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ANTIFUNGAL ACTIVITY OF SELECTED ESSENTIAL OILS AGAINST THE FUNGAL SPECIES OF THE GENUS *EUROTIUM* BY CONTACT VAPOUR

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ABSTRACT

The aim of this study was evaluation of the antifungal activity of 5 essential oils (EOs),we used concretely thyme, clove, basil, jasmine and rosemary, by vapour contact against the fungal species *Eurotium rubrum*, *E. chevalieri* and *Eurotium* sp.. Each fungus was inoculated in the centre on Czapek Yeast Autolysate Agar (CYA) plates. Plates were tightly sealed with parafilm and incubated for 7, 14, and 21 days at 25 ± 1 °C (three replicates were used for each treatment). Volatile phase effect of concentration 50 µl of the essential oils was found to inhibit on growth of *E. rubrum*, *E. chevalieri* and *Eurotium* sp.. Complete growth inhibition of the isolates by EOs of thyme and clove was observed. The essential oil (EO) of basil had antifungal activity on growth of *E. chevalieri* only after 7th and 14th days of the incubation, but in case of *Eurotium* sp. on all days of cultivation. Only *E. rubrum* was sensitive to jasmine EO and *E. chevalieri* to basil EO after all days of the incubation. Data was evaluated statistically by 95.0 % Tukey HSD test.
The conclusions indicate that volatile phase of combinations of thyme oil and clove oil showed good potential in the inhibition of growth of *Eurotium* spp. EOs should find a practical application in the inhibition of the fungal mycelial growth in some kind of the food.

Keywords: *Eurotium chevalieri*, *Eurotium rubrum*, *Eurotium* sp., essential oils, vapour, antifungal activity

INTRODUCTION

Fungi are significant destroyers of foodstuffs during storage, rendering them unfit for human consumption by retard their nutritive value and sometimes by producing mycotoxins (**Bajaj *et al.*, 1975**). In bakery processing, the most common type of microbial spoilage is mould growth and in many cases it is the major factor governing shelf-life (**Earle and Putt, 1984**). Xerophilic moulds are food spoilage organisms of low moisture food such as bakery products (**Abellana *et al.*, 1999**; **Hocking and Pitt, 1980**; **Samson, 1989**; **Vega *et al.*, 1998**), dried fruit (**Pitt and Christian, 1968**) and grain flours (**Beuchat and Hwang, 1996**).The number of resistant fungal pathogenic and toxinogenic species is rising (**Deising *et al.*, 2008**) and the need for a new antifungal substances and alternative treatments are becoming increasingly obvious. The food industry at present is facing a tremendous pressure from consumers for using chemical preservatives to prevent the growth of food borne and spoiling microbes. The increasing demand for safe and natural food, without chemical preservatives, led many researchers to investigate the antimicrobial effects of natural compounds (**Rasooli *et al.*, 2008**). Between these compounds belong essential oils (EOs). EOs of plants are of growing interest both in the industry and scientific research because of their antibacterial, antifungal, and antioxidant properties and make them useful as natural additives in foods (**Pattnaik *et al.*, 1997**). The antimicrobial activity of spices and EOs is well recognized. Several studies have reported results on their preservative action (**Mishra and Dubey, 1994; Paster *et al.*, 1995; Montes – Belmont and Carvajal, 1998; Vázquez *et al.*, 2001**).Eugenol, thymol and carvacrol, for example, are well known phenolic representatives found in clove, thyme and oregano oils and have been demonstrated to have an inhibitory activity against both bacteria (**Hao *et al.*, 1998**; **Ultee *et al.*, 1998**; **Didry *et al.*, 1994**) and fungi (**Scora and Scora, 1998**; **Mahmoud, 1994**; **Boonchird and Flegel, 1982**). The mechanism of phenolic toxicity towards fungi is based on the inhibition of fungal enzymes, which contain –SH groups in their active sites (**Cowan, 1999**; **Celimene *et al.*, 1999**). For this reason, these substances can be used as safe and effective alternatives to synthetic preservatives (**Wilson *et al.*, 1997**).
The objective of our study was evaluation of the antifungal activity of 5 EOs by vapour contact against the selected fungal species of the genus *Eurotium*.

MATERIAL AND METHODS

Species of microscopic fungi

A total of three isolates, were used. These isolates, two *Eurotium rubrum*, *Eurotium chevalieri* and one isolate *Eurotium* sp. were obtained from different Slovak wheat grains (*Triticum aestivum* L.). These isolates belong to the microorganisms' collection at the Department of Microbiology of the Slovak Agricultural University in Nitra. They were inoculated on Czapek Yeast Autolysate Agar (CYA) plates.

Essential plant oils

The essential oils used in this study were of thyme, clove, basil, jasmine and rosemary. They were all supplied by Calendula company a.s. (Nová Ľubovňa, 238 A, Slovakia). Essential oils were extracted by hydro distillation and its quality and stability were certified by suppliers.

Effect of essentials plants oil

Evaluation by filter paper was making by the method adapted from **Guynot *et al.* (2003)**. Plates were kept in an inverted position. A sterilized filter paper (square of 1 x1 cm) was placed in the centre of the lid and 50 µl of pure essential oil were added to the paper. Blank were made by adding 50 µl of water to it. Each fungus was inoculated in the centre on Petri plates with needle–inoculated. Plates were tightly sealed with parafilm and incubated for 7th, 14th, and 21st days at 25 ± 1 °C (three replicates were used for each treatment). Diameters of the growing colonies were measured weekly with the aid of ruler. Data was evaluated statistically by 95.0 % Tukey HSD test.

RESULTS AND DISCUSSION

The antimicrobial compounds in plant materials are commonly found in the EOs fractions obtained by steam or supercritical distillation, pressing, or extraction by liquid or volatile solvents. The traditionally most well – known antimicrobial species and herbs are clove, cinnamon, chilli, garlic, thyme, oregano and rosemary. But also bay, basil, sage, anise, coriander, allspice, marjoram, nutmeg, cardamom, mint, parsley, lemongrass, celery, cumin, fennel and many others

have been reported to have an inhibitory effect toward microorganisms (**Deans and Ritchie, 1987; Hili *et al*., 1997; Hammer *et al*., 1999; Elgayyar *et al*., 2001**)

In this study was evaluated the ability of 5 essential oils to inhibit bakery spoilage fungi – species of genus *Eurotium*. Although there have been many reports of the antibacterial and antifungal effects of EOs per se (**Al-Burtamani *et al*., 2005; Holley and Patel, 2005**) there are fewer on essential oils vapours. Vapours are generally more effective than the oils against fungi (**Lopez *et al*., 2005; Tullio *et al*., 2007**). The aim of our study was to find the activity of the volatile components of essential oils clove, basil, jasmine, thyme and rosemary against fungal growth of *Eurotium chevalieri*, *Eurotium rubrum* and *Eurotium* sp.. The combination of EOs had significant impact on the growth of all tested isolates (Table 1).

Table 1 Antifungal activity of tested essential oils to *Eurotium* spp.

Isolates	Days	Essential oils (mean colony diameter in mm ± SD)					Control
		Clove	Basil	Jasmine	Thyme	Rosemary	
Eurotium rubrum	**7th**	$0^{a} \pm 0$	$0^{a} \pm 0$	$0^{a} \pm 0$	$0^{a} \pm 0$	$0^{a} \pm 0$	$18.67^{b} \pm 1.53$
	14th	$0^{a} \pm 0$	$10.67^{b} \pm 0.58$	$0^{a} \pm 0$	$0^{a} \pm 0$	$26,67^{c} \pm 0$	$19^{c} \pm 0$
	21st	$0^{a} \pm 0$	$35^{b} \pm 0$	$0^{a} \pm 0$	$0^{a} \pm 0$	$35^{b} \pm 0$	$43.34^{c} \pm 2.89$
Eurotium chevalieri	**7th**	$0^{a} \pm 0$	$0^{a} \pm 0$	$3.67^{ac} \pm 1.53$	$0^{a} \pm 0$	$0^{a} \pm 0$	$6.67^{b} \pm 4.73$
	14th	$0^{a} \pm 0$	$0^{a} \pm 0$	$9.67^{b} \pm 4.51$	$0^{a} \pm 0$	$0^{a} \pm 0$	$7^{ac} \pm 4.58$
	21st	$0^{a} \pm 0$	$3^{a} \pm 1,73$	$26.67^{b} \pm 2.89$	$0^{a} \pm 0$	$0^{a} \pm 0$	$25^{b} \pm 5$
***Eurotium* sp.**	**7th**	$0^{a} \pm 0$	$0^{a} \pm 0$	$25.34^{b} \pm 6.43$	$0^{a} \pm 0$	$2.6^{a} \pm 1.15$	$28.67^{b} \pm 1.15$
	14th	$0^{a} \pm 0$	$0^{a} \pm 0$	$25.67^{b} \pm 5.86$	$0^{a} \pm 0$	$3^{a} \pm 1$	$28.67^{b} \pm 1.15$
	21st	$0^{a} \pm 0$	$0^{a} \pm 0$	$26.34^{b} \pm 5.69$	$0^{a} \pm 0$	$3^{a} \pm 1$	$29^{b} \pm 1$

Data in the column followed by different letters are significantly different in 95.0 % Tukey HSD test, P < 0.05

Among the EOs, the ones that totally inhibited growth of all isolates were clove and thyme regardless of time of the days of cultivation. Basil, jasmine and rosemary were also able to inhibit the growth of mould, but depending of the isolates and time of incubation. The most sensitive isolate was *Eurotium chevalieri*. The growth of *Eurotium chevalieri* was inhibited by 4 from 5 tested EOs. Clove, thyme and rosemary had antifungal activity against *Eurotium chevalieri* at all days (7^{th}, 14^{th} and 21^{st}) of the incubation. The essential oil (EO) of basil was effective only after 7^{th} and 14^{th} days of the incubation. On the other hand jasmine, in comparison with control, had positive or stimulating effect on the growth of this isolate after 14^{th} and 21^{st} days of cultivation (Figure 1). Isolate *Eurotium rubrum* was sensitive to clove, jasmine and thyme at all days of incubation, but basil and rosemary had antifungal activity only after 7^{th} days of incubation (Figure 2). *Eurotium* sp. was sensitive to clove and thyme at all days of the incubation, as well as *E. chevalieri* and *E. rubrum*, but only *Eurotium* sp. had shown sensibility to basil at all days of incubation. Result showed that EOs jasmine had no the notable effect on the growth *Eurotium* sp., but rosemary, in comparison with the control, had inhibiting effect on its growth (Figure 3).

Nielsen and Rios (2000) had proven that the volatile compounds from mustard, cinnamon, garlic and clove EOs were efficient in the control of common bread spoilage fungi. **Omidbeygi *et al.* (2007)** studied antifungal activity of EOs of thyme, summer savory and clove in culture medium and tomato paste against *Aspergillus flavus*. *A. flavus* were inoculated in SDB (Sabouraud Dextrose Broth) and tomato paste and then 0, 50, 200, 350 and 500 ppm of EOs were added to each sample and then kept at 25 ± 0.5°C for 2 months. Result showed that all EOs cloud inhibits the growth of *A. flavus* and the thyme oil and summer savory, showed the strongest inhibition at 350 ppm and 500 ppm, respectively. **Rasooli and Abyaneh (2004)** tested inhibition of *Aspergillus parasiticus* growth and its aflatoxin production exposed to the EOs extracted from two varieties of thyme. They concluded that substitution of currently used antifungal and aflatoxin inhibiting chemicals by natural compounds such as thyme is recommended. Also **Blooma and Etcheverry (2008)** in their study analysed five essential oils (anise, boldus, mountain thyme, clove and griseb) and their inhibitory effect on growth and accumulation of mycotoxins by *Aspergillus* section *flavi*. All of these essential oils showed significant impact on aflatoxins accumulation, but the most effective were boldus, poleo and mountain thyme EOs.

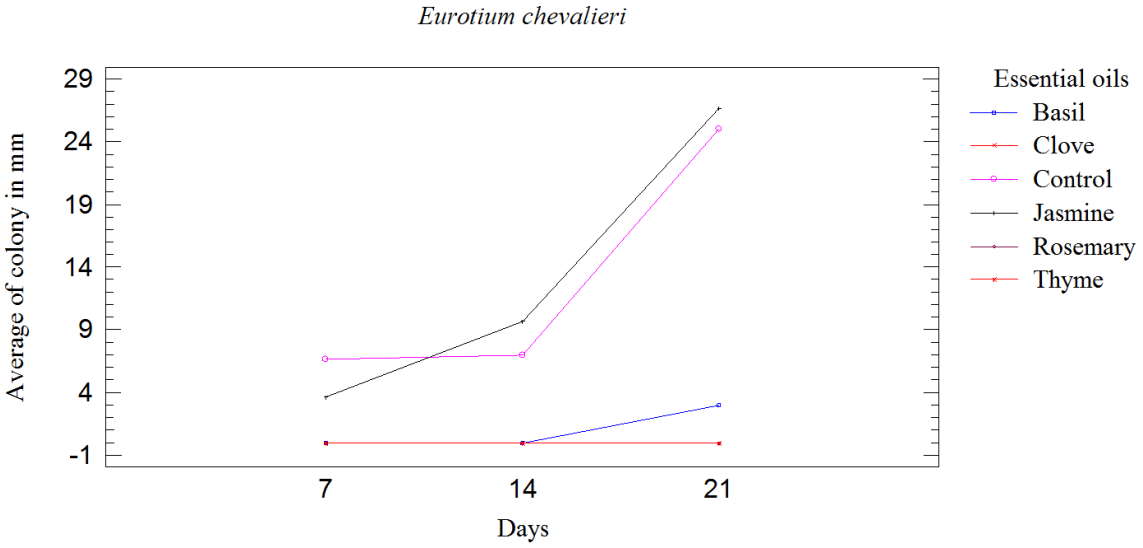

Figure 1 Effect of tested EOs on the growth of *Eurotium chevalieri*. Three replications were done for each treatment. *P < 0.05*, with the respect to the control.

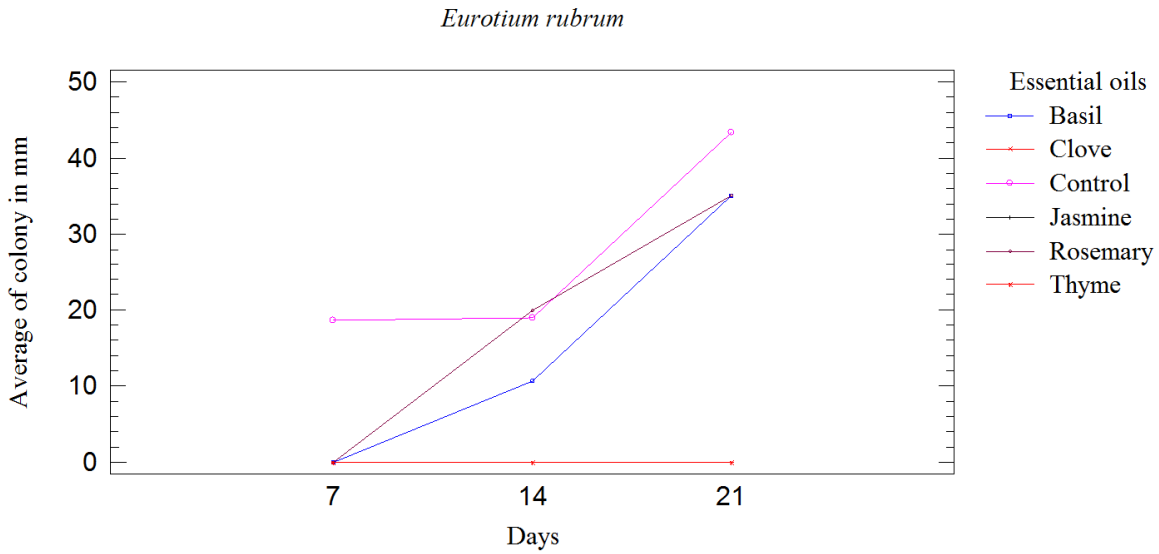

Figure 2 Effect of tested EOs on the growth of *Eurotium rubrum*. Three replications were done for each treatment. $P < 0.05$, with the respect to the control.

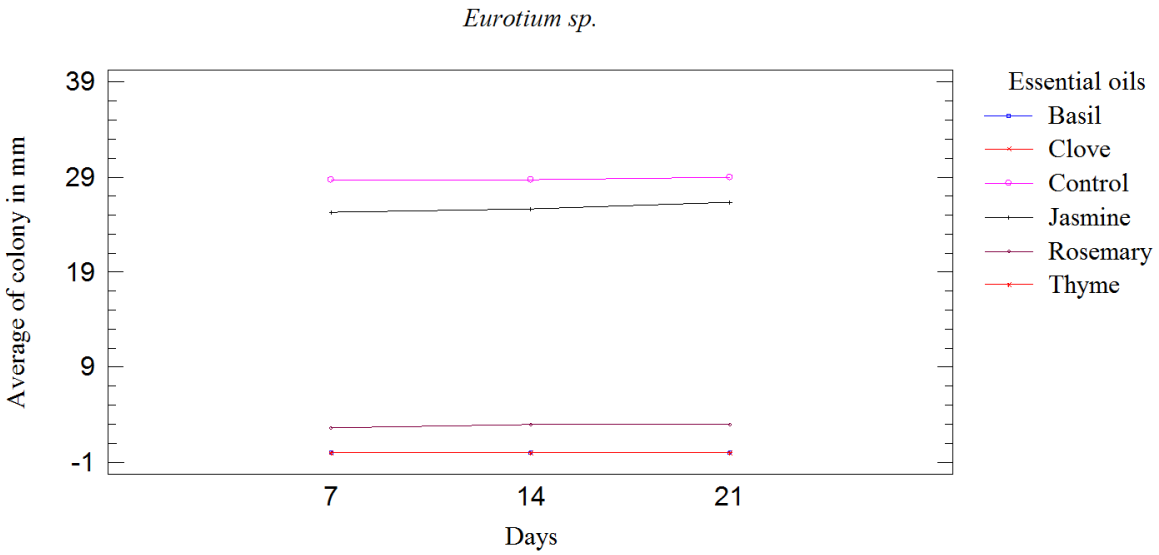

Figure 3 Effect of tested EOs on the growth of *Eurotium* sp. Three replications were done for each treatment. $P < 0.05$, with the respect to the control.

Guynot *et al.* (2003) studied volatile fraction of 16 EOs for activity against the more common fungi causing spoilage of bakery products (*Eurotium amstelodami, E. repens, E. rubrum, Aspergillus flavus, A. niger* and *Penicillium corylophilum*). They found that 5 EOs (cinnamon leaf, clove, bay, lemongrass and thyme) had potential antifungal activity against all species tested.

Our results showed that the most effective EOs are thyme and clove and the weakest EO was jasmine. Basil and rosemary appeared to be less effective, but many of authors had different results. **Basílico and Basílico (1999)** tested inhibitory effect of EOs of oregano, mint, basil sage and coriander, on the mycelial growth and ochratoxin A production by *Aspergillus ochraceus* NRRL 3174. Cultures were incubated on yeast extract-sucrose (YES) broth, at concentration 0, 500, 750 and 1000 ppm of essential oils during 7th, 14th and 21st days at 25 °C. They found oregano and mint completely inhibited *A. ochraceus* and its production of ochratoxin A up to 21 days, while basil was only effective up to 7 days. But **Atanda *et al.* (2007)** proved that EOs of sweet basil optimal protective dosage of 5 % (v/v) was fungistatic on *Aspergillus parasiticus* CFR 223 and aflatoxins produced *in vitro* and on fungal development on sorghum grains with a residual effect that lasted for 32 days. **Dambolena *et al.* (2010)** tested inhibitory effect of basil EO of *Ocinum basilicum* and *O. gratissimum* L. against *Fusarium verticillioides* and fumunosin production. Their result showed that the oils from different location had antifungal activity and antimycotoxinogenic property. **Soylu *et al.* (2010)** studied antifungal activity of EOs, such as origanum, lavender and rosemary against *Botrytis cinerea*. Volatile phase effects were consistently found to be more effective on fungal growth than contact phase effect. But rosemary and lavender essential oils were inhibitory at relatively higher concentrations (25.6 µg/ml).

However, comparison of the data obtained by different studies is difficult, because of differences in plants extract compositions, in methodologies followed to assess antimicrobial activity and in microorganisms chosen to be tested (**Hammer *et al.*, 1999**).

CONCLUSION

The conclusions indicate that volatile phase of combinations of thyme oil and clove oil showed good potential to inhibit growth of *Eurotium* spp. Even though that EOs such as basil, rosemary and jasmine had not antifungal activity like thyme and clove EOs, they should find a practical application in the inhibition of the fungal mycelial growth. In our study we tested the antifungal activity of EOs against common fungi causing spoilage of bakery products for 7th, 14th and 21st days of cultivation. Results showed that all of the tested EOs had antifungal activity after 7th days of cultivation, except jasmine, which appeared to be effective only to isolate *E. rubrum* after all days of culture. According to all, bakery products haven't longer shelf life, therefore EOs can be use as preservative materials on bakery products, but further safety studies are necessary.

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REFERENCES

ABELLANA, M., BENEDI, J., SANCHIS, V., RAMOS, A.J. 1999. Water activity and temperature effects on germination and growth of *Eurotium amstelodami*, *E. chevalieri* and *E. herbariorum* isolates from bakery products. *Journal of Applied Microbiology*, 87, 371-380.

AL-BURTAMANI S.K.S., FATOPE, M.O., MARWAH, R.G,, ONIFADE, A.K., AL-SAIDI, S.H. 2005. Chemical composition, antibacterial and anti fungal activities of the esencial oil of *Haplophyllum tuberculatum* from Oman. *Journal of Ethnopharmacology*, 96, 107-112.

ATANDA, O.O., AKPAN, I., OLUWAFEMI, F. 2007. The potential of some spice essential oils in the control of *A. parasiticus* CFR 223 and aflatoxin production. *Food Control*, 18, 601-607.

BAJAJ, B. S., GHOSH A. K. 1975. Antifungal antibiotics in perspective, p. 297-311. In RAYCHAUDHURI, S. P., VERMA, A., BHARGAVA, K. S. and MEHROTRA, B. S. (ed.), *Advances in mycology and plant patology*. Sagar Printers, New Delhi, India.

BASÍLICO, M.Z., BASÍLICO, J.C. 1999. Inhibitory effects of some essential oils on *Aspergillus ochraceus* NRRL 3174 growth and ochratoxin A production. *Letters in Applied Microbiology*, 29, 238-241.

BEUCHAT, L.R., HWANG, C.A. 1996. Evaluation of modified dichloran 18% glycerol (DG18) agar for enumerating fungi in wheat flour: a collaborative study. *International Jouranl of Food Microbiology*, 29, 161-166.

BLOOMA, R., ETCHEVERRY, M.G. 2008. Application of essential oils in maize grain: Impact on *Aspergillus* section *Flavi* growth parameters and aflatoxin accumulation. *Food Microbiology*, 25, 324-334.

BOONCHIRD, C., FLEGEL, T.W. 1982. In vitro antifungal activity of eugenol and vanillin against *Candida albicans* and *Cryptococcus neoformans*. Canadian Journal of Microbiology, 28, 1235-1241.

CELIMENE, C.C., MICALES, J.A., FERGE, L., YOUNG, R.A.1999. Efficacy of pinosylvins against white-rot and brown-rot fungi. *Holzforschung*, 53, 491–497.

COWAN, M.M. 1999. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12, 564-582.

DAMBOLENA, J.S., ZUNINO, M.P., LÓPEZ, A.G., RUBINSTEIN, H.R., ZYGADLO, J.A., MWANGI, J.W., THOITHI, G.N., KIBWAGE, I.O., MWALUKUMBI, J.M., KARIUKI, S.T. 2010. Essential oils composition of *Ocimum basilicum* L. and *Ocimum gratissimum* L. from Kenya and their inhibitory effects on growth and fumunosin production by *Fusarium verticillioides*. *Innovative Food Science and Emerging Technologies*, 11, 410-414.

DEANS, S.G., RITCHIE, G. 1987. Antibacterial properties of plant essential oils. *International Journal of Food Microbiology*, 5, 165-180.

DEISING, H.B., REIMANN, S., PASCHOVATI, S.F. 2008. Mechanism and significance of fungicide resistance. *Brazilian Journal of Microbiology*, 39, 286-295.

DIDRY, N., DUBREUIL, L., PINKAS, M. 1994. Activity of thymol, carvacrol, cinnamaldehyde and eugenol on oral bacteria. *Pharmaceutica Acta Helvetiae*, 69, 25-28.

EARLE, M.D., PUTT, G.J. 1984. Microbial spoilage and use of sorbate in bakery products. *Food Technology N. Z.* , 19, 25-36.

ELGAYYAR, M., DRAUGHON, F.A., GOLDEN, D.A., MOUNT, J.R. 2001. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. *Journal of Food Protection*, 64, 1019-1024.

GUYNOT, M.E., RAMOS, A.J., SETÓ, L., PURROY, P., SANCHIS, V., MARÍN, S. 2003. Antifungal activity of volatile compounds generated by essential oils against fungi commonly cusing deterioration of bakery products. *Journal of Applied Microbiology*, 94, 893-899.

HAMMER, K.A., CARSON, C.F., RILEY, T.V. 1999. Antimicrobial activity of essential oils and others plant extracts. *Journal of Applied Microbiology*, 8, 985-990.

HAO, Y.Y., BRACKETT, R.E., DOYLE, M.P. 1998. Efficacy of plant extracts in inhibiting *Aeromonas hydrophila* and *Listeria monocytogenes* in refrigerated, cooked poultry. *Food Microbiology*, 15, 367-378.

HILI, P., EVANS, C.S., VENESS, R.G. 1997. Antimicrobila action of essential oils: the effect of dimethylsulfoxide on the activity of cinanamon oil. *Letters in Applied Microbiology*, 24, 269-275.

HOCKING, A.D., PITT, J.I. 1980. Dichloran-glycerol medium for enumeration of xerofilic fungi from low-moisture foods. *Applied and Environmental Microbiology*, 39, 488-492.

HOLLEY, R.A., PATEL, D. 2005. Improvement of shelf- life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food Microbiology*, 22, 273-292.

LOPEZ, P., SANCHEZ, C., BATLLE, R., NERIN, C. 2005. Solid- and vapor – phase antimicrobial activities of six essential oils: susceptibility of selected foodborne bacterial and fungal strains. *Journal Agricultural and Food Chemistry*, 53, 6939-6946.

MAHMOUD, A.-L.E. 1994. Antifungal action and antiaflatoxigenic properties of some essential oil constituents. *Letters in Applied Microbiology*, 19, 110-113.

MISHRA, A.K., DUBEY, N.K. 1994. Evaulation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. *Applied and Environmental Microbiology*, 60, 1101-1105.

MONTES-BELMONT, R., CARVAJAL, M. 1998. Control of *Aspergillus flavus* in maize with plant essential oils and their components. *Journal of Food Protection*, 61, 616-619.

NIELSEN, P.V., RIOS, R. 2000. Inhibition of fungal growth on bread by volatile components from spices and herbs, and the possible application in active packing, with special emphasis on mustard essential oil. *International Journal of Food Microbiology*, 60, 219-229.

OMIDBEYGI, M., BARZEGAR, M., HAMIDI, Z., NAGHDIBADI, H. 2007. Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus* in liquid medium and tomato paste. *Food Control*, 18, 1518-1523.

PASTER, N., MENASHEROU, M., RAVID, U., JUVEN, B. 1995. Antifungal activity of oregano and thyme essentail oils applied as fumigants against fungi attacking stored grain. *Journal of Food Protection*, 58, 81-85.

PATTNAIK, S., SUBRAMANYAM, V.R., BAPAI, M., COLE, C.R. 1997. Antibacterial and antifungal activity of aromatic constituents of essential oils. *Microbios Journal*, 89, 39-46.

PITT, J.I., CHRISTIAN, J.H.B. 1968. Water relation of xerofilic fungi isolated from prunes. *Applied and Environmental Microbiology*, 16, 1853-1858.

RASOOLI, I., ABYANEH, M.R. 2004. Inhibitory effect of Thyme oil on growth and aflatoxin production by *Aspergillus parasiticus*. *Food Control*, 15, 479-483.

RASOOLI, I., FACOIOR, M.H., YADEGARINIA, D., GACHKAR, L., ALOLAMEH, A., REYAEI, M.B. 2008. Antimycotoxigenic characteristics of *Rosmarinum officinalis* and *Trachysperum copticum* L. essential oils. *International Journal of Food Microbiology*, 122, 135-139.

SAMSON, R.A. 1989. Filamentous fungi in food and feed. *Journal of Applied Microbiology*, 67, 27-35.

SCORA, K.M., SCORA, R.W. 1998. Effect of volatiles on mycelium growth of *Penicillium digitatum, P. italicum*, and *P. ulaiense*. *Journal of Basic Microbiology*, 38, 405-413.

SOYLU, E.M., KURT, Ş., SOYLU, S. 2010. *In vitro* and *in vivo* antifungal activities of the essential oils of various plants against tomato grey mould disease agent *Botrytis cinerea*. *International Journal of Food Microbiology*, 143, 183-189.

TULLIO, V., NOSTRO, A., MANDRAS, L., DUGO, P., BANCHE, G., GANNATELLI, M.A, CUFFINI, A.M., ALONZO, V. 2007. Antifungal activity of essential oils against filamentous fungi determined by broth microdilution and vapor contact methods. *Journal of Applied Microbiology*, 102, 1544-1550.

ULTEE, A., GORRIS, L.G.M., SMID, E.J. 1998. Bactericidal activity of carvacrol towards the food-borne pathogen *Bacillus cereus*. *Journal of Applied Microbiology*, 85, 211-218.

VÁZQUES, B.I., FENTE, C., FRANCO, C.M., VÁZQUES, M.J., SEPEDA, A. 2001. Inhibitory effect of eugenoul and thymol on *Penicillium citrinum* strains in culture median and cheese. *International Jouranl of Food Microbiology*, 67, 157-163.

VEGA, M.C., MARTINEY, M.C., ALBISU, M., PEREZ-ELORTONDO, F.J., SALMERON, J. 1998. Storage problems in bakery products during marketing. *Alimentaria*, 292, 77-80.

WILSON, C.L., SOLAR, J.M., EL GHAOUTH, A., WISNIEWSKI, M.E. 1997. Rapid evaluation of plant extract and essential oils for antifungal activity against *Botrytis cinerea*. *Plant Disease*, 81, 204-210.