



International Journal of Pharmacology

ISSN 1811-7775

science
alert

ansinet
Asian Network for Scientific Information

***In vitro* Antifungal Activity of Some Plant Essential Oils**

^{1,2}Abdallah M. Elgorban, ¹Ali H. Bahkali, ²Mohamed A. El-Metwally, ³Mohamed Elsheshtawi and ^{1,4}Mohamed A. Abdel-Wahab

¹Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

²Plant Pathology Institute, Agricultural Research Center, Giza, Egypt

³Department of Plant Pathology, College of Agriculture, Mansoura University, Mansoura 35516, Egypt

⁴Department of Botany, College of Science, Sohag University, Sohag, Egypt

ARTICLE INFO

Article History:

Received: October 18, 2014

Accepted: December 10, 2014

Corresponding Author:

Abdallah M. Elgorban,

Department of Botany and

Microbiology, College of Science,

King Saud University, P.O. Box 2455,

Riyadh 11451, Saudi Arabia

ABSTRACT

In the present study the antimicrobial activity of essential oils of allium bulb (*Allium cepa* L.), black cumin seeds (*Nigella sativa* L.) and eucalyptus (*Eucalyptus globulus* Labill) were evaluated against five fungi (*Fusarium oxysporum* f.sp. *melonis*, *Fusarium solani*, *Fusarium verticillioides*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani*). *Allium cepa* oils completely inhibited the mycelial growth of *Fusarium oxysporum* f.sp. *melonis*, *Fusarium solani* and *Sclerotinia sclerotiorum* at 500 ppm concentration. While, *E. globulus* oil completely inhibited the radial growth of *F. solani*, *S. sclerotiorum* *R. solani*. On the other hand, the percentage inhibition varied in case of *Nigella sativa* oil is 28.6-73.9% from (*F. oxysporum* f.sp. *melonis*, *F. verticillioides*) at 500 ppm concentration. Whereas, the spore germination of *F. oxysporum* f.sp. *melonis* and *F. solani* were completely inhibited by the application of *A. cepa* oil, while the oil of eucalyptus completely inhibited the spore germination of *F. solani* and highly effective against spore germination of *F. oxysporum* f.sp. *melonis*, *F. verticillioides* with 98 and 93%, respectively at 500 ppm concentration. Considering the inhibition in the growth and spore germination, it concluded that allium and eucalyptus essential oils could be used as possible bio-fungicides alternative to synthetic fungicides against phytopathogenic fungi.

Key words: Allium, black cumin, *Fusarium verticillioides*, eucalyptus

INTRODUCTION

The prevalence of pathogens resistant to drug is one of the most serious impediments to effective treatment of microbial diseases. Over the centuries extracts and other essential plants have evoked interest as sources of natural products. They have been examined for their potential uses as alternative therapy for the treatment of many microbial infection. It is known that many aromatic plants synthesize diverse bio-active compounds such as phenylpropanoids, sesquiterpenes and monoterpenes. These secondary metabolites as mixtures of different lipophilic and volatile constituents are known to play important role in defense

system of higher plants (Reichling, 1999). A large number of studies have revealed that medicinal plants can be considered as rich sources of antimicrobial agents (Ates and Erdogru, 2003; Mahesh and Satish, 2008; Al-Taisan *et al.*, 2014; Hatamleh *et al.*, unpublished). Essential oils derived from medicinal plants such as *Mentha arvensis* (Imai *et al.*, 2001), *Nigella sativa* (El-Kamali *et al.*, 1998), *Cryptomeria japonica* (Cheng *et al.*, 2005), *Ziziphora clinopodioides* (Sonboli *et al.*, 2006), *Thymbra capitata* (Salgueiro *et al.*, 2004), *Salvia sclarea* (Pitarokili *et al.*, 2002), *Pimpinella anisum* (Kosalec *et al.*, 2005), *Thymus pulegioides* L. (Pinto *et al.*, 2006) and *Tagetes patula* (Romagnoli *et al.*, 2005) were effective against numerous fungi.

Soil-borne pathogenic fungi i.e., *Fusarium* spp., *Rhizoctonia*, *Pythium*, *Phoma* spp., etc., that cause serious problem of sudden wilt (vine decline) are considered as hazardous organisms of minatory vegetable production in the nursery, protected agriculture and open fields, because of the decrease in plants number and quality (Gwynne *et al.*, 1997).

These pathogens of sudden wilt especially in some crops such as Muskmelon (El-Sheshtawi *et al.*, 2014) and watermelon (Anjorin and Mohammed, 2014) cause rotting, pre and post-emergence damping off, while the older plants are affected by wilting or bad growth during flowering or fruiting stages reflecting on plant growth and on the yield quantity and quality (Martyn and Miller, 1996). The recommended systemic or contact chemical fungicides for the control of such pathogens are extremely harmful in the short or long terms on the man health and on the environment, causing dangerous diseases i.e., cancer, kidney, liver diseases and others (Mansour, 1992). Furthermore, emergence of fungicide-resistant pathogens and increasing concerns over use of agrochemicals on storage products have highlighted the need of research for new antifungal substances derivative from several sources especially medicinal plants.

The purpose of this study was to evaluate the antifungal activity of essential oil derived from Allium bulb (*Allium cepa* L.), black cum in (*Nigella staiva* L.) and eucalyptus (*Eucalyptus globulus* Labill) leaves against the mycelial growth of five pathogenic fungi and spore germination of the three *Fusarium* spp. tested.

MATERIALS AND METHODS

Isolation and identification of soil borne pathogenic fungi:

Muskmelon plant roots with root rot characteristic were collected from the infected muskmelon fields. The samples were collected from Dakahliya and Demiat Governorates, Egypt. The root parts were examined under light microscope to verify the presence of pathogens and then cut into pieces (3-4 mm and surface sterilized with 0.1% sodium hypochlorite for 30 sec). The samples were washed three times with sterilized distilled water and transferred aseptically on Potato Dextrose Agar (PDA). The plates were incubated at 25±2°C and examined daily for emergence of the fungal hyphae. Pure cultures of the pathogens were stored at 4°C on PDA tubes. Isolated pathogens were identified as described by Barnett and Hunter (1998), Booth (1977) and Kora *et al.* (2005).

Isolation of essential oils: Allium bulb (*A. cepa*), black cum in seeds (*N. sativa*) and Eucalyptus leaves (*E. globulus*) were cleaned with distilled water and air-dried at room temperature (25±2°C). These samples (150 g each), in triplicate, were subjected to hydrodistillation for 6 h using a Clevenger-type apparatus (Chang *et al.*, 2001) followed by determination of oil contents. Leaf essential oils were stored in the refrigerator.

In vitro antifungal assay: *Allium cepa*, *N. sativa* and *E. globulus* oil were dissolved in 0.1% Tween 80 and added to the PDA medium which was autoclaved and cooled in a water bath to 45°C to obtain the final concentrations (10, 50, 100, 250 and 500 ppm) and 0.1% Tween 80 without essential oil was served as control. The mixed PDA medium was poured into 90 mm Petri plates with 20 mL for each plate. Mycelial disks of 5 mm diameter were cut out from the periphery of 7-day-old cultures of the pathogens. The disks were inoculated at the center of the plates. Four replicates were performed per treatment. All the pathogens were incubated at 25±2°C. The diameters of colonies were measured after five days. Experiments were performed three times.

Conidial germination: Plant Essential oils were dissolved in 0.1% Tween 80 and added into a 15 mL glass tube containing 5 mL Potato Dextrose Broth (PDB) to obtain the final concentrations (10, 50, 100, 250 and 500) and 0.1% Tween 80 without essential oil was added as control. One hundred microliters of spore (1×10^6 spores mL⁻¹) of the *Fusarium* spp. were added into each tube. After 24 h from incubation at 25±2°C, on shaker (200 rpm), 100 conidia per replicate were observed microscopically to determine the germination rate. Four replicates were used for every treatment and experiments were performed three times.

RESULTS

Effect of essential oils against the radial growth and spore germination of *Fusarium oxysporum* f.sp. *melonis*:

Data in Table 1 revealed that allium oil completely inhibited the mycelial growth of *F. oxysporum* f.sp. *melonis* at 500 concentration, while oil of eucalyptus reduced the mycelial growth of the pathogen with increasing concentrations reached to 67.2% inhibition at 500 ppm concentration. Black cum in oil inhibited the mycelial growth by 28.6% as related to the control. On the other hand, results showed that spore germination of *F. oxysporum* f.sp. *melonis* was completely inhibited by allium essential oil at 500 ppm concentration, this was followed by eucalyptus and black cum in essential oil that giving 98 and 72.5% reduction in spore germination at 500 ppm concentration, respectively (Table 1). The estimated LD₅₀ in the fungus for *A. cepa* and *E. globulus* essential oil was 75.75 and 128.31 ppm L⁻¹. While, LD₅₀ of *N. sativa* was 3499.13 ppm L⁻¹ and this value indicate a low toxicity of *N. sativa* to *F. oxysporum* f.sp. *melonis*.

Effect of essential oils against the radial growth and spore germination of *Fusarium solani*:

All the three essential oils tested showed varied degree of inhibition over control in the mycelial growth of the pathogen *F. solani* at different concentrations (Table 2). The maximum inhibition of the mycelial growth was recorded in *E. globulus* which completely inhibited the mycelial growth at 250 and 500 ppm

Table 1: Effect of essential oils against the radial growth and on spore germination of *Fusarium oxysporum* f.sp. *melonis* (Inhibition %)

Essential oils	0 (ppm)	10 (ppm)	50 (ppm)	100 (ppm)	250 (ppm)	500 (ppm)	LD ₅₀ (ppm L ⁻¹)	Slope±SE
Radial growth								
<i>A. cepa</i>	0.0	17.8	28.2	48.6	74.9	100.0	75.75	1.52±0.016
<i>N. sativa</i>	0.0	5.4	7.7	15.0	22.8	28.6	3499.13	0.68±0.018
<i>E. globulus</i>	0.0	10.4	44.4	48.0	58.7	67.2	128.31	0.91±0.015
Spore germination								
<i>A. cepa</i>	0.0	14.2	77.5	81.0	96.7	100.0	29.36	2.08±0.027
<i>N. sativa</i>	0.0	7.5	16.0	26.7	38.0	72.5	280.68	1.19±0.017
<i>E. globulus</i>	0.0	26.2	41.0	68.2	87.0	98.0	42.45	1.41±0.015

Table 2: Effect of essential oils against the radial growth and on spore germination of *Fusarium solani* (Inhibition %)

Essential oils	0 (ppm)	10 (ppm)	50 (ppm)	100 (ppm)	250 (ppm)	500 (ppm)	LD ₅₀ (ppm L ⁻¹)	Slope±SE
Against the radial growth								
<i>A. cepa</i>	0.0	44.3	53.2	63.7	66.7	100.0	26.22	0.83±0.011
<i>N. sativa</i>	0.0	0.8	0.8	9.7	29.1	43.5	594.53	1.70±0.046
<i>E. globulus</i>	0.0	34.2	63.3	78.5	100.0	100.0	22.94	1.62±0019
On spore germination								
<i>A. cepa</i>	0.0	30.0	45.5	77.5	81.8	100.0	34.65	1.34±0.014
<i>N. sativa</i>	0.0	28.0	41.2	45.2	57.8	68.5	110.68	0.61±0.110
<i>E. globulus</i>	0.0	64.8	80.0	90.2	100.0	100.0	6.23	2.45±0.0124

Table 3: Effect of essential oils against the radial growth and on spore germination of *Fusarium verticillioides* (Inhibition %)

Essential oils	0 (ppm)	10 (ppm)	50 (ppm)	100 (ppm)	250 (ppm)	500 (ppm)	LD ₅₀ (ppm L ⁻¹)	Slope±SE
Against the radial growth								
<i>A. cepa</i>	0.0	3.2	23.9	29.9	53.5	69.7	211.44	1.33±0.017
<i>N. sativa</i>	0.0	12.0	27.8	56.7	60.2	73.9	122.32	1.09±0.012
<i>E. globulus</i>	0.0	27.5	34.5	57.0	80.3	91.9	55.77	1.17±0.015
On spore germination								
<i>A. cepa</i>	0.0	14.8	33.2	65.0	67.5	79.5	83.75	1.13±0.014
<i>N. sativa</i>	0.0	25.0	43.8	67.0	68.0	84.8	54.32	0.96±0.011
<i>E. globulus</i>	0.0	33.5	39.0	76.2	81.5	93.0	36.22	0.12±0.012

concentrations and LD₅₀ of this oil was 42.45 ppm L⁻¹. Also, *A. cepa* essential oil was completely inhibited the mycelial growth of *F. solani* but at 500 ppm concentration. Reduction in percentage in spore germination increment is clear as essential oil concentration increased. Highest essential oil concentration of all tested oils caused most profound reduction of spore germination. The highest reduction in spore germination came from eucalyptus and allium oil which completely inhibited the spore germination of the fungus at 500 ppm and LD₅₀ of both oils were 6.23 and 34.65 ppm L⁻¹, respectively. Conversely, the essential oil of black cummin gave moderate reduction in spore germination with 68.50% at 500 ppm concentration and LD₅₀ of this oil was 110.68 ppm L⁻¹ (Table 2).

Effect of essential oils against the radial growth and spore germination of *Fusarium verticillioides*: The effects of different concentrations of the three essential oils tested on the radial growth of *F. verticillioides* are shown in Table 3. All three essential oils were found to inhibit *F. verticillioides* growth in a concentration-dependent manner. Eucalyptus essential oil showed the maximum inhibition in the mycelial growth of *F. verticillioides* with 91.9% when compared to control and the estimated LD₅₀ obtained by linear regression was 55.77 ppm L⁻¹. This was followed by *N. sativa* and *A. cepa* oil which significantly exhibited the radial growth of *F. verticillioides* giving 73.9 and 69.7% reduction in the mycelial growth of the pathogen and LD₅₀ were 122.32 and

211.44 ppm L⁻¹, respectively. Spore germination of *F. verticillioides* was inhibited by allium, black cummin and eucalyptus oils at all concentrations (Table 3). All three essential oils at 500 ppm concentration were highly effective against spore germination. The maximum inhibition of spore germination was recorded in *E. globulus* oil that giving 93% reduction in spore germination when compared with control. This was followed by black cummin and allium oils which produced 84.75 and 79.50% inhibition in spore germination of the fungus, respectively.

Effect of essential oils against the radial growth of *Sclerotinia sclerotiorum*: The *in vitro* results revealed that the growth of *S. sclerotiorum* was completely inhibited by the application of *A. cepa* and *E. globulus* at 500 ppm concentration and the estimated LD₅₀ were 19.84 and 147.03 ppm L⁻¹, respectively. While, black cummin oil showed moderate reduction in the mycelial growth of the fungus with 49.4% and LD₅₀ was 940.61 ppm L⁻¹ (Table 4).

Effect of essential oils against the radial growth of *Rhizoctonia solani*: *Eucalyptus globulus* oil was more effective against the pathogen tested which exhibited 100% mycelial inhibition of the fungus and LD₅₀ of oil was 71.67 ppm L⁻¹. This was followed by *A. cepa* essential oil which significantly inhibited the mycelial growth of *R. solani* with 97.1% when compared with control and LD₅₀ was 77.09 ppm L⁻¹ (Table 4).

Table 4: Effect of essential oils against the radial growth of *Sclerotinia sclerotiorum* and *Rhizoctonia solani* (Inhibition %)

Essential oils	0 (ppm)	10 (ppm)	50 (ppm)	100 (ppm)	250 (ppm)	500 (ppm)	LD ₅₀ (ppm L ⁻¹)	Slope±SE
<i>Sclerotinia sclerotiorum</i>								
<i>A. cepa</i>	0.0	4.5	8.1	36.7	55.4	100.0	147.03	2.17±0.031
<i>N. sativa</i>	0.0	12.3	20.2	25.6	29.5	49.4	940.61	0.63±0.016
<i>E. globulus</i>	0.0	48.5	61.7	57.2	73.2	100.0	19.84	0.79±0.010
<i>Rhizoctonia solani</i>								
<i>A. cepa</i>	0.0	11.2	15.2	60.1	89.9	97.1	77.09	2.01±0.024
<i>N. sativa</i>	0.0	2.3	5.7	8.6	10.3	34.5	2101.39	0.98±0.029
<i>E. globulus</i>	0.0	4.9	25.3	72.4	84.2	100.0	71.67	2.29±0.031

DISCUSSION

This study was conducted to assess the antifungal efficacy of essential oils from *A. cepa* bulb, *N. sativa* seeds and *E. globulus* leaves against soil borne pathogenic fungi. It was observed that *A. cepa* oil completely inhibited the mycelial growth *F. oxysporum* f.sp. *melonis*, *F. solani* and *S. sclerotiorum*, also significantly inhibited the mycelial growth of *F. verticillioides* and *R. solani* at 500 ppm concentration. Furthermore, this essential oil completely inhibited spore germination of *F. oxysporum* f.sp. *melonis* and *F. solani*.

The inhibitory activity of essential oil and extracts of Allium plants against fungi was reported by numerous authors, though, in general, essential oils are more effective inhibitors of fungi than of bacteria (Zaika, 1988; Hatamleh *et al.*, 2014). Antifungal activity of Allium plants was stated by Yin and Tsao (1999), who observed that *A. cepa* showed highest antifungal activity against three *Aspergillus* species tested. Phay *et al.* (1999) reported that *A. cepa* exhibited marked antifungal activities against numerous fungal species particularly *Penicillium roqueforti* and *Aspergillus oryzae* which showed high sensitivity. Also, the essential oil of Allium plants showed marked antimicrobial activity against *Staphylococcus aureus*, *Salmomella Enteritidis* and three fungi, *A. niger*, *Penicillium cyclopium* and *F. oxysporum* (Benkeblia, 2004). Kocic-Tanackov *et al.* (2012) showed that the essential oil of *A. cepa* and *A. sativum* had a stronger inhibitory effect on the *A. versicolor* mycelial growth and sterigmatocystin production. This antifungal activity of *A. cepa* probably depend on the major components dimethyl-trisulfide, methyl-propyl-trisulfide, diethyl-1, 2,4-tritriolol, methyl-(1-propenyl)-disulfide and methyl-(1-propenyl)-trisulfide (Kocic-Tanackov *et al.*, 2012).

As a result, essential oil of eucalyptus inhibited the mycelial growth in all of the tested fungi after 5 days. The most important soil borne fungi infected muskmelon, *F. solani*, *S. sclerotiorum* and *R. solani*, had 100% complete inhibition and highly effective against the mycelial growth and spore germination of *F. oxysporum* f.sp. *melonis* and *F. verticillioides*. These results in agreement with those obtained by Hur *et al.* (2000) and Katooli *et al.* (2011) that showed Eucalyptus oil inhibited the mycelial growth of three phytopathogenic fungi such as *Colletotrichum gloeosporioides*, *R. solani* and *Pythium* spp. Also, Hatamleh *et al.* (2014) demonstrated that eucalyptus extract

significantly inhibited the mycelial growth and spore germination of three *Fusarium* species and the mycelial growth of *S. sclerotiorum* and *R. solani*.

This high antifungal activity of eucalyptus oil probably related to the components such as 1,8-cineole (Saad *et al.*, 2006), citronellol (Su *et al.*, 2006), citronellal (Batish *et al.*, 2006), ρ -cymene (Su *et al.*, 2006), citronellyl acetate, limonene, eucamalol (Watanabe *et al.*, 1993), limonene, linalool, α -pinene (Sartorelli *et al.*, 2007), alloocimene, aromadendrene and α -terpineol (Duke, 2004; Liu *et al.*, 2008).

CONCLUSION

The use of essential oils as natural fungicides is of immense significance in view of the environmental and toxicological implications of the indiscriminate use of synthetic fungicides and reducing the problem of increasing fungi resistance.

ACKNOWLEDGMENTS

The researchers extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group no RGP-277.

REFERENCES

- Al-Taisan, W.A., A.H. Bahkali, A.M. Elgorban and M.A. El-Metwally, 2014. Effective influence of essential oils and microelements against *Sclerotinia sclerotiorum*. Int. J. Pharmacol., 10: 275-281.
- Anjorin, S.T. and M. Mohammed, 2014. Effect of seed-borne fungi on germination and seedling vigour of watermelon (*Citrullus lanatus* thumb). Afr. J. Plant Sci., 8: 232-236.
- Ates, D.A. and O.T. Erdogru, 2003. Antimicrobial activities of various medicinal and commercial plant extracts. Turk. J. Biol., 27: 157-162.
- Barnet, H.L. and B.B. Hunter, 1998. Illustrated Genera of Imperfect Fungi. 4th Edn., American Phytopathological Society Press, St. Paul, USA., ISBN-13: 978-0890541920, Pages: 240.
- Batish, D.R., H.P. Singh, N. Setia, S. Kaur and R.K. Kohli, 2006. Chemical composition and phytotoxicity of volatile essential oil from intact and fallen leaves of *Eucalyptus citriodora*. Zeitschrift Naturforschung, 61: 465-471.

- Benkeblia, N., 2004. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). LWT-Food Sci. Technol., 37: 263-268.
- Booth, C., 1977. *Fusarium*: Laboratory Guide Identification of the Major Species. Commonwealth Mycological Institute, Kew, ISBN-13: 9780851983837, Pages: 58.
- Chang, S.T., P.F. Chen and S.C. Chang, 2001. Antibacterial activity of leaf essential oils and their constituents from *Cinnamomum osmophloeum*. J. Ethnopharmacol., 77: 123-127.
- Cheng, S.S., H.Y. Lin and S.T. Chang, 2005. Chemical composition and antifungal activity of essential oils from different tissues of Japanese cedar (*Cryptomeria japonica*). J. Agric. Food Chem., 53: 614-619.
- Duke, J., 2004. Dr. Duke's phytochemical and ethnobotanical databases. <http://www.ars-grin.gov/duke/>.
- El-Kamali, H.H., A.H. Ahmed and A.A.M. Mohammed, 1998. Antibacterial properties of essential oils from *Nigella sativa* seeds, *Cymbopogon citratus* leaves and *Pulicaria undulata* aerial parts. Fitoterapia, 69: 77-78.
- El-Sheshtawi, M., A.H. Bahkali, W.A. Al-Taisan and A.M. Elgorban, 2014. Pathogenicity of *Fusarium oxysporum* f.sp. *melonis* to melon genotypes (*Cucumis melo* L.) and its biocontrol. J. Pure Applied Microbiol., 8: 317-324.
- Gwynne, B.J., T.R. Gordon and R.M. Davis, 1997. A new race of *Fusarium oxysporum* f. sp. *melonis* causing Fusarium wilt of muskmelon in the central valley of California. Plant Dis., 81: 1095-1095.
- Hatamleh, A.A., A.H. Bahkali, M. El-Sheshtawi, M.A. ElMetwally and A.M. Elgorban, 2014. Inhibitory influence of plant extracts on soil borne fungi infecting Muskmelon (*Cucumis melo* L.). Int. J. Pharmacol., 10: 322-327.
- Hur, J.S., S.Y. Ahn, Y.J. Koh and C.I. Lee, 2000. Antimicrobial properties of cold-tolerant eucalyptus species against phytopathogenic fungi and food-borne bacterial pathogens. Plant Pathol. J., 16: 286-289.
- Imai, H., K. Osawa, H. Yasuda, H. Hamashima, T. Arai and M. Sasatsu, 2001. Inhibition by the essential oils of peppermint and spearmint of the growth of pathogenic bacteria. Microbios, 106: 31-39.
- Katooli, N., R. Maghsodlo and S.E. Razavi, 2011. Evaluation of eucalyptus essential oil against some plant pathogenic fungi. J. Plant Breed. Crop Sci., 3: 41-43.
- Kocic-Tanackov, S., G. Dimic, J. Levic, I. Tanackov, A. Tepic, B. Vujicic and J. Gvozdanovic-Varga, 2012. Effects of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) essential oils on the *Aspergillus versicolor* growth and sterigmatocystin production. J. Food Sci., 77: M278-M284.
- Kora, C., M.R. McDonald and G.J. Boland, 2005. Epidemiology of *Sclerotinia* rot of carrot caused by *Sclerotinia sclerotiorum*. Can. J. Plant Pathol., 27: 245-258.
- Kosalec, I., S. Pepeljnjak and D. Kustrak, 2005. Antifungal activity of fluid extract and essential oil from anise fruits (*Pimpinella anisum* L., *Apiaceae*). Acta Pharmaceutica, 55: 377-385.
- Liu, X., Q. Chen, Z. Wang, L. Xie and Z. Xu, 2008. Allelopathic effects of essential oil from *Eucalyptus grandis* x *E. urophylla* on pathogenic fungi and pest insects. Front For. China, 3: 232-236.
- Mahesh, B. and S. Satish, 2008. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World J. Agric. Sci., 4: 839-843.
- Mansour, N., 1992. Integrated pest management and pesticide management: The future challenge in the Arab world. Proceedings of the Scientific Symposium on Pesticide Hazards: Effects on Human, Animal Health and Environmental Pollution, May 4-7, 1992, Beirut, pp: 241-266.
- Martyn, R.D. and M.E. Miller, 1996. Monosporascus root rot/vine decline: An emerging disease of melons worldwide. Plant Dis., 80: 716-725.
- Phay, N., T. Higashiyama, M. Tsuji, H. Matsuura, Y. Fukushi, A. Yokota and F. Tomita, 1999. An antifungal compound from roots of welsh onion. Phytochemistry, 52: 271-274.
- Pinto, E., C. Pina-Vaz, L. Salgueiro, M.J. Goncalves and S. Costa-de-Oliveira *et al.*, 2006. Antifungal activity of the essential oil of *Thymus pulegioides* on *Candida*, *Aspergillus* and dermatophyte species. J. Med. Microbiol., 55: 1367-1373.
- Pitarokili, D., M. Couladis, N. Petsikos-Panayotarou and O. Tzakou, 2002. Composition and antifungal activity on soil-borne pathogens of the essential oil of *Salvia sclarea* from Greece. J. Agric. Food Chem., 50: 6688-6691.
- Reichling, J., 1999. Plant-Microbe Interaction and Secondary Metabolites with Antiviral, Antibacterial and Antifungal Properties. In: Functions of Plant Secondary Metabolites and their Exploitation in Biotechnology, Wink, M. (Ed.). Vol. 3, Taylor and Francis, Sheffield, UK., ISBN-13: 9781841270081, pp: 187-273.
- Romagnoli, C., R. Bruni, E. Andreotti, M.K. Rai, C.B. Vicentini and D. Mares, 2005. Chemical characterization and antifungal activity of essential oil of capitula from wild Indian *Tagetes patula* L. Protoplasma, 225: 57-65.
- Saad, E.Z., R. Hussien, F. Saher and Z. Ahmed, 2006. Acaricidal activities of some essential oils and their monoterpenoid constituents against house dust mite, *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). J. Zhejiang Univ. Sci. B, 7: 957-962.
- Salgueiro, L.R., E. Pinto, M.J. Goncalves, C. Pina-Vaz and C. Cavaleiro *et al.*, 2004. Chemical composition and antifungal activity of the essential oil of *Thymra capitata*. Planta Med., 70: 572-575.
- Sartorelli, P., A.D. Marquioreto, A. Amaral-Baroli, M.E.L. Lima and P.R.H. Moreno, 2007. Chemical composition and antimicrobial activity of the essential oils from two species of *Eucalyptus*. Phytother. Res., 21: 231-233.

- Sonboli, A., M.H. Mirjalili, J. Hadian, S.N. Ebrahimi and M. Yousefzadi, 2006. Antibacterial activity and composition of the essential oil of *Ziziphora clinopodioides* subsp. *Bungeana* (Juz.) Rech. f. from Iran. *Zeitschrift Naturforschung C*, 61: 677-680.
- Su, Y.C., C.L. Ho, E.I.C. Wang and S.T. Chang, 2006. Antifungal activities and chemical compositions of essential oils from leaves of four eucalypts. *Taiwan J. For. Sci.*, 21: 49-61.
- Watanabe, K., Y. Shono, A. Kakimizu, A. Okada, N. Matsuo, A. Satoh and H. Nishimura, 1993. New mosquito repellent from *Eucalyptus camaldulensis*. *J. Agric. Food Chem.*, 41: 2164-2166.
- Yin, M.C. and S.M. Tsao, 1999. Inhibitory effect of seven *Allium* plants upon three *Aspergillus* species. *Int. J. Food Microbiol.*, 49: 49-56.
- Zaika, L.L., 1988. Spices and herbs: Their antimicrobial activity and its determination. *J. Food Saf.*, 9: 97-118.