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Growth Inhibition of *Aspergillus flavus* Isolated from Pistachio by Secondary Metabolites

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Abstract

Pistachio nut is a strategic product throughout the world, especially in Iran. There are some problems that reduce production and export of pistachios, for example, postharvest fungi, especially *Aspergillus* spp., that lead to production of mycotoxins. Nowadays the use of chemical and synthetic antifungals is discouraged and reduced because of health risks to mankind and nature. In present study we investigated the antifungal activities of carvacrol and allyl isothiocyanate (AITC) on conidia germination and mycelial growth in *Aspergillus flavus* at six concentrations and two observation times (3 and 7 days after inoculum). The results indicated that these compounds could inhibit spore germination and mycelial growth completely at 12 and 2.9 μL L^{-1} for mycelial growth and conidia germination, respectively. In addition, the ED_{50} and ED_{95} values were determined for all treatments. It was concluded that the natural compounds examined in the present study could be used as antifungal agents against food spoilage and mycotoxin producing fungi. Further studies on natural antifungal compounds to replace chemical fungicides are required.

Keywords: Allyl isothiocyanate, Carvacrol, Conidial germination, Mycelial growth.

Introduction

Pistachio (*Pistachia vera* L.) is a favorite tree nut worldwide especially in Iran. Pistachio nuts substantially contribute to the agricultural exports of Iran and are known as "green gold" (Aghdaie, 2009). Pistachio kernels are a rich source of oil and fatty acids such as the oleic acid (52-68%) and linoleic acid (12-27%) and protein (20% on average) that are essential for humans (Tsantili *et al.*, 2010). Unfortunately some contaminants can lead to significant economic losses of this important product (Herrman *et al.*, 2002).

Mycotoxin contamination of food is a serious health risk for humans and animals and has been regarded as a high priority food safety issue during the past three decades (Afsah-Hejri *et al*., 2013), especially in developing countries (Rustom, 1997). Mycotoxins are secondary metabolites produced by fungi that are distributed worldwide. Of the mycotoxins, the aflatoxins (AFs) are the most important and attract a significant amount of attention due to their mutagenic, carcinogenic and teratogenic effects (Bhat *et al.*, 2010). The aflatoxins are highly toxic compounds produced by some species of *Aspergillus*, such as *A. parasiticus* and *A. flavus* (Afsah- Hejri *et al*., 2013), and may be present in a wide range of food commodities, such as nuts.

Aspergillus family members are wound-invading pathogens. *Aspergillus* spores enter through the split hull and colonize between the kernel and coat, where they produce AFs (Mahoney and Rodriguez, 1996).

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Aflatoxin contamination of pistachio nuts significantly reduces the quality and value of pistachios and directly affects farmers and consumers, and especially leads to problems regarding international export of this nut. During the past 36 years there have been some cases where Iranian pistachios have been sold at low prices or sent back because of aflatoxin contamination.

The contamination of pistachio by *A. flavus* can be reduced by certain chemical, biological and physical methods. Application of chemical antifungal substances is commonly used for preserving agricultural products. However, most of these compounds are synthetic and expensive and have side effects on human beings. As a result, the use of chemical and synthetic fungicides has been restricted because of health concerns linked to chemical residues on products. For this reason, these problems have prompted an increase in research on cultural and biological alternatives for controlling this fungal contamination.

Natural substances with biocidal activity represent an interesting alternative, and essential oils (Tsao and Zhou, 2000), aromatic compounds and bioactive molecules produced by various plant defense systems are being studied for their fungicidal action (Neri *et al.*, 2006). Plant extracts with anti-microbial and antifungal properties can be used to prevent formation of aflatoxin in nuts.

In recent years many researchers have explored natural herbal compounds such as essential oils that exhibit little harm to human health and the environment. Plant extracts have demonstrated antimicrobial effects due to several compounds, including phenolics, flavonoids, allicin, thiosulfinates, betalain and phytoalexins (Harris *et al.*, 2001), and there is an ongoing and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action (Parekh and Chanda, 2007).

Omidbeygi *et al.*, (2007) showed that the essential oils of thyme and cloves had high inhibitory effect on *Aspergillus flavus*. Rasooli and Razzaghi (2004) studied the inhibition of *A. parasiticus* growth and its aflatoxin production on exposure to the essential oils extracted from two varieties of thyme. In their research, they found that the oils from these plants were strongly fungicidal, resulting in low aflatoxin production. They recommended the substitution of currently used antifungal and aflatoxin inhibiting chemicals by natural compounds. The high inhibitory effect of thyme may relate to its essential oil components that include thymol, carvacrol, eugenol, which may inactivate some essential enzymes of fungi (Davidson, 2001). Carvacrol is one of the important components of essential oils that have antifungal activities (Lima *et al.*, 2013; Elshafie *et al.*, 2015; Tsao and Zhou, 2000; Manuel *et al.*, 2011).

In addition of essential oils, there is another group of compounds that includes some molecules that come from the enzymatic hydrolysis of glucosinolates (GLs), namely isothiocyanates (ITCs) that can be used as 'biofumigants'.

GLSs are β–thioglycoside N-hydroxysulphates (also known as (Z)-N-hydroximinosulphate esters or S-glucopyranosyl thiohydroximates) with a side chain R and a sulphur-linked β–D-glucopyranose moiety which can be found in at least 16 higher plant families, particularly Brassicaseae, where a single GL is often dominant (Fahey *et al.*, 2001). These compounds are hydrolysed by the myrosinase (thioglucosideglucohydrolase E.C. 3.2.1.147), producing not only ITCs, but also a combination of nitriles, thiocyanates and oxazolidinethiones in varying amounts according to the hydrolysis conditions and the chemical nature of the GL.

Some ITCs are volatile substances and could potentially be successfully employed as gaseous treatments of fruit or vegetables before storage or in modified atmosphere packaging. For example, the treatment of pears for 24 h at room temperature with an allyl isothiocyanate (AITC) enriched atmosphere produced good control of blue mould (*Penicillium expansum*) (Mari *et al.*, 2002).

Allyl isothiocyanate (AITC), C_4H_5NS, a natural compound commonly found in the Brassicaceae fami-

ly (Tookey *et al.*, 1980), possesses a strong antimicrobial activity against bacteria and other pathogenic bacteria (Inoue *et al.*, 1983). The major pungent component of black mustard (*Brassica nigra*) and brown mustard (*B. juncea*), and of wasabi (*Eutrema wasabi* Maxim.), is allyl isothiocyanate (AITC). AITC is released from a naturally occurring glucosinolate, sinigrin, by the action of myrosinase. It has been used as a preservative and is considered safe for human consumption (Kermanshai *et al*., 2001).

It has been demonstrated that AITC effectively inhibits a variety of pathogenic microorganisms such as *M. laxa, R. stolonifer*, *Neofabraalba*,and *B. cinereae* (Mari *et al.*, 2002)

In this study we investigated the possibility of using some natural antifungal substances to inhibit the growth of aflatoxin-producing fungi. In this regard, we present this *in vitro* study in which we evaluate the antifungal activity of allyl isothiocyanate (AITC) and carvacrol on *Aspergillus flavus* isolated from contaminated pistachios.

Materials and Methods

Isolation, purification and identification of Aspergillus from pistachio

To isolate the contaminant fungi, pistachio nuts that showed obvious *Aspergillus* sporulation were surface-sterilized with 5% sodium hypochlorite solution for one minute before being rinsed three times with sterile distilled water, After three days shaking overnight and filtration of contaminated nuts solution by cheesecloth, all visually characterized *Aspergillus* isolates were sub-cultured on PDA (potato dextrose agar) containing antibiotics (streptomycin and neomycin) to obtain pure cultures. The Petri-dishes were incubated for 7 days at 25 °C and observed daily for the emergence of colonies, which were then counted. Isolates were purified through single-spore methods and then transferred to PDA slants. Species were determined using microscopic features.

Carvacrol and allyl isothiocyanate, as commercial preparations with purity of 95%, were purchased from Sigma–Aldrich.

Fungal spore suspension and inoculums preparation

To obtain spores of *Aspergilus flavus* inoculum, the fungus was sub-cultured by streaking spores on fresh potato dextrose agar (PDA) plates and incubated for 7 d at 25 °C. The seven-day-old spore cultures of the fungus were suspended in sterile distilled water containing 0.001 mL L^{-1} of Tween 80. The spore suspensions were then filtered with 6 layers of cheesecloth to remove debris such as mycelia and condensed agar fragments, and its concentration was adjusted to 10^3 and 10^6 fungal spores' mL^{-1} suspensions using a haemocytometer. This suspension was used to study the effect of carvacrol and AITC on mycelial growth and conidia germination of *A. flavus.*

Control of Aspergillus flavus by compounds

The effect of carvacrol on inhibition of conidia germination and mycelium growth of *Aspergillus flavus* was tested in vitro on malt extract agar at six concentrations.

Effects of AITC and Carvacrol on conidia germination

The inhibition of conidia germination was tested by spreading 100 µL of *A. flavus* conidia suspension (10^3 conidia mL^{-1}) onto 90 mm Petri dishes containing 20 mL of malt extract agar (MEA). In each case different aliquots of pure AITC and carvacrol (0, 0.01, 0.03, 0.06, 0.12 and 0.24 µL per plate, equivalent to 0.12, 0.37, 0.74, 1.5 and 3.0 µL L^{-1}, respectively, were placed, using a microsyringe, on a 90-mm diameter paper filter (Whatman No. 1), positioned on the inside cover of the dish. The dishes were quickly closed and sealed with Parafilm and incubated at 25 ºC (Bluma *et al*., 2008). To evaluate the fungicidal activity of AITC and carvacrol on conidia germination, the dishes were opened after two days and the paper filter removed, after which the fungus was allowed to grow for the next 3 and 7 days, respectively. Petri dishes inoculated with *A. flavus* but treated with distilled water were used as controls. Conidia germination was determined by observing the conidia directly with a light microscope.

Effects of AITC and carvacrol on mycelium growth

Mycelium growth inhibition was evaluated by placing a plug (6-mm diameter) from an actively growing culture (the margins of a 7-day-old culture) in the center of a MEA plate.

In each case different aliquots of pure AITC and carvacrol (0, 0.06, 0.12, 0.24, 0.48 and 0.96 µL, equivalent to 0.74, 1.5, 3.0, 5.9 and 11.8 µL L^{-1}, respectively) were placed, using a microsyringe, on a 90-mm diameter paper filter (Whatman No. 1), positioned on the cover of the dish. The dishes were quickly closed and sealed with Parafilm and incubated at 25 °C. The dishes were opened after 7 days to evaluate mycelium growth. Petri dishes inoculated with *A. flavus* but treated with distilled water were used as a control. Mycelium growth (colony diameter) was measured with a ruler (centimeter) Data were expressed as percent of conidia germination or mycelium growth inhibition compared with the control. In turn, Parafilm and filter papers were removed from the dishes and another assessment was carried out after 7 days at 25 ºC in order to evaluate whether the activity of the compounds was fungistatic or fungicidal. The experiments were performed twice.

When mycelial growth was asymmetrical, four diameter measurements were determined and averaged. Inhibition of mycelial growth (IG) was calculated as a percentage from the difference between growths of treated and control mycelium, as shown below:

GI (%) = 100 (C-T) / C

Where: C is mycelium diameter in control dishes and T ismycelium diameter in treated dishes.

Statistical analysis

The statistical effect of carvacrol and AITC concentration on *A. flavus* growth was studied with analysis of variance based on a completely randomized design. The means comparisons were made using Duncan's Multiple range tests at $P \leq 0.05$. All statistical analyses were carried out using the SPSS software.

For each compound, the effective dose (ED) for 50 and 95% inhibition (ED_{50} and ED_{95}, respectively) was calculated using probit analysis applied to the percentages of conidial germination and mycelial growth of *A. flavus* obtained from *in vitro* experiments. Regression lines between the logarithm of the compound concentrations and the effectiveness indices transformed in probit were calculated using the Statgraphics software.

Results

Effect of carvacrol on conidial germination and mycelial growth of A. flavus isolated from pistachio nuts

The results showed significant inhibitory effect of carvacrol on conidia germination of *A. flavus* isolated from pistachio nut. In the first control, after 3 days, carvacrol completely inhibited conidia germination of fungus at 3.0 µL L^{-1} (100%). In addition, carvacrol at 1.5 and 0.74 µL L^{-1} indicated 93% and 86% germination inhibition, but at 0.74 µL L^{-1} didn't show an acceptable inhibitory effect on conidia germination of *A. flavus* (13 %). After 7 days, approximately the same results were obtained for germination inhibition. The highest germination inhibition was at 3.0 and 1.5 µL carvacrol per liter (100% and 93 % respectively). But we observed large reduction of conidia germination inhibition at 0.37 and 0.12 (35% and 0.9%) after 7 days (Fig. 1).

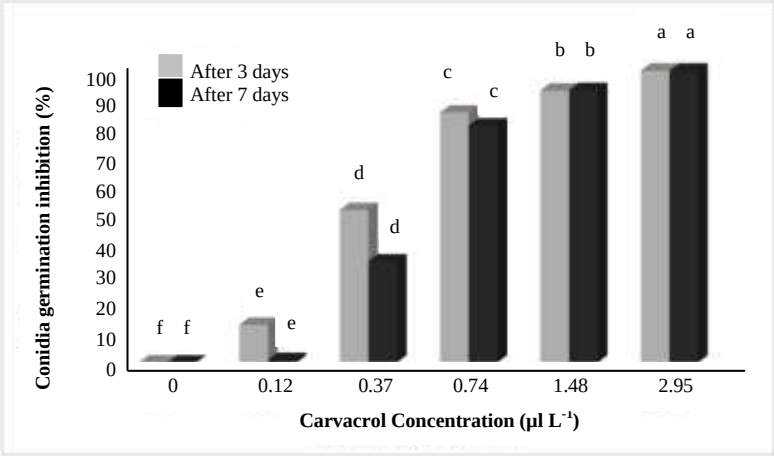

Fig 1. Effect of different concentrations of carvacrol on conidia germination of *A. flavus*

On the other hand results obtained for the effect of carvacrol on mycelium growth of *A. flavus* at 3 days and at 7 days showed that carvarol could inhibit completely mycelial growth of fungus at 12 µL L^{-1} in both cases. Different concentration of carvacrol had significant effect on *A. flavus* mycelial growth. After 3 days, complete inhibition of mycelium growth was found at 5.9 µL L^{-1}. However in the 7-day experiment, mycelial growth inhibition reduced and was less pronounced at the lower concentrations because of fungal growth (Fig. 2).

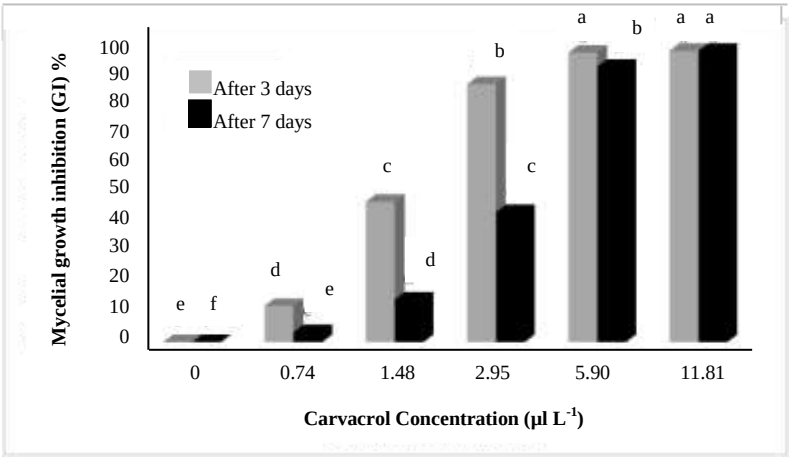

Fig 2. Effect of different concentrations of carvacrol on mycelial growth of *A. flavus*

Effect of Allyl isothiosyanate on conidial germination and mycelial growth of A. flavus isolated from pistachio nuts

AITC had significant a effect on conidia germination of *A. flavus* at 1.5, 0.74, 0.37 and 0.12 µL L^{-1} with 92, 69, 47 and 15% in the 3-day experiment. However 3.0 (µL L^{-1}) completely (100%) inhibited spore germination of this fungus after 3 days. We observed similar results in the second experiment, after 7 days of inoculum, plates containing MEA with AITC at different concentrations, we observed similar results, except that there was less inhibition at the lower concentrations. So at 1.5, 0.74, 0.37 and 0.12 µL L^{-1} AITC, there were 91, 72, 28 and 12% inhibition (Fig. 3).

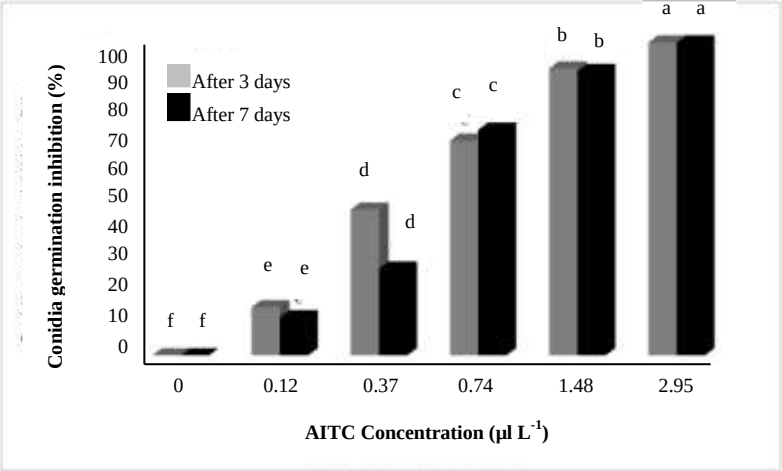

Fig 3. Effect of different concentrations of AITC on conidia germination of *A. flavus*

Our results showed a highly significant effect of AITC on mycelial growth of *A. flavus*. Our results showed that AITC at 12, 6.0 and 3.0 µL L^{-1} completely inhibited mycelium growth of *A. flavus* after 3 days. This growth inhibition was lower after 7 days from inoculum with *A. flavus*. However there was high percentage of growth inhibition at 12 and 6 µL L^{-1} (100%) whereas we observed reduced in mycelial growth inhibition at lower concentrations (3.0, 1.5 and 0.74) of AITC) (Fig. 4).

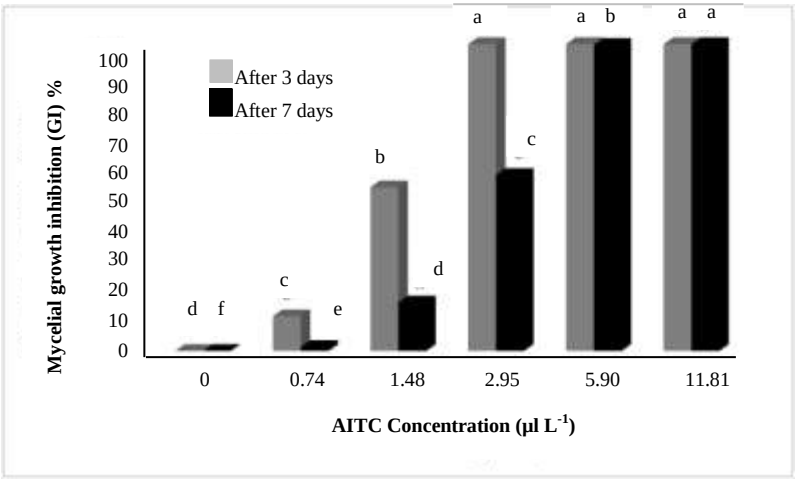

Fig 4. Effect of different concentrations of AITC on Mycelial growth of *A. flavus*

Logarithmic regression lines of the compound concentrations and the effectiveness indices transformed in probit were used to calculate the ED_{50} and ED_{95}. On basis of these results, AITC was a better mycelial growth inhibitor (ED_{50} = 2.18 µL/L, ED_{95} = 4.5 µL/L) than carvacrol (Table 1).

Table 1. ED50 and ED95 (µL L^{-1}) of treatments with Carvacrol and AITC measured by conidial germination and mycelial growth of *A. flavus*

Natural compound		ED50	ED95
Carvacrol	Mycelial growth	2.5882	5.6169
	Conidia germination	0.375	1.145
AITC	Mycelial growth	2.1817	4.4978
	Conidia germination	0.301	1.264

Discussion

The results of this experiment indicated that carvacrol and AITC have a significant inhibitory effect on conidia germination and mycelial growth of *A. flavus* isolated from pistachio nuts.

The antimicrobial and antifungal effect of aromatic plant EOs has been described in several studies (Arras and Usai, 2001; Alizadeh *et al.*, 2010). In particular, the major phenolic components of EOs, mainly carvacrol, have been suggested to have a potent antimicrobial activity *in vitro* (Ultee *et al.*, 2002; Bouchra *et al.*, 2003; Abbaszadeh *et al.*, 2014; Pérez-Alfonso *et al.*, 2012) against *Aspergillus* spp. (Bisht *et al.*, 2011; Fernanda *et al.*, 2012). Little literature exists on the effect of carvacrol on pathogenic food fungi (Morcia *et al*., 2012), although carvacrol was effective on inhibiting spore germination of *Botrytis cinerea* (Martinez-Romero *et al*., 2007) and mycelial growth of *Fusarium* spp. (Muller-Riebau *et al*., 1995). Abbaszadeh *et al*., (2014) have mentioned the potent inhibitory activity of thymol, carvacrol, eugenol and menthol against *Aspergillus* spp. In addition, Markovic *et al*., (2011) demonstrated that carvacrol has more remarkable antifungal potential against *Aspergillus* spp. than *Penicillium* spp. The above-mentioned studies are consistent with our findings.

Bouddine *et al*., (2012) revealed that *A. niger* growth was completely inhibited by carvacrol at concentrations of 0.025% MFC (minimal fungicidal concentration). The antifungal mechanism of carvacrol is not well understood although membrane and cell wall disruption with morphological deformation, collapse and deterioration of the conidia and/or hyphae have been hypothesized (Ahmad *et al*., 2011) Several studies have shown that phenols have very strong antifungal activity. On the other hand, The World Health Organization (WHO) has stated that carvacrol residue in food is without danger to the consumer as long as they do not exceed 50 mg/kg (WHO, 2002). Thus, it could be applied as post-harvest treatment for controlling food decay.

The antimicrobial activity of AITC is suggested to involve a reaction with thiol groups of glutathione or redox-active proteins, with subsequent inhibition of sulfhydryl enzyme activities and inhibition of redox-based defenses (Kolm *et al*., 1995; Jacob and Anwar, 2008). The addition of exogenous thiol groups can suppress the antimicrobial effect of AITC (Tajima *et al*., 1998). The activity of AITC varies with the concentration and pathogen.

Mari *et al*., (2002) demonstrated inhibition of conidial germination and mycelial growth of several post-harvest fruit pathogens by natural ITCs. A few years later, Manici *et al*., (1997) confirmed inhibition of fungal growth of eight plant pathogenic fungi from different taxonomical classes by four ITCs (benzyl, 3-methylsulfinylpropyl, allyl and 2-hydroxy-3-butenylisothiocyanates).

The mechanism by which ITCs inhibit fungal growth is not yet completely known. Nevertheless, some hypotheses suggest a non-specific and irreversible interaction of the ITC with the sulfidryl groups, disulfide bonds and amino groups of proteins and amino acid residues (Fahey *et al.*, 2001). Reactions with the sulfhydryl group and a decrease in the amount of free amino groups of the proteins' amino acid side residues were observed. The main target of toxic lipophilic compounds on eukaryotic cells is thought to be the cell membrane (sikkema *et al.*, 1995) AITC is a lipophilic compound, therefore it is possible it could react with some enzymes present at the plasma membrane level, causing fungi growth inhibition or cell death.

Reduction in fungal growth inhibition after 7 days compared by 3 days can be because of logarithmic control of fungal growth, or reduction of components after 7 days because of their volatility.

Our results showed an interesting antifungal potential of Carvacrol and allyl isothiocyanate against postharvest fruits spoilage fungi. Additionally, the properties of carvacrol and AITC as antibacterial, antifungal and anticancer compounds and their safety for human and environment, have increased their interest

as food supplements (Singh and Singh, 2012; Zhang, 2012). As pistachio is an important product of Iran and is strategic to export, we suggest further

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References

Abbaszadeh S, Sharifzadeh A, Shokric H, Khosravib AR, Abbaszadehd A (2014) Antifungal efficacy of thymol, carvacrol, eugenol and menthol as alternative agents to control the growth of food-relevant fungi. Journal of Medical Mycology. 24(2), 51–56.

Afsah-Hejri L, Jinap S, Hajeb P, Radu S, Shakibazadeh S (2013) A Review on Mycotoxins in Food and Feed: Malaysia Case Study. Comprehensive Reviews in Food Science and Food Safety. 12, 629-651.

Aghdaie AS (2009) Investigating Effective Factors on Iran's Pistachio Exportation. International Journal of Marketing Studies. 1(2), 35-40.

Ahmad A, Khan A, Akhtar F, Yousuf S, Xess I, Khan LA (2011) Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against *Candida*. European Journal of Clinical Microbiology & Infectious Diseases. 30, 41-50.

Alizadeh-Salteh S, Arzani K, Omidbeigi R, Safaie N (2010) Essential oils inhibit mycelial growth of *Rhizopus stolonifer*. European Journal of Horticultural Science. 75, 278–282.

Arras G, Usai M (2001) Fungitoxic activity of essential oils against four postharvest citrus pathogens: chemical analysis of *Thymus capitatus* oil and its effect in subatmospheric pressure conditions. Journal of Food Protection. 64(7), 1025-1029.

Bhat R, Rai RV, Karim A (2010) Mycotoxins in food and feed: present status and future concerns. Comprehensive Reviews in Food Science and Food Safety. 9, 57-81.

Bisht D, Pal A, Chanotiya CS, Mishra D, Pandey KN (2011) Terpenoid composition and antifungal activity of three commercially important essential oils against *Aspergillus flavus* and *Aspergillus niger*. Natural product research. 25(20), 1993-8.

Bluma R, Amaiden MR, Daghero J, Etcheverry, M (2008) Control of *Aspergillus* section Flavi growth and aflatoxin accumulation by plant essential oils. Journal of Applied Microbiology. 105, 203–214.

Bouchra C, Achouri M, Idrissi HLM, Hmamouchi M (2003) Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against *Botrytis cinerea* Pers: Fr. Journal of Ethnopharmacology. 89, 165-169.

Bouddine L, Louaste B, Achahbar S, Chai N, Chamri F, Remmal A (2012) Comparative study of the antifungal activity of some essential oils and their major phenolic components against *Aspergillus niger* using three different methods. African Journal of Biotechnology. 11, 14083-7.

da Silva FS, Chalfoun SM, de Siqueira VM, Botelho DMS, Lima N, Batista LR (2012) Evaluation of antifungal activity of essential oils against potentially mycotoxigenic *Aspergillus flavus* and *Aspergillus parasiticus*. Brazilian Journal of Pharmacognosy. 22(5), 1002-1010.

Davidson PM (2001) Chemical preservatives and naturally antimicrobial compounds. In M. P. Doyle, Beuchat LR, Montville TJ (Eds.), Food microbiology fundamentals and frontiers, Washington DC: ASM Press. (2nd ed., pp. 593-628).

Elshafie HS, Mancini E, Sakr S, De Martino L, Mattia CA, De Feo V, Camele I (2015) Antifungal Activity of Some Constituents of *Origanum*

vulgare L. Essential Oil against Postharvest Disease of Peach Fruit. Journal of Medicinal Food. In press.

Fahey JW, Zalcmann AT, Talalay P (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry. 56, 5–51.

Harris JC, Cottrell SL, Plummer S, Lloyd D (2001) Antimicrobial properties of *Allium sativum* (garlic). Applied Microbiology and Biotechnology. 57, 282-286.

Herrman TJ, Trigo-Stockli D, Pedersen JR (2002) Mycotoxins in feed grains and ingredients. Manhattan, KS: Cooperative Extension Service, Kansas State University.

Inoue S, Goi H, Miyauchi K, Muraki S, Ogihara M, Iwatani Y (1983) Inhibitory effect of volatile constituents of plants on the proliferation of bacteria– Antibacterial activity of plant volatiles. Journal of Antibacterial and Antifungal Agents. 11, 609–615.

Jacob C, Anwar A (2008) The chemistry behind redox regulation with a focus on sulphur redox systems. Physiology of Plants. 133, 469–48010

Kermanshai R, McCarry BE, Rosenfeld J, Summers PS, Weretilnyk EA, Sorger G (2001) Benzyl isothiocyanate is the chief or sole anthelmintic in papaya seed extracts. Phytochemistry. 57, 427–435.

Kolm RH, Danielson UH, Zhang Y, Talalay P, Mannervik B (1995) Isothiocyanates as substrates for human glutathione transferases: structure-activity studies. Biochemistry Journal. 311(2), 453–459.

Lima O, Pereira F, Araújo W, Lima E, Menezes EA, Afrânio Cunha FA, MeloDiniz M (2013) Antifungal activity and mode of action of carvacrol against *Candida albicans* strains. Journal of Essential Oil Research. 25(2), 138-142.

Mahoney N, Rodriguez SB (1996) Aflatoxin variability in pistachios. Applied Environmental Microbiology. 62, 1197-1202.

Manici ML, Lazzeri L, Palmieri S (1997) *In vitro* fungitoxic activity of some glucosinolates and their enzyme-derived products toward plant pathogenic fungi. Journal of Agricultural and Food Chemistry. 45, 2768–73.

Mari M, Rossi A, Brigati S, Pratella GC (2002) Antifungal vapourphase activity of allyl-isothiocyanate against *Penicillium expansum* on pears. Plant Pathology. 51, 231–6.

Markovic T, Chatzopoulou P, S˘iljegovic J, Nikoli N, Glamoclija J, C´iric A (2011) Chemical analysis and antimicrobial activities of the essential oils of *Satureja thymbra* L. and *Thymbra spicata* L. and their main components. Archive of Biological Sciences. 63, 457-64.

Martinez-Romero D, Guillen F, Valverde JM, Bailen G, Zapata PJ, Serrano M (2007) Influence of carvacrol on survival of *Botrytis cinerea* inoculated in table grapes. International Journal of Food Microbiology. 115, 144-8.

Morcia C, Malanati M, Terzi V (2012) In vitro activity of terpinen-4-ol, eugenol, carvone, 1, 8-cineole (eucalyptol) and thymol against mycotoxigenic plant pathogens. Food Additives Contamination. 29, 415-22.

Muller-Riebau F, Berger B, Yegen O (1995) Chemical composition and fungitoxic properties to phytopathogenic fungi of essential oils of selected aromatic plants growing wild in Turkey. Journal of Agricultural Food Chemistry. 43, 2262-6.

Neri F, Mari M, Brigati S (2006) Control of *Penicillium expansum* by plant volatile compounds. Plant Pathology. 55, 100-105.

Numpaque MA, Oviedo LA, Gil JH, García CM, Durango, DL (2011) Thymol and carvacrol: biotransformation and antifungal activity against the plant pathogenic fungi Collet *Ot-*

richum acutatum and *Botryodiplodia theobromae*. Tropical Plant Pathology. 36(1), 3-13.

Omidbeygi M, Barzegar M, Hamidi Z, Naghdibadi H (2006) Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus xavus* in liquid medium and tomato paste. Food Control. 18, 1518-1523.

Parekh J, Chanda S (2007) *In vitro* screening of anti-Bacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from enterobacteriaceae. African Journal of Microbiology Research. 6, 92-99.

Pérez-Alfonso CO, Martínez-Romero D, Zapata PJ, Serrano M, Valero D, Castillo S (2012) The effects of essential oils carvacrol and thymol on growth of *Penicillium digitatum* and *P. italicum* involved in lemon decay. International Journal of Food Microbiology. 158(2), 101-106

Rasooli I, Razzaghi AM (2004) Inhibitory effects of Thyme oils on growth and aflatoxin production by *Aspergillus parasiticus*. Food Control. 15(6), 479-483.

Rustom I (1997) Aflatoxin in food and feed: occurrence, legislation and inactivation by physical methods. Food Chemistry. 59, 57-67.

Sikkema J, de Bont JA, Poolman B (1995) Mechanisms of membrane toxicity of hydrocarbon. Microbiological Review. 59(2), 201-22.

Singh SV, Singh K (2012) Cancer chemoprevention with dietary isothiocyanates mature for clinical translational research. Carcinogenesis. 33, 1833–1842.

Tajima H, Kimoto H, Taketo Y, Taketo A (1998) Effects of synthetic hydroxyisothiocyanates on microbial systems. Bioscience, Biotechnology and Biochemistry. 62, 491–49510.

Tookey HL, Van Etten CH, Daxenbichler ME (1980) Glucosinolates. In I. E. Liener (Ed.), Toxic constituents of plant stuffs. Pp. 103–142. New York: Academic Press.

Tsantili E, Takidelli C, Christopoulos MV, Lambrinea E, Rouskas D, Roussos PA (2010) Physical, compositional and sensory differences in nuts among pistachio (*Pistachia vera* L.) varieties. Scientia Horticulturae. 125, 562-568.

Tsao R, Zhou T (2000) Antifungal Activity of Monoterpenoids against Postharvest Pathogens *Botrytis cinerea* and *Monilinia fructicola*. Journal of Essential Oil Research. 12(1), 113-121.

Ultee A, Bennik MHJ, Moezelaar R (2002) The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Applied Environmental Microbiology. 68(4), 1561-1568.

WHO (2002) Food safety and foodborne illness. World Health Organization Fact sheet 237.

Zhang Y (2012) The molecular basis that unifies the metabolism, cellular uptake and chemo preventive activities of dietary isothiocyanates. Carcinogenesis. 33, 2–9.