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ORIGINAL ARTICLE

Study on The Sensitivity Level of Some Pistachio Cultivars of Khorasan-E-Razavi Province To Aspergillus Flavus

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Leila Jalali, Hossein Afshari, Shahram Ashraf and Mehdi Mohammadi Moghadam: Study on The Sensitivity Level of Some Pistachio Cultivars of Khorasan-E-Razavi Province To Aspergillus Flavus

ABSTRACT

In order to evaluate the sensitivity level of various cultivars of pistachio from Khorasan e Razavi Province to aflatoxigenic *Aspergillus flavus* mold, 4 cultivars of popular pistachio of Khorasan-e-Razavi were selected and collected. For this research, an isolate of aflatoxigenic *Aspergillus flavus* separated from the pistachio was used. initially, 60 grams of pistachio kernels in 3 consecutive 20-gram sampling were selected and placed on Petri-dishes separately. 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 26 degrees centigrade.After 5 and 8 days of inoculation, growth rate and colonization of *A. flavus* on pistachio kernels measured in different cultivars. The average difference in colonization levels of various cultivars of pistachio were analyzed statistically by the help of Duncan's multiple range test.The results of the study demonstrated that among the examined cultivars, Daneshmandi had the highest sensitivity and in contrast, Garmeh addressed the lowest level of sensitivity.Undoubtedly, the most effective and useful approach to reduce the contamination level of the crop to *A. flavus* and aflatoxin is to select the most persistent ones to fungal growth and consequently the aflatoxin resulted by its growth.

Key words: Khorasan-e-Razavi Province; *Aspergillus flavus*; Pistachio; aflatoxin.

Introduction

Aflatoxins are secondary metabolites that are produced by strains of filamentous fungi, namely *A. falvus* and *A. prasiticus*. These compounds are extremely toxic (acute and chronic toxicity), teratogenic and carcinogen and are considered as mutagenic agents [10].

So far, 18 aflatoxins have been identified and reported; among which, 13 are compounds that are produced naturally [3]. The main types of these compounds are B_1, B_2, G_1 and G_2 aflatoxins and while exposed to ultra violet rays, B_1 and B_2 aflatoxins radiate blue florescent compared to G_1 and G_2 aflatoxins which radiate green florescent [1].

So far, various strains of *Aspergillus*, *Penecillium* and *Rhizopus* molds have been reported that produce aflatoxin; among which, *A. flavus* have been placed above all and is one of the major causes of aflatoxin [2].

The colony diameter of *A. flavus* on the Czapek Yeast extract Agar, CYA, is 50 to 70 mm. These colonies are flat, spread, or relatively dense and are velvet-like at least on edges. In most cases, the central area is convex-shaped and accumulated and floccose and in some others the central areas are depressed. Mycelium is only observed in floccose regions and is white in color. Conidial heads usually cover the whole colony surface except those regions that are floccose or in case of secretion of sclerotinia become rare or extinct [8]. Their color is olive green but was also identified as yellow which gradually changed their color to green.

Among the four major aflatoxins, i.e. B_1, B_2, G_1 and G_2, B_1 possesses the highest toxic level after which G_1, B_2 and G_2 aflatoxins have lower toxicities respectively ($B_1>G_1>B_2>G_2$). Also, M_1 and Q_1 aflatoxins are somehow toxic and the reason for higher toxicity in B_1, G_1, M_1 and Q_1 rather than B_2 and G_2 is the existence of a double bind of 8 and 9 dihydrouridine (or in other words 2 and 3 Vinyl Ether) [9].

The most important factors in aflatoxin production are the fungal properties, food environment, moisture of crop and the relative humidity and ambient temperature and time.

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In Iran 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 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.

Thus, 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 [1].

Mojtahedi *et al.,* stated that the minimum relative humidity required for infection to aflatoxin of pistachio in warehouses is %85 and the least interval needed for the creation of poison in this relative humidity is between 7 to 10 days depending on temperature from 20 to 27 degrees centigrade.

Aminshahidi, M., [1] studied aflatoxigenic *Aspergillus* molds in infected native Iranian Pistachio and reviewed the capability of aflatoxigenic properties. In his studies, he observed that most of the examined samples were contaminated with *A. falvus* and *A. parasiticus* molds and aflatoxin.

Kamimura *et al.,* did extensive research in regards with contaminated crops to aflatoxin. They reported the most contamination to B_1 aflatoxin in pistachios as 1382ppb.

Ghewande, M.P. *et al*., [4] analyzed the resistance level of peanut cultivars to fungus growth and aflatoxin formation and found out that there are significant differences between various cultivars of peanut regarding growth and colonization level of fungi and aflatoxin levels.

Gradziel, T.M. and D. Wang, [5] studied the sensitivity level of different cultivars of almonds from California America towards aflatoxigenic *Aspergillus flavus* and figured out that the sensitivity levels of various cultivars are different. They also analyzed the impacts of coating of almond kernel in prevention and reduction of penetration of fungus in the core and found out that it plays the role of a resistant barrier.

Materials and Methods

Select and Collect Different Cultivars of Pistachio For The Purpose of Evaluating Their Sensitivity Towards Aflatoxigenic Aspergillus flavus:

In order to evaluate the sensitivity level of various cultivars of pistachio towards aflatoxigenic *Aspergillus flavus* mold, 4 various cultivars of Khorasan-e-Razavi Province were selected and collected. While experimenting, it was tried to use cultivars which were among the most important and commercially available ones of the regions that possessed a great deal of cultivation. Therefore, 4 cultivars of pistachio named Daneshmandi, Red, Sefid Badamy and Garmeh were collected in the time of harvest for the purpose of the experiment. In order to minimize possible contamination of pistachios to *Aspergillus flavus mold* and aflatoxin, they were collected from trees at the time of sampling. After collecting the fresh pistachios, the pest-stricken ones and those with a potential to be contaminated were removed. Then, the outer soft layer of the pistachio was separated from the horny skin by hand to avoid any damage to the inner shell. After that, pistachios were dried under proper conditions and were used for laboratory purpose in vitro.

Fungus Isolate:

For this research, an isolate of aflatoxigenic *Aspergillus favus* mold separated from the pistachio was used and throughout all stages of isolate cultivation, subculture, or for the production of slant, the two medium MEA (Malt Extract Agar) and PDA (Potato Dextrose Agar) were used.

Evaluation of The Sensitivity of Cultivars of Pistachio To Aspergillus flavus:

Before the experiment, in order to ensure no *Aspergillus flavus* mold contamination for the nuts, initially, 60 grams of pistachio kernels in 3 consecutive 20-gram sampling were collected (completely randomized design in 3 replications). These 20 grams were sterilized by the help of %0.5 Sodium hypochlorite solution.

Then, they were thoroughly rinsed in sterile distilled water. After that, in order to absorb the primary moisture of kernels, they were soaked in sterile distilled water for 10 minutes. In the next stage, kernels were taken out from the sterile distilled water and were put in sterile Petri and 1 milliliter of sterile distilled water was added to it.

For the purpose of evaluating sensitivity of cultivars, firstly, the fungal isolate of *A. flavus* in the medium PDA inside the SLANT was implanted. After 8-10 days, along with the growth and sporulation of mold, a little sterile distilled water in addition to a few drops of Tween 20 were added to the slants and a uniform suspension of fungal spores was prepared.

To perform this experiment, 2×10^6 spore per milliliter is needed. Hemacytometer was used for the purpose of counting spores. For every cultivar, 3 repetitions alongside with an observant were considered and in control Petri, instead of adding spore suspension, sterile distilled water was added. After each surface disinfection and soaking pistachios in the sterile distilled water, one milliliter of the fungal spore suspension was added to each Petri including 20 grams of kernels.

By shaking the Petri, fungal spore suspension was thoroughly spread throughout the Petri until every surface was impregnated. To provide adequate moisture (up to saturation level), Petri containing the

moist kernels were put inside plastic containers with lids at the bottom of which a little sterile distilled water was poured and the plastic container lid was firmly closed and these dishes were incubated inside the incubator for a period of one week at 26 degrees centigrade. After growth of fungus and covering all the surfaces by the fungus, the amount of fungal colonization throughout the surfaces on the fifth and eighth days was calculated [4].

Results and Discussion

Results of The Study of Sensitivity Levels of 4 Cultivars of Pistachio To Isolates of Aspergillus flavus Mold:

In order to evaluate the sensitivity level of cultivars of pistachio to the growth of *Aspergillus flavus*, after the growth of fungus on inoculated pistachios, the criterion for measuring fungal growth was considered as the fungal colonization on pistachio kernel. After recording the percentage of fungal colonization on kernels on the fifth day after inoculation, the average difference of various cultivars of pistachios were analyzed by the help of statistical method of Duncan's multiple range test. Table 1 presents the variance analysis of colonization rate of *Aspergillus flavus* mold amongst various cultivars of pistachios on the fifth and eighth day after inoculation. Results of the statistical analysis illustrate a significant difference in average difference of fungal colonization rate in various cultivars of pistachio on the fifth and eighth day after inoculation (at %1).

As it is seen, the difference amongst of fungal growth rate (colonization) of various cultivars on the fifth and eighth day after inoculation is significant at a %1 level and Daneshmandi had the highest sensitivity and in contrast, Garmeh addressed the lowest level of sensitivity (Table 1 and 2). In Fig 1 and 2, the rate of fungal growth in pistachio cultivars of Daneshmandi, Sefid Badamy, Ghermez and Garmeh showed for 5 and 8 days after inoculation respectively.

Discussion:

Since the discovery of aflatoxins, *Aspergillus flavus* mold have always been mentioned as the most common source of mold contamination in food science which demonstrates the economical importance of this fungus. *A. flavus* illustrates a particular tendency to contaminate nuts and oil seeds. Peanuts, corn and pistachio are the major crops attacked by this fungus[7].

Undoubtedly, as this fungus attacks a large spectrum of agricultural products, one of the most effective and useful approaches to solve this concern is to analyze the resistance of various cultivars of a product and select the most persistent cultivars to fungal growth and consequently, from the aflatoxin caused by its growth which eases lowering contamination levels by the help of choosing the best cultivar in a reforming program so as to reduce contamination to aflatoxin.

In this study, the criterion measuring fungal growth of *A. flavus* was considered as the amount of colonization of fungus on 4 cultivars of pistachio. After recording the colonization percentage of fungus on nuts on the fifth and eighth day after inoculation, the average difference in colonization levels of various cultivars of pistachio were analyzed statistically by the help of Duncan's multiple range test.

As it was observed, the difference in the rate of fungal growth on nuts was significant at %1 whose cause goes back to its genotype. Applying crops that are sensible to the contamination of Aspergillus, pests, or other microbial agents increases the potential to be contaminated by aflatoxin. Therefore, resistance of the chosen cultivar should be considered and farmers need to consult with plant breeding professional and agricultural promotion experts to find the most suitable cultivar.

The amount of fat and sugar and elements such as zinc, manganese, magnesium, iron, etc are different for various cultivars of pistachio which may address the amount of sporulation of *Aspergillus flavus* for every genotype and naturally, the aflatoxin resulted from its growth.

In most regions of the world, extensive research are being done for the purpose of identifying various crops' resistance level to aflatoxigenic *Aspergillus flavus* whose reports imply success.

Mohammadi Moghaddam, M. *et al*., [6] studied the sensitivity level of 10 cultivars of pistachios cultivated in Kerman, Semnan and Ghazvin regions to *Aspergillus flavus* and aflatoxin. The findings suggested a significant difference in fungal growth and toxin production in different studied cultivars which is aligned with the findings of ours concerning pistachio cultivars in Khorasan-e- Razavi.

Ghewande, M.P. *et al*., [4] stated the resistance of host as one of the most critical and important aspects for lowering contamination levels based on the genetic diversity of different peanut cultivars.

Ghewande, M.P. *et al*., [4] performed studies regarding the resistance level of peanut cultivars relative to fungual growth of *Aspergillus flavus* and the consequent aflatoxin resulted from its growth. Their findings suggested a prominent correlation between resistance of variant cultivars and fungal growth.

Gradziel, T.M. and D. Wang, [5] analyzed the sensitivity level of various cultivars of almonds from California America towards aflatoxigenic *Aspergillus flavus* and figured out that the sensitivity level of various cultivars to this fungus are significantly different. They also studied the rate of fungal penetration to the core as a result of damage to the

cover of almonds and could demonstrate its role in reducing fungal growth. Throughout stages of performing this research, sensitivity levels to aflatoxigenic *Aspergillus flavus* were also studied and the rate of B_1 produced was analyzed. The findings represent a significant correlation between fungal growth on the surface of nuts and show that various cultivars react differently based on their sensitivity level towards this fungus.

Table 1: Variance analysis of colonization rate of *Aspergillus flavus* mold amongst various cultivars of pistachios on the fifth and eighth day after inoculation.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
The percentage of fungal colonization on nuts on the fifth day after inoculation	3	149.46	49.82	9.92**	19.55**
The percentage of fungal colonization on nuts on the eighth day after inoculation	3	500.07	166.69	/0001	0/2826

Table 2: Comparison of average colonization rate of *Aspergillus flavus* amongst various cultivars of pistachios on the fifth day after inoculation.

Type of pistachio	Average colonization rate Classification (∞ = 1%)	Dunken Statistical
1-Daneshmandi	38.790	a
2-Sefid Badamy	35.113	ab
3- Ghermez	34.100	b
4-Garmeh	28.917	b

Table 3: Comparison of average colonization rate of *Aspergillus flavus* amongst various cultivars of pistachios on the eighth day after inoculation.

Type of pistachio	Average colonization rate Classification (∞ = 1%)	Dunken Statistical
1-Daneshmandi	66.310	a
2-Ghermez	61.017	ab
3-Sefid Badamy	58.687	b
4-Garmeh	48.530	c

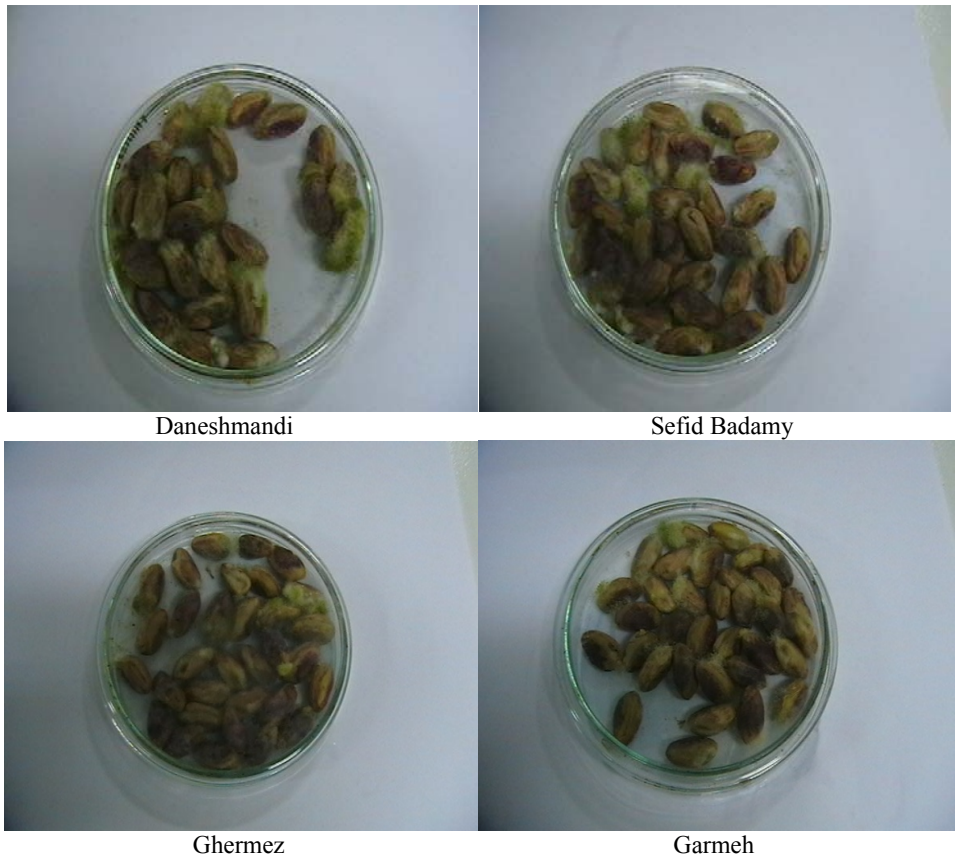

Fig. 1: The percentage of colonization of *A.flavus* on kernels on the fifth day after inoculation.

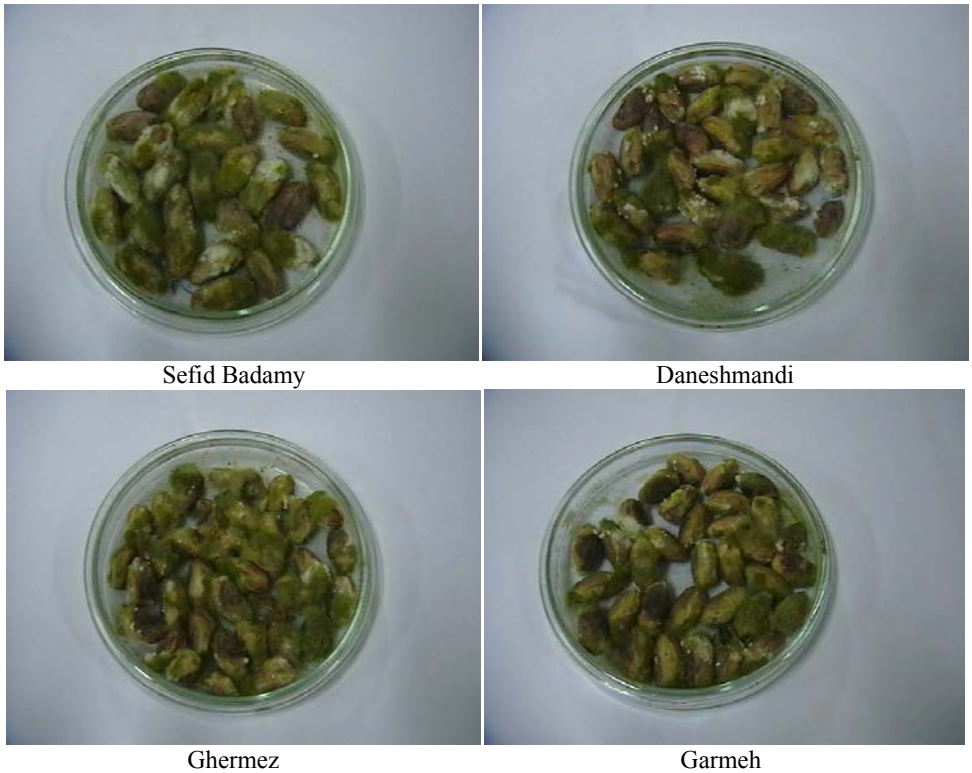

Fig. 2: The percentage of colonization of *A.flavus* on kernels on the eighth day after inoculation.

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