Effect of Aflatoxin produced by *Aspergillus flavus* in reduction of our pistachio marketing all over the world

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Abstract
Pistachio product makes a special importance in the agricultural production of Iran and is one of the main portion of non-petroleum exportation. Aflatoxin as secondary metabolites produced by the fungus *Aspergillus flavus* on pistachio fruits is toxic and reduces our competition in global pistachio markets.. It is necessary that Iran observe international standards and make investments in marketing . We have to find new target markets for this product, particularly by eliminating fungal infection and aflatoxin production . In this study, existing position and challenges of major pistachio producing and its exporting in Iran and other countries are considered and a number of strategies are recommended.

Keyword: Pistachio, Aflatoxin, Aspergillus, exporting

Introduction

Pistachio has good functional properties among other nuts due to its nutritious value and economical importance. Because of its high nutritional value and favorable taste, planting pistachio trees has become common in other parts of the world. Pistachio product as a commercial output has a special importance in the agricultural production of some countries especially in Iran. According to the latest statistics released by the World Food and Agriculture Organization (8) affiliated to the United Nations, the pistachio production in the world stood at 548,759 metric tones (MTs) in 2002 year. Generally, Iran ranking first with a production of 300,000 MTs and U.S. with a production of 127,010 MTs, Turkey with 40,000 MTs, Syria with 39,208 MTs, and China with 26,000 MTs took other places after our country Iran (7).

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Normally, Iran ranks first in the world in terms of pistachio production and harvested area but it does not enjoy such a high position in global marketing due to exporting challenges. Actually, USA as Iran's rival in pistachio global markets tries in many devices to improve its situation and gain in global war of pistachio production and export by defecting Iran. Of course, major markets in Europe, East Asia, Central Asia and the Persian Gulf littoral states use Iranian pistachios at present. But it is necessary that Iran observe international standards and make investments in marketing and exporting processing industries and find new target markets for this product.

Aflatoxin contamination of pistachio nuts is undoubtedly a serious problem for Iran and other producing countries (9,5). The European Union's 1997 ban on pistachio imports from Iran because of high levels of aflatoxin in some shipments, exemplifies the seriousness of this problem.

Commonly, aflatoxins are toxic, carcinogenic, mutagenic, immunosuppressive agents, produced as secondary metabolites by the fungus *Aspergillus flavus* and *A. parasiticus* on variety of food products (10,16) . Among 18 different types of

aflatoxins identified, major members are aflatoxin B1, B2, G1 and G2. Aflatoxin B1 (AFB1) is normally predominant in amount in cultures as well as in food products. Pure AFB1 is pale-white to yellow crystalline, odorless solid. Aflatoxins are soluble in methanol, chloroform, actone, acetonitrile. Usually, *A. flavus* produces AFB1 and AFB2, where as *A. parasiticus* produces AFG1 and AFG2 as well as AFB1 and AFB2. Four other aflatoxins M1, M2, B2A, and G2A which may be produced in minor amounts were subsequently isolated from cultures of *A. flavus* and *A. parasiticus.* A number of closely related compounds namely aflatoxin GM1, parasiticol and aflatoxicol are also produced by *A. flavus*. Aflatoxin M1and M2 are major metabolites of aflatoxin B1 and B2 respectively, found in milk of animals that have consumed feed contaminated with aflatoxins (1,11).

Fungal contamination and subsequent production of aflatoxin can occur in crops in the field, at harvest, during post harvest operations and in storage. The rate and degree of contamination are dependent on temperature, humidity, soil and storage conditions. Prevention, particularly by excluding or reducing toxigenic mould growth and toxin production in susceptible food crops, is the most effective way to restrict mycotoxin contamination. In practice, this can be accomplished by reducing fungal infections in growing crops through rapid drying and correct storage of the harvested crops (2).

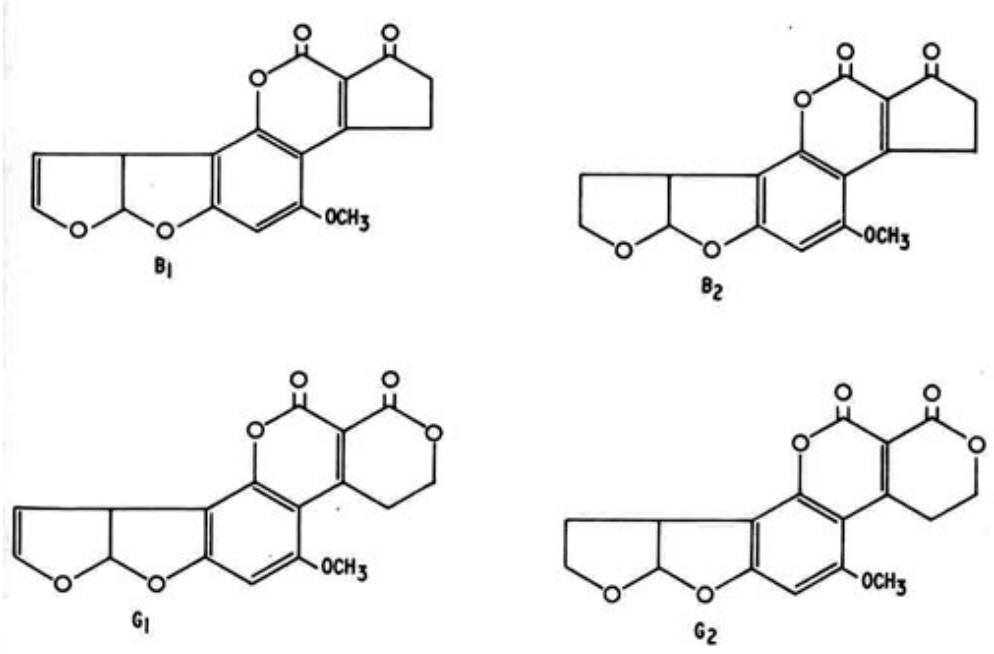

Fig. 1 Structures of aflatoxins B_1, B_2, G_1, and G_2.

Pistachio nuts are grown commercially in Afghanistan, Iran, Iraq and Turkey. In the first three countries the nuts are usually decupled very soon after harvest and the nuts in shell are then stored and processed. However, the nuts are stored in-hull, sometimes for many months, or even for years In Turkey. Early de-hulling has the advantage of avoiding staining of the shell, but has the disadvantage of exposing the split nuts at an early stage to *Aspergillus flavus* and *A. parasiticus*

spores which have the potential to produce aflatoxin (3,8).

Method and Materials

The marketing's problem in pistachio in Iran led us to study about the possibility of aflatoxin infection in pistachio. Pistachio growing areas were visited and infected fruits of plants were collected from orchards. The samples were collected and transferred to the laboratory of plant pathology for isolation and identification of the causal agents. Soil around the roots of infected plants were also removed and sent to Laboratory to detect population densities of the pathogen in soil.

Isolation and identification of the pathogen

Various samples were cut into 0.5-1.0-cm pieces, then dipped in sodium hypochlorite (0.5%) for 2 min. The pieces were rinsed in sterile distilled water, dried on sterile filter paper and plated on the media in Petri dishes. The samples were cultured in Potato Dextrose Agar (PDA) as common rich media. Soil dilution technique was also applied to isolate inoculums from soil around the infected plant roots and all cultures were incubated in light room. Morphological characteristics of isolated fungi including shape of conidiophores and conidia were examined in laboratory and light room.

Managements of pathogen

The possibility of infection by causal agent was surveyed to find any possible ways for uninfected pistachio nuts in growing regions. In fact the good quality agricultural procedure was important to reduce possible infection by *Aspergillus flavus* which produces aflatoxin in pistachio nuts. On the regulatory side, efforts should be made to establish, as soon as possible, internationally agreed guideline levels for aflatoxin in pistachio nuts which would be accepted by all parties so as to eliminate possible technical barriers to trade.

Further research is needed to explore other means of preventing mould infection and aflatoxin production. These could include prevention of contamination in the field through the use of biological control agents that are pathogenic to *Aspergillus flavus*. We concluded that using saprophytic fungi such as yeast or harmless bacteria could be helpful for controlling the agent of aflatoxin producers.

The experiments demonstrated that the yeast, *Pichia. anomala* can modulate spore production of fungi including *A. flavus* on the pistachio nut-fruits (12). Field spraying of yeasts to pistachio trees may decrease the population of *A. flavus* in the orchards and thus lower the number of nuts infected by this fungus. The outcome may be reduction of aflatoxin contamination in the edible nuts. Monitoring *A. flavus* and *A. parasiticus* on pistachio buds may provide the growers a way to evaluate the population of the aflatoxin-producing fungi in orchards and help them make decision on control strategy. Appreciative the importance of nuts infection by *A. flavous* was the main goal of the project.

Results and discussion

Different fungal species were isolated from samples in this study; however, most frequent isolate was *Aspergillus flavus*. The fungus was occurred in different studied areas in both orchards and nursery sites but its population density was more in relatively warm areas. Isolated species was produced conidiophores in culture (Fig. 1). The development of *Aspergillus* mould on pistachio litter has been related to the increase of *Aspergillus* inoculums in the orchard and results in greater numbers of moldy and mycotoxin-contaminated pistachio nuts. Certain practices such as burying or removing litter may decrease colonization by *Aspergillus flavus* moulds in the orchard. Another possible treatment for pistachio litter includes the

application of microorganisms that limit the development of *Aspergillus* mould.

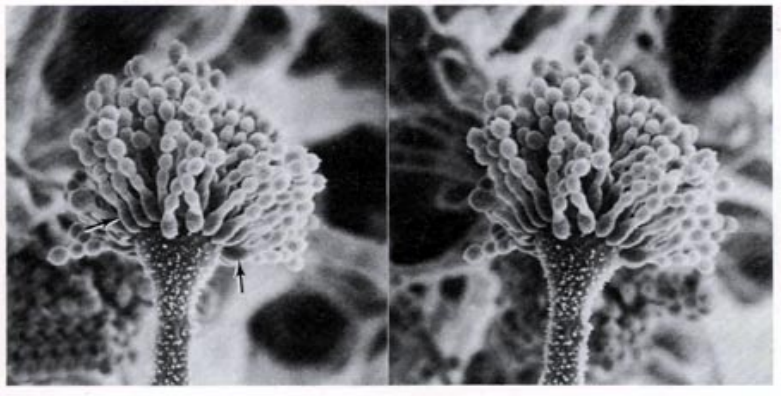

Figure 1: Conidiophores of Aspergillus flavus causal agent of aflatoxin production in Pistachio on orchards, (arrows). X 1000.

Symptoms on infected pistachio nuts

Collecting a representative sample of pistachio nuts for aflatoxin testing was particularly difficult because it has been established that the incidence of significantly contaminated nuts is usually very low, in the order of 1 nut in 10,000 to 1 nut in 30,000 (1). This means that even a 30 kg sample, as recommended by the European Union, may only contain a single contaminated nuts. The nuts are exported in a number of forms including: whole raw nuts for further processing; roasted and salted nuts with or without red. The shells of most pistachio nuts split naturally in the orchard prior to harvest. Fortunately, the hull covering the shell usually remains intact, protecting the kernel from invasion by moulds and insects (Fig. 2)).

a b

Figure 2: Pistachio nuts, open shells (a), kernels enclosed in brown seed coat (middle row, left) and shelled green pistachio kernels (middle row, right), infected nuts by *Aspergillus flavus* a main aflatoxin producer.

The major aflatoxin-producing fungi are *Aspergillus flavus* and *Aspergillus parasiticus*. These fungi grow on certain foodstuffs, most commonly ground nuts, dried fruit, walnuts and pistachios under favorable temperature and humidity conditions. Production of aflatoxin is optimal at relatively high temperatures, so contamination is most acute and widespread in warm, humid climates. Although contamination is generally considered to be a problem in tropical and subtropical regions of Africa, Asia and Latin America, aflatoxins have also been found in

temperate countries of Europe and North America (7).

In some countries the proportion of early splits is called an "early split" and can be as high as 30 percent. When early splits were examined in one study, about 20 percent of the samples were found to be contaminated with aflatoxin. On the other hand there was no contamination in nuts with hulls that remained intact in the orchard. Although the importance of early splitting for mould, aflatoxin and insect contamination is well established, very little is known concerning when early splits occur and become contaminated (15).This knowledge could aid in timing the application of either chemical or biological control treatments.

Early split nuts that are not infected in the orchard may become infected during transport and handling. Commonly, high humidity and high temperature within bulk bins provide ideal conditions for the infection of early split nuts. It can dramatically increase the incidence and aflatoxin contamination. By the way the nut is undoubtedly a serious problem for many producing countries since nuts are mycologically stabilized by drying or aflatoxin contamination of pistachio (6,15).

Prevention of mycotoxin contamination during storage is largely a matter of strict moisture control of the crops. There must be no insect activity, as insects can create favorable microclimates for toxigenic fungal growth; no moisture migration; no condensation or water leaks; and no rodent activity, as the moisture level could be increased by urination. In summary, conditions which restrict fungal growth will almost invariably limit or exclude mycotoxin production (16).

Aflatoxin contamination of pistachio nuts is undoubtedly a serious problem for many producing countries. The European Union's 1997 ban on pistachio imports from Iran because of high levels of aflatoxin in some shipments exemplifies the seriousness of this problem (11). This will not be possible without proper training of all agent in the chain: farmers, processors, warehouse keepers, traders and transporters. Food control officials and extension workers should be closely involved in this effort (1,14).

Saprophytic fungal yeast such as the, *P. anomala* modulate spore production of fungi including *A. flavus* on the pistachio nut-fruits. Field spraying of these effective yeasts to pistachio trees may decrease the population of *A. flavus* in the orchards and thus lower the number of nuts infected by this fungus. The outcome can be a reduction of aflatoxin contamination in the edible nuts. Monitoring *A. flavus* and *A. parasiticus* on pistachio buds may provide the growers a way to evaluate the population of the aflatoxin-producing fungi in orchards and help them make decision on control strategy.

On the regulatory side, efforts should be made to establish, as soon as possible, internationally agreed guideline levels for aflatoxin in pistachio nuts which would be accepted by all parties so as to eliminate possible technical barriers to trade. Such guideline levels should be based on sound scientific evidence and on realistic risk assessment. The recent JECFA work on this subject is a breakthrough and provides hope for a positive development in the near future.

Different varieties of pistachio trees are grown in Iran, Iraq and Afghanistan. Commonly, varieties are grown which tend to have large nuts with hulls which are relatively prone to early splitting, although climatic factors also have a bearing on this. In Turkey the pistachio varieties tend to yield smaller nuts with greener kernels and these have hulls which are not very susceptible to early splitting. The nuts are exported in a number of forms including: whole raw nuts for further processing; roasted and salted nuts with or without red staining; and kernels for the food manufacturing industry.

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تاثیر میزان آفلاتوکسین تولیدی توسط آسپرژیلوس فلاووس بر کاهش بازاریابی پسته در جهان

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چکیده

محصول پسته یکی از محصولات عمده کشاورزی در ایران به شمار می رود و بخش عمده صادرات غیر نفتی را به خود اختصاص می دهد . آفلاتوکسین به عنوان متابولیت ثانویه تولید شده توسط قارچ آسپرژیلوس فلاووس در پسته به عنوان یک سم بالقوه خطرناک در کاهش امکان رقابت بازاریابی پسته در جهان نقش دارد . بعضی از کشورها بخصوص آمریکا با مغتنم شمردن این وضعیت برای موقعیت خود در افزایش سهم صادرات پسته و کنار گذاشتن ایران تلاش می کنند . بنابراین ضرورت دارد تا ایران استانداردهای جهانی را مد نظر قرار داده؛ در صنعت فرآوری و تجارت پسته سرمایه گذاری کند .یکی از راههای مهم کاهش الودگی پسته به ا ین قارچ تولید کننده افلا تو کسین می باشد که علاوه بر تولید زهرابه زمینه را برای اقدامات سیا سی اقتصادی رقیبان فراهم می نماید. در این مطالعه موقعیت فعلی و اقدامات انجام گرفته توسط تولید کنندگان و صادرکنندگان عمده پسته ایران و دیگر کشورها بررسی و چندین راهکارهای پیشنهاد گردیده است .

کلمات کلیدی : پسته ، آفلاتوکسین ، آسپرژیلوس فلاووس ، صادرات

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