IDENTIFICATION OF THE MYCO-TOXIGENOUS FUNGI FROM PEANUT SEEDS (*ARACHIS HYPOGAEA L.*) FROM STORES

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Abstract. *Peanuts (Arachis hypogaea L.) can be attacked by many pathogens both in field and in deposits. Most of the time the peanuts are healthy, but during improper storage they can be contaminated with fungi. The myco-flora associated with the peanut seeds is formed mainly by fungi from the genera Aspergillus, Fusarium, Penicillium and Rhizopus. The mentioned fungi are very harmful for health because they are producing carcinogenic mycotoxins. Mainly the Aspergillus species can infect peanuts during the vegetation period, especially in the case very high humidity conditions in field. Thus, the infection can continue during the harvesting, transport and storage. Due to this fact, the problem of the accumulation of aflatoxins is very serious in the big peanut cultivating countries. Many researches show the presence of the aflatoxins in the sold peanuts. The approached topic in this research is actually and presents interests for the consumers in special. The identification if the myco - toxigenous from peanuts from supermarket was realised on seeds that were originating from four countries (Romania, Brasil, U. S. A. and Egypt). The main objective of this laboratory study was to identify the myco - toxigenous fungi, mainly those that grow on and from peanuts sold in supermarkets in Timisoara. During the sample collection there was considered the origin of the peanuts to comprise different parts of the world. The peanuts were bought from big supermarkets. The obtained results regarding the fungal contamination index of the peanuts seeds with fungi show the presence in myco - flora of some myco - toxigenous fungi harmful for health. Fusarium sp. is highlighted in all the samples by a quite great contamination rate comprised between 5.12% (the peanut crust samples from U. S. A.). The contamination of the seeds with the myco-toxigenous fungus Aspergillus flavus is obvious in the case of the peanuts from U. S. A. where was registered the greatest infection rate of 25.89% in seeds and 10.25% in crusts. The third fungus that was determined in quite great rates on the peanuts seeds was Penicillium sp. The greatest infection level was registered in the samples from China (24.56%) and U. S. A. (22.32%). Aside the three myco - toxigenous fungi was grown in a great rate in some samples the fungus Rhizopus sp., that usually is present in the peanuts seeds. The greatest infection level of the infection was determined in the peanuts from Egypt (65.85%) and those from Romania (64.56%).*

Key words*: Fusarium sp., Aspergillus flavus, Penicillium sp., Rhizopus sp., peanuts, Arachis hypogaea, myco-toxigenous fungi.*

INTRODUCTION

In Romania peanuts are cultivated on small surfaces, mainly in the southern part of the country (Dăbuleni), Constanţa and sporadically in west (Orăştie/Hunedoara, Timiş). The merchandised peanuts from the supermarkets in Romania are mostly imported from USA, Brasil, Egypt, China and Romania.

Peanuts (*Arachis hypogaea* L.) can be attacked by many phyto-pathogenous. Generally, the most frequent fungi found on the seeds are belonging to the general *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus.* Sometimes there can be developed randomly other fungi species,

respectively *Alternaria*, *Rhizoctonia*, *Chaetomium*, *Drechslera*, *Phoma* and *Cladosporium* [FRANCIELE DOS SANTOS *et al.*, 2016].

Many of the mentioned fungi are myco-toxigenous (*Aspergillus sp.*, *Fusarium sp.* and *Penicillium sp.*) are developing fast on peanuts. The myco-toxins from contaminated peanuts can affect seriously the human and animal health, mainly the aflatoxins produced by different species of *Aspergillus* [JOGEE S. P. *et al.*, 2012]. The *Aspergillus* can be infected during the vegetation period, mainly if in field are high humidity conditions. Thus, the infection can continue during the harvesting, transport and storage [AZAWEI ALAMENE, 2015]. The problem of aflatoxine accumulation is very serious in the main cultivating countries, e.g. Nigeria. Numerous studies have shown the presence of the aflatoxins in the merchandised peanuts, mainly the aflatoxins G_2 and B_2 [PADMA SINGH, 2017].

In the southern Etiopia are reported levels of the aflatoxins in peanuts that are passing over the international admitted levels. The presence of the aflatoxins is correlated with the infections produced by *Aspergillus flavus* and *Aspergillus parasiticus* during the vegetation period [CHALA A. *et al*., 2012; EPHREM GUCHI, 2015]. Peanuts are susceptible to the infections with myco-toxigenous fungi and other fungi during the storage period, especially when the storage conditions are improper. After EPHREM GUCHI (2015), the isolated *Aspergillus* species from the peanuts from the eastern side of Etiopia were the following: *Aspergillus nomius* (88.63%), *Aspergillus niger* (85.65%), *Aspergillus flavus* (81.80%) and *Aspergillus tamari* (63.57%). This research highlights the fact that in the eastern Etiopia the aflatoxins are frequent contaminants of the peanuts during the storage period. As the storage period is longer, the risk of aflatoxins grows. The contamination of the peanuts with aflatoxins produced by *Aspergillus flavus* represents the greatest quality and health problem that that characterises somehow the peanuts merchandising and consumption worldwide [NAYAK S. N. *et al*., 2017]. In a research from 1969 D. MCDONALD show shat the condition of the peanut shell influences the infections with *Aspergillus flavus* and later the presence of the aflatoxins in seeds. There are areas in the world (Nigeria) where the peanuts are harvested during the rainy season, being susceptible to the infection with *Aspergillus flavus*. Thus, the condition of the shell is very important in peanuts. There was noticed that if the shell is intact, undamaged, rarely the seeds are infected with *A. flavus*. The seeds from the perforated shells are attacked by the fungus *Aspergillus flavus.* Thus, is very important the peanuts to be dried after harvesting, especially if they have been harvested during rain. If the humidity is greater than 20% the seeds can be strongly infected.

The tests made in laboratory for the evidencing of the fungi from the peanut shells and seeds show that the dominant fungi on the shell were *Fusarium sp.* and *Macrophomina phaseoli*. Here are found also the fungi *Aspergillus sp.*, *Penicillium sp.*, *Botryodiplodia theobromae* and *Rhizopus sp.* From seeds were grown the fungi *Fusarium sp.*, *Aspergillus sp*. and *Penicillium sp*. [D. MCDONALD, 1970].

EMBABY E. M. and ABDEL-GALEL M. M. (2014) have shown that the peanuts seeds myco-flora was formed usually from fungi belonging to the genera *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus.* The researches evidenced that the fungi from the genus *Fusarium* are present. The analyses have determined the presence of the zearalenone toxin in peanuts. According with MEHAN *et al.* (1985), the frequent isolated *Fusarium* species from peanuts are *Fusarium oxysporum* and *Fusarium moniliforme*. Both species are producing zearalenone, an estrogenic toxin. The estrogenic syndrome can appear both in animals and humans (hyper-estrogenism, tumefiation of the mammary glands and vulva, uterus hypertrophy,

infertility) (MEHAN *et al*., 1981; GOODMAN *et al*., 1987). KRISHNA KISHORE G. *et al* (2002) have been reported infections of the peanut seeds with the fungi *Fusarium sp*. and *Aspergillus flavus*. In the evaluated samples the infection was comprised between 0 - 24% for *Fusarium sp.* and 0 - 38% for *Aspergillus flavus.* In all the analysed samples there were determined different concentrations of aflatoxins. From all the samples 20% had over 30 µg/kg aflatoxins content. From all the aflatoxins (B_1, B_2, G_1 and G_2), aflatoxine B_1 has been detected in all the analysed samples. Comparing the aflatoxins, zearalenone has been detected only in two samples from the total samples number. The determined zearalenone concentrations determined were 129.4 µg/kg and respectively 35.1 µg/kg.

ABDELA M. H. (2009) reports in the peanut myco-flora the presence of the myco-toxigenous fungi *Aspergillus flavus* and *Aspergillus niger* with infections rates that reach to 60%. Aside to the fungi from the *Aspergillus* genus there were determined too the following fungi *Penicillium sp., Rhizopus sp.* and *Sclerotium bataticola.*

The purpose of this research is to identify the myco-toxigenous fungi that are growing from and on the shell of the peanut seeds merchandised in the supermarkets fromTimişoara. During sampling there was considered the origin of the peanuts, in such a way that to have samples from different worldwide areas. The topic of the research is present day and of interest mainly for the consumers.

MATERIAL AND METHODS

The biological material is represented by peanuts raw decorticated and roasted in shell, bought from supermarkets. The peanuts were chosen in such a way to have samples from different countries, obtaining finally five samples:

- ***Sample A_1*** - 10 replicates - raw decorticated peanuts, country of origin ***Romania;***
- ***Sample A_2*** - 8 replicates – roasted decorticated peanuts; 4 replicates half pods (in shell); country of origin ***USA;***
- ***Sample A_3*** - 5 replicates – raw peanuts, country of origin ***Egypt ;***
- ***Sample A_4*** - 3 replicates - raw decorticated peanuts , country of origin ***Brazil;***
- ***Sample A_5*** - 6 replicates - raw peanuts, country of origin ***China***.

Preparation of the peanuts seeds for setting in humid chambers was done by respecting the following stages: seeds washing with tap water; disinfection by immersion in ethanol 96% for 1 minute; successive wash of the disinfected seeds in two baths with sterile water; seeds drying on sterile filter paper. The dried peanuts seeds have been placed with a flame sterilized pincers in the Petri plates on wet filter paper (humid chamber). The Petri plates prepared in this way have been incubated at 20 – 22^o C for 5 days.

The fungus *Aspergillus flavus* was isolated from the peanuts seeds and inoculated in the agar culture medium prepared in laboratory by dissolving 23 g agar in 1l distilled water. The obtained data have been processed by calculating the fungal contamination index.

The fungal contamination index of the peanut seeds was calculated using the following formula:

ICS% = number of contaminated sections/total sections number on plates x 100

Examination of the mycelia grown on seeds was realised at stereomicroscope, being completed with microscopic examination.

RESULTS AND DISCUSSION

The results regarding the fungal contamination index of the peanuts seeds with fungi

shows the presence in the myco-flora of some myco-toxigenous fungi harmful for health. Thus, on seeds were developed fungi from the genera: *Fusarium*, *Aspergillus*, *Penicillium* and *Rhizopus.*

Fusarium sp. is evidenced in all the samples by a quite high contamination rate comprised between 5.12% (sample of roasted peanuts shells from USA America) and 34.84% (sample of roasted peanuts seeds from USA). The peanuts from Brazil were contaminated with *Fusarium* having a rate of 28.57%, followed by the samples from China with 24.56%, Egypt with 23.17% and Romania with 22.83%. The low rate of *Fusarium sp.* in the shells sample can be due to the fact that these peanuts were roasted (without salt) and the microbial load decreased.

Table 1

Synthesis of the results regarding the peanuts seeds contamination with myco-toxigenous fungi

Sample	***ICS% Fusarium sp.***	***ICS% Aspergillus sp.***	***ICS% Penicillium sp.***
Sample A_1 Romania	**22.83**	**2.36**	**10.23**
Sample A_2 USA (seeds)	**34.84**	**25.89**	**22.32**
Sample A_2 USA (shells)	**5.12**	**10.25**	**10.25**
Sample A_3 Egypt	**23.17**	**2.43**	**9.75**
Sample A_4 Brazil	**28.57**	**2.04**	**12.24**
Sample A_5 China	**24.56**	**9.64**	**24.56**

Contamination of the seeds with the myco-toxigenous fungus *Aspergillus flavus* is obvious in the peanuts from USA in these samples being determined the highest infection rate of 25.89% in seeds and 10.25% in shells. Peanuts samples from China were contaminates in a rate of 9.64%. In the other samples the infection level didn't passed over 2% (Table 1). The third fungus determined in relatively high rates on the peanuts seeds was *Penicillium sp.* The highest infection rates were determined in the samples from China (24.56%) and USA with 22.32%. The other samples were infected in rates lower than 12% seeds with mycelium on surface.

With the three above mentioned myco-toxigenous fungi there was developed in a high rate in some samples the fungus *Rhizopus* sp. This fungus is usually present in the peanuts seeds, this fact being evidenced in many researches. From all the six samples *Rhizopus* was developed only in four. The highest infection rate was determined in the peanuts originating from Egypt (65.85%) and from Romania (64.56%). The peanuts from Brazil were infected with *Rhizopus* in a rate of 30.61% (Figure 1). The lowest *Rhizopus* infection rate was determined in the peanuts from China. *Rhizopus sp.* hasn't been developed on the peanuts from USA.

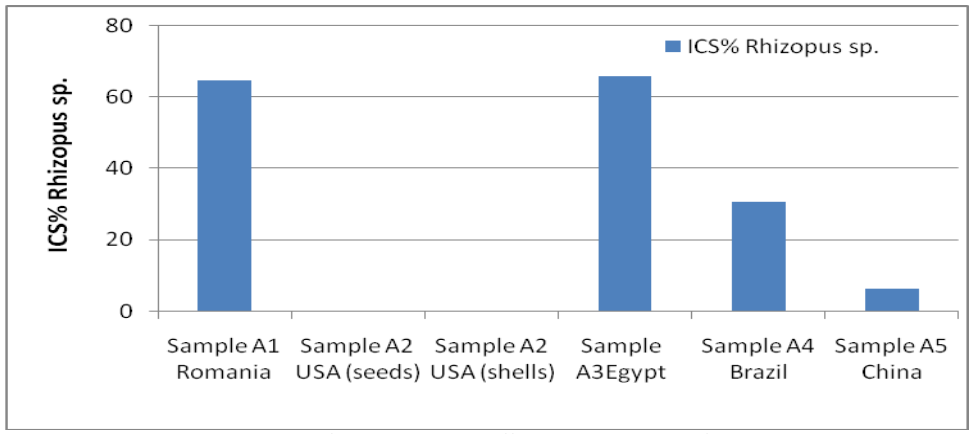

Figure 1. Contamination of the peanuts with different origins with the fungus *Rhizopus sp*.

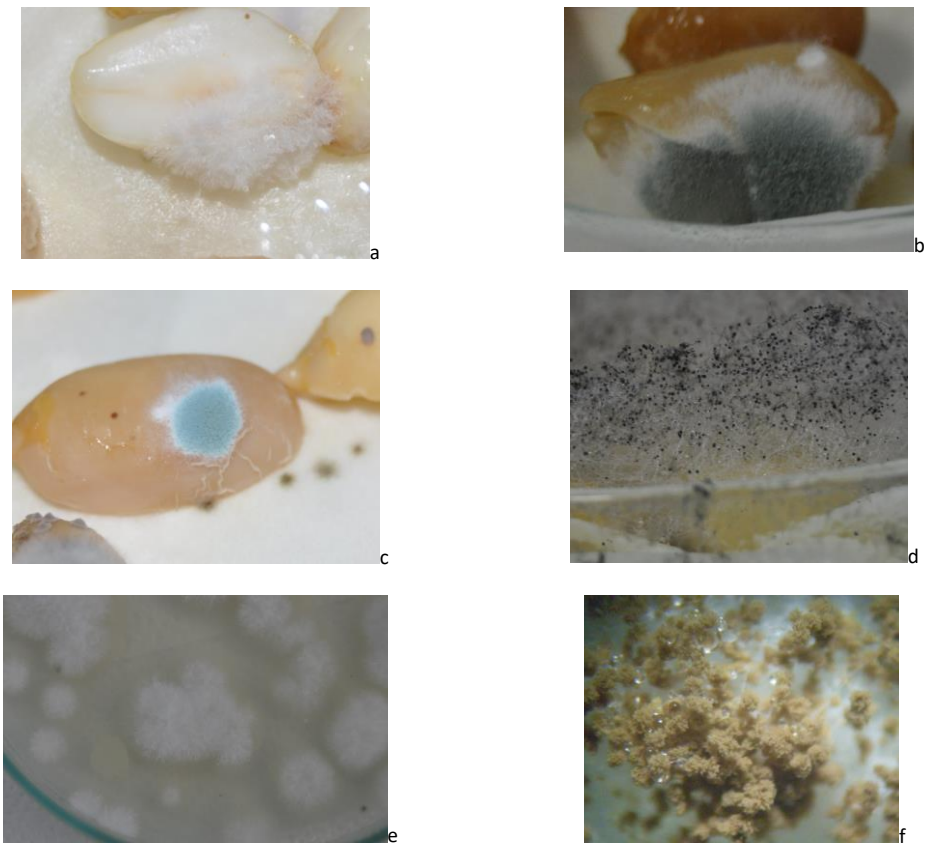

Figure. 2. a) mycelium of *Fusarium sp.* on peanuts seeds; b) mycelium of *Aspergillus sp*.; c) mycelium of *Penicillium sp*.; d) mycelium of *Rhizopus sp*.; e) mycelium of *Aspergillus sp*. isolated form peanuts and seeded on agar culture medium; f) mycelium of *Aspergillus sp.* with fialides and conidia isolated from peanuts seeds inoculated on agar culture medium (photo with stereo-microscope, original)

We can assume that the fungus *Fusarium sp.* was prevalent in all the six peanuts samples, having relatively high infection rates. Thus, *Aspergillus flavus* is present in myco-flora, but with lower infection rates. It is well known that this fungus is a major aflatoxins producer, the strongest and most harmful from health known until now, being carcinogenous. This fungus has been isolated from peanuts seeds on the culture medium (water with agar) where it has been developed, being more facile to identify it.

The species *Penicillium* hasn't been determined taxonomically in our research, but we can presume that it could be *Penicillium chrysogenum*. *Penicillium* species mustn't be ignored, even not of them are producing carcinogenous myco-toxins. Regarding *Penicillium chrysogenum*, there is well known that it produces ochratoxine, being carcinogen too.

The fungi that have grown on the peanuts seeds are considered pathogenous of this species, they being able to infect the plants during vegetation period, especially *Aspergillus flavus*. The critical fact is that those fungi can continue their development during the harvesting and particularly during the storage period.

CONCLUSIONS

The obtained results are evidencing the fact that the stored peanuts are susceptible to fungal infections, part of those fungi being myco-toxigenous. Prevalent on the peanuts seeds sampled from stores was determined the fungus *Fusarium sp.* Other identified fungi were belonging to the following genera: *Aspergillus sp., Penicillium sp.* and *Rhizopus sp.* The peanuts from Brazil were contaminated with *Fusarium* in 28.57% rate, those from China in 24.56% rate, those from Egypt in 23.17% rate and those from Romania in 22.83% rate. The contamination of the peanuts seeds with the myco-toxigenous fungus *Aspergillus flavus* was highlighted in the peanuts originating from USA where was determined the highest infection rate, respectively 25.89%. Other infection rate was determined in the peanuts originating from China, respectively 9.64%. In the other samples the infection rate was low, respectively below 2%. The third fungus determined in high rates on the peanuts seeds was *Penicillium sp.* the highest infection rate was determined in the samples originating from China (24.56%) and USA (22.32%). The fungus *Rhizopus* was developed only on four samples from all six. The greatest infection rate was determined in the peanuts originating from Egypt (65.85%) and from Romania (64.56%). The infection risk is great in peanuts during the storage period if there aren't respected the optimal temperature and humidity conditions. Thus, there is recommended to not store peanuts for long time periods.

BIBLIOGRAPHY

1. AZAWEI ALAMENE**,** 2015 - Effects of plant essential oils and biocontrol agents on the growth of and mycotoxin production by *Aspergillus spp.* on groundnut, PhD thesis, University of Nottingham., 173 p;

2. ABDELLA, M. H., 2009, Mycoflora of groundnut kernels from Sudan. Trans. Br. Mycol. Soc. 63: 353 - 359;

3. CHALA, A., MOHAMMED, A., AYALEW, A. AND SKINNES, H., 2012, Natural occurrence of aflatoxins in groundnut (*Arachis hypogaea* L.) from eastern Ethiopia. Food Cont. 30: 625 - 632;

4. EMBABY, E. M, ABDEL - GALEL, M. M., 2014, Detection of fungi and aflatoxins contaminated peanut samples (*Arachis hypogaea* L.). International Journal of Agricultural Technology 10(2):423 - 437;

5. EPHREM GUCHI, "Effect of Storage Time on Occurrence of *Aspergillus* species in Groundnut (*Arachis hypogaea* L.) in Eastern Ethiopia." Journal of Applied & Environmental Microbiology, vol. 3, no. 1 (2015): 1-5. doi: 10.12691/jaem-3-1-1;

6. FRANCIELE DOS SANTOS, PRISCILA FRATIN MEDINA, ANDRÉ LUIZ LOURENÇÃO, JOÃO JOSÉ DIAS PARISI, IGNÁCIO JOSÉ DE GODOY, 2016, Damage caused by fungi and insects to stored peanut seeds before processing, Bragantia, Campinas, Plant protection article, v. 75, n. 2, p. 184 - 192; DOI: http://dx.doi.org/10.1590/1678-4499.182;

7. GOODMAN, K. T., SCOTT, P. M. AND WATANABE, H. 1987, Risk assessment of the mycotoxin zearalenone. Regul. Toxicol. Pharmacol. 7:253 - 306;

8. JOGEE S. P., A.P. INGLE, I. R. GUPTA, S. R. BONDE AND M. K. RAI, 2012, Detection and Management of Mycotoxigenic Fungi in Nuts and Dry Fruits, Proc. IS on Mycotoxins in Nuts and Dried Fruits, Eds.: M. Razzaghi Abyaneh et al., *Acta Hort.* 963, ISHS 2012, 69 - 77;

9. KRISHNA G. KISHORE, S. PANDE, K. MANJULA, J. NARAYANA RAO AND D. THOMAS, 2002, Occurrence of Mycotoxins and Toxigenic Fungi in Groundnut (*Arachis hypogaea* L.) Seeds in Andhra Pradesh, India, Plant Pathol. J. 18(4) : 204 - 209 (2002);

10. MCDONALD D., 1969, *Aspergillus flavus* on groundnut (*Arachis hypogaea* L.) and its control in Nigeria, Volume 5, Issue 3, November 1969, Pages 275 - 280;

11. MCDONALD D., 1970, Fungal infection of groundnut fruit before harvest, Volume 54, Issue 3, June 1970, Pages 453 - 460;

12. MEHAN, V. K., BHAVANISHANKER, T. N. AND MCDONALD, D., 1985, Zearalenone production in groundnut. In: *Trichothecenes and other mycotoxins*, Proceedings of the International Mycotoxin Symposium. Sydney, Australia. ed. by J. Lacey. pp. 73-78. John Wiley & Sons Ltd., USA;

13. MEHAN, V. K., MCDONALD, D. AND RAO, V. R., 1981, Pod rot disease of peanut at ICRISAT, India. Proc. Am. Peanut Res. Educ. Soc. 13:91;

14. NAYAK S. N., AGARWAL G., PANDEY M. K., SUDINI H. K., JAYALE A. S., PUROHIT S., BAJAJ P., DESAI A., WAN L., GUO B., LIAO B., VARSHNEY R. K., 2017, IDT9-055 - Insights on host - pathogen interaction between groundnut (*Arachis hypogaea*) and *Aspergillus flavus*, p. 217;

15. PADMA SINGH, 2017, Isolation of *Aspergillus flavus* from *Arachis hypogea* & its aflatoxin production Padma Singh Gurukul Kangri University, India Virol-mycol 2017, 6:2 (Suppl) http://dx.doi.org/10.4172/2161-0517-C1-021;