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Study on Some of Pistachio Cultivars' Contamination of Khorasan-e-Razavi Province to Aspergillus *flavus*

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Abstract: In order to study on some of pistachio cultivars' contamination of Khorasan-e-Razavi province,eight cultivars of pistachio kernels were collected from the pistachio production areas of this province. Using serial dilution method, 10 grams of ground pistachio was moved to 90 ml water peptone and continuous dilutions 10^{-1}, 10^{-2} were prepared. Then 0.1 ml from each dilution was inoculated in three replicates on plates containing AFPA medium and was placed inside an incubator at 28 ° C for 72 hours. The characteristic of this environment is the advent of green yellowish colonies by Aspergillus flavus and Parasiticus that is distinguishing criteria of mentioned fungi. Since it has capacity to stop interfering bacteria and fungi growth causes the emersion of distinct and discrete colonies that are easily seperable and countable from each other. After three days, the number of colonies of *A.flavus* was identified and calculated in samples. The results of this research indicated that there was a significant difference in colonies number of these fungi in different pistachio cultivars (At 5% level). Among these cultivars Akbari Namavar had the highest contamination and against, Fandoghi 1 had the lowest contamination.

Keywords: *Aspergillus flavus*, Khorasan-e-Razavi province, Pistachio, Contamination.

INTRODUCTION

Pistachio has been long cultivated in different parts of Iran. Wild and self-growing forests of pistachio in northeast of Iran and borderline areas with Turkmenistan and Afghanistan have ancient backgrounds while pistachio tree is supposed to be domesticated and cultivated in Iran about 3-4 thousand years ago.

Aflatoxins are secondary mold metabolites produced by molds of *Aspergillus flavus* genus. They are severely toxic and carcinogenic. They are known as mutagenic factors. Consumption of foodstuffs contaminated with Aflatoxin may create severe side effects in human or the animal (Yabe *et al*., 1993).

In all cases of contamination, Aflatoxin rate lower than mortal rate would cause liver cancer and aflatoxenitic damages. Based on the importance of the subject broad researches have been done on recognition of pathogenic factors and its performance showing that *Aspergillus* mold has a significant role in production of Aflatoxin. For this reason broad studies have been performed in the country and internationally on the mold life, formation of Aflatoxin and methods of prevention and detoxication.

Aflatoxins belong to the groups of Bisfurtano-isocoumarin chemically. 18 types of Aflatoxins have been identified which 13 kinds of them are naturally produced. The main combinations are that of B_1, B_2, G_1 and G_2 aflatoxins. When subjected under ultraviolet light, B_1 and B_2 aflatoxins emit blue florescent while G_1 and G_2 aflatoxins emit green fluorescent light (Angle *et al*., 1982).

Many general of *Aspergillus*, *Penecillium* and *Rhizopus* molds have been reported which produce Aflatoxin among which *A. flavus* group molds are considered as the main and the most important molds producing aflatoxin (Bayman and Cotty, 1991).

A. flavus, *A. parasititicus* and *A. nomius* are main producers of aflatoxin in this group (IARC monographs, 1987).

Since 1971, United States of America confiscated some pistachio exported for Iran and Turkey for being contaminated to aflatoxin, Subject of foodstuffs contamination to Mycotoxins and especially aflatoxin were considered in our country regarding pistachio and broad range researches were started by State Research Institutes on the subject (Aminshahidi, 1997).

Sozangar and his colleagues executed a plan titled "Study of the problems related to Iran Pistachio contamination by Aflatoxin". The results of the said research showed that Rafsanjan and Isfahan pistachios may have been contaminated with aflatoxin when on the tree and before cropping. Aflatoxin producing mold spore seems to be transmitted by insects or is directly penetrated into the newly ripped and opened pistachio. No aflatoxin contamination has been seen before pistachio is ripen unless in those pistachios are pierced by the insects.

Based on the importance of Iranian pistachio contamination by aflatoxin, Javanshah and his colleagues have performed

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researches in State Pistachio Research Institute providing useful results in decreasing such contamination. Study of climatologic conditions and its effects on pistachio drying time showed that drying under the sun would increase for up to 120 hours, especially when there is precipitation which creates optimum conditions for quick germination of mold spore and production of aflatoxin. So using new methods and modern equipment for quick drying of pistachio product would prevent the required condition for spores germination and growth.

As contamination of pistachio by aflatoxin would create many problems in production, consumption and exportation of pistachio and for the importance of the subject, congestion of *Aspergillus niger* and *Aspergillus flavus* group molds in processing terminals and processed pistachio in such terminals (17 pistachio processing terminals) have been studied by Panahi and his colleagues during two year period.

The study of spore congestion in different terminals during pistachio processing showed that the congestion rate is higher in peeling and washing process than other stages.

Percentage of separation in different species of *Aspergillus* mold in the studied samples indicated that the pistachio going out of traditional terminals has higher level of contamination that semi-mechanized terminals. The comparison of different washing systems showed that using water shower would be more effective in lowering the contamination than fixed or running current pools. In our country, pistachio bring the highest currency income among non-oil exporting products and the main problem in exportation of pistachio during the current years has been that of aflatoxin contamination. Therefore considering the economic importance of this vital product in our country we decided to study contamination of pistachio in Khorasan-e-Razavi by *Aspergillus flavus* mold

MATERIALS AND METHODS

a) Sampling

As dried fruits are among highly susceptible materials to aflatoxin mold contamination for having high rate of fat and pistachio as a dried fruit has high economic value in our country, the study of aflatoxigenic fungi has critical value. The first step in this study is the selection of a suitable sample. To this end 8 types of pistachios from different pistachio areas of Khorasan-e-Razavi province were collected including Akbari Namavar, Sefid Feiz Abad, Sefid Badamy, Fandoghi 1, Fandoghi 2, Badamy Haji Torbati, Akbari and native Badami and tests were performed in vitro.

b) Preparation of the sample for cultivation

As distribution of toxin and its dispersion was different in various parts of the kernel, intensity of mold contamination requires a homogeny and totally uniform sample to be studied. So the pistachio kernel should be grinded. Then 10g of grinded pistachio of any type was added to 90 ml of 0.1% Pepton water and dilution of 10^{-1} and 10^{-2} were prepared. For big sizes of mold spores and other mold producing components, quick accumulation and precipitation would be likely. For this purpose after quick agitation of pipes, 0.1 ml of each concentration (three random selection) were cultured in specialized culture medium (AFPA) and they were spread on the plate level using sterile glass pipe. In fact the altered medium would be that of Aspergillus Differential Medium (ADM), The medium was identified with appearance of green-yellow collies by *Aspergillus flavus* and *Aspergillus parasiticus* which was diagnostic criteria for the said molds. Dichlorane as anti-mold prevented quick growth of the molds while chloramphenicol was used to prevent bacteria growth (Aminshahidi, 1997). Then plates are placed in incubator for 28 ° C for 72 hours.

Morphologic specifications of colonies:

Based on morphologic specifications, *A. flavus* and *A. parasiticus* clonies were identified on AFPA surface with appearance of greenish yellow colonies.

c) Counting colonies of *A. flavus* group

Among significant particulars of AFPA medium is that it prevents sporulation of *A. flavus* and *A. parasiticus* and no-load colonies or limited sporulation is appeared. As it could stop intervening quick-growing bacterium and molds, it would result in specific and separate colonies which were easily identifiable and countable. Relying on this specifications, the medium has been used as suitable medium for enumeration and estimation of contamination by this mold (General Enumeration). And finally the numbers of colonies in different concentration were enumerated.

RESULTS

The result from the study of contamination in 8 types of pistachio by *Aspergillus flavus*:

Contamination in 8 types of pistachio from Khorasan-e-Razavi including Akbari Namavar, Sefid Feizabad, Sefid Badamy, Fandoghi 2, Badamy Haji Torbati, Fandoghi 1, Akbari and Native Badamy by *A. flavus* were studied and statistical investigations showed that there was a significant difference between the average number of colonies of this mold in different types of pistachio (At 5% level).

Among the tested parameters, the figure for Akbari Namavar had the highest contamination while figures for Fandoghi 1 had the lowest contamination. Tables 1 and 2 show variance analysis and comparison of the average rate of contamination in 8 types of pistachio by *A. flavus*.

Table 1- Variance analysis for average number of *A. flavus* mold colonies in 8 types of pistachio

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
treat	7	176.00	25.14	2.22	0.08
Error	16	181.33	11.33		
Corrected Total	23	357.33			

Table 2- Comparison of average contamination in 8 types of pistachio by *A. flavus* mold

Type of pistachio	Average number of colonies per gram	Dunken Statistical Classification (∞ = 5%)
1- Akbari Namavar	8.66×10^3	a
2- Sefid Feizabad	5×10^3	ab
3- Sefid Badamy	3.33×10^3	ab
4- Fandoghi 2	1.33×10^3	b
5- Akbari	1×10^3	b
6- Native Badamy	1×10^3	b
7-Badamy Haji Torbati	0.66×10^3	b
8-Fandoghi 1	0.33×10^3	b

DISCUSSION

In most parts of the world broad and spread researches are in progress on recognition of resistant type of the products to aflatoxin producing *Aspergillus flavus*, of which successful results have been reported (Dickens, 1966). 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, is a good strategy 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 rigorously studying their resistance mechanisms with some brilliant results already reported (Gradziel and Wang, 1994).

Contamination of pistachio to aflatoxin is a main problem for public health and would result in returning pistachio cargoes by the users. Some members of *Aspergillus* group, especially *flavus* and *parasiticus* are known as producers of aflatoxin in pistachio (Yabe *et al*., 1993).

Economic shocks, in the same extent as health risks from dried fruits contamination to aflatoxin has made the researchers to perform studies for monitoring aflatoxin contamination in pistachio.

In a study by Rahimi (2007), separation of *Aspergillus* from the outer and inner skin and kernel of pistachio collected from the study gardens in Kerman, Rafsanjan and Esfahan showed that *Aspergillus* are generally distributed in pistachio growing areas in Iran. the results from the study showed that aflatoxin producing *Aspergillus* are more prevalent in pistachio.

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