

## MICROBIAL CONTAMINATION OF JUNGLANS REGIA L. WALNUTS STORED IN MOLDOVA

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**Abstract:** This paper includes an analysis of microbial contamination of *Juglans regia* L walnuts based on a bibliographic study of estimates made in the last 10 years by research workers from different countries. Existing studies show that nuts microbiota is quite diverse and is conditioned by many factors. If the conditions of fruit processing and storage are not respected, it leads to increasing the microbiological risk. This can affect the consumers health and can cause economic losses. The paper also presents an initial study of microbial contamination of walnuts grown in Moldova. The main objectives of the present research were to evaluate the mold, the total number of yeasts and other microorganisms of contamination in autochthonous *Juglans regia* L. walnuts, which were cultivated and stored at different stages of maturation. In the study (bibliographical and experimental), there are determined the factors which condition the microbiological risk, and are suggested ways to reduce microbial contamination of walnuts during storage.

**Key words:** microbial contamination, walnuts stored

### INTRODUCTION

Various nuts are used as a raw material in many industries as well as for a direct consumer. Walnuts *Juglans regia* L. are rich sources of unsaturated fatty acids, proteins, vitamins, minerals etc. They contain an important amount of protein and fat and their products have wide acceptance as food throughout the world. Due to the extremely high fat, protein and low water content of various nuts such as hazelnut, almonds, walnuts, these products are quite refractory to spoilage by bacteria. Molds can grow upon them if they are stored under conditions that permit sufficient moisture for their propagation [7]. So the bibliographic and experimental study of interest across the microbial contamination walnuts stored in commercial markets.

Walnut trees are a habitat for a wide variety of fungal and (to a lesser extent) bacterial taxon's, some of which can pose a serious threat to plant health. Measures should be taken to further prevent the accumulation of microbial inoculum, e.g. removal of leaf debris [1]. Microbial biodiversity associated with the walnut *Juglans regia* L. is reported in several studies [1-11]. Study [1] includes microflora analysis on walnuts trees in South Tyrol (Italy). From 3.800 isolates obtained, belonged 3.742 (96,4%) to fungi, only 138 (3,6%) were bacteria. Fungal isolates were classified into 30 genera, *Altermaria*, *Penicillium*, *Phoma*, *Botryosphaeria*, *Fusarium*, *Cladosporium*, *Phyllosticta* and *Epicoccum* being the most taxa. More isolates were obtained from leaves 45.3% than from twigs 31.8% and fruit 23.0%.

Microbial growth depends on a variety of environmental parameters. Among them temperature and relative humidity play a predominant role [6, 7].

This study was conducted with the following aims:

- Analysis of microbial contamination of *Juglans regia* L walnuts based on a bibliographic study;

- Investigation of microbial contamination of walnuts grown in Moldova.

#### MATERIALS AND METHODS

##### *Materials*

The study was conducted on autochthonous walnuts which were stored for 1 month, 1 and 2 years. Nuts *Juglans regia* L. were harvested in Telenesti, Moldova, during harvest 2010 and 2011. Storage took place at ambient temperature.

##### *Microbiological Analyses*

A twenty-five g analytical unit taken from the 100g sample unit was used in aerobic bacterial count, mold count. Plating for total bacterial count was carried on standard nutrient agar and for molds on Seabourad dextrose agar [9, 13]. Isolated colonies after incubation period of 3 days at 28°C for bacterial count and 7 days at the same temperature for mold count were studied.

Also, total mold, yeast contamination and bacterial counts of the samples were determined using a dilution plate method. About 25g walnut samples (whole nuts, kernels) were suspended in 225ml of 0.1% peptone solution and homogenized. Homogenized samples were diluted with 0.1 % peptone solution to concentrations of  $10^{-2}$  and  $10^{-3}$ . Each dilution (100 $\mu$ l) was dispensed and spread onto sterile BA/ Seabourad in Petri plates, followed by incubation at 28°C for 3/5 days. Total mold and yeast counts were estimated by back-calculation based on observed mold for each dilution and was expressed as the number of colony forming units (CFU) per gram in each sample.

#### RESULTS AND DISCUSSION

Bibliographic study indicates that the microbiota nuts depend on variety, geographical conditions, climate, the collection, processing and storage [1-12]. The climatic parameters (temperature, relative humidity, precipitations and UV irradiation), the composition of leaf surface (senescence) and the availability of nutrients all change over the microbial colonization [1]. Molds of many genera may be found on examining nuts [6]. That mould genera, occurring most frequently in shelled and unshelled nuts, were, *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor* and *Cladosporium*. *Aspergillus* and *Penicillium* species predominated in all the tested shelled nuts being (32-39%) *Aspergillus* and (25 to 31%) *Penicillium*. While *Aspergillus*, was dominant in all the unshelled nuts (41-50%). Other genera were found to be in low percentage (*Rhizopus*, *Mucor* and *Cladosporium*). The results [6] are in accordance with Smith and Arend [7] who stated that *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor* and *Cladosporium* represented the common genera in nuts.

Data reported by M.J. Sejny et al.[6] show the qualitative and quantitative distribution of different groups of bacteria present in the shelled and unshelled nuts. It was found that Gram positive spore forming bacilli, Gram positive Micrococci, Gram positive rods and Gram negative short rods were generally contaminating the samples. Their quantitative distribution varied through the tested nuts. The most dominant bacteria appearing in the unshelled nuts was Gram positive spore forming bacilli being 55 and 68% in shelled and unshelled walnuts respectively. While Gram positive Micrococcus were 24 and 29% in the respective value. The dominant groups of bacteria presented in shelled nuts showed nearly the same trend as those on unshelled nuts.

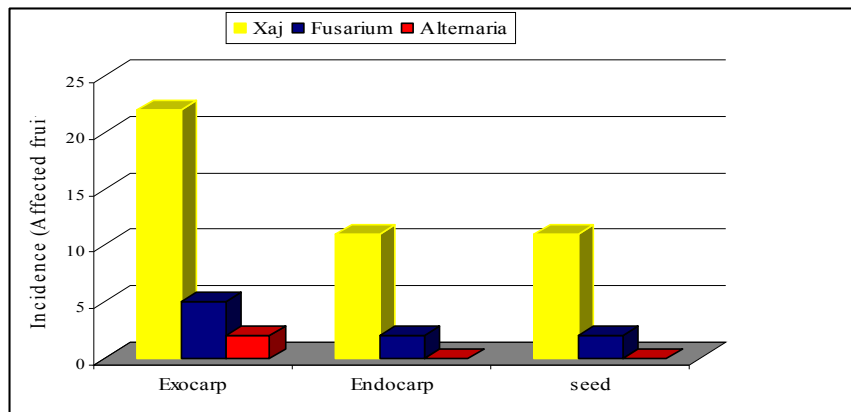
The distribution of Gram negative short rods were found very low in the nuts (2-10%), comparing with the other groups of bacteria. These results confirm the work of King et al [9] who reported that microorganisms associated with commercially shelled nuts were numerous and varied. They added that the genera of bacteria isolated from almonds includ

Bacillus, Micrococcus, Streptococcus, Brevibacterium, Escherichia and Aerobacter. These results are also in harmony with Hall [10] and Chypley and Heaton [11] who found that of Gram negative short rods as *Pseudomonas*, *Escherichia*, *Leuconostoc*, *Proteus* and *Aerobacter* were found associated with commercially shelled nuts.

Microorganisms associated to Brown Apical Necrosis (BAN) of walnut [8]:

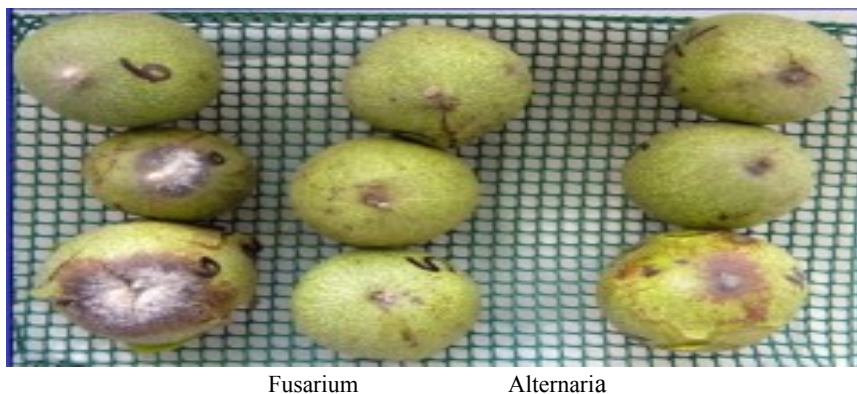
- *Xanthomonas arboricola* pv. *juglands*, isolated from all walnut fruit affected tissues in more than 36% fruits.
- *Fusarium* spp. (*F. moniliforme*, *F. solani*, ...) isolated from exocarp and sees in 4% fruits (depending on fruits tissue)
- *Alternaria* spp. only isolated from exocarp at the rate of 1% (more frequent in exocarp and mesocarp).

In Figure 1 we present incidence (Affected fruits) fruits walnuts *Juglans regia* L. under study [8].



**Fig. 1.** Microorganism isolated from walnut fruits with BAN symptoms [8]

Infection with microorganism walnuts *Juglans regia* L. can occur in Stage growth, ripening and harvest. In Figure 2 we present incidence walnuts caused by microorganism associated with BAN (*Fusarium* and *Alternaria*).



**Fig. 2.** Seed infections in walnut fruits caused by microorganism associated with BAN [8]

In Table 1 we present experimental data obtained from analysis of walnut *Juglans regia* L, grown in different years and stored at ambient temperature, shelf life 10 and 22 months.

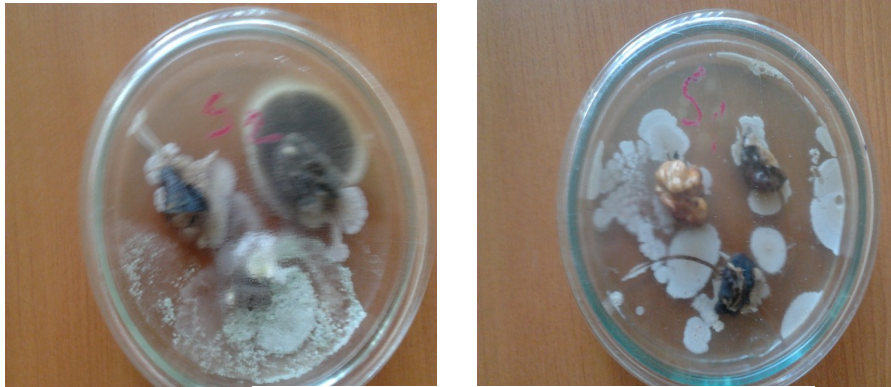
**Table 1.** Seasonal variation of fungi and bacteria of five experimental walnuts *Juglans regia* L in three different seasons

Harvest year	During storage, (months)	Sample, no.	This microorganisms		
			Fungi and yeasts	Bacteria	
				Gram positive	Gram negative
2010	22	1	++++	++	+++
		2	++++	++++	++
		3	+++	++++	-
		4	++++	+	-
		5	++	-	+++
		6	++++	+++	++
		7	+++	++	++++
		8	+	++++	+
		9	++++	++	-
		10	+++	-	++++
2011	10	1	++	+++	++++
		2	+	++++	++
		3	+++	+++	+
		4	++++	+	-
		5	++	-	-
		6	++	++	++
		7	+	++	-
		8	+	++	+
		9	++	++	-
		10	+++	-	++

(+) = minor infestation; (++) = average infestation; (+++) = major infestation; (++++) = heavy infestation; (-) = lack of infestation.

The results of microbiological control of walnut *Juglans regia* L., grown in Moldova are the limits reported in bibliographic study. It was found that longer storage of nuts contributes to increased microbial infestation. The rate of infestation abundance of walnuts harvested in 2010 amounted to fungi and yeasts 50% and 30% Gram positive and Gram negative 20%. Nuts harvested in 2011 were less infested. Heavy infestation rate being only 10% for fungi, yeasts, Gram positive and Gram negative bacteria.

In Figure 3 we present some images of colonies of microorganisms found in walnuts *Juglans regia* L., collected in Moldova, stored 22 months at ambient temperature.



**Fig. 3.** Isolated colonies after incubation period of 7 days at 28°C

When storing nuts should be taken into account Code of Practice for the prevention and reduction of aflatoxin contamination in nuts (*CAC/RCP 59-2005*):

- Storage facilities should be clean and dry (if possible keep the relative humidity < 70%), well-vented structures that provide protection from rain, entry of rodents and birds, drainage of ground water and have minimum temperature and humidity fluctuations. If possible, temperature should be kept between 0°C and 10°C to minimize fungal growth during storage.
- Good storage practices should be implemented to minimize the levels of insects and fungi in storage facilities. This may include the use of suitable, registered insecticides and fungicides or appropriate alternative methods. Nuts stored in sacks should be placed on pallets which are positioned to allow good ventilation and access.
- Water activity, which varies with moisture content and temperature, should be carefully controlled during storage. *Aspergillus flavus/A. parasiticus* cannot grow or produce aflatoxins at water activities less than 0.7.

### CONCLUSION

Bibliographic and experimental study shows that walnuts *Juglans regia* L. can be infected with fungi, yeasts and bacteria, which minimize their quality. Infection rate depends on climatic parameters (temperature, relative humidity, precipitations and UV irradiation), variety nuts and storage conditions. Good storage practices should be implemented to minimize the levels of insects and fungi in storage facilities.

To store walnuts should be taken into account Code of Practice for the prevention and reduction of aflatoxin contamination in nuts (*CAC/RCP 59-2005*).

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