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ORIGINAL ARTICLE

Aflatoxin Production Potential in *Aspergillus* Section *Flavi* Isolates Obtained from Almond Fruits in Different Regions of Iran

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K E Y W O R D S

Almond;
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A B S T R A C T

Almond is one of the most important nut fruits, which is widely consumed in Iran and the world due to its high nutritional value and importance in health. Investigations have shown that the contamination of nut fruits to *Aspergillus flavus* and aflatoxin can threaten the health of the community. Therefore, it is necessary to investigate the potential for aflatoxin production in *Aspergillus* section *Flavi* isolates in the main almond-growing areas of Iran. In this research, 95 almond fruit samples were collected from important almond-growing areas of Iran. The samples were cultured by serial dilution method in differential AFPA culture medium and 100 isolates of *Aspergillus* section *Flavi* were identified. In the next step, the toxin-producing capacity of the isolates were investigated. Three culture media including coconut agar medium (CAM), yeast extract sucrose (YES) medium modified with methyl-β-cyclodextrin, and YES medium exposed to ammonia vapor were applied to identify and screen the toxigenic and atoxigenic isolates. After the initial screening and the selection of toxigenic isolates, in order to measure the toxigenic potential of isolates, the fungus isolates were cultured on the medium of sterile crushed rice medium and toxigenic potential was done using thin layer chromatography (TLC) method. The results showed that out of 100 fungal isolates, 10 isolates were unable to produce any type of aflatoxin, while 90 isolates were able to produce one or more types of aflatoxin. Seven fungal isolates were capable of producing four types of aflatoxins (B_1, B_2, G_1, G_2), 10 isolates were able to produce three types of aflatoxins (B1, B2 and G1) and 33 isolates were able to produce two types of aflatoxins (B1 and B2). 40 fungal isolates were only able to produce aflatoxin B1. Among the toxin-producing isolates, the production range of aflatoxin B1 was between 24 and 17129, aflatoxin B2 between 43 and 8391, aflatoxin G1 between 132 and 2957 and aflatoxin G_2 between 61 and 212 ng g^{-1}.

Introduction

Almond (*Prunus dulcis*) is one of the most important nut fruit products in the world. Due to its favorable climate, Iran is one of the world's significant almond producers (Imani *et al*., 2021; Ansari and Gharaghani, 2019; Ziaolhagh, 2012). The area under cultivation of almond in Iran is 154,413

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hectares, of which 16,377 hectares are non-bearing orchards and 138,036 hectares are bearing orchards. Fars, Razavi Khorasan, Chaharmahal and Bakhtiari, Kerman and Isfahan provinces have the largest area under almond cultivation in Iran, respectively. The total production of almonds in Iran is 119,221 tons, and the average yield in irrigated and rainfed orchards is 1107 and 522 kg per hectare, respectively (Statistics of Horticultural Products of Iran (Anonymous, 2022). Australia, Spain, Italy, China, Turkey, Morocco and Iran have been the major almond producing countries in recent years. In 2022, the production of almonds in the world is reported to be 3,630,427 tons (in shell), and the United States of America has the highest production of almonds with 1,858,010 tons (FAOSTAT, 2020).

Aflatoxin contamination is one of the most serious food health problems worldwide (Abbass *et al*., 2005). Aflatoxins are highly toxic secondary metabolites that are responsible for many carcinogenic, mutagenic, and harmful effects in humans and animals (Mahbobinejhad *et al.*, 2019). The liver is the main target of aflatoxins and consumption of contaminated food over an extended period of time may lead to adverse consequences for the liver, including damage to hepatic tissues and cells (Valasi *et al*., 2021, Daliri, 2023, Razzaghi-Abyaneh, 2006). So far, 18 types of aflatoxins are known, and only aflatoxins B1, B2, G1 and G2 have been identified in agricultural products (Torre *et al*. 2015; Aydin and Ulvi 2019; Ehrlich *et al*., 2007). Among aflatoxins, aflatoxin B1, as a lipophilic metabolite has the most toxicity and is able to accumulate in liver tissues and cause cancer. While in the case of the other three aflatoxins, their carcinogenic properties have not been proven (Taghizadeh *et al*., 2018). These highly toxic secondary metabolites are produced by some species of fungi including *Aspergillus flavus*, *A. parasiticus*, *A. bombycis*, *A. nomius*, *A. pseudotamari*, *A. ochraceoroseus* and two anamorphic species with *Aspergillus*, *Emericella venezuelensis* and *E. astellata* (Frisvad and Samson, 2004; Frisvad *et al*., 2005; Klich *et al*., 2000). However, they are mainly produced by two species of *A. flavus* and *A. parasiticus* (belonging to section *Flavi*). Aflatoxin B1 is mainly produced by *Aspergillus flavus* fungi, however, belonging to the *A. flavus* group cannot be a definitive reason for its toxin production (Allameh and Razzaghi-Abyaneh, 1999) and the production of any type of mycotoxin depends not only on the species, but also on the isolate of the producing fungus.

Aflatoxin contamination of almonds has been reported in various countries. In Spain, in 1986 and 1987, out of 38 almond samples collected from retail stores, the aflatoxin contamination level of one sample was 110μg kg^{-1}, and all samples had fungal contamination (Jimenez *et al*., 1991). Investigations in Japan showed that 7 out of 12 samples of almonds imported to that country were contaminated with aflatoxin, but the contamination less than 10 ng g^{-1} was determined as the permissible threshold for that country (Adachi *et al*., 1991). In 1997, twelve of the 28 samples examined in Pakistan were contaminated with aflatoxin (Nizami & Zuber, 1997). In 1984, in Italy, 74% of different samples of sweets containing almond kernel had less than 0.5 μg kg^{-1} of aflatoxin B (Finoli *et al*., 1994).

In Iran, contamination of pistachios, peanuts, corn, wheat, figs, hazelnuts and some medicinal plants with aflatoxin has been reported. Almond fruit is attacked by pests in the pre-harvest stage and when it is still on the tree. Also, during the post-harvest stages, until it is transferred to suitable covered warehouses, it is damaged by several factors, and this causes its contamination with aflatoxigenic fungi (Fuller *et al*., 1977; Parcell *et al*., 1980; Schade *et al*., 1975). Almond kernels contain approximately 50-60% oil, predominantly unsaturated. As a result, they are prone to rancidity caused by auto-oxidation and fungal infestation during extended storage periods (Ziaolhagh, 2012).

Aflatoxin production is also affected by various factors such as the genetic characteristics of the producing fungi and the physicochemical environment in which they grow. Occurrence of environmental stress, wounding of hard skin (shell) and insect activity causes high contamination. Damaged kernels and discolored kernels have the most aflatoxin contamination (ABC, 2002). In the research, it was found that the contamination was high only in the parts of the fruit where the pests have paved the way for the spores of aflatoxin-producing fungi to enter the kernel (Schatzki and Ong, 2001, 2000).

Despite the fact that Iran is one of the most important almond-producing countries in the world, there has not been a comprehensive study on the contamination of almonds to *A. flavus* and aflatoxin. In recent years, the main and important problem of the country in the nut fruit export has been the issue of aflatoxin contamination, which has threatened this source of foreign exchange income and prevented us from competing in the global market. Therefore, in this study, in addition to the isolation and identification of *Aspergillus* section *Flavi* fungi from almond fruit kernel samples, their aflatoxigenic properties and intensity were also investigated.

Materials and Methods

In order to investigate the contamination status of almonds in different regions of Iran to *Aspergillus* section *Flavi* and aflatoxin, 95 samples from the provinces of Fars, Razavi Khorasan, South Khorasan, Chaharmahal and Bakhtiari, Isfahan, Zanjan, Lorestan, East Azarbaijan, Kermanshah, Hamedan, Semnan and Markazi were collected. To prepare each sample, 10 subsamples of one kilogram of almond fruit were prepared from each region and after mixing these 10 subsamples together, half a kilogram of it was considered as the main sample and the status of *Aspergillus flavus* contamination was investigated. Since the estimation of contamination to *Aspergillus* section *Flavi* fungus requires obtaining a homogenous and completely uniform sample, the kernels of the fruit samples were first ground.

The homogenous samples were then cultured using the serial dilution method in differential AFPA (*Aspergillus Flavus* and *Parasiticus Agar*) culture medium (Mohammadi Moghadam *et al*., 2020).

This differential culture medium is used to screen and identify fungal species belonging to *Aspergillus* section *Flavi* (Pitt and Hocking, 1997). In this culture medium, two species, *A. flavus* and *A. parasiticus*, can be identified by the production of yellow to olive green spores and formation a bright orange color on the back side of the growing colony (Raper and Fennel, 1965, Samson *et al*., 2006). AFPA culture medium has the feature of preventing the sporulation of colonies of species belonging to Flavi section, as a result, the fungus colonies are distinct and separate and can be easily separated from each other and can be counted.

For this purpose, 10 grams of each ground sample was added to 90 ml of 0.1% peptone water and shaken on a shaker for 20-40 minutes. 0.1 ml of 10^{-1} and 10^{-2} dilutions was spread on the surface of Petri dishes containing AFPA culture medium. Petri dishes were kept at a temperature of 28 ℃ and two to three days later, *Aspergillus flavus* fungi colonies were identified, counted and isolated. By counting the number of colonies on the surface of the Petri dishes, the level of contamination in each sample was determined.

Investigating the aflatoxigenic properties of Aspergillus section Flavi isolates

Considering the fact that all the isolates of *A. flavus* may not be able to produce aflatoxin, and the type and intensity of toxin production of different isolates of the species belonging to section Flavi are also very different from each other, evaluation of aflatoxin production potential in this group of fungi is very important. Therefore, after isolation of *Aspergillus* section *Flavi* fungi, coconut agar medium (CAM), Yeast Extract Sucrose (YES) medium

modified with methyl-β-cyclodextrin and YES medium exposed to ammonia vapor were used to identify and screen the toxigenic and atoxigenic isolates. Toxigenic and atoxigenic strains were screened using fluorescence detection (FD) and ammonia vapor (AV) assays. Thin layer chromatography (TLC) method was used in order to more accurately measure the toxigenecity potential and intensity of the isolates.

Coconut Agar Medium (CAM)

The isolates were grown for 72 hours in coconut agar medium at 28°C and kept in the dark. Toxin production of the isolates was investigated by observing the presence (no aflatoxin production) or absence (aflatoxin production) of fluorescent halo around the colonies when exposed to UV rays with a wavelength of 365 nm after three days.

YES medium ambedded with methyl-β-cyclodextrin

1.5 g methyl-β-cyclodextrin (Sigma-Aldrich, Germany) was dissolved in sterile distilled water and passed through a 22 µm filter and added to 500 ml sterilized YES culture medium (100 g sucrose, 10 g yeast extract and 10 g agar) and poured into Petri-dishes (Fente *et al*., 2001). The isolates were grown in modified medium at 28°C for three days and kept in the dark. The toxin production capacity of the isolates was investigated using the previously described method.

YES medium exposed to ammonia vapor

In order to differentiate between toxigenic and atoxiganic isolates, YES medium was exposed to ammonia vapor, so that the color of the colonies would change to pink or red after three days (Satio and Machida, 1999). It should be noted that isolates without a change in colony color to pink or red are considered atoxiganic (Fani *et al*., 2014). In this way, at this stage, the toxigenic isolates were identified and separated from the atoxiganic isolates through the initial screening.

Inoculation of Aspergillus section Flavi isolates on rice flour and Thin-layer Chromatography (TLC) assay

Studies have shown that natural substrate such as corn, rice and wheat are better substrates for *A. flavus* growth and toxin production than artificial media. Therefore, after the initial screening using the culture medium and the selection of toxigenic isolates, in order to ensure the toxigenic properties and intensity of the toxigenic isolates, the fungi isolates were cultured on rice flour as natural substrate. For this purpose, after preparing the fungus spore suspension from each isolate, 1 ml spore suspension (concentration of 10^3 spores per ml) was cultured on 22 g sterile rice flour and kept at 28 ℃ for one week (Magnoli *et al*., 1998). Then aflatoxin of rice samples was extracted using chloroform and methanol. The toxigenic capacity of the isolates was measured using Thin Layer Chromatography (TLC) method (Trucksess *et al*, 1990).

Statistical analysis

The used design was completely random with three replication and the obtained data were statistically analyzed by SPSS statistical software. Means were compared using Duncan's multiple range test at the 5% level.

Results

Investigating Aflatoxigenic properties of Aspergillus section Flavi isolates

For the initial screening of the *Aspergillus* section *Flavi* isolates, the isolates were examined in terms of appearance characteristics on differntial AFPA medium. In this culture medium, two species *A. flavus* and *A. parasiticus* could be identified by the production of yellow to olive green spores and formation a bright orange colors on the reverse side of the growing colony (Raper and Fennel, 1965; Samson *et al*., 2006) (Fig. 1). In total, 100 isolates belonging to *Aspergillus* section Flavi were separated from

almond samples of different regions of the country and identified based on their morphological characteristics (Klich, 2002).

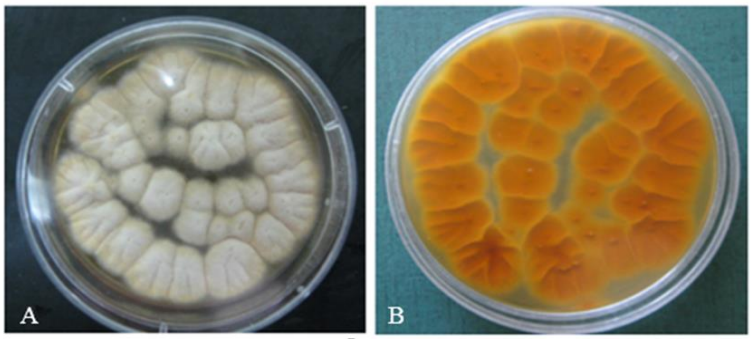

Fig. 1. A view of the growth and colonization *of A. flavus* on (A) and reverse (B) sides of differntial AFPA medium.

Identification of toxigenic isolates of Aspergillus section Flavi on CAM and YES media

In coconut agar medium (CAM) and YES medium modified with methyl-β-cyclodextrin, blue fluorescent light is observed around the toxigenic isolates under the wavelength of 365 nm UV rays (Fig. 2). In the culture method in YES medium exposed to ammonia vapor, isolates with a change in the color of the colony to pink or red were considered to be toxigenic (Fig. 3). In general, based on the color change of the colony to pink or red, out of 100 isolates, 16, 19 and 14 atoxigenic isolates were identified in coconut agar medium, YES culture medium modified with methyl-β-cyclodextrin and YES cultures medium exposed to ammonia vapor, respectively.

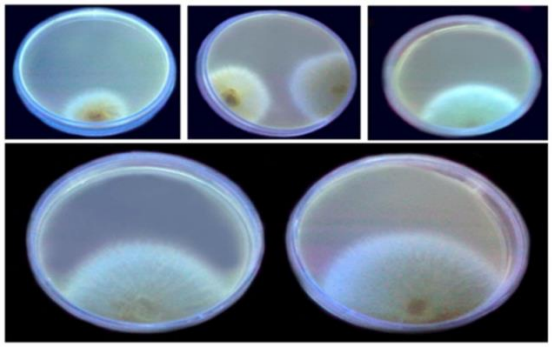

Fig. 2. Blue fluorescent light around the colonies of toxigenic isolates and the absence of fluorescent light in atoxigenic isolates on YES medium modified with methyl-β-cyclodextrin under the wavelength of 365 nm UV rays.

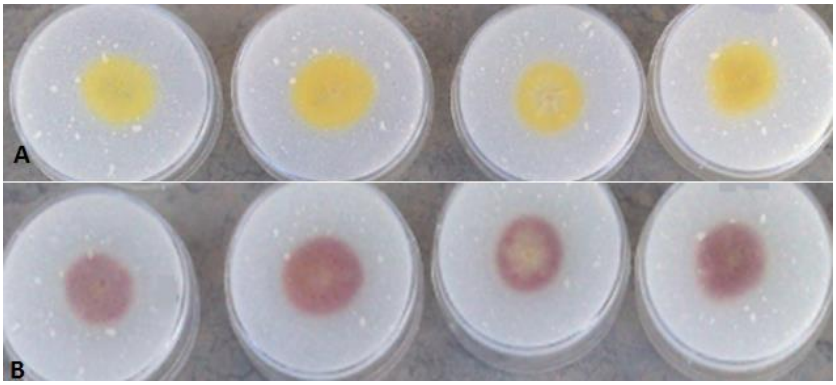

Fig. 3. Distinguishing toxigenic isolates of *A. flavus* from atoxigenic isolates, using the method of no change (A) or color change (B) of the reverse the colony to pink or red color in YES medium exposed to ammonia vapor

Determination of aflatoxin production capacity in isolates using culture in sterile rice flour medium and TLC assays

Determination of aflatoxin production capacity in isolates using culture on the sterile rice flour medium and TLC assays showed that only 10 isolates were not able to produce any type of aflatoxin. The frequency of the number of atoxigenic isolates obtained from the three previous culture media mentioned above compared to 10% (10 isolates) obtained in this method indicates a 4-9% error in the diagnosis of atoxigenic isolates. This issue indicates the relative ability of the culture media used to isolate atoxigenic isolates, which causes overestimation of the abundance of atoxigenic isolates. Therefore, it is necessary to use more accurate methods in addition to the culture medium for initial screening.

Out of 100 isolates of *A. flavus* studied, 90 isolates (90%) were able to produce one or more types of aflatoxin (G1, B2, B1 and G2) and 10 isolates (10%) were not able to produce any type of aflatoxin. Among the fungal isolates, 7% isolates (7 isolates) produced all four types of aflatoxin B1, B2, G1 and G2. 10% isolates (10 isolates) produced three types of aflatoxin B1, B2 and G1, 33% isolates (33 isolates) also produced aflatoxins B1 and B2, and 40% isolates (40 isolates) produced only aflatoxin B1. Among the toxigenic isolates of *Aspergillus* section *Flavi*, the strength and intensity of toxin production varied from strong to weak, so that the ranges of changes in the production of B1, B2, G1 and G2 aflatoxins were 24-17129, 43- 8391, 132-2957 and 61-212 ng g^{-1}, respectively (Table 1).

Table 1. Determination of aflatoxin production capacity of *Aspergillus* section *Flavi* isolates in almond fruit samples collected from different cultivation areas of the country

Aflatoxin type (ng g^{-1})				Isolates	Aflatoxin type (ng g^{-1})				Isolates
G_2	G_1	B_2	B_1		G_2	G_1	B_2	B_1	
ND	ND	+	+	1	ND	ND	ND	ND	51
ND	ND	+	+	2	+	+	+	+	52
ND	+	+	+	3	ND	+	+	+	53
ND	ND	ND	+	4	ND	ND	+	+	54
ND	ND	+	+	5	ND	ND	ND	+	55
ND	ND	+	+	6	ND	ND	ND	+	56
ND	ND	+	+	7	ND	ND	ND	+	57
ND	ND	ND	+	8	ND	ND	ND	ND	58
ND	ND	ND	ND	9	ND	ND	+	+	59
ND	+	+	+	10	ND	ND	+	+	60
ND	ND	+	+	11	ND	ND	ND	+	61
ND	+	+	+	12	ND	+	+	+	62
ND	ND	+	+	13	ND	ND	+	+	63
ND	ND	ND	+	14	ND	ND	ND	+	64
ND	ND	ND	+	15	ND	ND	+	+	65
ND	ND	ND	+	16	ND	ND	ND	+	66
ND	ND	+	+	17	ND	ND	+	+	67
ND	ND	ND	+	18	ND	ND	+	+	68
+	+	+	+	19	ND	ND	+	+	69
ND	ND	ND	+	20	ND	ND	ND	+	70
ND	ND	ND	+	21	ND	+	+	+	71

Aflatoxin type (ng g^{-1})				Isolates	Aflatoxin type (ng g^{-1})				Isolates
G_2	**G_1**	**B_2**	**B_1**		**G_2**	**G_1**	**B_2**	**B_1**	
ND	ND	ND	+	22	ND	ND	+	+	72
ND	ND	ND	ND	23	ND	ND	ND	+	73
ND	ND	+	+	24	ND	ND	+	+	74
+	+	+	+	25	ND	ND	ND	+	75
ND	+	+	+	26	ND	ND	+	+	76
ND	ND	+	+	27	ND	ND	ND	+	77
ND	ND	ND	+	28	ND	ND	+	+	78
ND	ND	ND	ND	29	ND	ND	ND	ND	79
ND	ND	ND	+	30	ND	ND	ND	+	80
ND	ND	ND	+	31	ND	ND	ND	+	81
ND	ND	ND	+	32	ND	ND	ND	+	82
+	+	+	+	33	ND	ND	ND	+	83
ND	ND	ND	+	34	ND	ND	ND	+	84
ND	ND	+	+	35	ND	ND	+	+	85
ND	ND	ND	+	36	ND	ND	ND	+	86
ND	ND	+	+	37	ND	ND	+	+	87
ND	ND	ND	+	38	ND	ND	ND	ND	88
ND	ND	+	+	39	ND	ND	+	+	89
ND	ND	+	+	40	ND	+	+	+	90
ND	ND	+	+	41	ND	ND	+	+	91
ND	ND	ND	+	42	ND	ND	ND	+	92
ND	ND	ND	ND	43	ND	ND	ND	ND	93
ND	+	+	+	44	ND	+	+	+	94
+	+	+	+	45	+	+	+	+	95
ND	+	+	+	46	ND	ND	ND	+	96
ND	ND	ND	+	47	ND	ND	ND	ND	97
ND	ND	ND	+	48	ND	ND	ND	+	98
+	+	+	+	49	+	+	+	+	99
ND	ND	ND	+	50	ND	ND	ND	+	100

ND = Not Detected

Discussion

One of the most important challenges for food security and the health of human society is the presence of mycotoxins, especially aflatoxins in agricultural products, especially nut fruits. This issue has caused the approval of strict laws and regulations and spended a lot of costs to manage contamination at different levels from production to consumption. Aflatoxins are carcinogenic and mutagenic secondary metabolites and polyketide derivatives that are produced by *Aspergillus flavus*, A. *parasiticus*, *A. nomius*, *A. pseudotamari and A. bombycis* (Bennett and Klich, 2003).

Considering that *A. flavus* fungus attacks a wide range of agricultural products, it is essential to isolate and identify *Aspergillus* section Flavi fungi from contaminated almonds and determine their aflatoxin production capacity. Therefore, in this research, the contamination status of almond fruits of Fars, Razavi Khorasan, South Khorasan, Chaharmahal and Bakhtiari, Isfahan, Zanjan, Lorestan, East Azarbaijan, Kermanshah, Hamedan, Semnan and Markazi

provinces and the toxigenic property of *A. flavus* isolates were investigated. Initial screening of toxigenic isolates from atoxigenic isolates was done by culturing on Coconut agar medium (CAM), Yeast Extract Sucrose (YES) medium modified with methyl-β-cyclodextrin and YES medium exposed to ammonia vapor. After that, toxigenic isolates were cultured in the sterile natural rice medium and their toxigenic ability was evaluated by thin layer chromatography. The comparison of initial screening methods for atoxigenic isolates indicated the existence of differences between them, and there was a 4-9 % error in the diagnosis of atoxigenic isolates. Therefore, it is necessary to use more accurate methods combined with the culture medium method for screening. According to the results of TLC, among the isolates of *Aspergillus* section Flavi, a small percentage of the investigated isolates were atoxigenic (10% of the isolates). Among the culture media, the method of changing or not changing the color on the reverse side of the growing colony to pink or red in the YES culture medium exposed to ammonia vapor was the most effective for detecting toxigenic isolates, followed by the media of Coconut agar medium, Yeast Extract Sucrose medium modified with methyl-β-cyclodextrin. It can be said one of the basic problems in the use of culture media such as YES modified with methyl-β-cyclodextrin and YES with the use of ammonia vapor is the low efficiency in identifying isolates with low aflatoxin generation ability and these culture media are not able to detect these isolates and they are considered as atoxigenic.

The results of investigating the toxinogenic properties of *Aspergillus* section Flavi isolates obtained from the kernels of almond fruits collected from different regions of the country indicate the existence of genetic diversity in the investigated isolates in terms of the ability to produce aflatoxin, which is consistent with the researches of Fani *et al*. (2014), Zamani (2009), Alibakhshi *et al* (2011) and Mohammadi *et al*. (2009). Out of the 9709 samples of raw almonds and semi-processed almonds that were examined for the presence of aflatoxin in the years 1993 to 1999 in the United States, 983 samples (10 percent) were contaminated with aflatoxin and had less than 5 μg kg^{-1} of aflatoxin. In another study in 1993, among 1547 almond samples, 19 broken fruit samples, 84 samples from factory warehouses and 8 ungraded samples had more than 1 μg kg^{-1} of aflatoxin (Schatzki, 1996). In another study, out of 74 unsorted almond samples in 1972, 14% were contaminated, and the amount of contamination in the samples was less than 20 ng g^{-1} (Schade *et al*., 1975). From 241 samples examined in Egypt that were stored in inappropriate conditions, the most contaminations were related to almonds. Aflatoxin was detected in moldy samples more than others, and among the four aflatoxins, aflatoxin B_1 was the most common (Qutet *et al*., 1983). The potential for toxin production in fungal isolates in other products has also been investigated. Zamani (2009) investigated the population structure of *A. flavus* based on the aflatoxin production ability of Canola and Peanut seeds isolates. The results showed that 63% of the isolates were aflatoxigenic and 36.58% were non aflatoxigenic. Alibakhshi (2011) investigated the effect of hyphal anastomosis in the transfer of genetic material between toxin-producing and non-toxin-producing isolates of *A. flavus* isolated from pistachio fruits. According to the ELISA test, 80% of the isolates produced aflatoxin and 19% were non-toxin. Fani *et al*, (2014) investigated the distribution of atoxigenic strains of *Aspergillus flavus* in pistachio cultivation areas. The results showed that among 524 isolates of *A. flavus*, 53 and 10 isolates from fruit and soil, respectively, were unable to produce aflatoxin. The frequency of atoxigenic isolates varied from 6.2 to 25%. Afshari *et al* (2022) investigated the *Aspergillus* growth and aflatoxin production in fresh pistachios stored in different temperatures and packagings. Aflatoxins were quantified by HPLC and the fungal population was monitored by dilution series method and AFPA medium. The results showed that aflatoxin

contamination started on the fourth day after storage in the ambient conventional temperature and increased over time. The results showed that in the 4°C treatment, the fungal population was significantly less than in ambient temperature treatments.

According to the results of TLC, among the isolates of *Aspergillus* section Flavi, a small percentage of the investigated isolates were atoxigenic (10% of the isolates). Various strategies of chemical, physical and biological control during before and post-harvest of agricultural and garden crops have been proposed to reduce the damage caused by toxigenic fungi. From the pre-harvest strategies that are very promising and many works have been done in this regard around the world, biological control using endophytic bacteria such as Bacillus mojarensis, *B. Subtilis* and atoxigenic isolates of *A. flavus* can be mentioned. The success in biological control of aflatoxin-producing fungi in most cases depends on the use of native isolates of each region. Therefore, it is essential to investigate the aflatoxin production properties and population diversity of *A. flavus* in terms of aflatoxin production in different regions of the country and to separate the atoxigenic isolates of each region for population management of toxin-producing strains. In the present study, 10% of *Aspergillus* section *Flavi* isolates from almond fruits obtained from different regions of Iran were atoxigenic isolates. This shows the potential of these atoxigenic isolates for the biological control of aflatoxigenic fungi, and they can be useful in additional research for field use in different almond-growing areas.

Conclusions

Investigation of the potential for aflatoxin production in *Aspergillus flavi* isolates in almond samples from different regions of Iran showed that among the toxin-producing isolates, the production range of aflatoxin B1 was between 24 and 17129, aflatoxin B2 between 43 and 8391, aflatoxin G1 between 132 and 2957 and aflatoxin G2 between 61 and 212 ng g^{-1}. In addition, all culture media estimate the population of atoxigenic isolates more than its actual value, but due to its simplicity, cheapness, high speed and no need for complex laboratory facilities and technical staff, it is considered as a quick method for the primary screening of *Aspergillus* section Flavi isolates. Therefore, due to the high cost of analytical methods such as TLC and HPLC, it is suggested to perform the initial screening of fungal isolates with a culture medium such as CMA and YES modified with methyl-beta-cyclodextrin and ammonia vapor, and in the next step, precise laboratory methods such as thin layer chromatography, ELISA and high-performance liquid chromatography (HPLC) should be used for the final diagnosis of toxin-producing isolates. Based on TLC analysis, out of 100 fungal isolates, 10 isolates were unable to produce any type of aflatoxin, while 90 isolates were able to produce one or more types of aflatoxin. Seven fungal isolates were able to produce four types of aflatoxins (B1, B2, G1, G2), 10 isolates were able to produce three types of aflatoxins (B1, B2 and G1) and 33 isolates were able to produce two types of aflatoxins (B1 and B2). 40 fungal isolates were only able to produce aflatoxin B1. These findings show that almond fruit samples collected from different regions of the country had aflatoxin contamination.

Conflict of interests

All the authors declare that there is no conflict of interest in the study.

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