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Full Length Research Paper

Application of the ELISA and HPLC test for detection of aflatoxin in Pistachio

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***Pistacia vera* L. grows successfully in South-Eastern Anatolia due to its own ecological properties. Because of a high price of *P. vera* L. in domestic and foreign markets it becomes a good source of income for the producers. The most faced problem in its exportation is 'aflatoxin'. Several methods are used for the detection of aflatoxin in *P. vera* L. However, for these methods, the developed laboratories, experienced personals and high-cost are needed. In this study, aflatoxin content of *P. vera* L. was investigated by using ELISA test which is used as a rapid, cheap and safe diagnostic technique in last decades. In addition, HPLC was applied for same samples and results obtained from ELISA were compared with those from HPLC. Plant materials used in this study were taken from *P. vera* L. Depots and wholesale trade stores and consisted of 38 different sources of *P. vera* L. samples. Aflatoxin B1 was determined over 2 ppb in 16 samples by ELISA. However there was no evidence about the total of aflatoxin. By HPLC, there were no evidence for aflatoxin B1 and also the total of aflatoxin. These results showed that the ELISA, is a molecular diagnostic test depends on protein content was more sensitive than HPLC. In this study ELISA test was used for the first time in the determination of alfatoxin content in *P. vera* L. kernels.**

Key words: *Pistacia vera* L., aflatoxin, enzyme-linked immunosorbent assay.

INTRODUCTION

In recent years, *Pistacia vera* L. exportation shows a decrease due to metabolite outcome *"Aflatoxins"* of *Aspergillus* sp. Aspergillus species generally cause the contamination in laboratories. *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger* are the most seen pathogen examples. These species cause a disease named aspergillosis in human and animals. Pulmonic aspergillosis is a serious and common disease which is seen especially in birds and mammals including human (Bicici, 1993). Aflatoxin firstly found in *A. flavus* may cause primer liver cancer, lung cancer and Reye's syndrome (Anonymous, 1979; Bullerman, 1986; Topal, 1987). Aflatoxin caused by incorrect Pistacia drying techniques and unsuited storage conditions decreases significantly *P. vera* L. crop and exportation. Such factors as storage duration, humidity in depot, water leak, insect activity, outside temperature, fault of air conditioning caused to product toxin in storage period (Coksoyler, 1991; Ozkaya, 2000). Studies related with aflatoxin in worldwide investigated aflatoxin quantity in nut, peanut and crops especially by HPLC (Asis ve Paola, 2002). The occurrence of aflatoxin in raw peanut kernels which were randomly collected from Malaysian supermarkets was examined by HPLC. It was determined that about 78.57% of the collected samples were contaminated with aflatoxin of which 10.71% exceeded the maximum tolerable limit (Arzandeh et al., 2010).

In this study, ELISA, one of the diagnostic test, depends upon protein content was firstly used to determine the analysis of aflatoxin content in *P. vera* L. In Turkey, ELISA test was used to determine aflatoxin content in red pepper, bird seed, fig and some spices before (Oruc and Sonal, 2001). Besides in this study

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Table 1. Pistachio sample locations, cultivation areas and production dates.

Sample no.	Sources of samples	Cultivation area	Date of production
1	Firm (Fistikcilar sitesi)	Barak	2002
2	Firm (Fistikcilar sitesi)	Barak	2002
3	Firm (Fistikcilar sitesi)	Nizip	2002
4	Firm (Fistikcilar sitesi)	Nizip	2002
5	Firm Fistikcilar sitesi)	Nizip	2000
6	Firm (Depot)	Nizip	2000
7	Firm (Depot)	-	2000
8	Nizip (Depot)	Nizip	2000
9	Firm (Depot)	Gaziantep	2000
10	Firm (Depot)	Gaziantep	2000
11	Firm (Depot)	Gaziantep	2002
12	Firm (Depot)	Gaziantep	2001
13	Firm (Depot)	Gaziantep	2001
14	Firm (Fistikcilar sitesi)	Nizip	2002
15	Barak (Farmer)	Barak	2002
16	Firm (Fistikcilar sitesi)	Nizip	2001
17	Firm (Fistikcilar sitesi)	Gaziantep	2001
18	Firm (Fistikcilar sitesi)	Nizip	2002
19	Nizip (Depot)	Nizip	2001
20	Islahiye-farmer	Islahiye	2001
21	Islahiye-farmer	Islahiye	2002
22	Araban-hisar village	Hisar village	2001
23	Araban-elif village	Elif village	2000
24	Firm (fistikcilar sitesi)	Oguzeli	2001
25	Firm (fistikcilar sitesi)	Nizip	2001
26	Araban-farmer	Araban	2001
27	Araban-farmer	Araban	2001
28	Araban-farmer	Araban	2001
29	Firm (Fistikcilar sitesi)	Oguzeli	2001
30	Firm (Fistikcilar sitesi)	Oguzeli	2001
31	Nizip	Nizip	2001
32	Firm (Depot)	Gaziantep	2000
33	Firm (Depot)	Gaziantep	2001
34	Firm (Depot)	Gaziantep	2001
35	Firm (Depot)	Gaziantep	2001
36	Firm (Depot)	Gaziantep	2001
37	Araban-farmer	Araban	2001
38	Firm (Fistikcilar sitesi)	Iran	2001

ELISA test results were controlled with the HPLC results and the result of the two methods was compared with each other.

MATERIALS AND METHODS

Plant material

The pistachio samples were taken from 37 different sources. The sources were chosen from Gaziantep provinces Nizip, Oguzeli, Araban and Islahiye and their villages in which *P. vera* L. production is very frequent and firms that manage Pistachio drying, hulling and breaking. Besides an Iranian Pistachio produced in 2001, sample was taken to trial. 3 samples from NIZIP distinct- Barak ova, 11 samples from NIZIP distinct, 2 samples from Islahiye distinct, 4 samples from Araban distinct, 1 sample from Araban distinct-Hisar village, 1 sample from Araban distinct-ELIF village, 3 samples from Oguzeli distinct and 12 samples from Pistachio firm stores in Gaziantep city center were taken. Production dates and locations of the taken samples are given in Table 1.

Materials used in ELISA and HPLC tests

Plant materials used in ELISA test were designated within the aforementioned details of inner part of the Pistachio samples.

Aflatoxin analysis was performed by using Ridascreen® fast aflatoxin kits (total aflatoxin, aflatoxin B1, aflatoxin colomn) owned by R-Biopharm (Germany), with sensitivity limits of 0 to 45 ppb. Inner parts of the Pistachio samples which are taken from investigated areas were also used in HPLC test studies.

Preparation of samples used in ELISA and HPLC tests

38 Pistachio materials have been harvested from Gaziantep and some of its provinces. Harvest process was performed according to the sampling method and then samples were placed in labeled and perforated plastic bags. Samples were kept in dark and wood cabinets at a room temperature (25°C) until analysis.

Aflatoxin analysis

ELISA studies

Inner part of *P. vera* samples was taken and was granulated as large as semolina. 5 g granulated sample was mixed with 70% methanol (1:5 ratio) and was infiltrated. 5 ml suspension diluted to 1:3 ratio. A total of 20 ml solution was placed into ELISA colon by injector. Immuno affinity colon (IAC) was used for detection of aflatoxin. This colon is composed of gel suspension which covalent binding with monoclonal antibodies and these antibodies are specific for aflatoxin B1, B2, G1, G2 and M1.

Standard preparing for total aflatoxin: 50 µl standard and 50 µl *P. vera* sample were mixed with 450 µl buffer solution respectively. Then conjugate and antibody were added to suspension and were incubated in darkroom for 30 min. After incubation washing step was applied 3 times using ELISA washer. Chromogen and substrate were placed into strip wells and were incubated for 30 min again. Following to incubation, 100 µl stop reagent was added for finishing reaction. Reading was performed at a wavelength 450 nm in ELISA reader.

Standard preparing for aflatoxin B1: Standard preparing procedure for total aflatoxin was applied also for aflatoxin B1. However incubation duration was extended to 2 h from 30 min. Ridasoft win programme was used for data obtained from reading results of total aflatoxin and aflatoxin B1. Numerical results were assessed at a limit of 5 ppb.

HPLC studies

25 g granulated *P. vera* sample was mixed with 5 g NaOH. In addition, 50 ml 100% methanol and 75 ml distilled water were added to suspension and then were infiltrated. 20 ml infiltrated suspension was filled into colon by injector. The colon used in HPLC test was same with ELISA test. Analysis for each sample in HPLC took 18 min and the results of aflatoksin B1, B2, G1, G2 were obtained graphical and also numerically.

RESULTS

In this study, aflatoxin analysis of 37 Gaziantep Pistachio and 1 Iranian Pistachio samples were analyzed by using ELISA and HPLC methods. Analyses were repeated two times. In HPLC and ELISA analyses, there was no evidence about the total aflatoxin. Despite in HPLC, there was no encounter of any of the aflatoxin B1, there was seen aflatoxin more than 2 ppb which is above the European standard in 16 samples of total 38 Pistachio samples in ELISA. ELISA and HPLC analysis results of total aflatoxin and aflatoxin B1 were given in Tables 2 and 3 respectively. There was no encounter of any of the total aflatoxin in ELISA and HPLC tests of 38 Pistachio samples as seen in Table 2. In Table 3 there encountered over 2 ppb aflatoxin B1 which is above the European standards in 16 samples of a total of 38 Pistachio samples in ELISA test results. ELISA results between 2 and 3 ppb, 3 and 4 ppb, 4 and 5 ppb and above the 5 ppb are shown with (+), (+ +), (+ + +) and (+ + + +) respectively. Thus over 2 ppb aflatoxin B1 values which is a peak value in most European countries was recorded and shown with (+) symbols according to its value in 16 samples. However in Table 3 there was no encounter of any aflatoxin findings with HPLC method in 38 Pistachio samples and the values were shown with (-) symbols.

DISCUSSIONS

In October 2003, firstly ELISA test was applied to the *P. vera* L. samples taken from Gaziantep Pistachio wholesale trade stores, Gaziantep city center and some of the provinces for the determination of aflatoxin content. After then HPLC test was applied to the same samples to check the results obtained from ELISA test. There was no encounter of any of the total aflatoxin according to ELISA and HPLC test results. Although aflatoxin B1 was not detected in HPLC test, it was detected in 16 samples in ELISA test. It was more remarkable that over 2 ppb aflatoxin B1 was detected in 16 samples by ELISA test. Although any findings about total aflatoxin was not determined. Determination of aflatoxin B1 in 16 samples can be explained as follows: columns used in aflatoxin analysis are very specific columns and these columns cannot be determined as aflatoxin rate in total aflatoxin because of its very low amount. On the other hand the aflatoxin could be brought by the specific columns because of its higher rate. In this study, in 15 of 37 pistachio samples, aflatoxin B1 dose was detected above the 2 ppb. In 9 of these 15 samples, aflatoxin B1 dose was detected above the 3 ppb. Aflatoxin B1 dose in 1 sample of Iranian 2001 production was detected above the 2 ppb. This study was the first for the determination of alfatoxin content in *P. vera* L. by ELISA. Similar methods were used for the other products like bird feeds. Aflatoxin in bird feeds was determined by using ELISA test in 2001 and total aflatoxin rate and aflatoxin levels were found approximately 72.72% and 0.0 to 9.2 ppb respectively (3). These results showed that the determination of aflatoxin by ELISA test was more sensitive than the other methods. Aflatoxin produced by *Aspergillus* sp. was reported to be the most effective secondary metabolite among mycotoxin because of being carcinogenic and also decreasing *P. vera* yield (Bicici, 1993). Our results

Table 2. ELISA and HPLC analysis results of total aflatoxin.

					Results	
sample no.	**HPLC**	**Europe std. (ppb)**	**ELISA**	**Europe std. (ppb)**	**HPLC**	**ELISA**
1	-	4	0.001	4	-	-
2	-	4	0.0106	4	-	-
3	-	4	0.0262	4	-	-
4	-	4	0.1834	4	-	-
5	-	4	0.0253	4	-	-
6	-	4	0.312	4	-	-
7	-	4	0.0769	4	-	-
8	-	4	0.1927	4	-	-
9	-	4	0.127	4	-	-
10	-	4	0.1332	4	-	-
11	-	4	0.585	4	-	-
12	-	4	0.4276	4	-	-
13	-	4	0.369	4	-	-
14	-	4	0.959	4	-	-
15	-	4	0.5007	4	-	-
16	-	4	0.3411	4	-	-
17	-	4	0.2866	4	-	-
18	-	4	0.5108	4	-	-
19	-	4	1.6047	4	-	-
20	-	4	0.6549	4	-	-
21	-	4	0.4626	4	-	-
22	-	4	0.4983	4	-	-
23	-	4	0.6828	4	-	-
24	-	4	0.4861	4	-	-
25	-	4	0.7123	4	-	-
26	-	4	0.3745	4	-	-
27	-	4	1.3527	4	-	-
28	-	4	1.0377	4	-	-
29	-	4	1.0377	4	-	-
30	-	4	1.0032	4	-	-
31	-	4	0.9698	4	-	-
32	-	4	0.8384	4	-	-
33	-	4	1.7855	4	-	-
34	-	4	0.3105	4	-	-
35	-	4	1.8449	4	-	-
36	-	4	1.9836	4	-	-
37	-	4	1.2887	4	-	-
38	-	4	0.7123	4	-	-

Table 3. ELISA and HPLC analysis results of aflatoxin B1.

					Results	
Sample no.	**HPLC**	**Europe std. (ppb)**	**ELISA**	**Europe std. (ppb)**	**HPLC**	**ELISA**
1	-	2	0.0938	2	-	-
2	-	2	0.0709	2	-	-
3	-	2	0.1157	2	-	-
4	-	2	0.1415	2	-	-
5	-	2	0.1568	2	-	-
6	-	2	0.2204	2	-	-

Table 3. Contnd

7	-	2	0.2026	2	-	-
8	-	2	0.1942	2	-	-
9	-	2	0.1695	2	-	-
10	-	2	0.1534	2	-	-
11	-	2	0.3072	2	-	-
12	-	2	0.2753	2	-	-
13	-	2	0.26	2	-	-
14	-	2	0.5832	2	-	-
15	-	2	0.4121	2	-	-
16	-	2	0.6491	2	-	-
17	-	2	0.5361	2	-	-
18	-	2	0.5703	2	-	-
19	-	2	1.631	2	-	-
20	-	2	1.9409	2	-	-
21	-	2	1.3326	2	-	-
22	-	2	2.3554	2	-	+
23	-	2	2.5408	2	-	+
24	-	2	3.7242	2	-	+ +
25	-	2	2.5408	2	-	+
26	-	2	2.8505	2	-	+
27	-	2	3.1538	2	-	+ +
28	-	2	4.4507	2	-	+ + +
29	-	2	4.1022	2	-	+ + +
30	-	2	4.2725	2	-	+ + +
31	-	2	5.0385	2	-	+ + + +
32	-	2	4.5618	2	-	+ + +
33	-	2	5.8141	2	-	+ + + +
34	-	2	3.2544	2	-	+ +
35	-	2	1.7652	2	-	-
36	-	2	2.5798	2	-	+
37	-	2	2.3202	2	-	+
38	-	2	2.0897	2	-	+

showed that ELISA test was more effective than HPLC for detecting aflatoxin in *P. vera.* We suggested that determining of *P. vera* contaminated with aflatoxin especially aflatoxin B1 had risk for health hazard for human and also economic losses.

Besides this, *P. vera* producers should make conscious of aflatoxin. Especially during *P. vera* drying and storing steps, aflatoxin formation has significance (Coksoyler, 1991; Ozkaya, 2000). To minimize such factors caused to produce aflatoxin will increase Pistachio quality and in this way will be a benefit for health and this product's exportation.

REFERENCES

Anonymous (1979). Environmental Health Criteria. Mycotoxins World Health Org., Geneva.

Arzandeh S, Selamat J, Lioe H (2010). Aflatoxin in Raw Peanut Kernels Marketed in Malaysia. J. Food Drug Anal., 18(1): 44-50.

Asis R, Paola RD (2002). Food Agric. Immunol., 14(3): 201-208.

Biçici M (1993). *Mikoloji* Ders Kitabı. Ç.Ü. Zir. Fak. Yay. p. 121.

Bullerman LB (1986). Mycotoxin and Food Safety: Food Techonology. A Scientific Status Summary by the IFT Expert Panel on Food Safety and Nutrition, 40(5): 59-66.

Coksoyler N (1991). The effects of sampling and analysis steps to results in determination of the mycotoxin level in food and feeds TKB. BURSA 2nd International Food Simp. Proceedings, pp. 284-295.

Oruc HH, Sonal S (2001). University of Uludag, Veterinary Faculty. Pharmacology and Toxicology Course Notes.

Ozkaya Ş (2000). Education Programme on "Analysis of Aflotxin in Food and Feeds" Ankara.

Topal M (1987). Several important mycotoxins and their characteristics, Soc. Food Technol., 12(5): 283-291.