum*

Table 1 Screening of some essential oils for fungitoxicity against *B. cinerea*

Essential oils	Family	Percent inhibition of growth at 500 ppm
Aegle marmelos Linn. (leaf)	Rutaceae	75
Ageratum conyzoides Linn. (leaf)	Asteraceae	60
Ammomum subulatum Roxb (leaf)	Zingiberaceae	55
Azadirachta indica A. Juss (leaf)	Miliaceae	65
Caesulia axillaries Roxb. (leaf)	Asteraceae	95
Callistemon lanceolatus DC (leaf)	Myrtaceae	25
Chenopodium ambrosioides Linn. (leaf)	Chenopodiaceae	100
Citrus medica Linn. (leaf)	Rutaceae	30
Citrus reticulata Linn. (leaf)	Rutaceae	45
Cymbopogon citrates (DC) Stapf (leaf)	Poaceae	95
Elettaria cardemomum Maton (leaf)	Zingiberaceae	75
Eucalyptus citriodora Hook. (leaf)	Myrtaceae	100
Eupatorium cannabinum Linn. (leaf)	Asteraceae	100
Hyptis suaveolens Linn. (leaf)	Lamiaceae	20
Lawsonia inermis Linn. (leaf)	Lytheraceae	100
Lippia alba Mill N. B. Br (leaf)	Verbenaceae	20
Melaleuca leucodendron Linn. (leaf)	Myrtaceae	40
Murraya koenigii Linn. (leaf)	Lamiaceae	80
Nepeta hindostana Roth Haines (leaf)	Lamiaceae	50
Ocimum basilicum Linn. (leaf)	Lamiaceae	80
O. canum Sims (leaf)	Lamiaceae	100
O. gratissimum Linn. (leaf)	Lamiaceae	100
O. sanctum Linn. (leaf)	Lamiaceae	100
Prunus persica Linn Stackes (leaf)	Rosaceae	100
Zingiber cassumunar Roxb (leaf)	Zingiberaceae	100
Zingiber officinale Rosc. (rhizome)	Zingiberaceae	100

canum, O. gratissimum, O. sanctum, Prunus persica, Zingiber cassumunar and *Z. officinale* showed absolute toxicity (100% growth inhibition) against the test fungus at 500 ppm (mg/l). The essential oils of *Aegle marmelos, Ageratum conyzoides, Ammomum subulatum, Azadirachta indica, Caesulia axillaries, Cymbopogon citrates*, *Elettaria cardemomum, Murraya Koenigii* and *Ocimum basilicum* exhibited moderate fungitoxicity (<100% but >50%). Poor fungitoxicity was observed by the oils of *Callistemon lanceolatus, Citrus medica*, *Citrus reticulata*, *Hyptis suaveolens*, *Melaleuca leucodendron, Lippia alba* and *Nepeta hindostana*. The essential oils of *O. sanctum, P. persica* and *Z. officinale* were selected for further investigation as the MIC of these oils were lower as compared to other fungitoxic oils.

Physicochemical properties of essential oils of *O. sanctum*, *P. persica* and *Z. officinale*

The yield of oils of *O. sanctum*, *P. persica* and *Z. officinale* were 0.4%, 0.5% and 0.8% respectively. These three essential oils were yellow in colour and pungent in odour. The oils were found to be soluble in all the tested organic solvents. The specific gravity of *O. santum*, *P. persica* and *Z. officinale* oil was found to be 0.9234, 0.985, and 0.8900 respectively. The phenolic contents were present in all the three oils (Table 2). The GLC of *O. sanctum* oil indicated it to be a mixture of 8 major and 12 minor components. The oil of *P. persica* was a mixture of 4 major and 30 minor components. The oil of *Z. officinale* was a mixture of 9 major and 16 minor components.

The MIC and nature of toxicity of *O. sanctum*, *P. persica* and *Z. officinale* essential oils

The MIC of *O. sanctum*, *P. persica* and *Z. officinale* oils was found to be 200, 100 and 100 ppm. respectively. The other fungitoxic oils showed MIC ranging between 250 ppm to 500 ppm. Due to lower MIC of essential oils of *O. sanctum*, *P. persica* and *Z. officinale* as compared to other oils, these were selected for further investigation.

It was found that all these essential oils were fungistatic at their respective MIC. Though the oils were fungistatic in nature at lower concentrations, they greatly reduced the formation of melanin in the culture plates. The reinoculated

Table 2 Physico-chemical properties of essential oils of *O. sanctum, P. persica* and *Z. officinale*

Parameters	*O. sanctum*	*P. persica*	*Z. officinale*
Yield	0.4%	0.5%	0.8%
Colour	Pale yellow	Pale yellow	Yellow
Odour	Pungent	Pungent	Aromatic Pleasant
Specific gravity	0.9234	0.985	0.8900
Optical rotation	(–)25°	(–)28°	(–)5°
Refractive index	1.4510 (at 24°C)	1.4420(at 24°C)	1.4840 (at 24°C)
Solubility			
Acetone	Soluble (1:1 conc)	Soluble (1:1 conc)	Soluble (1:1 conc)
Absolute alcohol	Soluble (1:1 conc)	Soluble (1:1 conc)	Soluble (1:1 conc)
90% alcohol	Soluble (1:1 conc)	Soluble (1:1 conc)	Soluble (1:1 conc)
Ethyl acetate	Soluble (1:1 conc)	Soluble (1:1 conc)	Soluble (1:1 conc)
Benzene	Soluble (1:1 conc)	Soluble (1:1 conc)	Soluble (1:1 conc)
Chloroform	Soluble (1:1 conc)	Soluble (1:1 conc)	Soluble (1:1 conc)
Hexane	Soluble (1:1 conc)	Soluble (1:1 conc)	Soluble (1:1 conc)
Methanol	Soluble (1:1 conc)	Soluble (1:1 conc)	Soluble (1:1 conc)
Acid Number	2.3	3.4 mg	4.48 mg
Saponification value	35.375	38.475 mg	23.84 mg
Ester value	33.235	35.235 mg	19.36 mg
Phenolic content	Present	Present	Present

plates showed weak and hyaline mycelial growth without sporulation indicating that the oils were also inhibitory to pigment synthesis as well as to spore formation. The fungicidal effects of the three oils appeared at higher concentrations (2000, 1500 and 2500 ppm respectively).

Fungitoxic properties of the essential oils of *O. sanctum*, *P. persica* and *Z. oficinale*

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 (Table 3). 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 *O. sanctum* remained active for 24 months while that *of P. persica* and *Z. officinale* could retain their toxicity up to 48 and 36 months respectively. The oils remained fungitoxic in nature at different temperatures between 5°C and 50°C showing the thermostable nature of their fungitoxicity.

The oils were found to exhibit a broad fungitoxic spectrum by inhibiting the mycelial growth of 15 common fruit rotting fungi at viz. *Alternaria citri*, *Aspergillus niger*, *A. flavus*, *Botryodiplodia theobromae*, *Ceratocystis paradoxa*, *Colletotrichum gloeosporioides*, *Fusarium roseum*, *Geotrichum candidum*, *Monilinia fructicola*, *Mucor pyriformis*, *Penicillium italicum*, *P. expansum*,

Table 3 Effect of increased Inoculum Density on fungitoxicity of the oils

Number of fungal discs	Approximate number of spores	Growth of the test fungus					
		O. sanctum		*P. persica*		*Z. officinale*	
		Treatment	Control	Treatment	Control	Treatment	Control
1	2358×10^3	–	+	–	+	–	+
2	47174×10^3	–	+	–	+	–	+
4	94348×10^3	–	+	–	+	–	+
8	188696×10^3	–	+	–	+	–	+
16	377392×10^3	–	+	–	+	–	+
32	754784×10^3	–	+	–	+	–	+
64	1509568×10^3	–	+	–	+	–	+

– indicates no growth of test fungus

+ indicates growth of test fungus

P. digitatum, *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), diphenylamine, 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 (Table 4). Thus the oils have been found to be more efficacious than the synthetic fungicides.

In vivo applicability of the oils of *O. sanctum*, *P. persica* and *Z. officinale*

The *P. persica*-oil-treated grapes showed enhancement of storage life up to 4 days, while the *O. sanctum*- and *Z. officinale*-oil-treated grapes showed enhancement of shelf life up to 5 days and 6 days respectively (Table 5). Thus the oil showed their antifungal efficacy in control of storage rotting of grapes during in vivo trials.

Discussion

The essential oils are thought to play a role in the plant defence mechanism against phytopathogenic microorganisms (Mihaliak et al. 1991). Most of the essential oils have been reported to inhibit post-harvest fungi in in vitro conditions (Bishop and Reagon 1998; Singh and Tripathi 1999; Bellerbeck et al. 2001; Hidalgo et al. 2002). 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 (Bishop and Thornton 1997). Recent findings on the success of essential oils as biodegradable and ecofriendly fungitoxicants have shown the possibilities for their exploitation as natural fungicides (Dixit et al. 1995; Tripathi et al. 2004).

Table 4 Comparative efficacy of the oils with some prevalent synthetic fungicides

Fungicides	Minimum inhibitory concentration against *B. cinerea* (ppm or mg/l)
Benzimidazole (benomyl)	3000
Diphenylamine	1000
Phenylmercuric acetate (Ceresan)	1000
Zinc dimethyldithiocarbamate(Ziram)	5000
Oils	
O. sanctum	200
P. persica	100
Z. officinale	100

Table 5 Efficacy of the oils against grey mould of grapes

	Initiation of rotting of fruits (in days)	Enhancement of storage life (in days)
Control	4	
O. sanctum	9	5
P. persica	8	4
Z. officinale	10	6

In the present investigation the essential oils of *O. sanctum*, *P. persica* and *Z. officinale* were 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 grey mould of grapes caused by *B. cinerea*. Such investigations are essential with most of the fungitoxic plant products and are also required to recommend them to agrochemical firms for their formulation.

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 (Panizzi et al. 1993).

The GLC of the essential oil of *O. sanctum* showed it to be a mixture of 8 major and 12 minor constituents. The *P. persica* oil was a mixture of 4 major and 30 minor components. The Z. *officinale* oil was a mixture of 9 major and 16 minor constituents. Thus the activity of the oils seems likely to be due to the synergistic effect of major and minor components of the oils.

The oils have a fungistatic action at their respective MIC, which is a positive indication that they 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.

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 be lower as compared to synthetic fungicides (Table 4).

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 *O. sanctum*, *P. persica* and *Z. officinale* have shown significant fungitoxic activity and enhanced the shelf life of grapes during storage by protecting them from grey mould. The fruits were fumigated by the essential oils at their respective MIC. The fumigated fruits with the oils of *O. sanctum*, *P. persica* and *Z. officinale* of treated sets showed enhancement of shelf life up to 5, 4 and 6 days, respectively. The oils did not showed 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 *O. sanctum*, *P. persica* and *Z. officinale* 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 inoculum 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 post harvest 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.

Acknowledgements Authors are grateful to the Head of the Department of Botany, Banaras Hindu University, Varanasi, India, for providing various laboratory facilities and Department of Science and Technology, New Delhi for financial assistance.

References

Alankararao GSJG, Baby P, Rajendra Prasad Y (1991) Leaf oil of *Coleus amboinicus* Lour: in vitro antimicrobial studies. Perfumerie Kosmetics 72:744–745

Barnett HL, Hunter BB (1972) Illustrated genera of imperfect fungi. Burgess Publishing Company, Minneapolis

Baruah P, Sharma RK, Singh RS, Ghosh AC (1996) Fungicidal activity of some naturally occurring essential oils against *Fusarium monoliforme*. J Essential oil Res 8:411–441

Bellerbeck VG, De Roques CG, Bessiere JM, Fonvieille JL, Dargent R (2001) Effect of *Cymbopogon nardus* (L) W. Watson essential oil on the growth and morphogenesis of *Aspergillus niger*. Can J Microbiol 47:9–17

Bishop CD, Reagan J (1998) Control of the storage pathogen *Botrytis cinerea* on Dutch white cabbage (*Brassica oleracea* var *capitata*) by the essential oil of *Melaleuca alternifolia*. J Essential Oil Res 10:57–60

Bishop CD, Thornton IB (1997) Evaluation of the antifungal activity of the essential oils of *Monarda citriodora* var. *citriodora* and *Melaleuca alternifolia* on the post-harvest pathogens. J Essential Oil Res 9(1):77–82

Caccioni DRL, Tonini G, Guizzardi M (2002) In vitro antifungal activity of some South African medicinal plants. South Afr J Bot 68:72–76

Chandra H (1984) Evaluation of some higher plants for volatile activity against blue mould rot of oranges. Ph.D. Thesis. Gorakhpur University, Gorakhpur, India

Chowdhury AR, Kapoor VP (2000) Essential oil from fruit of *Apium graveolens*. J Med Aromat Plant Sci 22:621–623

Deans SG, Ritchie G (1987) Antimicrobial properties of plant essential oils. Int J Food Microbiol 5:165–180

Dixit SN, Chandra H, Tiwari R, Dixit V (1995) Development of botanical fungicide against blue mold of mandarins. J Stored Prod Res 31:165–172

Domsch KH, Gams W, Anderson PH (1980) Compandium of soil fungi. Academic Press, London

Garcha HC, Singh Y (1980) Post-harvest diseases of fruits in Punjab. Indian Phytopathology 33:42–47

Gogoi R, Baruah P, Nath SC (1997) Antifungal activity of the essential oil of *Litsea cubeba* Pers. J Essential Oils Res 9:213–215

Hidalgo PJ, Ubera JL, Santos JA, LaFont F, Castelanos C, Palomino A, Roman M (2002) Essential oils in *Culamintha sylvatica*. Bromf. ssp. ascedens (Jorden) P.W. Ball wild and cultivated productions and antifungal activity. J Essential Oil Res 14:68–71

Ficker C, Smith ML, Akpugana K, Gbeassor M, Zhung J, Durst T, Assabgui R, Arnason T (2003) Bioassay-guided isolation and Identification of antifungal compounds from ginger. Pharmaceutical Biol 41:392–397

Meepagala KM, Sturtz G, Wedge DE (2002) Antifungal constituents of the essential oil fraction of *Artemisia drancunculus* L. var. *dracunculus*. J Agric Food Chem 50:6989–6992

Mihaliak CA, Gershenzo J, Croteau R (1991) Lack of rapid monoterpene turnover in rooted plants, implications for theories of plant chemical defense. Oecologia 87:373–376

Mohamed S,Saku S, El-Sharkawy SH, Ali AM, Muid S (1996) Antimycotic screening of 58 Malaysian plants against plant pathogens. Pesticide Sci 47:259–264

Molyar V, Pattisapu N (1987) Detoxification of essential oil components (Citral and menthol) by *Aspergillus niger* and *Rhizopus stolonifer*. J Sci Food Agric 39:239–246

Moss MO (2002) Mycotoxins review. 1. *Aspergillus* and *Penicillium*. Mycologist 16:116–119

Panizzi L, Flamini G, Cioni PL, Morelli I (1993) Composition and antimicrobial properties of essential oils of four Mediterranean Lamiaceae. J Ethnopharmacol 39:167–170

Perrucci S, Mancianti F, Ciont PL, Flamini G, Morelli I, Macchioni G (1994) In vitro antifungal activity of essential oils against some isolates of *Microsporum canis* and *M. gypseum*. Planta Medi 60:184–187

Phillips DJ (1984) Mycotoxins as a postharvest problem. In: Moline HE (eds) Post-harvest Pathology of fruits and vegetables: post harvest losses in perishable crops. Agricultural Experimental Station, University of California, Berkeley Publications, N E, pp 50–54

Pitarokili D, Tzakou O, Couladis M, Verykokidou E (1999) Composition and antifungal activity of the essential oil of *Salvia pomifera* subsp. *calycina* growing wild in Greece. J Esential Oil Res 11:655–659

Reuveni R, Fleischer A, Putievski E (1984) Fungistatic activity of essential oils from *Ocimum basilicum* Chemotypes. Phitopatol Z 10:20–22

Shahi SK, Shukla AC, Bajaj AK, Dikshit A (1999) Broad spectrum antimycotic drug for the control of fungal infection in human beings. Current Science 76:836–839

Sharma RC, Yadav PV (1996) Effect of fungicides on *Penicillium* rot and quality of chilgoza seed. Phytopathology 49:77–79

Singh J, Tripathi NN (1999) Inhibition of storage fungi of black gram (*Vigna mungo* L.) by some essential oils. Flavour Fragrance J 14:42–44

Tewari SN, Nayak MC (1991) Bioassay-guided isolation and identification of antifungal compounds from ginger. Tropical Agriculture 68:373–375

Thompson DP (1989) Fungitoxic activity of essential oil components on food storage fungi. Mycologia 81:151–153

Tripathi P, Dubey NK (2004) Exploitation of natural products as alternative strategy to control post-harvest fungal rotting of fruits and vegetables. Postharvest biology and Technology 32:235–245

Tripathi RD, Srivastava HS, Dixit SN (1978) A fungitoxic principle from leaves of *Lawsonia inermis* Lam. Experientia 34:51–52