



Biolog. Journal of Armenia, 4 (67), 2015

## DETECTION OF TOXICITY OF FILAMENTOUS FUNGI ISOLATED FROM DRIED VINE FRUIT BY BIOTEST METHOD

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Dried vine fruit is a favorable substrate not only for growth of toxigenic fungi but for toxin synthesis since it contains enough humidity and sugars. The results of our research have shown high contamination level of dried vine fruit by ochratoxigenic and aflatoxigenic fungi from genus *Aspergillus*. Toxigenic potential of 75 strains from *Aspergillus* genera: 30 - *A. carbonarius*, 15 - *A. niger*, 10 - *A. sclerotium*, 5 - *A. lacticoffeatus*, 3 - *A. ochraceus* and 12 - *A. flavus*, was studied. The biotest was carried out on Brine Shrimp (*Artemiasalina*) Larvae. 20 strains of *A. carbonarius*, 6 - of *A. niger* and 2 - *A. sclerotium* shown toxicity of different degree. All the rest did not show any toxic properties. Eight of 12 analyzed *A. flavus* strains were toxic.

*Aspergillus* genera – ochratoxigenic – aflatoxigenic – fungi – strains

Չորացրած խաղողը բարենպաստ սուբստրատ է հանդիսանում ոչ միայն տոքսիգեն սնկերի աճի, այլ նաև նրանց կողմից տոքսինների սինթեզի համար, քանի որ պարունակում է բավարար քանակությամբ խոնավություն և շաքարներ: Մեր հետազոտության արդյունքները ցույց տվեցին չորացրած խաղողի բարձրաստիճան աղտոտվածության՝ *Aspergillus* ցեղին պատկանող օքրատոքսիգեն և աֆլատոքսիգեն սնկերով: Ուսումնասիրվել է *Aspergillus* ցեղին պատկանող 75 շտամների. (30 – *A. carbonarius*, 15 – *A. niger*, 10 – *A. sclerotium*, 5 – *A. Lacticoffeatus*, 3 – *A. ochraceus* և 12 – *A. flavus*), տոքսիգեն պոտենցիալը *Artemiasalina* խոիկատոն խեցգետնակերպ թրթուրների նկատմամբ: Տոքսիկ հատկություններ դրսևորել են *A. Carbonarius*-ի 20, *A. Niger*-ի 6 և *A. Sclerotium*-ի 2 շտամներ: Մնացած շտամները որևէ տոքսիկ հատկություն չեն դրսևորել: Ուսումնասիրված *A. flavus*-ի 12 շտամներից 8 –ը եղել են տոքսիկ:

Ցեղ *Aspergillus* – օքրատոքսիգեն – աֆլատոքսիգեն – սնկեր – շտամներ

Сушеный виноград является благоприятным субстратом не только для роста токсигенных грибов, но и для их токсинообразования, так как содержит достаточное количество влаги и сахаров. Результаты наших исследований показали на высокую степень контаминации сушеного винограда охратоксигенными и афлатоксигенными грибами из рода *Aspergillus*. Исследован токсигенный потенциал у 75 штаммов из рода *Aspergillus*: 30 – *A. carbonarius*, 15 – *A. niger*, 10 – *A. sclerotium*, 5 – *A. Lacticoffeatus*, 3 – *A. ochraceus* и 12 – *A. flavus*. Биотестирование проводили на личинках жабраногого рачка *Artemiasalina*. 20 штаммов вида *A. carbonarius*, 6 штаммов *A. niger* и 2 штамма *A. sclerotium* проявили токсичность в разной степени. Остальные штаммы не проявили токсичных свойств. Из исследованных 12 штаммов *A. flavus* 8 оказались токсичными.

Под *Aspergillus* – охратоксигенные – афлатоксигенные – грибы – штаммы

Species from *Aspergillus* section *Nigri*: *A. niger*, *A. carbonarius*, *A. tubingensis* are the main fungi producing OTA (ochratoxin A) in grape and raisin. The problem of the

contamination of dried vine fruit by ochratoxin A is studied in many countries with developed viticulture and winemaking: in Spain [6], France [18], Italy [5], Portugal [21], Argentina [14] and Australia [13]. In these studies it is remarked that most of ochratoxigenic strains belong to *A. carbonarius* species. Small amount of strains belonging to *A. niger* species produced OTA. As it was shown in researches the presence of ochratoxin A in raisin samples is mainly associated with *A. carbonarius* species in California as well [15].

During mycological analyses of corinthian raisin (currant) spent in Greece the authors revealed a high contamination level of raisin by species *Aspergillus spp.* among which *A. niger* and *A. carbonarius* were dominated [22]. Most of *A. carbonarius* strains produced OTA. The results of analyses of dried vine fruit (currant, sultana and raisin) in Spain have shown domination of *A. niger* (98%) and *A. carbonarius* (58%) species [3]. 96.7% of all isolated *A. carbonarius* strains and just 0.6% of *A. niger* strains produced OTA.

Recently they repeatedly reported about contamination of dried vine fruit by aflatoxigenic species of *A. flavus* [4, 12]. In most cases *A. flavus* is considered to be one of the most frequent occurring after *A. niger* and *A. fumigatus* species contaminating dried vine fruit [10]. The presence of *A. flavus* species in dried vine fruit is a risk from point of view a possibility of product contamination by aflatoxins.

**Materials and methods.** 167 samples of dried vine fruit (white and black types of raisin and sultana) were analyzed. 87 of all analyzed samples were Armenian produced and 80 – imported from different countries. The samples were collected from different markets in Yerevan city according to GOST 1750-86 [2] and EC 2006b (No 401/2006) [9]. Following nutrient mediums were used: CYA (Chapek-Yeast Agar medium, HiMedia Ltd.), GYA (Glucose-Yeast Agar medium, HiMedia Ltd.), and MEA (Malt-Extract Agar medium, HiMedia Ltd.). Mycological analyses dried vine fruit samples were carried out with direct plating and dilution plating methods [16]. Identification of isolated micromycetes cultures was spent based on macro- and microscopic characteristics according to Beelay V.I. [1], Raper K.B. and Fennell D.I. [17], Samson R.A. et al. [19], [20].

Determination of toxicity of micromycetes extracts and culture fluid was spent on Brine Shrimp (*Artemiasalina*) Larvae which are sensitive to toxic metabolites of filamentous fungi [8], [11]. The toxicity degree of fungi extracts and culture fluid was assessed by percentage mortality of larvae. Nontoxic extracts caused death to 9% of the larvae, slightly toxic – 10 to 49%, toxic – from 50 to 89%, very toxic – from 90 to 100%. Death of 1% larvae is allowed in control version. For biotest on larvae three replications have been used.

**Results and Discussion.** As the results of mycological analyses of 167 dried vine fruit samples in different type realizing in Armenia 508 strains potential producers of OTA and aflatoxins from *Aspergillus* genera were isolated. These strains belong to *A. carbonarius*, *A. niger*, *A. sclerotium*, *A. ochraceus*, *A. lacticoffeatus* and *A. flavus* species. From total number of isolated strains 61.8% of *A. carbonarius* species and 46.7% of *A. niger* species respectively were isolated from Armenian dried vine fruit samples.

The toxigenic potential of 75 strains from *Aspergillus* genera was studied. The toxicity of their culture fluids and chloroform extracts was determined. Fungi extracts was prepared by incubating them on Chapek – Docs liquid nutrient medium. Toxigenic potential of 30 *A. carbonarius* strains isolated from dried vine fruit samples was detected (tabl. 1).

20 *A. carbonarius* strains of all 30 analyzed were isolated from Armenian black dried vine fruit. 16 strains showed different degree of toxicity. Six *A. carbonarius* strains of all 10 isolated from imported samples were nontoxic and 4 showed slightly toxic properties (table 1). Five *A. niger* strains of all isolated from imported samples were analyzed. Three showed slightly toxic properties and two were nontoxic (tabl. 1).

Ten strains of *A. niger*, 10 – *A. sclerotium*, 5 – *A. lacticoffeatus* and 3 – *A. ochraceus*, isolated from Armenian dried vine fruit samples were analyzed. Slightly toxic properties were detected in 3 *A. niger* strains and 2 *A. sclerotium* strains. All of *A. lacticoffeatus* and *A. ochraceus* strains were nontoxic (tabl. 1).

**Table 1.** Results of biotest of ochratoxigenic strains isolated from dried vine fruit samples on *Artemiasalina* Larvae

Number of analyzed strains	Strains	Number of strains with toxicity in different degree							
		Toxicity of culture fluid				Toxicity of chloroform extract			
Strains isolated from local produced sample		A	B	C	D	A	B	C	D
20	<i>A. carbonarius</i>	4	8	4	4	4	8	4	4
10	<i>A. niger</i>	0	0	3	7	0	0	3	7
10	<i>A. sclerotiniger</i>	0	0	2	8	0	0	2	8
5	<i>A. lacticoffeatus</i>	0	0	0	5	0	0	0	5
3	<i>A. ochraceus</i>	0	0	0	3	0	0	0	3
Strains isolated from imported samples		A	B	C	D	A	B	C	D
10	<i>A. carbonarius</i>	0	0	4	6	0	0	4	6
5	<i>A. niger</i>	0	0	3	2	0	0	3	2

Note hereinafter: according to Brown [8], Harwing, Scott [11] and Biji [7],

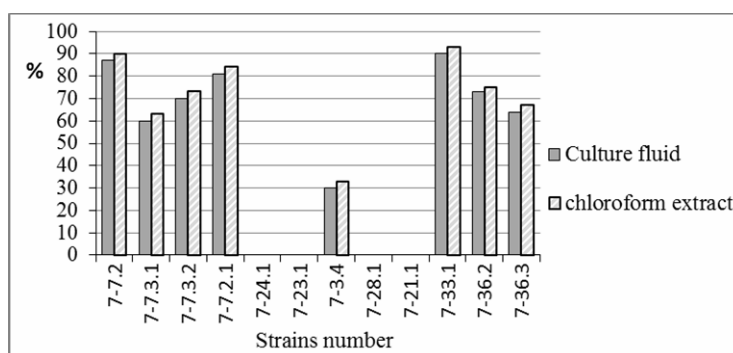
A= very toxic extracts of fungi cause the death of larvae from 90% to 100%,

B= toxic – from 50 to 89%,

C= slightly toxic – from 10 to 49%,

D= nontoxic – up to 9%.

67 *A. flavus* strains potential producers of aflatoxins were isolated from dried vine fruit samples. The presence of aflatoxin B<sub>1</sub> was studied in 12 *A. flavus* strains by biotest method (fig. 1). Very toxic properties were revealed in 3 *A. flavus* strains. Four strains caused the death of 75% of larvae. Slightly toxicity was detected in 1 strain. All the remainder 4 strains were nontoxic.



**Fig. 1.** Toxicity of extracts and culture fluid of *A. flavus* strains isolated from dried vine fruit by biotest

Our studies have shown that there is a risk of contamination of dried vine fruit by ochratoxigenic and aflatoxigenic fungi. Most of the *A. carbonarius* strains we analyzed produced ochratoxin A. The risk of contamination of product named above by *A. flavus* aflatoxigenic fungi is high either. Based on the fact that dried vine fruit is a good substrate for not only fungal growth but also toxin production, the risk of accumulation of

OTA and aflatoxin B<sub>1</sub> in dried product increases. Thus, controlling the contamination level of dried vine fruit by *A. flavus* and *A. carbonarius* species is a primary task for countries with developed viticulture including Armenia. Especially considering the fact that in our country there are no regulations for OTA and aflatoxins in grapes products.

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*Received on 16.09.2015*