IOSR Journal Of Pharmacy
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
www.iosrphr.org Volume 5, Issue 2 (February 2015), PP. 38-44

Occurrence of aflatoxins in some medicinal plants stored under different conditions

Vesna Kostik
Institute of Public Health of Republic of Macedonia, 50 Divizija No. 6 , 1000 Skopje, Republic of Macedonia, Assistant professor

Abstract : *Medicinal plants are widely used as home remedies and raw materials for the pharmaceutical industries. During harvesting, handling, storage and distribution, medicinal plants are subjected to contamination by various fungi, which may be responsible for spoilage and production of mycotoxins. The increasing consumption of medicinal plants has made their use a public health problem due to the lack of effective surveillance of the use, efficacy, toxicity and quality of these natural products.*

The aim of this study was to determine the content of aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2) and the sum of total aflatoxins (AFs) in medicinal plants which were stored in storehouses with controlled macro-environment such as humidity, temperature; modified macro-environment of 50% CO_2, 30% O_2 and 20% N_2 and in storehouses with air at 25 ^0C and relative humidity between 70% and 80%. The results of this study revealed that the content of AFs was the lowest in medicinal plants which were stored in modified macro-environment. This implies the necessity of continuous monitoring of the macro-environmental condition during the storage.

Keywords – *aflatoxins, macro-environment, medicinal plants*

I. INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by moulds such as Aspergillus, Penicillium, Fusarium and Alternaria [1]. Mycotoxins can be defined as ‘‘fungal metabolites that when ingested, inhaled or absorbed through the skin cause illness or human and animal death’’ [2]. Mycotoxins cause variety of toxic effects. They are carcinogenic, neurotoxic, teratogenic and immunotoxic [3].

Aflatoxins (AFs) are a class of mycotoxins produced mainly by toxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus* and rarely by *Aspergillus nomius* which can grow on various kinds of foods, beverages and medicinal plants. Aflatoxins are the most important human mycotoxins and are among the most toxic mycotoxins [4]. There are nearly 20 different types of aflatoxins of which the four major ones are aflatoxins B_1, B_2, G_1 and G_2. Aflatoxin B_1 (AFB_1) and aflatoxin B_2 (AFB_2) are produced by *Aspergillus flavus*, while all four isoforms (B_1, B_2, G_1, and G_2) are produced by *Aspergillus parasiticus* [5]. Aflatoxin B_1 is the most toxic of the aflatoxins and most potent naturally occurring hepatic carcinogen [6]. Aflatoxins are amongst the most powerful mutagens and carcinogens known. The International Agency of Research on Cancer (IARC) has classified AFs as Group 1 human carcinogen [7]. Aflatoxins and hepatitis B virus (HBV) infection synergistically develop approximately thirty-fold higher hepatocellular carcinoma risk in aflatoxin-exposed, HBV-positive individuals, as compared to HBV-negative persons [8]. Other toxic effects of aflatoxins include genotoxicity, teratogenicity and immunosuppressive activity. Aflatoxin B_1 letal dose 50 (LD_{50}) values for most animal species ranges from 1 to 50 mg/kg body weight [9].

The aflatoxin contamination of herbal drugs is controlled by legal limits. Maximum limits of 2 μg/kg for AFB_1 and 4 μg/kg for total aflatoxins in herbal drugs have been set by the European Pharmacopoeia [10]. However due to the fact that most herbs are processed before consumption, higher maximum limits of 5 μg/kg and 10 μg/kg for AFB_1 and total aflatoxins, respectively, have been proposed [11].

Medicinal plants are frequently contaminated with toxic fungi originating in the soil. During harvesting, handling, storage and distribution, these plants are subjected to contamination by various fungi, which may be responsible for spoilage and production of mycotoxins [12]. The condition is serious especially in developing countries, where there are poor production practices [i.e., good agricultural practices (GAP) and good harvesting practices (GHP) are inadequate]. Moreover, the poor conditions of storage and transportation contribute to fungal contamination and increase the risk of mycotoxin production [13].

The Republic of Macedonia has the exceptional geographical position with excellent climate, as well as relief, with a large number of sunny days, which allows growing of more than 3,000 species of higher plants of which 1,000 have medical properties. Nowadays, the consumption of medicinal herbs as home remedies and raw materials for the pharmaceutical industries is increasing**.** In order to monitor the influence of storage conditions safety of medical plants in respect of aflatoxins contamination, a pilot study was conducted. The aim of this study was to investigate the occurrence and concentrations of aflatoxins residues in some medicinal plants from domestic producers, which are stored in storehouses with controlled ambient conditions (humidity, temperature, the content of O_2, CO_2 and N_2) and medicinal plants which are stored in air at 25 °C and 80% relative humidity.

II. MATERIALS AND METHODS

2.1. Sampling

During 2014, a total of 176 samples of ten different medicinal plants: *Matricaria chamomilla* L. (flowers), *Tilia parvifolia* L. (flowers), *Verbascum thapsus* L. (flowers), *Taraxacum officinale* L. (roots), *Herniaria glabra* L. (aerial parts), *Hibiscus esculentus* L. (leaves), *Mentha piperita* L. (aerial parts), *Achillea milefolium* L. (aerial parts), *Rosa canina* L. (rose hips) and *Sambucus nigra* L.(flowers) were tested to detect the presence of residues of the alflatoxins AFB_1, AFB_2, AFG_1, AFG_2 and the sum of total aflatoxins (AF). All plants were cultivated in the Republic of Macedonia. The samples were obtained from storehouse with air at 22 °C and relative humidity below 40% (S1), storehouse with modified atmosphere of 50%, CO_2, 30% O_2, and 20 % N_2 (S2) and storehouses with air at 25 °C and relative humidity between 70% and 80% (S2 and S3). Blank samples of *Matricaria chamomilla* L. and *Achillea milefolium* L. were purchased from a local organic producer. Blank samples were used for the fortification experiment.

2.2. Reagents and standards

HPLC reagents (methanol, acetonitrile, water) and chemicals (benzene, KBr, NaCl, HNO_3) were purchased from Merck (Darmstadt, Germany). For clean-up purification immunoaffinity columns Aflaprep (R-Biopharm Rhône, Glasgow, Scotland) were used. Purified water was delivered by the Millipore water purification device Milli-Q Gradient A10 (Bedford, USA).

Reference pure standards of each individual aflatoxin were obtained from Sigma Aldrich/Fluka/Riedel-de-Haen (Zwijndrecht, The Netherlands). Stock standard solution mixture of aflatoxins above indicated was prepared in chloroform at a concentration of 10 μg/mL. From this, a dilution with a final concentration of 0.10 μg/mL solution was used for calibration standards purposes. Portions of 125, 250, 500, 750 and 1000 μL from this last standard were evaporated in 250 mL volumetric flasks, afterwards re-dissolved with 75 mL of methanol and filled up with water. The following concentrations were achieved: 0.05, 0.10, 0.20, 0.30, 0.40 in ng/mL. For recovery experiments, 5 mL of a 0.40 μg/mL total aflatoxins standard solution was used to fortify 1 kg of blank matrix the day prior to analysis, resulting in a level of fortification of 2 μg/kg.

2.3. HPLC equipment and reverse phase conditions

HPLC analysis was performed with Perkin Elmer (PE) chromatographic system equipped with binary pump (PE LC-250), manual injector (PE Rheodyne 7125) and fluorescence detector (PE LC-240). Aflatoxins were separated on Supelco column (250 mm x 4.6 mm, 5mm) at room temperature. The mobile phase was a mixture of water: acetonitrile: methanol (600:50:350, *V/V/V)* with addition of 119 mg KBr and 350 mL 4N HNO_3. The mobile phase was degasified in the ultrasonic bath before use. The flow rate was 1 mL/min and the injection volume was 100 mL. The detection was carried out at excitation of 360 nm and emission of 440 nm.

2.4. Sample preparation and clean up

The extraction and purification of aflatoxins from medicinal plant samples was done according to AOAC method [14]. High performance liquid chromatography (HPLC) with fluorescence detector (FlD) was performed according to ISO standard [15]. 25 g of tested sample with addition of 5 g NaCl and 125 mL 70% (*V/V*) methanol was mixed in a blender jar for 2 min at high speed. The mixture was filtered through a fluted filter paper. 30 mL of purified water was added to a 15 ml of filtrate and filtered again through microfiber filter paper. 15 mL of the second filtrate was quantitatively passed through the immunoaffinity column at flow rate of 1mL/min. The column was washed with 10 mL of water. Aflatoxins were eluted with 1 mL of methanol in an amber vial at flow rate of 1 mL/min. The elution step was repeated one more time with 1 mL of water. Then, 100 mL of methanol-water solution was applied to HPLC-FlD system, followed by derivatization with bromine in Kobra cell (R-Biopharm Rhône).

2.5. Statistical analysis

The results were expressed as the mean values and standard deviation. Statistical analysis was performed by analysis of variance (ANOVA) and included the Tukey´s test to evaluate significant differences amongthe means ($p < 0.05$).

III. RESULTS AND DISCUSSION

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated according to the formulas LOD = 3.3 · standard deviation (SD)/slope and LOQ = 10 · SD/slope. The computed values for all the tested AFs are shown in Table 1.

Table 1. Limit of detection (LOD) and limit of quantification (LOQ) obtained for the tested aflatoxins

Aflatoxin	LOD (µg/kg)	LOQ (µg/kg)
AFB_1	0.03	0.1
AFB_2	0.01	0.04
AFG_1	0.09	0.3
AFG_2	0.06	0.2
Total AFs	0.21	0.7

Representative chromatograms for AFs separation with HPLC – FlD method are shown in Fig. 1 and Fig. 2, respectively.

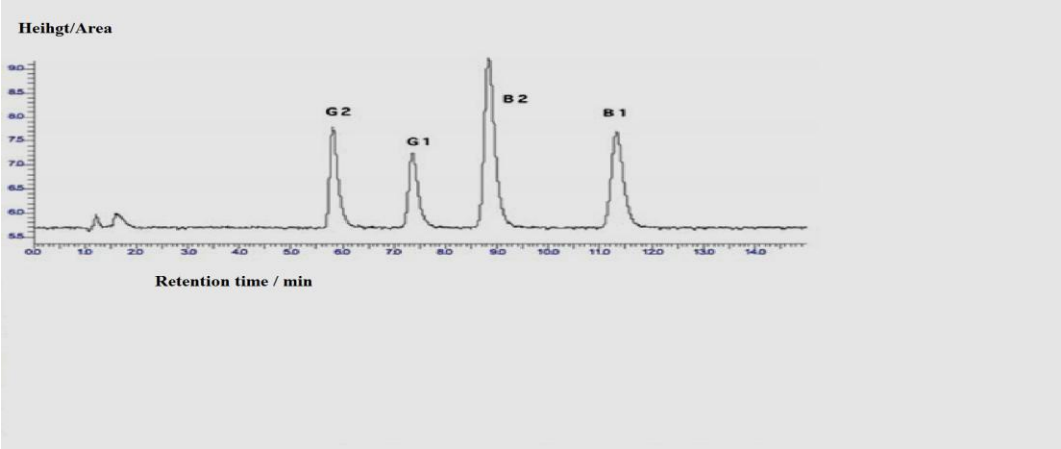

Figure 1. Chromatogram of standard solution of AFB_1, AFB_2, AFG_1, AFG_2 at 0.3 µg/L (methanol:water)

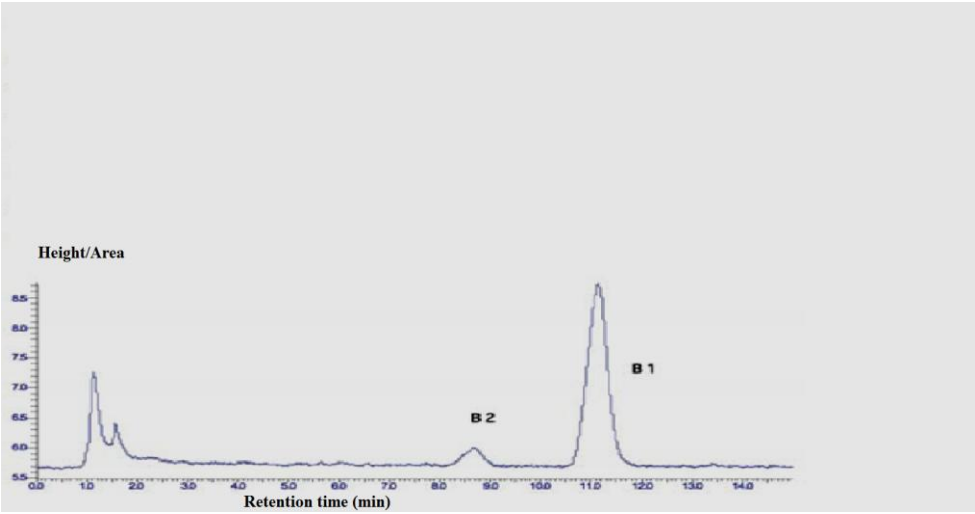

Figure 2. Chromatogram of the extract of the *M. chamomilla* sample

The content of aflatoxins found in the tested samples of medicinal plants is reported in Table 2. According to the obtained results 93.75%, 81.80% and 29% of all the tested samples were contaminated with AFB_1, AFB_2 and AFG_1 (Fig. 3) with the mean concentration of 3.1 µg/kg, 0.84 µg/kg and 0.59 µg/kg, respectively (Table 2). The percentage of tested samples that contained Afs below the calculated LOQ was found in 4% for AFB_1, 16% for AFB_2, 75% for AFG_1 and 2% for total AFs (Fig. 4). Interestingly, no one sample was contaminated with AFG_2, which might be due to the greater susceptibility of medicinal plants towards *Asp. flavus* than towards *Asp. Parasiticus*.

Table 2. Concentration of aflatoxins in medicinal plants

Plant	Sampling location	Number of samples	AFB_1 (µg/kg) mean ± SD	AFB_2 (µg/kg) mean ± SD	AFG_1 (µg/kg) mean ± SD	AFG_2 (µg/kg) mean ± SD	Total AFs (µg/kg) mean ± SD
Matricaria chamomilla	S1	3	1.23 ± 0.32^a	0.45 ± 0.10^a	<0.3	<0.2	1.68 ± 0.32^a
	S2	3	1.94 ± 0.48^b	0.23 ± 0.08^b	<0.3	<0.2	2.17 ± 0.52^b
	S3	4	3.44 ± 0.55^b	0.85 ± 0.22^b	0.55 ± 0.12^a	<0.2	4.84 ± 0.64^b
	S4	4	4.14 ± 0.68^a	0.75 ± 0.26^a	<0.3	<0.2	4.89 ± 0.72^a
Tilia parvifolia	S1	4	1.45 ± 0.72^a	0.15 ± 0.02^a	<0.3	<0.2	1.60 ± 0.28^a
	S2	3	1.05 ± 0.42^a	<0.04	<0.3	<0.2	1.05 ± 0.42^a
	S3	4	4.45 ± 0.55^b	0.77 ± 0.22^a	0.47 ± 0.12^a	<0.2	5.69 ± 0.66^b
	S4	5	5.12 ± 0.82^c	0.85 ± 0.28^b	<0.3	<0.2	5.97 ± 0.72^b
Verbascum thapsus	S1	4	1.85 ± 0.30^b	0.23 ± 0.06^a	<0.3	<0.2	2.08 ± 0.37^b
	S2	3	0.23 ± 0.12^a	<0.04	<0.3	<0.2	0.23 ± 0.12^a
	S3	4	4.15 ± 0.52^b	1.12 ± 0.32^a	<0.3	<0.2	5.27 ± 0.96^b
	S4	4	6.12 ± 0.62^b	1.05 ± 0.24^a	<0.3	<0.2	7.17 ± 0.92^b
Taraxacum officinale	S1	3	0.94 ± 0.22^b	<0.04	<0.3	<0.2	0.94 ± 0.22^b
	S2	4	0.82 ± 0.28^a	<0.04	<0.3	<0.2	0.82 ± 0.28^a
	S3	4	3.47 ± 0.35^b	1.55 ± 0.32^a	0.80 ± 0.22^a	<0.2	5.82 ± 0.76^b
	S4	3	5.78 ± 0.55^a	1.78 ± 0.42^a	0.55 ± 0.12^a	<0.2	8.11 ± 1.12^a
Herniaria glabra	S1	4	1.05 ± 0.18^b	0.12 ± 0.02^a	<0.3	<0.2	1.17 ± 0.33^b
	S2	3	<0.1	0.24 ± 0.04^a	<0.3	<0.2	0.24 ± 0.04^a
	S3	3	6.20 ± 0.82^b	0.89 ± 0.18^a	0.80 ± 0.18^a	<0.2	7.98 ± 1.10^b
	S4	4	5.84 ± 0.48^a	1.12 ± 0.22^a	0.45 ± 0.09^a	<0.2	7.41 ± 0.92^a
Hibiscus esculentus	S1	4	0.45 ± 0.18^a	<0.04	<0.3	<0.2	0.45 ± 0.18^a
	S2	3	0.87 ± 0.38^b	0.44 ± 0.12^a	<0.3	<0.2	1.31 ± 0.28^b
	S3	5	5.87 ± 0.92^c	1.65 ± 0.34^b	0.52 ± 0.18^b	<0.2	8.04 ± 1.22^b
	S4	3	5.45 ± 0.82^b	1.55 ± 0.36^a	0.60 ± 0.22^a	<0.2	7.60 ± 0.88^a
Mentha piperita	S1	4	1.45 ± 0.72^b	0.12 ± 0.03^a	<0.3	<0.2	1.57 ± 0.32^b
	S2	3	1.89 ± 0.34^b	0.18 ± 0.04^a	<0.3	<0.2	2.07 ± 0.28^b
	S3	4	2.92 ± 0.62^b	0.55 ± 0.12^a	<0.3	<0.2	3.47 ± 0.62^b
	S4	4	2.88 ± 0.72^b	1.15 ± 0.36^a	<0.3	<0.2	4.03 ± 0.34^b
Achillea milefolium	S1	3	0.45 ± 0.12^a	<0.04	<0.3	<0.2	0.45 ± 0.12^a
	S2	4	0.28 ± 0.10^a	<0.04	<0.3	<0.2	0.28 ± 0.10^a
	S3	3	5.50 ± 0.98^b	1.18 ± 0.22^a	0.62 ± 0.18^a	<0.2	7.30 ± 0.72^a
	S4	3	4.98 ± 0.72^b	1.05 ± 0.24^a	0.54 ± 0.12^a	<0.2	6.57 ± 0.62^a
Rosa canina	S1	4	1.65 ± 0.38^b	0.44 ± 0.12^a	<0.3	<0.2	2.09 ± 0.34^b
	S2	3	1.15 ± 0.22^b	0.32 ± 0.12^a	<0.3	<0.2	1.47 ± 0.22^b
	S3	4	7.45 ± 0.32^b	1.44 ± 0.42^a	0.80 ± 0.24^a	<0.2	9.69 ± 1.12^a
	S4	4	6.85 ± 0.36^b	1.58 ± 0.32^a	0.55 ± 0.14^a	<0.2	8.43 ± 0.92^a
Sambucus nigra	S1	5	1.45 ± 0.22^b	0.22 ± 0.08^a	<0.3	<0.2	1.57 ± 0.34^b
	S2	4	<0.1	<0.04	<0.3	<0.2	<0.7
	S3	3	2.95 ± 0.44^a	1.02 ± 0.08^a	<0.3	<0.2	3.97 ± 0.52^a
	S4	3	3.78 ± 0.56^a	0.90 ± 0.25^a	<0.3	<0.2	4.68 ± 0.58^a

^{a,b,c,d} Similar letters in each column show insignificant differences

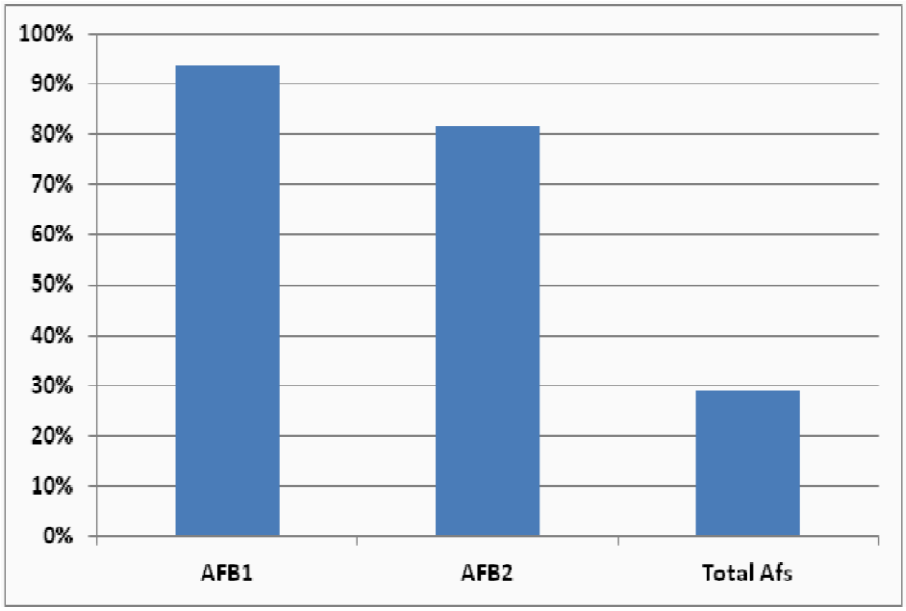

Figure 3. Percentage of contaminated samples with AFs

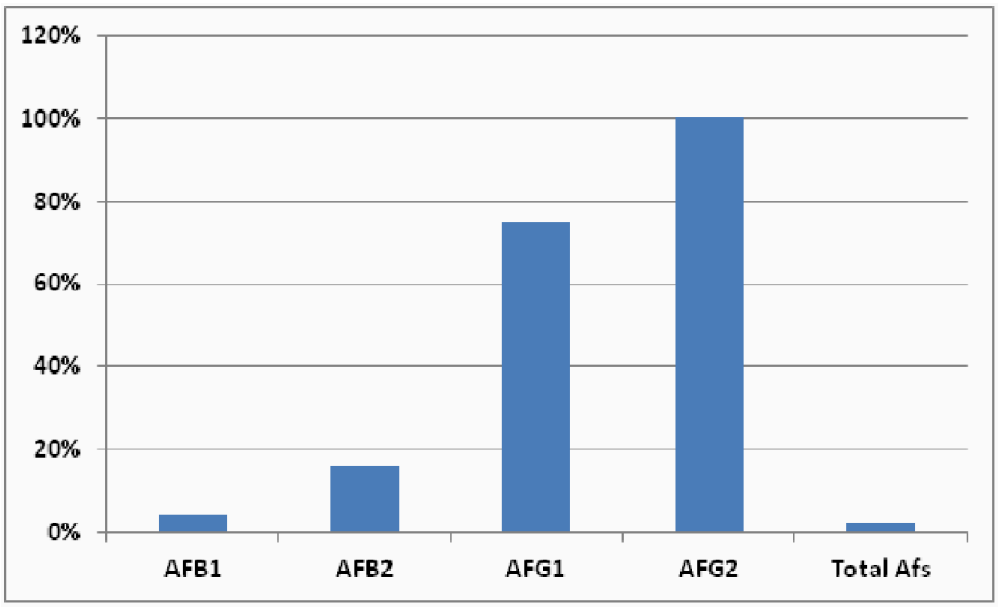

Figure 4. Percentage of samples with the content of AFs below MAC

In their study Roy and Chourasia found out that 81.1% of tested medicinal plants obtained from local Indian storehouses were contaminated with aflatoxins [16]. Our findings for AFs content in medicinal plants were in line with those reported by Roy and Kumari, 1991 [17], but far below the results obtained by Yang *et al* [18] and Liu *et al.* [11]. Namely, in their investigations of the AFs content in the traditional Chinese medicinal herb samples, they demonstrated the occurrence of AFs up to 32 μg/kg and 290.8 μg/kg, respectively. However in another study only 5.2% medicinal herbs samples from China were confirmed positive for aflatoxins [19]. Aflatoxin contamination in medicinal plants has been reported in the study conducted by Selim *et al.* [20]. They revealed that in Egypt, 29% of the 31 samples of herbs and medicinal plants were determined to contain AFB_1 with a mean concentration of 49 μg/kg. Contrary in another study in Egypt, different samples of medicinal plants, showing presence of moulds (A*spergillus, Penicillium, Fusarium* etc.), were tested to be free of aflatoxins [21].

High moisture content, relative humidity, temperature and environment rich in oxygen (O_2) are designated as the main factors for contamination of agricultural commodities with Afs [22]. The growth of fungi which produced AFs is directly related to the conditions of their storage, transport and commercialization**.** But, we couldn't find any data in the literature for correlation between the occurrence of AFs in medicinal plants and the content of moisture, temperature and oxygen during their storage. Therefore, in our study we investigated the content of AFs in medicinal plants which were stored under different atmospheric conditions as described above.

The results of our study revealed that the concentrations of AFB_1 in the all the tested samples from storehouses S3 and S4 were over the established maximum allowable concentration (MAC) of 2 μg/kg, ranging from 2.88 μg/kg to 7.45 μg/kg. At the 0.05 level of significance, the analysis of variance revealed that the population means for AFB_1 content showed a significant difference ($p<0.05$) between the analyzed samples of medicinal plants from storehouses S1 and S2 *vs.* the samples from the storehouses S3 and S4. The content of AFB_2 was also higher in all the tested samples from the storehouses S3 and S4 in comparison with the results obtained for the samples from the storehouses S1 and S2 ($p<0.05$). Significant differences at the 0.05 level were also observed for the total AFs content between the samples from the storehouses S1 and S2 compared with the samples from the storehouses S3 and S4. Namely, the content of total AFs was found to be higher in all the tested the samples from the storehouses S3 and S4 in the range from 3.47 μg/kg to 9.69 μg/kg.

The concentrations of aflatoxins in the samples from storehouses S1 and S2 were all below MAC, in the range from <0.1 μg/kg to 1.94 μg/kg (AFB_1), from <0.04 μg/kg to 0.45 μg/kg (AFB2) and from <0.7 μg/kg to 2.17 μg/kg (total AFs). Low content of AFs in the samples from the storehouses S1 and S2 is due to the lower content of moisture, lower temperature and modified atmosphere in storage (macro-environment). But, in spite of the low AFs content in the samples from the both storehouses, the analysis of variance at the 0.05 level of significance showed significant differences (Fig. 5) for the AFB_1 content (*M. chamomile*, *V. Thapsus*, *H. glabra*, *H. esculentus*, *R. canina* and *S. nigra*); AFB_2 content (*M. chamomile*, *V. Thapsus*, *H. esculentus* and *S. nigra*) and total AFs content (*M. chamomile*, *V. Thapsus*, *H. glabra*, *H. esculentus*, *R. canina* and *S. nigra*), which might be due to the natural contamination with AFs.

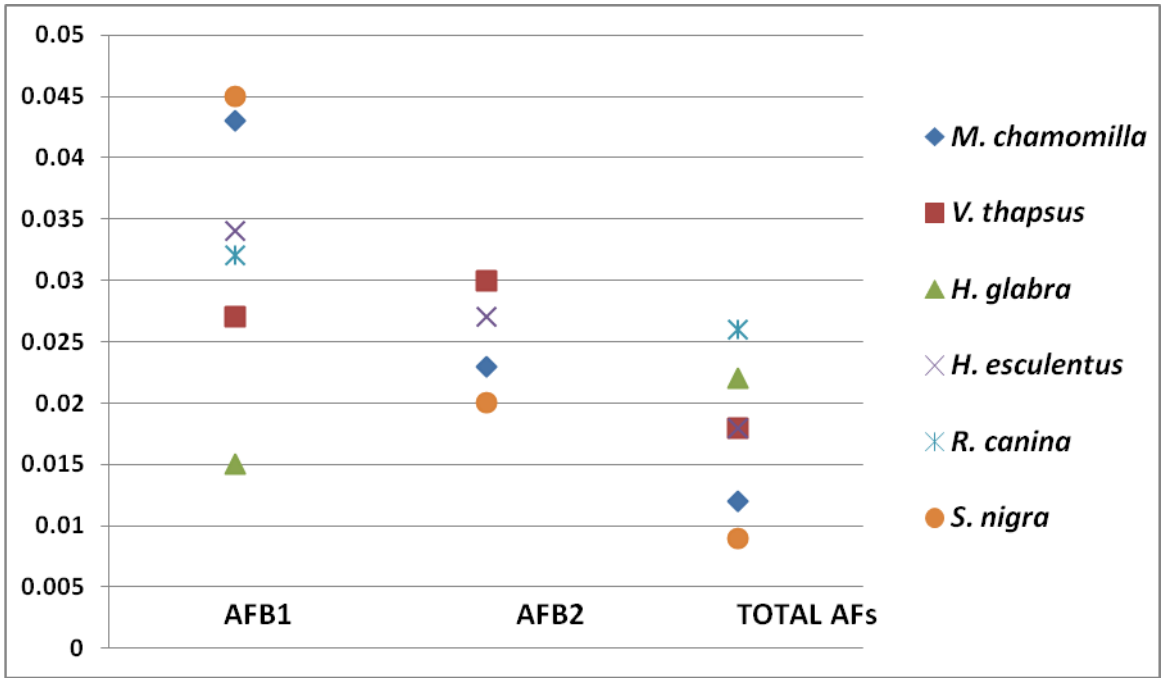

Figure 5. Statistical data for AFs content between the samples from S1 and S2

IV. CONCLUSION

The content of aflatoxins found in the tested samples of medicinal plants is directly influenced by the macro-environmental conditions during the storage. The lowest AFs content was observed in all tested samples which were stored under modified macro-environment which were consisted of 50% CO_2, 30% O_2 and 20% N_2. Humidity and temperature also have impact on the AFs occurrence in tested samples. Due to the importance and the widespread usage of the medicinal plants, special attention should be taken in terms of the conditions of their storage, as well as, during transportation and commercialization. Continuous surveillance of the AFs content also should be performed in the final products which are sold in the market.

REFERENCES

[1] M.E. Zain, Impact of mycotoxins on humans and animals, J. *Saudi. Chem. Soc*. *15*, 2011, 129–144.

[2] J. I. Pitt, What are mycotoxins, *Aust. Mycotoxin Newslett. ,7.1,* 1996.

[3] E. Petzinger, and .K. Ziegler, Ochratoxin A from a toxicological perspective, *J. Vet. Pharmacol. Ther.*, *23*, 2000, 91–98.

[4] M.A Passone, L.C Rosso, A. Ciancio, and M. Etcheverry, Detection and quantification of Aspergillus section Flavi spp. in stored peanuts by real-time PCR of nor-1 gene, and effects of storage conditions on aflatoxin production, *Int. J. Food Microbiol*. , *138*, 2010, 276–281.

[5] J.W. Bennett, and M. Klich, Mycotoxins. *Clin. Microbiol. Rev*. , *16*, 2003, 497–516.

[6] R. A. Squire, Ranking animal carcinogens: a proposed regulatory approach, *Science 214*, 1989, 887–891.

[7] IARC (International agency for research on cancer), Monographs on the evaluation of the carcinogenic risk of chemicals to humans: Some naturally occurring substances: *Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins, 56*, 1993, 245–521.

[8] J. D. Groopman, and W. Kensler, Temporal patterns of aflatoxin albumin adducts in hepatitis B surface antigen-positive and antigen negative residents of Daxin, Qidong County, People's Republic of China, *Cancer Epidemiol., Biomarkers Prev., 5(4),* 1996, 253–261.

[9] S. Leeson, G. J Diaz, and J. D. Summers, Poultry *Metabolic Disorders and Mycotixins* (University Books, Guelph, Ontario, Canada, 1995).

[10] EP (European Pharmacopoeia), Council of Europe. European Directorate for the Quality of Medicines (EDQM), 7th ed., 2011.

[11] L. Liu, H. Jin, L. Sun, S. Ma, and R. Lin, Determination of Aflatoxins in Medicinal Herbs by High-performance Liquid Chromatography-Tandem Mass Spectrometry, *Phytochem. Anal. 23*, 2012, 469–476.

[12] I. Rizzo, G. Vedoya, S. Maurutto, M. Haidukowski, and E. Varsavsky, Assessment of toxigenic fungi on Argentinean medicinal herbs, *Microbiol. Res., 159(2),* 2004, 113– 120.

[13] S. Z. Iqbal, I. A. Bhatti, M.R. Asi, H.N. Bhatti, and M.A. Sheikh, Aflatoxin contamination in chilies from Punjab Pakistan with reference to climate change, *Int. J. Agric. Biol. , 13*, 2011, 261–265.

[14] V. I. ARRANZ , E. SIZOO , H. VAN EGMOND , K. KROEGER , T. M. LEGARDA, P. BURDASPAL , K. REIF, J. STROKA, DETERMINATION OF AFLATOXIN B1 IN MEDICAL HERBS: INTERLABORATORY STUDY. *J AOAC INT., 89(3),* 2006 , 595-605.

[15] International standard ISO16050:2003 (E): Foodstufs-Determination of aflatoxin B1 and the total content of aflatoxins B1, B2, G1 and G2 in cereals, nuts and derived products – High-performance liquid chromatographyic method.

[16] A.K. Roy, K. K. Sinha, and H.K Chourasia, Aflatoxin contamination of some common drug plants, *Appl. Environ. Microbiol*. , *54*, 1988, 842–843.

[17] A.K. Roy, and S. Kumar, Occurrence of ochratoxin A in herbal drugs of Indian origin – a report, *Mycotoxin Res., 9*, 1993, 94–98.

[18] M.H. Yang, J.M. Chen, and X.H. Zhang, Immunoaffinity column clean-up and liquid chromatography with postcolumn derivatisation for analysis of aflatoxins in traditional Chinese medicine, *Chromatographia, 62*, 2005, 499–504.

[19] X. Zhang, H. Liu, and J.Chen, Immunoaffinity column cleanup with liquid chromatography using post-column bromination for aflatoxins in medicinal herbs and plant extracts, *J. Chromat. Sci., 43*, 2005, 47–51.

[20] M.I.Selim, W. Popendorf, , M.S. Ibrahim, S. El Sharkawy, and E.S. El Kashory, Aflatoxin B1 in common Egyptian foods, *J. AOAC Int. ,79 (5),* 1996, 1124–1129.

[21] M.A. Abou Donia, Microbiological Quality and Aflatoxinogenesis of Egyptian Spices and Medicinal Plants, *Global Vet., 2(4),* 2008, 175–181.

[22] V.M. Scussel, A.C Tanello, B.N. Giordano, D. Manfio, S. Galvão, and M.N.F. Rodrigues, Effect of oxygen reducing atmospheres on the quality and safety of stored shelled Brazil nut packs, *Julius-Kühn-Archiv, 425,* 2010, 560-565.