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ORIGINAL ARTICLE

The Effects of Deficit Irrigation on the Early Splitting of Pistachio Nuts and Aflatoxin Contamination

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Background: The cracking of pistachio nuts is the major source of mold contamination as well as pests and aflatoxins.

Materials and methods: The effects of deficit irrigation on pistachio-nut cracking were evaluated at two irrigation intervals (25 days and 45 days) and five omissions of the irrigation time. The frequencies of hull early splitting and cracking were determined in nuts.

Results: All and all, long irrigation intervals and deficit irrigation since late April until early June were critical and increased the early splitting rate (up to 90%) in comparison with regular irrigation. The highest frequencies of the early splitting of pistachios were observed due to the drought stress at early June ranging from 7.7 percent to 9.6 percent. Deficit irrigation in July increased the rate of pistachio hull cracking significantly. The averages of the hull early splitting and cracking formation were by 37 percent and 18.5 percent higher in 45 days of irrigation intervals compared with 25 days, respectively. The contents of B₁ and B₂ aflatoxins in hull early splitting and cracking were 223.4 $\mu\text{g.kg}^{-1}$ and 25.47 $\mu\text{g.kg}^{-1}$, respectively. In hull cracking fruits, aflatoxin B₁ and B₂ were 111.06 $\mu\text{g.kg}^{-1}$ and 9.71 $\mu\text{g.kg}^{-1}$, respectively.

Conclusion: In general, to reduce the risks of aflatoxins in pistachio nuts, it is critical to manage irrigation in April and June.

Keywords: Aflatoxin; Aspergillus; Mycotoxins; Pistachio health

1. Introduction

It has been suggested that Pistachio trees are drought tolerant and withstand low quantities of water, yet they require enough water during the growing season to produce the potential yield. Doster *et al.* (2001) indicated that the increase in the abnormal splitting of pistachio nuts is due to the insufficient spring irrigation. They also observed a higher frequency of early splitting in the no-irrigation case in June compared with regular irrigation [6]. Doster and Michailides (1995) reported that the omission of irrigation in the middle of April until the middle of June increased early splitting formation, while in late July until the middle of September, negative effects were observed being related to the shell hardening period [5]. No significant effects were observed among irrigation systems regarding the early splitting phenomenon. The frequencies of early splitting in orchards receiving surface, microsprinkler and sprinkler irrigation systems were 2.6 days, 2.8 days and 2.1 days, respectively [19]. Similar effects were observed where the orchards received deficit irrigation in late May that significantly led to an increase in the rate of early splitting formation [6].

Doster and Michailides (1993) demonstrated that cultural practices have no significant effects on the rate of early-split nut formation in the orchards [3].

In the pistachio growing area of Rafsanjan, the total evapotranspiration (ET_c) during late March to mid-October is about 690 mm, being equal to 4.8 mm per day in midsummer [7]. Flooded irrigation is used for most pistachio orchards at the ratio of 90 % and drip and bubbler irrigation systems are utilized for other orchards.

The contamination of pistachio nuts with molds and their mycotoxins is the major threat to human and animal health. Pistachio nut shells are naturally split and surrounded by hulls protected from airborne mold spores.



Fig. 1. Early split pistachio nuts

The early splitting and hull cracking of nuts are the major sources of aflatoxin in pistachios. Early split nuts (Fig. 1)

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happen in few nuts where both the hull and the shell are split exposing the kernel to infection with molds and aflatoxin productions [5].

The cracking of the hull (Fig. 2) occurs anywhere on the hull except the shell split, and it exposes the shell with the frequency of occurrence lower than 5 percent [9].



Fig. 2. Hull cracking pistachio nuts

The hull-cracking occurrence only happens quite close to the harvest time, so it does not lead to severe mold infections as against early split nuts. [8].

There is a scarcity of information on the effects of the irrigation management of the cracking of pistachio nuts under pistachio orchard conditions. Therefore, the current study is carried out mainly aimed at determining the effects of deficit irrigation as against full irrigation on the formation of the early splitting and cracking of the nut hull as well as the amount of aflatoxins.

2. Materials and Methods

This study was carried out in an area of nine hectares of commercial pistachio orchards (*Pistacia vera* cv. *Ohadi*) in Rafsanjan regions with sandy soil. The experiments were conducted in a randomized complete block design (a split plot) with ten treatments, including:

T₁: Control, at the irrigation interval of 25 days (regular irrigation);

T₂ to T₅: The omission of irrigation on April 14, May 10, June 5 and July 1, respectively, at the irrigation interval of 25 days;

T₆: Control, at the irrigation interval of 45 days (regular irrigation);

T₇ to T₉: The omission of irrigation in April 14, June 1 and July 15, respectively, at the irrigation interval of 45 days;

T₁₀: The omission of irrigation on April 14 and July 15, at the irrigation interval of 45 days. Based on the water requirements of pistachio trees during the omission of irrigation (Table 1), in each treatment, the amount of water applied has been demonstrated in Table 2.

All treatments received the same cultural practices during the experiments. At the harvest time, early split and hull cracking nuts were determined in all treatments. The data were analyzed using a one-way analysis of variance (ANOVA) using MSTATC software. The means were

compared via Duncan's multiple range test ($P \leq 0.05$). For the aflatoxin analysis, pistachio nuts in each treatment were classified into early splitting pistachios, hull-cracking pistachios and intact pistachios. The aflatoxin content was measured using TLC assays as described by Fani *et al.* (2014) [8].

3. Results

The results demonstrated that the splitting of the hull that is characteristic of early split nuts was significantly affected by deficit irrigation in late April to early June (Fig. 3).

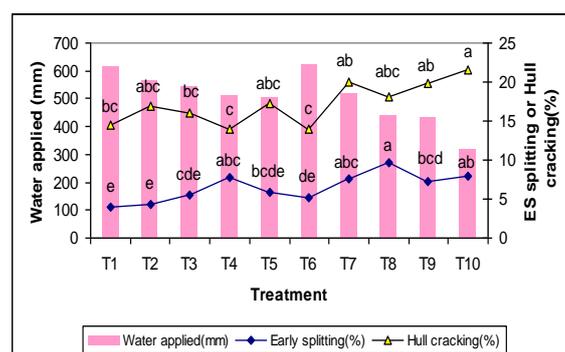


Fig. 3. Effect of deficit irrigation during the growing season on early splitting and hull cracking nuts at harvest in different treatments

Trees with deficit irrigation in early June (T₄ and T₈ treatments) received about 20% less water than the control treatment (T₁ and T₆ treatments). In these treatments, the early splitting formation rate was twice higher than the control treatment. Deficit irrigation in July (18-30% less than the control treatment) had lower effects (T₅ and T₉ treatments) on early splitting formation, i.e. 30% higher than full irrigation.

The frequency of irrigation is one of the effective factors in the occurrence of early split nuts. Observations showed that long irrigation intervals increased the amount of early split nuts. The formation of early split nuts in deficit irrigation was affected by the irrigation interval. In T₄ and T₈ treatments with irrigation intervals of 25 days and 45 days together with the omitted irrigation at early June, the early splitting nuts were 7.7 and 9.6 percent, respectively, where T₈ treatment rate was 25% higher than T₄. The averages of early splitting nuts in treatments at the irrigation interval of 45 days were 37.3% higher than the 25-day irrigation intervals.

Regarding hull cracking, no significant effects were observed among irrigation omission treatments at both irrigation intervals. However, deficit irrigation in July had the highest impact on the increase in the hull-cracking occurrence. By increasing the irrigation interval from 25 to 45 days, the hull cracking of pistachios increased by 18.5%, being indicative of a significant effect.

Table 1. Water requirements of mature pistachio trees in growing season [7].

Treatments with irrigation intervals of 25 days (T ₁ - T ₅)			Treatments with irrigation intervals of 45 days (T ₆ - T ₁₀)		
Period	Water requirement (mm)	Irrigation time	Period	Water requirement (mm)	Irrigation time
April 14-May 10	50	April 14	April 14-May 31	107.6	April 14
May 10-June 5	75.5	May 10	May 31-July 15	186	May 31
June 5-July 1	101.7	June 5	July 15-Sep. 1	197.1	July 15
July 1-July 26	112.8	July 1	Sep. 1-Oct. 16	134.3	Sep. 1
July 26-Aug. 22	108.1	Aug. 22	-	-	-
Aug. 22-Sep. 15	95.5	Aug. 22	-	-	-
Sep. 15-Oct. 11	70.9	Sep. 15	-	-	-
Total	614.5	*****	Total	625	*****

Table 2. Effect of Omission of irrigation during the growing season on the incidence of early splitting and hull cracking nuts at harvest in different treatments.

Irrigation interval	Treatment	Time of irrigation omission	Water applied (mm)	Early-split nuts (%)	Hull-cracking nuts (%)
25 days	T1	Control	614.5	3.9 e*	14.6 bc
	T2	April 14	564.5	4.3 e	16.9 abc
	T3	May 10	539	5.5 cde	15.9 bc
	T4	June 5	512.8	7.7 abc	14.0 c
	T5	July 1	501.8	5.8 bcde	17.3 abc
45 days	T6	Control	625	5.1 de	13.9 c
	T7	April 14	517.9	7.5 abc	19.9 ab
	T8	June 1	439.5	9.6 a	18.1 abc
	T9	July 15	428.4	7.2 bcd	19.8 ab
	T10	April 14 & July 15	320.8	7.9 ab	21.5 a

* Values followed by the same letter(s) are not significantly different ($P < 0.05$)

The measurement of aflatoxin in this study revealed that the contents of B₁ and B₂ aflatoxins in hull early splitting and cracking were 223.4 $\mu\text{g.kg}^{-1}$ and 25.5 $\mu\text{g.kg}^{-1}$, respectively. In hull cracking fruits, aflatoxins B₁ and B₂ were 111.1

$\mu\text{g.kg}^{-1}$ and 9.7 $\mu\text{g.kg}^{-1}$, respectively. No aflatoxin was observed in intact hull pistachio nuts. In some samples of early split pistachio nuts, the amount of aflatoxin was observed to be up to 550 $\mu\text{g.kg}^{-1}$ (Table 3).

Table 3. Occurrence of Aflatoxin B1 and B2 in pistachio nut samples during 4 years

Sample Type	Number of sample	Aflatoxin B1 ($\mu\text{g.kg}^{-1}$)			Aflatoxin B2 ($\mu\text{g.kg}^{-1}$)		
		Min	Max	Mean	Min	Max	Mean
Early Split	40	15.25	549.2	223.4	16.5	52.45	25.47
Cracked hull	40	78.87	145.28	111.06	7.05	15.56	9.71
Intact Hull	40	ND*	ND	ND	ND	ND	ND

*Not Detected

4. Discussion

The results of the current study demonstrated that deficit irrigation in late spring and an increase in the irrigation frequency from 25 to 45 days in pistachio orchards increased the early splitting of pistachio nuts. Doster *et al.* (2001) observed that deficit irrigation in April or May increased early split nuts [6]. Furthermore, it has been demonstrated that the time of irrigation is critical to infection especially in early splitting cultivars [4-5, 13-15]. It has been shown that the aflatoxin contamination rate in early-split nuts was 50 times higher than non-split nuts [17]. They also reported that the aflatoxin contamination rate in non-split nuts was less than $2.0 \mu\text{g.kg}^{-1}$, while the amount of aflatoxin in early split nuts was higher than $20 \mu\text{g.kg}^{-1}$ in some, and even higher than $1000 \mu\text{g.kg}^{-1}$ in some other, being similar to the results of this study.

Critical parameters such as cracking the hull, environmental factors, cultural practices, insect damage, the irrigation time, plant litter, animal manures, the frequency of toxigenic strains and the harvest date may affect the contamination of pistachio nuts [1-2, 4-5, 10-12, and 16-18]. Based on the results of the current study, cultivators are required to water pistachio orchards in spring to reduce the infection of pistachio nuts with various kinds of aflatoxins.

5. Conclusions

Based on the results, long irrigation intervals and irregular irrigation increase the frequency of early split pistachio nuts. All and all, the drought stress in late April to early June severely affects the frequency of early splitting pistachio nuts and consequently leads to aflatoxin contamination. Therefore, it is recommended that the appropriate irrigation interval be selected based on the soil texture, the water quality, soil salinity, the age of trees and the irrigation method.

Conflict of interest

The authors declare no conflicts of interest.

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