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Original Article

Evaluation of the effect of influential factors on the Aflatoxin levels of medicinal herbs in different climate regions

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ABSTRACT

In this study, 80 samples of herbs were prepared in order to measure the aflatoxin level in different regions of Iran. Aflatoxin was extracted, using 80% methanol and then purified via immunoaffinity column. Measurements were performed using high-performance liquid chromatography. According to the results, unfavorable conditions lead to the development of *Aspergillus* infection and the production of the associated toxins (e.g., aflatoxin). Given the role of various environmental factors in fungal growth and aflatoxin production (as a secondary fungal metabolite), improvement of these factors could significantly prevent the infection. In this study, a number of environmental factors, such as moisture content of the samples, temperature, and relative humidity, which could have an impact on the infection of medicinal herbs, were evaluated in different climate regions. The examination of factors affecting the aflatoxin level showed a significant difference between the samples in terms of moisture ($P=0.042$). No significant correlation was observed between the average temperature and aflatoxin levels ($P=0.374$). No significant association was observed between the mean of humidity and aflatoxin levels ($P=0.878$). On the other hand, changes in the moisture content of the samples during the storage period were almost stable in different regions. According to the results, there was insufficient moisture exchange between the sample and the surrounding environment to increase *Aspergillus* growth and aflatoxin production. This indicated that aflatoxin levels were not affected by environmental factors in this study. In fact, contamination of samples happened at previous stages, including culture, harvest, and storage, which could be increased in an unfavorable environment during the following stages.

Keywords: Mycotoxins, Aflatoxin, Aspergillus, HPLC, Herbs

Introduction

Over the past decade, prevention of mold contamination in some foodstuffs has become a public health concern. Production of highly toxic aflatoxins (AFs) may arise from negligence in the implementation of Good Agricultural Practice (GAP) regulations (for cultivating and harvesting) or desirable conditions for the invasion of toxicogenic *Aspergillus flavus* strains [1]. Mycotoxins are natural toxins that are produced by several fungal species and are associated with morbidity or even mortality in animals, plants, and humans. These compounds have diverse chemical structures and low molecular weight. Mycotoxins are often found in a large number of agricultural and food products throughout the world. In different stages, such as production, harvest, transport, and storage of agricultural products, mycotoxins can result in the contamination of human food or animal feed. Nevertheless, these fungi are not endemic to specific geographical areas or climates. In fact, fungal growth and toxin production occur only if the environment and conditions are suitable [2]. Aflatoxins are the most important group of mycotoxins, produced by different *Aspergillus* species, i.e., *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* [3]. Among recognized aflatoxins, Aflatoxin B1 is considered to be the most toxic [4].

Environmental factors have a significant impact on the fungal growth and production of aflatoxins. This study aimed to evaluate the effect of influential factors in supply on the level of aflatoxins in medicinal herbs.

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Materials and Methods

Sampling
In the present study, 80 samples of herbs were prepared.

Sample preparation
The samples were prepared using a laboratory grinding mill and passed through a 40-mesh sieve. In order to achieve a uniform composition, the samples were mixed by a mixer.

Aflatoxin measurement by HPLC
Aflatoxin level was determined using HPLC, equipped with a fluorescence detection system, using the C18 silica gel column and Kobra cell derivatization at excitation and emission wavelengths of 365 and 435 nm, respectively. The mobile phase consisted of water-acetonitrile-methanol (30:20:60 v/v/v) containing 120 mg/L KBr and 350 µl HNO3 4M, with a flow rate of 1 mL per min and an injection volume of 100 µL.

Statistical analysis
For statistical analysis, data were entered into SPSS version 18. The qualitative data were analyzed and the correlation between nominal variables was evaluated, using Chi-square. After confirming the normality of quantitative data, the ANOVA test was applied for making comparisons. In case the data were not normally distributed, non-parametric tests were applied. A P-value of less than 0.05 was considered statistically significant.

Results

Warehousing is one of the main stages in the production and supply chain of medicinal plants. The present study examined the effective parameters in fungal growth and aflatoxin production in 80 samples of medicinal plants obtained from 40 warehouses during the warehousing stage. Tables 1 and 2 show the effective factors in the warehousing of medicinal plants and also the prevalence of contamination with *Aspergillus*. This data was gathered from different regions of the country at different times. Based on the results, except for relative humidity and temperature, the factors under investigation showed no significant correlation with *Aspergillus* growth and aflatoxin production in the studied warehouses. The maximum relative humidity level was obtained as < 37%; in addition, the water activity and moisture of the medicinal plants were less than the critical range for fungal growth and aflatoxin production. Therefore, this could be the reason for the lack of correlation between the measured factors and *Aspergillus* fungus growth/aflatoxin production.

Table 1. The correlation coefficient of evaluated factors among warehouses in various regions of the country.

	Moisture sample (%)	Water activity	Relative moisture (%)	Temperature (°C)	*Aspergillus* contamination
Moisture sample (%)	1	0.15	0.14	0.05	0.01
Water activity	0.15	1	0.08	0.08	0.003
Relative moisture (%)	0.14	0.08	1	0.684	0.07
Temperature (°C)	0.05	0.08	0.684	1	0.06
Aspergillus contamination	0.01	0.003	0.07	0.06	1

The maximum level of water activity in the medicinal plants was lower than the critical range needed for the growth of *Aspergillus*. The investigation of average water activity at different times revealed that the moisture exchange between the medicinal plants and the surrounding environment was not at a level that could increase the water activity or moisture of the medicinal plants. Temperature changes during the warehousing stage were different in various regions of the country. However, the comparison of the temperature data and their association with *Aspergillus* growth and aflatoxin production in various warehouses revealed relatively comparable results. The region was not an influential factor in the diversity of *Aspergillus* species. Furthermore, contamination prevalence varied in different medicinal plants.

Table 2. Moisture of sample, water activity, relative moisture and temperature changes in various regions of the country.

	Mashhad	Tehran	Kerman	Bandar Abbas
Moisture sample (%)	3.9±0.8	4.1±0.9	4.0±1.1	4.1±0.9
Water activity	0.26±0.125	0.24±0.12	0.26±0.125	0.24±0.12
Relative moisture (%)	20.1±6.95	22.6±11.4	18.3±7.7	21.9±6.65
Temperature (°C)	23.3±10.0	23.8±10.05	26.1±10.2	25.2±10.3

The examination of factors affecting the aflatoxin level showed a significant difference between the samples in terms of moisture (P=0.042). No significant correlation was observed between the average temperature and aflatoxin levels (P=0.374). No significant association was observed between the mean of humidity and aflatoxin levels (P=0.878). In terms of the effect of water activity on the growth of *Aspergillus*, the maximum amount was less than the critical zone of fungal growth and aflatoxin production. This could suggest that moisture exchange between the samples and surrounding environment was not sufficient to increase water activity or moisture content of the samples, significantly affecting fungal growth and aflatoxin production. This could indicate that moisture exchange between the samples and environment could not lead to the increased moisture content in the samples, fungal growth, and aflatoxin

production. Environmental factors are among the most significant contributing factors of fungal growth and aflatoxin production.

Discussion

In a study by Giorni and colleagues in 2007, it was revealed that the aflatoxin level is strongly influenced by the moisture content of raw compounds [5]. Ellis Wo et al. (1994) demonstrated that fungal growth and aflatoxin production in storage conditions on the nutrient environment, such as dried fruits, might be affected by the gas composition of the environment, water activity, acidity, microbial interactions, and length of storage [6]. In addition, Magann et al. (1984) marked that *Aspergillus* could grow in an enriched environment (exposed to carbon dioxide gas in the presence of oxygen), leading to the production of aflatoxin. The resistance of different toxin agents of *Aspergillus* depends on low levels of oxygen (0.14%) and higher levels of carbon dioxide (15%), as well as temperature and water activity [7]. In another research, Georgiadou et al. (2012) claimed that the preservation of pistachio in the temperature of 5-7°C and relative humidity of 45-60% could prevent aflatoxin contamination [8]. According to the results obtained by Set and Erkmen (2010), drying of pistachio could reduce the moisture of the core from 50% to less than 5%, which is considered as the optimal storing condition for this product [9]. In this regard, Marin et al. (2012) stated that optimizing the storage condition by reducing humidity was experimentally deduced from the proposed models of fungal growth and aflatoxin production in pistachio [10]. Khazaeli et al. (2017) showed that among 120 tested samples, 37 (30.8%) were contaminated with aflatoxins (range: 0.2-57.5 µg/kg); based on the findings, all these samples were contaminated by AFB1 (0.7-57.5 µg/kg). The examination of factors affecting aflatoxin level showed a significant difference between the samples in terms of moisture ($P=0.046$). In addition, the aflatoxin level was not significantly influenced by the packaging of the samples ($P=0.578$) [11]. In another study, Khazaeli et al. (2017) showed that there was a significant difference in AF decomposition under different treatment conditions ($P<0.001$). Also, degradation of AFB1 was found to be both time and temperature-dependent [12]. Khazaeli et al. (2014) showed that mean aflatoxin contamination in the roasted samples (16.53 µg/kg) was significantly higher than the raw nuts (7.25 µg/kg) (P-value< 0.001). After measuring the moisture content of the samples and statistical analysis, there was a significant correlation between the aflatoxin and the amount of moisture in the samples (P-value< 0.001) [13].

According to the findings of previous studies, unfavorable conditions lead to the development of *Aspergillus* infection and the production of the associated toxins (e.g., aflatoxin). Given the role of various environmental factors in fungal growth and aflatoxin production (as a secondary fungal metabolite), improvement of these factors could significantly prevent the infection.

Conclusion

In this study, a number of environmental factors such as relative humidity, temperature, and moisture content of the samples, which could have an impact on the infection of medicinal herbs, were evaluated for twelve months. According to the results, there was insufficient moisture exchange between the sample and the surrounding environment to increase *Aspergillus* growth and aflatoxin production. This indicated that aflatoxin levels were not affected by environmental factors in this study. In fact, contamination of samples happened at previous stages, including culture, harvest, and storage, which could be increased in an unfavorable environment during different stages.

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