JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

THE INHIBITORY EFFECT OF ESSENTIAL OILS ON THE GROWTH OF GENUS *PENICILLIUM* ISOLATED FROM PEANUTS BY CONTACT VAPOR

Miroslava Císarová, Dana Tančinová, Marianna Brodová*

Address(es):
Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Microbiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, +421 376414433.

*Corresponding author: miroslava.cisarova@gmail.com

doi: 10.15414/jmbfs.2014.4.special1.6-11

ARTICLE INFO

Received 4. 12. 2014
Revised 15. 1. 2015
Accepted 17. 1. 2015
Published 2. 2. 2015

Regular article

OPEN ACCESS

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 EOs by vapor contact against the fungal species, namely *Penicillium citrinum* (P1 – P2), *P. crustosum* (P3 – P4) and *P. expansum* (P5 – P6) and their ability to affect production of mycotoxins. Each fungus was inoculated in the center on Czapek Yeast Autolysate Agar (CYA) dishes. Dishes were tightly sealed with parafilm and incubated for fourteen days at 25 ± 1 °C (three replicates were used for each treatment). Volatile phase effect of 50 µl of the essential oils was found to inhibit on growth of *Penicillium* spp.. Fungicidal and fungistatic concentracions (MFC) were determined by microathmosphere method. Complete growth inhibition of the isolates by EOs of thyme and clove was observed. The most sensitive isolate was *P. crustosum* (P4) ($P < 0.05$) The essential oils (EOs) of basil and rosemary had antifungal effect on growth of *P. citrinum* (P1 – P2) after 3 day of the incubation at concentration 100 % of EOs. The most resistant isolates were *P. expansum* (P5 – P6). Growth of these isolates was inhibited by thyme and clove EOs (100 %), like each other tested isolates, but with effective MFC concentration of 30 % (30/70; v/v) after all days of cultivation. Data were evaluated statistically by 95.0 % Tukey HSD test. In this stud, we also tested potential effect of EOs to affect production of mycotoxins of tested *Penicillium* isolates which are potential toxigenic fungi. After 14 days of incubation with EOs (100 %) with control sets, they were screened for a production of mycotoxins by TLC chromatography. Oils exhibited a various spectrum of fungal toxicity inhibit all tested species except the jasmine EO.
The present study demonstrated the potential food preservative ability of the thyme, clove, basil, jasmine and rosemary EOs. The jasmine EO has none antifungal or anti – toxic activity.

Keywords: *Penicillium* sp., essential oils, vapor, antifungal activity, mycotoxin

INTRODUCTION

Peanuts (*Arachis hypogaea*) are an inexpensive, nutritionally powerful food source for people worldwide (**Reese and Lehrer, 1999**). On the other hand peanut is one of the eight most common food allergens (**Wild and Gong, 2010**). After soybeans, peanut is a major oil seed crop of global significance for processing and consumption of peanuts is about 30.5 million tones (**CREA, 2011**). Peanuts are often invaded before harvest by *Aspergillus flavus*, *A. parasiticus* and *Penicillium* species, which produce mycotoxins (**Lisker *et al.*, 1993; Horn *et al.*, 2000**). Mycotoxins are secondary metabolites of certain filamentous fungi that can be produce in foods as a result of fungal growth (**Sweeney and Dobson, 1998**). Aflatoxins have been identified as the most toxic mycotoxins associated with peanuts. In addition to alfatoxins, another commonly occurring natural contaminant of peanut is the mycotoxin cyclopiazonic acid, produced by several species of *Aspergillus* and *Penicillium* (**Lansden and Davidson, 1983**), zearelenone (**El-Maghraby and El-Maghraby, 1987; Kishore *et al.*, 2002**) and trichothecene toxins (**Bhavanishankar and Shantha, 2006**), both produced by *Fusarium* sp., citrinin (**El-Maghraby and El-Maghraby, 1987**) produced mby *Penicillium* sp., *Aspergillus* sp. and ochratoxin A (**Magnoli *et al.*, 2007**) produced by *Aspergillus* sp.. The growing awareness of customers concerning the relation between food and health is revolutionizing food industry. New techniques such as high pressure, nanotechnology, irradiation, etc., are increasingly used to maximize the nutritional properties of foods, while new ingredients with functional properties contribute to improving health (**Martos *et al.*, 2008**). Currently, there is a strong debate about the safety aspects of chemical preservatives since they are considered responsible for many carcinogenic and teratogenic attributes as well as residual toxicity (**Skandamis *et al.*, 2001**). The elimination of additives use in a wide variety of foods is demanded, while natural additives are seen as a benefit for both quality and safety (**Martos *et al.*, 2008**). A renewed interest in natural preservation appears to be stimulated by present food safety concerns, growing problems with microbial resistance, and rice in production of minimally processed food, together with green image policies of food industry. Numerous studies have documented the antifungal (**Aligiannis *et al.*, 2001; Thompson, 1989**) and antibacterial (**Canillac and Mourey, 2001; Lachowicz *et al.*, 1998**) effects of plant essential oils. Although there have been many reports of the antibacterial and antifungal effects of EOs against fungi (**Al-Burtami *et al.*, 2005; Holley and Patel, 2005**), but less of essential oil vapors. In general, vapors are more effective than oils against fungi (**Lopez *et al.*, 2005; Tulio *et al.*, 2007**). The vapor phase appears to be promising as a control protocol and could be applied in active packing and creating of protective atmosphere with a minimum organoleptic alternation of the packaged foodstuffs (**Rodriguez *et al.*, 2007**). This way, antimicrobial concentrate at the surface of the products where noxious organisms grow and the interference with the food is avoided (**Tyagi *et al.*, 2012**).
The objective of our study was evaluation of the antifungal activity of 5 EOs by vapor contact against the selected fungal species of the genus *Penicillium* isolated from peanuts and their ability to affect production of selected mycotoxins.

MATERIAL AND METHODS

Fungal isolates

A total of six isolates, concretely two isolates of *Penicillium citrinum* (P1 – P2), two isolates of *P. crustosum* (P3 – P4) and two isolates of *P. expansum* (P5 – P6) isolated from peanuts were used. These isolates belong to the collection of microorganisms at the Department of Microbiology of the Slovak Agricultural University in Nitra. They were inoculated on Czapek Yeast Autolysate Agar (CYA) (**Samson *et al.*, 2002)** dishes.

Essential plant oils

The essential oils used in this study were extracts of thyme (*Thymus vulgaris* L.), clove (*Syzygnium aromaticus* L.), basil (*Ocinum basilicu*m L.), jasmine (*Jasminum officinale* L.) and rosemary (*Rosmarinus officinalis* L.). They were all

supplied by Calendula company a.s. (Nová Ľubovňa, 238 A, Slovakia). The gas chromatography analyze of main components of each essential oils were determined by Calendula company a.s. (Tab. 1). Essential oils were extracted by hydro distillation and its quality and stability were certified by suppliers.

Table 1 The major constituents of essential oils analyzed by Calendula company a.s.

Essential oils	Compound	Amount (%)
Thyme	ρ-cimene	40±3
	thymol	32±2
Clove	eugenol	85±3
Basil	metylchavicol	75±2
Jasmine	–	–
Rosemary	α-pinene	19±1
	campfene	9±1
	β-pinene	5±1
	cineol	25±1
	ρ-cimene	17±1
	campher	19±1
	α-terpineol	2.5±0.2
	bornylacetate	0 – 2.0
	borneol	2.0±0.5

Antifungal activity of essential oils

The antifungal activity of selected EOs was investigated by microatmosphere method. The following method allows the effect a volatile fraction of the EOs to be studied. The test was performed in sterile Petri dishes (Ø 90 mm) containing 15 ml of CYA. Isolates of potential toxigenic fungi were used for toxigenic analyze by thin layer chromatography (TLC) method adapted from **Samson *et al*** (**2002**), modified by **Labuda and Tančinová** (**2006**).

Microatmosphere method

Evaluation by filter paper was made by the method adapted from **Guynot *et al*.** (**2003**). Dishes were kept in an inverted position. A sterilized filter paper (square of 1 x1 cm) was placed in the center of the lid and 50 µl of pure EOs (100/0; v/v; oil/diluent) were added on it. Filter paper disks impregnated with dimethyl sulfoxide (DMSO) (50 µl) were only used as a control to confirm no solvent effect of bioactivity. Each fungus was inoculated in the center on Petri dishes with needle–inoculated. Dishes were tightly sealed with parafilm and incubated for fourteen days at 25 ± 1 °C (three replicates were used for each treatment). Diameters (Ø mm) of the growing colonies were measured at the 3rd, 7th, 11th and 14th day with a ruler. Essential oils able to inhibit each fungus (visible inhibition – non growth of fungus) were used in the following test.

Minimum fungicidal concentration (MFC)

The minimum fungicidal concentration (MFC) of the essential oils with the most significant activity was determined by method of graded concentration of oils. The essential oils dissolved in DMSO (dimethyl sulfoxide) were prepared at concentration of 90/10, 80/20, 70/30, 60/40, 50/50, 40/60, 30/70, 20/80 and 10/90 (v/v; oil/diluent). Cultivation was carried out the same way as before (at 25 ± 1 °C for 14 days, measured at the 3rd, 7th, 11th and 14th day).The test were performed in triplicate. The MFC was regarded as the lowest concentration of the oil that did not permit any visible growth in comparison with control sets. Data from both methods were statistically evaluated by 95.0 % Tukey HSD test.

Mycotoxins screening by modified agar plug method

After microatmosphere method of 14 days of cultivation with EOs (antifungal activity of 100 % of each tested essential oil with a control sets), all toxigenic isolates of the genus *Penicillium* were screened for potential production of citrinin, patulin and roquefortin C by TLC method. Three small pieces (each 5x5 mm) were cut from the colony growing on CYA and placed into 1.5 ml Eppendorf vials. Then 500 µl of extraction solvent (chloroform:methanol, 2:1, v/v) was added to vials containing the agar plugs and shaken on a vortex at least 2 minutes. Extracts (30 – 50 µl) were applied afterwards as spots to the TLC plate (Silicagel 60, Merck, Germany) 1 cm apart. Consequently, spots were dried and the plates were developed in toluene:ethylacetate:formic acid (6:3:1, v/v/v) solvent system that gave an average R_f value of 0–1.46 for citrinin, 0.98 patulin and 0.19 for roqurefortin C. Mycotoxin visualization was directly detectable as a coloured spots under UV- light (365 nm) for citrinin (yellowgreen tailed). Patulin was visualized by spraying 0.5 % methylbenzothiazolone hydrochloride (MBTH, Merc, Germany) in methanol, heated at 130 ºC for 8 min, and then was detected a yellow-orange spot. Roquefortin C was visualized by spraying $Ce(SO)_4$ and after drying detected as an orange spot in daylight. Mycotoxins standard, with the exception of patulin (Calbiochem, USA), were obtained from Sigma – Aldrich (Germany).

RESULTS AND DISCUSSION

In the food industry, after several decades of synthetics antimicrobials dominion, essential oils are once again being proposed as food preservatives (**Tiwari *et al*., 2009**). Screened experiment with 13 – 52 essential oils and major active components against 5 – 25 microorganisms (**Conner and Beuchat, 1984; Dorman and Deans, 2000**) have reported thyme, clove, cinnamon, bay, oregano, garlic and lemongrass to be some of the best broad spectrum candidates for inhibition of food – borne pathogens and spoilage organisms. Biological activity of essential oils depends on their chemical composition which is determined by the genotype and influenced by environmental and agronomic condition (**Marotti *et al*., 1992**). The major components of EOs are listed in Table 1.

This study was realized in two steps: firstly, antifungal activity of thyme, basil, clove, jasmine and rosemary oils were evaluated, using the microatmosphere method against two isolates of *P. citrinum* (P1 – P2), *P. crustosum* (P3 – P4) and *P. expansum* (P5 – P6). All tested EOs, except of jasmine EO, had potential ability to affect growth of tested isolates P1 – P6, but inhibition of growth depend on the concentrations of EOs, days of incubation and tested isolates.

The most effective tested EOs were thyme and clove oils, which totally inhibited all tested isolates for all days of incubation at 100 % concentration. Eugenol (the main component of clove oil) and thymol (from thyme oil), that are two of the most important representatives, their antifungal and antibacterial properties are well known (**Kim *et al*., 1995; Mansour *et al*., 1996; Outtara *et al*., 1997; Abbaszadeh *et al*., 2014**). **Markovic *et al*. (2011)** studied thymol and carvacrol antifungal activity on *Aspergillus* spp. and *Penicillium* spp, and they found that both, thymol and carvacrol have potential antifungal activity, but with more susceptibility of *Aspergillus* spp. than *Penicillium* spp.

The most sensitive isolate was *P. crustosum* (P4) (Table 2). This isolate was inhibited completely by thyme and clove EOs at concentration 100 % as same as isolate *P. crustosum* (P3). Additionally, in comparison with isolate *P. crustosum* (P3), the growth of *P. crustosum (*P4) (*P < 0.05*) was completely inhibited by rosemary EO (100 %) after 7 days of cultivation, over (Table 2). The second sensitive isolate was *P. citrinum* (P1) (*P < 0.05*) which was inhibited completely by thyme and clove EOs, as same as each tested isolate. On the other hand, the comparison with other tested isolates *P. crustosum* (P3 – P4) and *P. expansum* (P5 – P6), *P. citrinum* (P2) and control sets, its growth was lower inhibited by basil and rosemary EOs (100 %) after 3 days of cultivation (Table 2). The most resistant isolates were *P. expansum* (P5 – P6). Growth of these isolates was inhibited only by thyme and clove EOs with the concentration at 100 %.

In this study, the minimum fungicidal concentration (MFC) of the essential oils with the most significant activity was determined by method of graded concentration of oils. MFC value of clove and thyme essential oils was between 30 % (30/70; v/v) and 10 % (10/90; v/v) for all tested isolates. Isolates *P. crustosum* (P4 – P5) have MFC of thyme and clove EOs at concentration of 10 % after 7 days of cultivation (Fig. 1, Fig. 2). MFC values of thyme and clove EOs for *P. citrinum* (P1 –P2) were 10 % of tested EOs after 7 days of cultivation (Fig. 1, Fig. 2). The effective MFC concentration of thyme and clove EOs for the most resistance isolates *P. expansum* (P4 – P5) was 30 % after all days of cultivation. Concentration of 20 % of tested thyme and clove oil was not effective (*P < 0.05*).

Table 2 The antifungal activity of pure of tested essential oils (100 %) to *Penicillium* spp.

Isolates	Days	**Essential oils** (mean colony diameter in mm ± SD)					Control
		Thyme	Basil	Clove	Jasmine	Rosemary	
***P. citrinum* (P1)**	**3rd**	$0^a \pm 0$	$1.00^a \pm 0$	$0^a \pm 0$	$5.17^c \pm 1.15$	$2.67^b \pm 0.76$	$8.50^d \pm 0.50$
	7th	$0^a \pm 0$	$6.83^b \pm 1.04$	$0^a \pm 0$	$22.83^c \pm 2.84$	$9.00^b \pm 3.46$	$21.00^c \pm 1.00$
	11th	$0^a \pm 0$	$16.33^b \pm 7.08$	$0^a \pm 0$	$45.67^c \pm 4.25$	$20.67^b \pm 4.37$	$47.33^c \pm 2.52$
	14st	$0^a \pm 0$	$21.00^b \pm 11.17$	$0^a \pm 0$	$45.67^c \pm 4.25$	$29.83^b \pm 4.54$	$50.50^c \pm 2.50$

***P. citrinum* (P2)**	**3rd**	$0^{a} \pm 0$	$1.33^{b} \pm 0.76$	$0^{a} \pm 0$	$17.17^{c} \pm 0.29$	$1.50^{b} \pm 0.50$	$19.00^{d} \pm 1.00$
	7th	$0^{a} \pm 0$	$32.67^{b} \pm 9.83$	$0^{a} \pm 0$	$35.00^{b} \pm 3.97$	$29.67^{b} \pm 20.93$	$29.50^{b} \pm 2.50$
	11th	$0^{a} \pm 0$	$45.67^{b} \pm 5,48$	$0^{a} \pm 0$	$37.00^{b} \pm 6.08$	$38.33^{b} \pm 16.07$	$31.50^{b} \pm 2.50$
	14th	$0^{a} \pm 0$	$52.83^{c} \pm 5.86$	$0^{a} \pm 0$	$38.00^{bc} \pm 3.00$	$38.33^{bc} \pm 16.07$	$33.00^{b} \pm 3.00$
***P. crustosum* (P3)**	**3rd**	$0^{a} \pm 0$	$7.00^{b} \pm 0$	$0^{a} \pm 0$	$16.17^{c} \pm 1.00$	$6.50^{b} \pm 1.30$	$17.50^{c} \pm 2.50$
	7th	$0^{a} \pm 0$	$29.33^{c} \pm 4.01$	$0^{a} \pm 0$	$27.00^{c} \pm 0.50$	$18.50^{b} \pm 1.50$	$42.50^{d} \pm 2.50$
	11th	$0^{a} \pm 0$	$36.17^{d} \pm 1.04$	$0^{a} \pm 0$	$28.00^{c} \pm 0.50$	$24.33^{b} \pm 1.26$	$51.50^{e} \pm 1.50$
	14th	$0^{a} \pm 0$	$39.67^{bd} \pm 4.51$	$0^{a} \pm 0$	$34.50^{b} \pm 10.04$	$30.67^{b} \pm 2.93$	$51.50^{c} \pm 1.50$
***P. crustosum* (P4)**	**3rd**	$0^{a} \pm 0$	$7.50^{b} \pm 0.87$	$0^{a} \pm 0$	$16.67^{c} \pm 0.29$	$0^{a} \pm 0$	$18.00^{c} \pm 2.00$
	7th	$0^{a} \pm 0$	$29.17^{c} \pm 3.06$	$0^{a} \pm 0$	$29.17^{c} \pm 1.89$	$0^{a} \pm 0$	$22.50^{b} \pm 5.50$
	11th	$0^{a} \pm 0$	$42.83^{c} \pm 4.75$	$0^{a} \pm 0$	$36.33^{bc} \pm 2.84$	$28.67^{b} \pm 5.80$	$34.50^{bc} \pm 14.50$
	14th	$0^{a} \pm 0$	$47.67^{b} \pm 1.44$	$0^{a} \pm 0$	$42.50^{b} \pm 5.57$	$47.50^{b} \pm 5.50$	$38.00^{b} \pm 18.00$
***P. expansum* (P5)**	**3rd**	$0^{a} \pm 0$	$16.50^{a} \pm 18.62$	$0^{a} \pm 0$	$16.00^{a} \pm 6.61$	$17.33^{a} \pm 15.37$	$90.00^{b} \pm 0$
	7th	$0^{a} \pm 0$	$23.67^{ac} \pm 12.77$	$0^{a} \pm 0$	$25.50^{b} \pm 10.76$	$20.33^{ac} \pm 13.90$	$90.00^{c} \pm 0$
	11th	$0^{a} \pm 0$	$25,83^{b} \pm 10.54$	$0^{a} \pm 0$	$27.50^{b} \pm 11.03$	$23.83^{b} \pm 13.81$	$90.00^{c} \pm 0$
	14th	$0^{a} \pm 0$	$26,33^{b} \pm 10.15$	$0^{a} \pm 0$	$31.33^{b} \pm 7.97$	$23.50^{b} \pm 12.03$	$90.00^{c} \pm 0$
***P. expansum* (P6)**	**3rd**	$0^{a} \pm 0$	$10.60^{b} \pm 1.53$	$0^{a} \pm 0$	$18.83^{c} \pm 3.75$	$11.17^{b} \pm 3.75$	$90.00^{d} \pm 0$
	7th	$0^{a} \pm 0$	$20.83^{b} \pm 1.26$	$0^{a} \pm 0$	$26.00^{c} \pm 2.29$	$19.00^{b} \pm 3.12$	$90.00^{d} \pm 0$
	11th	$0^{a} \pm 0$	$23.50^{c} \pm 2.29$	$0^{a} \pm 0$	$32.50^{d} \pm 2.00$	$25.17^{c} \pm 2.36$	$90.00^{e} \pm 0$
	14th	$0^{a} \pm 0$	$25.17^{b} \pm 3.75$	$0^{a} \pm 0$	$35.83^{c} \pm 3.79$	$29.17^{b} \pm 2.08$	$90.00^{d} \pm 0$

Data in the column followed by different letters are significantly different in 95.0 % Tukey HSD test, $P < 0.05$, *P. – Penicillium*, SD – standard deviation

Our result accorded with results of other authors, where tested thyme and clove EOs, and found that they are the most effective oils. The antifungal activity of *Cymbopogon citratus*, *Ocinum gratissimum* and *Thymus vulgaris* EOs was tested in studies by **Nguefack *et al*. (2009)** against *Aspergillus ochraceus*, *Penicillium expansum* and *P. verrucosum*. Their results showed that all tested EOs have antifungal activity, but *T. vulgaris* and *C. citratus* were less active against tested *Penicillium* species and *A. ochraceus*. Similarly, these authors **Nguefack *et al*. (2012**) investigated the same EOs latter, and results showed that *O. gratissimum* was significantly ($P < 0.05$) more active against *P. expansum* than *C. citratus* and *T. vulgaris*. **Zabka *et al*. (2009**) studied antifungal effect of EOs from 25 species of medical plants against *Fusarium oxysporum*, *F. verticillioides*, *Penicillium expansum*, *P. brevicompactum* and some species of *Aspergillus*. Results showed that all of tested essential oils evidently affected growth of these fungi. In the study of **Silva *et al*. (2012**) the antifungal activity of fennel, ginger, mint and thyme was evaluated against *Aspergillus flavus* and *A. parasiticus*. All tested EOs showed antifungal activity, but the highest activity had EO of thyme. **Matan *el al*. (2011**) studied the antifungal activity of cinnamon, clove, anise and peppermint EOs against *Mucor dimorphosporus*, *Penicillium* sp., *Aspergillus niger* and *Rhizopus* sp. They reported than 100 and 450 $\mu g.ml^{-1}$ respectively, MICs of clove oil, anise oil and peppermint oil were higher than that for cinnamon oil, which had the strongest inhibitory effect. But **Daniel *et al*. (2015**) also studied antifungal activity of clove EO and garlic extract against postharvest decay by *Botrytis cinerea* and *Penicillium expansum*, and found that all treatments significantly reduced decay. Clove oils antimicrobial capacity has been demonstrated on bacterial and fungal pathogens (**Huang and Lakshman, 2010; Combric *et al*., 2011**).

The present study confirmed the antifungal activity of thyme and clove essential oils. Although, by applying of jasmine EO no growth inhibition was observed. Rosemary and basil EOs were found to delay fungal growth by vapor contact. **Dubey and Nundy (1983)** also demonstrated that volatile fraction of *Rosmarinus officinalis* strongly inhibited mycelial growth of all tested fungi isolated from apple (*Mortierella hyaline*, *Penicillium roqueforti* and *Phoma exigua*).

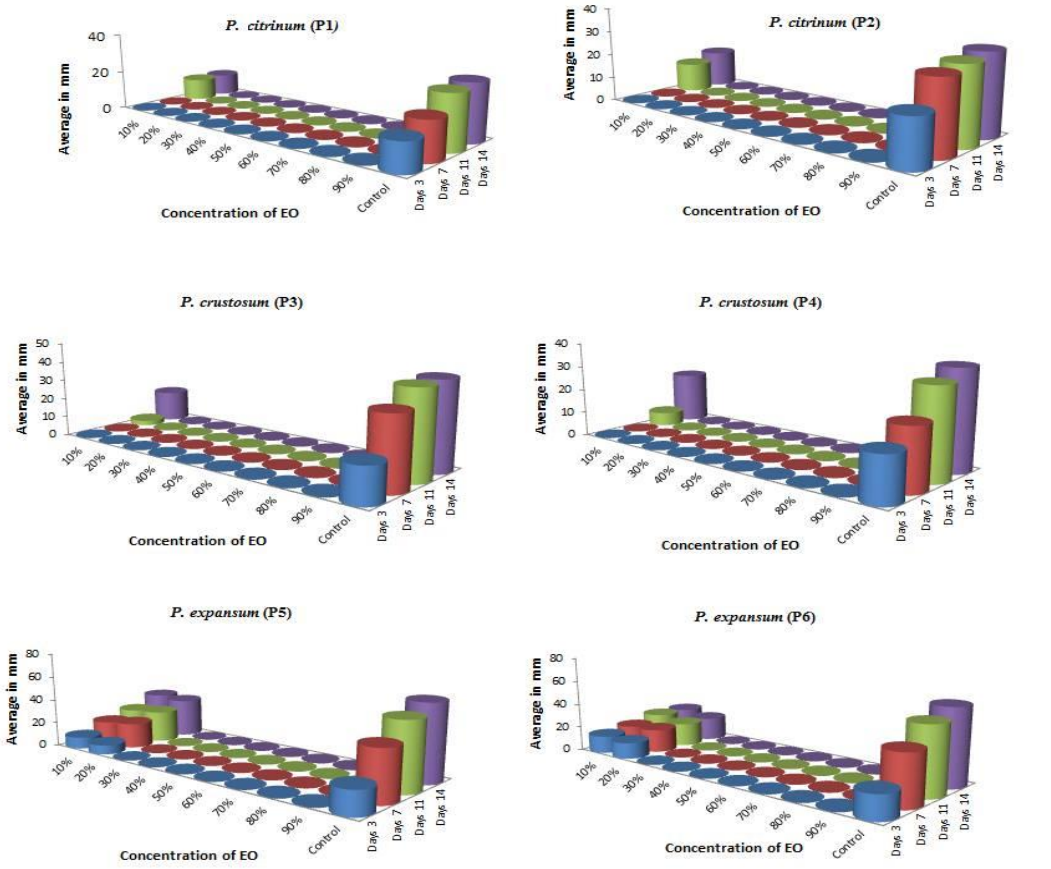

Figure 1 Minimum fungicidal concentration (MFC) of thyme essential oil to *Penicillium* spp.

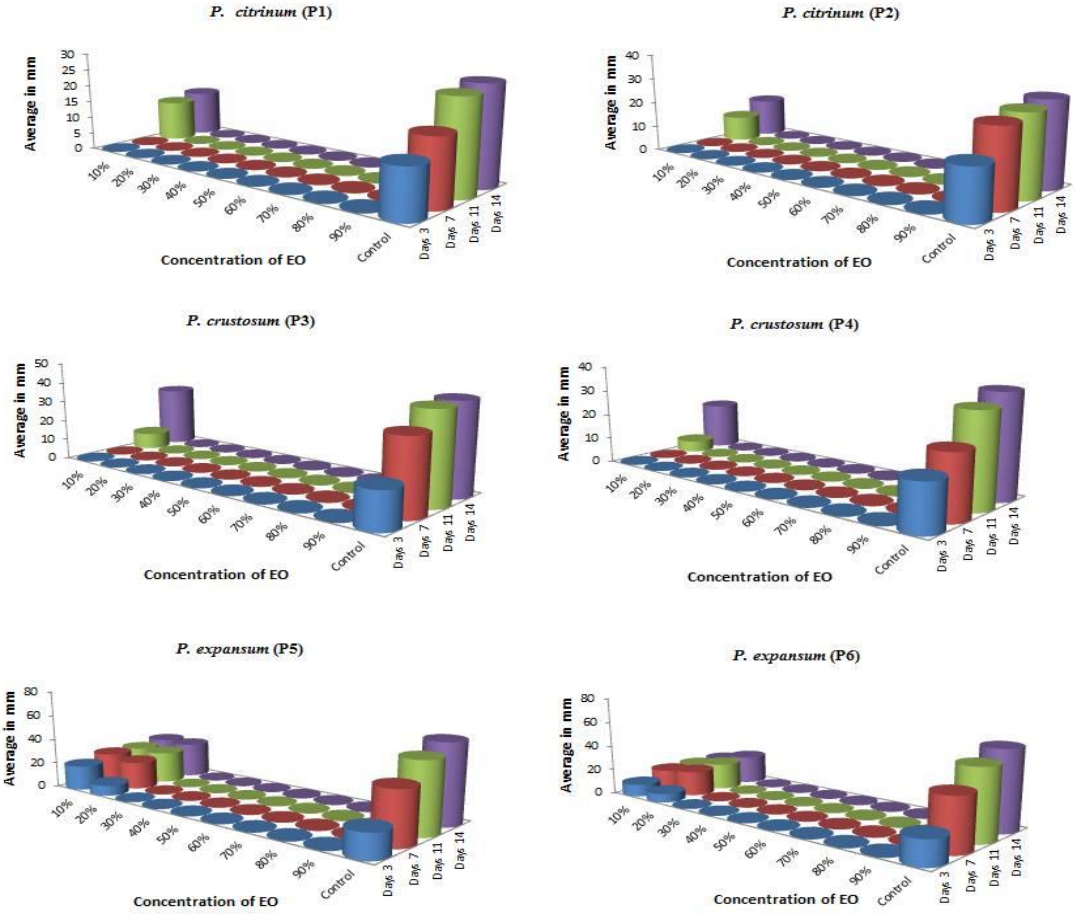

Figure 2 Minimum fungicidal concentration (MFC) of clove essential oil to *Penicillium* spp.

In the second step we tested potentially effect of EOs to affect mycotoxins production of tested *Penicillium* isolates which are potential toxigenic fungi. After 14 days of cultivation with EOs (100 %) with a control sets, isolates *P. citrinum* (P1 – P2) for citirnin, *P. crusto*sum (P3 – P4) for roquefortin C and *P. expansum* (P5 – P6) for patulin and roquefortin C were tested for a mycotoxins production by TLC chromatography. Oils exhibited a various spectrum of fungal toxicity inhibit all tested species except the jasmine EO (Table 3). Citrinin, roquefortin C and patulin were not detected only in the samples treated with thyme and clove EOs, because they completely inhibited mycelial growth of fungi. In treatments with jasmine EO, all tested mycotoxins, in comparison with controls, were produced. Treatments with rosemary and basil EOs showed some potential of fungal toxic inhibiting. Production of C produced by *P. citrinum* (P1) was completely inhibited by basil and rosemary EOs in all repetitions. Isolate *P. citrinum* (P2) did not produce citrinin only with treatments by basil EO in two repetitions and rosemary inhibited their production in one screened repetition. Production of citrinin by *P. expansum* (P5) was inhibited in one repetition by basil EO, but production of roquefortin C was inhibited completely. In case of isolate *P. expansum* (P6), production of citrinin was inhibited by basil EO in two repetitions, as same as production of roquefortin C. Rosemary EO did not have anti – toxic effect on isolates *P. expansum* (P5 – P6). Production of patulin was not affected by basil and rosemary EOs. **Rasooli and Owlia (2005)** also studied essential oils from *Thymus erioxalix* and *Thymus X-porlock* fir its inhibition of growth of *Aspergillus parasiticus* and aflatoxins production. They found that aflatoxins production was inhibited at 250 ppm of both oils. **Kedia *et al.* (2014)** tested potential of *Cuminum cuminum* EO to affect aflatoxins production and their results showed that minimum aflatoxin inhibitory concentration of EO were 0.6 and 0.5 $\mu l.ml^{-1}$. Essential oils from boldo, poleo, clove, anise and thyme were evaluated for their effect against *Asperigillus niger*, *A. carbonarius* and their accumulation of ochratoxin A (OTA) by **Passone *et al.* (2012)**. Their results suggest that boldo, poleo and clove oils affect the OTA biosynthesis pathway of both *Aspergillus* species. These authors, **Passone *et al.* (2013)** also reported the antifungal activity and antiaflatoxinogenic properties of same EO expect thyme EO (boldo, poleo and clove EOs) by vapor contact. The antifungal and antiaflatoxigenic effects of poleo and clove EOs were highly depended on a_w, but boldo and poleo volatile fractions can be used as effective non – toxic biopreservatives.

Table 3 Inhibitory effect of tested essential oils (100 %) on mycotoxins production by *Penicillium* sp.

Fungi	Screened mycotoxins	Essential oils (100%)									
		Thyme		Basil		Clove		Jasmine		Rosemary	
		Rep.	Control	Rep.	Control	Rep.	Control	Rep.	Control	Rep.	Control
***P. citrinum* (P1)**	**C**	NS*	$1^1/1^2$	$3^1/0^2$	$1^1/1^2$	NS*	$1^1/1^2$	$3^1/3^2$	$1^1/1^2$	$3^1/0^2$	$1^1/1^2$
***P. citrinum* (P2)**	**C**	NS*	$1^1/1^2$	$3^1/1^2$	$1^1/1^2$	NS*	$1^1/1^2$	$3^1/3^2$	$1^1/1^2$	$3^1/2^2$	$1^1/1^2$
***P. crustosum* (P3)**	**RC**	NS*	$1^1/1^2$	$3^1/3^2$	$1^1/1^2$	NS*	$1^1/1^2$	$3^1/3^2$	$1^1/1^2$	$3^1/0^2$	$1^1/1^2$
***P. crustosum* (P4)**	**RC**	NS*	$1^1/1^2$	$3^1/3^2$	$1^1/1^2$	NS*	$1^1/1^2$	$3^1/3^2$	$1^1/1^2$	$3^1/2^2$	$1^1/1^2$
***P. expansum* (P5)**	**C** **P** **RC**	NS*	$1^1/1^2$ $1^1/1^2$ $1^1/1^2$	$3^1/2^2$ $3^1/3^2$ $3^1/0^2$	$1^1/1^2$ $1^1/1^2$ $1^1/1^2$	NS*	$1^1/1^2$ $1^1/1^2$ $1^1/1^2$	$3^1/3^2$ $3^1/3^2$ $3^1/3^2$	$1^1/1^2$ $1^1/1^2$ $1^1/1^2$	$3^1/3^2$ $3^1/3^2$ $3^1/3^2$	$1^1/1^2$ $1^1/1^2$ $1^1/1^2$
***P. expansum* (P6)**	**C** **P** **RC**	NS*	$1^1/1^2$ $1^1/1^2$ $1^1/1^2$	$3^1/1^2$ $3^1/3^2$ $3^1/1^2$	$1^1/1^2$ $1^1/1^2$ $1^1/1^2$	NS*	$1^1/1^2$ $1^1/1^2$ $1^1/1^2$	$3^1/3^2$ $3^1/3^2$ $3^1/3^2$	$1^1/1^2$ $1^1/1^2$ $1^1/1^2$	$3^1/3^2$ $3^1/3^2$ $3^1/3^2$	$1^1/1^2$ $1^1/1^2$ $1^1/1^2$

P. – Penicillium, Rep. – repetition, * – no screened isolates (no visible growth), 1 – number of screened isolates, 2 – number of positive isolates, RC – roquefortin C, C – citrinin, P - patulin

The difference between the antifungal activities of essential oils studied in this study against *Penicillium* spp. Is permanence of the inhibitory effect over time and potential of EOs to inhibit fungal toxicity is directly related to chemical composition of the EO, microbial species, the mechanism of action and the method used to analyze the antifungal activity of EOs.

CONCLUSION

The present study demonstrated the potential food preservative ability of thyme, clove, basil, jasmine and rosemary EOs. We tested the antifungal activity of EOs against *Penicillium* spp. isolated from peanuts for 14 days with addition of pure EOs (100 %). These aromatic plants commonly used as spices or in beverage formulation are considered as to be safe. Our results suggest that the thyme and clove EOs might be a good natural fungicide, because of their MFC values, which were up to 20 % (20/80; v/v) for all tested isolates, except *P. expasum* (P5 – P6) with MFC 30 % (30/70; v/v). Jasmine EO have none antifungal or anti-toxic activity. Even though that EOs such as basil and rosemary had not antifungal activity like thyme and clove EOs, they should find a practical application in the inhibition of the fungal mycelia growth or mycotoxins production of fungi. These effects against food spoilage and mycotoxins producing fungi indicated the possible ability of each essential oil to be a food preservative. However, consumer sensory tests will be needed to determine concentrations of essential oils suitable for specific products.

Acknowledgments: This work was co-funded by VEGA 1/0611/14 and European Community under project no 26220220180: Building Research Centre Agrobiotech**.**

REFERENCES

ABBASZADEH, S., SHARIFZADEH, A., SHOKRI, H., KHOSRAVI, A.R., ABBASZDEH, A. 2014. Antifungal efficacy of thymol, carvacrol, eugenol and methol as alternative agents to control the growth of food-relevant fungi. *Journal de Mycologie Médicale*, 24, 51-56. http://dx.doi.org/10.1016/j.mycmed.2014.01.063

AL-BURTAMI, S.K.S., FATOPE, M.O., MARWAH, R.G., ONIFADE, A.K., AL-SAIDI, S.H. 2005. Chemical composition, antibacterial and antifungal activities of the essential oil of *Haplophyllum tubercul*atum from Oman. *Journal of Ethnopharmacology*, 96, 107-112. http://dx.doi.org/10.1016/j.jep.2004.08.039

ALIGIANNIS, N., KALPOUTZAKIS, E., CHINOU, I.B., MITAKOU, S., GIKAS, E., TSARBOPOULOS, A. 2001. Composition and antimicrobial activity of the essential oils of five taxa of Siderites from Greece. *Journal of Agricultural and Food Chemistry*, 49, 811-815. http://dx.doi.org/10.1021/jf001018w

BHAVANISHANKAR, T.N., SHANTHA, T. 2006. Natural Occurrence of *Fusarium* toxins in peanut, sorghum and maize from Mysore (India). *Journal of the Science and Food and Agriculture*, 40,327-332. http://dx.doi.org/10.1002/jsfa.2740400405

C. DA SILVA, F., CHALFOUN, S.M., DE SIQUERA, V.M., DEILA M. DOS S. BOTELHO, LIMA, N., BATISTA, L.R. 2012. Evaluation of antifungal activitxy of essential oils against potentially mycotoxigenic *Aspergillus flavus* and *Aspegillus parasiticus*. *Brazilian Journal of Pharmacology*, 22, 1002-1010. http://dx.doi.org/10.1590/s0102-695x2012005000052

CANILLAC, N., MOUREY, A. 2001. Antibacterial activity of the essential oil of *Picea excelsa* on *Listeria*, *Staphylococcus au*reus and *coliform* bacteria. *Food Microbiology*, 18, 261-268. http://dx.doi.org/10.1006/fmic.2000.0397

COMBRINK, S., REGNIER, T., KAMATOU, G.P.P. 2011. In vitro activity of eighteen essential oils and some major components against common postharvest fungal pathogens of fruit. *International Crops an d Products*, 33, 344-349. http://dx.doi.org/10.1016/j.indcrop.2010.11.011

CONNER, D.E., BEUCHAT, L.R. 1984. Effects of Essential Oils from Plants on Growth of Food Spoilage Yeasts. *Journal of Food Science*, 49, 429-434. http://dx.doi.org/10.1111/j.1365-2621.1984.tb12437.x

CREA (Consorcios Regionales de Experimentación Agrícola), 2011. Prensa y Difusión. Información CREA. El Secreto del Maní. Available at: http//www.redcrea.org.ar

DANIEL, CH.K., LENNOX, CH.L., VRIES, F.A. 2015. In vivo application of garlic extracts in combination with clove oil to prevent postharvest decay caused by *Botrytis cinerea*, *Penicillium expansum* and *Neofabrea alba* on apples. *Postharvest Biology and Technology*, 99, 88-92. http://dx.doi.org/10.1016/j.postharvbio.2014.08.006

DORMAN, H.J., DEANS, S.G 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, 88, 308-316. http://dx.doi.org/10.1046/j.1365-2672.2000.00969.x

DUBEY, P., NUNDY, S. 1983. Simultaneous measurement of gastric acid and duodenal alkali secretion by in situ titration in health and disease. *Gut BMJ Journal*, 24, 1126-1129. http://dx.doi.org/10.1136/gut.24.12.1126

EL-MAGHRABY, O.M.O., EL-MAGHRABY, M. 1987. Mycobiota and mycotoxins of peanuts (*Arachis hypo*gea L.) seeds in Egypt. 1.sugar fungi and

natural occurrence of mycotoxins. *Mycopathologia*, 98, 165-170. http://dx.doi.org/10.1007/bf00437651

GUYNOT, M.E., RAMOS, A.J., SETÓ, L., PURROY, P., SANCHIS, V., MARÍN, S. Antifungal activity of volatile compounds generated by essential oils against fungi commonly causing deterioration of bakery products. 2003. *Journal of Applied Microbiology*, 94, 893-899. http://dx.doi.org/10.1046/j.1365-2672.2003.01927.x

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. http://dx.doi.org/10.1016/j.fm.2004.08.006

HORN, B.W., GREENE, R.L., SORENSEN, R.B., BLANKESHIP, P.D., DORNER, J.W. 2000. Conidial movement of nontoxigenic *Aspergillus flavus* and *Aspergillus parasiticus* in peanuts fields following application to soil. *Mycopathologia*, 151, 81-92.

HUANG, Q., LAKSHMAN, D.K. 2010. Effect of clove oil on plant pathogenic bacteria and bacterial wilt of tomato and geranium. *Journal of Plant Pathology*, 92, 701-707.

KEDIA, A., PRAKASH, B., MISHRA, P.K., DUBEY, N.K. 2014. Antifungal and antiaflatoxigenic properties of *Cuminum cyminum* (L.) seed essential oil and its efficacy as a preservative in stored commodities. *International Journal of Food Microbiology*, 168-169, 1-7. http://dx.doi.org/10.1016/j.ijfoodmicro.2013.10.008

KIM, J., MARSHALL, M.R., WEI, C.H. 1995. Antibacterial activity of some essential oil components against five foodborne pathogens. *Journal of Agricultural and Food Chemistry*, 43, 2839-2845. http://dx.doi.org/10.1021/jf00059a013

KISHORE, K.G., PANDE, S., MANJULA, K., RAO, N. J., THOMAS, D. 2002. Occurrence of mycotoxins and toxigenic fungi in groundnut (*Arachis hypogaea* L.) seeds in Andhra Pradesh, India. *Plant Pathology Journal*, 18, 204-209. http://dx.doi.org/10.5423/ppj.2002.18.4.204

LABUDA, R., TANČINOVÁ, D. 2009. Fungi on wheat bran and their toxinogenity. *Annals of Agricultural and Environmental Medicine*, 16, 325-331.

LACHOWICZ, K.J., JONES, G.P., BRIGGS, D.R., BIENVENU, F.E., WAN, K., WILCOCK, A. 1998. The synergistic preservative effects of the essential oils of sweet basil (*Occinum basalicum* L.) against acid-tolerant food microflora. *Letters in Applied Microbiology*, 26, 209-214. http://dx.doi.org/10.1046/j.1472-765x.1998.00321.x

LANSDEN, J.A., DAVIDSON, J.I. 1983. Occurrence of cyclopiazonic acid in peanuts. *Applied and Environmental Microbiology*, 45, 766-769. LISKER, N., MICHAELI, R., FRANK, Z.R. 1993. Mycotoxigenic potential of *Aspergillus flavus* strains isolated from groundnuts growing in Israel. *Mycopathologia*, 122, 177-183. http://dx.doi.org/10.1007/bf01103479

LOPEZ, P., SANCHEZ, C., BATLLE, R., NERÍN, 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, 6939-6949. http://dx.doi.org/10.1021/jf050709v

MAGNOLI, C., ASTORECA, A., PONSONE, M.L., FERNÁNDEZ-JURI, M.G., BARBERIS, C., DALCERO, A.M. 2007. Ochratoxin and *Aspergillus* section *Nigri* in peanut seeds in different months of storage in Córdoba, Argentina. *International Journal of Food Microbiology*, 119, 213- 218. http://dx.doi.org/10.1016/j.ijfoodmicro.2007.07.056

MANSOUR, N., YOUSEF, A.E., KIM, J. 1996. Inhibition of surface growth of toxigenic and non-toxigenic *aspergilli* and *penicillia* by eugenol, isoeugenol and monolaurin. *Journal of Food Safety*, 16, 219-229. http://dx.doi.org/10.1111/j.1745-4565.1996.tb00162.x

MARKOVIC, T., CHATZOPOULOU, P., ŠILJEGOVIC, J., NIKOLI, N.,, GLAMOCLIJA, J., CIRIC, A. 2011. Chemical analysis and antimicrobial activities of the essential oils of *Satureja thymbra* L. and *Thymbra spicata* L. and theri main components. *Archives of Biological Sciences*, 63, 457-464. http://dx.doi.org/10.2298/abs1102457m

MAROTTI, M., DELLACECCA, V., PICCAGLIA, R., GIOVANELLI, E. 1992. Agronomic and chemical evaluation of three varieties of *Foeniculum vulgare* Mill. *Presented at First World Congress on Medicinal and Aromatic Plants for Human Welfare,*, 19-25. Maastricht, The Netherlands

MATAN, N., SAENGKRAJANG, W., MATAN, N. 2011. Antifungal activities of essential oils applied by dip-treatment on areca palm (*Areca catechu*) leaf sheath and persistence of their potency upon storage. *International Biodeterioration and Biodegradation*, 65, 212-216. http://dx.doi.org/10.1016/j.ibiod.2010.09.009

NGUEFACK, J., DONGMO, J.B., DAKOLE, C.D., LETH, V., VISMER, H.F., TORP, J., GUEMDJOM, E.F.N., MBEFFO, M., TAMGUE, O., FOTIO, D., AMVAN ZOLLO, P.H., NKENGFACK, A.E. 2009. Food preservative potential of essential oils and fractions from *Cymbopogon citratus, Ocinum gratissimum* and *Thymus vulgaris* against mycotoxigenic fungi. *International Journal of Food Microbiology*, 131, 151-156. http://dx.doi.org/10.1016/j.ijfoodmicro.2009.02.009

NGUEFACK, J., TAMGUE, O., DONGMO, J.B., DAKOLE, C.D., LETH, V., VISMER, H.F., AMVAN ZOLLO, P.H., NKENGFACK, A.E. 2012. Synergistic action between fractions of essential oils from *Cymbopogon citratus, Ocinum gratissimum* and *Thymus vulgaris* against *Penicillium expansum*. *Food Control*, 23, 377-383. http://dx.doi.org/10.1016/j.foodcont.2011.08.002

OUTTARA, B., SIMARD, R.E., HOLLEY, R.A., PIETTE, G., BÉGIN, A. 1997. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. *International Journal of Food Microbiology*, 37, 155-162. http://dx.doi.org/10.1016/s0168-1605(97)00070-6

PASSONE, M.A, GIRARDI, N.S., FERRAND, G.A., ETCHEVERRY, M. 2012. Invitro evaluation of five essential oils as botanical fungitoxicants for the protection of stored peanuts from *Aspergillus flavus* and *A. parasiticus* contamination. *International Biodeterioration and Biodegradation*, 70, 82-88. http://dx.doi.org/10.1016/j.ibiod.2011.11.017

PASSONE, M.A., GIRARDI, N.S., ETCHEVERRY, M. 2013. Antifungal and antiaflatoxigenic activity by vapor contact of three essential oil, and effects of environmental factors on their efficacy. *LWT Food Science and Technology*, 53, 434-444. http://dx.doi.org/10.1016/j.lwt.2013.03.012

RASOOLI, I., OWLIA, P. 2005. Chemoprevention by thyme oils of *Aspergillus parasiticus* growth and aflatoxin production. *Phytochemistry*, 66, 2851-2856. http://dx.doi.org/10.1016/j.phytochem.2005.09.029

REESE G., LEHRER, S. B. 1999. Food allergens. In M. Frieri, & B. Kettelhut (Eds.), *Food hypersensitivity and adverse reactions: a practical guide for diagnosis and managementn*, 69-97. New York: Marcel Dekker, Inc. http://dx.doi.org/10.1056/nejm200004133421519

RODRIGUEZ, A., BATLLE, R., NERÍN, C. 2007. The use of natural essential oils as antimicrobial solutions in paper packing. Part II. *Progress in Organic Coatings*, 60, 33-38. http://dx.doi.org/10.1016/j.porgcoat.2007.06.006

SAMSON, R.A., HOEKSTRA, E.S., LUND, F., FILTENBORG, P., FRISVAD, J.C. 2002. Method for the detection, isolation and charcterisation of food-borne fungi. Samson, R.A., Hoekstra, E.S., Frisvad, J.C., Filtenborg, O. (Eds.): *Introduction to food-and airborne fungi*, *CEntraalbureau voor Schimmecultures*, Utrecht, 283-297. http://dx.doi.org/10.1007/s11046-005-4201-1

SKANDAMIS, P., KOUTSOUMANIS, K., FASSEAS, K., NYCHAS, G.J.E. 2001. Inhibition of oregano essential oil and EDTA on *E. coli* O157:H7. *Italian Journal of Food Science*, 13, 55-65.

SWEENEY, M.J., DOBSON, A.D.W. 1998. Mycotoxin production by *Aspergillus, Fusarium* and *Penicillium* species. *International Journal of Food Microbiology*, 43, 141-158. http://dx.doi.org/10.1016/s0168-1605(98)00112-3

THOPSON, D.P. 1989. Fungitoxic activity of essential oil components on food storage fungi. *Mycologia*, 81, 151-153. http://dx.doi.org/10.2307/3759462

TIWARI, B.K., VALDRAMIDIS, V.P., ODONNELL, C.P., MUTHUKUMARAPPAN, K., BOURKE, P., CULLEN, P.J. 2009. Application of natural antimicrobials for food preservation. *Journal of Agricultural and Food Chemistry*, 57, 5987-6000. http://dx.doi.org/10.1021/jf900668n

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 vapour contact methods. *Journal of Applied Microbiology*, 102, 1544-1550. http://dx.doi.org/10.1111/j.1365-2672.2006.03191.x

TYAGI, A.K., MALIK, A., GOTTARDI, D., GUERZONI, M.E. 2012. Essential oils vapour and negative air ions: A novel tool for food preservation. *Trends in Food Science and Technology*, 26, 99-113. http://dx.doi.org/10.1016/j.tifs.2012.02.004

VIUDA-MARTOS, M., RUIZ-NAVAJAS, Y., FERNÁNDEZ-LOPÉZ, J., PÉREZ-ÁLAREZ, J. 2008. Antifungal activity oflemon (*Citrus lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.) and orange (*Citrus sinensis* L.) essential oils. *Food Control*, 19, 1130-1138. http://dx.doi.org/10.1016/j.foodcont.2007.12.003

WILD, C.P., GONG, Y.Y. 2010. Mycotoxins and human disease: a largely ignored global health issue. *Carcinogenesis*, 31, 71-82. http://dx.doi.org/10.1093/carcin/bgp264

ZABKA, M., PAVELA, R., SLEZAKOVA, L. 2009. Antifungal effect of *Pimenta dio*ica essential oil against dangerous pathogenic and toxinogenic fungi. *Industrial Crops and Products*, 30, 250-253. http://dx.doi.org/10.1016/j.indcrop.2009.04.002