

## Investigation of Aflatoxin Contamination in Indehiscence and Mechanical Splitting Pistachios

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**Abstract:** Iran is the biggest pistachio producer and exporter in the world. Surely, one of the most problems of pistachio industry is Aflatoxin contamination in recent years that treated exportation this valuable crop. This research was done in order to evaluate Aflatoxin contamination in indehiscence and mechanical splitting pistachios of Ouhadi cultivars in Rafsanjan region. Treatments of this research were indehiscence pistachios with soft irregular cracked hull (in proper harvest time), indehiscence pistachios with dry irregular cracked hull (in delay harvest time), indehiscence pistachios with soft irregular cracked hull delaying in dehulling after 72 hours, indehiscence pistachios with unstained shell, indehiscence pistachios with stained shell, mechanical splitting pistachios and indehiscence pistachios with intact hull (control). This research was done in 4 replications in complete randomized block design (CRBD). Results showed that mechanical splitting pistachio had the greatest Aflatoxin contamination; these pistachios had significant differences with other evaluated pistachios from the Aflatoxin point of view. The second rank of Aflatoxin contamination belonged to indehiscence pistachios with stained shell that had significant differences with other categories. Amount of Aflatoxin in indehiscence pistachios with dry irregular cracked hull (delay in harvest time) was negligible. Indehiscence pistachios with soft irregular cracked hull (in proper harvest time), indehiscence pistachios with soft irregular cracked hull (delaying in dehulling) and indehiscence pistachios with intact hull (control) had not detectable Aflatoxin contamination.

**Key words:** Aflatoxin • Contamination • Cracking • Harvest time • Pistachio

### INTRODUCTION

Pistachio is one of the major horticultural products with especial economic importance. In average, 70% of Iranian pistachio nuts are exported (FAO, 2008). Due to suitable climate condition, Iranian's pistachio holds high quality and it is unique compared its competitors. Contamination of pistachio fruit to Aflatoxin is one of the problems what threatens pistachio exportation and food safety. Aflatoxin a secondary metabolite of *Aspergillus flavus* and *A. parasiticus* contaminates many commodities, especially common is the contamination of various air-or sun -dried seeds (cereals, cotton, peanuts and tree nuts) and fruits (figs and dates) (Ciegler, 1975; Thomson *et al.*, 1978). Aflatoxin has commonly been found in pistachio nuts. Aflatoxin contamination can be minimized by rapid dehydration of the nuts and by

avoiding delays between harvest and dehydration. Rapid cooling and storage at low temperature (<5°C) can be employed if delays are unavoidable (Stoloff, 1974).

Different Factors influenced on infection of pistachio nuts to Aflatoxin which is include: cracking of pistachio nuts (especially early hull splitting pistachios) (Doster and Michailides, 1995, Sommer *et al.*, 1986), environmental factors (Campbell *et al.*, 2003; Emami *et al.*, 1977; Denizel *et al.*, 1976; Mojtahedi *et al.*, 1979; Heperkan *et al.*, 1994), cultural practices (Campbell *et al.*, 2003; Fooladi and Tafti, 2006; Tajabadipour *et al.*, 2006; Hosseini-fard and Panahi, 2006), frequency and time of irrigation (Sedaghati and Alipour, 2006), plant litter (Doster and Michailides, 1994b, Moradi *et al.*, 2004), animal manures (Moradi *et al.*, 2004), distribution of Aflatoxin in pistachio bulks (Pearson *et al.*, 1994; Moradi and Javanshah, 2006) and harvesting date (Crane, 1978; Panahi *et al.*, 2005).

Delay in pistachio harvesting will cause an increase in the percentage of cracked nut, increase of high maturing fruits and finally the increase of contamination of pistachio nuts with Aflatoxin. Delay in hulling of the harvested pistachios will increase the percentage of stained pistachios and will cause the increase of contamination. It should be noted that the effects of delay in hulling is less than the effects of delay in harvest (Doster and Michailides, 1995a).

Due to the increased number of cracked pistachios, fell down pistachios and pistachios in contact with orchard surface, the abundance of fungi of *A. flavus* and *A. niger* specie increase as the harvest time approaches. We can also mention to the longer time of exposure of pistachio nuts to the spores present in the orchard space as the; factors increasing the abundance of fungi. Adding all these up, we come to this conclusion that on-time harvesting will decrease the contamination of pistachio nuts (Moradi *et al.*, 2004; Doster and Michailides, 1995b).

The study of relative humidity of storage in Rafsanjan and analyzing the possibility of post-harvest contamination of pistachio nuts with Aflatoxin has shown that, due to the low relative humidity of storage during the storage of pistachio nuts, the spores of fungi *A. flavus*, which is present on most of the stored pistachios, will not have the opportunity for growth and production of toxin (Emami *et al.*, 1977; Ershad, 1973).

Several studies have suggested that some nuts contained Aflatoxin before harvest. Although they failed to find Aflatoxin. Thomson and Mehdy (1978) isolated *A. flavus* from freshly harvested nuts. They suspected that the lack of Aflatoxin contamination was the result of an inadequate number of samples. Also Mojtahedi *et al.*, (1979) reported Aflatoxin contaminated nuts in an orchard in Iran.

Aflatoxin is rarely detected in pistachio kernels with intact hulls (Stoloff, 1974). Pistachio nuts that split abnormally to expose the kernels were prone to infection by *A. flavus* or *A. parasiticus* and contamination with Aflatoxin. The incidence of Aflatoxin was more frequent if novel orange worm also infected the nuts. Nuts with tattered hulls found at the end of harvest as the hull tissues became senescent were subject to infestation by orange worm moths and Aflatoxin developed. Aflatoxin accumulated in tattered nuts with or without insect infestation but with higher frequency in the former (Mojtahedi *et al.*, 1979). The incidence of aflatoxin

contamination in tree nuts is low, but Aflatoxin levels can be variable and high levels can develop in a small percentage of harvested nuts. For example, it has been estimated that only 1 in 28250 walnuts 1 in 26500 almonds (Fuller *et al.*, 1977) and 1 in 25000 pistachios (Schatzki and Pan, 1997) may develop high levels of Aflatoxin contamination. However, a single pistachio with an aflatoxin concentration of 60000 ppb can contaminate an aflatoxin free lot of 4.5 kg (approximately 3000 nuts) to 20 ppb toxin (Doster and Michailides, 1994b). Both *A. flavus* and *A. parasiticus* could contaminate Iranian pistachio nuts under normal conditions and both can produce toxins. However, it was found that pistachios were contaminated more by *A. flavus* than by *A. parasiticus* (Mojtahedi *et al.*, 1979). Early-split nuts are usually present in orchards in a frequency of 1-5 % and vary from tree to tree and from orchard to orchard depending on nutrition and irrigation management (Tomson and Mehdy, 1978).

Some researcher (Person, 1993; Schatzki and Pan, 1997; Schatzki, 1995; Doster and Michailides, 1999; Moradi *et al.*, 2004) has documented the relationship between the stains on pistachio shells and Aflatoxin. For example, the amount of Aflatoxin B1 before, during and after harvesting was related to early splitting, cracking, floater, shell discoloration and small pistachios (Moradi *et al.*, 2004). Doster and Michailides (1999) reported a positive correlation between shell discoloration and contamination by *Aspergillus* species. The shells contained less than 1% of the Aflatoxin B1 found in the edible kernel and they accounted for 41.7-46.8% of the weight of the whole pistachio.

The aim of this project was evaluation of Aflatoxin contamination in indehiscence pistachios in various categories and effect of the mechanical splitting on Aflatoxin contamination of pistachio.

## **MATERIALS AND METHODS**

This study was done in pistachio orchard with Oudadi cultivar in Rafsanjan region. The selected orchard had 20-25 years old on Badami rootstocks. Spacing between rows were 6 meters and distance between trees in rows were two meters. Tree height was about 2 to 3 meter. Soil orchard had no problem of salinity and alkalinity (Soil EC: 6 dS.m<sup>-1</sup> and pH=7.3). Soil texture was sandy loam and growth condition of pistachio trees in this orchard was suitable.

Treatments: Seven Treatments Were Considered as Follow:

- Indehiscence pistachios with irregular cracked and soft hull (On time harvesting).
- Indehiscence pistachios with irregular cracked and shriveled hull ( delay on harvesting).
- Indehiscence pistachios with irregular cracked and soft hull (dehulling time delay for 72 hours)
- Indehiscence pistachios with none stained shell
- Indehiscence pistachios with stained shell
- Mechanical splitting pistachios
- Indehiscence pistachios with intact hull (control)

Sampling Procedures for Each Category:

**Indehiscence Pistachios with Irregular Cracked and Soft Hull, Indehiscence Pistachios with Intact Hull (Control):**

In proper time of pistachio harvesting (10 September), random sampling of pistachio with irregular cracked and soft hull and pistachio with intact hull separately in four replications (four consecutive days) from mentioned Pistachio orchard were harvested. Pistachio harvested immediately moved to the position of pistachio processing and dehulling them was done by machine metal dehulling. After Washing operation and removing the hull and drying in oven with temperature 60°C, indehiscence pistachios samples were isolated separately. Weight of each sample was 4 kg.

**Delay Time of Dehulling of Indehiscence Pistachios with Irregular Cracked and Soft Hull:** Samples of pistachio with irregular cracked and soft hull after 72 hours of harvest were dehulled and after drying, indehiscence pistachios were separated.

**Indehiscence Pistachios with Stained and No Stained Shell:** A part of pistachio crop of orchard in proper harvest time (September 10) was completely harvested. After the operations related to dehulling, washing and drying, samples from indehiscence pistachios with stained and no stained shell harvested and sampling was done separately.

**Indehiscence Pistachios with Irregular Cracked and Shriveled Hull (Delayed Harvest):** A month period after the appropriate time harvest (October 10), pistachio with irregular cracked and shriveled hull were harvested from fruit clusters. After dehulling, washing and drying processes, indehiscence pistachios were separated.

**Mechanical Splitting Pistachios:** Pistachios with intact hull were harvested. After dehulling, washing and drying processes, indehiscence pistachios were separated and mechanical splitting process was done on them.

**Aflatoxin Measurement:** The Aflatoxins B1, B2, G1 and G2 samples were measured for each treatment by using HPLC (Stroka *et al.*, 1997).

**Data Analysis Method:** The statistical analysis was performed using Mstatc software (Michigan State University, USA) and means were compared using Duncan's Multiple Range Test (DMRT).

## RESULTS

**The Total Amount of Indehiscence Pistachios Percentage in Selected Orchard:** Evaluation of the indehiscence pistachio in selected orchard showed that this rate was 22 percent.

**Indehiscence Pistachios Levels in Pistachios with Irregular Cracked Soft and Shriveled Hull:** The results revealed that small percentages (1.5 percent) of pistachios with irregular cracked soft and shriveled hull in the orchard were indehiscence pistachios.

**Weight of Indehiscence Pistachios:** The results showed that the weight of indehiscence pistachios with irregular cracked hull in the orchard, are significantly higher than indehiscence pistachios with intact hull (Fig. 1).

**Aflatoxin Contamination:** Results of Aflatoxin measurements showed that the highest aflatoxin contamination belonged to mechanical splitting pistachios and there was significant difference with other samples that was investigated ( $P=0.05$ ). Second highest amount of Aflatoxin related to indehiscence pistachios with stained shell that with the other samples had significant differences. Aflatoxin contamination of indehiscence pistachios that harvested one month after the proper time to harvest (irregular cracked with shriveled hull) and the difference was no significant with indehiscence pistachios with intact hull, indehiscence pistachios at the proper time of harvest (irregular cracked with soft hull) and the indehiscence pistachios without stained shell. No Aflatoxin detected in indehiscence pistachios with intact hull, indehiscence pistachios at the proper time of harvest and indehiscence pistachios without stained shell (Table 1). 78.5 percent of measured total Aflatoxin was related to Aflatoxin B1 group.

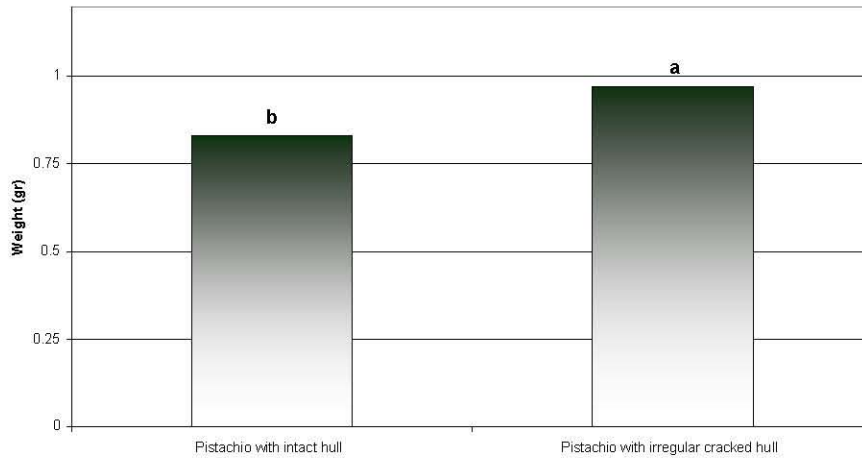


Fig. 1: Comparison of dry weight of indehiscence pistachios with irregular cracked hull with Indeiscence pistachios with intact hull (gr)

Table 1: Aflatoxin amount in types of indehiscence and Mechanical splitting pistachios (ng. kg<sup>-1</sup>)

| Pistachio samples                                                                                                         | Aflatoxin (ng. kg <sup>-1</sup> ) |
|---------------------------------------------------------------------------------------------------------------------------|-----------------------------------|
| Indehiscence pistachio with intact hull                                                                                   | 0 c                               |
| Indehiscence pistachios at the proper time of harvest (irregular cracked with soft hull)                                  | 0 c                               |
| Indehiscence pistachios that harvested one month after the proper time to harvest (irregular cracked with shriveled hull) | 0.18 c                            |
| delay 72 hour time of dehulling of Indeiscence pistachios with irregular cracked and soft hull                            | 0 c                               |
| Mechanical splitting pistachios                                                                                           | 253.5 a                           |
| Indehiscence pistachios with stained shell                                                                                | 14.94 b                           |
| Pistachio indehiscence pistachios with no stained shell                                                                   | 0 c                               |

Number followed by the same letter are not significantly different ( $P=0.05$  by Duncan's multiple range test)

## DISCUSSION

The results showed that Aflatoxin contamination in indehiscence pistachios with intact hull was not detectable. Pistachio with intact hull acts as a barrier against penetration of fungal spores and even some insects. If cracking occurs in hull, fungal spores penetrate in pistachio fruits. In favorable conditions into fruits, fungal spores growth and produce toxin (Dehghani, 1999). The results showed that the indehiscence pistachios that their hulls were cracked had also no Aflatoxin. Shell alleviated as a barrier against the fungus penetration as hull. Based on research findings, even if time of dehulling of indehiscence pistachios delayed for 72 hours, Aflatoxin did not exist in kernel. Some shells never split, in such nuts the kernels have not developed fully and are small. They do not appear to be a likely locus for infection by Aflatoxin-producing fungi because both the intact hull and the indehiscence shell protect them. Of course, this point should be noted that very small percentage of pistachios with irregular cracked hull are indehiscence pistachios, so if the delaying occurs in

dehulling of pistachios, Aflatoxin contamination in split pistachio with irregular cracked hull would be very likely. Usually larger Pistachios ripen earlier than others do and so probability cracking in their hull is greater than others are. The results showed that indehiscence pistachios with cracking in hull considerably larger and had a weight more than indehiscence pistachios that are having intact hull.

It is thought that the impossibility of Aflatoxin contamination of indehiscence pistachios, Mahoney *et al.*, (1998) showed that after inoculation of indehiscence pistachios with spore *A. flavus* and maintenance in favorable conditions, fungal growth in the kernel of indehiscence pistachios is observed. The results showed that indehiscence pistachios with stained shell had Aflatoxin contamination.

However, this contamination may be related to of shell surface, Tajabadipur and Moradi (2003) showed that range of total Aflatoxin (B1, B2, G1, G2) in shell is variable from 1.44 to 385.4 ng. kg<sup>-1</sup>. Amount of aflatoxin kernel in Ouhadi and Ahmadaghaei and Kalleh-gouchi cultivars were respectively 12, 25 and 21 times greater than the amount of Aflatoxin in their shell. Evaluation of processed

Pistachio to *Aspergillus flavus* and *A. niger* fungi group contamination, showed that there is relationship between stained shell and infection fungi. Shell characteristics can be used as a factor in order to separation of contaminated Pistachio and Aflatoxin contaminated pistachios.

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 (Moradi and Javanshah, 2005).

Index of stained shell surface can occur in separation of contaminated pistachios during processing (Kader *et al.*, 1980). The results showed that indehiscence pistachios were been spitted by water, had significant amount of Aflatoxin. Mahoney *et al.*, (1998) showed that after an *A. flavus* spore inoculation of indehiscence pistachios and maintenance in favorable conditions, fungal growth in the kernel of indehiscence pistachios was observed.

These studies proved that the kernel of indehiscence pistachios can also be contaminated with Aflatoxin. It is possible that *Aspergillus* fungi penetrate through the connection place of pistachio fruit to axis. So in mechanical splitting process of pistachio should be avoided from its hydration. Variation between amount of Aflatoxin samples (replication) may due to the scattering of Aflatoxin-producing fungi spores and other none toxigenic species in the air and causing randomly establishment of the fungal spores on Pistachio fruit (Tajabadipur and Fooladi, 2006).

### CONCLUSION

Aflatoxin contamination in indehiscence pistachios with intact hull was not detectable also the indehiscence pistachios that their hulls were cracked, had no Aflatoxin even if time of dehulling of indehiscence pistachios delayed for 72 hours. It is thought that the impossibility of Aflatoxin contamination of indehiscence pistachios kernel, partial contamination may be related to of shell surface. It is possible that *Aspergillus* fungi penetrate through the connection place of pistachio fruit to axis in during re hydration. So in mechanical splitting process of pistachio should be avoided from hydration.

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