THE MYCOFLORA AND PUBLIC HEALTH RISKS OF SOME FOOD MATERIALS SOLD IN LUGBE MARKETS, FEDERAL CAPITAL TERRITORY (FCT), ABUJA, NIGERIA

Abstract

Mycotoxins are secondary metabolites produced by many species of fungi. They are natural contaminants of food and (feed) commodities where they cause significant risks to human and animal health. In this work, some food items; Roasted Groundnut Kernel, Dried smoked fish, Grounded melon, *Ogbono* (*Irvingia gabonensis*) and Date palm fruits were directly cultured on modified Potato Dextrose Agar (PDA) (Chloramphenicol; 0.1g/l and Cycloheximide; 0.5mg/ml) and the respective food-associated molds were isolated and identified microscopically by the use of Lacto-phenol cotton blue for their morphological characterisation. The results showed the presence of *Aspergillus spp.*, *Penicillium spp.*, *Rhizopus*, *Mucor*, *Fusarium* and *Cladosporium* with *Cladosporium* having the highest occurrence in Melon flour (38%) followed by Aspergillus spp. on ground nut (25 %) and Mucor spp. having the lowest on cashew nuts (5 %). The presence of indicator organisms such as Aspergillus (*A. flavus and A. parasiticus*) renders foods potentially unwholesome because of the likelihood production of one of the most poisonous mycotoxins (aflatoxin). Poor storage, moisture conditions cause growth and production of unacceptable aflatoxin levels. Some foods are consumed without further processing or under processed. Based on our study, proper food drying and protection from spores are recommended safety preventive measures. This research should create awareness on the dangers associated with the consumption of such food materials especially the ready-to-eat ones.

Key words*: mycotoxins, food items, metabolites, moisture*

Introductions

Several Low moisture content foods materials, are sold and consumed in Nigeria as a quick source of recipe either as fruit, nut, or as recipe for cooking. These materials are either eaten partially washed, unwashed or partially cooked [1].

Groundnut, melon seeds, ogbono nuts (*Irvingia*), dries *catfish*, dried water leaf, cashew nut and stock fish are various food materials that are known to contribute substantially to food intake of Nigerian and are readily available in the open markets [2]. These materials are produced in substantial quantities but are usually seasonal, as such, they are often dried or remove part of their moisture contents in order to preserve them for the time of non-availability. However, the modes of drying and subsequent exposures often raise concerns about their tendency to harbour molds and hence mycotoxin production [3]. Many fungi groups are capable of surviving freely outside their hosts including hash environments such as dry soil, air or water [4]. They are capable of reproducing when conditions become favourable. The growth of some species is usually accompanied with the production of some deadly metabolites called mycotoxins [5]. This phenomenon has been a concern because of the negative impact they have on human and livestock around the world. Both *Aspergillus*

flavus and *Aspergillus parasiticus* molds have the tendency of producing the toxins (aflatoxins) and they are found distributed in many food products as contaminants. Some of these foods are consumed directly or partially cooked. Unfortunately, even the cooking processes, either at domestic or industrial levels do not eliminate the toxins completely [6].

Groundnut (*Arachis hypogaea* L.) is a popular plant-based proteinous, snack and recipe for many food material that are produced and consumed in several African countries due to their healthy benefits such the presence of monounsaturated and polyunsaturated fats that keeps the heart healthy. However, they are prone to growth of aflatoxin-producing *Aspergillus* spp., the growth and full expressions of which occur either in the field or during storage [7].

Melon (*Colocynthis citrullus L.*) seeds locally refer to as *egusi* remain an important recipe for thickening soup in many parts of West [8]. During harvest, the seeds are heaped in the broken exocarp (thick rind) for fleshy endocarp to ferment for easy extraction of the melon seeds. They are then washed, sun-dried and bagged with the shell. The seeds may be stored in-shell till their market price is favourable or sold as shelled seeds after seed-coat removal. During storage and transportation, seeds, especially the broken ones often grow molds [10]. Also, depending on the prevailing market and environmental conditions, the shelled seeds could be grounded and kept till customers come to bye. This time also give room for mold contamination and toxin production [9].

Bush mango (*Irvingia gabonensis*) seeds (locally referred to as *ogbono* are obtained from bush mango fruits, a non-tree forest plant. The entire fruits are usually allowed to undergo spontaneous fermentation by heaping them together. The seeds are then extracted, sun-dried and bagged for transportation and storage {13]. Depending on the marked need, the dried bush mango seeds are ground into powdery form prior to sales. Expectedly, the long storage time before use often expose the product to mold contamination under humid tropical condition and when the seeds are not dried enough before storage [8].

Cashew nut is a widely consumed as well a good raw material for some other industries. They are rich in protein, minerals and fat. Their wide acceptability makes them popular in Africa and the world over [12]. Although they are extremely high in fat, protein and low water content thereby them refractory to spoilage by microorganisms as such they could be preserved for longer period, however, cashew nut are subject to mold growth when the moisture conditions become compromised [14].

Drying water leaves is a way of preservation to minimise post harvest loss as well as making the foods available at off-seasons which are great challenges in many Tropical countries [15]. It is the most ancient way of preserving food and is based on the concept of lowering the availability of water in order to deter the activity of microorganisms and enzymes in food which in turn increases the shelf life of foods. Mostly the perishable crops are dried to increase the shelf life and promote food security.

Date palm (*Phoenix dactylifera L.*) is a palm in the genus Phoenix which are cultivated for their edible sweet fruit. They are widely distributed by being dispersed far and wide owing to their high calorie and long-keeping qualities [16]. However, despite their low water contents, they are affected by some microbial spoilage agents especially during storage and processing [17].

Stockfish (*Gadus morhua*) is unsalted fish especially cod, dried by sun and wind or in special drying houses. Due to the specialised modes of drying, they usually retain all the nutrients of the fresh fish but are only concentrated. Stockfish are prone to microbial deterioration and source of contamination by mycotoxin which are difficult to remove by heat [18].

MATERIALS AND METHODS

Sampling

Sampling was carried out randomly on the following food products, groundnuts, stockfish, dried fish, cashew nuts, dried water leaves, grounded *ogbono* seeds, grounded melon seeds and Date palm fruits. These products were usually displayed outside, in metal or plastic containers or wooden boxes in order to attract the would-be consumers. In all, a total of five samples of each product were collected randomly from different markets within Abuja Metropolis to prepare one composite sample. The samples were sealed in sterile polyethylene bag and kept in the refrigerator until needed for mycological analysis [19].

Determination of Moisture Contents of Samples

One (1g) A known quantity of each sample were taken, kept in a hot-air oven at 105^0C and dried to a constant weight (AOAC, 1990) and readings were taken for triplicate samples per food sample. The percentage of each sample was calculated;
Initial wt.-final wt/Initial wt.x100. [20]

Isolation of Fungi

Direct surface plating method was applied for the isolation of fungi present in the food samples. From each sample, 1g was homogenised in 9ml sterile peptone water and serially diluted. Aliquot 0.1ml 10^{-4} taken after proper mixing and spread-plated out in duplicate on Potato Dextrose Agar (PDA) modified with Chloramphenicol (0.1g/l) and Cycloheximide (0.5mg/ml to suppress the growth of bacteria and yeasts respectively [21]. The plates were incubated 28°C for 5 days. Distinct fungal colonies were thereafter counted and transferred to freshly prepared and stored on PDA slants at 4°C for further studies [22].

Characterization of Fungal Isolates

All fungal isolates obtained previously and kept at refrigerated temperature were re-activated on PDA and assessed for macroscopic and microscopic characteristics [23]. They were then compared with descriptions in appropriate mycology databases.

Results

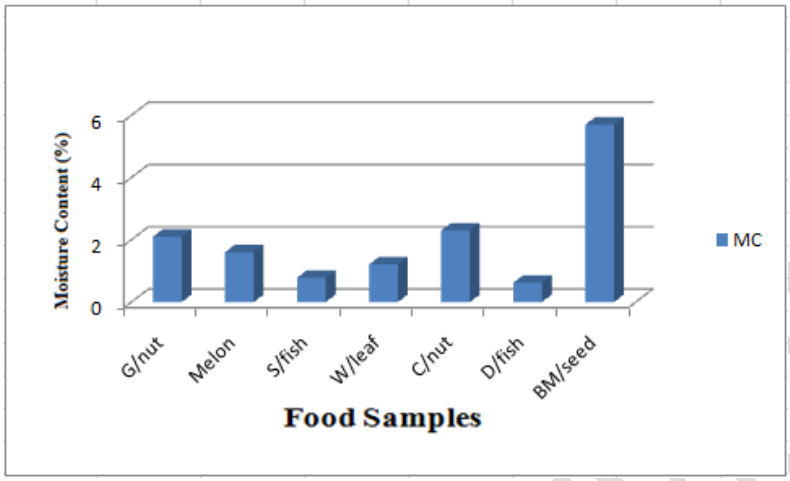

Fig. I: Bar-Chart Showing the Mean Moisture Content of the Food Samples

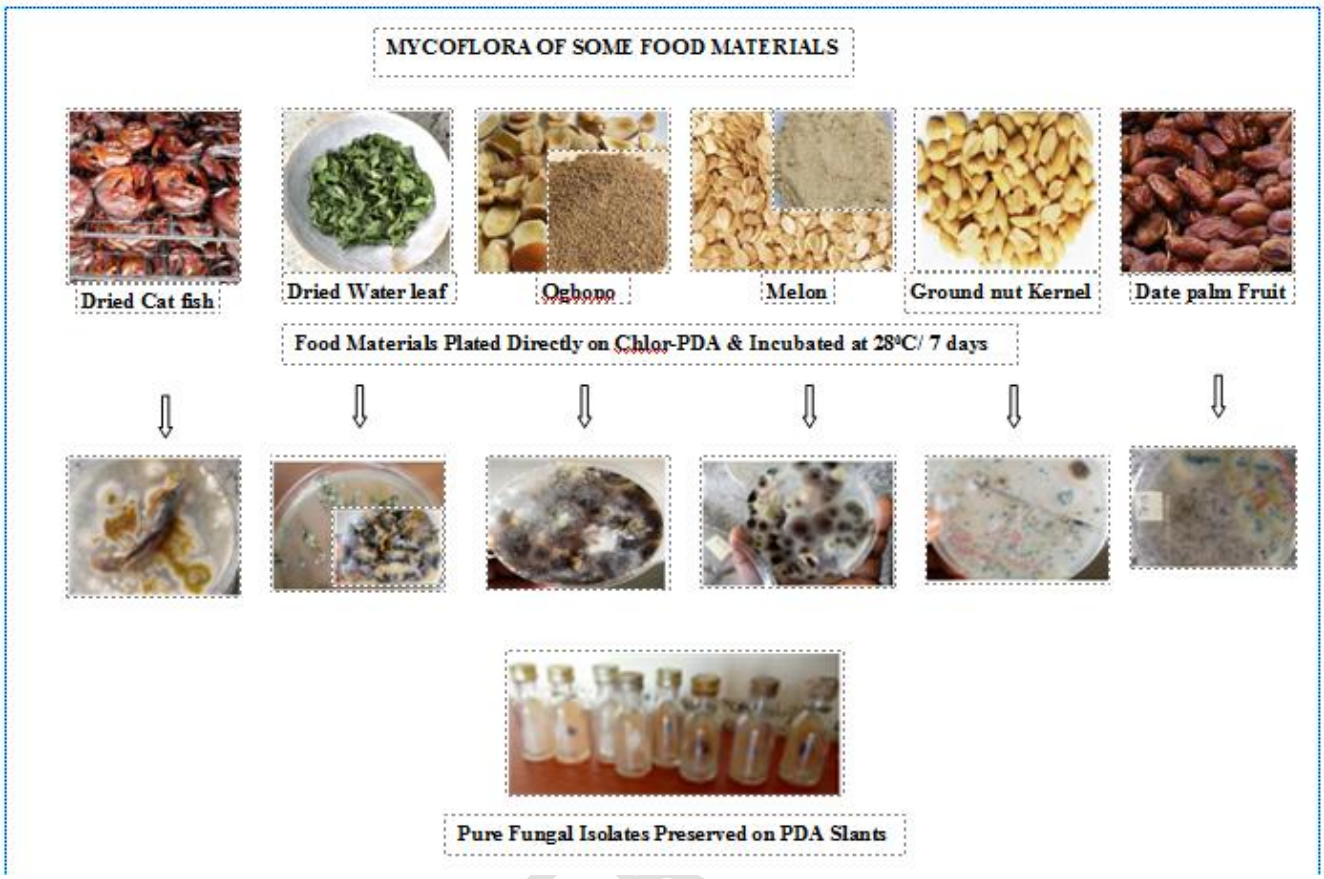

Fig. II: Flow Diagram of the Isolation of Fungi

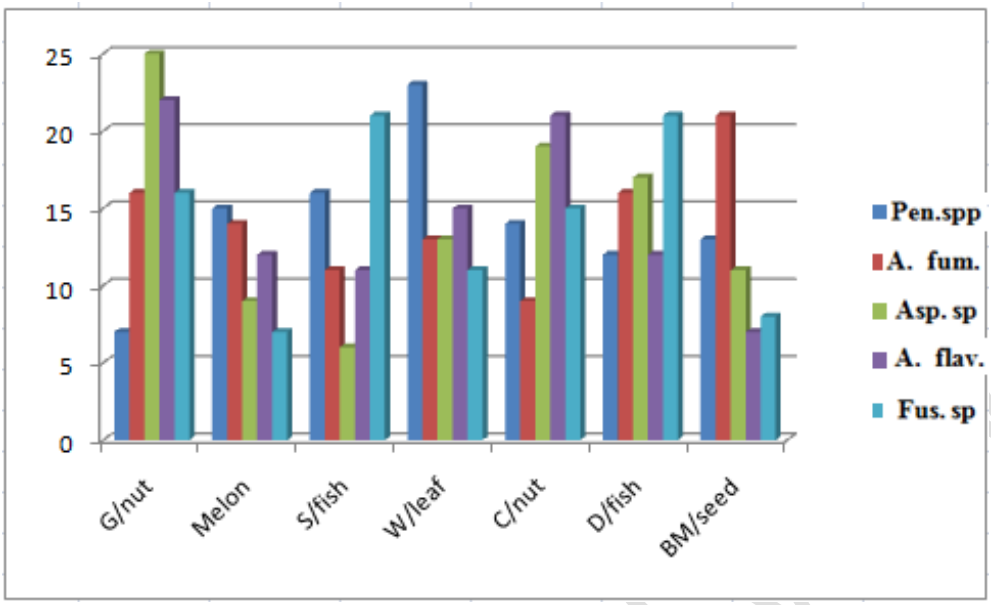

Fig. III: ~~**Frequency (%)**~~ **Percentage frequency of Fungi Species Most Implicated in Mycotoxins Production**

One critical factor in food contaminations due to microorganisms like fungi is the level of moisture content [20].

Fig I shows the mean moisture content and a generally low moisture contents ranging from 0.62-5.7 % (mean: 2.01%) were recorded in the foods analyzed in this study. The lowest mean moisture levels of 0.62% was recorded for Date palm fruit while 5.7% was the highest level recorded for Bush mango seeds (Ogbono). The high moisture content might be attributed to insufficient drying before being packaged for sale.

From the figure (Fig.I), the bar-chart presents the moisture content of the food samples, the highest is BM/ seeds and the lowest is dried fish.

Figure II shows the pictorial flow diagram of the isolated fungi from each of the food items.

Figure III shows the percentage incidence of fungal occurrence on the various food samples. Out of all the food types studied, only stockfish and water leaf had no incidence of Cladosporium (Fig. III).

This study indicates that the food types were grossly contaminated by fungi (Figure III). The percentage occurrence was 5% in cashew nut to 25 % in G/nut. This might be due to the nature of the pre-market processing as well as their conditions in the market which include being openly displayed, usually without protective packaging.

The percentages of fungi usually implicated in mycotoxin production are also presented. The group include *Aspergillus*, *Penicillium* and *Fusarium* (Fig. II and Fig. II). The highest is from ~~G/nut~~ Groundnut (25 %) followed by water leaf (23 %).

Discussions

Aside the fungal strain variation, genetic susceptibility of the host plant or commodity, commodity composition, temperature, aeration, microbial population and stress factors, moisture content is an important ant Conditions that affect toxin production in materials including foods [24]. Although the moisture content of the food samples in this study were generally moderate, gross contamination by fungi still occurred. This could be due to the fact that, these foods were made available in the open market and usually displayed opened for end users (Customers). They are therefore prone to flies perching on them, high human/ animal traffic and being exposed to different types of airborne spores. It is obvious that all molds and mold spores in the indoor environment as well as in the market where the materials are being sold cannot be eliminated; moisture control to a high degree is the most important strategy for reducing mold growth [25].

The difference in the incidence of fungi in the food materials might be due to differences in the climatic conditions occurring in different areas where these foods where processed or sold. Prevailing humidity is an extrinsic factor that may affect moisture content, aside the intrinsic factor of the type of food itself [28]. These factors, probably coupled with improper drying, poor handling, poor processing techniques, and storage methods accounted for the disparity in the study. Several authors like Bragulat *et al* (2001) had similar reports of dense population of fungi in similar food samples [26] which also corroborated the findings of this research work.

The lower viable fungal count in the cashew nuts may be attributed to the presence of anacardic acids and phenolic compounds they contain which are inhibitory to many fungi. As we have recorded in this research and is similar to the report of Nayara *et al* (2018). They equally reported that Anacardic acids have been demonstrated to be potent against some fungi. Lower fungi count against the recommended limit might have been recorded for some of the food materials, however, these number might increase as times passes by while waiting for customers because the items are either being hawked in the sun which provides favourable temperature, or partially being covered with polythenes which also conserve moisture and temperature [29] .

The occurrence of *Aspergillus spp.* (25 %) in Groundnut despite being packaged in polythene might be due to pre-packaging handlings which might have exposed them to fungal contamination by spores and might ~~which~~ probably developed in the materials providing good growth conditions [30].

Aspergillus, *Fusarium* and *Penicillium* have been implicated with varying degrees of mycotoxins production depending on the species. These were predominantly present in this study thereby making the situation somewhat worrisome [26].

Conclusion~~s~~

Various reports of the presence of mycotoxins in many agricultural commodities is a worldwide challenge because almost all commodities are potentially susceptible to contamination giving ideal conditions. However, the presence of the genera such as Aspergillus, *Penicillium* and *Fusarium*, needed to be given due attention owing to the

potential toxin production abilities. Therefore, adequate drying, storage and packaging of the food material are needed since toxins are highly heat stable.

Recommendations

- Although the potential for toxin production varies considerably within any given species, the presence of any form of fungi should be avoided.
- Packaging of these food materials after their hygienic processing should immediately be considered because, exposure of food materials will result in massive food contamination by diverse fungi;
- Proper drying should as a matter of rule, precede bagging as well as occasional re-drying as the case made be;
- Common sources of moisture in buildings such as leaks of all sorts, flooding, condensation on cold surfaces, poor draining system, dampness of any type or wet foundations should be avoided at all cost where these foods are processed or stored.
- It is reported that processing at temperatures greater than 150^0C are needed to give good reduction of zearalenone, moderate reduction of alfatoxins, variable to low reduction of deoxynivalenol and good reduction of fumonisins. Obviously this temperature is not achieved during normal domestic cooking. Therefore, preventive measures are highly recommended;
- Accurate identification of fungal species is a fundamental step to understand the role of fungi in relation to food poisoning due to mycotoxin production. Therefore, molecular tools should be included in fungi characterization;
- Proposed Guidelines for Mycotoxin Risk Assessment and training should be in place and made compulsory for all stakeholders in food processing.

References

[1] Ezekiel, C. N., Sulyok, M., Ogara, I. M., Abia, W. A., Warth, B., Šarkanj, B., et al. (2019). Mycotoxins in uncooked and plate-ready household food from rural northern Nigeria. *Food Chem. Toxicol.* 128, 171–179.

[2] Egbuta, M. A., Mwanza, M., Njobeh, P. B., Phoku, J. Z., Chilaka, C. A., and Dutton, M. F. (2015). Isolation of filamentous fungi species contaminating some Nigerian food commodities. *J. Food Res.* 4:38.

[3] Chibundu N. E., Oluwawapelumi A. O., Bart K., Kolawole I. A., Michael S., Jos H. and Rudolf K. (2020): Diversity and Mycotoxins in Low Moisture Content Ready-To-Eat Foods in Nigeria; Front. Microbiol.

[4] Laura, S., Eleonora, E., Daniela, I. and Silvano, O. (2013): Biodiversity, evolution and adaptation of fungi in extreme environments; Plant Biosystems 147(1).

[5] Ezekiel, C. N., Sulyok, M., Babalola, D. A., Warth, B., Ezekiel, V. C., and Krska, R. (2013): Incidence and consumer awareness of toxigenic *Aspergillus* section *Flavi* and aflatoxin B1 in peanut cake from Nigeria. *Food Control* 30, 596–601.

[6] Dojin, R., Andreia, B. & Lloyd, B. B. (2008): Effects of processing on mycotoxins; Stewart Postharvest Review 4(6):1-7.

[7] Oluwawapelumi A., Oyedele[a] Chibundu Ezekiel, N., Michael, Sulyok[b] Modupeade C.Adetunji[c],Benedikt Warth[bd] Olusegun O.Atanda[c] Rudolf Krska[b] (2017): Mycotoxin risk assessment for consumers of groundnut in domestic markets in Nigeria; International Journal of Food Microbiology; Volume 251, 19 June 2017, Pages 24-32.

[8] Chibundu N. E.,Michael,S., Yinka, S., Odutayo, F.I., Nwabekee, S.U. & A. T. Balogun (2014): Mould and mycotoxin exposure assessment of melon and bush mango seeds, two common soup thickeners consumed in Nigeria; International Journal of Food Microbiology; 237:83-91.

[9] Obani, F. T123*., Atehnkeng, J. 2 , Ikotun, B1 . and Bandyopadhyay, R2 (2019): Natural Occurrence of Aflatoxin in Different Egusi Types found in Nigeria; IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 12, Issue 1 Ser. II, PP 15-20.

[10] Chiejina, Nneka V. (2006). Studies on Seed-Borne Pathogens of some Nigerian Melons. Journal of Agriculture, Food, Environment and Extension 5(1): 13-16.

[11] Christianah Adetunji, C., Aroyeun, S.O., Michael, O. & Michael S. (2019): Fungal metabolite and mycotoxins profile of cashew nut from selected locations in two African countries; Food Additives & Contaminants: Part A 36(1):1-13.

[12] Adebajo, L. O. and Diyaolu, S. A. (2003): "Mycology and spoilage of retail cashew nuts," *African Journal of Biotechnology*, vol. 2, no. 10, pp. 369–373, 2003.

[13] Adebayo-Tayo 1 , A.A. Onilude 2 , A.A Ogunjobi 2 , J.S. Gbolagade 2 and M.O. Oladapo4 (2006): Detection of fungi and aflatoxin in shelved bush mango seeds (Irvingia spp.) stored for sale in Uyo, Nigeria B.C.; African Journal of Biotechnology Vol. 5 (19), pp. 1729-1732, 2.

[14] Oladapo, A.S. Abiodun, O.A. Akintoyese, O. and Adepeju, A. (2014): "Effect of packaging materials on moisture and microbiological quality of roasted cashew nut (Anarcardium Occidentale L)," *Research Journal in Engineering and Applied Sciences*, 2014.

[15] Loreno, E. T., Eustáquio, M. D., Castagnara, P. B., Costa, M. A., N. M., Bottoni HornPaulo Sérgio Rabello de OliveiraCristiane Claudia Meinerz (2013): Dehydration curve, fungi and mycotoxins in Tifton 85 hay dehydrated in the field and in shed; Forage Crops • R. Bras. Zootec. 42 (6).

[16] Atia, M.M.M. (2011): Efficiency of physical treatment and essential oil in controlling fungi associated with some stored date palm fruits;. Australian Journal of Basic Applied Science, 5(6):1572.

[17] Zeki Berk (2018): Food Process Engineering and Technology (Third Edition).

[18] Junaid, S. A., Olarubofin, F. and Olabode, A. O.(2010): Mycotic contamination of stockfish sold in Jos, Nigeria, Journal of Yeast and Fungal Research Vol. 1(7). pp. 136 - 141, Academic Journals Full Length Research Paper.

[19] Bankole, S., Schollenburger, M., and Drochner, W. (2006). Mycotoxins in food systems in sub Saharan Africa: a review. *Mycotoxin Res.* 22, 163–169.

[20] AOAC, (1990). *Official Methods of Analysis*, 15th Edn. Arlington, VA: Association of Official Analytical Chemists.

[21] Vargas Gil[a]S.Pastor[b] G.J.March[a] (2009): Quantitative isolation of biocontrol agents *Trichoderma* spp., *Gliocladium* spp. and actinomycetes from soil with culture media; Microbiological Research, Volume 164, Issue 2, , Pages 196-205.

[22] Abekhti A. , Zarour K., Boulal A., Benmechernene Z., Kihal M. (2013): Evaluation of Microbiological Quality of the Date Fruit Product *"Btana"* Produced in Adrar South Algeria; Journal of Microbiology Research, 3(5): 163-170.

[23] Sohail A. A.and Bayan M. B. (2018): Morphological and Molecular Identification of Fungi Isolated from Different Environmental Sources in the Northern Eastern Desert of Jordan; Jordan Journal of Biological Sciences; Volume 11, Number 3, Pages 329 – 337.

[24] Abdallah H., El-Shanawany, A.A., Nageh F. A. & Ahmed A. (2017): Influence of Different Moisture Contents and Temperature on Growth and Production of Aflatoxin B1 by a Toxigenic Aspergillus flavus Isolate in Wheat Flour; Journal of Ecology of Health & Environment 5(3):77-83.

[25] Milani, J.M. (2013): Ecological conditions affecting mycotoxin production in cereals. Veterinarni Medicina, 58(8), 405-411.

[26] Bragulat, M.R., Abarca, M.L. and Cabanes, F.L. (2001): An easy screening method for fungi producing ochratoxin A in pure culture. International Journal of Food Microbiology, 71, 139-144.

[27] Nayara, Z.T. G., Giselle, F. B., Matias, R. & Denise, R. P. (2018): Antifungal potential of cashew nut shell liquid in the control of plant pathogens, Bioscience Journal 34(1):95-103

[28] Fojan, B., Farahnaky. A. & Homa, B. (2014): Effect of Storage Relative Humidity on Physical Stability of Dried Fig; Journal of Food Processing and Preservation 38(1)

[29] Linke M, Geyer M (2013). Condensation dynamics in plastic film packaging for fruits and vegetables. J. Food Eng. 116:144-154.

[30] Rostami, R. Naddafi, K., Aghamohamadi, A., Najafi S. A. & Fazlzadeh D. M. (2009): Survey of peanut fungal contamination and its relationship with ambient conditions in the Bazar of Zanjan; Iran. J. Environ. Health. Sci. Eng., 2009, vol. 6, no. 4, pp. 295-300 295.