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Usage UV Irradiation for Reducing Fungal Contamination of Loose Nuts in Al Jouf Markets

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ABSTRACT

Aspergillus flavus, *Aspergillus niger*, *Aspergillus Parasiticus*, *Aspergillus terreus*, and *Rhizopus stolonifera* were isolated from the seeds' surfaces of loose nuts that are sold in markets of Sakaka city, Saudi Arabia. The ammonia detection experiment showed that isolates of *A. flavus*, *A. parasiticus* and *A. niger* have the ability to release aflatoxins rather than the other isolated species. Loose nuts were exposed to UV wavelengths (240-380 nm) for different times (180, 120, 90, 60, 40, 20 minutes) on both sides of the nut. The highest inhibition rate of fungi growth was 90 minutes at 100%. Also, the heavy metal, as well as the ether contents, were studied both for the preserved and the loose samples to detect the effect of the UV irradiations and the pathogenic microorganism.

INTRODUCTION

Many agricultural crops, Nuts, and food are suitable media for the growth of fungi, including pathogenic fungi that produce toxins (Moustafa and Abdelzaher, 2016, Moustafa, 2018). It is known that very small amounts do not exceed parts per billions of these toxins to the blood of humans or animals cause many pathological problems. Mycotoxins are toxins produced by fungi and can cause toxic effects on human, animal tissues and organs (kazemi, 2003). There are currently more than 300 types of mycotoxins, but only 10 of them pose a significant risk to human and animal health, including Aflatoxin, Ochratoxin, Fumonisis, Trichothecenes, Oleoxynivalenol, and Zearalenone (Wu *et al.*, 2011).

The infection of fungi begins first in the field by infecting crops and then infecting a seed product before and during the harvest period (Jarvis, 1971). The fungal damage is not limited to agricultural crops in the field but goes beyond the possibility of infection during storage (hesseltine, 1976; Sinha, 1990). Agricultural crops are susceptible to diseases of fungi, which are associated with seeds borne fungi from the field to the store and include several species.

The most important of which are *Aspergillus, Rhizopus, Penicillium, Clasiodiplodia, Fusarium, Alternaria, Cladosporium,* and *Helminthosporium* (Rodrigues *et al.,* 2012; Tournas *et al.,* 2015; Adeniyi and Adedeji, 2015)

Due to the high composition of fat, protein and water content of many nuts such as hazelnuts, almonds, walnuts, pistachios and cashews these products are damaged by microorganisms. They can grow on them if stored in conditions that allow sufficient humidity to spread. Therefore, the availability of the appropriate temperature for the growth of the fungi, the availability of minimum moisture content of the seeds, relative humidity of the surround atmosphere in the store are the most important factors help spread of fungi associated with the extracts stored, especially *Aspergillus* sp that able to produce aflatoxins (Reddy, 1992). Mycotoxins, especially aflatoxins, are the most serious toxins that have a significant relationship with liver and kidney cancer (Collee *et al.*, 1996).

Ultra-violate irradiation is one of the types of non-ionized radiation that is used in the disinfection and elimination of microorganisms and have different wavelengths between 210-380 nm, and is used in sterilizing hospitals and eliminating different types of toxins-producing fungi, especially *Aspergillus sp*, *Fusarium sp*, and *Botrytis* sp. (Amit., *et al.,* 2003). These rays have short wavelengths and high depletion within the living material, which leads to clot protein in the body of the pathogen and leads to the rapid killing of these organisms.

Irradiation is a fast disinfection process that is low in costs and does not cause harm to humans and animals without raising the temperature. Therefore, this study aims to isolate and identify the fungi associated with some types of loose nuts, which play an important role in the events of many diseases. Furthermore, it aims to assess the inhibitory effect of ultraviolet rays on the growth of fungi and their production of aflatoxin.

MATERIALS AND METHODS

Collection of Samples and Isolation of Fungi:

Loose nuts were brought from five different local markets of Sakaka KSA All samples were planted in Petri dishes containing sterilized Potato Dextrose Agar (PDA) medium supplemented with rose bengal (0.001 gm/L). The palates were incubated at 25oC for 7 days and the growing fungal isolates were purified by transferring a disc from each colony into a new dish containing PDA media. The process was repeated three times to confirm the purification of the selected isolate.

Identification of Fungi:

Isolated fungi were identified based on their morphological characters according to the identification key of Ainsworth., *et al.,* 2001)

Testing the Ability of Isolates to Produce Aflatoxins:

To determine the ability of isolated fungi to produce aflatoxins, the following steps were followed:

Coconut extract medium was prepared to determine the ability of isolated fungi to produce toxins. Approximate weight of 100gm of coconut grated was added to 300 ml of distilled water, the mixture was heated for 20 minutes and filtered by a clean piece of gauze, and 1.5% of agar-agar material was added to the filtrate followed by autoclaving. After sterilization, the medium was aseptically poured into Petri plates and 5 mm disc of the purified and selected isolates were inoculated to the plates' center followed by incubation for 7 days. After incubation, all the plates were turned over so that the lid of the dish was put down allowing the addition of 0.2 ml of 20% ammonia solution to each lid all plates were sealed with parafilm and then incubated at 28 ° C for 24-48 hours. (Lee and Hagler 1991).

Assessment of the Effect of Ultraviolet Radiation Exposure on Loose Nuts Fungi Spreading:

Inside the isolation cabinet, samples of

nuts (hazelnuts, pistachios, and almonds) were placed inside a sterile Petri dish and both sides of each nut type were exposed to ultraviolet radiation for a period of (180, 120, 90, 60, 40, and 20 minutes) at a wavelength of 240-380 nm.

After irradiation, a seed of each type of nuts was aseptically placed on the surface of rose bengal- containing PDA plates. All the plates were sealed with parafilm and incubated at 23±2ºC until the appearance of fungal colonies. These experiments were repeated 3 times for more confirmation of the obtained results and allowing proper statistical analysis.

Statistical Analysis:

Data were statistically analyzed using the GraphPad Prism 2.01 program; the results were expressed as mean values ± standard error. A significant difference between control and different UV exposure times were carried out using one-way ANOVA and unpaired t-test.

Oil Extraction:

Each sample (control and the collected samples) was ground (particle size ≤ 0.5 mm) in a mill. The samples were homogenized by a mixer and later analyzed to determine oil content. Lipid extraction from the nut samples was carried out by ether extraction under the operating conditions specified in IUPAC methods no.1.121 and expressed as a percentage by mass of the product as received (IUPAC, 1987). The samples were analyzed in triplicate, and then the mean was calculated. The obtained oil was stored at 4ºC for further investigation.

Chemical Analysis:

Heavy Metals Content (Al, Ni, and Cu):

Samples (5g each) were digested with a concentrate HNO_3-H_2O_2 digester mixture at 120°C for three hours in a Teflon digestor. Digested samples were diluted with distilled water to 100 cm^3. Examined elements were measured by complexometric (Ni and Cu) or gravimetric determinations (Al).

Free Fatty Acids Determination:

Free fatty acids (FFA) were measured by direct titration of the nuts' oil extract with (0.1N) NaOH using phenolphthalein as an indicator. Free fatty acid contents of oil samples were determined in accordance with methods no.2.201of IUPAC (1987).

RESULTS AND DISCUSSION

Detection and Isolation of Pathogenic Fungi:

Three types of nuts were investigated for their fungal contamination through direct inoculation into PDA plates. After incubation, the macroscopic examination showed the cultivation of three fungal genera that belonging to *Aspergillus*, *Penicillium*, and *Rhizopus*. As shown in table 1, three-quarters of the isolated species were almost belonging to genus *Aspergillus* with an exact percentage of 75.6%. After microscopic examination, four *Aspergillus* species were dominating as *A. Parasiticus A. flavus*, *A. niger* and *A. terreus* with percentages of (24.90%, 24.47%, 15.93%, and 10.31%), respectively. However, the other two genera showed the growth of the species *Penicillium chysogenum* and *Rhizopus stolonifera* with percentages of 15.25 and 9.15%, respectively (Table 1).

We could attribute the detected fungal contamination may be due to bad storage conditions, which is one of the most important factors that assist in the growth of fungi especially pathogenic ones. Fungi are well known for their ability to grow on different nutrients due to their ability to produce a wide range of digestive enzymes (Riba *et al.*, 2010; Abdulla ,2013; Abed, 2017). These results are matched with previous studies that isolate and diagnose some fungi from nuts which offered for human usages such as *Rhizopus* spp, *A .niger*, *Penicillium* spp, *Rhizoctonia* spp, *Chaetomium* spp, *Trichoderma* spp and *Fusarium* spp (AL-Rawi , 2009). Our results are in good agreement with the study results of Mimoune (2016) who showed that *Aspergillus* sp. and *Penicillium* sp. were the most common and frequencies fungal on various nuts. They attributed this to the ability of fungi to produce a wide range of enzymes that facilitated them to grow on different nutrients (Riba *et al*., 2010 ; Tournas *et al.*, 2015).

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Table 1: Total counts of fungal genera and species recovered from 5 samples of local markets by dilution plates, number of cases of isolation of fungi (NCl; out of 5 cases), occurrence remarks (OR), and percentage of total counts (TC%) on rosebengal-PDA at 28°C after 7 days**.**

Genera and species	Isolation remarks		
	NCI	OR	TC(%)
Different types of loose nut* from 5 different places			
Aspergillus flavus	82.33 ± 1.45	5H	24.47
A. Parasiticus	83.78 ± 7.8	5H	24.90
A. niger	53.6 ± 2.18	4H	15.93
A. terreus	34.67 ± 2.9	2M	10.30
Penicillium chysogenum	51.33± 4.97	2M	15.25
Rhizopus stolonifera	30.67 ± 3.11	1L	9.11
Control" nut nitrogen flushed"	0	0	0
Gross total counts	336.38		
No. of genera	3		
No. of species	6		

OR = Occurrence remarks; H = 60% -100.0%, M = 33 - 59.0%, L = 20 - 32%, and R = 7 – 19%.
*different types of nuts contain: walnuts, pistachios and Cashews

Detection of Aflatoxins Production:

The ability of fungal isolates to produce aflatoxins was detected through the use of ammonia solution as an indicator. As shown in table 2, the isolates *Aspergillus flavus, A. parasiticus,* and *A. niger* have been detected to produce aflatoxins as indicated by their colony's color change. The normal colony color of each species was observed to be changed to light orange-yellow color. These changes were qualitatively diagnosed as aflatoxin producing fungi. The un-changed colony color of the other species indicated an inability to produce aflatoxins.

Table 2: Detection of aflatoxin production by the fungal isolates**.**

Fungal isolates	Aflatoxin production
Aspergillus flavus	+
Aspergillus Parasiticus	+
Aspergillus niger	+
Aspergillus terreus	-
Penicillium chysogenum	-
Rhizopus stolonifera	-

+ = Positive aflatoxin production, - = Negative aflatoxin production

UV irradiation for Decontamination of Nuts-Inhabiting Fungi:

All the tested nuts were exposed to UV radiation for different times to investigate suitable time exposure that may result in the absolute inhibition of fungal growth. As shown in table 3 and figure 1, the time of exposure is a significant factor for microbes' decontamination. It has been detected that the genus *Aspergillus* was the most tolerant isolates compared with genera *Penicillium* and *Rhizopus*. All the tested fungal isolates were dramatically decreased in growth in progress with long times of exposure. Heavy fungal growth was detected after 20 min of UV exposure; while, reduced growth was detected after 40 min followed by 60 min of exposure. After 60 min of exposure, 33.3% of the tested isolates were completely inhibited (genera *Penicillium* and *Rhizopus)*; while 66.6% were almost detected in small amounts (genus *Aspergillus)*. On the other hand, all the tested isolated failed to grow after 90 min of UV exposure (240-380 nm).

Table 3: The Effect of radiation exposure periods of UV on the emergence and growth of fungi in nut samples at 25ºC

Isolated fungi	0 min	20 min	40 min	60 min	90 min
A. flavus	82.33 ± 1.45	74.67±2.4	57± 1.5	47.17± 4.5	0
A. Parasiticus	83.78 ± 7.8	53.22± 2.05	38.89± 6.97	1.33 ± 0.88	0
A. niger	53.6 ± 2.18	36.33± 2.4	20.89± 3.4	5± 3.2	0
A. terreus	34.67 ± 2.9	15± 4.7	2± 2	0.6 ± 0.6	0
Penicillium chysogenum	51.33± 4.97	36.33± 3.28	25.67± 4.33	0	0
Rhizopus stolonifera	30.67 ± 3.11	16.17± 2.9	5.33± 2.8	0	0

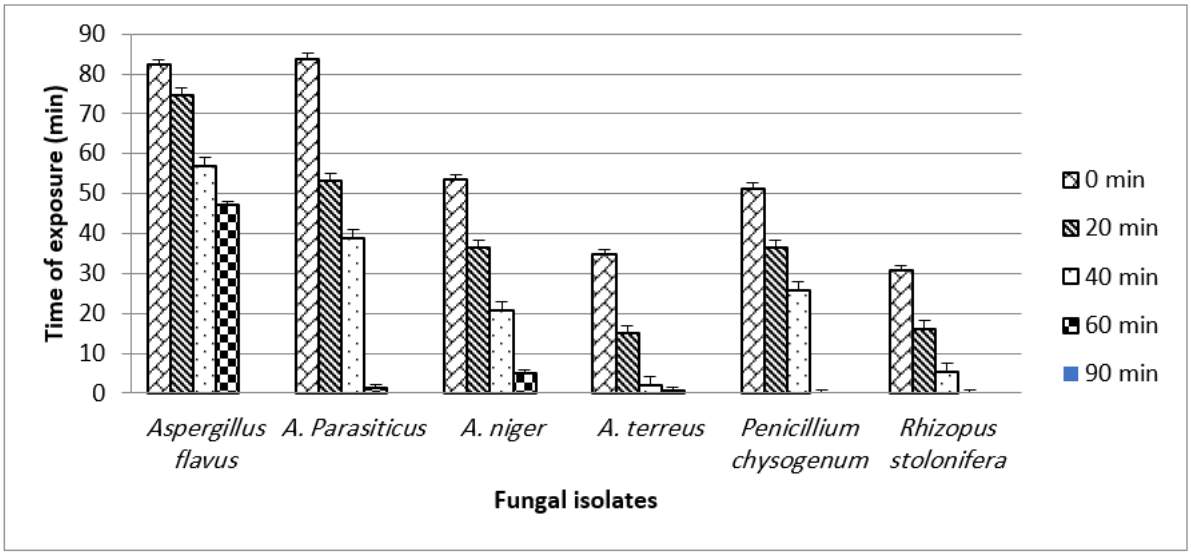

Fig.1. Measurements of the growth of tested fungal isolates after exposure to UV radiation for different times, all the results are the mean of three replications and Bar on each column represents the standard deviation.

Determination of the Heavy Metal Contents:

The heavy metal content of the deferent samples was determined either by complexometric (Ni and Cu) or gravimetric (Al) determination. By a careful comparison of the content of the control sample and the other samples before and after irradiation, it was found that there is no variation in terms of the heavy metal contents were observed among all the samples (Fig.2). These results indicate that the irradiation by the UV does

not affect the heavy metal content. It is well known that heavy metals are inorganic elements that neither biodegradable nor thermodegradable. However, fungal biomass is biological entities affected by external factors including UV radiation. These concepts explain the successful ability of UV radiations to stop the fungal growth while failed to remove or reduce the existed concentrations of heavy metals (Umit *et al.*, 2011 and Gholamhossein *et al.*, 2013).

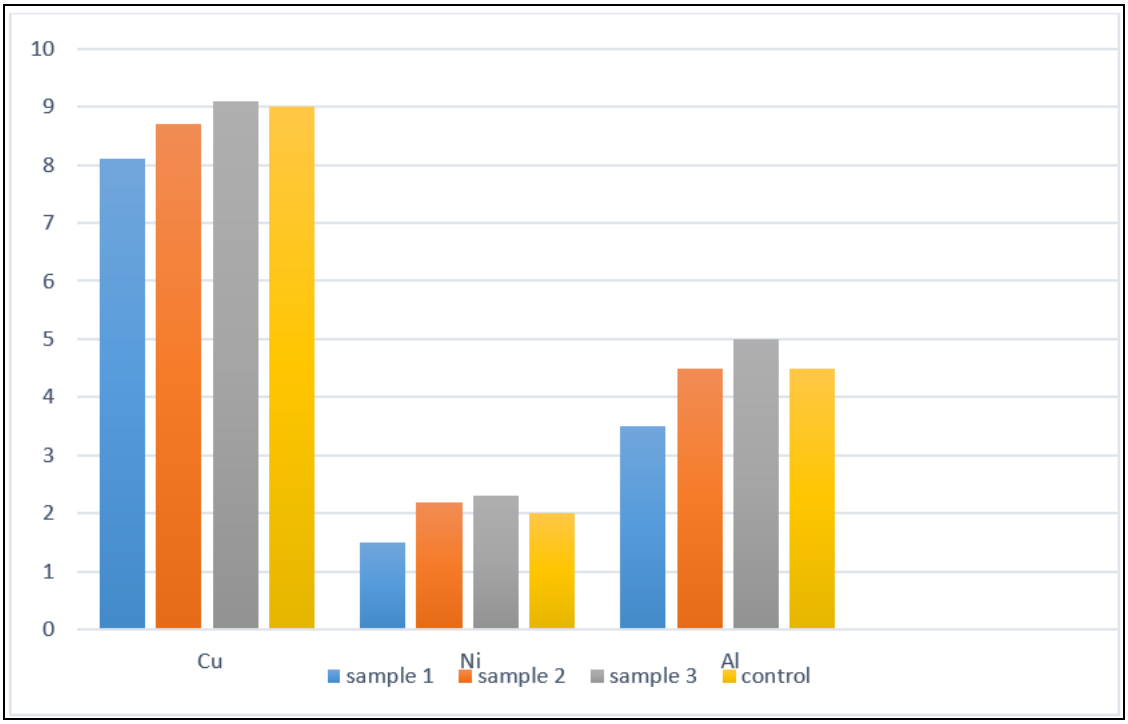

Fig.2: Comparison of heavy metals content of the control and tested samples before and after UV irradiation

Ether Content:

Ether content means the extraction of all soluble substances that can be extracted by ether or other organic solvents.

These substances include fats, oils, dyes, waxes Lipids, triglycerides, and carbohydrates. It can also dissolve all the oxidized and non-oxidized fats in the sample. In contrast to the results obtained for the content of the heavy metals, it was found that there is a great variation between the control and the collected samples. As shown in figure 3, the organic content of the control sample was much higher than tested samples indicating the negative effect of the presence of the pathogenic fungi which decrease the concentration of the essential lipids and carbohydrates (Marcília *et al.*, 2017). These results indicate the ability of pathogenic fungi to use the organic substances of the examined nuts to boost their nutrients and energy requirements.

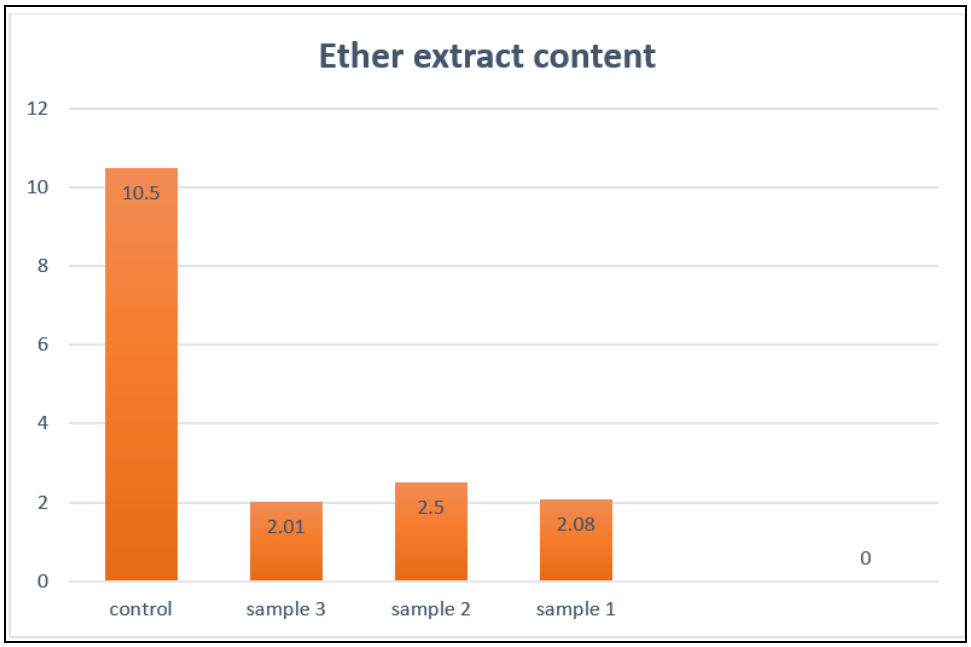

Fig.3. Comparison of Ether content of the control and test samples

Conclusion:

It would be concluded that the nuts are nutrient-rich compounds that act as a good medium for the growth of pathogenic and aflatoxin-producing fungi. One of the potent methods to eliminate the fungi is exposing these nuts into UV radiations for a period not less than 90 min.

We recommend companies to take advantage of the design (Fig. 4) to sterilize nuts using ultraviolet radiation with continuous stirring for not less than 90 minutes before being presented to the consumer to make sure to eliminate the fungi that may be related to nuts.

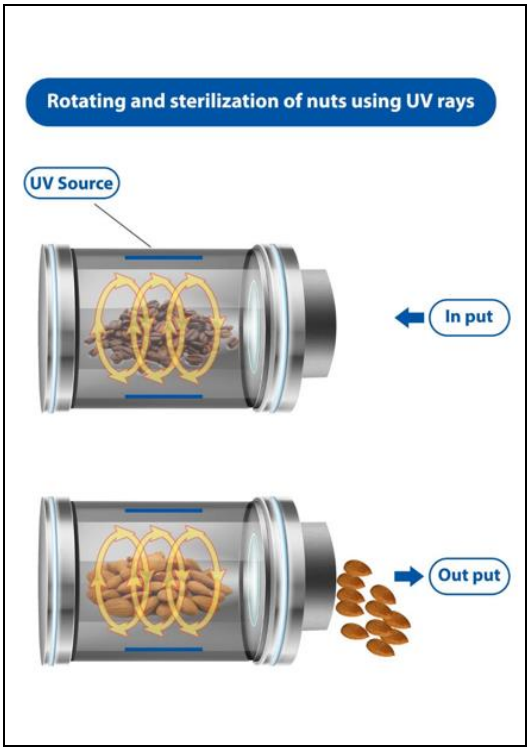

Fig.4: Proposed form for the design containing an ultraviolet source.

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REFERENCES

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.

Abed. R.M., (2017): Investigation of Fungal Contamination in Local and Imported Nuts Which Displayed in Markets of Diyala , Iraq. Food Science and Quality Management, Vol.59: 1-5.

Adeniyi, 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.

Ainsworth, G. C., Bisby, G. R., Kirk, P. M., and CABI Bioscience. (2001): Ainsworth & Bisby's dictionary of the fungi / by P.M. Kirk ... [et al.]; with the assistance of A. Aptroot ... [et al.]. Wallingford, Oxon, UK: CABI Pub.

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.

Collee, J. G.; Fraser, A. G.; Marmion, B. P. and Simmons, A., (1996): Practical Medical Microbiology. 4th edn., Churchill Livingstone, pp. 695-717.

Gholamhossein DAVARYNEJAD, Mehdi ZAREI, Peter Tamas NAGY (2013) Identification and Quantification of Heavy Metals Concentrations in Pistacia, Not Sci Biol, 5(4):438-4;644.

Hesseltine, C. W. (1976): Conditions Leading to Mycotoxins Contamination of Food and Feeds. In: Advance in Chemistry Series. No.149 Mycotoxins and Other Fungal Related Food Problems. Edited by: Joseph,V. Rodricks.

Jarvis, B., (1971): Factor Affecting the Production of Mycotoxins. J. Appl. Bact., 34(1): pp. 199-213.

Kazemi, A. (2003): Carcinogenic mycotoxins; The 1st Student's Congress of Cancer; Shahed Uni., Tehran, Iran. p. 90.

Lee, Y.J. and W. M. J, Hagler. (1991): Aflatoxin and Cyclopiazonic Acid produced by Aspergillus flavus isolated from contaminated. J. Food Sci., 56 :871-872.

Marcília M. Barbosa, Edenio Detmann, Sebastião C. Valadares Filho, Kelly S.C. Detmann, Marcia O. Franco, Erick D. Batista And Gabriel C. Rocha (2017) Evaluation of methods for the quantification of ether extract contents in forage and cattle feces. Anais da Academia Brasileira de Ciências, 89(2): 1295-1303.

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.

Moustafa, S. M. N. 2018. Role of Hydrolytic Enzymes of Some Fungi in Rotting Fruits and a Possibility of Retard Spoilage. Journal of Pure And Applied Microbiology, Vol. 12(3), p. 1525-1535.

Moustafa, S. M. N. and Abdelzaher, H. M. A. (2016): Occurrence of Hemolytic Fungi Mounted on Wheat Grains in the main Silo of Sakaka, Saudi Arabia. Journal of Pure and Applied Microbiology, 10 (3): 1817-1824.

Reddy,T. V. 1992. Aflatoxins in Feed: An Enemy to Poultry Producers in Tropics. Misset-World poult., 8: pp. 19-22.

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.

Sinha, K. K., (1990): Incidence of Mycotoxins in Maize Grains in Bihar State, India.Food Contam., 7: pp. 55-61.

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.

Umit Gecgel, Tuncay Gumus, Murat Tasan, Orhan Daglioglu, Muhammet Arici (2011): Determination of fatty acid composition of g-irradiated hazelnuts, walnuts, almonds, and pistachios, Radiation Physics and Chemistry 80, 578–581.

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.