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Antifungal activity of essential oils and their volatile constituents against respiratory tract pathogens causing Aspergilloma and Aspergillosis by gaseous contact

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Abstract: Aspergillosis is an acute chronic and rapidly fatal disease which is not contagious. Invasive Aspergillosis is often found in severely immuno-suppressed patients, and is characterized by invasion of blood vessels which can result into dissemination to other organs. Aspergilloma is a fungal ball that develops in previous cavitary lung lesions. Essential oils and their volatile constituents have been used as antifungal, anti-infectious and antimicrobial agents. Inhalation of vapours of the essential oils kill invaders attached to the inner respiratory lining and work synergistically with the body defences. In this study, 16 essential oils were used against *Aspergillus niger* and *A. fumigatus* of which about 14 oils proved to be effective. Results showed that the most effective oils against both *Aspergillus* species were found to be of *Cinnamomum zeylanicum* (Cinnamon), *Syzygium aromaticum* (Clove), *Carum carvi* (Caraway), *Cymbopogon citrates* (Lemongrass), *Foeniculum vulgare* (Fennel) and *Myristica fragrans* (Nutmeg). Moderately effective oils were of *Gaultheria procumbens* (Wintergreen), *Pinus palustris* (Turpentine), *Sesamum indicum* (Sesame), *Trachyspermum ammi* (Ajowain) and *Origanum vulgare* (Oregano). The oils of *Lavandula augustifolia* (Lavender), *Elletaria cardamomum* (Cardamon) and *Cymbopogon nardus* (Citronella) showed minimum activity. *Azadirachta indica* (Neem) and *Linum usitatissimum* (Linseed) showed no activity giving no inhibition zones.

Keywords: Antifungal activity, Aspergillosis, Aspergilloma, Essential oils

INTRODUCTION

The genus Aspergillus includes over 185 species which are ubiquitous and are especially common in soil and decaying vegetation. Around 20 species of the genus Aspergillus have been reported as the causative agents of opportunistic infections in human beings. Among these, *Aspergillus fumigatus* is the most commonly isolated species, followed by *A. flavus*, *A. niger*, *A. clavatus*, *A. glaucus*, *A. nidulans*, *A. oryzae*, *A. terreus*, *A. ustus* and less commonly *A. versicolor*. Aspergillosis is an opportunistic infection which can attack the lungs, ears, eyes, digestive system, kidney and brain (Chakraborty *et al*., 2006; Dubey *et al*., 2006). It develops mainly in individuals who are immuno-compromised either from disease or from immunosuppressive drugs and is a leading cause of death in acute leukemia and hematopoetic stem cell transplantation. Conversely, it may develop as an allergic response. Aspergillosis develops in the body either by inhalation or by penetration of inoculum (conidia) through surgical interventions and colonization of wounds. The most common causative species is *A. fumigatus* followed by *A. niger*. Aspergilloma also known as mycetoma or fungus ball is a clump of fungi which exists in the body cavity such as lungs and is generally associated with members of the genus *Aspergillus* but certain members of genus *Fusarium* and Class Zygomycetes may also form similar structures.

Resistant property of Aspergillus to some clinically used antifungal brings a worrying clinical prognostic in people attacked by Aspergillosis (Canuto and Rodero, 2002; Curtis *et al.*, 2005). Essential oils have been traditionally used for treatment of infections and diseases all over the world for centuries (Rios and Recio, 2005). The oils are used for example, in the food and beverage industries and as fragrances in perfumes and cosmetics. In addition to this, the oils also cover a broad spectrum of biological activity which has led to an increased interest among researchers. In recent years, there has been extensive research to explore and determine the antimicrobial activity of essential oils. Thymol, carvacrol, linalool, and eugenol are main constituents of some plant essential oils that have been shown to have a wide spectrum of activity against microbes (Kalemba and Kunicke, 2003; Dorman and Deans, 2000). The mechanism of action is still unclear but some studies suggest that compounds penetrate the cell, where they interfere with cellular metabolism (Guynot *et al*., 2003). Other studies show

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that phenols such as carvacrol and eugenol, disturb the cellular membrane and react with active sites of enzymes (Guynot *et al.*, 2003).

Although there have been numerous reports on the antifungal activity of essential oils applied directly to fungus (Alizadeh *et al*., 2010; Bansod and Rai, 2008; Tullio *et al*., 2007; Cavaleiro *et al*., 2006; Pinto *et al*., 2006; Pawar and Thaker, 2006), the studies concerning the antifungal activity of volatile vapours of the essential oils are relatively limited. (Jain and Agrawal, 2002) and few studies concerning inhibitory mode of essential oils have been reported. This study was conducted using essential oils of 16 different plants viz. *Trachyspermum ammi* (Ajowain), *Azadirachta indica* (Neem), *Foeniculum vulgare* (Fennel), *Linum usitatissimum* (Linseed), *Elletaria cardamomum* (Cardamon), *Gaultheria procumbens* (Wintergreen), *Syzygium aromaticum* (Clove), *Sesamum indicum* (Sesame), *Carum carvi* (Caraway), *Cymbopogon nardus* (Citronella) and *Myristica fragrans* (Nutmeg) which were earlier reported to be effective against different diseases (Singh *et al*., 1980; Inouye *et al*., 2001; Annussek, 2001; Nakahara *et al*., 2003; Lloyd *et al*., 2005; Soyulu *et al*., 2006; Schadler and George, 2006). Essential oils and their volatile vapours were investigated for their inhibitory effect on spore germination and mycelial growth of the two concerned fungi i.e. *Aspergillus fumigatus* and *Aspergillus niger*. The aim of this study was to evaluate the antimicrobial activity of plant volatile oils and to determine how the inhibition was affected by different exposure times to the essential oil vapour.

MATERIALS AND METHODS

Procurement and maintenance of culture: Fungal cultures of *A. niger* (MTCC No-2196) and *A. fumigatus* (MTCC No-3070) were procured from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh for the study. The cultures were maintained on Sabouraud dextrose agar plates and tubes at 25^0C.

Extraction procedure: Sixteen aromatic plants viz. *Azadirachta indica, Foeniculum vulgare, Linum usitatissimum, Elletaria cardamomum, Gaultheria procumbens, Syzygium aromaticum, Sesamum indicum, Carum carvi, Cymbopogon nardus, Myristica fragrans, Cinnamomum zeylanicum, Cymbopogon citratus, Origanum compactum, Trachyspermum ammi, Pinus palustris* and *Lavandula angustifolia* were collected from different regions of Dehradun District. Identification of plant specimen was done by Forest Research Institute, Dehradun. The air dried aerial parts were hydro-distilled in Clevenger's apparatus for 6 hrs. The aqueous phase was extracted with Dichloromethane and organic phase was dried with Sodium sulphate, filtered and the solvent evaporated until dryness by air dryer. The oils were *stored* in a refrigerator at 4^0C until required.

Antifungal assays:

Agar well diffusion assay: Preliminary analysis of antifungal activity was conducted using Agar well diffusion assay as described by Smania *et al.* (1995). Fungal inoculum was prepared in Tween 80 saline solution. Each fungal suspension was poured into the sterilized petriplates. After that molten SDA medium was poured into the petriplates containing inoculum and rotated to mix the inoculum and the medium uniformly and kept for solidification. After solidification wells of 6mm diameter were bored with the help of sterilized borer. The wells were filled with 20 µl/ml of essential oils. 20% DMSO was used as a solvent to dissolve the essential oils. Amphotericin B, Fluconazole, Clotrimazole and Nystatin were used as positive controls. The plates were incubated at 25^0C for 2-3 days. The results were expressed in terms of the diameter of the inhibition zone. All experiments were carried out in triplicates.

Gaseous contact method: Gaseous contact assay was done using the method described by Lopez *et al.*, 2005 with some alterations. For this method, 250 ml of SDA media was autoclaved and cooled to 40^0C. The small amount (70 µl) of medium was taken with the help of micropipette and poured on the inner wall of sterilized eppendorf tube's cap. The fungal cultures were inoculated in the media of the eppendorf tube's cap with the help of inoculation loop. The control tube used in this experiment contained only media and inoculum. No oil was used for control. After this, the essential oils were added in the eppendorf tubes containing different fungal inoculum and the lids of the tubes were closed. They were incubated at 25^0C for 3-4 days. The inhibition of spores and growth of fungal culture was observed and recorded.

Gas liquid chromatography of essential oil constituents: The GC analysis of essential oil was carried out using a GC apparatus (Agilent technologies, 6890 N). The analysis was performed with a DB-5 column (0.32 mm X 30m). Nitrogen gas was used as carrier gas with a flow rate of 1ml/min. The column was raised from 60 to 220^0C at a rate of 3^0C/min. The identification of oil components was based on their retention time with available literature values.

Statistical analysis: The inhibitory zones of essential oils were expressed as the mean $\pm$ Standard deviation and compared using Student Waller Ducan test at $P \leq 0.05$.

RESULTS

The results of antifungal assay showed that the essential oils and their volatile constituents possess antifungal activity against *A. niger* and *A. fumigatus*. In Agar well diffusion assay, out of total sixteen oils, the oil of *C.*

zeylanicum was found to be most effective against both the Aspergillus species showing inhibition zone of 50mm against both *A. niger* and *A. fumigatus* which was highest among all the oils used followed by oils of *Syzygium aromaticum* showing inhibition zone of 50mm against *A. niger* and 40 mm against *A. fumigatus*, *C. carvi* exhibiting inhibition zone of 45 mm against *A. niger* and 17 mm against *A. fumigatus*, *C. citratus* showing inhibition zone of 27 mm against *A. niger* and 32 mm against *A. fumigatus*, *M. fragrans* showed inhibition zone of 20 mm against *A. niger* and 35 mm against *A. fumigatus* and *F. vulgare* showing inhibition zone of 35 mm against *A. niger* and 20 mm against *A. fumigatus*. Moderate activity was shown against five oils with *G. procumbens* showing inhibition zone of 25 mm against both *A. niger* and *A. fumigatus* followed by *Sesamum indicum* showing zone of inhibition of 20 mm against *A. niger* and 30 mm against *A. fumigatus*, *Pinus palustris* showed inhibition zone of 25 mm against both *A. niger* and *A. fumigatus*, *Origanum vulgare* showing inhibition zone of 27 mm against *A. niger* and 20mm against *A. fumigatus*, *Trachyspermum ammi* showing inhibition zone of 20 mm against *A. niger* and 27 mm against *A. fumigatus*. Minimum activity was shown by essential oils of *Elletaria cardamomum* exhibiting 20 mm inhibition zone against *A. niger* and 10 mm against *A. fumigatus* followed by oil of *C. nardus* showing 15 mm and 13 mm inhibition zones against *A. niger* and *A. fumigatus* and *L. augustifolia* showing 20 mm inhibition zone against *A. niger* and 15mm against *A. fumigatus*. Oils of *A. indica* and *Lm. usitatissimum* were found to be inactive showing no inhibition against both the fungi. Amphotericin B, Fluconazole, Clotrimazole and Nystatin were used as positive controls and were found to be less effective than some of the used oils.

In Gaseous contact (Indirect contact), the antifungal and sporicidal activity of volatile constituents (vapour) of essential oils were observed against both the filamentous species. In this assay, oils of *Azadirachta indica*, *Sesamum indicum* and *Pinus palustris* produced complete growth inhibition in case of *A. niger* and *A. fumigatus* both after two days of incubation period at 25^0C. The vapours of these oils completely inhibited the growth after 2 days. Oils of *Syzygium aromaticum*, *Cinnamomum zeylanicum*, and *Lavandula augustifolia* showed spore inhibition in case of *A. niger* and growth inhibition in case of *A. fumigatus*. In other cases all the

Table 1. GC analysis of essential oils constituents.

S. No	Essential oils	Oils constituents (%)
1.	*C. zeylanicum*	Cinnamaldehyde (95.29 %)
2.	*M. fragrans*	α-pinene (3.06 %) β-pinene (2.20 %) sabinene (2.12 %) limonene (79.60 %)

Table 2. Agar well diffusion assay mean zone of inhibition (in mm) against *A. niger* and *A. fumigatus.*

S. No	Botanical name	Essential oils	Family	Zone of inhibition (in mm)	
				A. niger	*A. fumigatus*
1	*T. ammi*	Ajowain	Apiaceae	20±1.5	27±1.0
2	*A. indica*	Azadirachta	Meliaceae	-	-
3	*F. vulgare*	Fennel	Apiaceae	35±1.1	20±1.5
4	*L. usitatissimum*	Linseed	Linaceae	-	-
5	*E. cardamomum*	Cardamom	Zingiberaceae	20±1.5	10±1.1
6	*G. procumbens*	Wintergreen	Pyrolaceae	25±1.1	25±1.0
7	*S. aromaticum*	Clove	Myrtaceae	50±1.5	40±1.5
8	*S. indicum*	Sesame	Pedaliaceae	20±1.5	30±1.1
9	*C. carvi*	Caraway	Apiaceae	45±1.5	17±1.0
10	*C. nardus*	Citronella	Poaceae	15±1.0	13±1.0
11	*M. fragrans*	Nutmeg	Myristicaceae	20±1.5	35±1.5
12	*C. zeylanicum*	Cinnamon	Lauraceae	50±1.5	5±1.5
13	*C. citratus*	Lemongrass	Poaceae	27±1.0	32±1.1
14	*O. vulgare*	Oregano	Lamiaceae	27±1.0	20±1.5
15	*P. palustris*	Turpentine	Pineceae	25±1.1	25±1.5
16	*Lavandula angustifolia*	Lavender	Lamiaceae	20±1.1	15±1.0
17	AmphotericinB (100µg/disc)	-	-	10±0.0	10±0.0
18	Nystatin (100µg/disc)	-	-	15±0.0	15±0.0
19	Chloramphenicol (10µg/disc)	-	-	8±0.0	10±0.0
20	Fluconazole (10µg/disc)	-	-	-	-

Table 3. Gaseous contact assay to study the antifungal action of volatile constituents of oil against *A. niger* and *A. fumigatus.*

S. No	Botanical name	Oils	*A. niger* (growth pattern observed after 2 days)	*A. fumigatus* (growth pattern observed after 2 days)	Control
1	*T. ammi*	Ajowain	SI	SI	++++
2	*A. indica*	Azadirachta	-	-	++++
3	*F. vulgare*	Fennel	SI	+	++++
4	*L. usitatissimum*	Linseed	SI	+	++++
5	*E. cardamomum*	Cardamom	SI	SI	++++
6	*G. procumbens*	Wintergreen	SI	SI	++++
7	*S. aromaticum*	Clove	SI	-	++++
8	*S. indicum*	Sesame	-	-	++++
9	*C. carvi*	Caraway	-	SI	++++
10	*C. nardus*	Citronella	SI	SI	++++
11	*M. fragrans*	Nutmeg	-	SI	++++
12	*C. zeylanicum*	Cinnamon	SI	-	++++
13	*C. citratus*	Lemongrass	SI	+	++++
14	*O. vulgare*	Oregano	-	SI	++++
15	*P. palustris*	Turpentine	-	-	++++
16	*L. angustifolia*	Lavender	SI	-	++++

SI: Spore Inhibition,+: Less Growth, ++++: Complete Growth, -: no growth

oils generally showed spore inhibitory activity for both the fungi (Table 3). Distilled water taken as negative control showed complete growth of both the fungi at 25^0C for 2 days. This assay showed the antifungal and sporicidal activity of the vapours of the essential oils which are active in the gaseous state.

The identification of oil constituents was carried out by GC analysis of oil samples. Oils were analyzed among which one oil i.e. *Cinnamomon zeylanicum* (Cinnamon) oil was active while another oil of *Myristica fragrans* (Nutmeg) was less active.

The major constituent of Cinnamon oil was identified as Cinnamaldehyde (95.29%) through this analysis. The four constituents from Nutmeg oil were limonene (79.60%) while other three constituents i.e. α-pinene, β-pinene and sabinene were found to be less than 4%.(Table 1).

DISCUSSION

The traditional use of plants as medicines provide the basis for indicating which essential oils and plant oils may be useful for specific medical conditions. Historically, many plant oils and extracts such as tea tree, myrrh and clove have been used as topical antiseptics or have been reported to have antimicrobial properties (Hoffman, 1987; Lawless, 1995). It is important to investigate scientifically those plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds (Mitscher *et al.*, 1987). Various publications have documented the antimicrobial activity of essential oils and plant extracts including Rosemary, Peppermint, Bay, Basil, Tea tree, Celery seed and Fennel (Morris *et al.*,1979; Ross *et al.*, 1980; Yousef and Tawil, 1980; Hili *et al.*, 1997; Lis-Balchin and Deans, 1997). All the oils tested exhibited different degrees of antifungal activity against *A. fumigatus* and *A. niger*. Bansod and Rai (2008) reported antifungal activity of some essential oils against toxigenic Aspergillus species. Pawar and Thaker (2006) demonstrated *in vitro* efficacy of 75 essential oils against *A. niger.* Aggrawal *et al.*(2000) reported antimycotic activity of *C. martini* against *A. niger*. Quale *et al.* (1996) treated infections caused by *Candida* in AIDS patients with a drug based on Cinnamon. In our study we also found that essential oils extracted from *C. zeylanicum* demonstrated strong antifungal activity on both the species of Aspergillus. Plant oils are important source of fungitoxic compounds and they may provide a renewable source of useful fungicides that can be utilized in antimycotic drugs against *A. fumigatus* and *A. niger* infection in patients suffering from respiratory diseases. Among the plant oils tested, *Cinnamomum zeylanicum* (Cinnamon), *Syzygium aromaticum* (Clove), *Carum carvi* (Caraway), *Cymbopogon citratus* (Lemongrass), *Foeniculum vulgare* (Fennel) and *Myristica fragrans* (Nutmeg) showed high antimycotic activity. Most of the oils vapours inhibited the spore formation and also the growth of both the filamentous fungi. These results support the notion that plant essential oils have a role as

pharmaceuticals and preservatives. It was concluded that volatile vapours of essential oils possessed fungicidal activity at high dose level, preventing to resume growth after removal of essential oils. Therefore antifungal activity of volatile vapours of essential oils could be widely applicable in the variety of fungal infections, treatment and prevention.

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REFERENCES

Aggrawal, K.K., Ahmad, A., Santha, T.R., Jain, N., Gupta, V.K., Sushil, K. and Khanuja, S.P. (2000). Antimicrobial activity spectra of *Pelargonium graveolens* L. and *Cymbopogon winterians* Jowitt. oil constituents and acyl derivatives. *Journal of Medicinal and Aromatic Plant Sciences*., 22: 544-548.

Alizadeh, A., Zamani, E., Sharaifi, R., Javan-Nikkah, M. and Nazari, S. (2010). Antifungal activity of some essential oils against toxigenic *Aspergillus* species. *Commununications in Agricultural and Applied Biological Sciences*, 75(4): 761-7.

Annussek, G. (2001). Sesame oil. In: Gale encyclopedia of alternative medicine.GaleGroupandLooksmart. http://www.hort.purdue.edu/newcrop/ncnu.html accessed in July, 2006.

Bansod, S. and Rai, M. (2008). Antifungal activity of essential oils from Indian medicinal plants against human pathogenic *Aspergillus fumigatus* and *A. niger*. *World Journal of Medical Sciences*, 3(2): 81-88.

Canuto, M. M. and Rodero, F. G. (2002). Antifungal drug resistance to azoles and polyenes. *The Lancet Infectious Diseases*, 2: 550-563.

Cavaleiro, C., Pinto, E., Goncalves, M. J. and Salgueiro, L. R. (2006). Antifungal activity of Juniperus essential oils against dermatophyte, *Aspergillus* and *Candida* strains. *Journal of Applied Microbiology*., 100: 1333-1338.

Chakraborty, A., Marak, R.S.K., Sing, S., Gupta, S.O., Hurst, S. F. and Padhye, A. A. (2006). Brain abscess due to *Aspergillus nidulans*. *Journal of Medical Mycology*, 16 : 100-104.

Curtis, L., Conroy, L., Coli, S., Baker, K., Our, C.H., Hershow, R., Norlock-Cruz, F. and Scheff, P. (2005). *Aspergillus* surveillance project at a large tertiary core hospital. *Journal of Hospital Infections*, 59 : 188-196.

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

Dubey, A., Patwardhan, R.V., Sampth, S., Santoshi, V., Kolluri, S. and Nanda, A. (2006). Intracranial fungal granuloma: analysis of 40 patients and review of the literature.*Surgical Neurology*, 63 : 254-260.

Guynot, M. E., Ramos, A. J., Seto, L., Purvoy, P., Sanchis, V. and Marin, S. (2003). Antifungal activity of volatile compounds generated by essential oils against fungi commonly causing deterioration of bakery products. *Journal of Applied Microbiology*, 94: 893-899.

Hili, P., Evans, C.S. and Veness, R.G. (1997). Antimicrobial action of essential oils: the effect of dimethylsulfoxide on the activity of Cinnamon oil. *Letters in Applied Microbiology*, 24: 269-275.

Hoffman, D.L. (1987). The Herb User's Guide. Wellingborough, UK: Thorsons Publishing Group.

Inouye, S., Takizawa, T. and Yamaguchi, H. (2001). Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. *Journal of Antimicrobial Chemotherapy*, 47: 565-573.

Jain, S. K. and Agrawal, S.C. (2002). Fungistatic activity of some perfumes against otomycotic pathogens. *Mycoses*, 45: 88.

Kalemba, D. and Kunicke, A. (2003). Antibacterial and antifungal properties of essential oils. *Current Medicinal Chemistry*, 10 : 813-829.

Lawless, J. (1995). The illustrated Encyclopedia of Essential oils. Shaftesbury, UK: Element Books Ltd.

Lis-Balchin, M. and Deans, S.G. (1997). Bioactivity of selected plant essential oils against *Listeria monocytogenes*. *Journal of Applied Bacteriology*, 82 : 759-762.

Lloyd, A.C.C., Menon, T. and Umamaheshwari, K. (2005). *Indian Journal of Pharmacolgy*, 37 (6): 387-389.

Lopez, P., Saanchez, C., Battle, R. and Nerain, C. (2005). Solid and vapour phase antimicrobial activities of six essential oil: susceptibility of selected food borne bacterial and fungal strains. *Journal of Agricultural Food Chemistry*, 53: 6939-46.

Mitscher, L.A., Drake, S., Gollapudi, S.R. and Okwute, S.K.(1987). A modern look at folkloric use of anti-infective agents. *Journal of Natural Products*, 50 : 1025-1040.

Morris, A., Khettry, A. and Seitz, E.W. (1979). Antimicrobial activity of aroma chemicals and essential oils. *Journal of American Oil Chemist's Society*, 56 : 595-603.

Nakahara, K., Alzoreky, N. S., A., Yoshihashi, T., Nguyen, H. T. T. and Trakoontivakorn, G. (2003). Chemical composition and antifungal activity of essential oil from *Cymbopogon nardus*. *Japan Agricultural Research Quaterly*, 37 (4) : 249-252.

Pawar, V.C. and Thaker, V.S. (2006). *In vitro* efficacy of 75 essential oils against *A. niger*. *Mycoses*, 49 : 316-323.

Pinto, E., Pina-Vaz, C. and Salgueirro, L. (2006). Antifungal activity of the essential oil of *Thymus pulegioides* on *Candida*, *Aspergillus* and Dermatophyte species. *Journal of Medical Microbiology*, 55 : 1367-1373.

Quale, J. M., Landman, D., Zaman, M.Z., Burney, S. and Sathe, S. (1996). *In vitro* activity of *Cinnamomum zeylanicum* against azole resistant and sensitive *Candida* species and a pilot study of Cinnamon for oral Candidiasis. *Americal Journal of Chinese Medicine*, 24 : 103-109.

Rios, J. L. and Recio, M.C. (2005). Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology*, 100: 80-84.

Ross, S.A., El-Keltawi, N.E. and Megalla, S.E. (1980). Antimicrobial activity of some Egyptian aromatic plants.

Fitoterapia, 51 : 201-205.

Schadler, D. L. and George, A. A. (2006). Synthesis and bioassay of a volatile fungistatic agent. *The plant health Instructor*, DOI 10.1094/PHI-I-2006-0717-02.

Singh, A. K., Dikshit, A. and Sharma M. L. (1980). Fungi toxic activity of some essential oils. *Economic Botany*, 34 (2) : 186-190.

Smania, A., Monache, D. F., Gil, M. L., Benchetrit, L.C. and Cruz, F.S. (1995). Antibacterial activity of substance produced by the fungus *Pycnoporus sanguineus* (Fr) merr. *Journal of Ethnopharmacology*, 45 : 177-181.

Soyulu, E. M., Soyulu, S. and Kurt, S. (2006). Antimicrobial activities of the essential oils of various plants against Tomato Late blight disease agent *Phytophthora infestans*. *Mycopathologia*, 151 (2) : 119-128.

Tullio, V., Nostro, A., Mandras, N., Dugo, P., Banche, G., Cannatelli, M.A., Cuffinii, A.M., Alonzo, V. and Carlene, N.A.(2007). Antifungal activity of essential oils against filamentous fungi determined by broth microdilution and vapour contact methods. *Journal of Applied Microbiology*, 102 : 1544-1550.

Yousef, R. T. and Tawil, G.G. (1980). Antimicrobial activity of volatile oils. *Die Pharmazie*., 35 : 698-701.