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Evaluation of Pistachio Contamination to *Aspergillus flavus* in Semnan Province

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Abstract: In order to evaluate pistachio contamination to *Aspergillus flavus* in Semnan province; samples of pistachio kernels were collected from the last processing pistachio stage in traditional and semi-mechanized processing plants in 2010. They were examined for the presence *A. flavus* fungi. The samples were cultured on AFPA media. All plate were incubated for 3 to 7 days. At the end of incubation period, the plate were evaluated and isolates of *A. flavus* were identified and relative density of them were recorded. The isolates of *A. flavus* were evaluated for their ability to produce aflatoxins, when grown on natural substrate (autoclaved rice flour). The aflatoxins were extracted with chloroform and analyzed by TLC and densitometer. The results indicated that population density of *A. flavus* is varied with the type of processing plants. Traditional processing plants have more population density of *A. flavus* than semi-mechanized processing plants. 55 isolates of *A. flavus* out of the 86 tested isolates (%63.95) were able to produce one or several types of aflatoxins, while 31 isolates (%36.04) were unable to produce any type of aflatoxin. Among evaluated isolates, %9.30 of isolates produced all types of the aflatoxins, %8.13 produced AfB_1,AfB_2 and AfG_1, %34.88 of isolates produced AfB_1 and AfB_2 and %11.62 of isolates only produced AfB_1. Among aflatoxigenic isolates, the strength of toxin production varied from weak to strong.

Key words: Pistachio, *Aspergillus flavus* , Semnan province,

INTRODUCTION

Aflatoxins are a large group of mycotoxins counted among secondary fungal metabolites produced by species such as *Aspergillus flavus*, *A. parasiticus, A. tamari, A. bombycis*, and *A. nominus* (Wilson and Payne,1994).

Due to their remarkable abundance in nature as well as their toxigenic 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& 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 Razaghi.,2002).

In the United States food or pharmaceutical materials containing more than 20 ppb of aflatoxins are legally banned 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 470000 hectares of pistachio orchards and produces about %57 of the world pistachio. More than %60 of the world pistachio export is done from Iran to other countries, showing well the economic significance of this product for the country. Iran is also recognized as the biggest and most important producer and exporter of pistachio in the world, among other pistachio producing countries (FAO Stat, 2008).

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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. Factors influencing infection of pistachio nuts 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; Kader *et al.,* 1982; Panahi *et al.,* 2005)

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 evaluation of pistachio contamination to *Aspergillus flavus* in Semnan province.

MATERIALS AND METHODS

In order to study the rate of contamination of pistachio kernels to *A. flavus* and determining aflatoxigenic potential of isolates, sampling of pistachio was done in some pistachio growing area of Damghan and the suburbs such as Amerieh, Mehmandust and Forat. Samples of pistachio kernels were collected from the last processing pistachio stage in 2010 in the traditional and semi mechanized processing plants.

In order to prepare each sample 10 sub samples of 500 grams of each processing plants were taken . The 10 sub samples totally mixed and then 500 grams of it was taken as the main sample. Thus 42 main samples were collected.

10 grams of each sample once were grinded and then were added to 90 ml of peptone water %./1.This mixture was shaken and diluted to final concentrations of 10^{-1} and 10^{-2}. From each dilution, 0.1 ml of inoculums was spread (completely randomized design with 3 replication) on the surface of selective medium AFPA. This culture medium was used for the differential detection of the *Aspergillus flavus* and *A. parasiticus* group by production of typically yellow to olive green conidia and a bright orange reverse coloration. The Petri dishes were kept at 28 ºC, 3 to 7 days after inoculation. The *A. flavus* colonies were counted and isolated. The rate of contamination of pistachio to *A. flavus* in different samples was compared with each other.

In the next phase, the capacity to produce aflatoxins by 86 isolates of *A. flavus* was determined. One ml of spore suspension from each isolate (density of 10^3 spore/ml) was grown on 22 grams of autoclaved rice flour (natural substrate). 7 days after inoculation, the aflatoxin content in the rice medium was extracted by chloroform and measured by thin layer chromatography (TLC) and densitometer.

RESULTS

In table 1 the results of *A. flavus* colony counting in different pistachio samples is shown. As shown in table 1, the rate of contamination of pistachio kennels of different samples to *A. flavus* is different. In some samples no sign of contamination to *A. flavus* could be observed while in other samples, there was serious contamination to *A. flavus*

The statistical findings showed that the average difference of colony number of *A. flavus* in different pistachio samples is meaningful in statistical level of %1.

The results indicated that pistachio samples that belong to traditional processing plants have more population density of *A. flavus* than semi-mechanized processing plants.

Table 1. Comparison of colony number of *A. flavus* in different pistachio samples of Semnan province.

Number of sample	**Colony number of *A. flavus* (CFU/G)**
1	8.5×10^3
2	1.5×10^4
3	ND
4	5×10^3
5	7.6×10^3
6	1.5×10^4
7	ND
8	5.5×10^4
9	1.5×10^4
10	4×10^3
11	3.5×10^3
13	ND
14	3×10^3
15	8×10^3
16	3.3×10^3
17	ND
18	7.6×10^3
19	3×10^4
20	2.5×10^3
21	5.3×10^3
22	4.3×10^3
23	3.3×10^4
24	2.3×10^3
25	ND
26	5.3×10^3
27	4.6×10^3
28	1.3×10^3
29	3.2×10^4
30	1.2×10^4
31	ND
32	ND
33	2.3×10^3
34	5×10^3
35	4×10^3
36	ND
37	ND
38	2.5×10^3
39	ND
40	3.6×10^3
41	3×10^3
42	3.3×10^4

ND = Not Detected

Table 2. Analysis of variance table for colony number of *A. flavus*

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr >f**
Treat	40	31364049187	784101230	177778	<.0001
Error	82	361667	4411		
Corrected Total	122	31364410854			

R-Square	**Coeff Var**	**Root MSE**	**_flavus Mean**
0.999988	0.603546	66.41212	11003.66

Also as pointed out in the materials and methods section, the isolates of *A. flavus* were evaluated for their ability to produce aflatoxins, when grown on natural substrate (autoclaved rice flour). Among 86 isolates of *A. flavus* that their aflatoxigenic properties were studied, 55 isolates (%63.95) were able to produce one or several types of aflatoxins (B_l, B_2, G_l, G_2), while 31 isolates (%36.04) were unable to produce any type of aflatoxin. Among evaluated isolates, 8 isolates (%9.30) produced all types of the aflatoxins (B_l, B_2, G_l, G_2), 7 isolates (%8.13) produced AfB_1, AfB_2 and AfG_1, 30 isolates (%34.88) produced AfB_1 and AfB_2 and 10 isolates (%11.62) only produced AfB_1. Among aflatoxigenic isolates, the strength of toxin production varied from weak to strong.

DISCUSSION

In general, the production of aflatoxin is affected by different factors, such as genetic properties of the producing fungi and the physiochemical medium that fungi grow. The production of any type of mycotoxin, besides the species, depends on the toxigenic isolate. (Allameh & Razaghi ,2002).

Although the aflotoxins are only produced by *A. flavus* and *A. parasiticus*, there are isolates belonging to *A. flavus* group that are unable to produce aflatoxin. Even if several fungus isolates can genetically produce aflotoxin , the type and amount of produced aflotoxin by these isolates are different from each other (Allameh & Razaghi ,2002).

Pear and Richard (1992) suggested that further studies be done on pistachio contamination in each region seriously because of the toxin producing property of different isolates of *A. flavus*.

Amin Shahidi (1996) has investigated aflatoxigenic *A. flavus* isolates in contaminated pistachio in Iran and studied about the capacity to produce aflatoxin by isolates and observed that most of the samples were contaminated with *A. flavus* and *A. parasiticus*.

Magnoli *et al* (1998) studied on the aflotoxin producing property of different isolates after identification and isolation of *A. flavus* from foods and observed that the property and power of producing toxin in these isolates were very different from each other so that only %74 of *A. flavus* isolates produced toxin.

Moratazavi (1989) studied the aflatoxin producing property of *A. flavus* isolated from cereals and oil seeds. Among the fungi which were studied for their aflatoxigenic property, some of the isolates had gained the property of producing toxin and were able to produce toxin up to 2000 ppb, while some isolates were able to produce toxin only up to the determining level and the intensity and strength of toxin producing fungi were spread over a wide range.

Moghadam *et al.*, (2006) studied aflatoxigenic properties of the *A. flavus* isolated from contaminated pistachio. The results showed that out of 80 isolates of *A. flavus* , 70 isolates (%87.5) were able to produce one or more types of Alflatoxin (G2,G1,B2,B1), while 10 isolates (%12.5) were not able to produce any type of Aflatoxin .

The results obtained from the present study indicated that the belonging of a fungus isolate to the *A. flavus* group on its own can not be taken as evidence that it produces aflatoxin. It was shown that 31 out of 86 isolates used in this study were unable to produce any type of aflatoxin. Also the aflatoxigenic properties and their intensities were scattered on a wide range from severe to weak.

In addition to the kind of *A. flavus* isolates , the combinations and the type of the culture medium and the used substrates also had considerable effect on the ability of producing toxin by isolates. In general, natural substrate (such as autoclaved rice flour) are far better environments in comparison to the artificial media (such as SLS).

The result of this study show that the rate of pistachio contamination to *A. flavus* in samples taken from traditional processing plants were more than the samples taken from semi mechanized processing plants which is compatible to the studies done by Moradi and Javanshah(2006). Some of the reason may be that traditional processing plants do not use drying machines while the semi mechanized processing plants make use of these machines which can be effective in reducing the contamination of pistachio to *A. flavus* .

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