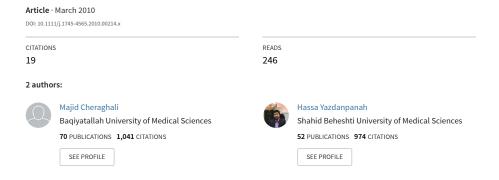
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# Interventions to control aflatoxin contamination in pistachio nuts: Iran experience



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## INTERVENTIONS TO CONTROL AFLATOXIN CONTAMINATION IN PISTACHIO NUTS: IRAN EXPERIENCE

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> > Accepted for Publication May 13, 2009

### **ABSTRACT**

Pistachio nut is an important agricultural and economical commodity in Iran. Annually, several hundred tons of pistachio nuts are produced in Iran. Therefore, the country earns substantial amount of hard currency from exporting pistachio nuts. In addition, livelihood of some million people in pistachiogrowing area depends on this crop. Traditionally, the European Union (EU) has been one of the major destinations of pistachio exported from Iran. Pistachio nuts are rich sources of fat, and contain linoleic and linolenic fatty acids. However, they are among the commodities with the highest risk of aflatoxins (AF) contamination. As a consequence of detection of unacceptable level of AF in pistachio consignments arriving in EU ports in 1997, special conditions were imposed on Iran pistachio entering the EU. Therefore, extensive efforts were made to overcome the problem. Iranian authorities in collaboration with EU authorities planned a multi-approach intervention to prevent and control AF contamination of Iran pistachio nuts. Iran experience to prevent and control AF contamination in pistachio nuts was fruitful, and Iran pistachio nuts now have significantly lower level of AF contamination. In addition to economic aspects, these improvements have greatly contributed to protection of both local and international consumers from possible health hazard caused by AF contamination.

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#### PRACTICAL APPLICATIONS

Despite the nutritional value of pistachio nuts, their susceptibility to aflatoxins (AF) contamination is a potential health hazard. This might cause substantial economical losses to the producers as well. In the past decade, Iran, as the largest pistachio producer in the world, has implemented effective interventions to control AF in pistachios. Implemented interventions such as establishing an efficient decision-making system, focusing on preventive methods, applying Hazard Analysis and Critical Control Point, good agricultural practice and good storage practice guidelines, and using accurate and sensitive sampling and analytical methods proved to be effective. As a consequence, in regard to AF contamination, Iran pistachio nut is now substantially safer. Obviously, this has contributed to the protection of both local and international consumers from possible health hazard caused by AF contamination. From the economical point of view, increasing volume of consumable pistachio nuts will also improve the country's earning from pistachio trade without culturing more orchards.

#### INTRODUCTION

Pistachio nuts as an important member of tree nuts crop are cultivated in several countries including Iran, USA, Turkey, Greece, Syria and China. Pistachio nuts are rich in fat, and contain linoleic and linolenic fatty acids essential for the human diet. On the other hand, pistachio nuts are among the commodities with the highest risk of aflatoxins (AF) contamination (Pittet 1998). The incidence of AF contamination in tree nuts is low, and their levels are quite variable. High levels of contamination may develop in a small percentage of nuts (Schatzki 1995). AF are known to be mutagenic, teratogenic, carcinogenic and immunosuppressive compounds. Because of known health hazard of AF to humans, many of the national and international competent authorities have established control measures and set maximum tolerable levels for AF in food and feed. In Iran, the maximum acceptable levels for AFB1 and total AF in pistachio nuts are 5 and 15 µg/kg, respectively (ISIRI 2002). Codex Alimentarius Commission in its 31st session agreed on maximum tolerable level (MTL) of 10 and 15 µg/kg for total AF in almonds, hazelnuts and pistachios "ready-to-eat" and for further processing, respectively (FAO and WHO 2008).

In addition to health risks to consumers, contamination of foods with AF may create significant direct and indirect economic consequences both for producer and consumer countries. The cost of rejected consignments at the port of entries is one of the direct economic impacts on producer countries.

Although the exact figures on pistachio nuts affected by AF and its impact on trade are not collected in a regular and systemic way in Iran or around the world, estimated numbers predict a very high cost to the producers.

In 1971, for the first time, the U.S. Food and Drug Administration (FDA) reported AF contamination in pistachio nut consignments arriving into the U.S.A. A more systematic testing on consignments arriving from Iran and Turkey led to rejection of 36 consignments out of total of 48 tested. In past decades, health authority of European Union (EU) has also observed AF contamination in pistachio nuts arriving at EU entry ports, and in 1997 the community imposed a temporary ban on importation of pistachio nuts from Iran for 2 months (Commission Decision 1997a,b). This was a precautionary measure following report of findings by Dutch authorities indicating that substantial numbers of pistachio nut consignments from Iran were contaminated with unacceptable levels of AF. Subsequent analyses showed that some of the consignments contained AF ranging from 11 to 400 µg/kg (MAFF 1997). Although ban on Iran pistachio nuts was removed later, based on 1997 decision of European Commission (EC), special condition imposed on Iran pistachio nuts entering EU (Commission Decision 1997b). This decision was also re-stated again in 2000 (Commission Decision 2000), 2003 (Commission Decision 2003), 2005 (Commission Decision 2005) and 2006 (Commission Decision 2006). According to the decision "the competent authorities in each EU member states shall take a sample for analysis from each consignment of pistachio nuts, and certain products derived from pistachio nuts originating in or consigned from Iran for analysis of AFB1 and total AF before release onto the market from the point of entry into the community" (Commission Decision 1997a).

Similar contaminations were also found in pistachio nut consignments from Turkey, and EU imposed testing conditions for pistachio nut consignments originating from Turkey (Commission Decision 2002). Despite the fact that EU traditionally has been the major market for Iran pistachio, following EC stringent regulatory measures imposed on Iran pistachio nuts, the importation of Iran pistachio nuts into EU has been substantially decreased (Fig. 1). Substantial decrease in exportation of Iran pistachio nuts into Europe in the past decade reduced the country's earning from pistachio nuts. Consequently, the livelihood of considerable numbers of people including farmers has been negatively affected.

The economic impact of AF contamination is not limited to crop losses. Additional costs are derived from supplementary efforts by the producers, distributors and national authorities to counteract their initial losses, the cost of samples for analysis and sophisticated analytical equipments and reagents for AF analysis, and most importantly consumer confidence loss. Because of heterogeneous distribution of AF in pistachio nuts, all sampling plans

Pistachio export to EU

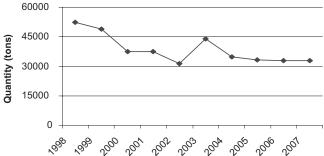


FIG. 1. QUANTITY OF EXPORTED IRAN PISTACHIO INTO THE EUROPEAN UNION (DATA FROM: HTTP://FAOSTAT.FAO.ORG/)

including EU method require substantial amount of samples, and this again will add to economic loss.

Because of susceptibility of pistachio nuts to AF contamination, in order to protect both consumers' health and producer benefits, it is important to implement interventions which could reduce AF contamination to as low as practically feasible. Failure to do this may create devastating economic consequences to producers, and also deprive consumers from a very valuable source of nutrient and pleasure. To overcome this problem, in past decades intensive researches on mycotoxins were initiated in research centers in Iran. Investigations revealed that contamination to mycotoxins starts when pistachio nuts are still on the trees, after ripening, but before harvesting. It was also found that AF contamination in pistachio nuts may not be solely from improper shipping and storage (Dikens and Welty 1975; Commission Decision 2002). Therefore, it is expected that preventive methods would be the most cost-effective approaches to curb AF contamination in pistachio nuts.

FDA investigations showed that yearly variation in AF contamination is directly related to the amount of rainfall during period of ripening to harvesting of the nuts. It is during this crucial period that AF contamination of the pistachio nuts occurs (Mojtahedi et al. 1978; Danesh et al. 1979).

More than 85% of ripe Iran pistachio nuts have outer splits which allow the entry of airborne spores of AF-producing fungi. However, only during rainfall, the relative humidity rises sufficiently to allow Aspergillus flavus spores to germinate and produce AF. This may also happen during storage or shipping. It has been reported that 82% relative humidity is the minimum humidity required for production of AF by A. flavus (Denizel et al. 1976). This is well above the maximum relative humidity (62%) recorded for any month in

the pistachio nuts growing area in Iran, except when it rains. In Iran, picked pistachio nuts are dried using either sunshine or dryer equipments. This needs 48 h of sunshine for drying and might be increased to 120 h during rainy period and would favor fungal spore germination. Rainy period during harvesting might also drastically increase population of insects that damage pistachio nuts (Danesh et al. 1979). It has been reported that the extent of AF contamination in pistachio nuts was affected more by the amount of precipitation during the post-ripening and postharvesting period than by storage and shipment conditions. It was reported that AF contamination, and storage and shipping time were not positively correlated (Danesh et al. 1979). Some published reports indicated that AF contamination of Iran pistachio nuts was more correlated with rainfall after ripening and before harvest time (Mojtahedi et al. 1978; Danesh et al. 1979). Therefore, it is clear that only combined interventions which include farming, harvesting, processing, storage and shipping, and possibly decontamination procedures might be able to curb AF contamination in pistachio nuts (Boutrif 1998; Olsen 1999).

#### CHRONOLOGICAL BACKGROUND

Following importation ban on Iran pistachio nuts into EU in 1997 (Commission Decision 1997a), Iran intensified its activities on improving hygienic condition of pistachio nuts production, processing, packaging and shipping. From 1997 to 2005, several expert teams of EC visited Iran, and in their reports provided recommendations for improving the situation. The first EC expert team visited Iran in October 1997 to inspect the hygienic conditions of the production of pistachio nuts. The report indicated that pistachio nuts production should be subjected to enhanced hygienic conditions. The main EC recommendations were as following (Report of EC Mission 1997):

- (1) Emphasizes on traceability of the primary production;
- (2) Avoid use of poultry manure and hulls in the orchards;
- (3) Avoid use of recycled water in the pistachio nut processing plants;
- (4) Elimination of "early splits" pistachio nuts by means of physical sorting;
- (5) Providing hygienic and controlled conditions (humidity and temperature) for drying of pistachio nuts;
- (6) Hygienic and controlled conditions for storage of processed pistachio nuts;
- (7) Good hygienic conditions for transport of the processed pistachio nuts;
- (8) Improve sampling procedure and test protocols for AF analysis;
- (9) National Food and Drug Control Laboratories (FDCL) of Ministry of Health (MOH) should take responsibility in sampling, analyzing and issuing health certificates for all pistachio consignments exported to EU.

Following these recommendations and in order to improve pistachio nuts production and its safety, "Pistachio Office" of the Ministry of Jihad-e-Agriculture (MOA) was established. This office in collaboration with other parties including producers' associations and MOH implemented following activities during 1998–2002 (Report of Pistachio Office 2001; Report of Iran Mission 2002):

- (1) Establishing "National Pistachio Committee" comprising of all stakeholders and decision makers from different sections of government and private sectors. Delegates from different organizations including the MOH, Customs, Ministry of Foreign Affairs, Institute of Standard and Industrial Research of Iran, Ministry of Commerce and producers participated in the debates and decisions;
- (2) Training workshops for local MOA experts in order to update their information on latest methods of good agricultural practice (GAP) for pistachio nuts;
- (3) Training workshops and seminars for pistachio nut producers and processors on GAP and good storage practice (GSP);
- (4) Preparing printed materials on different aspects of GAP including gardening, soil fortification, disease control, harvesting, processing and storage of pistachio nuts for distribution among producers;
- (5) Using local TV and radio stations to convey GAP methods to the farmers:
- (6) Investment on developing new processing facilities for freshly harvested pistachio nuts in order to facilitate mechanization of the processing;
- (7) Promoting use of fresh water for washing pistachio nuts in processing plants;
- (8) Use of "dig in" method instead of surface spreading of the fertilizers;
- (9) Promoting the use of cotton sheets under trees during the harvesting in order to avoid soil contact with fresh pistachio nuts;
- (10) Promoting timely harvesting of the pistachio nuts in order to avoid "early split" phenomena;
- (11) Using EU sampling procedure for sampling of pistachio consignments;
- (12) Establishing a unified validated HPLC method (AOAC 999.07) for analysis of AF in pistachio nuts by FDCL and its affiliated laboratories;
- (13) Designing joint research projects between Iranian and European researchers, to investigate possible effect of shipping conditions on increasing levels of AF in pistachio nut consignments during transportation.

The Commission Decision 97/613/EC regarding ban on Iran pistachio nuts was replaced by Commission Decision 97/830/EC of December 1997 (Commission Decision 1997b). This decision lifted the ban on importation,

but imposed special condition on the import of pistachio nuts and certain products derived from pistachio nuts originating in or consigned from Iran. According to this decision, all pistachio nut consignments from Iran should be tested both by Iran FDCL before exportation and on arrival to the ports of entry in EU. In October 1998, a second team of experts from EC visited Iran in order to assess the progress made by Iran government (Report of EC mission 1998). The team recognized substantial improvements and also provided some new recommendations. The team recognized that "the authorities of Iran made considerable efforts to increase hygienic quality of pistachio nuts." MOA was very much involved in three main topics including crop mechanization, horticulture and postharvest. The "National Pistachio Committee" in MOA had the mandate to resolve difficulties, coordination of training and information to all socioeconomic parties, including information and education through the media and a profound analysis of recommendations. The involvement of all ministries in the committee stimulates the socioeconomic operators to increase the quality of the products, and all pistachio nut producers and operators are under control of the authorities." The team also reported that following these improvements, fewer pistachio nut consignments from Iran were rejected at EU borders.

However, the EC mission team has concluded that improvements were not satisfactory enough to lift special conditions on Iran pistachio nuts, and recommended new actions to Iran. The most important of those were focused on traceability of pistachio nuts, improving hygienic conditions from production to the exit port and use of uniform sampling and analytical methods (Report of EC mission 1998).

The third EC mission to Iran took place in February 2001. The objectives of the mission were to verify whether the facilities and measures were in place to control AF contamination in foodstuffs. With regard to pistachio nuts, an assessment was carried out that public AF exposure from the consumption of these foodstuffs originated in Iran was within specified EU limits, and compiled with the Commission Decision 97/830/EC. The main conclusions and findings of EC mission team were as follows (Report of EC mission 2001):

- (1) There is no single competent authority with overall responsibility for AF contamination in pistachio nuts. However, The Pistachio Committee provides a good structure for decision making and communication.
- (2) The sampling method of pistachio nuts intended for export into the EU was in accordance with Commission Directive 98/53/EC.
- (3) All of the visited laboratories in MOH, except one, with some identified minor improvements, were adequate to undertake routine AF analysis for the purpose of export.

- (4) There are no formalized procedures for the follow-up of nonconforming lots.
- (5) The warehouse for intermediate storage of pistachio nut consignments is well structured.
- (6) An increase in the number of fully equipped processing plants and investment in modern processing machinery was noted.

The general conclusion of the report was that "the Iranian authorities have the will to initiate change in relation to AF contamination, and there are a number of steps being implemented. However, these are generally not completed and require greater coordination and leadership, especially in laboratory analysis issue. On the other hand, it is unlikely that any deficiencies noted in sampling and methods of analysis would explain the differences between the results of analysis between the Iranian labs and those of laboratories in EU. There is inadequate evidence to identify whether the contamination occurs during the export process in Iran, the sea transport or storage at a European port" (Report of EC mission 2001).

The EC mission team recommended that the competent authorities in Iran should address the shortcomings and deficiencies in order to improve the official control system of the foodstuffs concerned intended for export into the EU. The report also recommended to the EC to continue with the requirement for AF certification of Iran pistachio nut consignments. Given the possibility of AF contamination during export or transport, AF analysis should also be continued to be undertaken by the importing member states (Report of EC mission 2001).

Following these recommendations and in order to improve the situation, the Iranian authorities designated FDCL as the supervising and coordinating laboratory. FDCL implemented a unified method of analysis in all laboratories under its supervision. In order to determine the capabilities of the laboratories involved in AF analysis of pistachio nuts, FDCL has made the necessary arrangements for participation of its all three labs in international proficiency tests performed by CSL in UK (FAPAS). FDCL also made arrangements to play the role of reference laboratory in AF analysis. In this regard, for the purpose of performing national proficiency tests and controlling the results of pistachio nut analysis in FDCL and in its satellite laboratories, FDCL sends unknown samples every 2 months to those labs. It was formulated that those labs send some of routine analyzed pistachio nut samples along with the test results to the reference lab.

The next mission of EC was undertaken as part of the SPS-related technical assistance program in March 2003. The objectives of the mission were to assess the situation as found, to offer advice and to report the necessary modifications and improvements. The main conclusions and findings of the mission team were as follows (Report of SPS 2003):

- (1) Participation of FDCL in FAPAS which started in 2001 up to January 2003 showed that AF analysis in FDCL even at the lowest level of contamination is very good.
- (2) Iran MOA is very active in communicating with farmers. To stimulate this, in every village a representative of the MOA is responsible for this task.
- (3) The Iranian Pistachio Research Institute (PRI) has many projects running on mold prevention and control in the orchards. Nevertheless, these efforts have never been combined in an attempt to solve the problem together. It is necessary that an organization at the national level takes the responsibility of coordinating research in different aspects.
- (4) Combining the information of this visit and the 2001 Food and Veterinary Office mission leads to the conclusion that AF sampling and analysis are not differing between Iran and EU. Therefore, attention should be focused on the point that AF can be present in Iran pistachio nut lots in EU, as well at arrival as at a later stage during merchandizing. This phenomenon can only be explained if spores or molds are present in the lot, which can grow at a later stage, which in turn leads to AF production. To handle this at short term, it is advised as to change transport and storage conditions.
- (5) Research on AF prevention in orchard, during harvest and storage, needs to be continued, to minimize rejected lots as much as possible. Considering the number of rejections as released by the FDCL, any positive result of this type of research will increase the amount of consumable pistachio nuts without cultivation of more orchards.

The Iranian authorities on their ongoing efforts to improve the situation started new initiatives in 2003. These activities were mainly focused on improving the horticulture condition and prevention, and were as following:

- (1) MOA in collaboration with MOH prepared the Hazard Analysis and Critical Control Point (HACCP) guidelines for production and processing of pistachio nuts, and communicated these guidelines to the interested parties for implementation.
- (2) MOH designed a project for national survey and management of mycotoxins in foodstuffs. In early 2003, the Food and Agriculture Organization (FAO) approved a joint project proposed by MOA and MOH on "Control, Management and Analysis of Mycotoxins in Food and Feed in Iran." This project is mainly designed to evaluate the effect of implementing GAP and HACCP principles on reduction of mycotoxin contamination in food including AF in pistachio nuts.

The EC expert team on its mission to Iran in November 2005 to assess the facilities and measures in place for the control of AF contamination in pistachio concluded that "a good legal framework is in place for controls on

pistachio exports to the EU. Research on the subject of AF contamination in pistachios has revealed a number of measures, which were demonstrated to be efficient to reduce or eliminate the problem. Producing and processing companies visited had implemented these measures to various extents with good results. However, competent authorities do not enforce GAP and GMP principles systematically in this industry, and Rapid Alert System for Food and Feed (RASFF) notifications are not followed up at the exporters concerned. Overall, there is a good framework control system in place for exporting pistachio nuts to the EU." The mission of 2005 also had some recommendations for improving the situation. The most important of those were the following (Report of EC mission 2005):

- (1) The competent authorities should ensure the application of GAP and GMP principles identified in Iran to be effective to avoid or reduce AF contamination in pistachio nuts by all operators concerned as soon as possible.
- (2) The competent authorities should continue the HACCP project in 2006, and widen the project's scope in so far as it should address small-scale farmers and processors.

In order to further improve the situation, Iranian authorities together with private sectors also designed and implemented several research projects to examine the effect of different conditions on reduction of AF contamination in pistachio nuts. One of these projects is called the Green Corridor Project (GCP). This project is a joint and ongoing project between Iran and International Nut Council designed to restore confidence in Iranian pistachio nuts. The project was also evaluated by the EC team in its last visit to Iran. The mission reported that "GCP is an initiative setup in 2004 by private entities and companies in order to establish GAP and GMP throughout the production chain of pistachio nuts, and to reduce the rejection rate of pistachio nut consignments at EU borders. In order to become an approved participant in the project, farmers and processors have to fulfill certain prerequisites regarding GAP and GMP. Project participants are obliged to follow the SOPs, monitored by resident GCP inspectors."

#### RESULTS AND DISCUSSION

After the first report of the presence of AF contamination in Iran pistachio nuts by the FDA in 1971, Iranian authorities undertook several actions to control this contamination. These activities were mainly based on results of research projects designed to find critical points in preventing AF contamination. However, with increasing incidence of contamination in pistachio nut

consignments imported to EU, in 1997 the community imposed a temporary ban on export of Iran pistachio nuts into Europe. The ban was later changed to a "special condition" requesting testing of all consignments both on exporting and importing ports. Pistachio nut is a valuable commodity for Iran, and the country earns substantial amount of hard currency from exporting pistachio nuts. In addition, the livelihood of many people in pistachio nuts growing area (mainly central parts of Iran) depends on this crop. Therefore, extensive efforts were made by the government of Iran and private sector to overcome the problem. EC expert teams also made valuable contribution to these activities through providing technical expertise and assistance. The performed interventions could be categorized as follows:

- (1) Structure an efficient decision-making system. It was clear that because of the involvement of several governmental and private organizations such as MOA, Ministry of Commerce, Customs, Ministry of Industry and private sectors in pistachio nut affairs, uncoordinated efforts will not do any good. Therefore, a national committee comprising representatives of all stakeholders was set up in MOA. The committee also involved representatives from the MOH and Ministry of Foreign Affairs. In order to increase its authority, the committee later changed to "Pistachio High Council," which comprises several ministers involved in the production and trade of pistachios.
- (2) Focus on prevention. It was clear that most of the contaminations start on the orchards. Therefore, major activities were focused on contamination prevention especially at the farm. This was managed by promoting GAP principles in the orchards, and HACCP, GSP and GMP principles in storage and processing plants. Although the critical role of these regulations in improving the situation is obvious, the actual effect of these activities on reduction of contamination needs to be scientifically evaluated.
- (3) Apply quality assurance in sampling and analyzing AF in pistachio nut consignments. Laboratories in MOH followed very strict guidelines on analyzing the samples, and this provided very reliable test results. Results from participation of these labs in international proficiency testing scheme such as FAPAS proved their accuracy.
- (4) Improve storage and shipment conditions. Processing and storage sites were regularly inspected by MOH inspectors, and their compliance with regulations of GSP were reported. Measures were taken to reduce shipment period to destination ports to as short as possible.

These coordinated activities have drastically improved the situation. Published data regarding the incidence of AF contamination in Iran pistachio nut consignments intended for exportation to the EC showed an acceptable level of

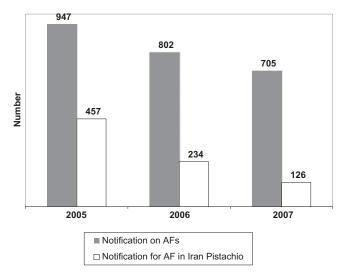


FIG. 2. EUROPEAN COMMISSION RAPID ALERT SYSTEM FOR FOOD AND FEED NOTIFICATIONS ON AFLATOXINS FOR 2005–2007 (DATA FROM: HTTP://EC.EUROPA.EU/FOOD/FOOD/RAPIDALERT/)

contamination in majority of the samples. According to the reported data, among 10,068 samples analyzed, the total AF was detected in 2,852 samples (28.3% of the total) with the mean and median of 7.3 and 0.4 ng/g, respectively. The mean contamination level (7.3 ng/g) was lower than the MTL of AF in pistachio nuts in Iran (15 ng/g), as well as lower than the level of Codex Alimentarius Commission for AF (10 ng/g) (Cheraghali *et al.* 2007).

In recent years, the EC has established RASFF in order to notify consumers and member states of any possible contamination found in food consignments entering the community. A substantial number of these notifications are concerned with AF in food commodities. The recent statistics published by the EC regarding RASFF for AF contamination in Iran pistachio nuts confirms a significant reduction in AF contamination in pistachio nuts imported from Iran (Fig. 2). According to annual reports of RASFF, while in 2005 there were 457 (48%) notifications about pistachio nuts from Iran, the figure has reduced to 234 (29%) in 2006, and 126 (18%) notifications in 2007. But, the quantity of imported pistachio nuts in the period 2005–2007 remained constant (approximately 33,000 tons/year). This means that the level of AF contamination in pistachio nuts imported from Iran is improving. This is also reflected in the rejection rates observed. While in 2005, approximately 25% of all consignments offered for import in the EU was found to be noncompliant with EU AF contamination level, this rate was decreased to approximately 10% in 2007

(RASFF 2005). This a clear indication of the effectiveness of measures implemented by Iranian authorities to control AF contamination in Iran pistachio nuts.

Although preharvest strategies have shown the best effects on reduction of AF contamination in food commodities, there are also effective strategies to minimize AF in the food chain (Magan and Aldred 2007). Despite many improvements regarding control of AF contamination in Iran pistachio nuts, it seems that reaching zero AF contamination in pistachio nuts would both be unnecessary and impractical, and a low level of contamination should always be expected in raw pistachio nuts. Therefore, it is important that by means of processing before consumption further reduce any residual risks to the customers. It has been reported that ozonation or even a simple roasting of pistachio nuts will greatly reduce AF contamination in pistachio nuts (Yazdanpanah *et al.* 2005; Akbas and Ozdemir 2006).

However, despite extensive efforts and significant improvements, AF contamination in pistachio nuts is still a matter of concern for both producers and consumers. It appears that the following facts are contributed to this:

- (1) Although the Pistachio High Council has played valuable coordinated role in harmonizing decisions and activities, it needs more executive power to implant its decisions. It seems that some of the public and private parties do not implement council decisions if there is any conflict with their interests. Therefore, it is recommended that an independent and competent authority should supervise all activities related to the pistachio nuts from production to trade.
- (2) The fact that Iranian laboratories using very accurate and reliable sampling and testing methods suggest that discrepancies between lab results in Iran and EU would possibly originate from a postshipping contamination. This only might be possible if pistachio nuts harboring *Aspergillus* spores and desirable conditions during shipping allow spores to germinate and produce AF. Therefore, most of the activities should be focused on improving farming condition to prevent contamination, and there are still lots of rooms for activities in this section. Recently, some investigators have examined measures to increase natural products with antioxidant properties in tree nuts, which might thereby reduce or eliminate the ability of *A. flavus* to biosynthesize AF (Molyneux *et al.* 2007). In addition, a rapid and functional cleanup method for the elimination of AF-producing fungus from shelled and unshelled nuts, including pistachio nuts, has been investigated as a suitable fungal decontamination method (Basaran *et al.* 2008).
- (3) The fact that cultivation of pistachio in Iran is mainly carried on by a large number of farmers with small orchards makes implementation of corrective interventions cumbersome. Pistachio nut trees are part of people's life in pistachio nuts growing area, and every family has few numbers of trees

- in its small garden or backyard. Therefore, it would be very difficult to train these huge numbers of farmers, and supervise them for implementing GAP principles.
- (4) Because of difficulties mentioned above, it is not always practical to track a contaminated consignment to its origin in order to fix the problem. Each consignment might comprise of crops of several small gardens which makes traceability very difficult.
- (5) Because of the importance of research on pistachio, in 1960 two departments of plant protection and horticulture started research projects on Iran pistachio nuts in Rafsanjan City, the main pistachio-growing area in Iran. Later, in 1993, the PRI was established by combining these departments with four new departments. PRI is currently undertaking several research projects on different aspects of pistachio nuts, including production and processing, horticulture, irrigation, plant nutrition and plant biotechnology and breeding (http://www.pri.ir). However, the fact that Iran is the number one pistachio nut-producing country in the world provides more room for research projects on different aspects of pistachio nut contamination with AF and methods to prevent and/or control the contamination. There is not yet any published scientific evaluation of implementation of GAP principles on reduction of AF contamination. Also to date, no scientific data have been presented to show the effect of shipping condition on contamination, and examine the use of different conditions such as packaging on preventing further contamination during shipment.

In conclusion, Iran experience to prevent and control AF contamination in pistachio nuts was fruitful, and Iran pistachio nuts now have significantly lower level of contamination with AF. This has greatly contributed to the protection of both local and international consumers from possible health hazard caused by AF contamination. However, further researches are necessary to shed light on other aspects of producing healthy pistachio nuts. Although it might be difficult to convince both national authorities and private sectors to spend more resources on research in these area, the importance of pistachio nuts for Iran economy and costs incurred because of rejection of consignments would create a logic base for any development in this area. It should be mentioned that any positive result of this type of research will increase the amount of consumable pistachio nuts without culturing more orchards.

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