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Contamination of Armenian Red Pepper by Microfungi and Aflatoxin B_1

Karine Grigoryan
Varduhi Hovsepyan

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***Key words**: paprika, hot red pepper, filamentous fungi, aflatoxin B_1, strains, contamination*

Introduction

Aflatoxins are often found on different types of red peppers during harvest and processing. According to the studies conducted in the UK [2], Portugal [13], Spain [20], India [18], Hungary [6] and Ireland [15] a high frequency of aflatoxin occurrence was observed in ground red pepper, which is a serious problem for public health.

Aflatoxins are produced by fungal strains of *Aspergillus flavus, A. nomius, A. parasiticus* and others under favorable conditions for their development.

According to Dimicr [3], from paprika and black pepper have been isolated species basically from genus *Aspergillus*: *A.glaucus, A. restrictus, A. flavus, A. ochraceus*, including the strains – producers of aflatoxins. The author noticed that contamination of spices by fungi occurs at the raised moisture content or storage of spices' samples in warehouse with high level of relative humidity. Species of *Cladosporium* were identified in 40 % of the analysed samples, *Candida* - in 40 %, *Penicillium* - in 18 %, *Acremonium* - in 18 %, *Fusarium* - in 18 %, *Aspergillus*-in 12 %. The total quantity of diaspors of mould in 1 g of red pepper was $8x10^3$ cfu/g. Only one strain of *A. flavus,* in experimental conditions, demonstrated the ability to produce aflatoxins.

According to Shundo [21], totally 70 samples of the red pepper were selected in Brazil for analyzing aflatoxins (AFs) and ochatoxin A (OTA) content. AFs were found in 82.9 % of samples, and AF in 61.4 %, among the analyzed samples, in range from 0.5 to 7.3 μg/kg, OTA - in 85.7 %, in range from 0.24 to 97.2 μg/kg. Out of the 182 analyzed samples of chili pepper selected from markets in India, 59 % were found to be contaminated with aflatoxin B_1.In 18% of the samples, the toxin levels exceeded the acceptable permissible limits. A high concentration of AFB_1 measuring 970 μg/kg, was found in a single sample of red pepper [18].

Unsatisfactory hygienic conditions during drying, transportations and storages of the red pepper, can lead to contamination by bacteria and toxigenic

microfungi [9]. Ardic et al. [1], has carried out monitoring of 75 samples of the red pepper on the maintenance AFB_1. 72 samples out of 75 analyzed, (i.e. 96 %) contained AFB_1 in quantity from 0.11 to 24.7 μg/kg. The content of AFs in eleven samples, i.e. in 14.7 %, exceeded the marginal levels established by legislative structures of EU. It is established that observance of hygienic conditions at all stages (processing, packing and storing) are the important factors influencing microbiological and chemical safety of spices. Zinedine et al., [23] identified AFB1 in samples of black pepper at the level of 0.09 mkg/kg, red pepper - 2.88 μg/kg, and caraway seeds - 0.03 mkg/kg. In separate samples of red pepper the maintenance of AF B_1 exceeded 9.68 μg/kg. The toxicological analysis of 70 strains isolated from soil and red pepper has shown that 16 strains (i.e. 36.6 %) from 44 strains *A. flavus*, allocated from soil and 25 % strains *A. flavus* - from 26 samples of red pepper chilli, produced AFs. A survey of 90 chilli products sold in Australia showed extensive contamination with aflatoxins. Overall only 9% of samples complied with the 5μg/kg maximum level as set out by Australian standards and another 12% were considered marginal. On the whole spice powders performed worse compared with minced and sauce samples having higher passing rates. Whole and crushed fruit also performed better than ground samples. The mean level of AFB_1 recorded was 19μg/kg with a maximum of 89μg/kg [12].

The European Union has set strict limitations on aflatoxins levels in various foodstuffs, such as groundnuts, nuts, dried fruits, cereals, milk and spices, including paprika and chili [5].

Contamination of pepper by aflatoxin-forming fungi has been of increasing concern in international trade circles [12, 22]. The incidence of *A. flavus* and the occurrence of aflatoxin in black and red pepper have been well documented [10].

The aim of this work was to assess the intensity and frequency of contamination of dry red pepper by moulds and mycotoxins in Armenian markets.

Material and Methods

Totally 45 local samples of red pepper were analyzed. The samples have been taken from the markets of Yerevan between 2019-2020 years.

Sampling

Samples were collected at least 100 gm from each of 3 containers in sterile jars and were tightly sealed. If spices were in small packages, they had been delivered to the laboratory in unopened packages.

Fungal Isolation: Dilution Plating

Two-gram samples of ground spices were blended in 99 ml of 0.1% (w/v) peptone broth in a wiring blender for 2 minutes at high speed. Serial dilution down to 10 was made, and 1 ml of each dilution was used on sterile Petri-dishes with four replicates per dilution. Plates were swirled to ensure even distribution of conidiospore (ISO 21527-2:2008) [11].

Fungal Isolation: Direct Plating

Whole pepper corns were sterilized in 25 (v/v) sodium hypochlorite , which include 0.5 % active chlorine for two minutes and rinsed twice in sterile distilled water before being plated onto tap Petri dish with Czapek –Yeast extract agar (CYA), glucose-yeast agar GYA [19].

Identification of pure culture of fungi was done according to the Manuals: Pitt and Hocking, Samson 1996, Elis 1976. [4, 16, 17,19].

Determination of the AFB_1 in fungal extracts from isolated strains of microfungi and samples of red pepper was conducted by using the method of TLC.

Results and Discussion

45 samples of red ground pepper were analyzed. 23 from the analyzed samples have been packed in to paper bags: it is worth noting that 22 were without packaging. 1126 strains of filamentous fungi, which belong to 13 species of micromycetes from five genera, have been isolated from samples of red pepper. It was noticed that *Mucor plumbeus* and *Absidia corymbifera* were predominant species from class *Zygomycetes* isolated from red pepper. In most cases, contamination by the mentioned species has been noticed in unpacked samples. Filamentous fungi from family *Moniliaceae* were represented by *Aspergillus* (7 spp.) and *Penicillium* (3 spp.) genera (Table 1).

Table 1

Systematic list of fungal species, contaminating red pepper

Classes	Femaly	Genus	Quantity	
			species	strains
Zygomycetes	*Mucoraceae*	*Absidia*	1	15
		Mucor	1	57
Deuteromycetes	*Moniliaceae*	*Aspergillus*	7	1012
		Penicillium	3	23
	Dematiaceae	*Alternaria*	1	19
Total: 2	**3**	**5**	**13**	**1126**

From identified fungal species, *A. niger* and *A. flavus* were prevalent in red pepper (Fig. 1). *A. niger* occurred in 88% and *A. flavus* in 79% of observed samples of red pepper. Frequency of occurrence of other species from genus *Aspergillus* can be expressed in the following order: *A. fumigatus (18%), A.ochraceus (11%), A. carbonarius (10%).* The rest of the species from genus *Aspergillus* was characterized with less frequent occurrence. The genus *Penicillium* was represented by the following 3 species: *P. citrinum (3%), P. lanosum (3%)* and *P. verrucosum var. cyclopium (2%).* The abovementioned species were being isolated mainly from packaged samples of red pepper.

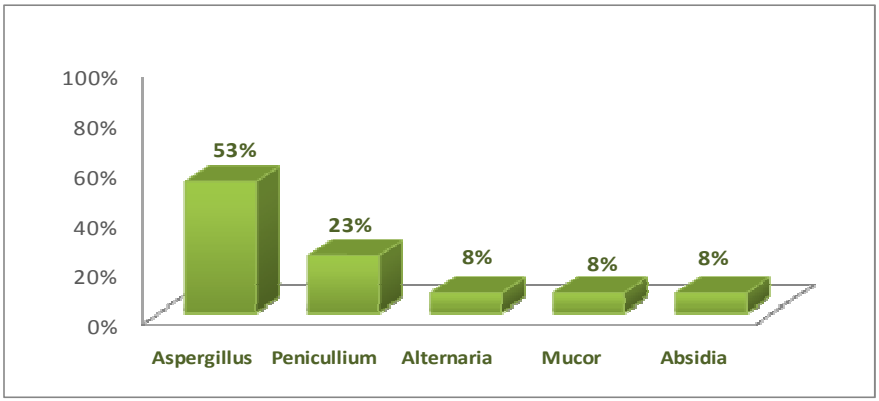

Figure 1. Percentage of filamentous fungi genus isolated from red pepper

The family *Dematiaceae* was represented by genus *Alternaria,* which includes one species of *A. alternata. A. alternata* often contaminates chilli pepper, both during vegetation and storage periods [16].

The total count of colony was assessed - forming units of microfungi in 1 g spices (CFU.g-1).

In accordance with (Technical Regulation TR/TS 021) acceptable values of filamentous fungi in 1 g of ready to use spices, do not have to exceed $1x10^3$ cfu/g. Contamination degree of red pepper by microfungi in 91% of analyzed samples surpassed maximum values. The content of fungal conidiospores in different samples was more than $1x10^3$ cfu/g and reached $1.4x10^4$ cfu/g. Only in two samples of red pepper, contamination level had very low values. Results of contamination of red pepper are presented in Table 2.

Table 2

Composition and occurence frequency of species of fungi determination in red pepper

Species of fungi	Freqiency of occurence of fungi in red pepper*
Absidia corymbifera Saccardo	26 %
Alternaria alternata Keissler	31 %
Aspergillus candidus Link	8 %
Aspergillus carbonarius (Bainier) Thom	13 %
Aspergillus flavus Link	82 %
Aspergillus fumigatus var. griseobrunes Rai et Singh	56 %
Aspergillus niger Thom	88 %
Aspergillus ochraceus Wilhelm	15 %
Aspergillus terricola Marshall et Raper	13%
Mucor plumbeus Bon	12 %
Penicillium citrinum Thom	3 %
Penicillium verrucosum var. Cyclopium	4 %
Penicillium lanosum Westl.	27 %

>50% - high frequency of occurrence
25-49% - moderate frequency of occurrence
12-24% - low frequency of occurrence
< 12% - rare frequency of occurrence [14]

The determination of aflatoxin B_1 was carried out in 19 fungal extracts of *A. flavus* strains isolated from local red pepper, by TLC method. In 13 extracts (68.4%) of examined strains of *A. flavus* it was occurrence of aflatoxin B_1. Its quantity varied from 5 to 55μg/100ml of liquid media. There was no occurrence of aflatoxins in six extracts of *A. flavus* isolated generally from red pepper or paprika. 45% of *A. flavus* strains isolated from red pepper were producers of aflatoxins (Table 3).

Table 3

Content of AFB1 in extracts of strains A. flavus, isolated from local samples. Paprika and chilli pepper

Numbers of *A. flavus strains*	Samples	AFB1 ng/100ml
7-3	Paprika	55
1-5	Hot red pepper	50
4-1	Paprika	45
1-2	Hot red pepper	34
1-8	Hot red pepper	32
6-4	Hot red pepper	31
15-4	Paprika	20.1
2-2	Paprika	10
65	Hot red pepper	7.3
1-1	Paprika	5.5
34'	Paprika	5.5
4-2	Paprika	5.5
2-3	Paprika	5
18	Hot red pepper	ND
15-5	Paprika	ND
15-1	Paprika	ND
11	Paprika	ND
64	Hot red pepper	ND
1-14	Paprika	ND

Numerous surveys carried out by foreign researchers, shown that all varieties of dry red pepper were exposed by aflatoxin producing fungi. There is much evidence of detection of aflatoxin B_1 in the following quantities: 525 μg/kg in Ethiopia [8], 48 μg/kg in pepper from India [21], 243 μg/kg from Italy [7].

It was carried out the chemical analysis of 17 samples of powdery dry red pepper. The results show that in 9 samples of red pepper it was detection of aflatoxin B_1. In 8 samples concentration of aflatoxin B_1 varied from 10 to 50 μg/kg, exceeding acceptable values (5μg/kg). In samples of chilli concentration of aflatoxin B_1 reached 50 μg/kg.

Conclusions

The present work indicated that dried examined red pepper were contaminated with several fungi especially with members of *Aspergillus, Penicillium* and *Mucor*. A number of these fungi are capable of producing mycotoxins such as aflatoxin B_1. These findings indicate that there may be a risk of human exposure to mycotoxins through the consumption of dry rep pepper. This indicates that continuous monitoring of ground red pepper for toxigenic fungi is necessary to minimize mycotoxin contamination.

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Հայկական կարմիր պղպեղի աղտոտումը միկրոսկոպիկ սնկերով և աֆլատոքսին B₁-ով

Կարինե Գրիգորյան
Վարդուհի Հովսեփյան

Ամփոփում

Հանգուցային բառեր. *պապրիկա, կծու կարմիր պղպեղ, միցելիալ սնկեր, աֆլատոքսին B₁, շտամ, աղտոտվածություն*

Այս հետազոտության նպատակն էր ուսումնասիրել աղացած կարմիր պղպեղի աղտոտվածությունը միկոտոքսիգեն սնկերով: 2019-2020 թվականների ընթացքում ուսումնասիրվել է հանրապետության տարբեր մարզերից վերցված 45 նմուշ: Աղացած կարմիր պղպեղից անջատվել են միկրոմիցեների 13 տեսակներ, որոնք պատկանում են 5 ցեղերի: *Aspergillus niger, A. flavus, Aspergillus fumigatus var. griseobrunes, A. ochraceus, Penicillium lanosum* և *Absidia corymbifera* տեսակները առավել հաճախ անջատվել են կարմիր պղպեղի նմուշներից 2 % ագար պարունակող Czapek-Dox միջավայրում: Կատարվել է աղացած կարմիր պղպեղի 17 նմուշի քրոմատոգրաֆիկ անալիզ՝ պարզելու աֆլատոքսին B₁-ի պարունակությունը: Ուսումնասիրված 9 նմուշում հայտնաբերվել է աֆլատոքսին B₁: Պապրիկայի և կծու կարմիր պղպեղի մեջ աֆլատոքսինի կոնցենտրացիան եղել է 10-50 մկգ/կգ: Կարմիր պղպեղի նմուշներում աֆլատոքսինի պարունակությունը կախված չէր դրա կծու լինելուց: Այս հետազոտության ընթացքում այլ միկոտոքսիններ չեն հայտնաբերվել: Հետազոտությունները ցույց են տալիս, որ միկոտոքսինների ազդեցության վտանգ կարող է նկատվել չորացած կարմիր պղպեղի օգտագործման ժամանակ:

Загрязнение армянского красного перца микроскопическими грибами и афлатоксином B_1

Карине Григорян
Вардуи Овсепян

Резюме

Ключевые слова: паприка, острый красный перец, мицелиальные грибы, афлатоксин B_1, штаммы, контаминация

Целью настоящего исследования было изучение контаминации молотого красного перца микотоксигенными грибами. В течение 2019-2020 гг. проанализировано 45 образцов, взятых из разных регионов республики. Из молотого красного перца выделено 13 видов микромицетов, принадлежащих к пяти родам. Виды *Aspergillus niger, A. flavus, A. fumigatus var. griseobrunes, A. ochraceus, Penicillium lanosum и Absidia corymbifera* наиболее часто выделяли из образцов красного перца на агаризованной среде Чапека-Докса. Проведен хроматографический анализ 17 образцов молотого красного перца на наличие афлатоксина B_1. Афлатоксин B_1 был обнаружен в 9 образцах. Концентрация афлатоксина в паприке и остром красном перце находилась на уровне от 10 до 50 мкг/кг. Содержание афлатоксина в образцах красного перца не зависело от его остроты. Другие микотоксины не были идентифицированы в ходе представленного исследования. Результаты показывают, что существует риск воздействия микотоксинов на человека при употреблении молотого красного перца.

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