Australian Journal of Crop Science AJCS
AJCS 4(9):744-749 (2010) ISSN:1835-2707

Study on the effect of pistachio testa on the reduction of *Aspergillus flavus* growth and aflatoxin B_1 production in kernels of different pistachio cultivars

Mehdi Mohammadi Moghadam and Hossein Hokmabadi*

Iran's Pistachio Research Institute, P.O. Box: 17175-435, Rafsanjan, Iran

***Corresponding authors: Hokmabadi@pri.ir**

Abstract

In order to evaluate the effect of testa (pistachio seed coat) on reduction of fungal growth and aflatoxin B_1 production, ten cultivars of pistachio were collected from different parts of the pistachio production area (i.e. Rafsanjan, Damghan and Ghazvin provinces in Iran). One section of the testa and 1 mm of endosperm were scraped, then 20 g of wounded kernels and 20 g of unwounded kernels were surface sterilized and placed on Petri-dishes separately (completely randomized design in 3 replications). 1 ml of the spore suspension of aflatoxigenic *Aspergillus flavus* added to each Petri-dish (spore suspension adjusted to contain of 2×10^6 spore/ml). The plates placed over water in plastic boxes and then placed inside an incubator at 26ºC. After 2.5, 5 and 8 days of inoculation, growth rate and colonization of *A. flavus* on wounded and unwounded pistachio kernels measured in different cultivars. In addition, aflatoxin content of inoculated kernels extracted by BF method and estimated by TLC and densitometer. The average percentage of *A. flavus* growth on the surface of wounded and unwounded kernels compared with t-student test. Results of this research indicated a significant difference in fungus growth rate and aflatoxin B_1 production between wounded and unwounded kernels of pistachio cultivars. In other words, testa in unwounded kernels could be considered as a resistant barrier against the penetration of fungus into kernels, reducing *A. flavus* growth and aflatoxin B_1 production as compared with wounded kernels.

Keywords: Pistachio, Cultivars, *Aspergillus flavus*, Aflatoxin.

Introduction

Aflatoxins are a large group of mycotoxins counted among secondary fungal metabolites produced by species such as *Aspergillus flavus*, *A. parasiticus*, *A. tamarii*, *A. bombycis*, and *A. nomius* (Wilson and Payne, 1994). Due to their remarkable abundance in nature as well as their intoxicating and carcinogenic properties, aflatoxins have been recognized as the leading mycotoxins. So far several aflatoxins have been identified, the most renowned being aflatoxin B_1, B_2, G_1 and G_2 (Trial et al., 1995).Among four main groups of aflatoxins(B_1, B_2, G_1, G_2), aflatoxin B_1 have the highest amount of toxicity (Moghaddam et al.,2006). Today one of the biggest problems of the world health community is the contamination of agricultural crops with aflatoxins. Various countries have put in order special regulations for production, consumption and import of food and drug materials to counter the serious risks posed by mycotoxins (Allameh and Razzaghi, 2002). In the United States, food or pharmaceutical materials containing more than 20 ppb of aflatoxins legally is banning for sales, import and export (Trial et al., 1995; Gourama and Bullerman, 1995). Since the discovery of aflatoxins in the 1960s, the *A. flavus* has been widely reported in scientific sources as the most common fungus affecting food products. This is more than sufficient to show its economic significance. This fungus is common all over the world as an air and soil mycoflora found in live and dead animal and plant organisms. It is particularly interested in colonizing nut kernels and oily cereals. Peanut, corn, wheat, rice, pistachio and almond are the major products infected by this fungus. Iran has about 440 hectares of pistachio orchards and produces about 57% of the world pistachio. More than 60% of the world pistachio exports are from Iran to other countries, showing well the economic significance of this product for the country. Iran is also recognizing as the biggest and most important producer and exporter of pistachio in the world, among other pistachio producing countries (FAO Stat, 2008). The economic value of pistachio exports to 66 countries is about one billion dollars/year, ranking second among the nation's sources of income after oil (FAO Stat, 2008). This alone is more than enough to show the strategic significance of this product and of course, the dire need to protect and optimize it to keep the edge in global commerce. Contamination of pistachio nut by *Aspergillus* species and their mycotoxins are the most serious challenge to pistachio production, consumption and exportation in the world. Different Factors influenced on infection of pistachio nuts to aflatoxin which is include: cracking of pistachio nuts (especially early hull splitting pistachios) (Doster and Michailides, 1995), Sommer et al**.,** 1986), environmental factors (Campbell et al., 2003; Emami et al., 1977; Denizel et al., 1976; Mojtahedi et al., 1979; Heperkan et al., 1994), cultural practices (Campbell et al., 2003; Fooladi and Tafti, 2006; Tajabadipour, et al., 2006; Hosseinifard and Panahi, 2006), frequency and time of irrigation (Doster et al., 2001; Sedaghati and Alipour, 2006), plant litter (Doster and Michailides , 1994); Moradi et al., 2004), animal manures (Moradi et al., 2004), distribution of aflatoxin in pistachio bulks (Pearson et al., 1994; Moradi and Javanshah, 2006) and harvesting date (Crane, 1978, Panahi et al., 2005). Tajabadipour et al., (2006) studied the effect of rootstock and scion on the frequency of early splitting formation. They also reported that the early splitting in Ouhadi scion cultivar is significantly less than the Kaleh Ghouchi scion, while, the

frequency of cracking pistachios Ouhadi and Ahmadaghaie scions are significantly higher than Kaleh Ghouchi scion.In artificial inoculation with *A. flavus*, the susceptibility of cultivars differed in kernel colonization and aflatoxin concentrations. The highest kernel colonization belonged to the Ahmadaghaie and Ouhadi cultivars, while the lowest ones were Akbari and Kaleh Ghouchi cultivars. The Kalkhandan and Fakhri, and Shahpasand and Abbasali cultivars had the lowest and highest content of aflatoxin kernels, respectively (Moghaddam et al., 2006). It is obvious that all different aspects of contamination by *A. flavus* and aflatoxin must be studied and considered in a comprehensive and integrated manner. This paper is dedicated to the effects of *pistachio testa* on the reduction of *A. flavus* growth and aflatoxin B_1 production in different pistachio cultivars.

Materials and methods

Collection of Different Pistachio Cultivars

To study the effects of *pistachio testa* on reduction of *A. flavus* growth and aflatoxin B_1 production, ten major pistachio cultivars were collected from different pistachio areas of the country. Rafsanjan provided cultivars of Akbari, Kaleh ghouchi, Ouhadi and Ahmadaghaie . Damghan provided Shahpasand, Abbasali, Khanjari and FAS-13-73 and Ghazvin provided Kalkhandan and Kaleh bozi cultivars. To prevent wounding of *pistachio testa* and to minimize *A. flavus* and aflatoxin contamination, sampling was done at harvest time, i.e. from the trees. Then the pistachio hulls toke from the shells by hand to prevent any damage to the kernel testa. The pistachios were then dried under the sun and used for *in vitro* experiment. For this purpose one isolate of *A. flavus* isolated from contaminated pistachio was used for the experiments. The isolate was capable of producing large amounts of aflatoxin B_1 and B_2.

The Effect of Testa on A. flavus Growth Reduction and Aflatoxin B_1 Production in Pistachio Cultivars

Given the fact that pistachio testa serves as a barrier against fungal invasions into the kernel, hence reducing the production of aflatoxin resulting from fungal growth, a part of the testa was wounded with 1 mm of the endosperm or pistachio kernel to facilitate the penetration of the fungus into the kernel: 20 g of unwounded and 20 g of wounded kernel of both cultivars (in a completely randomized design in 3 replications) were surface sterilized by 0.5% sodium hypochlorite and then soaked in distilled water for about 10 minutes to absorb the primary moisture. The kernels of different cultivars then placed inside separate Petri-dishes and inoculated with 1ml of spore suspension (2×10^6 spores/ml). Petri-dishes placed inside plastic containers having sterilized distilled water on their bottoms to provide the required moisture at saturation limit. They kept at 26°C. After 2.5, 5 and 8 days of inoculation, average percentages of fungal growth and colonization on wounded and unwounded pistachio cultivar kernels measured (based on colonized surface of kernels). Once the percentage of colonization by *A. flavus* was measured for different cultivars, the difference of average *A. flavus* colonization percentages were compared and analyzed for wounded and unwounded pistachios cultivars by SPSS software and t-student test. In addition, to calculate the rate of sporulation of *A. flavus*, on the 8^{th} day the colonized pistachios mixed with 100 ml of distilled water, poured inside erlenmyer flasks, and placed on a shaker for 24 hours so that the spores totally washed off the pistachio surfaces. Then the spores existing in 100 ml of distilled water

Fig 1. Comparison of *A. flavus* growth on unwounded (right) and wounded (left) pistachio kernels of Akbari cultivar (2.5 days after inoculation)

Fig 2. Comparison of *A. flavus* growth on unwounded (right) and wounded (left) pistachio kernels of Shahpasand cultivar (2.5 days after inoculation)

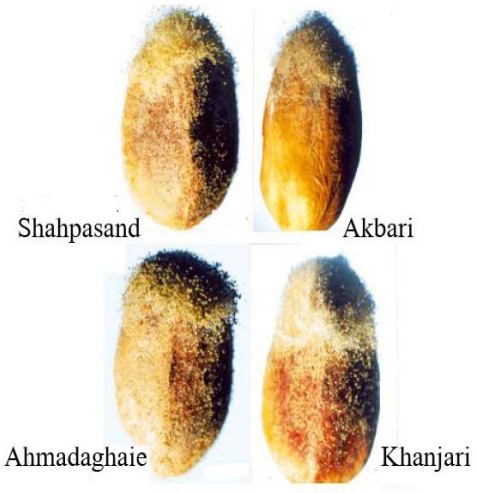

Fig 3. Comparison of *A. flavus* growth on wounded kernels of different pistachio cultivars (2.5 days after inoculation)

Table 1. Comparison of Average Colonization Percentage by *A. flavus* on Wounded Pistachio Kernels from Different Cultivars (2.5 days after inoculation)

Pistachio Cultivar	Average Colonization Percentage of pistachio kernels	Statistical Grouping (α=5%)
Ahmadaghaie	41.38	A
Shahpasand	29.80	B
Ouhadi	26.59	C
Khanjari	23.58	D
Abbasali	22.59	D
FAS-13-73	19.83	E
Kaleh ghouchi	19.33	E
Kaleh bozi	19.33	EF
Akbari	17.56	FG
Kalkhandan	16.49	G

Similar letters after average percentages on each column indicate no significant difference at a 5% level (Duncan's multiple range test method).

Table 2. Comparison of Average Colonization Percentage by *A. flavus* on Wounded and Unwounded Pistachio Kernels from Different Cultivars (5 days after inoculation)

Pistachio Cultivar	Average Colonization Percentage of unwounded Pistachios	Average Colonization Percentage of wounded Pistachios
Ahmadaghaie	A 55.21	B 84.73
Shahpasand	CD 24.13	A 87.45
Ouhadi	B 41.83	D 75.86
Khanjari	CD 26.45	C 78.39
Abbasali	D 19.72	E 69.75
FAS-13-73	CD 26.31	F 56.60
Kaleh ghouchi	D 20.38	G 52.69
Kaleh bozi	CD 24.49	G 53.56
Akbari	D 18.90	H 49.15
Kalkhandan	BC 31.44	F 57.26

Similar letters after average percentages on each column indicate no significant difference at a 5% level (Duncan's multiple range test method).

Table 3. Sporulation of *A. flavus* on Unwounded and Wounded Kernels of Pistachio Cultivars

Pistachio Cultivar	Sporulation (spore/ml) on Unwounded Pistachios	Sporulation (spore/ml) on Wounded Pistachios
Ahmadaghaie	3.73×10^7	7.11×10^7
Shahpasand	4.38×10^7	8.91×10^7
ouhadi	1.01×10^8	1.36×10^8
Khanjari	1.04×10^8	1.49×10^8
Abbasali	2.85×10^7	5.56×10^7
FAS-13-73	1.13×10^8	1.57×10^8
Kaleh ghouchi	2.38×10^7	5.33×10^7
Kaleh bozi	9.74×10^7	1.03×10^8
Akbari	6.78×10^7	9.59×10^7
Kalkhandan	2.17×10^7	7.19×10^7

Table 4. T-student Test for Comparison of Average *A. flavus* Colonization Percentage Difference on Wounded and Unwounded kernels of pistachio cultivars

Degree of Liberty	Calculated τ	Standard Deviation	Averages Variance	Average Colonization on Unwounded Pistachios	Average Colonization on Wounded Pistachios
18	-6.49**	5.80	33.68	28.89	66.54

** Significant at α=1%

Table 5. T-student Test for Comparison of Aflatoxin B1 Production in Unwounded and Wounded Kernels of Pistachio ultivars (8 days after inoculation)

Degree of Liberty	Calculated τ	Standard Deviation	Averages Variance	Average Aflatoxin production in Unwounded Pistachios	Average Aflatoxin production in Wounded Pistachios
18	-2.743*	111089	1234089.21	27261.7	30308.6

* Significant at α=5%

Table 6. Comparison of Aflatoxin B_1 Production in Unwounded and Wounded Kernels of Pistachio Cultivars (8 days after inoculation)

Pistachio Cultivar	Aflatoxin B_1 produced in Unwounded Pistachios (μg/kg)	Aflatoxin B_1 produced in Wounded Pistachios (μg/kg)
Ahmadaghaie	29429	30171
Shahpasand	31171	32300
Ouhadi	29823	32810
Khanjari	24811	30672
Abbasali	30754	32307
FAS-13-73	25393	29012
Kaleh ghouchi	26218	30423
Kaleh bozi	26410	27439
Akbari	26880	29940
Kalkhandan	21728	27912
Means	27261.7b	30308.6a

counted by Toma slides and considered as the spores produced due to the growth of fungus in 20 g pistachio kernels of each Petri-dish.

Extraction and Measurement of Aflatoxin B_1 Produced in Contaminated Pistachios

8 days after inoculation and calculation of fungal colonization percentage of contaminated pistachio kernels, the pistachios were dried inside an oven to prevent further growth of *A. flavus* and aflatoxin production. Then the aflatoxin content of pistachio samples was extracted by BF method and was measured by thin layer chromatography (TLC) and densitometer. Comparison of difference of average aflatoxin B_1 production in different cultivars was done by SPSS software and Duncan's multiple range tests.

Results

As explained under materials and methods, after 2.5, 5 and 8 days from inoculation, the average percentage of *A. flavus* growth and colonization on kernels of wounded and unwounded pistachios of different cultivars (based on colonized surface of kernels) were calculated. Results indicated that 2.5 days after inoculation, none of the pistachio cultivars with unwounded testa showed significant fungal growth, but on wounded pistachios, the fungus had colonized the surface of pistachio kernels (Fig 1). Results of statistical reviews show a significant difference in the rate of fungal colonization on kernels of pistachio cultivars (Table 1). In Fig 2 and 3, the rate of fungal growth in wounded and unwounded pistachio cultivars showed for 2.5 days after inoculation. Fungal colonization on pistachios with intact and wounded testas on days 5 and 8 were recorded and compared as well. As can be seen in Table 2, the fungal colonization on wounded pistachio kernels on the 5^{th} day was far more than unwounded pistachios. In addition, 8 days after inoculation all wounded pistachios totally colonized by the fungus, while none of the unwounded pistachios completely colonized.

Study on Sporulation of A. flavus on Unwounded and Wounded Kernels of Pistachio Cultivars (8 days after inoculation)

Once the fungus had grown, the spores produced on kernels of unwounded and wounded kernels of different pistachio cultivars were calculated and compared according to the same method described under materials and methods section, results presented in Table 3. As can be seen, the wounding of the testa of pistachio kernels facilitates *A. flavus* penetration into the kernel, which in turn translates into increased fungal growth (colonization) and in turn increased spore production on surfaces of wounded kernels as compared with unwounded ones.

Results of Study on Difference in A. flavus Growth on Unwounded and Wounded Kernels of Pistachio Cultivars (5 days after inoculation)

Statistical reviews show a significant difference between fungal colonization on unwounded and wounded kernels of pistachio cultivars at a 1% level (Table 4). In other words, kernels having wounded testas infected more easily colonized by the fungus, which is capable of growing more rapidly, and colonized most of the kernels. Thus, testa can be consider as a resistance barrier against fungal penetration into pistachio kernels, which at least delays the penetration and colonization process and prevents the fungus from growing too much.

Results of Study on Difference of Aflatoxin B_1 Production on Unwounded and Wounded Kernels of Pistachio Cultivars (8 days after inoculation)

Results of statistical reviews confirm that the difference between aflatoxin B_1 production rates on unwounded and wounded kernels of pistachio cultivars is indeed significant (Table 5). In other words, pistachios with intact testas have a strong barrier against fungal penetration and colonization, which is the testa. By delaying fungal growth and reducing colonization on unwounded pistachio kernels in comparison with wounded ones and because of the relative correlation between fungal growth and aflatoxin B_1 production, testa results in reduced aflatoxin B_1 production on unwounded pistachios in comparison with wounded ones (Table 6).

Discussion

Given the fact that *A. flavus* and aflatoxin contamination process is too complex and requires total destruction or serious control of toxin contamination, there is need for several approaches to the problem. Thus research on identification of resistant cultivars to *A. flavus* and aflatoxin production, as well as studying the physicochemical effects of *Pistachio testa* on the prevention of *A. flavus* growth and toxin production, may be good strategies to create a suitable knowledge base for controlling aflatoxin contamination of agricultural products, in this case pistachio. Most countries around the world have undertaken wide research projects aimed at identifying agricultural and horticultural products resistant to *A. flavus* and aflatoxin production reactions and are rigorously studying their resistance mechanisms with some brilliant results already reported (Gradziel and Wang

1994). Most studies focus on corn (Wallin, 1986) and almond (Gradziel and Wang, 1994). Results of studies by Ghewande et al., (1993) indicate a significant difference in the resistance of peanut cultivars to *A. flavus* growth. They have also studied aflatoxin B_1 production in different cultivars and observed that its production rates are totally different for different cultivars. While the most resistant cultivar produced 3900 µg/kg aflatoxin, production in the most susceptible cultivar was about 90000 µg/kg. They also found that there was a significant correlation and relationship between rates of *A. flavus* growth and aflatoxin production. Results of this research effort on the correlation of sugar content of kernels of different peanut cultivars and the growth rate of *A. flavus* and aflatoxin production indicate that there is not a logical and significant correlation, and that many physical and chemical factors may be involved (Ghewande et al., 1993). Permelata et al., (1990) measured the contents of sugar, protein and phenol in 38 cultivars of cereal plants to study their roles in susceptibility or resistance of such cultivars against *A. flavus* growth and aflatoxin production. Their results demonstrated that the protein and phenol contents of resistance cultivars were higher than susceptible cultivars, while the sugar content of susceptible cultivars was higher than resistant ones .Another study on peanut by Lata et al., (2007) came to the conclusion that among 21 different genotypes of peanut tested, the four genotypes IC-48, J-11, ICGV 89104, and ICGS-76 had the lowest rates of aflatoxin production (<25 ppb) and highest rate of phenol (>1300 µg/g). Aflatoxin production had a negative correlation with phenol contents of peanut kernels (r^2 = - 0.42) and leaves (r^2 = - 0.37, $p<0.05$). Burdaspal and Govostidi (1986) also studied contamination by aflatoxin B_1, B_2, G_1 and G_2 in 424 pealed and non-pealed peanut and pistachio samples and showed that pealed peanuts had higher rates of contamination. Hence they recognized the peanut testa as a resistant barrier to fungus penetration. Wallin et al., (1986) wounded the pericarp of corn seeds to study and demonstrate the effects of that layer in prevention of *A. flavus* penetration into corn seeds. They observed that aflatoxin produced in wounded seeds was far more than intact seeds. So the Aleron and pericarp layer of corn was recognized as a resistant barrier to *A. flavus* penetration. Gradziel and Wang (1994) studied the susceptibility of California almond cultivars to aflatoxigenic *A. flavus* growth. They found out that susceptibility of different cultivars to *A. flavus* were different. They also wounded the almond testa and studied the effects of the layer on reducing *A. flavus* penetration. Results of their research indicated that almond testa was capable of serving as a barrier to fungal invasion. Gradziel and Wang (1994) concluded that the kernel testa of all varieties of almond studied so far had strong resistance against *A. flavus* colonization. It also seemed some cultivars such as Ne Plus, Ruby and Carrion had cotyledon resistance. Studies have demonstrated that the wounding of testa increases the possibility of rapid invasion of *A. flavus* on peanut seeds, which in turn increases the chance of aflatoxin production. Damaging of pod too increases the chance of *A. flavus* to receive its necessary nutrients for growth (Mehan et al., 1987). Researches of Rodrigues et al., (1996) showed that pistachios with intact cuticles are resistant to *A. flavus* colonization. It must be noted anyway that any damage to the cuticle results in rapid colonization by *A. flavus* inside hull of the pistachio (Mahoney, et al., 1996). Mahoney and Rodrigues (1996) failed to trace any aflatoxin accumulation in hulls despite the rapid colonization by *A. flavus*. Intact pistachio kernels having testa had slight amounts of colonization by *A. flavus* and aflatoxin production. It is possible that the cuticle layer plays a limited role in resistance of kernels against colonization by *A. flavus*. During different phases of this study the effects of pistachio kernel testa on the prevention of A. flavus growth and aflatoxin B_1 production in pistachio cultivars were evaluated. Results indicated that pistachio testa could act as a resistant barrier against fungal invasion into the kernels and at least delay the A. flavus growth and colonization process, which in turn translates into reduced aflatoxin production. Such results were in total harmony with those of Gradziel and Wang (1994) on the effects of almond testa.

References

Allameh A, Razzaghi M (2002) Mycotoxins. First edition. Teheran. Emam Hossein University Press (In Persian).

Burdaspal PA, Govostidi A (1986) Data on aflatoxin contamination in peanut and other nuts. Alimentari. 192,51-53.

Campbell BC, Molyneux RJ, Schatzki TF (2003) Current research on reducing pre and postharvest aflatoxin contamination of the U.S. almond, pistachio and walnut. J. Toxicol. Toxin Rev. 22, 225–266.

Crane JC (1978) Quality of pistachio nuts as affected by time of harvest. J. Amer. Soc. Hort. Sci, 103(3): 332-333.

Denizel T, Jarvis B, Rolfe E (1976) A field survey of pistachio (*Pistacia vera*) nut production and storage in Turkey with particular reference to aflatoxin contamination. J. Sci. Food Agric. 27: 1021–1026.

Doster MA, Michailides TJ (1994) Development of Aspergillus molds in litter from pistachio trees. Plant Dis. 78: 393–397.

Doster MA, Michailides TJ (1995) The development of early split pistachio nuts and their contamination by molds, aflatoxin and insects. Acta Hort. (ISHS) 419:359-364.

Emami A, Suzangar M, Barnett R (1977) Contamination of pistachio nuts with aflatoxins while on the trees and in storage, Zesz. Probl. Postepow Nauk Roln 189:135–140.

FAO-STAT (2008): FAO Production Resource Statistics, available online at: http://faostat.org

Fooladi MH, Tafti AG (2006) Effect of preharvest spraying of pistachio trees with sodium diacetate as mold inhibitor. Acta Hort. (ISHS) 726:495-502.

Ghewande MP, Nagaraj G, Desai S, Narayan P (1993) Screening of groundnut bold seeded genotypes for resistance to *Aspergillus flavus* seed colonization and less aflatoxin production. Seed Science and Technology 21: 45-51.

Gourama H, Bullerman L (1995) *Aspergillus flavus* and *Aspergillus parasiticus*: Aflatoxigenic fungi of concern in foods and feeds: A review. Journal of Food Protection. 58: 1395-1404.

Gradziel TM, Wang D (1994) Susceptibility of california almond cultivars to aflatoxigenic *Aspergillus flavus*. Hort Science 29:33-35.

Heperkan D, Aran N, Ayfer M (1994) Mycoflora and aflatoxin contamination in shelled pistachio nuts. J. Sci. Food Agric. 66: 273–278.

Hosseinifard J, Panahi B (2006) The effect of different mineral nutrients on early splitting in pistachio. Acta Hort. (ISHS) 726:325-328.

Latha P, Sudhakar P, SreenivasuluY, Naidu PH, Reddy PV (2007) Relationship between total phenols and aflatoxin production of peanut genotypes under end-of-season drought conditions. Acta Physiology of Plant. 29: 563-566.

Mahoney ME, Rodrigues SB (1996) Aflatoxin variability in pistachios. Appl Environ Microbiol 62:1197-1202.

MehanVK, McDonald D, Rajagopalan K (1987) Resistance of Peanut Genotypes to Seed Infection by *Aspergillus Flavus* in Field Trials in India1. Peanut Science 14(1): 17-21.

Moghaddam MM, Goltapeh EM, Hokmabadi H, Haghdel M, Mortazavi AM (2006) Evaluation of susceptibility of pistachio cultivars to aflatoxigenic *Aspergillus flavus* and aflatoxin B1 production. Acta Hort. (ISHS) 726:655-658.

Mojtahedi H, Rabie CJ, Lubben AMS, Danesh D (1979) Toxic aspergilli from pistachio nuts. Mycopahtologia 31:123–127.

Moradi M, Javanshah A (2006) Distribution of aflatoxin in processed pistachio nut terminals. Acta Hort. (ISHS) 726: 431-436.

Moradi M, Ershad D, Mirabolfathi M, Panahi B (2004) The role of plant debris, soil and manure on population density of *Aspergillus flavus* and *Aspergillus niger* groups in pistachio orchards of Kerman province (in Persian). Iran. J. Plant Pathol. 40: 221-234.

Panahi B, Mirdamadiha F, Talaie A (2005) Determination of the best time of harvest in different commercial Iranian pistachio nuts. In *XIII GREMPA Meeting on almonds and pistachios,* ed. M. M. Oliveira, 215–19. Zaragoza: CIHEAM-IAMZ.

Pearson TC, Slaughter DC, Studer HE (1994) Physical properties of pistachio nuts. Trans. ASAE, 37, 913–918.

Singh P, Bhagat S, Ahmad SK (1990) Aflatoxin elaboration and nutritional deterioration in some pulse cultivars during infestation with *A. flavus*. Journal of Food Science and Technology. 27(1): 60-62.

Rodrigues SB, Mahoney ME, Irwing DW, King AD (1996) Aflatoxin production in pistachios, pp: 41. In Aflatoxin elimination workshop. U.S. Department of Agriculture, Fresno, Calif.

Sedaghati N, Alipour H (2006) The effect of different time of irrigation on occurrence of early split (ES) of pistachio nuts. Acta Hort. (ISHS) 726:582-586.

Sommer NF, Buchanan JR, Fortlage RJ (1986) Relation of early splitting and tattering of pistachio nuts to aflatoxin in the orchard. Phytopathology 76:692–694.

Tajabadipour A, Panahi B, Zadehparizi R (2006) The effects of rootstock and scion on early splitting and cracked hull of pistachio. Acta Hort. (ISHS) 726:193-198.

Trial F, Mahanti N, Linz J (1995) Molecular biology of aflatoxin biosynthesis. Microbiology. 141: 755-765.

Wallin GR (1986) Production of aflatoxin in wounded and whole maize kernels by *Aspergillus flavus*. Plant Diseases 10: 429-430.

Wilson DM, Payne GA (1994) Factors affecting *Aspergillus flavus* group infection and aflatoxin contamination of crops, In The Toxicology of Aflatoxins. Human Health, Veterinary, and Agricultural Significanse (David L. Ethon and John D.Groopman, San Diego: Academic Press).