

Investigation of Fungal Contamination in Local and Imported Nuts Which Displayed in Markets of Diyala , Iraq

Rabab Majeed Abed

Department of Biology ,College of education for pure science , University of Diyala , Diyala , Iraq

Abstract

This study aimed to investigation the fungal contamination in local and imported Nuts which processed for human consuming that have been collected from local markets in Diyala – Iraq . The study showed that 8 from 11 Nuts product were contaminated with fungi and bacteria , The total fungal isolates were 48 isolate belong to 3 fungi Genus which were *Aspergillus niger* , *Penicillium* spp and *Rhizoctonia* spp , The number of fungal isolates were 26 isolate of *Penicillium* spp , 18 isolate of *Aspergillus niger* and 4 isolate of *Rhizoctonia* spp . The occurrence percentage of *Aspergillus niger* ranged from 50 % to 20 % , for *Penicillium* spp ranged from 100% to 50% and for *Rhizoctonia* spp the occurrence was 80% in one sample only , Each of *Aspergillus niger* and *Penicillium* spp were occurrence in all tested samples except one sample which was contain *Rhizoctonia* spp fungus only . Also this study showed that local Nuts products were less fungal contamination than imported Nuts products .

Keyword: Nuts , *Aspergillus niger* , *Penicillium* spp , Fungal contamination

1. Introduction

nuts are known to foster the growth of various microorganisms including toxigenic and pathogenic fungal species , nuts in particular have been often reported to contain potentially toxigenic molds (Bayman *et al.*,2002), Due to the extremely high fat, protein and low water content of various nuts such as hazelnut, almonds, walnuts, pistachio and cashewnut, these products are quite refractory to spoilage by microorganisms. Molds can grow upon them if they are stored under conditions that permit sufficient moisture for their propagation (Frank, 1981). According to the previous studies the most common fungi in nuts are *Aspergillus*, *Rhizopus*, *Penicillium*, *Clasiodiplodia* , *Fusarium*, *Alternaria* , *Cladosporium* , *Helminthosporium* . (Bayman *et al.*, 2002; Rodrigues *et al.*, 2012 ; Tournas *et al.*, 2015 ; Adeniyi and Adedeji , 2015) . Molds naturally yield a wide range of metabolites which are called mycotoxins. Mycotoxins can cause toxic effects on human and animal tissue and organs (kazemi ,2003). Currently there are more than 300 species of mycotoxins, but only 10 of them can be of great danger to human and animal health and which include Aflatoxin , Ochratoxin , Fumonisin , Trichothecenes , Oleoxynivalenol , Zearalenone (Wu *et al.*, 2011). The present study aimed to 1. investigate the level of fungi contamination in local and imported nuts . 2. Isolate and diagnose the fungi associated with the nuts 3. ability of fungal isolates to produce aflatoxin

2. Material and methods

2.1 Samples

Nuts Samples were collected from different markets of Diyala , Iraq . These nuts samples included a number of local and imported products from different origins as shown in Table (1) . each of samples put in sterile packets and stored at 3-5°C until mycoflora determination and mycotoxin tested . all samples were intended for human consumption .

2.2 Total fungal counts

Dilution method was used to determine total fungal counts in nut products samples. One ml of 10^{-3} dilution were used to inoculate Petri dishes each containing Potato Dextrose Agar (PDA). Petri dishes were then incubated for 7 days at 28°C. Three replicates plates per medium were used for each sample and the developing fungi were counted and identified according to several key processes. After incubation, the results were expressed in Colony-Forming Units (CFU) /g of samples; all plates were examined visually, directly and with a microscope (Aziz *et al.*, 1998; Suleiman and Taiga, 2009).

Table 1. Details of each nuts sample

No	Trade name	Content	Origin
1	Samira	Different type*	Turkey
2	Lazeena	Sunflower seed	Turkey
3	Kastina	Different type*	Lebanon
4	Penguin	Sunflower seed	Iraq
5	Gambo	pistachio	China
6	Sultan	Different type*	Turkey
7	Zigi	pistachio	United Arab Emirates
8	Pasa	Sunflower seed	Turkey
9	Archi	Pumpkin seeds	Lebanon
10	Panuts	pistachio	China
11	Queen	Pumpkin seeds	Iraq

*different type of nuts contain : pistachios, hazelnuts , Cashews , chickpeas

2.3 Identification of the fungal genera

The fungal isolates were transferred to sterilized plates for purification and identification. The grown fungi were stained with lactophenol cotton blue to detect fungal structures (Basu, 1980), and examined under microscope and identified on the basis of their colony morphology and spore characteristics according to (Rajankar *et al.*, 2007; Ronhede *et al.*, 2005 ; Pittand and Hocking , 2009). Percentage of appearance are calculated according to the following formula:

The Percentage of appearance (%) =

$$\frac{\text{Number of sample which has the same genera or species}}{\text{Total number of samples of genera or species}}$$

2.4 Statistical Analysis

For statistical analysis SPSS version 17.0 was used , The comparison between the averages using less significant difference test (LSD) Least Significant Deference at the level of probability of 0.05 .

3. Results and dissection

3.1 Mycological analysis

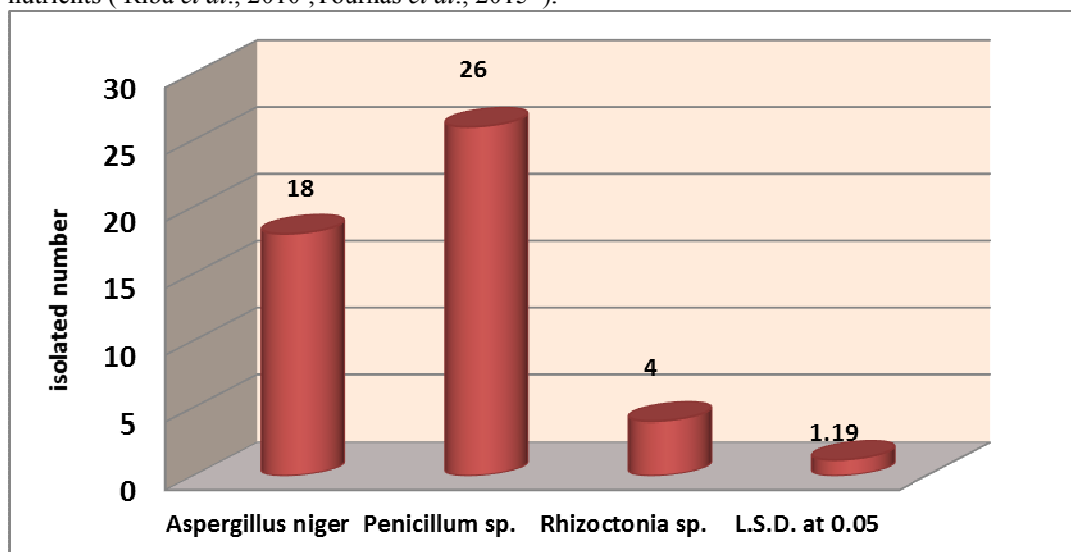
The mycological analysis revealed that 8 out of 11 nut products samples (72.73%) which collected from Diyala markets were contaminated with fungi . the results in table (2) showed that three samples are free from any fungal contamination these samples number are 1 , 2 and 4 , the samples number 6 , 7 , 9 , 10 , 5 , 8 , 3 and 11 showed fungal contamination which were (6×10^{-3} , 2.66×10^{-3} , 2.33×10^{-3} , 1.66×10^{-3} , 1.33×10^{-3} , 0.66×10^{-3} , 0.66×10^{-3} and 0.66×10^{-3}) respectively . Results refers to a discrepancy in the number of isolates of fungi in the contaminated samples , the highest number of fungal isolates in product number 6 which was 18 fungi isolated , in the other samples which has numbers 7 , 9 , 10 , 5 , 8 , 3 and 11 the isolated number of fungi were (8 , 7 , 5 , 4 , 2 , 2 and 2) respectively . the high contamination may be due to bad storage conditions which is one of the most important factors of fungi contamination fungi that characterized by their ability to grow on different nutrients for their ability to produce a wide range of digestive enzymes this result agree with (Riba *et al.*, 2010 ; Abdulla ,2013) .

Fig (1) Shown The number of fungal isolates isolated from local nut products which taken from the Diyala markets , 48 fungal isolates belonging to 3 fungi genera (*Penicillium* sp. , *Aspergillus niger* and *Rhizoctonia* sp.), *Penicillium* sp. (26) isolation , *Aspergillus niger* (18) isolation, while the number of isolates of the fungus *Rhizoctonia* sp. was (4) isolation. The result in Fig (2) showed the percentage of the appearance of fungi isolated which were ranged from 75 % to 50 % for *Penicillium* sp. , for *Aspergillus niger* was ranged from 50 % to 20 % , while the percentage of the appearance for *Rhizoctonia* sp. was 80% only in one sample . *Penicillium* spp appeared in all samples except sample number 11 , while *Aspergillus niger* appeared in all samples except sample number 10.

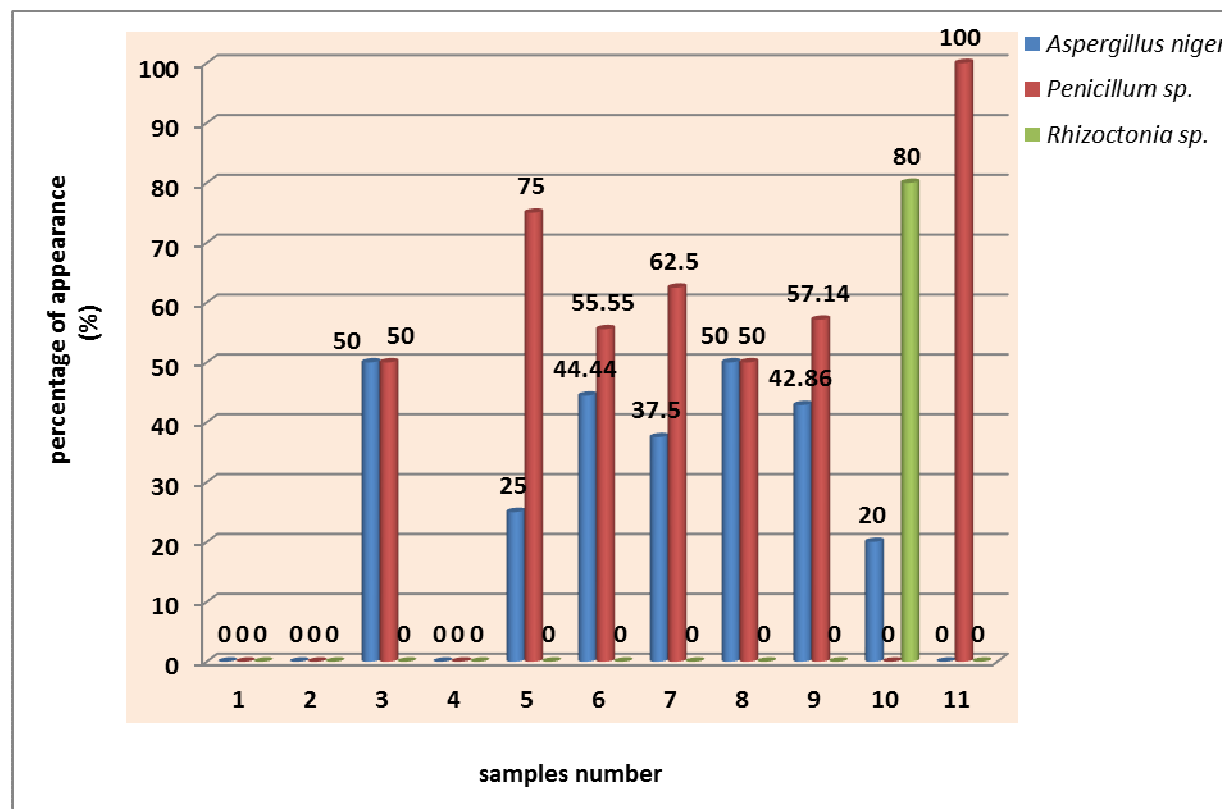
Table 2. Total fungi counts (Colony-Forming Units), number of isolated from 11 nut products samples from local markets in Diyala , Iraq.

Samples number	Total fungi count CFU /g	Isolated number
1	0	0
2	0	0
3	0.66×10^3	2
4	0	0
5	1.33×10^3	4
6	6×10^3	18
7	2.66×10^3	8
8	0.66×10^3	2
9	2.33×10^3	7
10	1.66×10^3	5
11	0.66×10^3	2
L.S.D at 0.05	1.127	1.332

The fungi isolated in this study considered as a fungi associated with pumpkin seeds and other plant (nuts tree) this due to infested of plant with fungi during the storage period or harvest (Nawar , 2007) . Pistachio was the most nut contamination with different genera of fungi this due to an injury in the field or when transport or packaging (Mehdi et al., 2000) . this results agree with several studies which isolate and diagnose some fungi from nuts which intended for human consumption such as *Rhizopus* spp, *A .niger*, *Penicillium* spp, *Rhizoctonia* spp, *Chaetomium* spp, *Trichoderma* spp and *Fusarium* spp (AL-Rawi , 2009) , and the result of Mimoune (2016) study showed that *Aspergillus* sp. and *Penicillium* sp. the most common and frequencies fungal on various plants this due to the ability of both fungi to produce a wide range of enzymes that enable them to grow on different nutrients (Riba *et al.*, 2010 ;Tournas *et al.*, 2015).



Figurer 1. The number of fungi isolated from 48 isolated total nut samples that collected from local markets in Diyala , Iraq.



Figuer (2) The percentage of appearance (%) for all fungi isolated in nut samples that collected from local markets in Diyala , Iraq . L.S.D. at 0.05 for fungi isolated = 4.99 , nut samples = 2.608 , interaction = 8.65

4.Reference

- Abdulla , N.Q.F. (2013)Evaluation of Fungal Flora and Mycotoxin in Some Important Nut Products in Erbil Local Markets . *Research Journal of Environmental and Earth Sciences* . 5(6): 330-336 .
- Adeniya , D.O. and Adedeji , A.R. (2015) Evaluation of fungal flora and mycotoxin potential associated with postharvest handlings of Cashew Nut . *Archives of Applied Science Research* . 7 (2):30-33 .
- Al-Rawi , A.A. (2013) Seed Borne Fungi in some Kinds of Isolation and Identification of Imported Nuts With Special Emphasis to Aflatoxigenic Fungi . *Rafidain journal of science* .20(2):46-57 .
- Aziz, N.H., Youssef, Y.A , El-Fouly, M.Z. and Moussa, L.A. (1998) Contamination of some common medicinal plant samples and spices by fungi and their mycotoxins. *Bot Bull. Acad. Sinica.*, 39(4): 279-285.
- Bayman, P., Baker, J.L. , and Mahoney, N.E. (2002) *Aspergillus* on tree nuts: incidence and associations. *Mycopathologia* .155:161–169.
- Frank, H.K. (1981) Moulds and mycotoxins in nuts and nut products. Schimmelpilze und mycotoxin in Nuessen und daraus hergestellten producten. *Mycotoxin in lebensmitteln, federal Republic of Germany*, pp: 397-414 .
- Kazemi, A. (2003) Carcinogenic mycotoxins; The 1st Student's Congress of Cancer; Shahed Uni., Tehran, Iran. p. 90.
- Mehdi, F. S. , Siddiqi, I. A. , Ali, N. I. and Afzal, M .(2000). Rhizosphere Mycoflora of Black Mangrove Seedling at Karachi Cost. *Pakistan J. Bio. Sci.*, 3: 1352-1353.
- Mimoune , N.A. , Riba , A. , Varheecke , C. , Mathieu , F. and Sabaou , N. (2016) Fungal contamination and mycotoxin production by *Aspergillus* spp. In Nuts and Sesame seeds . *Journal of Microbiology , Biotechnology and Food Sciences* , 5(4):301-305 .
- Nawar, L. S. (2007). Pathological and Rhizospherical Studies on Root Rot Disease of Squash in Saudi Arabia and it's Control. *African J. Biotech.*, 6(3): 219-229.
- Pitt, J. I. and Hocking, A. D. (2009) Fungi and food spoilage. Third Edition. Springer Science and Business Media, London, New York .
- Rajankar, P.N. , Tambekar, D.H. and Wate, S.R. (2007) Study of phosphate solubilization efficiencies of fungi and bacteria isolated from saline belt of purna river basin. *Res. J. Agric. Biol. Sci.*, 3(6): 701-703.
- Riba , A. , Bouras , N. , Mokrane , S., Mathieu , F. , Lebrihi , A. and Sabaou , N. (2010) *Aspergillus* section

- Flavi* and aflatoxins in Algerian wheat and drived products . *Food and Chemical Toxicology* , 48(10):2772-2777.
- Rodrigues, P., Venancio, A. and Lima, N. (2012) Aflatoxigenic fungi and aflatoxins in Portuguese almonds. *Sci World J.*, 47:1–9.
- Ronhede, S., B. Jenesen, S. Rosendahl, B.B. Kragelund, R.K. Juhler and Amand, J. (2005) Hydroxylation of the herbicide isoproturon by fungi isolated from agricultural soil. *Appl. Environ. Microbiol.*, 71(12): 7927-7932.
- Suleiman, M.N. and Taiga, A. (2009) Efficacy of aqueous extracts of neem and sharf for the control of fungi associated with milled and unmilled stored rice grains. 5th Proceeding of the Humboldt kellog Annual Agric. Conference, pp: 71-73.
- Tournas , V.H. , Niazi, N.S. and Kohn, J.S. (2015) Fungal Presence in Selected Tree Nuts and Dried Fruits . *Microbiology Insights* ,8: 1-6 .
- Wu, F., Narrod, C., Tiongo, M. and Liu, Y. (2011) The health economics of aflatoxin: global burden of disease. Afla Control Internal Food Policy Research Institute, working paper 4.