

RISK ASSESSMENT OF CONTAMINATION OF GRAPE PRODUCTS BY OCHRATOXIN A IN ARMENIA

Karina Grigoryan^{1*}, Lusine Hakobyan¹, Mariam Sargsyan¹

¹Yerevan State University Laboratory of Food Control, Alex Manoogyan 1, Yerevan, Armenia

*e-mail: foodlab@inbox.ru

Abstract

Mycotoxins produced by microscopic fungi in the grape producing system and grape products have received much recent attention. The aim of the work is to assess the real risk of presence of OTA in grape and grape products.

Species from section *Aspergillus Nigri* have been detected in 75% of the investigated samples from Ararat, 58% of raw grapes from Vayk and 88% from Kotayk regions. Among species from this group, percentage *A. carbonarius* makes 25-27%.

The species frequently were allocated from the damaged overripe berries. Primary contamination of grapes occurs during vegetation since the soil is the basic reservoir of these species. In particular, the period from 15 days pre-harvest until harvest was the critical time for rapid development of *Aspergillus* rots. Also, bad hygienic conditions at winemaking or juice processing plants promote the further growth of a level contamination of fungi from section *A. Nigri*.

Fungal growth on grapes, grapes musts, equipment, in storage and its processing is another major source of contamination by OTA. Studying of toxigenic potential of isolated strains has shown that about 45% strains of *A. carbonarius* are producing OTA. Other related species of *A. niger* have also been reported reliably as producers. However, the results of our research have shown that all the toxigenic strains belong to "black" *Aspergillus* not produced of OTA, exclude *A. carbonarius*.

Key words: Mycotoxins, OTA, *Aspergillus*, grape, contamination.

1. Introduction

In the latest years ochratoxin A (OTA) has attracted the attention of health authorities. OTA is a mycotoxin that possesses a risk to human health due to its nephrotoxic, immunotoxic, mutagenic, teratogenic and carcinogenic effects (Plestina [1] and Walker [2]). OTA production in grape and grape products is mainly

ascribed to species from *Aspergillus* section *Nigri*, especially *A. carbonarius* and *A. niger* (Leong *et al.* [3] and Battilani *et al.* [4]). Species from genus *Alternaria* spp, *Aspergillus* spp, *Botrytis cinerea*, *Cladosporium* spp, *Eurotium* spp, *Penicillium* spp and *Rhizopus* spp are regarded as the main natural contaminants of grapes (Varga *et al.* [5]). *A. carbonarius* probably plays a relevant role, because the percentage of positive strains and the amount of OTA produced in vitro were generally higher than those found in the other black *Aspergilli* (Magnoli *et al.* [6] and Cabañes *et al.* [7]). Ponsone [8] have shown that OTA was detected in 24% of all isolated strains of *A. carbonarius*. In the other researches Melki Ben Fredj *et al.* [9] have recently shown that in spite of the higher frequency of occurrence of *A. niger* in comparison with *A. carbonarius* in table grapes the highest levels of OTA production were detected in strains of *A. carbonarius* (80% of them), while only 5% of *A. niger* aggregate produced OTA. As shown in investigations carried out in Italy (Lucchetta *et al.* [10]) within 2003 – 2007 years contamination level of grape by ochratoxigenic fungi and OTA production is depend on environmental condition, climate and even the year of harvesting. The maximum permissible level fixed by The European Community is 2.0 µg/L of OTA for wines, grape must and grape juice (European Commission [11]).

The central grape area in Armenia comprises vineyards located in Ararat and Vayk districts, which represent over 85% of the Armenian wine production area. Previous studies showed that the presence of potentially OTA producer species has been detected in raisin (Hakobyan *et al.* [12]). No data are available on the dynamics of ochratoxigenic fungi through different stages of grape development in the field and the possible OTA contamination.

In order to improve the knowledge on the epidemiology of black *Aspergilli* in grapes, a survey was carried out during 2008– 2009.

To evaluate the dynamic of fungal populations from *Aspergillus* section *Nigri* on grapes from the vineyards during harvest, the level of content of conidiospores in soil and ochratoxigenic potential of isolated strains were detected.

2. Material and Methods

2.1. Grape sampling

The survey was carried out from 2008 to 2009 in Ararat, Vayk and Kotayk vineyards on Areni black grape sort. Samples were taken during the one growth stage: ripe berry (harvest time). 20 bunches of grape were collected from each vineyard.

2.2. Mycological analysis of grapes

Five randomly chosen berries from each bunch were put onto the surface of the DRBC (Dichloran Rose Bengale Chloramphenicol, HiMedia) agar and plates were incubated at 25 °C for 7 days. For identification of *Aspergillus*, cultures were grown on Czapek Yeast Extract Agar (CYA) at 25 °C±1 °C and 37 °C±1 °C. Identification of fungal strains was done according to (Varga and Kozakiewicz [13], Klich and Pitt [14], Pitt and Hocking [15] and Samson *et al.* [16]).

2.3 Mycological analysis of soil

During mycological analysis of soil serial dilution method were used in accordance with Hocking *et al.* [17].

2.4 Determination of toxigenic potential of fungi from *A. Nigri* section

It was carried out determination of ochratoxin A in fungal extracts received during incubation of tested strains on liquid Chapeck-Dox medium in accordance with Spradaro *et al.* [18].

3. Results and Discussion

In Table 1 and Figure 1 are shown the results of mycological analyses of grape samples of from three vineyards areas in Armenia: Ararat, Vayk and Kotayk, at harvest period. Isolated fungi belong to 6 genera and 19 species. 430 strains were totally isolated and identified. During harvest period the genus *Aspergillus* was only introduced with species from *Aspergillus* section *Nigri*. Specie *A. carbonarius* was isolated more often in grapes collected from Kotayk region. From 20 analyzed samples the mentioned specie was detected in 15 (75 %) samples. 25 -27 % (over 35 strains) of isolated strains from grape samples from Kotayk region were identified as *A. carbonarius*. The low frequency

of occurrence of this specie is noticed in Vayk region. Among 20 analyzed samples from Vayk region *A. carbonarius* is detected in 3 (15 %) samples. 87 strains of filamentous fungi were detected in mentioned region during harvest time from which 8 % were identified as *A. carbonarius*.

Table 1. Fungal species ccontaminating of Black Areni during harvest period in three regions

Species of fungi	Ararat n=20	Vayk n=20	Kotayk n=20
<i>B. cinereae</i>	10 (13%)*	3 (3.4%)	15 (7.3%)
<i>A. niger</i>	20 (26%)	25 (29%)	75 (36.5%)
<i>A. carbonarius</i>	12 (15.6%)	7(8.0%)	35 (17.1%)
<i>A. japonicus</i>	3 (3.9%)	4 (4.5%)	5 (2.4%)
<i>A. awamori</i>	2 (2.6%)	x**	4 (1.9%)
<i>A. acuelatus</i>	1 (1.3%)	x	3 (1.5%)
<i>A. pulverulentum</i>	2 (2.6%)	1 (1.1%)	3 (1.5%)
<i>A. phoenicus</i>	15 (19.5%)	8 (9.2%)	1 (0.6%)
<i>A. tubingensis</i>	6 (7.8 %)	2 (2.3%)	8 (3.9%)
<i>A. uvarum</i>	3 (3.9%)	x	5 (2.4%)
<i>A. sclerotioniger</i>	8 (10.4%)	x	11(5.4%)
<i>Alternaria alternata</i>	4 (5.2%)	7 (8.0%)	3 (1.5%)
<i>Caldosporium herbarum</i>	9 (11.7%)	4 (4.5%)	5(2.4%)
<i>P. claviforme</i>	1 (1.3%)	x	x
<i>P. expansum</i>	3 (3.9%)	1 (1.1%)	1 (0.5%)
<i>P. granulatum</i>	1 (1.3%)	x	x
<i>P. viridicatum</i>	1 (1.3%)	x	x
<i>Rh. stolonifer</i>	35 (45.5%)	25 (35.7%)	30 (14.6%)
<i>Rh. oryzae</i>	2 (2.6%)	x	1 (0.5%)
Total	138 (100%)	87 (100%)	205 (100%)

** Not detected; *Quantity of strains and their frequency of occurrence

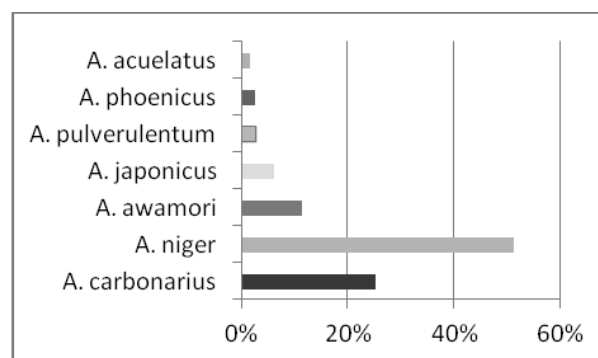


Figure 1. The frequency of occurrence of species from 'black' Aspergillus section isolated from grapes Areny during harvest period

The results of mycological analyses of samples of soil taken from different layers in harvest period are given in Figure 2. The high level of contamination was

detected in samples from upper layer of soil (0-2 cm) by *A. carbonarius* in harvest period. In particular, the period from 15 days until harvest is the critical time for rapid development of *Aspergillus* rots. *A. carbonarius* is one of the causative agents of mentioned disease. In addition, higher contamination level by *A. carbonarius* is noticed at this maturity stage. The most often mentioned specie occurs on damaged and overripe berry. The contamination of upper layers of soil from contaminated berries by *A. carbonarius* is a crucial issue. The results of analyzed soil, taken under Areni sort in earlier stages of maturation, have shown quite low level of contamination by *A. carbonarius*.

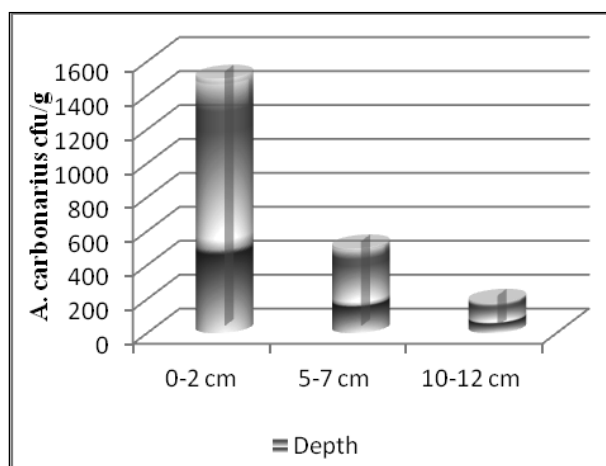


Figure 2. Distribution of *A. carbonarius* in soil layers of different depths

The chromatographic analyses of 117 fungal extracts from section *Aspergillus Nigri* were carried out. Twenty seven from all analyzed strains produced ochratoxin A, from which twenty five strains belong to *A. carbonarius* and two strains belong to *A. tubingensis*. OTA was not detected in extracts of all other strains (Table 2).

Table 2. The occurrence of OTA-producing strains from *Aspergillus* section *Nigri*, isolated from Armenian vineyards

Species of fungi	Number of analyzed strains	Ochratoxin A producing strains (%/number of strains)
<i>Aspergillus niger</i>	20	0
<i>Aspergillus carbonarius</i>	55	25/45
<i>Aspergillus japonicus</i>	5	0
<i>Aspergillus acuelatus</i>	10	0
<i>Aspergillus foetidus</i>	4	0
<i>Aspergillus sclerotioniger</i>	3	0
<i>Aspergillus tubingensis</i>	15	2/13
<i>Aspergillus phoenicus</i>	3	0
<i>Aspergillus awamori</i>	2	0
Total	117	27/23

4. Conclusions

The results of risk assessment of contamination of raw grape by ochratoxin producing fungi have shown high frequency of occurrence of fungi from section *A. Nigri* section. Thus, special attention must be paid to correct introduction of good agricultural practice in vineyard and harvesting, as well as to modern methods of sorting of raw grape material used in wine making, juice production and other products of grape processing.

5. References

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