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ORIGINAL ARTICLE

An Overview of the Risks of Latent Aflatoxin Contamination in Pistachio Products (from Production to Export): A Comprehensive Analysis

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ABSTRACT: Aflatoxin contamination of products is a worldwide food safety concern and a major challenge in food safety and security, mainly produced by two closely related fungi, *Aspergillus flavus* and A. *parasiticus*. Despite receiving a health certificate at the origin, many export shipments are rejected at the destination due to aflatoxin contamination, which is a result of latent contamination by fungi. Research has shown that *Aspergillus* is soil-borne in pistachio cultivation areas, with its populations in the soil influenced by the irrigation cycle and time. As harvest timing nears, the population density of aflatoxin-producing fungi on pistachio fruits rises. Investigations into pistachio processing terminals have revealed that traditional terminals have higher contamination levels compared to semi-mechanized ones. Comparing various washing systems, it has been found that using a water shower system is more effective than ponds with static or flowing water in reducing contamination. Fungi spores on pistachio fruits during storage and transportation can lead to aflatoxin contamination, especially in improper storage conditions. This is particularly crucial during the sea transportation of exported pistachios, as high humidity can promote the growth of fungi spores. These instances highlight how even seemingly healthy pistachios can be affected by latent infections. The available information about the latent risks of aflatoxin contamination in different stages of pre-harvest, during harvesting, transportation, processing and its effect on the export and marketing of pistachio product is reviewed. Finally, new perspectives on how to successfully control contamination by implementing these strategies are explored.

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INTRODUCTION

Pistachio is one of the most important agricultural products of Iran, the export of which brings significant currency to the country. In the world pistachio production in 2022, the US ranks first with 400070 tons and Iran ranks second with 241668 tons of production [1]. In terms of cultivated area, Iran ranks first with 497484 hectares and US ranks second with 173207 hectares [1].

Aflatoxin contamination of crops is a worldwide food safety concern and poses a significant challenge in food safety and security as it affects both health of consumers and supply chains. Despite receiving a health certificate at the origin, many export shipments are rejected at the destination due to aflatoxin contamination, which is a result of latent contamination by fungi. One of the factors contributing to pistachio fruit contamination, especially post-processing, is the presence of *Aspergillus flavus* spores on the fruit's surface. Aflatoxins refer to a group of four mycotoxins (B_1, B_2, G_1 and G_2) produced primarily by two closely related fungi, *Aspergillus flavus* and *Aspergillus parasiticus*. Predictions associated with global warming suggest that A. flavus is likely to infect more crop plants, and will show increased expression of the aflatoxin biosynthetic genes, enhancing the risk of crop contamination by aflatoxin [2].

Aflatoxins are a group of small molecular weight fungal toxins that threaten world food safety by contaminating~25% of the world's crops [2, 3]. Aflatoxins as secondary metabolites are commonly produced in pistachios before the harvesting process and in the orchard and may enter the feed and food chain at any time point from pre-harvest to human consumption. These toxins are mutagenic, teratogenic, genotoxic, and carcinogenic under long-term exposure and affect the physiological processes of animals and humans disadvantageously [4 -6]. Consuming high doses of aflatoxin in a short period can cause acute aflatoxicosis, leading to death [7, 8]. Intake of low to moderate doses of aflatoxins over a prolonged period results in immunity suppression to all age groups, children's impaired growth and liver cancer [8, 9]. The contamination of pistachios with aflatoxin can prevent the producers from competing in the global market. It has been estimated that a pistachio with the aflatoxin concentration of 60000 ppb can infect as much as 4.5 kg of healthy pistachios [10 - 13]. Due to health impacts associated with aflatoxin contamination, countries have set standards and restrictions for importing food crops and animal feed, resulting in greater economic losses to farmers, transporters, and crop processors [8]. Strains of *A. flavus* show a great variation in their ability to produce aflatoxins. Toxigenic strains of *A. flavus* typically produce only two aflatoxins, B1 and B2, but most strains of A. parasiticus could produce all the four toxins [14].

Aflatoxin biosynthesis can be affected by various genetic and environmental factors. A positive regulatory gene, aflR, encoding a sequence-specific zincfinger DNA-binding protein, is required for transcriptional activation of most, if not all, of the aflatoxin structural genes [15]. Also, chromosomal location and some global transcription factors (such as those mediating nitrogen, carbon and pH regulation) affect expression of aflatoxin structural genes. Excluding these genetic factors, carbon and nitrogen sources, pH, temperature, water activity and plant metabolites also influence aflatoxin synthesis significantly [16].

The abundance of *A. niger* fungi in pistachio orchards is much higher than *A. flavus.* Moradi et al. and Mojthahedi et al. [17, 18] also proved this in their studies that the abundance of A. niger group fungi is significantly higher than *A. flavus* group fungi. . However, the presence of *A. flavus* fungi is of particular importance because they can produce aflatoxin.

The results of the research have shown that pistachio fruit contamination with fungi started from the orchard and can develop in the next stages despite suitable conditions. Although contamination with fungal spores is not the reason for the presence of aflatoxin in pistachios, it can be a potential cause of contamination in the next stages of transportation or storage. The difference in fungus spore density in different processing methods or different planting areas indicates which areas need more attention or which methods should be improved so that the microbial load of the product is low. Among Aflatoxins, aflatoxin B_1 is the most potent carcinogen present in nature and is produced mainly by the

ubiquitous soil filamentous fungus *Aspergillus flavus* [2, 19, 20].

Tajabadipur and Moradi [21] showed that range of total Aflatoxin (B_1, B_2, G_1, G_2) in shell is variable from 1.44 to 385.4 ng. kg $^{-1}$. At present, the maximum allowed levels for aflatoxin B1 and total aflatoxin are 1-20 ng g^{-1} and 0-35 ng g^{-1}, respectively. The Iranian Institute of Standard and Industrial Research has set the highest acceptable levels of aflatoxin B1 and total aflatoxin in pistachios at 5 and 15 ng g-1, respectively [10]. The European Union has a maximum level of 2 µg/kg for B1 and 4 µg/kg for total aflatoxins in crops [22].

Among the factors that can contribute to post-harvest contamination of pistachio fruit, it can be mentioned the temperature, relative humidity of the environment, and humidity of pistachio fruit during processing, storage, and transportation [23 - 25]. Any harvest delays, lack of proper processing, and improper storage conditions are likely to result in the increased fungal growth and aflatoxin production [10].

The spores in the pistachio processing terminal space showed that during the process of peeling the fruit, a large number of spores are released and spread in the terminal space. These spores can colonize in the processing residues [26, 18]. Jones et al. showed [27] that factors such as planting date, moisture stress, pest infestation, late-season rain, delay in harvest, and pericarp damage play a role in corn seed contamination by aflatoxin. Below, a series of studies related to latent contamination and its importance in the production and export of pistachios will be discussed.

Factors that affect the density of Aspergillus flavus spores in the orchard

Irrigation

Excessive irrigation of fruits is one of the factors that increase the risk of aflatoxin. In 2001, fluctuations in the population density of *A. flavus* in the soil of an orchard with periodic irrigation in Rafsanjan city showed that irrigation causes an increase in the population of the fungus periodically in the soil. However, the effects of irrigation systems and cycles on *A. flavus* populations in pistachio orchards are not known and need to be researched. For example, we can mention the effect of different irrigation systems and cycles on the changes *in A. flavus* population in plant residues and soil. In some leaf samplings, a relationship between irrigation cycle and the fluctuation of Aspergillus populations in the autumn season was observed. Considering that the amount of rainfall in the pistachio farming areas of Kerman province (Iran) is low in the autumn season, it seems that irrigation is the factor of providing moisture required for the colonization of plant residues.

Temperature

Michailides and Morgan [28] reported that the thermal requirement of a number of fungi indicates their seasonal abundance, and the density of *A. niger* inoculum in pistachio orchards increases during the summer season. The results show that the trend of spore density increases during the spring and summer seasons in pistachio orchards and decreases in autumn, which indicates the matching of pistachio fruit growth period and the increasing trend of spore density of the studied fungi. The most important factor in reducing the density of fungal spores during the autumn and winter seasons is temperature. The investigation of factors affecting corn contamination with aflatoxin showed that when the average minimum temperature is less than 16 ˚C, the conditions are not suitable for the spread of aflatoxin fungus, and in these conditions, aflatoxin is undetectable. The comparison of temperature fluctuations with spore density shows an important relationship between these two factors. It does not seem that the temperature factor plays a limiting role during the summer season due to the wide temperature range that is suitable for the growth of Aspergillus. Rather, it can be considered as a positive factor in pistachio fruit infection with Aspergillus [27, 29, 30]. Aspergillus flavus grows and produces mycotoxins at a wide range of temperature and water activity (aw), although the ideal conditions are 28 to 30°C, at high aw values [31, 32]. Temperatures around 30◦C, relative humidity between 80% and 85%, water activity between 0.81–0.99, soil pH at optimum range of 3 to 7 and other factors are ideal for Aspergillus to grow and produce aflatoxin [8]. Water activity is defined as the ratio of the vapor pressure of water in a material to the vapor pressure of pure water at the same temperature.

Since kernel moisture varies between 30 and 40% [29] and the temperature is suitable for contamination, *A. flavus* can successfully increase its population in competition with other microorganisms, which can act as a secondary inoculum. The effects of temperature and humidity of the environment on the growth and colonization of different substrates have been investigated by several researchers [27, 33- 39].

Soil plant residues, animal manures and fungal contamination

Plant residues play an important role in increasing the inoculum of *Aspergillus* in pistachio orchards. The spread of processing residues during the winter season causes the release of a large number of spores in the air, and when they are mixed with the surface soil, they cause an increase in the population of fungi, and these spores play the role of the primary inoculum in the following seasons. Different species of *Aspergillus* can colonize pistachio fruits that are dropped on the ground during spring and summer. Observations in the orchard did not show the surface colonization of the pistachio fruits dropped on the ground (with the naked eye), but in the moist culture environment in the laboratory, after a few days, their surface was colonized by different species of *Aspergillus* fungi, which indicates high contamination in these pistachios. The contamination of pistachios spilled on the ground is related to the irrigation cycle of plant residues and organic fertilizers in the orchard. Pistachios spilled on the ground, like cracked pistachios, act as sources of primary and secondary inoculum (in spring and summer) and play a role in the wintering of various species of *Aspergillus* fungi in pistachio orchards.

Leaves thrown on the ground during autumn and winter can be a suitable substrate for the growth and increase of *A. flavus* and *A. niger* population in pistachio orchards and act as one of the primary inoculum sources. Especially when they are mixed with surface soil during gardening operations and cause an increase in organic matter on the soil surface. The residues resulting from hull processing were heavily colonized by *A. flavus* and *niger* in the orchard, and their spore masses were easily visible to the naked eye. Doster and Michailides [40] while investigating the role of plant residues in pistachio fruit infection with *Aspergillus* fungus, reported that pistachio fruits on the tree and the ground may play a role in the wintering of *Aspergillus* species and inoculum production in the next season. They reported that *Aspergillus* species colonize pistachio plant residues such as pistachios that have fallen on the ground and tree blossoms, and the population of *Aspergillus* species in the orchards is related to them. The results of colonization of residues from processing and leaves showed that *Aspergillus* species can colonize these residues in the early stages, and irrigation may play an important role in the colonization of plant residues during the autumn season. The results showed that during the peeling of the fruit, the spores of *Aspergillus* fungi are released and they contaminate the processing residues, which develop in the next stages when they are transferred to the orchard. Observations in the orchard showed that the colonization of the mass of pistachio husk is local, so that most of the centers are free of different species of *Aspergillus* fungi, and around the mass, most of the colonies of *Aspergillus* fungi are observed. Among its reasons, we can point out high humidity and temperature and the presence of other microorganisms in the center of the mass of processing residues, which prevent the growth of *Aspergillus* fungi (*A. flavus* and *A. niger*). However, this issue needs further investigation. The role of plant residues in increasing the population of different species of Aspergillus and their wintering on different crops has been investigated by researchers.

Because all kinds of animal manures increase the organic matter in the soil, they can play a significant role in increasing the population of different species of Aspergillus fungi and cause them to overwinter and increase their population in the soil in the following seasons. The population density of *A. favus* and *A. niger* decreases in sheep, cow and chicken manure, respectively.

Gardening operations in the following seasons will release a large number of spores from the soil surface, so surface application of animal manures should be avoided. In an experiment, the effects of surface and deep application of poultry manure in an orchard in the winter and summer of 2001 on the changes in population density of *A. flavus* were investigated. The initial

contamination of the tested orchard soil and poultry manure in the winter season was 8.2×10^2 and 4.6×10^2 propagules per gram, respectively. In the summer season, propagule density in the deep fertilization method, without fertilization, and surface fertilization was 1.1×10^3, 9.7×10^2, and 1.5×10^3 per gram of soil, respectively. These results show the effect of surface application of animal fertilizers in increasing the population density of *A. flavus* fungi. The increase in the fungal population in the deep method and without fertilization treatments in the summer season may be related to the sampling time and the effect of the irrigation cycle. The higher population density of fungi in some soils is caused by the surface use of animal fertilizers. It is a bit difficult to compare the soil of different regions concerning different plant residues because there is no time limit for the colonization of plant residues. Also, other effective factors such as the composition of microorganisms, the specific climatic conditions of the sampling location and the volume of the pistachio mass can be mentioned.

Fruit skin traits and fungal contamination

The most important factor for the entry of aflatoxin producing fungi into pistachio fruit and their growth and development and ultimately the production of aflatoxin is the cracking of the outer skin of pistachio in the orchard [10]. Intact hull acts as a barrier against penetration of fungal spores and even some insects. If cracking occurs in the hull, fungal spores penetrate the pistachio fruits. In its natural state, the green skin protects the kernels as a physical barrier against external factors, especially fungi, but if the green skin is cracked and the kernel is exposed to air, it may lead to pistachio kernel rot and aflatoxin production. Pistachio fruit cracking is influenced by various factors such as irrigation, harvest time, bird damage, soil texture and structure, the balance of nutrients in the soil and several other factors that increase or decrease it [17, 18, 29, 41- 43].

The findings show that different species of *Aspergillus* fungi can grow on pistachio fruit kernels whose green skin cracks during the fruit development period. In some cases, the colonization of different species of *Aspergillus* fungi (spore masses) on the pistachio kernel was easily visible with the naked eye. The results show that cracked pistachio fruits are the sources of primary and secondary inoculum in pistachio orchards, which increase as the harvest time approaches. Cracked pistachios act as a source of fungal inoculum during the summer season, especially in processing terminals, which can play an important role in increasing the contamination of new pistachios. This can cause many problems in the next stages (storage and transfer). The role of cracked pistachios in the contamination of pistachio fruit with different types of *Aspergillus* fungi and mycotoxins caused by them has been investigated by several researchers [17, 29, 41, 42, 44, and 45]. Studies have indicated that pistachios with early splitting are likely to expose pistachio kernels to fungal spores with high levels of aflatoxin contamination. Early splitting is the most dangerous type of splitting where both green and shell (hard skin) crack at the same time, and the kernel is exposed to the influx of aflatoxin-producing fungi [10].

It seems that the humidity of the pistachio kernel and the temperature of the environment play a role in the successful competition of different species of *Aspergillus* fungi with other microorganisms. Doster and Michailides [29] reported that when the green husk of the pistachio fruit cracked, the moisture content of the pistachio fruit decreased, which may be beneficial for the development of different species of *Aspergillus* fungi.

Tillage and fungal contamination

Considering that *Aspergillus* species are native to pistachio cultivation areas and their spore density in orchards is greatly influenced by their population in the soil, any tillage operation in the orchard leads to an increase in spore density in the space. The results of the research conducted in this regard have shown that the passage of flocks of sheep in the fall and winter seasons in the pistachio orchards causes the production of dust and subsequently the number of spores in the air increases (Mojtahedi, unpublished). Spores of fungi can be carried everywhere by wind and air currents. With this transport process, spores begin to synthesize mycotoxins by reproducing and developing when suitable conditions occur [46].

The results showed that *Aspergillus* fungus is mainly soil-borne in pistachio farming areas and its population can be influenced by gardening operations, irrigation

cycles, plant residues and organic matter on the soil surface. For example, any type of operation that produces dust in the orchard plays a role in spreading fungal spores to the aerial parts of trees in the orchard. In an orchard in Zarand city, small pits with a depth of about 20 cm were dug and Petri dishes were placed upside down on the support. The results showed that by creating dust, a large number of spores are scattered in space. In this regard, the dispersion of spores in the dust created during the operation of soil disturbance by a disk in pistachio orchards, [40] a strong relationship between the contamination of fruit with *A. niger* fungus and dust on the leaves and an increase in the density of *A. flavus* spores in the pistachio orchards of Iran with the passage of sheep flocks has been reported (Mojtahedi, unpublished). Based on the research, tillage operations should be avoided as much as possible during the sensitive period of pistachio fruit infection with Aspergillus fungus (August and September) so as not to allow the release of spores and increase the population density of aflatoxin-producing fungi in the orchards. The comparison of pistachio fruits at a height of zero to one meter and more than one meter indicates that the population density of *A. flavus* and *A. niger* fungi in pistachio fruits in contact with the ground surface is higher than at a height of one meter. The reasons for this can be wounding of the pistachios in contact with the ground by the wind, the contact of these pistachios with irrigation water and the provision of necessary moisture at the junction of the green husk with the soil and their rotting, and the presence of dust on the surface of the pistachios in contact with the surface of the earth. The presence of spores of *Aspergillus* fungi in the dust caused by disking pistachio orchards in California [47], the relationship between fruit contamination and the presence of dust on the leaves [28] and the increase in the population density of *Aspergillus* fungi spores in pistachio orchards of Iran due to the passage of sheep flocks [38] have been previously reported, so pruning the branches in contact with the ground surface can play a role in reducing pistachio fruit contamination by different types of *Aspergillus* and aflatoxin.

Fruit harvesting and fungal contamination

Fruit harvesting is the most important part in the formation of aflatoxin. If aflatoxin has formed in the pistachios during the harvest period, it will be a critical control point to prevent the infected pistachios from contaminating the uninfected product [46]. The population density of *A. flavus* and *A. niger* on pistachio varieties increases as the harvest time approaches. Doster and Michailides [29] reported that the highest inoculum of *Aspergillus* fungi should be expected to be related to the harvest time. Delay in harvesting time increases contamination or spore density, which can play an important role in increasing the contaminating inoculum during the harvesting season. The reasons for this can be (a) the increase in abundance and contamination of cracked pistachios thrown on the ground and in contact with the ground, (b) longer exposure of the above pistachios to the spores in the orchards, and (c) spore density in orchards. The effects of harvesting time on the contamination of pistachio fruit and several other products have been investigated by different researchers, and the most inoculum was related to the harvesting time, and the delay in the harvesting time can increase the density of contamination, create new contamination and increase the amount of aflatoxin in the product. The delay in harvesting until the end of September and October, because fruits are exposed to the spores of *A. flavus* fungus for a longer time in addition to increasing the number of cracked pistachios with green skin, plays an important role in their contamination with aflatoxin [36, 48]. The delay in harvesting time also causes the damage of fruits by birds, pests, and the coloring of shell and finally the fall of the product, which plays an important role in increasing the density of spores in the orchards. In the USA, a 12-day delay in pistachio harvest increased aflatoxin contamination approximately 3-fold [46, 49]. Esmailpour et al. [50] reported that the delay in harvesting and peeling the fruit increases the percentage of pistachio staining, and the maximum stained pistachios are observed at the end of October with a 48-hour delay in peeling. Doster and Michailides [29] stated that the first cracked pistachios have more time for fungal contamination and growth. Jones et al. [27] stated that factors such as planting date, moisture stress, pest

infestation, delay in harvesting, late season rainfall and damage to the pericarp play a role in corn seed contamination with aflatoxin, and as the harvest time approaches, the inoculum population increases.

Pistachio processing terminals

The results show that although the conditions are suitable for aflatoxin contamination and production in the orchard, the processing and storage conditions are not suitable for recontamination and are affected by its sources in a pile of pistachios. The study of the population density of *A. flavus* and *A. niger* fungi shows that contamination in different terminals is affected by various factors such as the type of processing terminal (traditional or mechanized), the washing system and pistachios entering a terminal. The abundance of cracked pistachios, pistachios spilled on the ground, and infested pistachios in a pile of pistachios entering the processing terminal are influencing factors that increase the potential for contamination. Population density of *Aspergillus flavus* in pistachio fruit kernels in traditional and semi-mechanized terminals was studied in Semnan province. The pistachio kernel samples were cultured on AFPA. The results indicated that the population density of *A. flavus* varies with the type of terminals. Traditional terminals showed more population density of *A. flavus* than semi-mechanized terminals. [51] Among its reasons, we can mention the longer processing time of the pistachio fruit, the lower volume of water used for washing, the different washing system, and the greater possibility of mixing contaminated and healthy pistachios in traditional terminals. The results show that the population density of *A. flavus* and *A. niger* fungi is different among different semi-mechanized terminals and different traditional terminals. In this regard, the capacity of the terminal, the volume of transferred pistachio, the amount of water used for washing, the washing system, the percentage of cracked pistachios spilled on the ground and in contact with the ground play a role in increasing or decreasing the concentration of contamination in a terminal.

Comparing the population density of fungi on the pistachio fruit before and after processing shows that the efficiency of some terminals to provide a product without contamination is negative and their processing increases the contamination of the imported nuts compared to the exported ones. In other words, the index of contamination ratio of imported to exported nuts into the terminal is less than 1. In some terminals, the index is greater than 1, and they have reduced the contamination of imported pistachios.

The comparison of different washing systems shows that the best system to reduce fungal contamination is using a water shower. One of the reasons is the less mixing of pistachios together and the washing method, which reduces the transfer of contamination from contaminated to healthy pistachios. This system is influenced by the amount of water coming out of the showers, the angle of the showers to the transmission axis, the time of exposure of the pistachio fruits to the washing system, the volume of the incoming pistachios, and the number of showers. Anyway, one of the main disadvantages of this system is its inability to separate pistachios contaminated with aflatoxin from other pistachios. The water used for washing the fruit should be of potable quality and water analyses should be done regularly [46]. The water basin system is superior to the above system because it can partially separate pistachios contaminated with fungi and aflatoxin from healthy pistachios. But often, this system increases the contamination of outgoing pistachios compared to incoming pistachios. Among the factors that affect the basin washing system, we can mention the volume of water in the basin, the height or depth of the water, the flow rate of the water used, the amount of pistachios entering it, and how to separate the pistachios from each other. The combination of the above two systems is useful in creating a suitable system for washing and separating contaminated pistachios from healthy ones.

The drying process in pistachios is a critical quality control step in preventing the risk of aflatoxin. It seems that the conditions for further growth of fungi by latent contamination do not arise in the processed pistachios during drying in the field, but the contamination is influenced by the presence and abundance of its resources in the processed pistachio mass. Denizel [24] reported that initial storage, peeling, sun drying and mass storage of the product create many opportunities for fungal contamination. Although the humidity is reduced to about 10% under the conditions of sun drying, but this

process takes 3 to 4 days. Therefore, in the next stages (mass storage in the warehouse) suitable conditions for fungal contamination are created and if the pistachios are not completely dried, there will be enough opportunity for moisture migration and fungal growth. Also, in this stage and subsequent stages and during transportation, there will be more time for aflatoxin production. However, the results of the research show that in solar drying, suitable conditions for the subsequent growth of fungi and the production of aflatoxin will not be created, because in most terminals, a dehumidifier or dryer is also used, and the temperature in August and September in the field is not suitable for the subsequent growth of *A. flavus*. In the processing terminals, most of the contamination sources are separated from the pistachio mass. However, during transportation, if suitable conditions for fungi growth are provided, contamination can be caused due to the latent contamination of propagules of aflatoxin-producing fungi. For this reason, several export shipments may receive a health certificate at the origin, but be rejected at the destination due to aflatoxin contamination.

Shell characteristics can be used as a factor to the separation of contaminated pistachio and aflatoxin-contaminated pistachios. The results of the examination of processed pistachios in terms of contamination with different fungi of the *A. flavus* and *A. niger* groups and aflatoxin showed that there is a relationship between shell color and their contamination with fungi and aflatoxin [52]. The highest population density of fungi spores Group *A. flavus* and *A. niger* in to pistachio processing stages belonged to stained, yellowish, small and floated water pistachios [53]. The results also show that it is difficult to find a relationship between spotted and small pistachios in terms of fungal contamination. Because the contamination in spotted pistachios is high, while in small pistachios, the contamination is random, that may be high or undetectable. Aflatoxin levels were not detected in pistachio nuts with shells without color. Studies proved that the kernel of indehiscence pistachios can be contaminated with Aflatoxin. Aspergillus fungi may penetrate through the connection place of pistachio fruit to the axis [52]. The amount of aflatoxin in the kernel of Owhadi, Ahmadaghaei and Kalleh-gouchi cultivars was respectively 12, 25, and 21 times greater than the amount of Aflatoxin in their shell. However, the highest amount of fungi and aflatoxin contamination in processed pistachios was related to spotted pistachios. In yellow pistachios, the population density of *A. flavus* and *A. niger* fungi was different, so that in some samples, high density was detected and, in some others, it was not detected. However, the amount of aflatoxin in these samples was high. The results of the population density of *A. flavus* and *A. niger* fungi and aflatoxin in small pistachios also showed that in some samples the contamination is high and in some others it is low. So the color or spots of the fruit shell can be used as a factor to separate infected pistachios from healthy ones. Improper storage of the crops, nuts, and grains further contributes to increased levels of aflatoxins [2]. The storage should be in a cool and dry place. If the warehouse environment is not adequately ventilated, storage areas should be ventilated naturally or mechanically, and appropriate ventilation systems should be installed in the warehouse [46].

Aflatoxin decontamination

Prevention methods, including good agricultural and manufacturing practices (like deep plowing and grain sorting) and appropriate storage conditions (cold, dry environment), are regarded as the best choices to reduce the aflatoxin contamination in pre- and postharvest stages. However, prevention methods are not always possible [54].

Aflatoxins are very stable and do not decompose quickly. Therefore, preventing the growth of aflatoxin-producer fungi and detoxification of contaminated crops is essential [55, 56].

Chemical (eg, fungicides) and physical (eg, ionizing radiation, mechanical separation, thermal inactivation) detoxification methods may be used to reduce or eliminate aflatoxins. Food irradiation is a physical method that is performed at the lowest temperature, unlike thermal pasteurization, food safety can be improved by irradiation, and in most cases, the shelf life of the product is maintained without significant changes in its nutritional, chemical and physical properties [10]. Biological control appears to be the most promising approach for control of aflatoxin in both pre- and post-harvested crops. Different organisms, including bacteria,

yeasts and nontoxigenic Aspergillus fungi, have been tested for their ability in controlling aflatoxin contamination. Great successes in reducing aflatoxin contamination have been achieved by application of nontoxigenic strains of *A. flavus* and *A. parasiticus* in the fields of cotton, peanut, maize and pistachio. The nontoxigenic strains applied to soil occupy the same niches as the naturally occurring toxigenic strains. Therefore, they can compete and displace toxigenic strains [57]. In many field experiments, particularly with peanut and cotton, significant reductions in aflatoxin contamination in the range of 70%~90% have been observed consistently using nontoxigenic Aspergillus strains [14, 57, 58]. This strategy is based on applying nontoxigenic strains to competitively exclude naturally toxigenic strains in the same niche and compete for crop substrates.

CONCLUSIONS

Aflatoxin contamination of products is a worldwide food safety concern and a major challenge in food safety and security, mainly produced by two closely related fungi, *Aspergillus flavus* and *A. parasiticus*. Despite receiving a health certificate at the origin, many export shipments are rejected at the destination due to aflatoxin contamination, which is a result of latent contamination by fungi. Fungi spores on pistachio fruits during storage and transportation can lead to aflatoxin contamination, especially in improper storage conditions. This is particularly crucial during the sea transportation of exported pistachios, as high humidity can promote the growth of fungi spores. These instances highlight how even seemingly healthy pistachios can be affected by latent infections. Undoubtedly, the reduction in the population of aflatoxin-producing fungus spores will reduce aflatoxin contamination during transportation. Any operation that reduces latent contamination will decrease aflatoxin level during transportation and export of pistachios, especially when exporting by sea or in ports with relative humidity above 85%. An investigation into weather conditions along the route of transporting and exporting pistachios revealed that in Bandar Abbas (the departure point for pistachio shipments from Iran), the port of Dubai, the route way to the Indian Ocean, and the port of Hamburg (an important destination for pistachio exports to Europe), the relative humidity and temperature increase the growth of aflatoxin-producing fungi all year round. Therefore, the presence of spores in these export shipments leads to initial fungal growth and aflatoxin production. On the other hand, the complete removal of spores from the export shipments and the environment is challenging. Therefore, it is recommended to use moisture and oxygen impermeable covers when transporting pistachios by sea to minimize contamination. Many organisms have been investigated for their potentials in the reduction of aflatoxin contamination of crops. The most successful biological control approach to date is the application of nontoxigenic strains of *A. flavus* and *A. parasiticus* to soils where they competitively exclude naturally toxigenic strains. Combinations of physical and biological (natural) methods are expected to improve aflatoxin decontamination efficiency, both pre- and post-harvest.

CONFLICT OF INTERESTS

NO conflict.

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