Հայաստանի Գիտությունների Ազգային Ակադեմիա Национальная Академия Наук Армении National Academy of Sciences of Armenia	Հայաստանի Կենսաբանական Հանդես Биологический Журнал Армении Biological Journal of Armenia

•Фпрастриции и профильмов профильмов и теоретические статьи-•Experimental and theoretical articles•

Biolog. Journal of Armenia, 3 (66), 2014

FUNGI SPECIES FROM PENICILLIUM GENERA IN RAISINS CONSUMED IN ARMENIA

L.L. HAKOBYAN

Yerevan State University, Department of Microbiology lusinehl@yahoo.com

Raisin is widely used as dried fruit with high food value. It is also used as ingredient in such products ready to use as muesli and various confectionery products. Mycological safety of raisin is an actual problem from the point of view of its potential possibility of contamination by mycotoxins. 63 samples of Armenian and imported raisins are investigated. 31 species of filamentous fungi are isolated, 12 of which belonged to *Penicilliumgenera*. *P. expansum*, *P. grisofulvum*, *P. velutinumand P. ciatophora* of all isolated species had average frequency of occurrence. All the rest showed rare frequency of occurrence.

Raisin – fungi – Penicillium – filamentous – mycological safety

Չամիչը լայնորեն օգտագործվում է որպես բարձր սննդային արժեքով չոր մթերք։ Այն նաև օգտագործվում է որպես բաղադրիչ այնպիսի սպառման ենթակա սննդամթերքների մեջ, ինչպիսիք են մյուսլիները և հրուշակեղենի զանազան տեսակներ։ Չամիչի սնկաբանական անվտանգությունը հանդիսանում է արդիական խնդիր՝ միկոտոքսիններով նրա աղտոտվածության հավանականության տեսանկյունից։ Ուսումնասիրվել են հայկական և ներմուծված չամիչների 63 նմուշներ։ Անջատվել են միցելիալ սնկերի 31 տեսաներ, որոնցից 12- ը պատկանել են *Penicillium* ցեղին։ Բոլոր անջատվես սնկերից *P. expansum, P. grisofulvum, P. velutinum* և *P. ciatophora* տեսակները ունեցել են միջին հանդիպման հաճախականություն։ Իսկ մնացածների մոտ նկատվել է ցածր հանդիպման հաճախականություն։

Չամիչ – սնկեր – Penicillium – միցելիալ – սնկաբանական անվտանգություն

Изюм широко используется в качестве сухофрукта с высокой пищевой ценностью. Он также используется в качестве ингредиента в таких продуктах, готовых к употреблению, как мюсли и разнообразные кондитерские изделия. Микологическая безопасность изюма является актуальной проблемой, с точки зрения потенциальной возможности его контаминации микотоксинами. Исследовано 63 образца армянского и импортированного изюма. Изолирован 31 вид мицеллиальных грибов, 12 из которых принадлежали роду *Penicillium*. Из всех выделенных видов *P. expansum, P. grisofulvum, P. velutinumu P. ciatophora* имели среднюю частоту встречаемости. У остальных частота встречаемости редкая.

Изюм – грибы – Penicillium – мицеллиальные – микологическая безопасность

Research in different countries of over the world have shown that there is considerable information on the contamination of raisin by filamentous fungi-potential producers of mycotoxins. Dried fruits including raisin often are affected by fungi from *Aspergillus* and *Penicillium* genera. It is reported in many researches.

As a result of analyses of 60 dried fruit samples (raisins, dates and figs) in Yemen Republic [13] 23 species of filamentous fungi from 15 genera were isolated. *Penicillium chrysogenum* is considered to be among dominated species. As a result of the study by Argentinian scientists toxigenic species of filamentous fungi were detected even after disinfection of samples [12]. Fungi from *Aspergillus* genera were dominated. The frequency of occurrence of species from *Penicillium* genera was 13.5 %. All isolated strains of *P. citrinum* were strong producers of citrinin. According to Magan and Aldred [9], ochratoxigenic fungi *P. verrucosum* was found in samples of raisin in Europe. In other mycological study [7] of raisin 29 species of filamentous fungi were revealed. *Penicillium* species have high frequency of occurrence after *A. flavus, A. niger and A. fumigatus*.

Thurty-six species from 12 genera were identified from analyzed 100 samples of dried vine fruit taken from different supermarkets in 10 provinces in Egypt [17]. Along with the species of the genus *Penicillium*: *P. chrysogenum* and *P. oxalicum* were the most encountered species. In some researches [18] the genera *Penicillium* is reported as the most common and is found in all samples of dried figs, prunes, apricots and grapes. This genera is presented with four species from which *P. chrysogenum* is the most common fungi in last tree dried fruits from listed above.

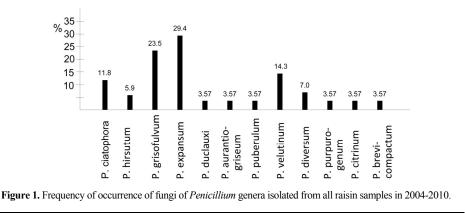
The aim of this research is: identification of fungi species from genus *Penicillium*, determination of their frequency of occurrence in different varieties of raisins and contamination degree of raisin samples by filamentous fungi from genus *Penicillium*.

Materials and methods. In order to identify fungi-contaminant of dried vine fruits realizing in markets and supermarkets in Yerevan 63 samples of Armenian and imported (mostly from Iran) raisin were analyzed. The sampling was carried out from 2004 to 2010 by dot method [5]at the markets as well as production areas.

The analyses were carried out by direct plating and serial dilution methods (1:10 dilution). For isolation of filamentous fungi CYA (Chapek-Yeast Agar medium, HiMedia Ltd.) and GYA (Glucose-Yeast Agar medium, HiMedia Ltd.) were used. Dilution method was done according to Pitt and Hocking [11]. After incubation of plats the contamination levels (CFU/g (colony forming units))[2, 3]and the frequencies of occurrence of fungi were determined [1, 4]. Identification of isolated fungi was carried out by microscopy method on the basis of morphological characteristics [10, 14].

Results and Discussion. The results of mycological analyzes of Armenian and imported samples of raisin have shown that 12 of all 31 isolated species of filamentous fungi belonged to the *Penicillium* genera.

On frequency of occurrence *P. expansum* (29.4%) specie occupies the first place which is followed by *P. grisofulvum* (23.5%), *P. velutinum* (14.3%) and *P. ciatophora* (11.8%). The values of frequency of other isolates were between 3.57 % - 7% (fig. 1).



L.L. HAKOBYAN

While summarizing the results of research it is revealed that the most frequently occurring species in Armenian raisin samples only are *P. grisofulvum* (32.2%) and *P. velutinum* (25.8%). The frequency of *P. expansum* (13.3%) is reduced. Besides the frequencies of occurrence of *P. diversum* (13%) and *P. grisofulvum* (32.2%) increased (Figure 2). This can be explained by the fact that these two species are not detected in imported samples of raisin. If considering the Armenian samples only, one should note increase in their percentage ratio along with reducing the number of investigated samples.

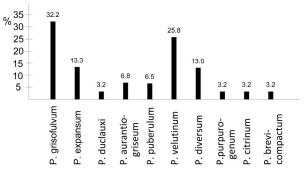


Figure 2. Frequency of occurrence of fungi of *Penicillium* genera isolated from Armenian raisin samples in 2004-2010.

	and imported raisins.

Raisin samples	Species from Penicillium		
	Genera		
	P. duclauxi Delacr.		
	P. aurantiogriseum Dierckx		
	P. puberulum Bainier		
Armenian raisin	P. velutinum Beyma		
	P. grisofulvum Dierckx		
	P. diversum Raper and Fennell		
	P. citrinum Thom		
	P. purpurogenum Stoll		
	P. brevicompactum Dierckx		
	P. expansum Link ex Gray		
Iranian raisin	P. duclauxi Delacr.		
	P. ciatophofora		
	P. hirsutum Dierckx		
Kazakh raisin	P. ciatophora		
	P. expansum Link ex Gray		
Uzbek raisin	P. puberulum Bainier		
Californian raisin	P. velutinum Beyma		
	P. expansum Link ex Gray		

Some detected species of *Penicillium* genera are considered to be potential producers of mycotoxins. For example species *P. grisofulvum*, *P. velutinum* and *P. citrinum* are producers of citrinin [15, 16], *P. purpurogenum* can produce rubratoxin [25], *P. aurantiogriseum* and *P. puberulum* – cyclopiazonic acid and penicilic acid [6, 15], *P. brevicompactum* – mycophenolic acid and *P. duclauxi*-patulin [6].

Raisin is imported into Armenia mainly from Iran and sometimes from other countries of the world. With level of contamination by filamentous fungi imported varieties of raisin is significantly differ from local samples too. Armenian raisin is comparably more contaminated than Iranian and other varieties (Table 2). This can be explained by presence of sulfur dioxide (SO₂) in Iranian raisin. SO₂ is often used to prevent contamination of raisin by microscopic fungi. In local samples SO₂ is detected in low content and there was high level of contamination by fungi respectively [8]. That is to say SO₂ has inhibitory influence on the growth of filamentous fungi. Three species of identified *Penicillium;*

P. duclauxi, P. hirsutumand P. expansum, have high resistance against sulfur dioxide due to their capability to make coremia. And in spite of presence of SO_2 these species are main contaminants of Iranian raisin.

Samples	Number of analyzed samples	Number of species from genus <i>Penicillium</i>	Levels of contamination in CFU/g
Armenian raisin	28	10	20 - 70
Iranian raisin	18	3	10 - 30
Californian raisin	7	2	20 - 40
Uzbek raisin	5	1	10 - 30
Kazakh raisin	5	2	10 - 40

Table 2. Contamination levels of raisin samples from different countries by species genus from *Penicillium*

The contamination level of Armenian samples of raisin by species from genera exceeded 70 CFU/g.

There is very few number of researches about contamination of dried vine fruits by fungi species from *Penicillium* genera. But the results of these analyzes have shown that the problem is more serious, because there are potential producers of mycotoxins species among isolated fungi such as *P. citrinum*, *P. purpurogenum*, *P. expansum*.

REFERENCES

- 1. Мирчинк Т.Г. Почвенная Микология, М., МГУ, 1988.
- Controle de la qualite des produits alimentaires controle microbiologique. NF ISO 7954-88. Direktivesgenerales pour le denombrement des levures et moisissures. 1993.
- Controle de la qualite des produits alimentaires controle microbiologique. NF ISO 7698-91. Direktivesgenerales pour le denombrement des levures et moisissures. 1993.
- El-Kady I.A., Abdel-Hafez J.N., El-Maraghy S.S. Contribution to the fungal flora of cereal grains in Egipt. In Journal Mycopathologia, 77, p. 103-109, 1982.
- European Commission. Guidance document for competent authorities for the control of compliance with EU legislation on aflatoxins. SANCO/1208/2005-rev.1. 2006.
- Frisvad J.C. and Samson R.A. Polyphasic taxonomy of Penicillium subgenus Penicillium A guide to identification of food and air-borne terverticillatePenicillia and their mycotoxins. Studies in Mycology, 49,p. 1-17, 2004.
- Giridhar P. and Reddy S.M. Incidence of mycotoxigenic fungi on raisins. Current Advances in Plant Science 14, p. 291-294, 2001.
- Hakobyan L., Grigoryan K., Kirakosyan A. Contamination of raisin by filamentous fungi – potential producers of ochratoxin A. Potravinarstvo. ISSN: 1337-0960, 4, 4, p. 28-33, 2010.
- Magan N., Aldred D. Conditions of formation of ochratoxin A in drying, transport and in different commodities. In Food Additives & Contaminants: part A, 22, Issue 1, p. 10-16, 2007.
- 10. *Pitt J.I.* The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. London, Academic press, 1979.
- 11. *Pitt J.I., Hocking A.D.* Fungi and Food Spoilage. Blackie Academic and Professional. London, p. 350, 1997.
- Romero S.M., Comerio R.M., Larumbe G., RitieniA., Vaamonde G., Fernandez P.V. Toxigenic fungi isolated from dried vine fruits in Argentina. In International journal of food microbiology, vol. 104, no.1, p. 43-49, 2005.

L.L. HAKOBYAN

- 13. Saeed M.S., Alghalibi M.S., Abdul-Rahman M.S. Mycoflora and mycotoxin contamination of some dried fruits in Yemen Republic. In Univ. Bull. Environ. Res., 7, 2, p. 235-243, 2004.
- 14. Samson R.A., Hoekstra E.S., Frisvad J., Filtenborg O. In Introduction to Food-borne fungi. 4thed. Central bureau Voor Schimme l cultures, Baarn, Nether-Lands. p.450. 1995.
- 15. Smith J.E., Henderson R.S., Mycotoxins and Animal Foods. ISBNO-8493-4904-4, p. 868, 1991.
- 16. Weidenborner M. Encyclopedia of food Mycotoxins, Berlin, 2001.
- 17. Youssef M.S., Abo-Dahab, N.F., Abou-Seidah A.A. Mycobiota and mycotoxin contamination of dried raisins in Egypt. African Journal of Mycology and Biotechnology, 8, 3, pp. 69-86, 2000.
- Zohri, A.A., Abdel-Gawad, K.M. Survey of mycoflora and mycotoxins of some dried fruits in Egypt. J. Basic Microbiology, 4, p. 279-288, 1993.

Received on 01.09.2014