Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
an open access publisher
http://ansinet.com

Plant Pathology Journal 7 (2): 174-178, 2008
ISSN 1812-5387

Efficacy of Essential Oils on the Conidial Germination, Growth of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc and Control of Postharvest Diseases in Papaya (*Carica papaya* L.)

Laura Leticia Barrera-Necha, Silvia Bautista-Baños,
Hilda Elizabeth Flores-Moctezuma and Abel Rojas Estudillo
Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional,
Carr. Yautepec-Jojutla Km. 8.5 San Isidro, Yautepec,
Morelos, México CP 62731

Abstract: The efficacy of nine essential oils were investigated *in vitro* on conidial germination and mycelial growth inhibition of *Colletotrichum gloeosporioides* isolate from papaya (*Carica papaya* L.). In general, better antifungal effect was observed with *Cinnamomum zeylanicum* and *Syzygium aromaticum* oils which had strong inhibition of conidial germination of *C. gloeosporioides* at 50, 100, 150, 200 and 250 μg mL^{-1} and a dose dependent inhibition mycelial growth was caused by these oils. *Teloxys ambrosioides*, *Mentha piperita* and *Ruta chalepensis* oils exhibited a moderate action at 150, 200 and 250 μg mL^{-1} on conidial germination and mycelial growth inhibition. *Allium sativum*, *Citrus aurantifolia* and *Eucalyptus globulus* oils had no antifungal activity at different concentration. Taking into account the *in vitro* results, *C. zeylanicum* and *S. aromaticum* oils were evaluated on papaya fruit during storage at ambient temperature and 14°C. The lowest infection percentage were for papaya fruits treated with *S. aromaticum* at 50 μg mL^{-1} at both temperature tested, nevertheless did not overcome the activity of synthetic fungicide. After storage at both temperature, values of Soluble Solids Content (SSC) was not significantly different. *S. aromaticum* oils may be a possibility to control *C. gloeosporioides* of papaya fruit.

Key words: *Carica papaya*, mycelial growth, percentage infection, antifungal activity, natural control

INTRODUCTION

Chemical control remains the main measure to reduce the incidence of postharvest diseases in various fruits and vegetables. Anthracnose disease caused by *Colletotrichum gloeosporioides* (Penz.) Penz. y Sacc. is a worldwide serious diseases in many fruits. The papaya provides a cheap source of vitamins and minerals in the daily diet of people. High postharvest losses due to development of anthracnose during storage and distribution of papaya fruit is being reported (Alvarez and Nishijima, 1987; Paull *et al*., 1997). Gamagae *et al.* (2003) reported use of sodium bicarbonate at 2% with the antagonist *C. oleophila* to control anthracnose in papaya how a promising alternative to chemicals. The essential oils produced by plants have long been known to have fungicidal properties (Wilson *et al*., 1997; Gogol *et al*., 1997; Pitarokili *et al*., 1999; Meepagala *et al*., 2002) safer environment to consumers and for the control of postharvest disease than synthetics. Moreover there are interest in the possibility of the application of essential oils to control plant pathogens and reduction in the use of chemical in agriculture. Systematic investigation of the antifungal activities of essential oils and their constituents has been reported by different authors. Kurita *et al*. (1981) screened 40 such compounds against seven species of fungi and Singh *et al.* (1980) similarly screened five essential oils against 22 species of fungi, including both human and plant pathogenic types. More recently, Muller-Riebau *et al.* (1995) screened nine essential oils against four species of plant pathogenic fungi, whereas Wilson *et al.* (1997) screened 49 essential oils against *Botrytis cinerea*. Daferera *et al.* (2003) screened eight essential oils against two species of fungi. The antifungal activity was strongly associated with monoterpenic phenols, especially thymol, carvacrol and eugenol, in the oils. Most of the essential oils have been reported to inhibit postharvest fungi in *in vitro* conditions (Bishop and Reagan, 1998; Singh and Tripathi, 1999; Bellerbeck *et al*., 2001; Hidalgo *et al*., 2002).

Corresponding Author: Laura Leticia Barrera-Necha, Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional. Carr. Yautepec-Jojutla Km. 8.5 San Isidro, Yautepec, Morelos, México CP 62731

However, the *in vivo* efficacy and practical activity of only a few of the essential oils have been studied. There are some reports on essential oils in enhancing storage life of fruit and vegetables by controlling their fungal rotting. The specific objectives in the present research were: to determine the effectiveness of nine commercial essential oils on the inhibition of conidia germination and mycelial growth of *C. gloesoporioides* and to evaluate postharvest disease development on *Carica papaya* treated with *S. aromaticum* and *C. zeylanicum*.

MATERIALS AND METHODS

Essential oils: In this study nine commercial essential oils (Company of Oils and Essences S.A., México, D.F.) were evaluated for their fungicidal activity: herb epazote (*Teloxys ambrosioides*), peppermint (*Mentha piperita*), fringed rude (*Ruta chalepensis*), thyme (*Thymus vulgaris*), cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*), garlic (*Allium sativum*), Mexican lime (*Citrus aurantifolia*) and eucalyptus (*Eucalyptus globules*).

Test microorganism: *Colletotrichum gloeosporioides* was isolated from papaya fruit with anthracnose symptoms, harvested in the state of Guerrero, México. Pure monosporic cultures were maintained on Potato-Dextrose-Agar (PDA). To maintain pathogenicity of the fungus, periodic inoculations and reisolations from infected papaya were carried out.

Activity of essential oils: To collect conidia for germination tests, Petri dishes with *C. gloeosporioides* were rinsed with 10 mL sterile distilled water, the surface was scrapped with a glass rod and the suspension was filtered through cotton wool. A conidial suspension of 1×10^{-5} mL was prepared in sterile distilled water. Essential oils dissolved in ethanol were tested at concentrations of 50, 100, 150, 200 and 250 μg mL^{-1}. Two hundred and seventy microliter of the conidia suspension and 30 μL of the essential oils were pipetted into wells of a row of a 96 multi-well microtitration plate. The control was a row containing sterile water with conidial suspension. After 10 h incubation at 28°C, 50 μL of the conidia-oil suspension was placed in 20 mm diameter agar disks and after 4 h at ambient temperature stained with lactophenol acid fushsin. The percent conidial germination was determined at 40X with light microscopy.

After sterilization of media of PDA essential oils were added at concentrations of 100, 150, 200, 250 and 300 μg mL^{-1} and poured in Petri plates (60×15 mm). A 5 mm agar disc containing the pathogen was placed at the center of each plate. They were stored at 25°C for seven days. Mycelial growth (colony diameter) was measured at the end of the incubation time. Six replications were considered simultaneously for each concentration of essential oils. Control Petri plates contained only PDA. Tests were finished when mycelium of the control plates reached the edge of the dishes. The experiment was repeated twice.

Fruit preparation: Papaya fruits were obtained from the wholesale market at Cuautla state of Morelos, México. Ripening stage of fruits was at the green to yellow color (index 2). Once in the laboratory fruits were dipped in essential oils of *C. zeylanicum* and *S. aromaticum* at concentrations of 50 and 250 μg mL^{-1} for 10 min and dried. Control fruits were dipped in commercial fungicide Mirage 45 CE (1 mL/2 L) (PROCLORAZ) after dried. They were storage at ambient temperature 28±3°C for five days and 14°C in humidified chambers for 8 days.

Activity of essential oils on fruits: After the storage period, percentage infection, disease severity, isolate of pathogen, SSC (hand refractometer Atago 0-32°), weight loss and firmness (Newton) were evaluated. Ten papayas per treatment were used as experimental units.

Statistical analysis: Treatments were arranged in a completely randomized design. All parameters evaluated were analyzed through ANOVA in statistical program Sigma Stat 2.0. For conidia germination, percentage infection, SSC and weight loss date transformation (vx) was carried out before ANOVA.

RESULTS AND DISCUSSION

Conidia germination: There were significantly different between treatments. Conidial germination after a 10 h incubation varied according to each essential oils. *C. zeylanicum* and *S. aromaticum* oils presented the least conidial germination on *C. gloeosporioides* compared with the remainder essentials oils (Table 1). At concentration of 50 at 250 μg mL^{-1} germination percentage was almost (1 at 0.3%) compared with control (96%). Inhibit effect of these essential oils were relevant on conidia germination. *A. sativum*, *T. ambrosioides*, *M. piperita* and *R. chalepensis* oils showed a moderate action (50%) conidia germination at concentrations of 150 a 250 μg mL^{-1} their effects is only partial and they do not kill all the conidia. *T. vulgaris*, *C. aurantifolia* and *E. globulus* oils showed no activity on *C. gloeosporioides* germination at different concentrations probably must be the volatility of their

Table 1: Effect of plant essential oils on percentage conidial germination of *C. gloeosporioides* at 10 h after incubation

	Conidial germination (%)					
	Oil concentration (μg mL^{-1})					
Essential oil	Control	50	100	150	200	250
Allium sativum	96±0.1^{a}	62±0.3^{b}	64±0.4^{b}	45±0.4^{c}	52±0.9^{c}	49.0±2.1^{c}
Cinnamomum zeylanicum	92±0.2^{a}	2±0.2^{b}	1±0.3^{b}	1±0.3^{b}	1±0.4^{b}	0.3±0.4^{b}
Citrus aurantifolia	92±0.1^{a}	99±0.4^{a}	96±1.1^{a}	92±1.7^{a}	96±1.4^{a}	94.0±1.1^{a}
Eucalyptus globulus	92±0.9^{a}	99±1.6^{a}	97±1.1^{a}	99±1.3^{a}	73±1.0^{b}	90.0±1.3^{a}
Menta piperita	95±0.1^{a}	55±2.1^{c}	79±2.2^{b}	56±3.3^{c}	35±4.0^{d}	37.0±2.4^{d}
Ruta chalepensis	96±1.0^{a}	94±2.3^{a}	92±2.4^{a}	82±2.6^{b}	46±2.7^{c}	34.0±2.2^{e}
Syzygium aromaticum	92±0.6^{a}	2±1.6^{b}	2±1.4^{b}	2±0.6^{b}	2±0.7^{b}	0.3±0.5^{b}
Teloxis ambrosiosides	95±0.2^{a}	78±0.4^{b}	79±0.6^{b}	75±0.4^{b}	60±0.8^{c}	79.0±0.5^{b}
Thymus vulgaris	96±0.8^{a}	96±1.4^{a}	92±1.8^{a}	98±1.9^{a}	98±1.6^{a}	90.0±1.5^{a}

Means followed by different letter(s) in each row are significantly different by to Duncan Test ($p \leq 0.05$)

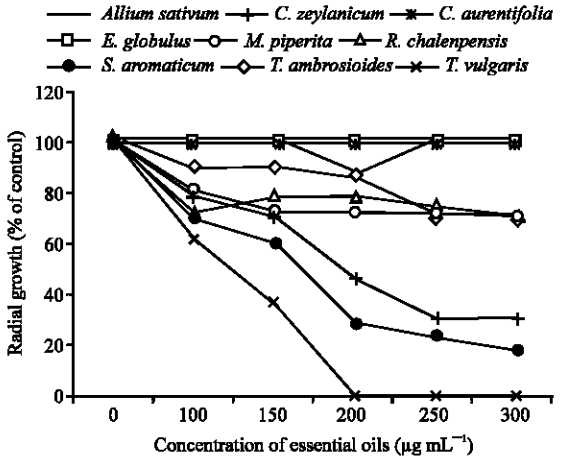

Fig. 1: Effect of plant essential oils on mycelial growth of *C. gloeosporioides* during seven days incubation

respective active principles. Wilson *et al.* (1997) found that *C. zeylanicum* had strong inhibitory effect on spore germination of *B. cinerea* at 1.56%. Bravo-Luna *et al.* (1998) reported highest inhibitory effect on sporulation of *Fusarium moniliforme* Sheld with *T. ambrosioides* (1000 ppm), *C. zeylanicum* (300 ppm), *S. aromaticum* (300 ppm) and *T. vulgaris* (150 ppm).

Mycelial growth: There was significantly different between treatments. *C. zeylanicum*, *S. aromaticum* and *T. vulgaris* oils had strong activities. A dose dependent inhibition of *C. gloeosporioides* mycelial growth was caused by these essential oils. Mycelial growth was totally inhibited by *T. vulgaris* at 200, 250 and 300 μg mL^{-1} (Fig. 1). *M. piperita*, *R. chalepensis* and *T. ambrosioides* oils exhibited a moderate action while *A. sativum*, *C. aurantifolia* and *E. globulus* oils presented null activity. These results agree with those of Montes and Carvajal (1998) who reported that *A. flavus* was totally inhibited for *C. zeylanicum T. ambrosioides* and *M. piperita*. Wilson *et al.* (1997) found that among the 49 essential oils tested, *Cymbopogon martini*, *Thymus zygis*, *Cinnamomum zeylanicum* and *Eugenia caryophyllata* demonstrated the most antifungal activity against *Botrytis cinerea*. Iscan *et al.* (2002) reported that essential oils of *Mentha piperita* strongly inhibited plant pathogenic microorganisms. Daferera *et al.* (2003) found that the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* was completely inhibited by *Origanum vulgare* and *Thymus capitatus* essential oils at relatively low concentrations (85-300 μg mL^{-1}). The essential oils as antimicrobial agents present two main characters: the first is their natural origin which means more safety to the people and the environment and the second is that they have be considered at low risk for resistance development by postharvest pathogenic fungi.

Control postharvest: After the storage period at room temperature (28°C), the lowest fruit weight loss value was 10.0% for treatment with *S. aromaticum* at 50 μg mL^{-1} and SSC were greater (7.8%). Firmness varied between treatments the lowest values were in fruit treated with *S. aromaticum* at 250 μg mL^{-1} (4.0 N) and the highest with *S. aromaticum* at 50 μg mL^{-1} (11.8 N). The lowest infection percentage (13.2 %) was for treatment with *S. aromaticum* at 50 and 250 μg mL^{-1}, nevertheless it did not overcome the activity of chemical fungicidal (1.6%). Severity index not varied significantly between fruit treated with essential oils and control (Table 2).

In fruit storage at 14°C the lowest weight loss values were (2.4%) for treatment with fungicidal and the greater in control fruit (3.0%). The firmness lowest values were for treatment with *C. zeylanicum* at 250 μg mL^{-1} (21.3 N) and the greatest with *S. aromaticum* at 50 μg mL^{-1} (44.9 N). SSC ranges were 6.5 at 7.5% in all treatments. The fruit treatment with fungicidal presented 0% infection and fruit treatment with *S. aromaticum* at 50 μg mL^{-1} had 3.3% infection. The lowest severity index values were in these treatments (Table 3). In *in vivo* evaluations the postharvest pathogenic fungi isolate from papaya

Table 2: Effect of essential oils on percentage weight loss, firmness, percentage solid solubles content, percentage infection and severity index of papaya at ambient temperature for 5 days

Treatments	Weight loss (%)	Firmness (N)	Solid solubles content (%)	Infection (%)	Severity index
Control	14.7±1.16^{a}	9.3±8.74^{a}	6.0±0.29^{a}	33.2±0.00^{c}	2.1±0.31^{b}
Fungicide	12.7±0.37^{a}	8.4±9.31^{a}	7.2±0.33^{a}	1.6±1.82^{a}	1.1±0.31^{a}
C. zeylanicum 50 μg mL^{-1}	12.8±0.24^{a}	7.8±7.64^{a}	6.3±0.24^{a}	23.1±0.48^{c}	1.9±0.73^{b}
C. zeylanicum 250 μg mL^{-1}	11.8±0.24^{a}	6.1±4.77^{a}	6.8±0.20^{a}	17.3±2.27^{b}	1.6±0.51^{b}
S. aromaticum 50 μg mL^{-1}	10.0±0.27^{a}	11.8±9.06^{a}	7.8±0.17^{a}	13.2±0.02^{b}	1.4±0.51^{b}
S. aromaticum 250 μg mL^{-1}	12.4±0.33^{a}	4.0±5.25^{a}	6.8±0.25^{a}	13.2±0.02^{b}	1.6±0.84^{b}

Means followed by different letter(s) in each column are significantly different by Duncan Test ($p \leq 0.05$), Severity index: 1 = 0%, 2 = 1-25%, 3 = 26-50%, 4 = 51-75% and 5 = 76-100%

Table 3: Effect of essential oils on percentage weight loss, firmness, percentage solid solubles content, percentage infection and severity index of papaya at 14°C for 8 days

Treatments	Weight loss (%)	Firmness (N)	Solid solubles content (%)	Infection (%)	Severity index
Control	3.0±0.53^{a}	35.8±9.86^{a}	7.0±0.21^{a}	33.3^{c}±0.00^{c}	2.0±0.00^{b}
Fungicidal	2.4±0.17^{a}	42.0±8.12^{a}	7.5±0.30^{a}	0.0±0.00^{a}	1.0±0.00^{a}
C. zeylanicum 50 μg mL^{-1}	2.7±0.17^{a}	40.9±11.17^{a}	7.3±0.22^{a}	13.2±0.02^{b}	1.4±0.16^{b}
C. zeylanicum 250 μg mL^{-1}	2.8±0.40^{a}	21.3±11.52^{a}	7.5±0.32^{a}	12.4±1.33^{a}	1.4±0.16^{b}
S. aromaticum 50 μg mL^{-1}	2.4±0.16^{a}	44.9±9.56^{a}	6.6±0.16^{a}	3.3±2.58^{a}	1.2±0.13^{a}
S. aromaticum 250 μg mL^{-1}	2.6±0.11^{a}	42.5±14.83^{a}	6.5±0.15^{a}	13.2±0.02^{b}	1.4±0.16^{b}

Means followed by different letter(s) in each column are significantly different by Duncan Test ($p \leq 0.05$), Severity index: 1 = 0%, 2 = 1-25%, 3 = 26-50%, 4 = 51-75% and 5 = 76-100%

were *Rhizopus stolonifer*, *C. gloeosporioides* and *Fusarium* sp. In this study, the essential oils tested no affected quality of fruit at two temperatures. Fruit treatment with *S. aromaticum* had the lowest infection percentage at 28 and 14°C. The composition, structure as well as functional groups of the oils play an important role in determining their antimicrobial activity. Usually compounds with phenolic groups are most effective (Deans *et al*., 1995; Dorman and Deans, 2000). Among these, the oils of clove, oregano, thyme and vanillin have been found to be most consistently effective against microorganisms. Awuah and Ellis (2002) reported the effective use of powders of leaves of *O. grattisimum* and cloves of *Syzygium aromaticum* combination with some packaging materials to protect groundnut kernels artificially inoculated with *A. parasiticus*. Bhaskara *et al.* (1998) reported potential of thyme oil volatiles as an antifungal preservative for strawberry fruits that are quire susceptible to decay caused by *B. cinerea* and *R. stolonifer*. Mature Embul bananas were treated with emulsions of either cinnamon bark or leaf (*Cinnamomum zeylanicum*) or clove (*Syzygium aromaticum*) oils to control postharvest diseases, packed under Modified Atmosphere (MA). Treatments with cinnamon bark and leaf oils controlled crown rot, whereas clove oil treatment did not affect crown rot development (Ranasinghe *et al*., 2005). Present study gives support for the application of certain essential oils to control postharvest pathogens fungi such as *Rhizopus stolonifer*, *C. gloeosporioides* and *Fusarium* sp.

CONCLUSION

The essential oils of *C. zeylanicum* and *S. aromaticum* inhibited the conidial germination and reduce growth of *C. gloeosporioides*. Infection by postharvest pathogenic fungi was also reduced by these essential oils. Because oils like these have low mammalian toxicity, are biodegradable and non-persistent in the environment, the possibility of developing essential oils for use in control postharvest diseases may be an attractive alternative.

ACKNOWLEDGMENTS

This research was supported by the Secretary of Postgraduate and Investigation, (Project: 20070433) and by the Commission of Operation and Support to Academic Activities from the National Polytechnic Institute.

REFERENCES

Alvarez, A.M. and W.T. Nishijima, 1987. Postharvest diseases of papaya. Plant Dis., 71 (8): 682-686.

Awuah, R.T. and W.O. Ellis, 2002. Effects of some groundnut packaging methods and protection with *Ocimum* and *Syzygium* powders on kernel infection by fungi. Mycopathologia, 154 (1): 29-26.

Bellerbeck, V.G., C.G. De Roques, J.M. Bessiere, J.L. Fonvieille and R. Dargent, 2001. Effect of *Cymbopogon nardus* (L.) W. Watson essential oil on the growth and morphogenesis of *Aspergillus niger*. Can. J. Microbiol., 47 (1): 9-17.

Bhaskara, M.V., P. Angers, A. Gosselin and J. Arul, 1998. Characterization and use of essential oil from *Thymus vulgaris* against *Botrytis cinerea* and *Rhizopus stolonifer* in strawberry fruits. Phytochemistry, 47 (8): 1515-1520.

Bishop, C.D. and L. Reagan, 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 (1): 57-60.

Bravo-Luna, L., K. Bermúdez-Torres and R. Montes-Belmont, 1998. Growth mycelial inhibition and sporulation of *Fusarium moniliforme* Sheld by plant essential oils and some their chemical components. Mex. J. Phytopathol., 16 (1): 18-23.

Daferera, J.D., N.B. Ziogas and G.M. Polissiou, 2003. The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis*. Crop Prot., 22 (1): 39-44.

Deans, S.G., R.C. Noble, R. Hiltunen, W. Wuryani and L.G. Penzes, 1995. Antimicrobial and antioxidant properties of *Syzygium aromaticum* (L.) Merr. and Perry: Impact upon bacteria, fungi and fatty acid levels in ageing mice. Flavour Fragrance J., 10 (5): 323-328.

Dorman, H.J.D. and S.G. Deans, 2000. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J. Applied Microbiol., 88 (2): 308-316.

Gamagae, S.U., D. Sivakumar, R.S. Wilson Wijeratnam and R.L.C. Wijesundera, 2003. Use of sodium bicarbonate and *Candida oleophila* to control anthracnose in papaya during storage. Crop Prot., 22 (5): 775-779.

Gogol, R., P. Baruah and S.C. Nath, 1997. Antifungal activity of the essential oil of *Litsea cubeba* Pers. J. Essential Oils Res., 9 (2): 213-215.

Hidalgo, P.J., J.L. Ubera, J.A. Santos, F. LaFont, C. Castelanos, A. Palomino and M. Roman, 2002. Essential oils in *Culamintha sylvatica*. Broma. ssp. ascendens (Jorden) P.W. Ball wild and cultivated productions and antifungal activity. J. Essential Oil Res., 14 (1): 68-71.

Iscan, G., N. Kirimer, M. Kürkcüoglu, K. Hüsnü Can Baser and F. Demirci, 2002. Antimicrobial screening of *Mentha piperita* essential oils. J. Agric. Food Chem., 50 (14): 3943-3946.

Kurita, N., M. Miyaji, R. Kurane and Y. Takahara, 1981. Antifungal activity of components of essential oils. Agric. Biol. Chem., 45 (4): 945-952.

Meepagala, K.M., G. Sturtz and D.E. Wedge, 2002. Antifungal constituents of the essential oil fraction of *Artemisia drancunculus* L. var. *dracunculus*. J. Agric. Food. Chem., 50 (24): 6989-6992.

Montes, B.R. and M. Carvajal, 1998. Control of *Aspergillus flavus* in maize with plant essential oils and their components. J. Food Prot., 61 (5): 616-619.

Muller-Riebau, F., B. Berger and O. Yegen, 1995. Chemical composition and fungitoxic properties to phytopathogenic fungi of essential ois of selected aromatic plants growing wild in Turkey. J. Agric. Food Chem., 43 (8): 2262-2266.

Paull, R.E., W. Nishijima, M. Reyes and C. Cavaletto, 1997. Postharvest handling and losses during marketing of papaya (*Carica papaya* L.). Postharvest Biol. Technol., 11 (3): 165-179.

Pitarokili, D., O. Tzakou, M. Couladis and E. Verykokidou, 1999. Composition and antifungal activity of the essential oil of *Salvia pomifera* subsp. *calycina* growing wild in Greece. J. Essential Oil Res., 11 (5): 655-659.

Ranasinghe, L., B. Jayawardena and K. Abeywickrama, 2005. An integrated strategy to control postharvest decay of *Embul banana* by combining essential oils with modified atmosphere packaging. Int. J. Food Sci. Technol., 40 (1): 97-103.

Singh, A.K., A. Dikshit, M.L. Sharma and S.N. Dixit, 1980. Fungitoxic activity of some essential oil. Econ. Bot., 34 (2): 186-190.

Singh, J. and N.N. Tripathi, 1999. Inhibition of storage fungi of black gram (*Vigna mungo* L.) by some essential oils. Flavour Fragrance J., 14 (1): 42-44.

Wilson, C.L., J.M. Solar, A. El Ghaouth and M.E. Wisniewski, 1997. Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. Plant Dis., 81 (2): 204-210.