Mohammad Esmaeil Zadah. ***et al.*** **/** ***International Journal of Pharmacy & Therapeutics*****, 7(2), 2016, 90-96.**

e- ISSN 0976-0342
Print ISSN 2229-7456

International Journal of Pharmacy & Therapeutics

Journal homepage: www.ijptjournal.com

IJPT

EFFECTS OF DEHYDROACETIC ACID AND OZONATED WATER ON ASPERGILLUS FLAVUS COLONIZATION AND AFLATOXIN B1 ACCUMULATION IN PISTACHIOS

Mohammad Esmaeil Zadah[1], Abbas Ali Jafari[2], Samaneh Sedighi[1], Seyed Morteza Seifati [1]

[1]Dep. of Biology, Ashkezar Branch, Islamic Azad Univesity, Ashkezar, Iran.
[2]Dep. of Medical Parasitology and Mycology, Medical school, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

ABSTRACT

Aflatoxin is the most prevalent group of carcinogenic mycotoxins, which produced mainly by *Aspergillus flavus*, on pistachio. The aim of present study was to use Dehydroacetic acid (DHA) and ozonated water to control *A. flavus* growth and accumulation of aflatoxin B1 on pistachios. In current experimental study, pistachio samples were firstly treated with DHA and ozonated water solutions and contaminated with 1×10^6 A. *flavus* cell suspensions. The chemical inhibitory properties were evaluated using enumeration of viable and cultivable fungal conidia on pistachios samples. The minimum inhibitory concentration and minimum fungicidal activity of DHA and ozonated water was also measured by broth dilution method. Finally Aflatoxin accumulation in treated samples was measured using HPLC technique. There wasn't seen any fungal growth on pistachios treated with DHA. There was seen a statistically significant differences between the mean viable conidia on pistachios treated with DHA and ozonated water (P=0.0001). MFC and MIC values for DHA was measured as 1/64 and 1/128, whereas for ozonated water obtained 1/4 and 1/16. There were seen statistically significant differences between the aflatoxin values in treated pistachios with two solutions. The inhibitory effect of DHA on the growth of fungi and production of aflatoxin was much more than ozonated water in present study.

Key Words:- Control, *Aspergillus flavus*, Aflatoxin, Pistachio, Dehydroacetic acid, Ozonated water.

INTRODUCTION

Aflatoxins (AFs) are known as potent sources of health risks to both humans and animals, naturally produced by the food borne *Aspergillus* species especially *A. flavus (*Sheikh-Ali *et al.*, 2014). They are primarily contaminated a wide range of human food and animal feeds, exhibited acute and chronic consequences including mutagenic, carcinogenic and teratogenic effects. Aflatoxins are secondary fungal metabolites mixes including Aflatoxin B1, B2, G1 and G2 in nature and several synthesized forms such as Aflatoxin M1 and M2 in dairy products. However the regulation for aflatoxin in food has been regulated in more than 120 countries (Williams *et al.* 2004), it has been estimated that over 5 billion people worldwide are exposed to dietary aflatoxin (Strosnider *et al.*, 2006).

The International Agency for Research on Cancer (IARC) has graded aflatoxin B1 (AFB1) as a Group I carcinogen, primarily affecting liver (Esteve *et al.*, 1993). There are seen also a synergism effect between aflatoxin and chronic hepatitis B virus (HBV) for developing cancer in endemic area resulted up to 30 times higher risk of liver cancer in individuals exposed to each risk factor alone (Kar, 2014).

Corresponding Author

Abbas Ali JAfari
Email: jaabno@gmail.com

Various sources of human foods, agricultural commodities, fruits, and tree nuts in tropical and subtropical areas of the world may contaminate with aflatoxin (Bandyopadhyay *et al.,* 2007). Maize, peanut, cottonseed, Brazil nut, pistachio nut, fig, spice and copra are reported as the most common agricultural commodities with the risk of aflatoxin contamination (Jelinek *et al.,* 1989)

Pistachio is one of main tree nuts that disposed to dietary aflatoxin exposure, reported as 7 to 45% of all sources humans' total aflatoxin exposure (Bui-Klimke *et al.,* 2014). In Iran, pistachios are considered as a major crop covering approximately 210000 ton (Abbaszadeh *et al.,* 2006), production of dried nuts and it was played an important role in the agricultural economy of several areas in central parts of Iran. Pistachio was one of the main Iranian export commodities, which banned by the European Union (EU) in 1997 as a result of aflatoxin levels ranging from 11–400 mg/kg in imported pistachio consignments. There are many reports in literature for Natural occurrence of AF in pistachio nuts. In turkey analysis of 523 pistachio nut samples showed the mean of AFB1 ranged 1–3.78 mg/g and the maximum level detected was 113ng/g during 1998–2002 (Set and Erkme 2010).

Aflatoxins (AFs) are known as the main contaminants in pistachio nuts mostly produced by Aspergillus flavus .A survey on 50 samples of pistachio nuts in Spain showed contamination with *A. flavus* in 30% of samples, that 70.8% of these isolates were able to produce aflatoxin B1 and B2. Aflatoxins were also detected in 10% of samples, all exceeding the maximum legal limit set for aflatoxin B1 or for total aflatoxin, with one sample having a very high level of contamination (1134.5 µg/ kg) (Fernane *et al*., 2010).

In Tunisia a study on the effect of the storage period on AFB1 accumulation on pistachio nuts showed that the contamination of pistachio nuts has occurred clearly after two years of storage ranged from 2.7±0.3 to 12.7±2.2 µg/kg for AFB1 (Bensassi *et al*., 2010). In Sweden, 9.5% pistachio nut samples had AFB1 higher than 2ng/g (Thuvander *et al.*, 2001).

There are reported several methods for control of aflatoxin in crops and tree nuts either by prevention of aflatoxin producer fungi and detoxification of toxin. Rodriguze *et al* used four surfactants, Triton X-100, Tergitol NP-10, Triton X-301, and Latron AG-98, all at 1% (wt/vol) in CYA agar, inhibited AFB1 production by 96 to 99% (Rodriguez and Mahoney 1994).

Patel *et al* (1989) used the combination of Hydrogen Peroxide and Gamma Radiation in order to detoxification and reduction of the toxin levels in artificially contaminated groundnuts 40% inactivation of aflatoxin (Patel *et al*. 1989).

Akbas and Ozdemir used ozone treatments for the degradation of aflatoxin in pistachio kernels and ground pistachios. They reported that AFB1 and total aflatoxin could be reduced by 23 and 24%, respectively, when pistachio kernels were ozonated at 9.0 mg L^{-1} ozone concentration for 420 min (Akbas and Ozdemir, 2006).

Several organisms have been tested for biological control of aflatoxin contamination, including bacteria, yeasts, and non-toxigenic strains o the causal organisms, *A. flavus* and *A. parasiticus* (Yin *et al.*, 2008).

In order to control aflatoxin accumulation in tree nuts such as pistachios, it is first necessary to prevent the growth of mycotoxins producing fungi. However the use of chemical antifungal agents to control mycotoxins accumulation has been intensively investigated (Velluti, 2003; Edlayne, 2009; Miller, 2001; Ismaiel, 2014), their use must be intently considered with provision because of ecological problems which may develop later. Dehydroacetic acid sodium salt is used in cosmetics and clinical tests show little to no evidence regarding the ingredient's toxicity and potential for irritation (Gazzaniga *et al.,* 1994). Durakovi *et al.* (2011) used synthesized analogues of Dehydroacetic acid to prevent the growth and vomitoxin accumulation of *Fusarium graminearum* (Durakovi *et al*., 2011).

The aim of present study was to used Dehydroacetic acid and Ozonated water to control *A. flavus* growth and accumulation of aflatoxin on pistachios.

MATERIAL AND METHODS:

Inoculums :

A stable Aflatoxin producing strain of *Aspergillus flavus* (PTCC 5004) was obtained from Persian-type culture collection in Iran (Tehran, Iran) in a lyophilized vial. The fungi was cultured on slants of Potato dextrose agar (Oxoid, Uk) and stored at 25°C. Isolated new colonies of *A. flavus* used for inoculation of PDA plates about 10 days at 25ºC until they were well sporulated. A spore suspension of 0.5 McFarland (1×10^6 CFU/ml) was harvested in sterile PBS and Tween 20 (PBST).

Treatment of Pistachio samples in chemicals:

Two kg of freshly de-hulled dried pistachios was prepared. Five 500 g Pistachios samples were then maintained and separately immersed in 1N Dehydroacetic acid, ozonated water at the final concentration of 10mg/lit (O3aq) for 15 minutes at room temperature, A 1+1 combination of 1N Dehydroacetic acid (DHA) and ozonated water (10mg/lit) as 3 test groups, sterile distilled

water and a 500mg/lit Cyclohexamide solutions as negative and positive control groups respectively. The immersed Pistachios samples were incubated on a 100 RPM reciprocal shaker (Labtron, Iran) at room temperature for 2 hours in other to treat with chemicals.

Growth inhibitory testing (viable cell counts):

Each treated samples were divided in five 100 g and transferred in a 500ml flask containing 250 ml Yeast extract broth (Merck, Germany) and inoculated with 5 ml of *A. flavus* conidial suspensions (final concentration of 5000 CFU/ml), incubated for 72 hours on a reciprocal shaker (Labtron, Iran) at room temperature. 5gr of each pistachio samples were transferred in sterile Petri dishes and put on a humid sterile gauze and incubated at 25ºC for one week (figure 1). All pistachio samples were firstly analyzed for fungal growth and then washed with PBST for determination absorbance (optical density) using Spectrophotometer (Perkin-Elmer, Germany).

A 10µl of each sample was mixed with 90 µl of normal physiologic serum and spread on Sabouraud dextrose agar (Merck, Germany) plates containing 50mg/ lit Chloramphenicol for viable conidial counting. The probable isolated colonies were enumerated and defined as viable conidia cell counts per milliliter (CFU/ml). The optical density (OD) of the broth is directly related to the number of fungal cells present and can be used to examine the antifungal efficacy of the treatment solutions 50 µl washings were transferred on the sterile 96-well plates, and the measurements were performed after the first 24 hour incubations.

Aflatoxin B1 analysis by HPLC:

After analyzing of growth inhibitory tests each pistachios samples was washed with PBST, the treated pistachios were separately dried at 50ºC for 24 hours and sent to Farough reference laboratory (Tehran, Iran) for Aflatoxin B1 analysis with HPLC method using immunoaffinity column.

STATISTICAL ANALYSIS

The colonization rate, spectrophotometric and Aflatoxin results were analyzed using one-way ANOVA and Tukey T-tests. The mean difference is significant at the 0.05 level, and $P < 0.05$ was considered to be statistically different

RESULTS:

The means of viable cell counting and the OD (optical density) values of treated pistachios showed in table 1 and 2 respectively. As can be seen from table 1, 1N Dehydroacetic Acid and 1+1 combination of Dehydroacetic Acid and ozonated water completely inhibited the growth of *A. flavus* similar to positive gold standard Cyclohexamide solutions (figure 1), whereas 8716.7±796.03 CFU/ml (mean±SD) was isolated from washing solutions of pistachio samples treated with ozonated water. The untreated negative control group revealed the highest colonization and OD values in all measurements.

Ozonated water caused 73.6% reduction in the growth of *A. flavus* on treated pistachios in compare with negative control, showed the lower antifungal properties than DHA that prohibited 100% growth of *A. flavus*.

One way ANOVA statistical test shows the effectiveness of 1 N DHA and combinations of DHA and ozonated water for complete control of *A. flavus* colonization on treated pistachio samples similar to positive control (P=0.0001)

As can be seen from table 3 there wasn't detectable amounts of Aflatoxin B1 in untreated pistachios and pistachio samples treated with Cyclohexamide as positive control. The highest Aflatoxin B1 contamination was seen in Negative control pistachios and pistachios treated with ozonated water. The least Aflatoxin contaminations was seen in pistachio samples treated with DHA (p=0.0001) and combinations of DHA and ozonated water (p=0.0001) as expected.

According to comparison of the groups in pairs showed that DHA and combinations of DHA and ozonated water had the most antifungal activity (P=0.0001) than the ozonated water (P=0.001).

Table 1. Mean and SD of viable *A. flavus* conidia on treated pistachio samples (CFU/ml)

Treatment solutions	Mean	SD
1N DHA	0	0
1+1 DHA and Oz. Water	0	0
10 mg/lit of Oz. Water	8716.7	796.03
Cyclohexamide (Pos. Cont)	0	0
Distilled water (Neg. Cont)	33000	4381.8

P=0.0001

Table 2. Mean and SD of Optical Density values from treated pistachio samples

Treatment solutions	Mean	SD
1N DHA	0.129	0.008
1+1 DHA and Oz. Water	0.143	0.012
10 mg/lit of Oz. Water	0.308	0.017
Cyclohexamide (Pos. Cont)	0.113	0.005
Distilled water (Neg. Cont)	0.341	0.215

P=0.0001

Table 3. Mean and SD of Aflatoxin B1 (µg/kg) values in pistachio samples

Treatment solutions	Mean	SD
Untreated Pistachios	ND	ND
1N DHA	1.5	0. 21
1+1 DHA and Oz. Water	1.59	0.21
10 mg/lit of Oz. Water	6.10	0.32
Cyclohexamide (Pos. Cont)	ND	ND
Distilled water (Neg. Cont)	6.19	0.33

Figure 1. Colonization of *A. flavus* on treated pistachio samples
From left to right: Pistachios treated with Cyclohexamide (Pos. Cont.), DHA, ozonated water and Distilled water (Neg. cont.)

DISCUSSIONS

The global pistachio market is provided by Iran and the United States included over 70% of the world's pistachio exports, which 47% come from Iran and 25% come from the US(Shahvardi, 2011; FAOSTAT, 2010). However the report from Joint FAO/WHO Expert Committee on Food Additives (2007) showed a big diversity between Iranian and US pistachio qualities as reported an average of 54ng/g aflatoxin, whilst the majority of US pistachios embrace average levels below the EU standard of 10ng/g (JECFA, 2007). Unfortunately this level of Aflatoxin contamination in Iranian pistachios caused the losses of global market. Therefore, effective methods are continually needed for control of aflatoxigenic fungal pathogens in exported pistachio to achieve the foregone world's trade share.

There are several physical, chemical (Bluma *et al.*, 2008), and novel biological (Rahaie *et al.*, 2010) methods in literature, which investigated in order to control of Aflatoxin producer fungi in food and tree nuts commodities. Certain antifungal agents also were used for control of aflatoxin producer fungi in agricultural commodities; however there is ecological concern, which may develop later (Kohiyama *et al.*, 2015).

Dehydroacetic acid that used in present study as a chemical agent for in vitro inhibitor is a safe chemical compound, which used in food industry, which showed potentially antifungal and antimycotoxigenic properties (Durakovi *et al.*, 2010,). Ozonated water as an antifungal agents (Arita *et al.*, 2005) and also used for degradation of aflatoxin B1 in dried figs (Zorlugenc *et al.*, 2008).

A stable Aflatoxin B1 producer (Mojtahedi *et al*., 1979) standard strain of *A. flavus* (PTCC 5004) was used for contamination of pistachio samples in present study.

Results of current study showed that 1N Dehydroacetic acid (DHA) and 1+1 combination of DHA and ozonated water completely inhibited the growth of *A. flavus* on pistachios nuts and there wasn't seen any

growth of *A. flavus* after treatment with these two chemicals. HPLC analysis of treated and contaminated pistachios showed 1.5 and 1.59 µg/kg Aflatoxin B1 in pistachios treated with DHA and the combination of DHA and ozonated water respectively.

This antifungal property of DHA in present study was in agreement with the results of Durakovi *et al.* (2010) as they showed Antifungal and Anti-aflatoxigenic Action of DHA against *A. flavus* and complete inhibition of Aflatoxin B1 production the same as the current study (Durakovi *et al.,* 2010).

Durakovi also reported complete inhibition of *Fusarium graminearum* and generation of vomitoxin in treated maize (Durakovi *et al.,* 2011).

Profit of DHA for Removal of aflatoxin M1 from artificially contaminated yoghurt was also used and the percentage loss of the initial AFM1 amount in yoghurt was estimated by about 22 to 45 % by up to28 day storage, respectively. The toxicity of DHA was investigated on brine shrimp (Artemia salina) larvae as a screening system for the determination of their sensitivity (Durakovic *et al.,* 2015). However this chemical (DHA) was used as a preservative in agricultural food and wine industry, there wasn't reported any toxicity and irritation disorders by clinical investigations (Daniels *et al.,* 1983).

Ozonated water caused 73.6% reduction in the growth of *A. flavus* on treated pistachios in compare with negative control, showed the lower antifungal properties than the other two test chemicals that prohibited 100% growth of fungi

HPLC test showed only contamination of Aflatoxin B1 in contaminated pistachios because A. flavus PTCC 5004 that used in present study solely produced Aflatoxin B1 .

However 10 mg/lit of Ozone in water reduced the growth of *A. flavus* on treated pistachios up to 73.6%, but there wasn't seen any statistically differences in the concentration of Aflatoxin B1 in treated pistachios in comparison with negative control. This result isn't in concordance with the results of Bashiri *et al* (Bashiri *et al.,* 2013) as they reported 32-47% reduction in Aflatoxin B1 on pistachio treated with 4 and 8 mg/ml Ozone in water respectively. It seems that this mislead activity may results from the 1N Choleric Acids, which they added to the ozonated water to regulate the pH at 5 as they reported. In current study when the 10 mg/ml ozone was added to the water, its pH was normally at 5 and there wasn't needed the addition of Choleric Acids.

CONCLUSION:

The increased concern on mycotoxins in agricultural commodities such as pistachios nuts has recently motivated the efforts for development of new chemical antifungal compounds. Among them, Dehydroacetic acid (DHA) and ozonated water are effective antifungal and antimycotoxigenic agents. The purpose of this study was to examine the effectiveness of the DHA and ozonated water for the control of growth of toxigenous mould *A. flavus* PTCC 5004 and accumulation of Aflatoxin B1 in Pistachios nuts in an in vitro qualification. We found that using different concentrations of DHA showed more antifungal activity than ozonated water for the treatment of pistachios nuts in order to control the growth of *A. flavus* and the production of Aflatoxin in compare with ozonated water .

Application of DHA may be a potential means of preventing the growth and aflatoxin accumulation by *A. flavus* in the investigated Pistachios nuts .

AUTHORS' CONTRIBUTIONS:

Study concept and design: Abbas Ali jafari and Samaneh Sedighi Khavidak. Sample collection and laboratory examinations: Mahmoud Esmaeilzadeh and Hossein Jafari; data interpretation: Seyed Morteza Seifati; manuscript drafting: Abbas Ali Jafari; manuscript revision: all the Authors. Statistical analysis: Abbas Ali Jafari and Seyed Morteza Seifati.

FUNDING/SUPPORT

This study designed as a MsC student thesis of Mahmoud Esmaeilzadeh. This study was financially supported by the Ashkezar Branch of Islamic Azad Univesity.

REFERENCES

Abbaszadeh, G, Seiedoleslami, H, Samih, MA, Hatami, B, 2006. Bioecology of pistachio twig borer moth Kermania pistaciella Amsel, in Rafsanjan and Isfahan-Iran. Commun Agric Appl Biol Sci.; 71:563-9.

Akbas, MY, Ozdemir, M, 2006. Effect of different ozone treatments on aflatoxin degradation and physicochemical properties of pistachios. J. Sci. of Food and Agri.; 86:2099-2104.

Arita M, Nagayoshi M, Fukuizumi T, Okinaga T, Masumi S, Morikawa M, *et al* 2005. Microbicidal efficacy of ozonated water against Candida albicans adhering to acrylic denture plates. Oral Microbiol Immunol.;20:206-10.

Bandyopadhyay, R, M. Kumar, Leslie JF, 2007. Relative severity of aflatoxin contamination of cereal crops in West Africa. Food Additives and Contaminants; 24:1109-1114.

Bashiri P, H.-K.M., Sedaghat N, Tabatabaei Yazdi F, Nasiri M, 2013. Effect of ozonated water on control of Aflatoxin in Owhadi Pistachios. Iranian Food Science and Technology. 9: 215-221

Bensassi JF, Rhouma A, Ghrab M, Bacha H, Rabeh Hajlaoui M, 2010. Evaluation of cultivar susceptibility and storage periods towards aflatoxin B1 contamination on pistachio nuts. Mycotoxin Res.;26:199-203. doi: 10.1007/s12550-010-0054-7. Epub 2010 May 6.

Bluma R, Amaiden MR, Daghero J, Etcheverry M, 2008. Control of Aspergillus section Flavi growth and aflatoxin accumulation by plant essential oils. J Appl Microbiol; 105:203-14.

Bui-Klimke TR, Guclu H, Kensler TW, Yuan JM, Wu F, 2014. Aflatoxin regulations and global pistachio trade: insights from social network analysis. PLoS One.; 26;9:e92149. doi: 10.1371/journal.pone.0092149.

Daniels DH, Warner CR, Selim S, Joe FL Jr, 1983. Gas-liquid chromatographic determination of dehydroacetic acid in squash and wine. J Assoc Off Anal Chem. ;66(4):893-6.

Durakovi L, Sikora S, Delaa F, Skelin A, Tudi A, Mrkonji Fuka M, Boanjak M, 2010. A Study of Antifungal and Antiaflatoxigenic Action of Newly hesized Analogues of Dehydroacetic Acid. Hrvat. Prehrambenu tehnol. Biotehnol. Nutr; 5:127-135.

Durakovi L, Skelin A, Sikora S, Del F, Mrkonji-Fuka M *et al*, 2011. Impact of new synthesized analogues of dehydroacetic acid on growth rate and vomitoxin accumulation by Fusarium graminearum under different temperatures in maize hybrid. African Journal of Biotechnology; 10: 10798-10810.

Durakovic L, Tudic A, Huic-Babic K, Redzepovic, 2012. Removal of aflatoxin M1 from artificially contaminated yoghurt by using of new synthesized dehydroacetic acid analogues. Mljekarstvo 2012; 62, 179-191.

Edlayne G, Simone A, Felicio JD, 2009. Chemical and biological approaches for mycotoxin control: a review. Recent Pat Food Nutr Agric.; 1:155-61. Review.

Esteve, J., *et al*., 1993. Facts and figures of cancer in the European Community.: International Agency for Research on Cancer.

FAOSTAT, Top Production - Pistachios 2010. In: FAO, editor. 2011.

Fernane F, C.-S.G., Sanchis V, Mar S, Ramos AJ, 2010. Aflatoxins and ochratoxin A in pistachios sampled in Spain: occurrence and presence of mycotoxigenic fungi. Food additives & contaminants. Part B, Surveillance." Food Addit Contam Part B Surveill.; 3:185-92.

Gazzaniga A, Sangalli ME, Giordano F, Conte U, Semenzato A, Bettero A, 1994. Controlled release of dehydroacetic acid sodium salt for the stability improvement of cosmetic formulations. Int J Cosmet Sci.; 16:105-12.

Ismaiel AA, Tharwat NA, 2014. Antifungal activity of silver ion on ultrastructure and production of aflatoxin B1 and patulin by two mycotoxigenic strains, Aspergillus flavus OC1 and Penicillium vulpinum CM1. J Mycol Med.; 24:193-204. doi: 10.1016/j.mycmed.2014.02.009.

(JECFA), J.F.W.E.C.o.F.A., 2007. Evaluation of certain food additives and contaminants: sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO technical report series 947.

Jelinek CF1, Pohland AE, Wood GE, 1989. Worldwide occurrence of mycotoxins in foods and feeds--an update. J Assoc Off Anal Chem. ; 72:223-30.

Kar P, 2014. Risk factors for hepatocellular carcinoma in India. J Clin Exp Hepatol.;4:34-42.

Kohiyama CY, Yamamoto Ribeiro MM, Mossini SA, Bando E, Bomfim Nda S, *et al*, 2015. Antifungal properties and inhibitory effects upon aflatoxin production of Thymus vulgaris L. by Aspergillus flavus Link. Food Chem.; 15;173:1006-10.

Miller, R., *et al*., 2001. Ketenes. Ullmann's Encyclopedia of Industrial Chemistry, John Wiley and Sons, Inc,.

Mojtahedi H, Rabie CJ, Lübben A, Steyn M, Danesh D, 1979. Toxic aspergilli from pistachio nuts. Mycopathologia.; 31;67:123-7.

Patel, U.D., P. Govindarajan, and P.J. Dave, 1989. Inactivation of aflatoxin B1 by using the synergistic effect of hydrogen peroxide and gamma radiation. Appl Environ Microbiol.; 55:465-7.

Rahaie S, Emam-Djomeh Z, Razavi SH, Mazaheri M, 2010. Immobilized Saccharomyces Cerevisiae as a potential aflatoxin decontaminating agent in pistachio nuts. Braz J Microbiol.; 41:82-90.

Rodriguez SB1, Mahoney NE, 1994. Inhibition of aflatoxin production by surfactants. Appl Environ Microbiol.; 60:106-10.

Set E, Erkmen O, 2010. The aflatoxin contamination of ground red pepper and pistachio nuts sold in Turkey. Food Chem Toxicol.; 48:2532-7.

Shahvardi S, Jamzad Z, Nejad satari T, 2012. Extraction and determination of essential oil compounds Origanum strobilaceum. Iran. J. medicinal and aromatic plants.; 28: 23-31(persian).

Thuvander, A., Moller, T., Barbieri, H.E., Jansson, A., Salomonsson, A.C., Olsen, M., 2001. Dietary intake of some important mycotoxins by the Swedish population. Food Addit. Contam. 18, 696–706

Velutti A, Sanchiz V, Ramos AJ, Egido J, Marin S, 2003. Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarosa essential oils on growth and deoxynivalenol production by Fusariumgraminearum in maize grain. Int. J. Food Microbiol., 42: 1132-1137

Yan-ni Yin, Lei-yan Yan, Jin-hua Jiang, and Zhong-hua Ma, 2008. Biological Control of Aflatoxin Contamination of Crops J Zhejiang Univ Sci B.; 9(10): 787–792.

Yu J, Payne GA, Campbell BC, Guo B, Cleveland, TE, Robens JF., *et al*. (2008). Mycotoxin production and prevention of aflatoxin contamination in food and feed.In G. H. Goldman, & S. A. Osmani (Eds.), The Aspergilli: Genomics, medical aspects, biotechnology, and research methods (pp. 457e472). Boca Raton: CRC Press

Zorlugenc, B., Kiroglu Zorlugenç, F., Ztekin, S., & Evliya, IB., 2008, The influence of gaseous ozone and ozonated water on microbial flora and degradation of aflatoxin B1 in dried figs. Food and Chemical Toxicology, 46(12), 3593-3597.