Legume Research, 42(6) 2019: 862-866
Print ISSN:0250-5371 / Online ISSN:0976-0571

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
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Presence of some mycotoxins in peanuts from harvest to storage

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Received: 13-08-2018 Accepted: 27-04-2019 DOI: 10.18805/LR-446

ABSTRACT

In this study, aflatoxin and cyclopiazonic acid levels on the second crop of peanuts collected from 102 different research areas of Osmaniye and Adana, Turkey in 2016 was determined from harvest, drying, pre-storage and storage. During the periods of the research, it was found that aflatoxin levels in 86 out of 102 contaminated samples were ranged from 0.2 to 2177.2 µg/kg. However, cyclopiazonic acid, another mycotoxin investigated in this study has not been found in any of the 102 peanut samples. When aflatoxin contamination compared in different periods; it was observed that the aflatoxin level in storage and drying (97%) periods were found to be higher than the harvest (66%) and pre-storage (71%). The statistical analysis showed that significant differences in the aflatoxins in drying, and storage periods. Therefore, for preventing grains from aflatoxins damage control management strategies need to be improved.

Key words: Aflatoxin, Contamination, Mycotoxins, Second crop peanut, Storage.

INTRODUCTION

Aflatoxins (AFs) are the most known mycotoxin which is naturally synthesized by *Aspergillus flavus* and *Aspergillus parasiticus* and lead to contamination in poor agricultural practice during harvest and post harvest treatments of peanuts (Latha *et. al.*, 2011). Therefore, peanuts are considered to be a high–risk product with aflatoxins, since they are frequently contaminated with fungi. (Singh *et. al.*, 2017). Furthermore, the researches showed that, the incidence of aflatoxigenic *A. flavus* strains was higher in peanut (69%) than wheat (13%) or soybean (5%) (Vaamonde *et. al.*, 2003). Aflatoxin-producing fungi are stimulated by poor agricultural practices, harvesting practices, post-harvest processing and storage methods, despite the fact that peanuts and other agricultural products are natural contaminants (Ahammed *et. al.*, 2008; Aschalew, 2010).

Also, especially *A. flavus* can produce cyclopiazonic acid (CPA), and indole-tetramic acid besides aflatoxins. Because of this, CPA can be seen naturally in peanuts as co–contaminant with aflatoxins (Ferna´ndez Pinto *et. al.*, 2001). While aflatoxin is always the most important toxin for peanut because of its toxicity, CPA presence in peanuts cause necrotic foci in internal organs such as the liver and, exerts neurotoxic effects (Vaamonde *et. al.*, 2003). Therefore in peanuts, aflatoxins and CPA should be investigated together.

For this reason, in this study; aflatoxin and cyclopiazonic acid presence were investigated together and evaulated in the second peanut crops at harvest, drying, pre-storage and the storage periods.

MATERIALS AND METHODS

Research areas: This study was conducted in Adana and Osmaniye provinces of Turkey in 2016. research areas were conducted according to the systematic sampling method based on 1% (Bora and Karaca,1970). Total of 102 second crop peanut samples from harvest, drying, pre-storage (eliminated soil) and storage periods were collected from 72 areas of Adana and Osmaniye provinces. Furthermore, 30 peanut samples were taken from the storage.

Peanut samples: Each sample (about 5 kg) obtained from different research areas were cracked manually and homogeneously (about 1 kg) for mycotoxin assays. Peanut samples were stored at +4°C (Lavkor, 2013).

Total AFs assay: Firstly, 25 g of the peanut sample with 5 g of NaCl were blended with 125 ml of methanol D water (70:30) for 2 min. and then filtered using fluted filter paper. After filtering, 15 ml of the extract was diluted with 30 ml of water. Then, 15 ml of the extract had been passed from the IAC containing monoclonal antibody specific for AFB_1, AFB_2, AFG_1, AFG_2. The column was washed with 20 ml of deionised water at a rate of about 2 drops/second. All of the samples eluted from the column with 1.0 ml of methanol, and the eluted fraction was diluted twice with deionised water. Then AFB_1, AFB_2, AFG_1, AFG_2 were determined by HPLC (Arzandeh and Jinap, 2011).

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CPA assay: The method of analysis used in this study is modified by Somuncuoglu (2007) for analysis of CPA in peanut. 45-g sample was weighed and blended with 150 ml of 20% methanol in chloroform and 3 ml of 2 % sodium bicarbonate acid were added. The extract was filtered then 80 ml was taken from the fitrate. To the filtrate 30 ml of 0.05 M solution of lead acetate was added and stirring. The plates were spotted with 60-80 µl of the respective extract and developed in the solvent ethyl acetate/2 propanol/sodium hydroxide (50:15:10, v/v) for 35-40 min. After the plates were being heated at 35-40°C for 1 hour and cooled. CPA was viewed after spraying with Ehrlich's reagent (1.0 g of 4-dimethylaminobenzaldehyde in 25 ml of HCl and 75 ml ethanol) with subsequent development of a purple color in daylight. Calculations were performed with the following formula:

$$\mu/kg=(S\times Y\times V)/(X\times W),$$

Where, S is the unknown microliter amount of CPA standard, Y is the concentration of CPA standard, V is the microliter amount of sample extract after the final dilution, X is the microliter amount of sample extract with intensity equal to X (CPA standard) and W is the gram amount of sample in 100 ml of initial extract.

Climatic data: Climatic data for June-November in year of 2016 in Adana and Osmaniye are given in Table 1.

Statistical analysis: In order to provide normal distribution of data logarithmic transformation were applied. After that, Statistical analysis were performed by using one-way ANOVA and then Duncan parametric multiple comparison test.The data were analyzed using SPSS, version 21.0 (IBM Corp., Armonk, NY, USA). Differences between mean values of periods were evaluated using significant difference at a 5% significance level.

RESULTS AND DISCUSSION

Aflatoxin contamination was determined in 19 (66%) out of 29 peanut samples with levels ranging from 0.2-318.0µg/kg collected from harvest; while during drying period, it was found that 28 out of 29 peanut samples (97%) with levels ranging from 0.4-1572.7 µg/kg (Table 2). The pre-storage period, aflatoxin contamination was determined in 10 (71%) out of 14 peanut samples with levels ranging from 0.2-1071.1µg/kg. In 29 (97%) out of 30 samples taken from storage were found to 0.4 to 2177.2 µg/kg.

The aflatoxin contamination in second crop peanuts collected in different periods from the research areas; the amount of aflatoxin in the samples of drying (97.0%) was higher than in harvest (66.0%) and pre-storage (71.0%) (Fig 1). According to Ding *et. al.*, (2012), low AFs contamination may find in peanuts after harvest, but AFs levels might be higher during storage. It is therefore, necessary to monitor the AFs contamination of peanuts during growth and storage. Moreover, high levels of contamination with AFs were detected in commercial peanut samples as a consequence of insufficient postharvest handling practices (Mutegi *et. al.*, 2013).

In addition, the aflatoxin contamination may occur in peanuts from the fields because of the field fungi and then could continue during ineligible drying. Besides this, aflatoxin contamination could develop due to the effects of climatic conditions in drying period such as humidity and temperature (Lavkor, 2013). The climate and soil conditions for peanut production of Adana and Osmaniye provinces are suitable for the infection of fungi, such as *A.* section *Flavi.* The average temperature is 25-30°C in the summer of 2016 in these provinces (Table 1). According to Table 1 of climate data (June to September in 2016), soil temperatures in the root zone were measured at about 30°C and more. It has been determined that inadequate irrigation and low temperature in the root zone in this period (Lavkor, 2013) and 29.6°C temperature causes to increase of *A. flavus* colonization and aflatoxin production (Oktay *et. al.*, 2011). Generally, aflatoxin production may occur above 24-28°C (Aschalew, 2010). For that reason, under dry conditions, if the soil temperature is in 25-32°C, aflatoxin production can be seen even in undamaged peanuts. Also, the controlling of aflatoxin contamination of the peanut in the field is very difficult due to the climatic conditions, especially the relative humidity and temperature. In addition, insects, pathogenic fungi and environmental factors such as adequate nutrition, irrigation, harvest time, drying problems have being leaded

Table 1: Some climatic data for Adana and Osmaniye (2016).

Months	Average Temper -ature	Above Ground Temper -ature	Soil Average Temper -ature (10 cm)	Average Moisture	Total Rain
		(°C)		(%)	(mm)
June	27.3	20.2	30.0	63.8	9.1
July	29.5	23.3	35.2	67.5	0.2
August	29.9	23.8	35.1	67.4	8.2
September	26.2	19.7	29.2	59.9	7.8
October	22.9	13.8	24.2	55.2	0.00
November	15.2	4.3	15.6	51.8	4.0

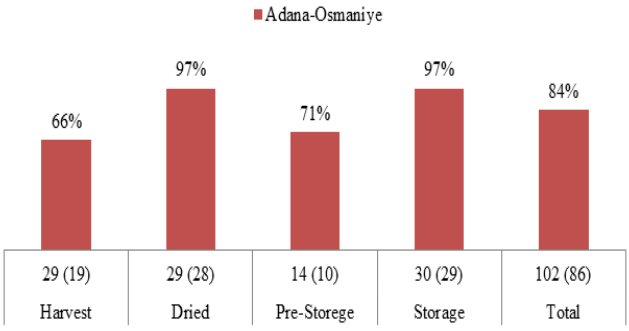


Fig 1: Distribution of the number of aflatoxins found in all posi tive samples in Adana and Osmaniye.

Table 2: Results of aflatoxin level in peanut samples from different periods in Adana and Osmaniye provinces in Turkey.

Periods	Samples (positive samples)	Percent -age (%)[b]	Range of AFs (µg/kg)	Samples >10 (µg/kg) (Per -centage %)[b]	Range of AFs (µg/kg)	Samples >100 (µg/kg)(Per -centage %)[b]	Range of AFs (µg/kg)	Samples >1000 (µg/kg)(Per -centage %)[b]	Range of AFs (µg/kg)
Harvest[a]	29 (19)	66	0.2-318.0	2 (7)	60.9-63.4	1 (3)	318.0	-	-
Drying[b]	29 (28)	97	0.4- 1572.7	5 (17)	16.6-97.8	6 (21)	118.3-515.1	3 (10)	1227.7-1572.7
Pre-storage[ab]	14 (10)	71	0.2-1071.1	1 (7)	76.5	1 (7)	202.6	1 (7)	1071.1
Storage[b]	30 (29)	97	0.4-2177.2	7 (23)	14.3-74.2	8 (27)	163.7-561.2	2 (7)	1082.5-2177.2
Total	102 (86)	84	0.2-2177.2	15 (15)	14.3-97.8	16 (16)	118.3-561.2	6 (6)	1071.1-2177.2

[a]Significant differences ($p<0.05$).
[b]Percentage related to the total number of samples in each period.

to contamination of aflatoxin-producing fungi in agricultural crops (Paterson and Lima, 2011).

Usually, in Adana and Osmaniye provinces, the second peanut harvest has continued from September to the end of November. During the harvest and drying periods in September-October-November, the temperature was above 20°C and the humidity was above 50% with rainfall (Table 1). When the harvesting has done in warm climate and humid weather, *A. flavus* invades seeds and produces micelles in cotyledons (Subrahmanyam *et. al.*, 1992).

Timely harvest and rapid drying regardless of the weather conditions also reduce the aflatoxins present. Genereally, in drying process for peanuts in Adana and Osmaniye provinces, harvested peanuts are leaving like a pile on the ground, and is carried out the mixing with a shovel by sightseeing on pile by the farmers in our research areas. Therefore, peanuts which hold on the soil surface is damaged and broken. Besides, the lack of enough ventilation, all these make the peanut product vulnerable to pathogens (Lavkor, 2013; Lavkor and Bicici, 2015; Lavkor *et. al.*, 2017).

In present research, aflatoxin content of peanut samples was decreased in the pre-storage period (Table 2, Fig 1). Because, aflatoxin contamination may be less in soil-sifted peanuts and other impurities (Okello, 2010a). According to Kacmaz (2006), the content of peanut contained 10-15% of impurities (stones, earth, garbage, fiber, hernia, etc.). As peanuts come from field, they contaminated with foreign material like rocks, moisture contended soil particles, and outer shells of raw peanuts, and for that reason these must be removed from pods and avoid optimum conditions for the aflatoxin development before entering the storage (Okello *et. al.*, 2010b).

The samples taken at harvest, aflatoxin contamination continued in the storage conditions and therefore it is clear that aflatoxin contamination increased (Lavkor *et. al.*, 2017). In a study conducted inthe main crop in Turkey, Osmaniye province, the amount of aflatoxin, in the samples of in harvest (0.0-1.9 µg/kg) and post-harvest samples (1.7-4.0 µg/kg) were found to be lower than the samples collected during drying (5.4-9.0 µg/kg) and the samples from pre-storage (4.0-8.6 µg/kg). Nevertheless, the high aflatoxin contamination (0.80-19.9µg/kg) was detected during the storage period. (Lavkor and Bicici, 2015). Ding *et. al.*,(2012) reported that low AFs contamination was found in peanuts after harvest, but AFs levels might be higher during storage and processing.

Of the naturally occurring AFB_1 is the most toxic (Alinezhad *et. al.*, 2011). In Turkey, the legal tolerance limits of AFB_1 applied to be greater than 10 µg/kg (Anonymous, 2011). The level of AFB_1 was determined in 19 (33.3%) peanut samples in harvest, drying, pre-storage periods, and in 17 (36.3%) peanut samples in storage period to be greater than 10 µg/kg. Results showed that the total aflatoxin level at 2177.17µg / kg on the peanut is 108 times higher than the tolerance level of 20µg/kg determined by an international organization such as the FDA (Table 2).

The CPA presence of the samples it was found that there was no finding about CPA in 102 peanut samples analyzed by TLC. Although, it does not mean that there might not be CPA in peanuts in the other studies. CPA level was determined in the different studies in peanuts. The frequency of detection was 18% for the Adana and Osmaniye, with mean levels ranging from 16.6 to 44.44µg/kg in 2015. Especially, CPA (30%) mostly produced during storage term (Lavkor *et. al.*, 2017). Other studies investigating the production of CPA in peanuts also reported high rates of 89% (Blaney, *et. al.*, 1989), 93% (Horn, *et. al.*, 1996) and 97% (Resnik *et. al.*, 1996). In general, CPA is produced by *A. flavus*, alone or in combination with type B aflatoxins, but not in conjunction with type G aflatoxins. *A. flavus* and *A. parasiticus* are closely related species belonging to the *Aspergillus* section *Flavi*. Both species can produce aflatoxins, but not all isolates of either species do so. Although Vaamonde *et. al.* (2003) were found that isolates of *A. parasiticus* consistently produce both B and G aflatoxins, but they do not produce CPA according to Oktay *et. al.* (2011). As seen in some studies, that the mutants can produce aflatoxin is independent of the CPA synthesis. It was observed in this study that aflatoxin is produced but CPA was not produced. Competition between these fungi

may explain the lack of detection of CPA in samplings. Besides, competition between these fungi may explain the lack of detection of CPA in samplings (Zorzete, 2013).

CONCLUSION

When the results of aflatoxin contamination at the storage and drying stages was compared with the harvest period, it was found that the amount during storage and drying periods was higher than that at harvest time. Thus, it showed that aflatoxin formation started during the harvest period and increased during the drying process. Partial removal of the product from the soil and/or other foreign bodies provided a reduction in the amount of aflatoxin during the pre-storage period. For it cannot be a matter of doubt that all these problems will continue to increase in the future, as long as measures are not taken to control aflatoxin contamination in this region where peanut harvesting, drying and storage periods must be important.

ACKNOWLEDGMENT

This work was financially supported by Tubitak of Turkey (Project no:115O007). The auther thank Tubitak for their contributions.

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