*Plant Archives* Vol. 20, Supplement 1, 2020 pp. 274-276 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

# ISOLATION AND IDENTIFICATION OF FUNGI FROM SOME NUTS PRODUCTS

**Alaa M. Alrudainy and Ahmed Mshari**
Department of Medical Laboratory Technology, College of Health and Medical Technology,
Southern Technical University, Basrah, Iraq
Corresponding authors: alaa.abdulamer@stu.edu.iq, ahmed.mshari@stu.edu.iq

## Abstract

This study aimed to isolate and identification of plant pathogenic fungi from nuts which imports for Iraq, the nuts included Pistachio, sunflower, Corn, Pumpkin, Almond, Peanut and Cashew, the result shown the maximum number of fungi isolates cause of nuts contamination by *A. niger*, *A. flavus* and *Penicillium* spp. were 43, 37 and 32 isolate respectively, while *Fusarium* spp. record 2 isolate as minimum number, and the result mention the highest presence rate were in Peanut, by *A. niger, A. flavus* and *Penicillium* spp., record 22.22, 20 and 15.15% respectively, while the less presence rate were in Peanut by *Fusarium* sp (4.44 %), in Cashew by *Alternaria* spp. (6.66 %) and in Sun flower by *Cunninghamella bertholletiae* (8.88%), while the maximum frequency ratio record in Pumpkin by A. *niger* (35%), in corn by *Rhizopus* spp. (33.33 in) and Pumpkin by A. *flavus* (30% in) respectively, while minimum frequency rate were in Peanut. by *Mucor* spp., *Fusarium* spp., *A. fumigatus* and *Rhizopus* spp.

***Keywords*** **:** isolate, identification, contamination, presence rate, frequency rate.

## Introduction

Nuts are used as direct consumption or in many industries, which contain to an important of fats and proteins their products coincident wide acceptances throughout the world (Watt and Merrill, 1964). nuts contamination by Fungal in increasing challenge to nuts quality, safety, and the contaminated nuts after consumption considered as dangers for human health (Al-Rifaie, and Maqtoofi, 2018) Wide range of fungi such as *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp. and *Mucor* spp., responsible to contamination of nuts like almond, cashewnut, hazelnut, Peanut and pistachio (Abdulla, 2013). Contamination of nuts maybe occur in one or more of the three different ways, which include, during the prior to harvest when the nuts on the trees, or after harvesting, or in storage (Boutrif, 1998; Alsuhaibani, 2018). Nuts are considered more crops that can be contamination by mycotoxins which led to a major problem in many countries (Bhatnagar *et al*., 2004), *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. were observed as most common fungi can producing mycotoxins (Eltariki *et al*., 2018). Mycotoxins considered secondary metabolites of fungi which responsible to adverse effects on crops, animals and humans, and led to illnesses and many economic losses. Aflatoxins, trichothecenes, ochratoxins, zearalenone, tremorgenic toxins, fumonisins, and ergot alkaloids are the most agro-economic importance mycotoxins (Zain, 2011). Many diagnosis studies focus on the local products, so this study aimed to isolation and diagnosis of fungi from different nuts products are imports from some countries, for Iraq.

## Materials and Methods

### Sample collection

In the present study, different nuts products (which imports from some brands) were collected randomly from Basra markets and brought to the laboratory, including sunflower, Pistachio, Pumpkin, Corn, Almond, Cashew and Peanut.

### Isolation and identification of fungi

The isolation from all samples include sterilize of sample with 1% of sodium hypo-chloride for 2 minutes followed by wishing once used distilled water and dried on sterile filter paper, and place 3 seeds on Petri dishes containing PDA, all plates incubated in 25°C for five days (Pitt and Hocking, 1997), after that, pure cultures prepared from each growth for diagnoses, which depended on the morphology and microscope features (Klich, 2002; Domsch *et al*., 2003). Appearance and frequency ratios of fungi isolates calculated following Eq. 1 and 2:

$$\text{(1) Ratio of appearance (\%)} = \frac{\text{No. of samples that the fungus appeare on it}}{\text{Total No. of samples}} \times 100$$

$$\text{(2) Ratio of appearance (\%)} = \frac{\text{No. of fungus isolates}}{\text{Total No. of isolates}} \times 100$$

## Results and Discussion

The current study record several fungi species that isolated from different types of nuts, and the result shown 169 different fungal isolate (Table 1) the highest number of isolates record by *A. niger* (43 isolate) followed by *A. flavus* (37 isolate) and *Penicillium* spp. (32 isolate) while the minimum number record by *Fusarium* spp. (2 isolates).

**Table 1:** Number of fungal species that isolated from different types of nuts.

| No. | The fungal isolates. | No. of isolates |
|---|---|---|
| 1 | *Penicillium* spp. | 32 |
| 2 | *A. niger* | 43 |
| 3 | *A. flavus* | 37 |
| 4 | *A. terreus* | 6 |
| 5 | *A. fumigatus* | 11 |
| 6 | *Alternaria* spp | 3 |
| 7 | *Rhizopus* spp. | 22 |
| 8 | *Mucor* spp. | 9 |
| 9 | *Cunninghamella bertholletiae* | 4 |
| 10 | *Fusarium* spp. | 2 |
| | Total | 169 |

*Aspergillus* spp. the more fungi effect on nuts, result of the appearing ratios of fungi (Table 2) shown *A. niger, A. flavus, Penicillium* spp. and *Rhizopus* spp. most fungi appear in all types of nuts, The maximum presence rate record in Peanut by *A. niger* (22.22%), *A. flavus* (20 %) and *Penicillium* spp. (15.15%) respectively, while *Fusarium* spp. (4.44 % in Peanut), *Alternaria* spp. (6.66 % in Cashew) and *Cunninghamella bertholletiae* (8.88 in Sun flower) record the minimum presence rate.

**Table 2:** Appearing ratios of fungi(%) isolated from different nuts.

| No. | Types of nuts | *Penicillium* spp. | *A. niger* | *A. flavus* | *A. terreus* | *A. fumigatus* | *Alternaria* spp. | *Rhizopus* spp. | *Mucor* spp. | *Cunninghamella bertholletiae* | *Fusarium* spp. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Sun flower | 13.33 | 8.88 | 6.66 | 0.00 | 6.66 | 0.00 | 6.66 | 0.00 | 8.88 | 0.00 |
| 2 | Pistachio | 8.88 | 8.88 | 8.88 | 6.66 | 4.44 | 0.00 | 8.88 | 11.11 | 0.00 | 0.00 |
| 3 | Pumpkin | 6.66 | 15.15 | 13.33 | 0.00 | 4.44 | 0.00 | 4.44 | 0.00 | 0.00 | 0.00 |
| 4 | Corn | 6.66 | 11.11 | 8.88 | 0.00 | 0.00 | 0.00 | 13.33 | 0.00 | 0.00 | 0.00 |
| 5 | Almond | 8.88 | 13.33 | 11.11 | 0.00 | 4.44 | 0.00 | 4.44 | 4.44 | 0.00 | 0.00 |
| 6 | Cashew | 11.11 | 15.15 | 13.33 | 6.66 | 0.00 | 6.66 | 6.66 | 0.00 | 0.00 | 0.00 |
| 7 | Peanut | 15.15 | 22.22 | 20.00 | 0.00 | 4.44 | 0.00 | 4.44 | 4.44 | 0.00 | 4.44 |

Result of the frequency of fungi isolates (Table 3) record the maximum ratio of the A. *niger* (35% in Pumpkin) followed by *Rhizopus* spp. (33.33 in corn) and A. *flavus* (30% in Pumpkin) respectively, while the minimum frequency rate shown by *Fusarium* spp., *Mucor* spp., *Rhizopus* spp. and *A. fumigatus* which record 5.88% in Peanut.

**Table 3:** Frequency of fungi isolates(%) isolated from different nuts.

| No. | Types of nuts. | *Penicillium* spp. | *A. niger* | *A. flavus* | *A. terreus* | *A. fumigatus* | *Alternaria* spp | *Rhizopus* spp. | *Mucor* spp. | *Cunninghamella bertholletiae* | *Fusarium* spp. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Sun flower | 26.08 | 17.39 | 13.04 | 0.00 | 13.04 | 0.00 | 13.04 | 0.00 | 17.39 | 0.00 |
| 2 | Pistachio | 15.38 | 15.38 | 15.38 | 11.53 | 7.69 | 0.00 | 15.38 | 19.23 | 0.00 | 0.00 |
| 3 | Pumpkin | 15.00 | 35.00 | 30.00 | 0.00 | 10.00 | 0.00 | 10.00 | 0.00 | 0.00 | 0.00 |
| 4 | Corn | 16.16 | 27.77 | 22.22 | 0.00 | 0.00 | 0.00 | 33.33 | 0.00 | 0.00 | 0.00 |
| 5 | Almond | 19.04 | 28.57 | 23.8 | 0.00 | 9.52 | 0.00 | 9.52 | 9.52 | 0.00 | 0.00 |
| 6 | Cashew | 18.51 | 25.92 | 22.22 | 11.11 | 0.00 | 11.11 | 11.11 | 0.00 | 0.00 | 0.00 |
| 7 | Peanut | 20.58 | 29.41 | 26.47 | 0.00 | 5.88 | 0.00 | 5.88 | 5.88 | 0.00 | 5.88 |

These result agreement with Abbas, *et al*. (2019), which reported *A. niger* and *A. flavus* most contamination of nuts, Alhussaini, (2012) shown in his result *A. niger* and *A. flavus* were isolated from all nuts samples (corn, Almond, Cashew and Pistachio),while Hamed *et al*. (2016) record *Aspergillus* spp. most isolates contamination of nuts, *A. flavus* shown highest presence rate in peanut (80%), whereas, in sunflower, pistachios, pumpkin seeds and almond (60%), while *A. niger* shown maximum percentage in peanut (70%) and in walnut (60%), *Penicillium* spp. record highest rate of appearance In sunflower (90%) and peanut (80%), and mention the maximum frequency ratio by *A. niger* in peanut and corn (50%) and *Penicillium* spp., in sunflower seeds and peanut (68.75%).

The different potential of contamination for the food products depends to nature of the material suitable food for the growth of the fungi as well as environmental conditions of function of hydrogen and temperature (Rostami *et al*., 2019) *Aspergillus* spp. are able to utilize a wide variety of substrates and adapt well to a high range of ecological conditions (Cray *et al*., 2013; Rhodes, 2006) and able to produce a large number of conidia which have high sensitivity to withstand critical environmental (Hagiwara *et al*., 2017).

## Conclusion

This study shown, *A. niger, A. flavus, Rhizopus* spp. and *Penicillium* spp. most fungi responsible to contamination of all nuts products (Pistachio, Corn, Pumpkin, sunflower, Peanut and Cashew and Almond) and Peanut most product that contamination by fungi.

## References


Abbas, M.; Naz, S.A.; Shafique, M.; Jabeen, N. and Abbas, S. (2019). Fungal contamination in dried fruits and nuts: a possible source of mycoses and mycotoxicoses. Pak. J. Bot, 51: 4.

Abdulla, N.Q.F. (2013). Evaluation of fungal flora and mycotoxin in some important nut products in Erbil local markets. Res J Environ Earth Sci, 5(6): 330-336.

Alhussaini, M.S. (2012). Mycobiota and mycotoxins of nuts and some dried fruits from Saudi Arabia. Journal of American Science, 8(12): 525-534.

Al-Rifaie, A.A. and Al-Maqtoofi, M.Y. (2018). Immunodetection and risk assessment for *Aspergillus* contamination in nuts using a highly specific monoclonal antibody. Biomedical Research, 29(21): 3807-3814.

Alsuhaibani, A.M. (2018). Effects of Storage Periods and Temperature on Mold Prevalence and Aflatoxin Contamination in Nuts. Pakistan Journal of Nutrition, 17(5): 219-227.

Bhatnagar, D.; Payne, G.A.; Cleveland, T.E. and Robens, J.F. (2004): Mycotoxins: Current issues in USA. In: *Meeting the mycotoxin menace*, Barug D., van Egmond H., López-García R., van Osenbruggen T. and Visconti A. (eds). Wageningen Academic Publishers, The Netherlands, 17-47.

Boutrif, E. (1998). Prevention of aflatoxin in pistachios. Food Nutrition and Agriculture, 32-37.

Cray, J.A.; Bell, A.N.; Bhaganna, P.; Mswaka, A.Y.; Timson, D.J. and Hallsworth, J.E. (2013). The biology of habitat dominance; can microbes behave as weeds. Microbial biotechnology, 6(5): 453-492.

Domsch, K.H.; Gams, W. and Anderson, T.H. (1980). Compendium of soil fungi Academic Press London 809.

Eltariki, F.E.M.; Tiwari, K.; Ariffin, I.A. and Alhoot, M.A. (2018). Genetic Diversity of Fungi Producing Mycotoxins in Stored Crops. Journal of Pure and Applied Microbiology, 12(4): 1815-1824.

Hagiwara, D.; Sakai, K.; Suzuki, S.; Umemura, M.; Nogawa, T.; Kato, N. and Kamei, K. (2017). Temperature during conidiation affects stress tolerance, pigmentation, and trypacidin accumulation in the conidia of the airborne pathogen *Aspergillus fumigatus*. PloS one, 12(5): e0177050.

Hamed, S.; Murad, A.F. and Abdul-Rahim, E.A. (2016). Molecularly diagnostic of aflatoxigenic *Aspergillus flavus* isolated from nuts. Res J Environ Toxicol, 10(1): 39-49.

Klich, M.A. (2002). Identification of common Aspergillus species (p. 116). Utrecht: Centraalbureau voor schimmel cultures.

Pitt, J.I. and Hocking, A.D. (1997). Fungi and Food Spoilage. 2nd Edn., Springer, USA., ISBN-13: 978-0412554605, Pages: 593.

Rhodes, J.C. (2006). *Aspergillus fumigatus*: growth and virulence. Medical mycology, 44 (Supplement_1), S77-S81.

Rostami, R.; Nadafi, K.; Aghamohammadi, A.; Najafi, S.H. and Fazlzadeh, D.M. (2009). Survey of peanut fungal contamination and its relationship with ambient conditions in the bazar of zanjan. Journal of Environmental Health Science & Engineering, 6(4): 295-300.

Watt, B.K. and Merrill, A.L. (1964). Composition of foods: raw, processed, prepared (No. 8). Consumer and Food Economics Research Division, Agricultural Research Service, US Department of Agriculture.

Zain, M.E. (2011). Impact of mycotoxins on humans and animals. Journal of Saudi Chemical Society, 15(2): 129-144.