Available online at www.sciencedirect.com
SCIENCE DIRECT
Phytochemistry 66 (2005) 2851–2856
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Chemoprevention by thyme oils of *Aspergillus parasiticus* growth and aflatoxin production

Iraj Rasooli [a,*], Parviz Owlia [b]

[a] *Department of Biology, Shahed University, P.O. Box 18151-159, Tehran-Qom Highway, Tehran, Iran*
[b] *Department of Microbiology, School of Medicine, Shahed University, P.O. Box 18151-159 Tehran, Iran*

Received 13 June 2005; received in revised form 22 September 2005
Available online 9 November 2005

Abstract

The essential oils from *Thymus eriocalyx* and *Thymus X-porlock* obtained by hydrodistillation were analyzed by GC/MS. The major components of *T. eriocalyx* and *T. X-porlock* oils were thymol (63.8, 31.7%), β-phellandrene (13.30, 38.7%), *cis*-sabinene hydroxide (8.1, 9.6%), 1,8-cineole (2, 1.7%), and β-pinene (1.31, 2%), respectively. Antifungal activities of the oils were studied with special reference to the inhibition of *Aspergillus parasiticus* growth and aflatoxin production. Minimal inhibitory (MIC) and minimal fungicidal (MFC) concentrations of the oils were determined. Static effects of the above oils against *A. parasiticus* were at 250 ppm and lethal effects of *T. eriocalyx* and *T. X-porlock* were 500 and 1000 ppm of the oils, respectively. Aflatoxin production was inhibited at 250 ppm of both oils with that of *T. eriocalyx* being stronger inhibitor. Transmission electron microscopy (TEM) of *A. parasiticus* exposed to MIC level (250 ppm) of the oils showed irreversible damage to cell wall, cell membrane, and cellular organelles. It is concluded that the essential oils could be safely used as preservative materials on some kinds of foods at low concentrations to protect them from fungal infections.

Keywords: *Thymus eriocalyx*; *Thymus X-porlock*; *Aspergillus parasiticus*; Essential oils; Aflatoxin; Ultrastructure

1. Introduction

Various species of thyme have been reported to possess antifungal properties (Pina-Vaz et al., 2004). There is no report on fungicidal activity of essential oils from *Thymus eriocalyx* and *Thymus X-porlock* on *Aspergillus parasiticus*. The essential oil of *Thymus striatus* and its major component, thymol, was analyzed for potential antifungal activity against plant, animal, and human pathogenic fungi from different genera including *Aspergillus*. The oil showed a strong inhibitory effect against all fungi investigated (Couladis et al., 2004). The presence and growth of fungi in food may cause spoilage and result in a reduction in quality and quantity. The use of natural antimicrobial compounds is important not only in the preservation of food but also in the control of human and plant diseases of microbial origin (Baratta et al., 1998). Some *Aspergillus* species are xerophilic fungi and are responsible for many cases of food and feed contamination (Abarc et al., 1994; Katta et al., 1995). Aflatoxin-producing moulds are widely distributed in nature and frequently contaminate human food resources. Aflatoxins are secondary metabolites produced by toxigenic strains of *Aspergillus flavus* and *A. parasiticus*. These fungi grow rapidly on a variety of natural substrates and consumption of contaminated food can pose serious health hazards to human and animals. They are able to produce aflatoxins in food and feedstuffs (Guo et al., 1996). Aflatoxin B1 (AFB1) is produced by *Aspergillus* species on agricultural commodities (Leontopoulos et al., 2003). Chemicals are currently used to limit the growth of hazardous fungi like *A. parasiticus* in stored foods. Namazi et al. (2002) demonstrated that 0.9–1% ammonia

* Corresponding author. Tel.: +98 21 6003260; fax: +98 21 5277645/6024006.
E-mail address: rasooli@shahed.ac.ir (I. Rasooli).

0031-9422/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2005.09.029

inhibited fungal growth together with aflatoxin production. The use of spices and their non-toxic derivatives as alternative preservative agents is considered by consumers as safe. The limited knowledge concerning antimicrobial activity and the mechanism of action of plant extracts has led us to address such issues. Most of these studies have been conducted using essential oils in microbiological media; consequently, little is understood about mechanism of their effectiveness. *A. parasiticus*, as food contaminant, has still received little attention as far as essential oils are concerned. Allameh et al. (2001) reported more than 50% inhibition of aflatoxin production at 50% (v/v) neem extract concentration. In this report, we decided to study the mechanism of fungicidal activity of essential oils from *T. eriocalyx* and *T. X-porlock* on *A. parasiticus* both in vitro and at ultrastructural level.

2. Results

Essential oils extracted from *T. eriocalyx* and *T. X-porlock* yielded 1.2% and 1.0% w/w oil, respectively. The oil contents were in expected range. Chemical analysis of the components of the oils led to identification of 18 and 19 components in *T. eriocalyx* and *T. X-porlock* oils, respectively

Table 1
Chemical composition of essential oils from *T. eriocalyx* and *T. X-porlock*

No.	*T. eriocalyx* compounds	Kovats	%	*T. X-porlock* compounds	Kovats	%
1	Tricyclene	909	0.70	Tricyclene	909	1.80
2	α-Thujene	916	0.40	α-Thujene	916	1.10
3	α-Pinene	929	0.30	α-Pinene	929	0.80
4	Jerbenene	949	0.30	Jerbenene	949	2.20
5	β-Pinene	967	1.31	Sabinene	967	0.30
6	Myrcene	982	0.30	β-Pinene	967	2.00
7	α-Phellandrene	995	1.20	Myrcene	982	0.30
8	β-Phellandrene	1005	13.30	β-Phellandrene	1005	39.4
9	1,8-Cineole	1009	2.00	1,8-Cineole	1009	1.70
10	*cis*-Sabinene hydroxide	1036	8.10	*cis*-Sabinene hydroxide	1036	9.60
11	*n*-Octanol	1039	0.90	*n*-Octanol	1039	1.0
12	*trans*-Thujene	1110	0.30	*cis*-Linalool oxide	1066	1.50
13	(*z*)-Tagetone	1134	0.80	(*z*)-Tagetone	1134	1.50
14	*cis*-Sabinene hydrate acetate	1214	0.70	Sabina ketone	1147	0.50
15	(*E*)-Cinnamaldehyde	1256	0.80	*cis*-Sabinene hydrate acetate	1214	0.20
16	*cis*-Sabinene hydrate	1096	0.30	Thymol	1267	31.7
17	Thymol	1267	63.8	*trans*-Sabinyl acetate	1271	1.80
18	Germacrene D	1472	1.2	Longifolene	1393	1.20
19	–	–	–	Germacrene	1468	0.20

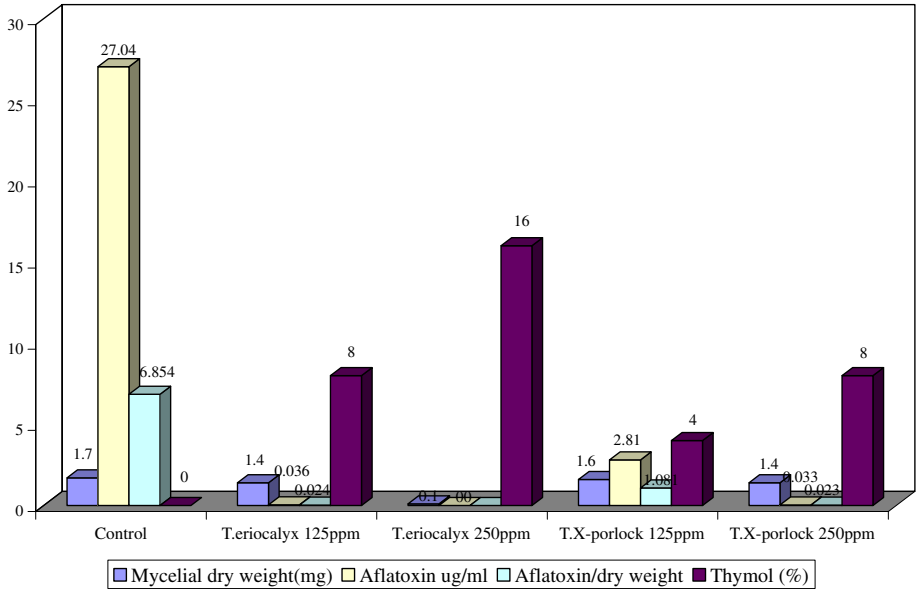

Fig. 1. Effect of thyme essential oils on *A. parasiticus* growth and aflatoxin production.

(Table 1). The major components of *T. eriocalyx* and *T. X-porlock* oils were thymol (63.8, 31.7%), β-phellandrene (13.30, 38.7%), *cis*-sabinene hydroxide (8.1, 9.6%), 1,8-cineole (2, 1.7%), and β-pinene (1.31, 2%), respectively. The chemical constituents are similar to other varieties of thyme but at different concentrations. Preliminary experiments were carried out in vitro using the disc diffusion and broth dilution methods to investigate antifungal action of the essential oils. Various concentrations of essential oils from *T. eriocalyx* and *T. X-porlock* were tested on potato dextrose agar plates and broth tubes showed a very strong antifungal property (Fig. 1). The oils strongly inhibited fungal growth at 2000, 1000, and 500 ppm levels. *A. parasiticus* growth inhibition zones of 12 and 10 mm were observed at 250 and 125 ppm of *T. eriocalyx* oil and 45, 25, 10, and 7 mm at 1000, 500, 250, and 125 ppm of *T. X-porlock* oil, respectively. MIC and MFC techniques were employed to assess fungistatic and fungicidal properties of the oils. Static effects of the above oils against *A. parasiticus* were at 250 ppm and lethal effects were observed at 500 and 1000 ppm of *T. eriocalyx* and *T. X-porlock* oils, respectively. Aflatoxin production was inhibited at 250 ppm of both oils with that of *T. eriocalyx* being stronger inhibitor (Fig. 1). Transmission electron microscopy (TEM) of *A. parasiticus* exposed to MIC level (250 ppm) of the oils showed severe damage to cell wall, cell membrane, and cellular organelles such as mitochondria which seem to be destroyed (Figs. 3 and 4).

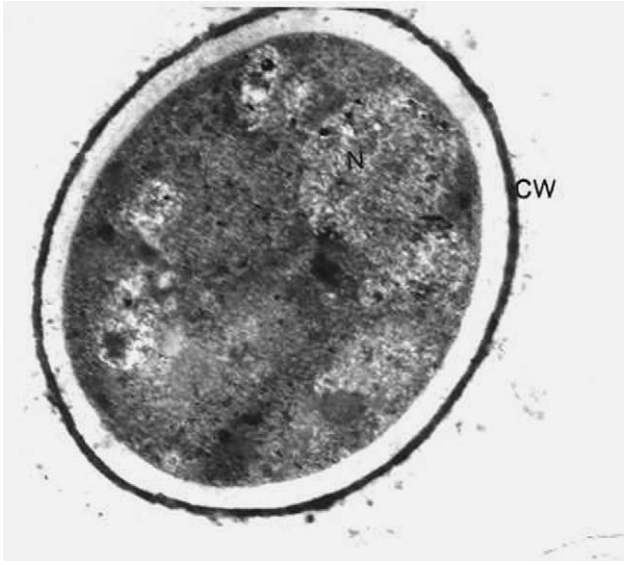

Fig. 2. EM graph 8000×, *A. parasiticus* (control), CW, cell wall and N, nucleus.

3. Discussion

Preliminary results indicate antifungal efficacy of the essential oils of *T. eriocalyx* and *T. X-porlock*. The essential oils of *T. eriocalyx* and *T. X-porlock* were inhibitory on fungal development and aflatoxin production at 250 ppm. The extent of inhibition of fungal growth and mycotoxin production was dependent on the concentration of essential oils used (Fig. 1). Soliman and Badeaa (2002) reported complete inhibition of *Aspergillus flavus*, *A. parasiticus*, and *A. ochraceus* by the oils of thyme and cinnamon (<500 ppm), marigold (<2000 ppm), spearmint, basil, (3000 ppm). However, they did not specify chemical composition of their thyme oil. Growth of *A. parasiticus* NRRL 2999 was reported to be completely inhibited by thyme (wild), thyme (black), oregano and savory extracts at the 2% level in Czapek-Dox Agar (Ozcan, 1998). Our results conducted in YES medium, more nutritious medium than Czapek-Dox Agar, indicate complete inhibition of *A. parasiticus* at 0.05% (500 ppm) and 1% (1000 ppm) of *T. eriocalyx* and *T. X-porlock* oils, respectively. This indicates that highly nutritious medium such as YES could not support fungal cells resistance against the oils. The antifungal effect of the thyme oils could be related to several components known to have biological activities, such as thymol as the most prevalent component (Soliman and Badeaa, 2002). Thymol (63.8, 31.7%) and β-phellandrene (13.30, 38.7%) accounted for the most abundant components of essential oils of *T. eriocalyx* and *T. X-porlock* oils, respectively. It may be deduced that fungal growth inhibition and subsequent mycotoxin production were related mostly to thymol content of the oils (Fig. 1). It should be noted that there was a gradual increase in inhibition due to the increased concentration of thymol present in the essential oils (Fig. 1). Transmission electron microscopy (TEM) of untreated *A. parasiticus* revealed intact mycelia with healthy structure, smooth cell wall, and cell membrane (Fig. 2). The mycelium treated with thyme oil showed alterations in the morphology of the hyphae, which appeared severely collapsed, and a reduction in conidiation and aflatoxin production (Figs. 1 and 3). Kale et al. (1996) attributed the loss of aflatoxigenic capabilities in the nonaflatoxigenic variants of *A. parasiticus* to the alterations in the conidial morphology of the fungus, suggesting that the regulation of aflatoxin synthesis and conidiogenesis may be interlinked. Fungal growth inhibition was reported to be associated with the degeneration of fungal hyphae after treatment with *Thymus vulgaris* L., *Lavandula* R.C., and *Mentha piperita* L. essential oils with the oil of thyme being more effective than that of lavender or mint (Zambonelli et al., 1996). Our observations show that the main target of the oils were cell wall and cell membrane (Figs. 3 and 4). The plasma membrane of *A. parasiticus*, in the presence of thyme essential oils at 250 ppm, was seen to be irregular, dissociated from the cell wall, invaginated and associated with the formation of lomasomes (Fig. 3). These lomasomes are usually found in fungi treated with imidazole components (Mazabrey et al., 1985, Scott et al., 1986). The marked action of oil components might have conferred lipophilic properties and the ability to penetrate the plasma membrane (Knobloch et al., 1989). A synthetic antifungal alcohol, econazole, has been reported to find its target in the plasma membrane of *Microsporum canis* cells which

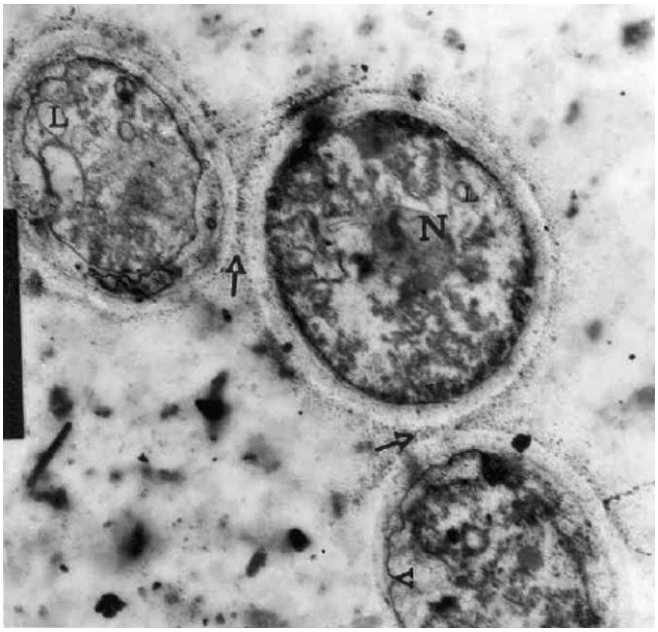

Fig. 3. EM graph 10,000×, *A. parasiticus* exposed to 125 ppm of essential oil from *T. eriocalyx* showing damage to the organelles, destruction of cytoplasm, folding of cell membranes (arrow head). The cells seem to be approaching each other by sharing their membranes (arrows). N, nucleus and L, lomasomes.

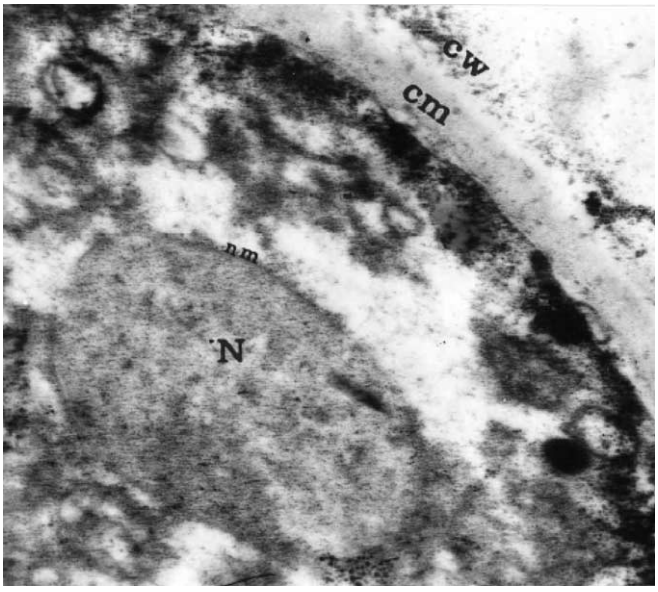

Fig. 4. EM graph 32,000×, *A. parasiticus* exposed to 250 ppm of essential oil from *T. X-porlock* showing damage to the cell wall (cw) and nuclear membrane (nm). cm, cell membrane.

dissociated from the wall after a 4 h treatment at 100 mg/L (Mazabrey et al., 1985). The thyme oils under this study showed shorter effective time at lower concentrations. TEM observations by Watanabe et al. (1998) on the effect of benacomicin A, a synthetic antifungal agent with mannan affinity, which therefore affects wall synthesis, revealed disruption of *Saccharomyces cerevisiae* plasma membrane. In this study, we noted a marked alteration of the *A. parasiticus* wall in the presence of thyme essential oils. The cells approach closer to each other by sharing their membranes (arrows in Fig. 3). This phenomenon was evident everywhere on the TEM sections scanned for destructive effect of the oils. The reason to explain this phenomenon needs further investigations. The cell wall (CW) exhibited degenerative changes (Figs. 3 and 4). The membrane being targeted by the essential oils is further evident in the nucleus. The nuclear membrane (nm) was also affected (Fig. 4). Ghfir et al. (1997) showed that *Hyssopus officinalis* essential oil affected the wall synthesis of *Aspergillus fumigatus*. The presence of the oil in the culture medium induced marked changes in the content of galactose and galactosamine. The alterations were related to changes in the structure of the cells. Such modifications induced by essential oils may be related to the interference of essential oil components with enzymatic reactions of wall synthesis, which affects fungal morphogenesis and growth. Cell wall degradation due to a lack of polysaccharide constituents of the fungal wall has also been observed on *Candida albicans* cells treated with *Carica papaya* latex sap (Giordani et al., 1996). Belanger et al. (1997) found growth inhibition, cell wall thinning and cell membrane degradation on cells of *Candida* sp. treated with voriconazole and fluconazole. Kurita et al. (1981) suggested that the antifungal activity of essential oil components, particularly aliphatic aldehydes, might be due to their ability to form charge transfer complexes with electron donors in the fungus cell.

4. Conclusions

The findings indicate that essential oils should find a practical application in the inhibition of mycotoxin production in food products. Essential oils could be safely used as preservative materials on some kinds of foods, such as thyme oil which inhibited the growth and aflatoxin production of *A. parasiticus* at low concentrations, leading to deleterious cellular morphological alterations, which become irreversible at 250 ppm, and could be added to food stuffs in storage to protect them from fungal infections.

5. Experimental

5.1. General

Clevenger, GC (9-A-Shimadzu), GC/MS (Varian-3400), Microbial cuture media (Merck), Silica gel–GF pre-coated sheets, Shimadzu UV-2501PC spectrophotometer, JEOL-100 Transmission Electron Microscope (TEM), and the fungal culture of *A. parasiticus* (NRRL-2999) were employed in this study. Other chemicals from Merck were of analytical grade.

5.2. Plant materials

The plants, *T. eriocalyx* and *T. X-porlock*, were collected from National Botanical Garden of Iran during May–June 2004.

5.3. Oil extraction and analysis

The fresh aerial parts, i.e. the leaves, were hydrodistilled for 90 min in full glass apparatus. The oils were isolated using a Clevenger-type apparatus. The extraction was carried out for two hours after a four-hour maceration in 500 mL of water. The oils were stored in dark glass bottles in a freezer until they were used. Methanol was selected as a diluting agent for the oils as it did not exhibit antifungal activity when tested with *A. parasiticus*. 1/2, 1/4, 1/8, and 1/16 dilutions of oils were made with methanol. These dilutions were used in antifungal analysis. Undiluted oil was taken as dilution 1. This solvent also served as control. The essential oils were analyzed by GC (9-A-Shimadzu) and GC/MS (Varian-3400) column: {DB-1 (dimethyl polysiloxane), 60 m × 0.25 mm fused silica capillary column, film thickness 0.25 μm film thick)} using a temperature program of 50–250 °C at a rate of 4 °C/min, injector temperature 250 °C, carrier gas: helium (99.99%), inlet pressure 3 kg/cm^2. The constituents were identified by comparison of their mass spectra with those in the computer library and with authentic compounds. The identifications were confirmed by comparison of their retention indices with those of authentic compounds or with literature data.

5.4. Microbial strain and growth media

A. parasiticus (NRRL-2999) was maintained on Potato Dextrose Agar. Spore suspensions were prepared and diluted in sterile yeast extract sucrose (YES) broth to a concentration of approximately 10^8 spores/ml. Spore population was counted using haemocytometer. Subsequent dilutions were made from the above suspension, which were then used in the tests. YES broth also served as aflatoxin production medium.

5.5. Antifungal analysis

Antifungal analysis and determination of the minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of the oils were performed by broth dilution method in test tubes as follows: 50 μl from each of various dilutions of the oils was added to 5 ml of YES broth tubes containing 10^7 spores/ml. The tubes were then incubated on an incubator shaker as to evenly disperse the oil throughout the broth in tubes. The highest dilution (lowest concentration), showing no visible growth, was regarded as MIC. Cells from the tubes showing no growth were subcultured on potato dextrose agar plates to determine if the inhibition was reversible or permanent. MFC was determined as the highest dilution (lowest concentration) at which no growth occurred on the plates.

5.6. Determination of mycelial weight

Flasks containing mycelia were filtered through Whatman filter No. 1 and then were washed with distilled water. The mycelia were placed on pre weighed Petri plates and were allowed to dry at 60 °C for 6 h and then at 40 °C overnight. The flasks containing dry mycelia were weighed. Percent growth inhibition on the basis of dry weight is calculated as:

$$\text{Control weight} - \text{Sample weight} \div \text{Control weight} \times 100.$$

5.7. Measurement of aflatoxins

Aflatoxin extraction was performed routinely with solvent extraction (Allameh et al., 2001; Razzaghi et al., 2000). Silica gel–GF precoated sheets were used for analysis of aflatoxins produced by the fungal strain. The toxin was measured spectrophotometrically in aflatoxin fraction eluted from silica gel according to the procedure described by Nabney and Nesbitt (1965).

5.8. Transmission electron microscopy

500 μl of *T. eriocalyx* and *T. X-porlock* oils at 125 and 250 ppm, respectively, was added to 50 ml of each spore suspension containing 10^7 spores/ml and were then incubated on a shaker at 30 °C for 72 h. Samples were then taken and processed for transmission electron microscopy (TEM). Mycelial samples were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate-buffer (pH 7.2) for 2 h at room temperature. They were washed three times, each time for 15 min, in cacodylate-buffer. Specimens were then post-fixed for 2 h in 1% osmium tetroxide (OsO_4) dissolved in cacodylate-buffer at room temperature and washed in cacodylate-buffer (three times, 15 min each). Samples were dehydrated in a graded series of ethanol (40%, 60%, 75%, 80%, and 95%, two times for 15 min each and two times for 30 min each in 100% ethanol). Fixed mycelia were processed in graded propylene oxide: araldite and finally were embedded in araldite. The polymeration of araldite to form specimen blocks was accomplished in an oven at 45 °C for 24 h and then at 70 °C for 48 h. The specimen blocks were hand trimmed with a razor blade and sectioned with an ultramicrotome with 1 μm thickness (sections appearing blue color under ultratome) for light microscopic observations and 0.1 μm (sections appearing blue color under ultratome) for transmission electron microscopic observations. The ultrathin sections were placed on 200 mesh copper grids. The sections were stained with 12.5% alcoholic uranyl acetate [$UO_2(OH_3COO)_2 \cdot H_2O$] in methanol for 20 min and then with lead citrate [25 mg lead citrate [$Pb_3(C_6H_5O_7)_2 \cdot 3H_2O$] dissolved 1 ml of 1 N sodium

hydroxide and the final volume was made to 10 ml by adding 9 ml double distilled water], and then were washed with double distilled water for 1 min, dried under reading lamp for 30 min and viewed with a JEOL 100 (Japan) Transmission Electron Microscope (TEM) operating at 80 kV.

Acknowledgments

The authors thank Shahed University (Tehran-Iran) for the sanction of research grants to conduct the present study. Special thanks to Mr. Mohammad Habibi and Ms. Shakiba Darvish Alipoor for their laborious works in our Microbiology laboratory.

References

Abarc, M.L., Bragulat, M.R., Castella, G., Cabanes, F.J., 1994. Mycoflora and aflatoxin-producing strains in animal mixed feeds. Journal of Food Protection 57, 256–258.

Allameh, A., Razzaghi, A.M., Shams, M., Rezaei, M.B., Jaimand, K., 2001. Effects of neem leaf extract on production of aflatoxins and fatty acid synthetase, citrate dehydrogenase and glutathione *S*-transferase in *A. parasiticus*. Mycopathologia 154, 79–84.

Baratta, T.M., Dorman, D.J.H., Deans, G.S., Figueiredo, C.A., Barroso, G.J., Ruberto, G., 1998. Antimicrobial and antioxidant properties of some commercial oils. Flavour and Fragrance Journal 13, 235–244.

Belanger, P., Nast, C.C., Fratti, R., Sanati, H., Ghannoum, M., 1997. Voriconazole (UK-109, 496) inhibits the growth and alters the morphology of fluconazole-suceptible and -resistant *Candida* species. Antimicrobial Agents and Chemotherapy 41, 1840–1842.

Couladis, M., Tzakou, O., Kujundzic, S., Sokovic, M., Mimica-Dukic, N., 2004. Chemical analysis and antifungal activity of *Thymus striatus*. Phytotherapy Research 18, 40–42.

Ghfir, B., Fonvieille, J.L., Dargent, R., 1997. Effect of essential oil of *Hyssopus officinalis* on the chemical composition of the walls of *Aspergillus fumigatus*. Mycopathologia 138, 7–12.

Giordani, R., Cardenas, M.L., Moulin-Traffort, J., Regli, P., 1996. Fungicidal activity of latex sap from *Carica papaya* and antifungal effect of *D*-(+)- glucosamine on *Candida albicans* growth. Mycoses 39, 103–110.

Guo, B.Z., Russin, J.S., Brown, R.L., Celveland, T.E., Widstrom, N.W., 1996. Resistance to aflatoxin contamination in corn as influenced by relative humidity and kernel germination. Journal of Food Protection 59, 276–281.

Katta, S.K., Eskridge, K.M., Bullerman, L.B., 1995. Mold content of commercial popcorn. Journal of Food Protection 58, 1014–1017.

Knobloch, K., Pauli, P., Iberl, B., Weigand, H., Weiss, N., 1989. Antibacterial and antifungal properties of essential oil components. Journal of Essential Oil Research 1, 119–128.

Kurita, N., Miyaji, M., Kurane, R., Takahara, Y., 1981. Antifungal activity of essential oil components. Agricultural Biology and Chemistry 45, 945–952.

Leontopoulos, D., Siafaka, A., Markaki, P., 2003. Black olives as substrate for *Aspergillus parasiticus* growth and aflatoxin B1 production. Food Microbiology 20 (1), 119–126.

Mazabrey, D., Nadal, J., Seguela, J.P., Linas, M.D., 1985. Scanning and transmission electron microscopy: study of effects of econazole on *Microsporum canis*. Mycopathologia 91, 151–157.

Nabney, J., Nesbitt, B.F., 1965. Aspectrophot ometric method for determining aflatoxins. Analyst 90, 155–160.

Namazi, M., Allameh, A., Aminshahidi, M., Nohee, A., Malekzadeh, F., 2002. Inhibitory effects of ammonia solution on growth and aflatoxin production by *Aspergillus parasiticus* NRRL-2999. Acta Poloniae Toxicologica 10 (1), 65–72.

Ozcan, M., 1998. Inhibitory effects of spice extracts on the growth of *Aspergillus parasiticus* NRRL2999 strain. Z Lebensm Unters Forsch A 207, 253–255.

Pina-Vaz, C., Gonçalves Rodrigues, A., Pinto, E., Costa-de-Oliveira, S., Tavares, C., Salgueiro, L., Cavaleiro, C., Gonçalves, M.J., Martinez-de-Oliveira, J., 2004. Antifungal activity of *Thymus* oils and their major compounds (*JEADV*). Journal of European Academy of Dermatology and Venereology 18, 73–78.

Razzaghi, A.M., Allameh, A., Shams, M., 2000. Screening of aflatoxin producing mould isolates based on fluorescence production on a specific medium under ultraviolet light. Acta Medica Iranica 38, 67–73.

Scott, E.M., Gorman, S.P., Millership, J.S., Wright, L.R., 1986. Effect of miconazole and clotrimazole on K+ release and inhibition of ergosterol biosynthesis in *Trichophyton mentagrophytes* and related ultrastructural observations. Journal of Antimicrobial Chemotherapy. 17, 423–432.

Kale, S.P., Cary, J.W., Bhatnagar, D., Bennett, J.W., 1996. Characterization of experimentally induced, nonaflatoxigenic variant strains of *Aspergillus parasiticus*. Applied and Environmental Microbiology 62 (9), 3399–3404.

Soliman, K.M., Badeaa, R.I., 2002. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food and Chemical Toxicology 40, 1669–1675.

Watanabe, M., Nishiyama, Y., Inouye, S., Yamaguchi, H., Kondo, S., Takeuchi, T., 1998. Morphological alterations of *Saccharomyces cerevisiae* induced by benacomicin A, an antifungal antibiotic with mannan affinity. Microbiology and Immunology 42, 365–370.

Zambonelli, A., D'Aurelio, A.Z., Bianchi, A., Albasini, A., 1996. Effects of essential oils on phytopathogenic fungi. Journal of Phytopathology 144, 491–494.