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Aflatoxin Risk in Pepper Crops and Flaked Pepper in Southeastern Anatolia, Turkey

Hayrettin ÖZER [1], Fatma KAPLAN [2*], Ayşin BİLGİLİ [3]
[1] Turkish Scientific and Technical Research Council, Marmara Research Centre Food Institute, Kocaeli
[2] Harran University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Şanlıurfa
[3] GAP Agricultural Research Institute, Department of Plant Health, Şanlıurfa
[*]Corresponding author: fatmakaplan@harran.edu.tr

Abstract
The formation of aflatoxins in agricultural products, especially in peppers used as spices, has been determined that the products are affected by various stages from the field to the spice. These toxins are formed when fungi belonging to the genus *Aspergillus* spp. colonize the plant during the growing stage and produce aflatoxins. Post-harvest processes, especially drying and storage, significantly affect aflatoxin levels. Toxin accumulation can increase when drying processes are not carried out under appropriate conditions, while during storage, factors such as humidity and temperature can promote aflatoxin production. The aim of this study was to determine the risk of aflatoxin in pepper plants and chili peppers used as spices. Various aflatoxin species were detected in the analysis of samples taken from pepper fields and spice shops in Southeastern Anatolia, Turkey. While AFG1 and AFG2 were found in one of the 15 chili pepper samples, AFB2 was detected between 0.0125 and 0.1875 ppb in 11 samples and AFB1 was detected between 0.1125 and 3.95 ppb in 13 samples. Total aflatoxin content ranged from a minimum of 0.15 ppb to a maximum of 4.1375 ppb with an average of 0.9455 ppb. In addition, when the aflatoxin contents of *Aspergillus* spp. fungi obtained from pepper plants taken from the field were analyzed, the presence of AFB1 and AFB2 was detected in some samples. These findings suggest that aflatoxin formation starts at the growing stage of peppers and that harvesting, drying and storage processes can increase toxin levels. Therefore, these processes need to be carefully managed and controlled to minimize aflatoxin risk.

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1. Introduction

Microorganisms can be present in the flora of agricultural products and various foodstuffs, or they can be transmitted to these products in different ways (Bilgili, 2022). Foods undergo changes under favorable conditions by contact with microorganisms in their natural environment or in unhygienic conditions. Fungi constitute a large part of these changes. The presence of fungi in foods and their consumption by humans adversely affects health (Baysal, 1984). These fungi have the ability to produce chemical substances with carcinogenic, teratogenic, mutagenic, and toxic content for humans and animals. These substances are defined as mycotoxins (Vıladımır, 1989). Mycotoxins are considered a major problem worldwide public health, agriculture, international trade, and the economy (Özer et al., 2012; Özer et al., 2017b). Approximately 10 % of the world's crops are destroyed each year due to fungal infections (Zhang et al., 2012). Losses due to mycotoxins are only a part of this. Aflatoxins in agricultural products are the most toxic mycotoxins and one of the most important microbiological problems of recent years (Bilgili, 2022).

Aspergillus spp. fungal species are widespread worldwide and can easily contaminate foodstuffs. This is especially a problem in tropical and subtropical climatic zones where environmental conditions such as high humidity and temperature prevail (Adeyeye, 2019). *Aspergillus flavus* fungus can infect field and soil crops such as barley, maize, cottonseed, rice, wheat, peanuts, cocoa, pistachios, hazelnuts, spices, olives, figs, sunflowers and capsicum and can multiply during storage. When environmental conditions are favorable, toxigenic strains of these organisms can produce aflatoxins (Öksüztepe and Erkan, 2016).

Mycotoxins are secondary metabolites of some sub-fungi that grow and multiply in agricultural products at all stages from field to consumption depending on ecological conditions. These toxins are produced by some fungi such as *Aspergillus*, *Penicillium*, and *Fusarium* under certain humidity and temperature conditions (Kumar et al., 2008). Aflatoxins are metabolites of *Aspergillus flavus* and *Aspergillus parasiticus* species known as *Aspergillus flavus* group. Aflatoxins, as a type of mycotoxins, are a major concern and pose a serious global health problem due to their high toxicity and widespread availability (Özer et al., 2012; Özer et al., 2017a). The most common mycotoxins are aflatoxin (AF), ochratoxin, trichothecene, zeranol, patulin, cyclopiazonic acid, and fumonisin (Kumar et al., 2008; O'Riordan and Wilkinson, 2008).

The color, taste, and shape of the peppers supplied for the production of red powder and chili peppers, which are consumed as a popular spice in Turkey, broadly (Ateş et al., 1992). The production of pepper products in our country is especially widespread in the Southeastern Anatolia Region, Gaziantep, and its surroundings (Ayda, 2020). In addition, various pepper products are produced from pepper with traditional methods in Şanlıurfa (Altun et al., 2020).

Dried spices are susceptible to fungal growth and toxin formation due to their growing conditions, processing characteristics and sensitivity during storage (Peter and Cotty, 2017). Pepper production is carried out under primitive conditions, increasing the risk of fungal contamination. Peppers are collected from the fields where pepper is grown, cut into small pieces, laid on the soil and dried, then pulverized in the mill and sold in sacks. Peppers produced under these primitive conditions are frequently contaminated with soil-borne fungi with toxicogenic effects and toxin formation (Yıldırım, 1996). However, in developed countries, contamination is kept to a minimum since the drying process is carried out using special devices (Semple et al., 1989). Especially *Aspergillus flavus* can cause problems in many products grown in tropical and subtropical climates. Various studies have indicated that fungi cannot survive in unsuitable conditions after producing toxins, but the toxins they produce can remain in the product (Taydaş, 1993).

Studies have shown that pre-harvest aflatoxin production is caused by climatic stresses such as drought, high temperature and rainfall, climatic conditions that are not suitable for the genotype of the plant and insect damage (Wu and Khlangwiset, 2010). Post-harvest toxin contamination can occur as a result of improper agricultural practices during storage, transportation and in the food processing industry (Halkman, 2013). In red pepper, fungal contamination starts while the fungus is still on the plant and continues until the end of the harvest period and then during the drying process. It has been determined that aflatoxin starts to form when the pepper fruit is on the branch (Ünlütürk and Turantaş, 1998).

Among aflatoxins, aflatoxin B1 is known to have the highest toxic effect (Gürhayta and Çağındı, 2015). Aflatoxins (B1, B2, G1 and G2) have been found to have acute toxic effects on animals used as test subjects. However, aflatoxins have carcinogenic properties rather than acute toxic effects. It was determined that aflatoxin B1 caused liver cancer in test animals. Consumption of aflatoxin-containing foods at certain intervals causes liver tumors in humans and aflatoxins have been found to cause teratogenic and mutagenic effects in a study on animals (Betına, 1989). According to the Turkish Food Codex Regulation on Contaminants (TGK, 2011) and the European Union food legislation (EU, no 165/2010), the permissible limit for aflatoxin B1 is set at 5 ppb and 10 ppb for total aflatoxin (Anonymous, 2010). However, aflatoxin limits vary from country to country, making international trade difficult (Özer et al., 2017b).

This study aims to evaluate the presence of aflatoxins, known for their carcinogenic, teratogenic, hepatotoxic, and mutagenic effects, in red pepper and to assess their transmission from field to table. Fungal samples collected from the roots of pepper plants in the provinces of the Southeastern Anatolia region of Turkey were analyzed, and soil-borne fungi, including *Aspergillus* spp., *Aspergillus niger*, and *Aspergillus flavus*, were identified (Bilgili, 2017; Bilgili, 2022). Additionally, chili pepper samples were gathered from the spice market in Balıklıgöl, Şanlıurfa. Aflatoxin levels were measured in both the fungal samples taken from the pepper plant roots and in the chili powder samples. The results indicate that fungi originating in the root zone of the pepper plant can be transferred to the fruit through transport and contamination processes. The initial contamination is likely to occur due to conditions in the root zone and can proliferate under favorable environmental conditions. Consequently, this study focuses on the investigation of four types of aflatoxins B1, B2, G1, and G2 which have carcinogenic potential in both pepper plants and chili peppers.

2. Materials and Methods

2.1.Experimental materials and tools

In this study, 9 fungal samples from diseased plants collected from pepper cultivation fields in different provinces of the Southeastern Anatolia region of Turkey were used (Figure 1). In addition, 15 chili pepper samples were taken from spice shops by random sampling method in Balıklı Lake spice shops bazaar in Şanlıurfa province and 50 grams of chili pepper for each sample was used in this research (Figure 2).

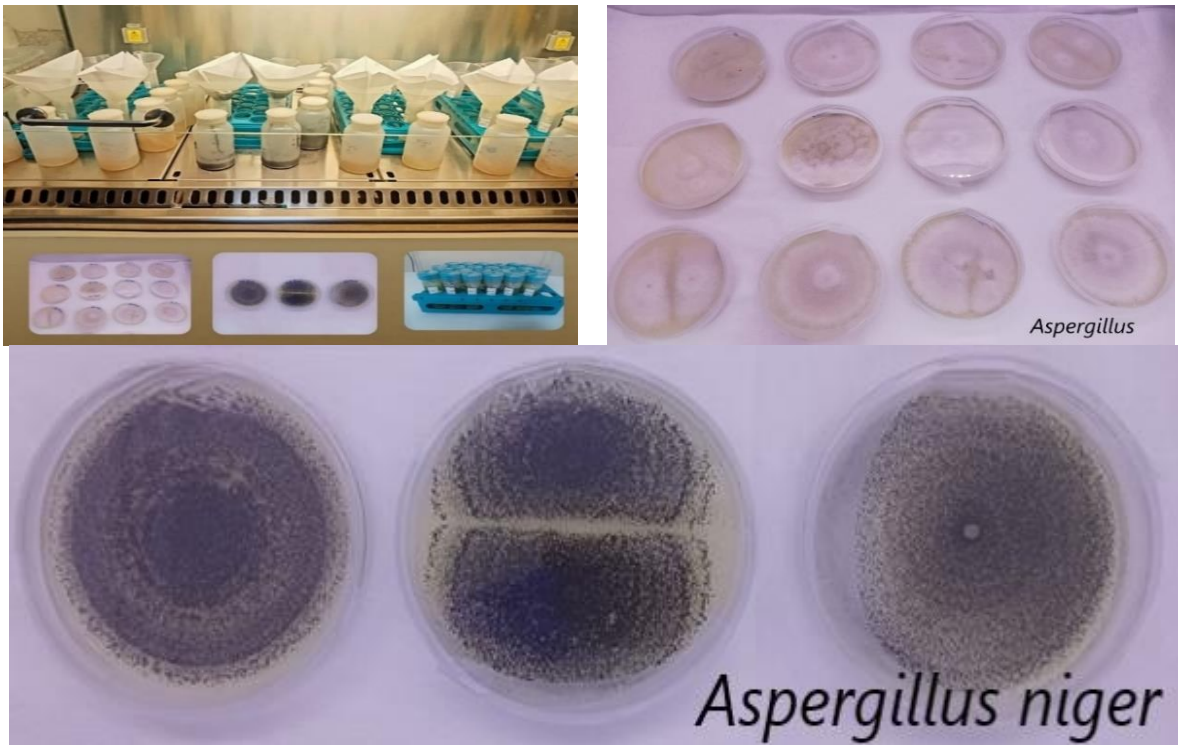


Figure 1. Growth status of fungal isolates 1 week after inoculation on PDA and images from extraction

Figure 2. A sample from the sampling points of chili pepper samples sold in the open and a view from the extraction stage

2.2. Methods

2.2.1. Aflatoxin analysis

Aflatoxin analysis was performed using the HPLC system according to AOAC Official Method 999.07 (Anonymous, 2000). There are three main steps in this analysis: extraction, cleaning, and injection into HPLC.

2.2.2. Preparation of samples for analysis and methods used

The samples taken from Balıklı Lake Spice Bazaar were brought to the laboratory in 100 gram transparent plastic bags and 50 grams of these samples were used for analysis. Fungal isolates were cultured of PDA and incubated at 26 ^{0}C for 7 days. In the study, cultivation on PDA, extraction and aflatoxin reading by HPLC method were performed. Sowing of fungal isolates on potato dextrose agar (PDA) was performed according to (Bilgili, 2017). For toxin extraction of the isolates, the method

of Aydın et al. (2008) was used as modified. In this process, *Aspergillus* spp. fungi cultured on agars were transferred to 250 ml tubes. Then 100 ml of 60% (v/v) methanol/water solution was added to the tubes and mixed with a stomacher for 30 min at room temperature. The mixture was filtered using Whatman no.1 filter paper. After filtration, the samples were diluted 1/5 with 60% (v/v) methanol/water and filtered through a 0.45 µm filter attached to the syringe tip. Finally, the samples were submitted to the HPLC-FLD for analysis to determine aflatoxin ratios. Extraction, HPLC conditions and quantification of aflatoxin in chili peppers were performed according to (Atasoy et al., 2017). The HPLC-FLD used in the analysis is SHIMADZU Nexera XR 20 series and this device measures in the wavelength range of 365-435 nm. *Aspergillus* spp. fungi taken from pepper plants for aflatoxin analysis and the aflatoxin species investigated are given in Table 1. In Table 2, the points where the chili pepper spices sampled for aflatoxin analysis were taken and the aflatoxin species investigated are given.

Table 1. *Aspergillus* spp. and aflatoxin species obtained from the plant for aflatoxin analysis

Sample No	Sample Name	Provinces of Pepper Plant	Aflatoxin Analyzed
1	7 ASPN	Şanlıurfa Center Kepirli Village	B1, B2, G1, G2
2	14 ASP	Şanlıurfa Umut Village	B1, B2, G1, G2
3	30 ASPN	Şanlıurfa Bozova Yaylak	B1, B2, G1, G2
4	33 ASP	Diyarbakır Bismil	B1, B2, G1, G2
5	37 ASPN	Diyarbakır Çınar Yuvacık	B1, B2, G1, G2
6	51 ASP	Diyarbakır Çermik	B1, B2, G1, G2
7	57 ASPN	Batman Kozluk	B1, B2, G1, G2
8	85 ASPF	Gaziantep Yavuzeli, Halilbaş	B1, B2, G1, G2
9	ASP-Urfa	Şanlıurfa	B1, B2, G1, G2

ASP: *Aspergillus* spp., ASPN: *Aspergillus niger*, ASPF: *Aspergillus flavus*

Table 2. Points of collection of chili pepper spices sampled for aflatoxin analysis and aflatoxin species investigated

Sample No	Sample Name	Where chili pepper samples were taken	Aflatoxin Analyzed
1	Sweet chili pepper1	Spice market, workplace number 1	B1, B2, G1, G2
2	Hot chili pepper 2	Spice market, workplace number 2	B1, B2, G1, G2
3	Sweet chili pepper 3	Spice market, workplace number 3	B1, B2, G1, G2
4	Hot chili pepper 4	Spice market, workplace number 4	B1, B2, G1, G2
5	Hot chili pepper 5	Spice market, workplace number 5	B1, B2, G1, G2
6	Sweet chili pepper 6	Spice market, workplace number 6	B1, B2, G1, G2
7	Hot chili pepper 7	Spice market, workplace number 7	B1, B2, G1, G2
8	Hot chili pepper 8	Spice market, workplace number 8	B1, B2, G1, G2
9	Sweet chili pepper 9	Spice market, workplace number 9	B1, B2, G1, G2
10	Hot chili pepper 10	Spice market, workplace number 10	B1, B2, G1, G2
11	Sweet chili pepper 11	Spice market, workplace number 11	B1, B2, G1, G2
12	Hot chili pepper 12	Spice market, workplace number 12	B1, B2, G1, G2
13	Hot chili pepper 13	Spice market, workplace number 13	B1, B2, G1, G2
14	Sweet chili pepper 14	Spice market, workplace number 14	B1, B2, G1, G2
15	Hot chili pepper 15	Spice market, workplace number 15	B1, B2, G1, G2

3. Results and Discussion

The aflatoxin contents of *Aspergillus* spp. fungi obtained from pepper plants are given in Table 3. When the contents of *Aspergillus* spp. fungi from different provinces of the Southeastern Anatolia region were analyzed, AFB1 and AFB2 were detected in 2 samples. The presence of *Aspergillus* spp. fungi in pepper plants indicate that aflatoxins begin to form at the stage when the peppers grow in the field. Aflatoxin formation may also continue with the harvesting method, drying and storage processes. These stages may cause an increase

in aflatoxin levels. İnanç (2024), aflatoxin formation in spicy red peppers starts while the fruits ripen on the plant due to the effect of fungi transmitted from air or soil. The harvesting method also affects this situation; because for economic reasons, all peppers are usually harvested at one time. This can cause early ripe fruits to remain on the plant for a long time and increase aflatoxin formation. Furthermore, aflatoxin content is directly related to subsequent processing such as storage, drying, and packaging.

Table 3. Aflatoxin amounts of *Aspergillus* spp. fungi obtained from pepper plants

	AFB1 (ppb)	AFB2 (ppb)	AFG1 (ppb)	AFG2 (ppb)	TOTAL AFs (ppb)
7ASPN	-	-	-	-	-
14 ASP	-	-	-	-	-
30ASPN	-	-	-	-	-
33ASP	-	-	-	-	-
37ASPN	-	-	-	-	-
51ASP	-	-	-	-	-
57ASPN	-	-	-	-	-
85ASPF	1094.38	23.35	-	-	1117.73
ASP-Urfa	2034.43	18.35	-	-	2052.78

ASP: *Aspergillus* spp., ASPN: *Aspergillus niger*, ASPF: *Aspergillus flavus*, -: not detected

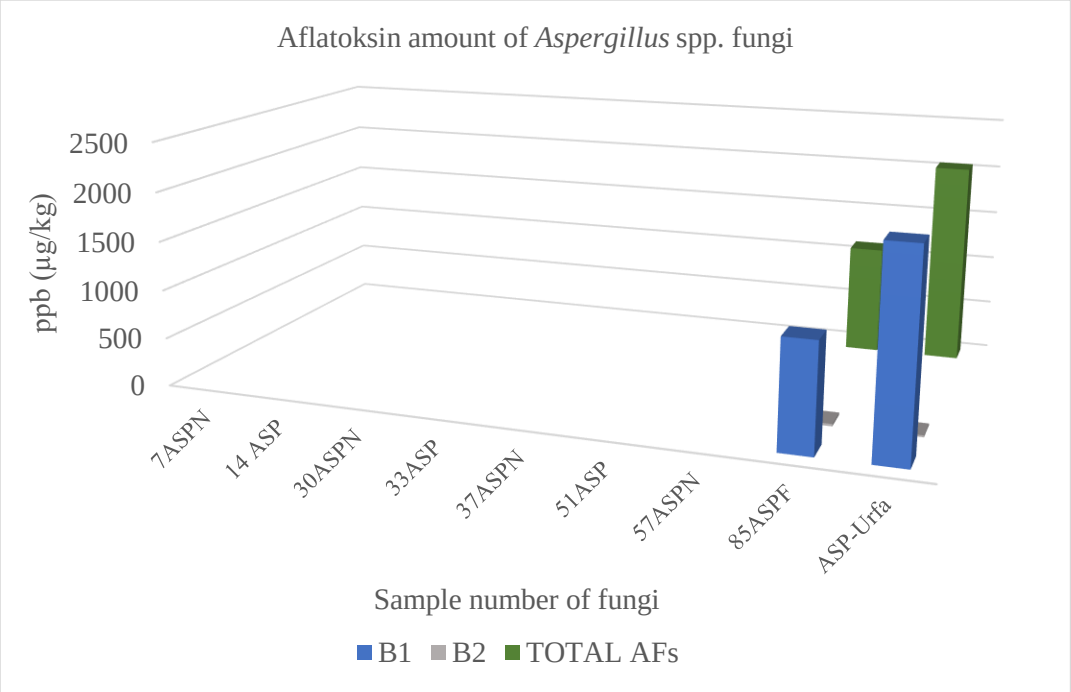


Figure 2. Graph of aflatoxin content of *Aspergillus* spp. fungi obtained from pepper plants

The aflatoxin contents of the chili pepper samples analyzed are given in Table 4. It was determined that 1 of the samples did not contain any aflatoxin. The presence of AFG1 and AFG2 was detected in 1 of the 15 chili pepper samples taken from the spice bazaar. AFB2 was detected in 11 of the analyzed samples and their amount was determined to be between 0.0125 and 0.1875 ppb. AFB1 content of chili pepper samples was found to vary between 0.1125- 3.95 ppb. Total aflatoxin content of chili pepper spices was found to be at least 0.15 ppb, maximum 4.1375 ppb and average 0.9455 ppb. Ardıç et al. (2008) investigated the presence of aflatoxin in 75 isot samples and found that 72 samples contained AFB1 and these amounts ranged between 0.11 and 24.7 ppb. In a study conducted by Erdoğan (2004) using thin layer chromatography, 20 isot samples were searched for aflatoxin and aflatoxin (B+G) was detected in only 1 sample and its value was reported as 13.8 ppb. Atasoy

et al. (2017), in a study conducted with 20 isot spices, no aflatoxin was detected in 1 sample, and the presence of G1 and G2 was not detected in any of the 20 isot samples. B2 was detected in 6 samples and was determined to be between 0.02 -1.09 ppb and B1 content in 19 samples ranged between 0.02 - 8.45 ppb. Total aflatoxin content ranged between 0.02 - 9.54 ppb and the average aflatoxin content was 1.52 ppb.

Table 4. Detected aflatoxin amounts of chili pepper spice

	AFB1 (ppb)	AFB2 (ppb)	AFG1 (ppb)	AFG2 (ppb)	TOTAL AFs (ppb)
Pepper1	0.3625	0.0375	-	-	0.4
Pepper2	0.3375	0.025	-	-	0.3625
Pepper3	0.525	0.0375	-	-	0.5625
Pepper4	1.3	0.025	-	-	1.325
Pepper5	3.95	0.1875	-	-	4.1375
Pepper6	0.2125	0.0125	-	-	0.225
Pepper7	0.5125	0.025	-	-	0.5375
Pepper8	0.15	-	-	-	0.15
Pepper9	-	-	-	-	-
Pepper10	0.225	-	-	-	0.225
Pepper11	1.275	0.025	-	-	1.3
Pepper12	0.7625	0.0625	-	-	0.825
Pepper13	0.5875	0.0125	-	-	0.6
Pepper14	1.3625	0.1	0.9625	0.1625	2.5875
Pepper15	0.1125	-	-	-	0.1125
Minimum	0.1125	0.0125	0.9625	0.1625	0.1125
Maximum	3.95	0.1875	0.9625	0.1625	4.1375
Average	0.7783	0.0366	0.9625	0.1625	0.89

-: not detected

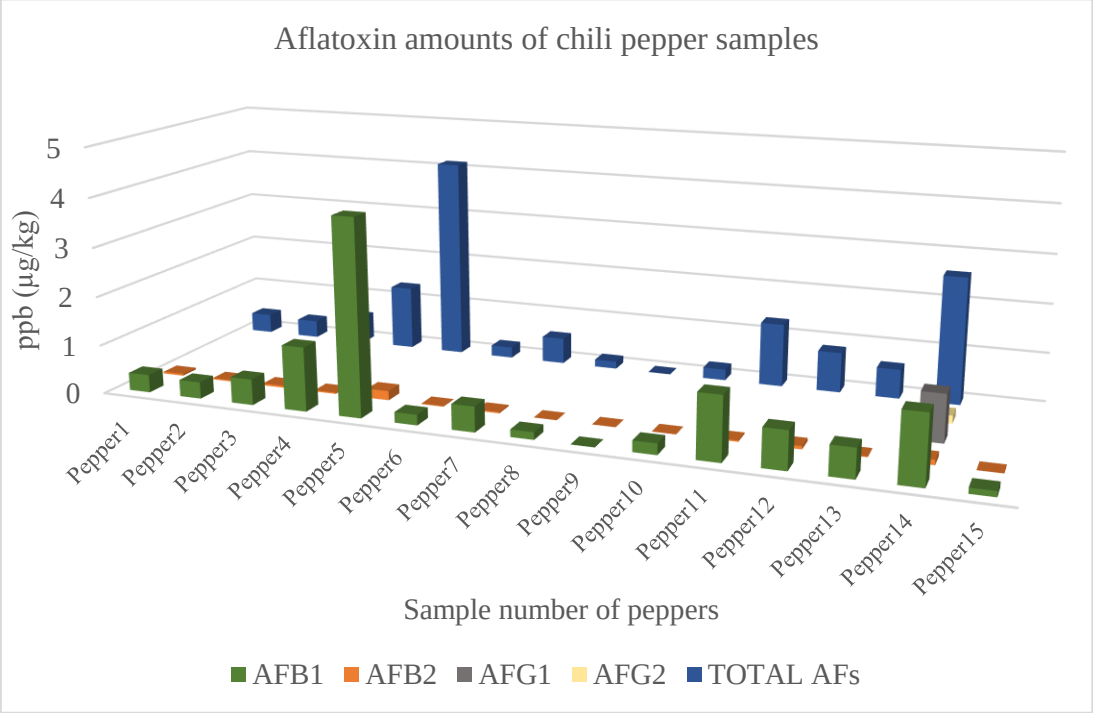
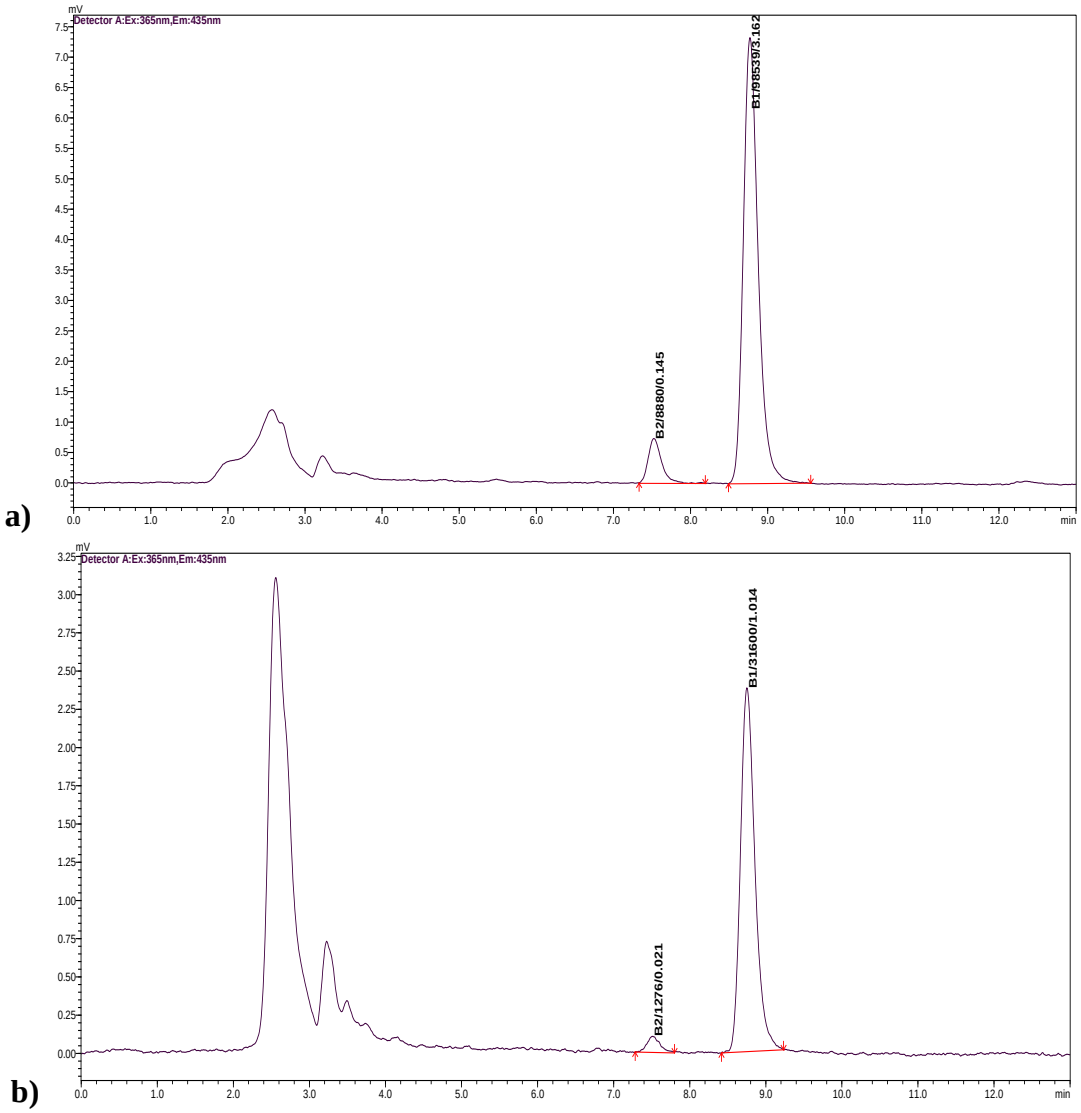


Figure 3. Graph of detected aflatoxin content of chili pepper spice

Graphs showing the aflatoxin types and amounts of fungal samples taken from the root of pepper plant and chili pepper samples are given in Figure 2 and Figure 3. When the graphs of fungal samples taken from pepper root are analyzed, aflatoxins in 85 ASPF and ASP- Urfa samples were B1: 1094.38 ppb, B2: 23.35 ppb, total AFs: 1117.73 ppb and B1:

2034.43 ppb, B2: 18.35 ppb, total AFs: 2052.78. When the graph of chili pepper samples is analyzed, it is seen that while aflatoxin was detected in all 14 samples, it did not exceed the limit value determined for B1 and total AFs. However, this situation has the potential to change over time depending on the ambient conditions of aflatoxin. Accordingly, depending on the ambient conditions, afla toxin content may decrease or increase. Chromatogram graphs showing aflatoxin peaks of some samples are presented in Figure 4.



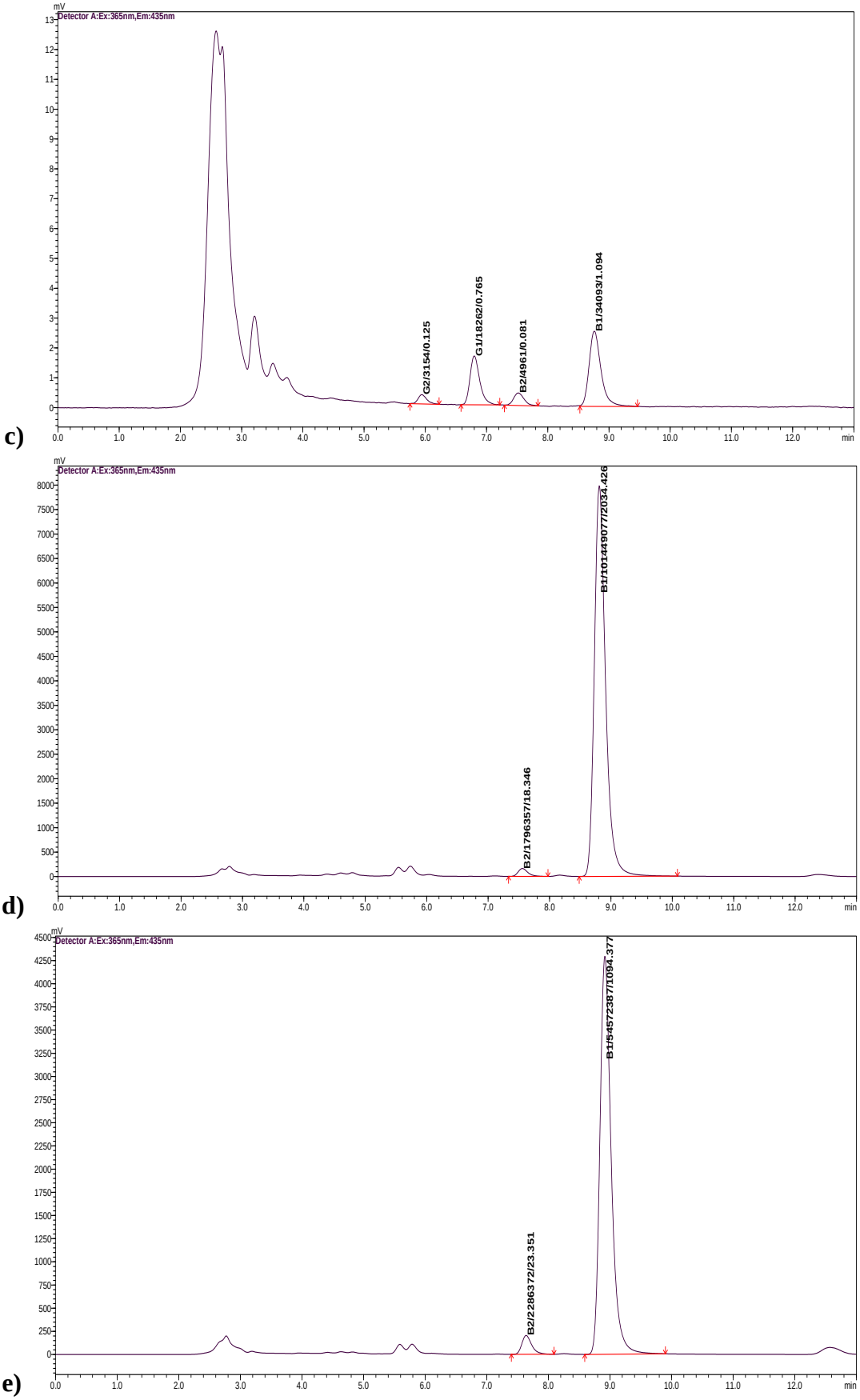


Figure 4. Chromatogram plots showing aflatoxin peaks of some samples (Chromatogram plots a) Pepper 5, b) pepper 11, c) pepper 14, d) ASP-Urfa, e) 85ASPF)

In general, the aflatoxin content of chili peppers is quite low; however, the fact that some samples are close to the limit values may be due to the variety and quality of the fresh pepper used, the place and method of production. In addition, the difference in production times and the duration of production stages may also affect these differences. Atasoy et al. (2016) reported that the aflatoxin content in dry flaked isot spice produced under controlled conditions was very low. According to the Turkish Food Codex Regulation on Contaminants (TGK, 2011) and the European Union food legislation (EU, no 165/2010), the permissible limit for Aflatoxin B1 is set at 5 ppb and for total aflatoxin at 10 ppb (Anonymous, 2010). It was determined that chili pepper samples did not exceed the limit values in terms of AFB1 and total AFs. Atasoy et al. (2017), none of the analyzed samples exceeded the legal limits in terms of total aflatoxin amount. In a study conducted on 75 isot samples, it was found that 14.7% of the samples did not comply with the standards set in terms of AFB1 (Ardıç et al. 2008). Fresh peppers used in the production of chili pepper spice have a high water content and need to be dried for a long time to obtain the original color of isot. However, aflatoxin formation is kept under control as the water activity of the peppers decreases rapidly during the drying process. In addition, the concrete floor used during production accelerates the drying process by increasing the temperature of the peppers due to sunlight. Atasoy et al. (2016), In their study, they supported the current study and stated that aflatoxin content in dry flaked isot seasoning produced under controlled conditions was very low. The amount of aflatoxin in pepper spices can vary depending on many factors. Among these, the type and water activity of the dried pepper (Marin et al., 2009), drying temperature and climatic conditions (Cho et al., 2008) play an important role. It is also stated that prolonged drying time is also effective in aflatoxin formation and the amount of aflatoxin may increase with increasing drying time (Inan et al., 2007). In recent years, extensive research has been conducted on the detoxification of toxins in foods. Pankaj et al. (2018) reviewed the methods applied to reduce aflatoxin levels in various foods. These methods include conventional heating, microwave, chemical methods (e.g. lactic acid, hydrogen peroxide, ozone or ozonated water), radiation (such as X, ultraviolet or gamma rays), electrolyzed water and cold plasma treatments. Park et al. (2007) reported the complete degradation of AFB1 on glass substrate within 5 seconds using microwave argon plasma at atmospheric pressure. However, these methods are not yet economically efficient and are generally not sufficiently successful. The most effective method to ensure food safety for consumers is government-led inspections. Out-of-bounds levels of aflatoxins in food pose a similar problem not only in Turkey but also in many other countries (Koutsias et al., 2021; Tsehaynesh et al., 2021). There are also studies on similar issues related to red pepper in Turkey (Demir et al., 2019; Coşkun and Ünsal, 2020). Aflatoxin B1 levels were found between 0.20-79.37 µg/kg and total aflatoxin levels were found between 0.22-93.05 µg/kg in red pepper samples taken from spice trading companies in Malatya province (Uğur, 2022). In a systematic review and meta-analysis of the presence of Aflatoxin B1 in red pepper, the lowest AFB1 concentration was 0.14 µg/kg in Korea and the highest concentration was 31.13 µg/kg in Turkey (Sevdin et al., 2021). It was determined that 28% (7 samples) of the red chili pepper samples offered for sale in Çukurova and Eastern Mediterranean Region did not meet the standards for AFB1 levels. In addition, total aflatoxin was detected in 16% (4 samples) of these samples (Hepsağ and Hayoğlu, 2022).

4. Conclusion

In conclusion, AFB1 was detected in 14 chili powder samples at concentrations ranging from 0.1125 to 3.95 ppb, and AFB2 was found in 11 samples at levels between 0.0125 and 0.1875 ppb. In one sample, AFG1 and AFG2 were detected at 0.9625 ppb and 0.1625 ppb, respectively. AFB1 and AFB2 were also detected in the fungal samples taken from the pepper roots, specifically in the 85ASPF and

ASP-Urfa samples. The 85ASPF sample contained 1094.38 ppb of AFB1, 23.35 ppb of AFB2, and a total aflatoxin (AFs) concentration of 1117.73 ppb. Similarly, the ASP-Urfa sample contained 2034.43 ppb of AFB1, 18.35 ppb of AFB2, and a total of 2052.78 ppb AFs. Based on these findings, the chili powder samples did not exceed legal aflatoxin limits. However, the high levels of AFB1 and AFB2 detected in the fungal samples from the pepper roots indicate the presence of aflatoxins originating in the field and persisting under favorable conditions. The formation of aflatoxins in agricultural products, especially in peppers used as spices, is affected by various stages in the process from field to spice. Aflatoxins are formed as a result of molds belonging to the genus *Aspergillus* spp. colonizing on the plant during the growing phase of the plants and producing aflatoxins as metabolic products. Post-harvest processes also significantly affect the levels of these toxins; in particular, drying processes are a determining factor in the presence and concentration of aflatoxins, and drying under inappropriate conditions can increase the accumulation of toxins. In the storage process, factors such as humidity and temperature can promote aflatoxin production. Although the methods applied in the processing stages have the potential to reduce the presence of aflatoxins, their effectiveness depends on the processing conditions. Therefore, the presence of aflatoxins in food poses a serious risk to both public health and food safety. To prevent the formation of aflatoxins, all processes from agricultural production to final consumption must be carefully managed and controlled. At this point, it is recommended that studies on factors such as production and storage conditions should be carried out and planned in the future.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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