Investigation the Effect of Different Agricultural Factors on Pistachio Aflatoxin Content

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Information

Article Type:
Original Article

Article History:
Received: 6 Sep. 2018
Accepted: 2 Nov. 2018
DOI: 10.22123/phj.2019.154478.1019

Keywords:
Pistachio
Aflatoxin
Agricultural Factors

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Abstract

Introduction*:* Pistachio is the most important agricultural product in Iran, especially in Kerman province. It is also important in the domain of export. However, exporting this important item has confronted serious challenges in recent years due to increased aflatoxin contamination in Iran. Exclusive genetic and environmental factors affect the amount of aflatoxin contained in pistachio. Controlling agricultural factors to reduce the amount of aflatoxin can be effective in overcoming the export problem.

Materials and Methods: The present study was conducted to investigate the effect of some agricultural factors on aflatoxin content. The present research was carried out in a commercial garden in Zangiabad village, 17 kilometers north of Kerman, in the 2011-2012 crop year. The research was conducted in seven separate designs in the form of a Completely Randomized Design with three treatments and replications in each design.

Results: The lowest amount of aflatoxin was obtained at the time harvested from September 5 to September 20. The removal of weeds significantly reduced the amount of aflatoxin. The lowest amount of aflatoxin was obtained when no spraying was carried out for pests and diseases. The traditional processing of pistachio significantly increased the amount of aflatoxin. The use of nitrogen and phosphate fertilizers at 400 kg ha^{-1} was the best solution for reducing the amount of aflatoxin. Less irrigation with an 84-day irrigation interval resulted in the highest reduction in aflatoxin content.

Conclusion: By controlling the agricultural factors and optimizing the conditions, it is possible to control and reduce the amount of pistachio aflatoxin.

►Please cite this article as follows:

Baghizadeh A, Tajadini H. Investigation the effect of different agricultural factors on pistachio aflatoxin content. Pistachio and Health Journal. 2018; 1 (4): 48-59

1- Introduction

Pistachio (*Pistacia vera L.)* belongs to the Anacardiaceae family. Itis one of the most prominent tree nuts [1- 3]. Due to the fact that a large area of Iran is located in desert regions with unfavorable conditions such as salinity of water and the deficiency of soil and water, cultivating pistachio, as a plant resistant to these conditions, has been of paramount importance [4]. As an important export item, pistachio struggles with contamination caused by different species of *Aspergillus* during the course of the cultivation which, in addition to apparent damage to the fruit, causes secondary metabolites, such as mycotoxins, and reduced nutritional and export value of this fruit [5]. Aflatoxin is one of the most important types of mycotoxins; it has attracted attention because of the different biochemical and biological effects and serious problems it causes for humans and animals. Aflatoxin is produced by two species of *Aspergillus flavus* and *Aspergillus parasiticus* [6, 7]. Aflatoxin is a common food contaminant, generally produced at temperatures of 24-35°C and a humidity of at least 18% [7]. Therefore, it has the potential of infecting agricultural products during the production, harvest, processing, and storage [7, 8, 9]. High levels of humidity and high temperatures at the end of the growing season are among the factors influencing the transition to the growth of fungi. The amount of aflatoxin is another major contamination of pistachio in the garden. Some other factors include the presence of early splitting pistachio nuts, pest attack, and delay in harvesting [8, 10].Given the importance of early splitting in the formation of aflatoxin-contaminated pistachios, it is absolutely necessary to pay attention to the amount and correct irrigation intervals in the pistachio garden according to the conditions of the region. Inappropriate irrigation in late spring increases the number of early splitting pistachios. One solution to reduce early splitting and aflatoxin contamination is to carry out regular irrigation operations. Contamination with aflatoxin-producing factors starts from the garden and expands during processing and storage. Early splitting varies from one garden to another and from year to year, depending on the type of soil and nutrition, variety, weather conditions, and irrigation diets [11, 12]. According to the cultivar, soil texture and weather conditions, it is recommended to harvest each individual crop at the right time and to avoid the maintenance of the product on the tree for any reason. Delay in harvesting provides more time for pistachio seeds to have contact with spores suspended in the garden. Thus, early harvesting will reduce pistachio contamination [11, 13]. Early splitting nuts with dry, wrinkled green skin are at the risk of fungal contamination three times more than the ordinary seeds that have a fresh green skin without any wrinkles. Aflatoxin levels in sunflower seeds are 50 times higher than natural pistachios [11, 14]. The starting point for contamination with aflatoxin in garden conditions is before harvesting. In regard with Fandoghi variety, which accounts for 60% of Iran's pistachios, over 65% of fruits are totally ripe by the first third of September, and the skin of the nuts crack afterwards and

conditions for subsequent contamination such as insect damage, growth of fungi, and the production of aflatoxin becomes more and more favorable [15]. Due to easy access to the nuts, the population of insects and mites on pristine pistachios with cracked skin is relatively high. In addition to providing moisture conditions for the growth of the fungus, weeds provide a winter accommodation period for some of the pests and spores of the fungus, and they might complicate the harvesting process and increase the risk of contamination. The growth and development of aspergillus in the plants and the remnants of the processing result in increased fungus densities and the contamination of pistachios with aflatoxin. Thus, it is recommended to reduce the amount of aflatoxin by removing vegetation residues from the gardens [16, 17]. Processing refers to an operation during which newly harvested pistachios are peeled, washed, dried, separated, and stored. The population of *Aspergillus flavus* fungi in pistachio fruit processing terminals is affected by various factors such as terminal type (traditional or mechanized), type of washing system, type of pistachio input, and terminal management [18,19]. The population of aspergillus toxin fungi in traditional storage is 2.6 times higher than semi-mechanized terminals. Also, Comparison of different washing systems shows that the use of a water shower system is more effective than a constant water reservoir or water in the flow to reduce the population of fungi and that the combination of the two methods is useful in creating a system for washing and separating infected pistachios from healthy nuts. [18, 19]. Early splitting pistachios which have not been contaminated in the garden might be contaminated during transport and processing operations. Humidity and high temperatures inside pistachio masses provide the best condition for contamination of early splitting pistachios, making the level of contamination quite unacceptable [11, 19, 20]. The present study was conducted to investigate the effect of harvesting time, weed removal, spraying rate, type of processing, the amount of nitrogen and phosphate fertilizers, and proper irrigation intervals on decreasing the amount of aflatoxin.

2. Materials and Methods

The present research was carried out in a commercial garden in Zangiabad village, 17 kilometers north of Kerman, with a height of 1700 meters above sea level, in the 2011-2012 crop year. The studied samples included Fandoghi pistachio trees, 25 years old, which were irrigated for 56 days. The research was conducted in seven separate designs in the form of a Completely Randomized Design with three treatments and replications in each design. The effects of harvesting time, weed removal, number of spraying, type of processing, nitrogen and phosphate fertilizers, and irrigation interval were investigated. It should be noted that the selection of treatments in each design was based on the local convention and the review of scientific resources.

2.1. The Effect of harvesting time:

The effects of harvesting was reviewed on the three dates 5th of September, the 20st of September, and the 5th of October.

2.2. The Effect of weed removal:

Effect of weed removal using mechanical methods and herbicide was examined. The state of non-removal of weeds was also examined.

2.3. Effect of spraying frequency:

The present research examined the effects of three treatments of six spray, three spray, and no spray paths. Washing was done with soap and water.

2.4. The Effect of processing type:

The present research studied the effects of traditional, semi-mechanized and mechanized processes; In traditional processing peeling to drying stages were done manually, which lasted 3 to 4 days. In semi-mechanized processing, the fruits were peeled by a machine and washed in a flooded pond. However, in the mechanized process, all steps were performed by the machine and the washing was carried out using a water shower, all of which were carried out in a few hours.

2.5. Nitrogen fertilizer treatments (46% urea):

800 kg ha^{-1} of N fertilizer was fed to the first row and 400 kg ha^{-1} to the second row as a superficial spread at the beginning of the vegetative season, once when the fruit and leaves appeared completely and then during mid-vegetative season, when the nuts were being filled.The third row did not receive any fertilizer; the row were irrigated after providing the required fertilizer.

2.6. Phosphate fertilizer application (Triple phosphate 46%):

In this treatment, the effect of three doses of phosphate fertilizer was investigated; the first row received 800 kg ha^{-1}phosphate fertilizer, the second row 400 kg ha^{-1} of phosphate fertilizer in a choral manner, and the third row receive no fertilizer. Each row was divided into three equal parts and were considered as three repetitions. The phosphate fertilizer was given to the ground during hibernation.

2.7. Irrigation cycle treatments:

In this treatment, three irrigation rounds of 28, 56, and 84 were examined; during the growing season, the first row was irrigated every 28 days, the second row every 56 days, and the third row every 84 days.

2.8. Aflatoxins measurement

Sampling was done completely randomly. In each block, 3 kg were collected from trees and transferred to the laboratory of Negin Gostar Kerman Company. The level of aflatoxin in the HPLC system was measured by the WATERS model of the United States of America, according to standard number 6872 (standard aflatoxin of the Bureau of Standards and Industrial Research of Iran). The level of aflatoxin was measured in three distinct stages in HPLC method**:**

1- Extraction or preparation of aflatoxin involving the separation of aflatoxin from fat and nuts.

2- Purification of aflatoxin.

3. Determining the amount of aflatoxin by the device.

Initially, the pistachios are first washed, and the fruits are extracted out of the skin and measured in 100 gr units. Then, the fruits are grinded and turned into a special form of dough. Then, 50 grams of the powdered fruit is weighed, to which 5 grams of sodium chloride is added to separate aflatoxin from the fruit. 200 ml of 80% methanol and 100 ml of N-hexane 100% are added to the mixture and,

then, the resulting product is completely mixed with a high speed blender for three minutes. The extract is Then filtered. Then, 20 ml of the solution is distilled off with a volume of 150 ml. The resulting mixture is poured into the test tube and shaken to be thoroughly mixed. The mixture is then straightened using the what man GF/F filter paper. 70 ml of the extracts prepared and diluted in the previous step is passed through *immune affinity clean up column* at a rate of 2 mL/min. Then, the column is washed with 15 ml of HPLC water. After that, the column is dried with mild air flow for 10 to 15 seconds, and 1500 µl methanol is added to the column. The resulting material is collected in the special vial of the device and 1500 ml of HPLC water is added to the vial after 1 minute and mixed with a vortex. Then, a 0.45 µm filter is used to stir the mixture. It is then injected into a special vial. Water, acetonitrile, and methanol mixture (60:20:20, v/v/v) with a flow-rate of 1 mL/min were used for the mobile phase. Conditions of analysis were C18, column 300 x 4.6 mm with guard column, and Fluorescence detection was performed at excitation and emission wavelengths of 365 and 435 nm, respectively. Finally, the amount of aflatoxin is reported by the machine in the form of a curve and computed in the Excel software.

2.9. Statistical analysis

Analysis of variance was done on the measured aflatoxin values. Duncan test was performed to compare the mean of treatments. The above analyses were performed with the SPSS software and the graphs were plotted with the Excel software.

3. Results

3.1. The effect of the harvest time

According to the F obtained from the analysis of variance of harvest time, the difference between the data is highly significant, indicating that different harvesting times have different effects on the amount of aflatoxin in pistachios (Table 1).To determine the best harvest time, Duncan test was used to examine the time needed for harvesting, as shown in the Fig. 1. The results show that pistachio harvesting is appropriate until September 20, and the delay in harvesting increases the amount of aflatoxin.

Table1. Variance analysis of agricultural factors affecting the amount of aflatoxin

Sources of variations	Degree of freedom	Mean of squares						
		Harvest time	Weed removal	Spraying cycle	Processing type	Nitrogen fertilizer	phosphate fertilizer	Irrigation round
Treatment	2	1413.74**	4.94**	243.1**	615.1**	77.75**	12.6**	40.1**
Error	6	1.93	0.003	0.17	0.11	0.009	0.004	0.004
CV %		11.88	14.83	14.41	9.02	8.16	8.23	18.65

** significant at a level of 1%

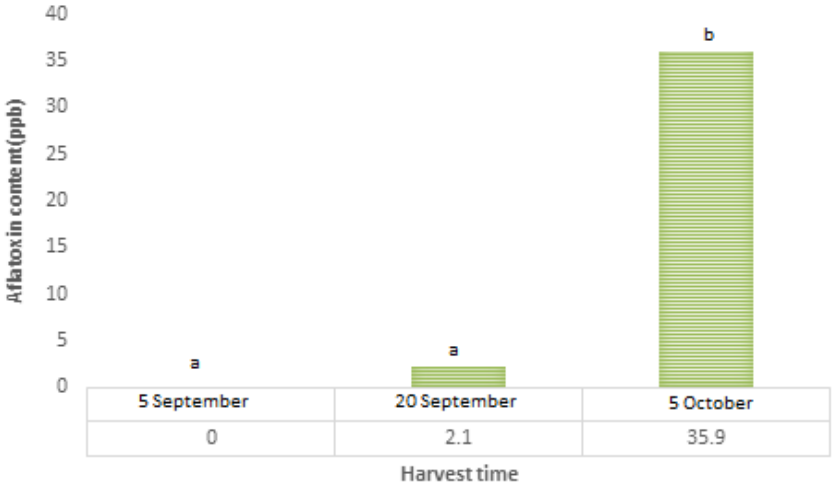

Fig 1. Comparison of different treatments of harvest time on aflatoxin content

3.2. The effect of weed removal:

According to the F obtained from weed removal table variance analysis, the difference between data is significant, indicating that different weed removal methods lead to different amounts of aflatoxin in the pistachio fruit (Table 1). To determine the best weed removal method, Duncan test was used to examine the weed removal as shown in the Fig 2. The removal of weeds, both mechanically and chemically, reduces the amount of aflatoxin as compared with the control.

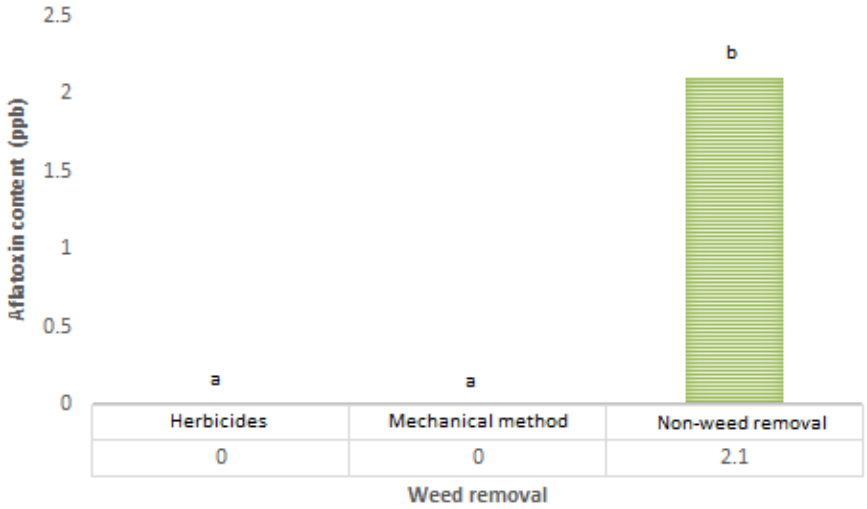

Fig 2. Comparison of different treatments of weed removal on aflatoxin content

3.3. The effect of spraying cycle:

According to the F obtained from spraying cycle table variance analysis, the difference between data is significant, indicating that different spraying cycles lead to different amounts of aflatoxin in the pistachio fruit (Table 1). To determine the best spraying cycle, Duncan test was used to examine the spraying cycle, whose results are shown in Fig 3. Spray with water and soap not only does not reduce the amount of aflatoxin, but also increases it. This is probably due to the creation of good moisture conditions for the growth of fungi. The highest levels of aflatoxin were observed in three rounds of spraying. Aflatoxin levels decreased with increasing spraying. However, it is not recommended to wash with soap and water to reduce the amount of aflatoxin.

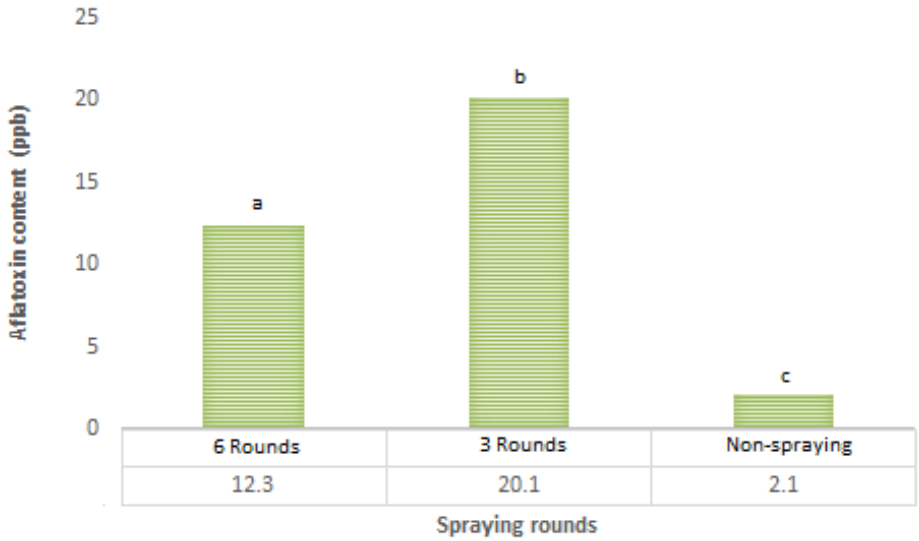

Fig 3. Comparison of different treatments of Spraying rounds on aflatoxin content

3.4. The effect of the type of processing:

According to the F obtained from processing table variance analysis, the difference between data is significant, indicating that different processing techniques lead to different amounts of aflatoxin in the pistachio fruit (Table 1). To determine the best processing strategy, Duncan test was used to examine the type of processing, whose results are shown in the Fig 4. The mechanized harvesting method is the best way to reduce aflatoxin levels. In the next step, the semi-mechanized method is recommended. The results showed that traditional harvesting increases the level of aflatoxin

3.5. The effect of nitrogen fertilizer:

According to the F obtained from nitrogen fertilizer table variance analysis, the difference between data is significant, indicating that

different doses of nitrogen fertilizer lead to different amounts of aflatoxin in the pistachio fruit (Table 1). To determine the best dose of nitrogen fertilizer, Duncan test was used to examine nitrogen fertilizer, whose results are shown in the Fig 5. The use of nitrogen fertilizer (400 kg ha-1) reduces the amount of aflatoxin, while the lack of fertilization and the use of high levels (800 kg ha-1) increase the amount of aflatoxin.

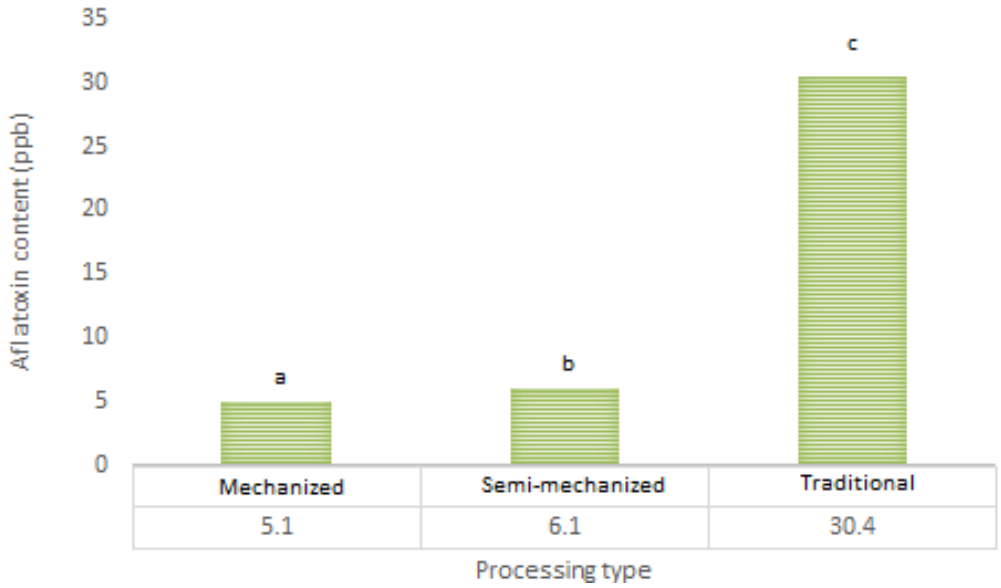

Fig 4. Comparison of different treatments of Processing type on aflatoxin content

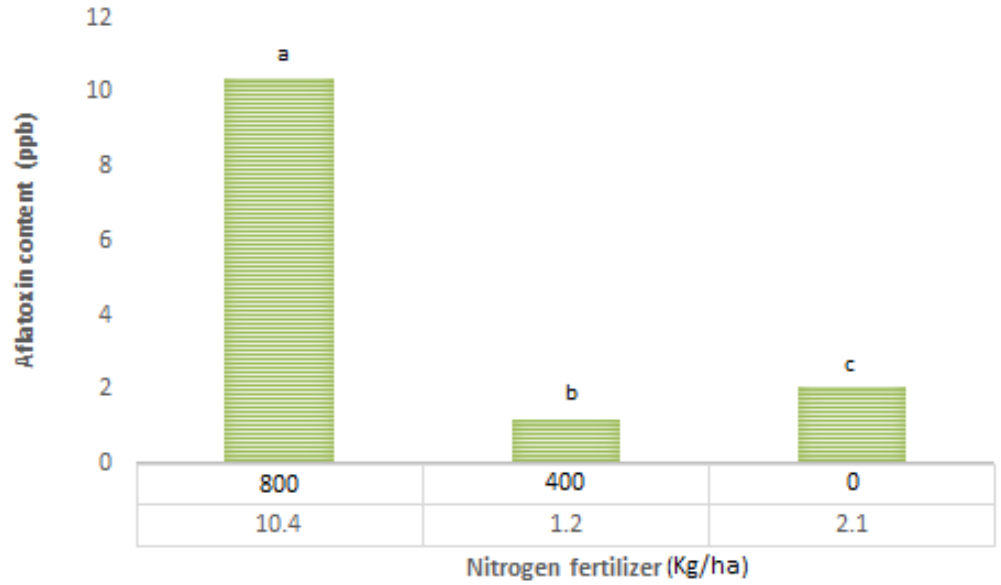

Fig 5. Comparison of different treatments of Nitrogen fertilizer on aflatoxin content

3.6. The effect of Phosphate fertilizer:

According to the F obtained from phosphate fertilizer table variance analysis, the difference between data is significant, indicating that different doses of phosphate fertilizer lead to different amounts of aflatoxin in the pistachio fruit (Table 1). To determine the best dose of phosphate fertilizer, Duncan test was used to examine the Phosphate fertilizer, whose results are shown in the Fig 6.The use of phosphate fertilizer (400 kg ha-1) reduces the amount of aflatoxin, while the lack of fertilization and the use of high levels (800 kg ha-1) increase the amount of aflatoxin.

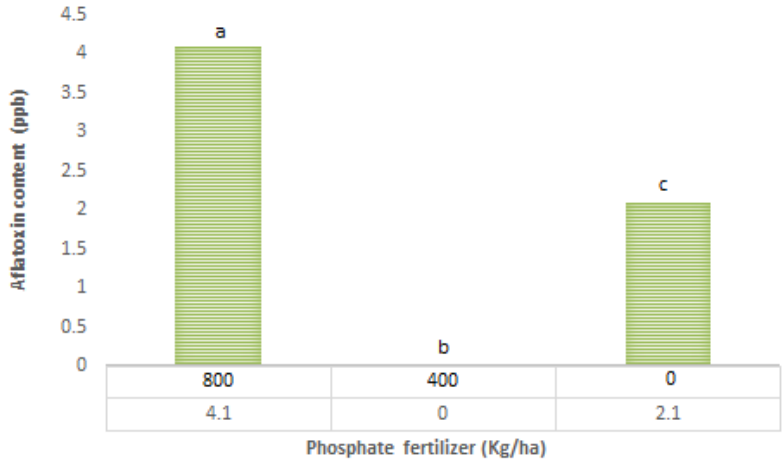

Fig 6 Comparison of different treatments of Phosphate fertilizer on aflatoxin content

3.7. The effect of irrigation round:

According to the F obtained from irrigation table variance analysis, the difference between data is significant, indicating that different irrigation rounds lead to different amounts of aflatoxin in the pistachio fruit (Table. 1). To determine the best irrigation round, Duncan test was used to examine the irrigation round, whose results are shown in the Fig 7. The lowest levels of aflatoxin have been produced in irrigation over 86 days. The highest aflatoxin level was seen in round 26 days.

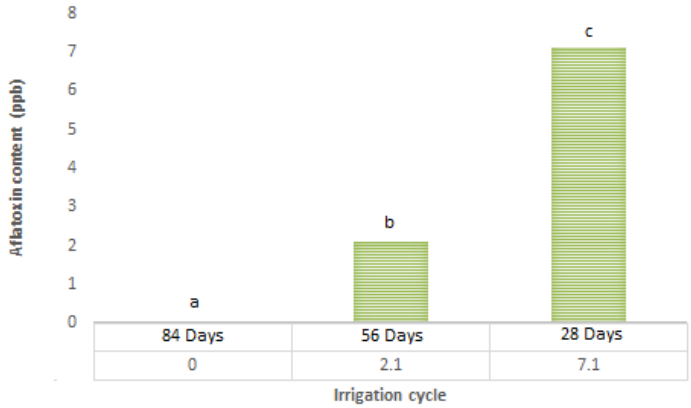

Fig 7. Comparison of different treatments of Irrigation cycle on aflatoxin content

4. Discussion

As we can see, the data of the harvesting time is in two categories, and there is no significant difference between the harvesting date of September 5th and September 20th, and the amount of aflatoxin is close to zero. However, the amount of aflatoxin is very high on October 5th, and the percentage of fruit in the sample of October 5th was higher than that of the other samples. At harvest time, it was observed that the percentage of split pistachios with cracked shells was very high in the sample of October 5; samples belonging to 5th of September were all healthy and with no crack in the shell. Fanny and colleagues Showed that the highest amount of Aflatoxin in pistachio was observed in October 10th.Delay in harvesting leads to an increase in the percentage of cracked fruits and, finally, an increase in the amount of pistachio fruit contaminated with aflatoxin [11, 12]. As harvesting was delayed, the amount of aflatoxin in the studied pistachios increased by 2 to 30 times [21].

As we can see, the data of weed removal is classified into two categories, and there is no difference between weed removal either using the mechanical method or herbicide, and the amount of aflatoxin is equal to zero. Also, the percentage of healthy fruit in mechanical weed removal is higher than other specimens.

Thus, removing weeds mechanically is recommended because herbicides have many side effects for the plant and soil. Weeds, in addition to providing moisture conditions of the garden for fungal growth, are also the location of wintering of some pests, so it is essential to manage fighting [11, 22].

As we can see, the data of spraying cycle is categorized into three groups, and the lowest amount of aflatoxin is related to a sample that has not been sprayed. The highest amount of aflatoxin is related to those samples that have been sprayed three time. Any factor that causes the soft skin of the pistachio to damage can increase the risk of aflatoxin. The best way to fight pests is timely control [11, 22].

As we can see, the data of the type of processing is categorized into three groups. The lowest amount of aflatoxin is related to the mechanized processing sample; due to the rapid processing stages that reduce the chance of growth and degradation of healthy pistachios, the amount of aflatoxin is reduced. The highest amount of aflatoxin is related to the traditional processing sample due to the slow processing stages, providing the suitable opportunity for the fungi to grow and infect healthy pistachios, resulting in an increase in the level of aflatoxin. The content of aflatoxin B_1 in stained, small, floater, and open shell pistachios was 21, 4, 15, and 2 times higher than unstained, large, sinker, and closed shell pistachios, respectively. With the identification and isolation of contaminated resources in the early stages and proper pistachio processing, aflatoxin levels can be reduced [23].

The data of nitrogen fertilizer is categorized into three groups. The lowest amount of aflatoxin is related to the sample receiving 400 kg ha^{-1} nitrogen fertilizer and the highest amount is related to 800 kg ha^{-1}. It was observed that the group receiving 800 kg ha^{-1} nitrogen fertilizer were infected with

cracking, soft shell, and early splitting at the harvest time. Thus, the fungi found their way easily into the fruit and infected the nuts. Nitrogen deficiency stress contributes to the accumulation of aflatoxin [24, 25]. A negative relationship was observed between nitrogen percentage and growth rate of Aspergillus fungus in different pistachio cultivars [26].

As it can be seen, the data of Phosphate fertilizer is classified into three categories, and the lowest amount of aflatoxin is related to the sample receiving 400 kg/ha phosphate fertilizer. The highest amount is related to the sample receiving 800 kg / ha. It was observed that the group receiving 800 kg ha^{-1} phosphate fertilizer were infected with cracking, soft shell, and early splitting at the harvest time. Thus, the fungi found their way easily into the fruit and infected the nuts. There was a negative relationship between the amount of aflatoxin production and the amount of phosphorus in the 5% level [26].

As it can be seen, for the data of irrigation round, the minimum amount of aflatoxin is observed in samples with 84-day irrigation cycle, and the highest amount of aflatoxin is related to the specimen with 28-day cycle. It was observed that the samples with 28-day irritation cycle had higher frequencies of early splitting, with completely cracked soft shells creating chances for fungal growth and contamination. Regarding the importance of early planting of aflatoxin-contaminated pistachios and the role of irrigation in causing this complication, it is absolutely necessary to observe the correct amount and duration of irrigation in the pistachio garden in accordance with the conditions of the area [11, 22].

5. Conclusions

By controlling some agricultural factors and optimizing the conditions, it is possible to control and reduce the amount of pistachio aflatoxin. The harvesting time of September 20, weed removal, non-spraying, mechanized harvest, the use of chemical fertilizers with standard values, and more irrigation intervals are the best conditions for reducing aflatoxin in pistachios.

Conflicts of interest

The authors declare no conflicts of interest

References

1- Zohary M. A monographical study of the genus Pistacia. Palestine Journal of Botany (Jerusalem Series). **1952**; 5(4):*187-228.*

2- Abrishami MH. Iranian Pistachios, Historical Recognition. Tehran University Press Publishing Center. **1994**.

3- Noroozi S, Baghizadeh A, Jalali M. The genetic diversity of Iranian pistachio (*Pistacia vera* L.) cultivars revealed by ISSR markers. Biol Diver Conserv. **2009**; 2: *50-56.*

4- Ak B E, Karadag S, Sakar E. Pistachio production and industry in Turkey: current status and future perspective. XVI GREMPA Meeting on Almonds and Pistachios.**2016**; *323-329.*

5- Azizi J, Yazdani S. Exports of pistachios in Iran. Extractors and foundries. Journal of Economic Research (Tahghighat-E-Eghtesadi). **2006**; 41(3):*217-247*.

6- Cheraghali AM, Yazdanpanah H, Doraki N, Abouhossain G, Hassibi M, Ali-abadi S, et al. Incidence of aflatoxins in Iran pistachio nuts.Food Chem Toxicol. **2007**;45(5):812-816.

7- Kurtzman CP, Horn BW, Hesseltine CW. Aspergillus nomius, a new aflatoxin producing species related to *Aspergillus flavus* and *Aspergillus tamarii*. Antonie van Leeuwenhoek.**1987**;53(3):*147-158*.

8- ahoney A, Noreen E, Susan B. Rodriguez. Aflatoxin variability in pistachios. Applied and environmental microbiology. **1996**; 62.4: *1197-1202*.

9- Khosravi AR, Shokri H, Ziglari T. Evaluation of fungal flora in some important nut products (pistachio, peanut, hazelnut and almond) in Tehran, Iran. Pak. J. Nut. **2007**; 6:*460-462*.

10- Doster W A, Michailides TJ. The relationship between date of hull splitting and decay of pistachio nuts by aspergillus species. plant disease. **1995**; 79:*766-769*.

11- Hadavi E, Several physical properties of aflatoxin-contaminated pistachio nuts: Application of BGY fluorescence for separation of aflatoxin-contaminated nuts. Food Additives & Contaminants.**2007**;22(11): *1144-1153*.

12- Carbonell-Barrachina AA, Memmi H, Noguera-Artiaga L, Gijón-López Mdel C, Ciapa R, Pérez-López D. Quality attributes of pistachio nuts as affected by rootstock and deficit irrigation. J Sci Food Agric. **2015**; 95(14):*2866-2873*.

13- Setamou M, Cordwell K F, Hell K. Aspergillus flavus infection and aflatoxin contamination of preharvest maize-in Benin. Plant Diseases.**1997**; 81: *1323-1327*.

14- Ephrem G. Implication of aflatoxin contamination in agricultural products." American Journal of Food and Nutrition. **2015**;3(1): *12-20*.

15- Denizel T, Jarvis B, Rolfe E J. A field survey of pistachio (Pistacia vera) nut production and storage in Turkey with particular reference to aflatoxin contamination. J Sci Food Agric. **1976**; 27(11):*1021-1026*.

16- Jones R. K, Duncan H E, Payne GA, Leonard K J. Factors influencing infection by Aspergillus flavus in silk-inoculated corn. Plant Dis. **1980**; 64:*859–863*.

17- Doster MA, Michailides TJ. Relationship between shell discoloration of pistachio nuts and incidence of fungal decay and insect infestation. Plant Disease. **1999**; 83(3):*259-264*.

18- Moradi M, Mirabofathy M. Population density of Aspergillus flavus and Aspergillus niger at different kinds of pistachio processing terminals. In: Kerman. Pajoohesh va Sazandegi Journal. **2007**;77:*104-109*.

19- Hadavi E, Feizi H, Gheibi B. Aflatoxin-contaminated nut separation by applied machinery and processing stages in fresh pistachio processing plant. Front Microbiol. **2017**; 8: *2404*.

20- Tajabadipour A, Panahi B, Zadehparizi R. The effects of rootstock and scion on early splitting and cracked hull of pistachio. Acta Hort. (ISHS) **2006**; 726:*193-198*.

21- Fani S, Javanshah J, Moradi M. Prevalence of aflatoxin in rafsanjan processed pistachios during 2011-12, and its relation with time of harvest. TB. **2014**; 12 (4) :*175-189*.

22- Waliyar F, Traore A, Fatondji D, Ntare B R. Effect of irrigation interval, planting date, and cultivar on Aspergillus flavus and aflatoxin contamination of peanut in a sandy soil of Niger. Peanut Science.**2003**; 30:*79–84*.

23- DargahiR, Masoumi H, Moradi M, Fani S. Evaluation of aflatoxin B1 in different parts of pistachio fruit and effects of processing stages. Food Hygiene. **2014**; 4, 3 (15):*21-31*.

24- Payne GA, Kamprath EJ, and Adkins C.R. Increased aflatoxin contamination in nitrogen stressed corn. Plant Dis. **1989**; 73:*556*.

25- Tubajika KM, Muscagni HJ, Damann KE, Russin JS. Nitrogen fertilizer influence on aflatoxin contamination of corn in Louisiana. Journal of Agricultural and Food Chemistry.**1999**; 47:*5257–5260*.

26- Mohammadi Moghadam M, Sobhanipour A, Hokmabadi H. Evaluation on correlation between aflatoxigenic Aspergillus flavus growth and aflatoxin B1 production with elements and protein percentage in pistachio kernels of different cultivars. Journal of Horticultural Science.**2010**; 24(2): *216-222*.