Microbiological Decontamination of Aflatoxin B1 in Peanuts by Backer's Yeast

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ABSTRACT

Background: Peanuts are one of the commonly consumed snacks but with poor storage practices and handling can make them prone to food borne infections.

Objective: The study aimed to measure the level of aflatoxin B1 in peanuts, which are contaminated with Aspergillus flavus before and after decontamination by yeast to detect the effect of biological decontamination of aflatoxin B1 in peanuts by yeast in selected locations in Qalubia Governorate in Egypt.

Materials and Methods: This study was carried out on 30 peanut samples from 3 different cities.Fungal Counts was determined by the dilution plate technique, Isolation of Fungi was done by direct plating method on SDA and incubated at 25 and 30°C, for 7 days, suspected aflatoxigenic fungi (A. flavus) that identified from peanuts were taken for aflatoxin analysis using Aflatoxin B1 ELISA Assay Kit then decontamination of peanuts by baker's yeast for 6 h and 24 h at 37 °c then requantitation of aflatoxin B1 after 6 h and 24 h by ELIZA method.

Results: There was significant strong positive correlation between fungal count and aflatoxin concentration with the samples from "Qalub" had the highest fungal count (30 $\times 10^3$ cfu/g) while "Benha" had the lowest count of (5 $\times 10^3$ cfu/g). Also we found that the aflatoxin concentration in "Benha" and "Kaha" less than the concentrations in "Qalub". The aflatoxin B1 concentration is decreased after microbiological decontamination with prolonged contact with backer's yeast.

Conclusion: This study showed that the peanuts were contaminated with toxigenic fungi, and we observed that yeasts have a huge potential application in aflatoxin degradation in foodstuffs.

Keywords: Aflatoxin B1, Microbiological decontamination, Peanuts, Yeasts.

INTRODUCTION

Mycotoxins formed primarily by **Aspergillus, Penicillium and Fusarium spp**., which are natural contaminants in different food staffs [1]. Mycotoxins are the main causes of toxicities to animals and humans. Presence of contamination by mycotoxins in foods is more in the subtropical and tropical countries leading to acute and chronic mycotoxicoses in animals and humans [2]. Aflatoxins are powerful, cancerous, toxic secondary metabolites formed mainly by **Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius** in different foodstuff as maize, grains, peanuts and cereals [3].

Aflatoxins ingested by man can cause many forms of diseases as cirrhosis, cancer, other liver diseases, immunosuppression, spontaneous abortion, and stunted growth. Aflatoxin B1 (AFB1) exhibits the highest toxicity of the discovered aflatoxins and it is one of the strongest of all mycotoxins. It has a strong genotoxic and carcinogenic effect, to which the liver is particularly susceptible [4]. Peanuts are very delicious so received by most of the people due to their taste and high nutritive value [5]. Peanuts are also important for many industrial goals such as obtaining oils for industrial and human uses [6].

Peanuts are rich in fats, proteins, minerals, and have little water inside it; so, they are very highly susceptible to fungal attack invasion more than other bacterial invasion at different stages and time [5], as while peanuts still on the trees and this often occurs when the shells of the peanuts are split open so the insects attack the seeds, which forms rooms for the fungal spores to get inside the developing seeds. There are other sources of invasion such as sorting, harvesting and peanuts washing before storage. Due to bad handling of peanuts at this stage, it leads to fungal growth especially when seeds are not properly dried to the appropriate moisture degree before storage [6,7]. There are other sources of fungal invasion, which can happen during storage as when peanuts are stored in conditions as humidity and high temperature [8].

Both fungi and bacteria are capable of causing damage of food crops so can lead to economic and food losses, thus leads to reduction of the exports of the food crops [9]. The proper management of food collection, transportation, storage and handling can help in decreasing the risk of exposing peanuts to invasion by mycotoxigenic moulds [10].

Decontamination of mycotoxin involves; physical methods such as ultraviolet light, thermal inactivation, extraction with solvents or ionizing radiation; chemical methods, which are based on materials that destruct the structure of mycotoxin, such as oxidizing agents (hydrogen peroxide, sodium disulfide and ozone), treatment with chlorine (chlorine gas or sodium hypochlorite), or hydrolytic agents (ammonia, acids and alkalis) but, these two methods have disadvantages, as inefficient removal, high costs or loss of nutritional value of the product [11]. So, there is other methods as biological methods that depend on the effect of microorganisms on mycotoxins produced. These microorganisms include different types as, filamentous fungi, algae, bacteria and yeasts, and their mechanisms of action may be based on interactions, competition by space and nutrients, and antibiosis [12].

Biodegradation by microorganisms of aflatoxins provide an excellent alternative for the control or degradation of aflatoxins in animal feed and foods,

Received: 21/2/2022
Accepted: 20/4/2022

protecting their safety and quality [13], their use provide more "natural" methods of decontamination, as the consumers don't prefer the chemical treatments [14].

Biological methods of decontamination are being largely studied and may be an alternative promising choice, as they are specific, efficient, cost-effective, and are environmentally safe [15]. Lactic acid bacteria (LAB) and yeasts are the main types of microorganisms, which are available and can be used to remove aflatoxins from any contaminated medium, and the most studied ones, by showing the excellent promising results.

Though the yeasts are considered to be potential biocontrol agents for decontamination of aflatoxins, Saccharomyces cerevisiae (SC) is the most well-known and commercially important species of yeast, and SC strains are widely used in the production of alcoholic drinks and in the baking industry. SC cells have been studied to evaluate their ability to remove aflatoxins from contaminated media[16], as the possible binding mechanisms between mycotoxins and yeast cell wall were studied, and authors suggested that β-D-glucans are the components of the cell wall that are responsible for forming the complex with the toxin, and that the reticular organization of β-D-glucans and their distribution in β-(1,3)-D-glucans and β-(1,6)-D-glucans have an important role in the efficiency of the bond [17]. Further field experiments are necessary to test their efficacies in reducing aflatoxin contamination under field conditions and also in vivo.

The aim of this study was to measure the level of aflatoxin B1 in peanuts, which are contaminated with *Aspergillus flavus* before and after decontamination by yeast to detect the effect of biological decontamination of aflatoxin B1 in peanuts by backer's yeast.

MATERIALS AND METHODS

This cross sectional study was carried out on 30 peanut samples from 3 different cities (Benha, Kaha, Qalub) in Qalubia Governorate in the period between June 2021 and January 2022.

Ethical approval:

The study was approved by the Clinical Research Ethical Committee of Benha University Hospital.

Samples were collected randomly from ten different traders in each city. The samples were aseptically blended by Electronic Mixer Grinder (China) and 50 g portions were kept in paper envelopes. Samples were kept at −80°C till time of analysis.

1. Firstly: Fungal count was determined by the dilution plate technique [18]. 9 ml of sterile water were added to 1 gram of each peanut in a test tube and the solution was decimally diluted. Twenty milliliters of sterilized Sabouraud Dextrose Agar (SDA) plates supplemented with 0.01% chloramphenicol and the plates were incubated 28°C for 48 h for determination of fungal counts. The total fungal counts were determined by the following formula: cfu/g = Number of colonies× reciprocal of the diluting factor divided by plating volume (1 ml) [6].

2. Secondly: Isolation of fungi was done by direct plating method. This was determined using the method described by **Adebajo and Diyaolu**[19]. 5 whole peanuts were obtained randomly. The 2 cotyledons were separated by hand, and the surfaces were disinfected with a solution of sodium hypochlorite 2% for 120s. Then they were rinsed in sterile distilled water, then four cotyledons were plated at constant spaces on the medium (SDA agar with 0.01% chloramphenicol). The cultures on SDA were incubated at 25 and 30°C, for 7 days. The resulted colonies were sub cultured on SDA agar plates to give pure cultures. Then their identification was based on the examination of the microscopic characteristics (conidia size and morphology) and macroscopic characteristics (colony morphology, colour, and size) on specific media and comparison with available identification keys [20-22]. Isolates of black **Aspergillus** on SDA was identified as **A. niger** and the green **Asprigellus** was identified as **A. Flavus**[23]. **The Penicillium and Fusarium** isolates were also identified to genus level using appropriate references and manuals [18, 24, 25].

3. **Thirdly:** determination of aflatoxin B1 by ELISA method; suspected aflatoxigenic fungi (A. flavus) that identified from peanuts were taken for aflatoxin analysis using Aflatoxin B1 ELISA Assay Kit.

Sample Preparation/Extraction:

Five grams of the crushed peanut sample were weighed into a suitable container to which 25 ml methanol (70%) was added, this suspension was shaken intensively for 3 minutes to extract the aflatoxin. The suspension was left to allow settling of the solids for 5 minutes. The suspension was then filtered via a folded filter for quantitative analysis. The filtrate (sample extract) was diluted in a new container with a 1:10 ratio with the sample diluent as 100 µL and filtrate was added to 900 µL AFB-SAMPLE-BUF so 1 mL of diluted filtrate equaled 0.1 g solid sample = dilution factor 50. Each of the fifty microlitres of the aflatoxin standards (AFB1-0 to AFB1-5) and filtrate were pipetted in separate dilution wells and was mixed with 50 µl AFB1-HRP conjugate. The microplate was covered with adhesive foil and briefly shaken on the microplate shaker and incubated for 30 minutes at room temperature, protected from light. Then 300 µL of reconstituted washing solution was pipetted into each cavity and again aspirated out and repeated twice. Hundred microlitres of substrate solution was then pipetted into all cavities for the colour reaction. The microplate was covered with adhesive foil again, briefly shaken and leaved to incubate for 15 minutes at room temperature (20°C- 25°C) protected from light and twenty-five microlitres of the stop solution was added to each well to stop the reaction and this converted the blue end point to yellow. The OD value measurement

of the cavities was undertaken by using a microplate reader at 450 nm.

Quantification of aflatoxin B1:

The result was read using ELISA plate reader at 450 nm. Optical densities of standards (0 ngg−1, 5 ngg−1, 10 ngg−1,25 ngg−1, 50 ngg−1 and 120 ngg−1) and those of samples were also recorded. The values for the mean optical density of the standards and samples were divided by the mean value of the zero standard (AFB1-0). Multiplying by 100 then gives an OD value percentage in reference to the zero standard (100%)

4. Finally:

Decontamination of peanuts by baker's yeast for 6 h and 24 h at 37 °C and pH 3 and then requantitation of aflatoxin B1 after 6 h and 24 h by ELIZA method described above then the results were compared.

Statistical analysis:

The collected quantitative data were summarized in terms of mean ± standard Deviation (SD). Comparisons between the different study groups were carried out using ANOVA (F-test). Correlation analysis to determine the association between variables was done using Pearson correlation coefficient (r). All tests were two sided. The accepted level of significance in this work was ($p \leq 0.05$) and $p \leq 0.001$ was considered highly statistically significant.

RESULTS

Fungal count and aflatoxin B1 Concentration:

Samples from "Qalub" had the highest fungal count (30 ×10³ cfu/g) while "Benha" had the lowest count of (5 ×10³ cfu/g), and there was significant strong positive correlation between fungal count and aflatoxin concentration as shown in table 1 and figure 1.

Table (1):Correlation between fungal count and concentration of aflatoxin B1 (ng/g)

	Benha	Kaha	Qalub
Pearson correlation coefficient	+0.94	+0.96	+0.94
P value	0.019*	0.007*	0.019*

*significant

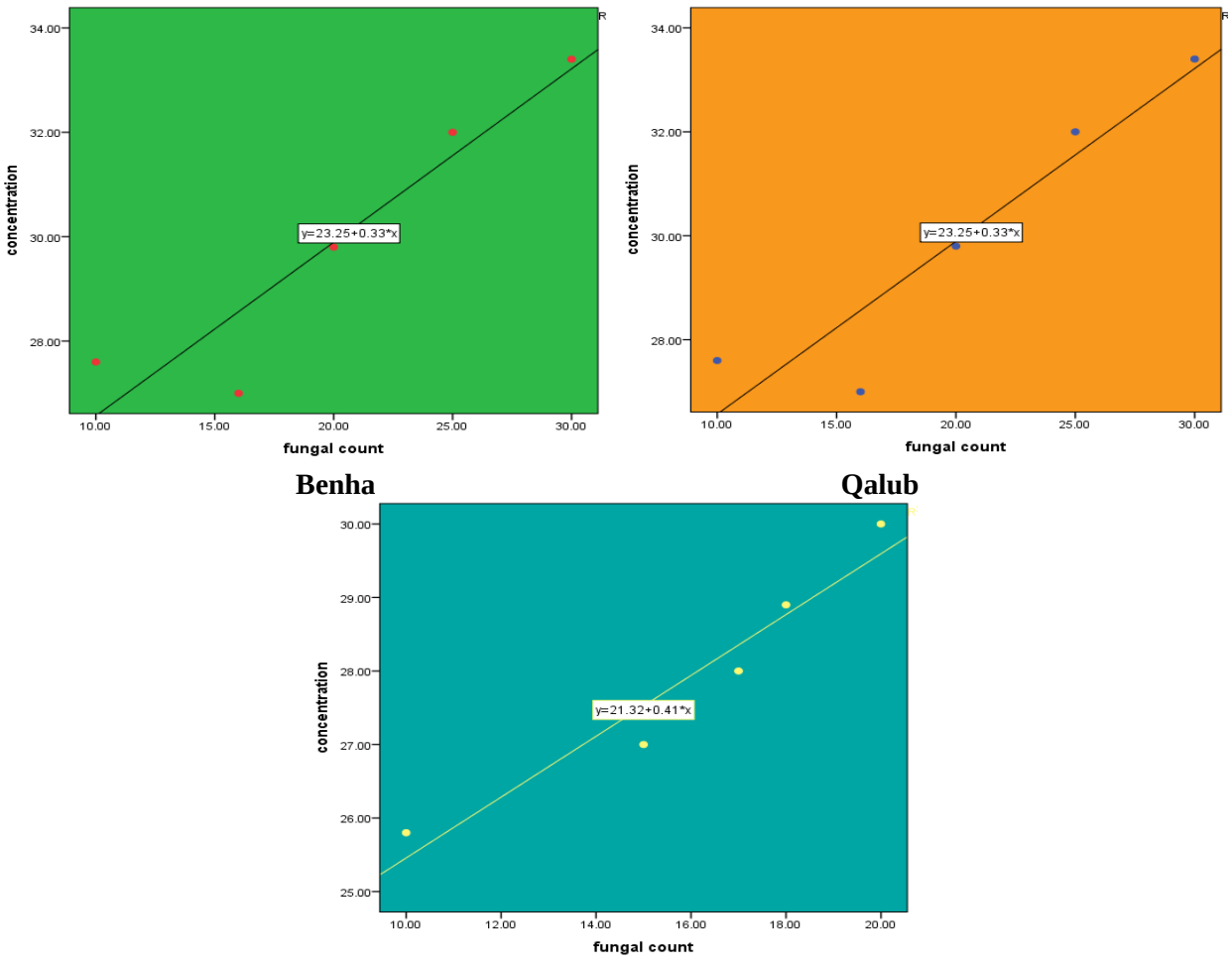

Fig. (1): Correlation between fungal count and concentration of aflatoxin B1 (ng/g)

Also we found that the aflatoxin concentration of the peanuts ranged from (20.5 ngg−1) to (33.4 ngg−1) with "Benha" and "Kaha" having the least concentrations, with "Qalub" having the highest concentration and this concentration decreased after microbiological decontamination with backer's yeast (Table 2).

Table (2): Comparison between samples from different sources

Variables	Banha	Kaha	Qalub	P-value
Fungal count *10^3 cfu/g	8.60±2.30	16.0±3.8	20.2±7.7	0.012*
Concentration of aflatoxin B1 (ng/g)	23.58±2.71	27.94±1.63	29.96±2.76	0.004**
Concentration of aflatoxin B1 after decontamination by yeast after 6 h	11.28±2.49	12.70±1.99	15.74±2.56	0.032*
Concentration of aflatoxin B1 after decontamination by yeast after24 h	3.86±1.34	4.64±1.06	5.32±1.77	0.3

*significant, **highly significant, data are presented as mean±standard deviation

Also we found that the higher the AFB1 concentration in the medium, the lower is the ability of AFB1 removal by SC strain. Also the inverse relation between the level of aflatoxin B1 and the time of contact with yeast that is represented in **(Fig. 2)** that showed the significant decrease in the level of aflatoxin B1 with prolonged contact with yeast.

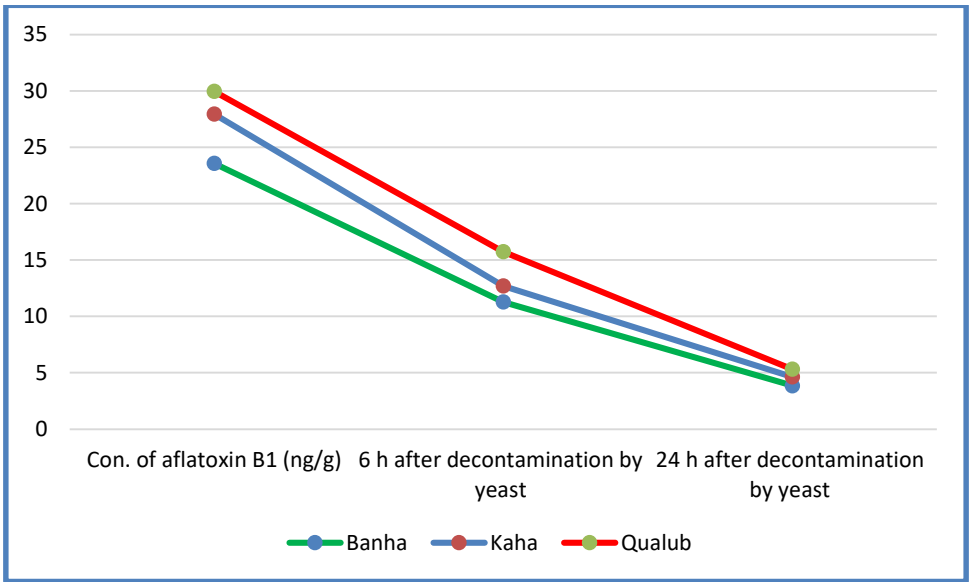

Fig. (2): Comparison between samples from different sources in aflatoxin B1 levels before and after 6 h and 2 4h decontamination with yeast

DISCUSSION

The improper management of storage conditions such as humidity and temperatures leads to growth of moulds that produce aflatoxins resulting in major problems in food safety and mycotoxicosis. **Aspergillus spp**. can easily grow at optimal conditions of pH range of 5-6 and 27–33°C, water humidity of 0.82–0.99 [26]. Contamination of peanuts by moulds especially **A. flavus, A. niger, Fusarium spp , Penicillium spp** occur early in the field and leads to deterioration during prolonged periods of storage leading to the production of high amount of mycotoxins [27]. The high incidence of **A. flavus, A. niger** that is reported in our peanuts as the report of previous authors[6], who reported the same results of fungal occurrence; however, **Oyedele *et al.*[20]** reported an increased incidence of **Fusarium spp.** (45%) in samples of groundnut in Nigeria.

There was higher aflatoxin concentration for peanut samples from Qalub (Max; 33.4 ngkg−1) than Kaha (30 ngkg−1), and Benha (26.9 ngkg−1) as reported by **Oyedele, *et al.*** [20] in Nigeria. This correlation between aflatoxin concentrations and toxigenic isolates confirms the fact that important requirements must be met before the production of aflatoxins in the food stuffs such as water activity, suitable temperature, storage environment and relative humidity [26].

Both β-D-glucans and chitin chains affect the plasticity of the cell wall. The external layer of the wall of the yeast cell is formed by mannoproteins, which have a role in the exchanges with the external medium. This structure is highly polymorphic and may vary according to the phase of the cell cycle, yeast strain, and culture conditions, such as temperature, pH, oxygenation rate, concentration and nature of the carbon source and nature of the medium. Thus, these differences in the structure of the cell wall in yeast strains are related with their ability to bind to the mycotoxin [17].

Chu *et al.* [28] reported a significant decrease in AFB1 concentration during production of beer, probably due to the bond between SC cell and mycotoxins. This hypothesis was obtained by other studies [3, 29]. Also, a 19% reduction in AFB1 during fermentation of dough in bread production was observed [30].

The adsorption process shows an inversely relationship with the concentration, as, the higher the AFB1 concentration in the medium, the lower the ability of AFB1 removal by SC strain as reported by **Shetty *et al.*** [31] who observed a decrease in toxin adsorption as the concentration increased, and concluded that adsorption is not a linear phenomenon.

The decreased AFB1 adsorption that observed as the toxin concentration increased may be caused by saturation of the adsorption sites on the SC cell. There are other factors, as pH, length of incubation, methods of analysis, and method of biomass purification.

In contrast to that reported by **Rahaie *et al.*** [32]. who used immobilized SC cells (ATTC 9763) that was investigated for their ability to decrease AFB1 concentration from pistachio seeds, and it was reported that the amount of toxin that removed was dependent on its concentration in the medium (40% and 70% of removal for concentrations of 10 ng/mL and 20 ng/mL AFB1, respectively) as the higher the AFB1 concentration in the medium, the higher the ability of AFB1 removal by SC strain. They also concluded that this ability to remove the toxin was greater in SC exponential phase of growth, and that the process was a quick one, being saturated after 3 hours of contact. In contrast to our results that showed the significant decrease in the level of aflatoxin B1 with prolonged contact with yeast. Continuous removal of aflatoxin, even after use of acid and heat treatments, confirms that yeast cell viability is not a significant factor for the removal of aflatoxin from the medium [33].

Dogi *et al.* [33], analyzed the ability of SC to remove AFB1 from a contaminated medium at different pH values (3.0, 6.0, and 8.0), and observed that the three strains analyzed showed great ability to remove the toxin (41.6% to 94.5%), as we do the test of decontamination at acidic ph.

In vitro studies aren't always good indicators of the in vivo behaviour, as in vivo studies are affected by physiological conditions, such as pH, gastric and intestinal secretions, and peristaltic movements. **Raju and Devegowda** [34] reported that the components of the cells wall of SC are able to adsorb mycotoxins, as the immune system, and compete for binding sites in the enterocytes, inhibiting intestinal colonization by pathogens. Therefore, SC strains acted both as mycotoxin adsorbents and probiotics (co-aggregation and inhibition of pathogenic bacteria).

Further studies are necessary to determine the behavior of yeasts in the different environmental conditions before they are used commercially. However, new studies are necessary to identify bacterial species with greater binding potential with aflatoxins, once there are differences in sensitivity and selectivity, besides the influence of factors that are intrinsic and extrinsic to the bacteria in the decontamination process.

After this step of choosing species with greater efficiency has been overcome, new production technologies that are economically viable to be applied to human and animal foods may be developed.

CONCLUSION

This study showed that the peanuts were contaminated with toxigenic fungi, resulting in aflatoxin contamination of the products, thereby posing health risks to their consumers. Yeasts have a huge potential application in aflatoxin B1 degradation in foodstuffs.

Acknowledgments: To all members of Clinical Microbiology and Immunology Department.
Conflict of interest: The authors declare no conflict of interest.
Sources of funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
Author contribution: Authors contributed equally in the study.

REFERENCES

1. **Reddy K, Salleh B, Saad B *et al.* (2010):** An overview of mycotoxin contamination in foods and its implications for human health. Toxin Rev., 29: 3-26.
2. **Shetty P, Jespersen L *et al.* (2006):** Saccharomyces cerevisiae and lactic acid bacteria as potential mycotoxin decontamination agents. Trends Food Sci Technol., 17: 48-55.
3. **Bennett W, Klich M (2003):** Mycotoxins. Clinical Microbiology Reviews, 16(3): 497–516.
4. **Abbas A, Baldwin D, Ma Y *et al.* (2005):** Immune response in silico (IRIS): Immune-specific genes identified from a compendium of microarray expression data. Genes & Immunity, 6: 319–331.
5. **Bhat R, Vasanthi S (2003):** Food safety in food security and food trade. Mycotoxin Food Safety Risk in Developing Countries IFPRI. https://core.ac.uk/download/pdf/48024134.pdf
6. **Abdulla N (2013):** Evaluation of fungal flora and mycotoxin in some important nut products in Erbil local markets. Research Journal of Environmental and Earth Sciences, 5: 330–336.
7. **Adetunji M, Atanda O, Ezekiel C *et al.* (2014):** Fungal and bacterial metabolites of stored maize (Zea mays, L.) from five agro-ecological zones of Nigeria. Mycotoxin Research, 30: 89-102.
8. **Abdel-Hafez A, Saber S (1993):** Mycoflora and mycotoxin of hazelnut (Corylus avellana L.) and walnut (Juglans regia L.) seeds in Egypt. Zentralblatt f ur Mikrobiologie, 148(2): 137–147.
9. **Oluwafemi F, Ewelukwa U, Okuwa G (2009):** Outbreak of E.coli 0157: h7 infections associated with ready-to eat cashew nuts in a Nigerian university community. African Journal of Biomedical Research, 12: 113–119.

10. **Alhussaini M (2012):** Mycobiota and mycotoxins of nuts and some dried fruits from Saudi Arabia. Journal of American Science, 8: 525–534.
11. **El-Nezami H, Kankaanpaa P, Salminen S *et al.* (1998):** Ability of dairy strains of lactic acid bacteria to bind a common food carcinogen, aflatoxin B1. Food and Chemical Toxicology, 36: 321-26.
12. **Fazeli M, Hajimohammadali M, Moshkani A *et al.* (2009):** Aflatoxin B1 binding capacity of autochthonous strains of lactic acid bacteria. Journal of Food Protection, 72(1): 189-192.
13. **Alberts J, Gelderblomb W, Botha A *et al.* (2009):** Degradation of aflatoxin B1 by fungal laccase enzymes. International Journal of Food Microbiology, 9: 135-47.
14. **Bata A, Lasztity R (1999):** Detoxification of mycotoxin-contaminated food and feed by microorganisms. Trends in Food Science & Technology, 10: 223-29.
15. **Wu Q, Jezkova A, Yuan Z *et al.* (2009):** Biological degradation of aflatoxins. Drug Metabolism Reviews, 41(1): 1-7.
16. **Joannis-Cassan C, Tozlovanu M, Hadjeba-Medjdoub K *et al.* (2011):** Binding of zearalenone, aflatoxin B1, and ochratoxin A by yeast-based products: a method for quantification of adsorption performance. Journal of Food Protection, 74(7), 1175-1185.
17. **Jouany J, Yiannikouris A, Bertin G (2005):** The chemical bonds between mycotoxins and cell wall components of Saccharomyces cerevisiae have been identified. Archiva Zootechnica, 8: 26-32.
18. **Samson R, de Boer E (1995):** Introducction to food-borne fungi. eBook: 4th ed., Baarn : Centraalbureau voor Schimmelcultures, Pp. 233-252. https://www.worldcat.org/title/introduction-to-food-borne-fungi/oclc/680002633#relatedsubjects
19. **Adebajo L, Diyaolu S (2003):** Mycology and spoilage of retail cashew nuts. African Journal of Biotechnology, 2(10): 369–373.
20. **Oyedele O, Ezekiel C, Sulyok M *et al.* (2017):** Mycotoxin risk assessment for consumers of groundnut in domestic markets in Nigeria. International Journal of Food Microbiology, 251: 24–32.
21. **Tambekar P, Wate S (2007):** Study of phosphate solubilization efficiencies of fungi and bacteria isolated from saline belt of Puma river basin. Research Journal of Agriculture and Biological Sciences, 3: 701–703.
22. **Ronhede S, Jensen B, Rosendahl S *et al.* (2005):** Hydroxylation of the herbicide isoproturon by fungi isolated from agricultural soil. Applied and Environmental Microbiology, 71(12): 7927–7932.
23. **Pitt J, Hocking A (2009)** Fungi and Food Spoilage. Blackie Academic and Professional, London. http://dx.doi.org/10.1007/978-0-387-92207-2
24. **Frisvad J, Samson R (2004):** Polyphasic taxonomy of Penicillium subgenus Penicillium: A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. Studies in Mycology, 49(1): 173-177.
25. **Leslie J, Summerell B (2006):** Fusarium laboratory workshops - A recent history. Mycotoxin Research, 22(2): 73-74.
26. **Richard J (2007):** Some major mycotoxins and their mycotoxicoses: An overview. International Journal of Food Microbiology, 119: 3–10.
27. **Freire F, Offord L (2002):** Bacterial and yeast counts in brazilian commodities and spices. Brazilian Journal of Microbiology, 33: 145–148.
28. **Chu F, Chang C, Ashoor S *et al.* (1975**): Stability of aflatoxin B1 and ochratoxin A in brewing. Applied Microbiology, 29(3): 313-316.
29. **Celyk K, Denly M, Savas T (2003):** Reduction of toxic effects of aflatoxin by using baker yeast (Saccharomyces cerevisiae): in growing broiler chicken diets. Revista Brasileira de Zootecnia, 32: 615-19.
30. **El-Banna A, Lau P, Scott P (1983):** Fate of mycotoxins during processing of foodstuffs. II- Deoxynivalenol (vomitoxin) during making of Egyptian bread. Journal of Food Protection, 46: 484-88.
31. **Shetty P, Hald B, Jespersen L (2007):** Surface binding of aflatoxin B1 by Saccharomyces cerevisiae strains with potential decontaminating abilities in indigenous fermented foods. International Journal of Food Microbiology, 113: 41-46.
32. **Rahaie S, Emam-Djomeh Z, Razavi S *et al.* (2010):** Immobilized Saccharomyces cerevisiae as a potential aflatoxin decontaminating agent in pistachio nuts. Brazilian Journal of Microbiology, 41: 82-90.
33. **Dogi C, Armando R, Ludueña R *et al.* (2011):** Saccharomyces cerevisiae strains retain their viability and aflatoxin B1 binding ability under gastrointestinal conditions and improve ruminal fermentation. Food Additives and Contamiantes, Part A, 28(12): 1705-1711.
34. **Raju M, Devegowda G (2000):** Influence of esterified-glucomannan on performance and organ morphology, serum biochemistry and haematology in broilers exposed to individual and combined mycotoxicosis (aflatoxin, ochratoxin and T-2toxin): British Poultry Science, 41(5): 640-650.