SHANYA BAQI SADIQ DETERMINATION OF AFLATOXIN AND OCHRATOXIN IN COMMONLY CONSUMED FOOD NEU 2020

DETERMINATION OF AFLATOXIN AND OCHRATOXIN IN COMMONLY CONSUMED FOOD

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DECLARATION

Hereby, I declare that this thesis study is my own study, I had no unethical behaviors in all stages from planning of the thesis until writing there for, I obtained all the information in this thesis in academic and ethical rules, I provided reference to all of the information and comments which could not be obtained by this thesis study and took these references into the reference list; and, had no behavior of breeching patent rights and copyright infringement during the study and writing of this thesis

Shanya Baqi Sadiq Sadiq

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LIST OF ABBREVIATIONS

AFB1	**Aflatoxin B1**
AFB2	**Aflatoxin B2**
AFG1	**Aflatoxin G1**
AFG2	**Aflatoxin G2**
AFTs	**Aflatoxins**
AFTs-B	**Aflatoxin B**
AFT-B1	**Aflatoxin B1**
AFT-B2	**Aflatoxin B2**
AFT-G1	**Aflatoxin G1**
AFT-G2	**Aflatoxin G2**
AFTs-M1	**Aflatoxin M1**
AFTs-M2	**Aflatoxin M2**
AFIR	**Aflatoxin Regulatory Gene**
AFM1	**Aflatoxin M1**
AFM2	**Aflatoxin M2**
ALP	**Alkaline Phosphatase**
CAR	**Cordillera Administrative Region**
CDC	**Centres for Disease Control**
CDCP	**Centres for Disease Control & Prevention**
DNA	Deoxyribonucleic Acid

DON	**Deoxynivalenol**
ELISA	**Enzyme Linked Immunosorbent Assay**
FAO	**Food and Agricultural Organisation**
FTIR	**Fourier Transform Infrared Spectroscopy**
HBV	**Hepatitis B Virus**
HCC	**Hepatocellular Carcinoma**
HCV	**Hepatitis C Virus**
HIV	**Human Immunodeficiency Virus**
MSP	**Mouldy Sugar Poisoning**
HPLC	**High Performance Liquid Chromatography**
IARC	**International Agency for Research on Cancer**
IgA	**Immunoglobulin A**
IgG	**Immunoglobulin G**
IgM	**Immunoglobulin M**
LD50	**Lethal Dose 50**
NADPH	**Nicotinamide Adenine Dinucleotide Phosphate**
OD	**Optical Densities**
OTA	**Ochratoxin A**
OTB	**Ochratoxin B**
PPB	**Parts Per Billion**
Phe	**Phenylalanine**
USA	**United States of America**

USFDA	**United States Food and Drug Administration**
USAID	**United States Agency for International Development**
VHA	**Versiconal hemiacetal acetate**
VONE	**Versicolorone**
WHO	**World Health Organisation**
ZEN	**Zearalenone**

ÖZET

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Mantarlar ökaryotik hücre yapısına sahiptirler, görünümlerine, ve üredikleri ısıya göre küf ve maya olarak iki gurupta incelenirler.Çok sayıda mantar bitki, insan ve hayvanlarda hastalıklara neden olur. Bitki hastalıkları, mahsul veriminde ve kalitesinde azalmaya yol açar. Mantarlar mikotoksinler olarak bilinen ikincil metabolitler üretir. Hayvanlar ve insanlar tarafından zararlı mikotoksinlerin ağız yolu ile alınması, halk sağlığı üzerinde büyük etkilere sahiptir. Bu nedenle, bu çalışma, Enzim Bağlantılı İmmünosorbent Deneyi (ELISA) kullanılarak yaygın olarak tüketilen çeşitli gıdalarda aflatoksin ve okratoksin insidansını araştırmıştır. Irak, Süleymaniye'deki yerel pazarlardan ve süpermarketlerden yüz yetmiş dört (174) farklı gıda örneği alındı. Üreticinin talimatlarına göre, gıda örnekleri mikotoksin ekstraksiyonu için hazırlandı. Ekstraktlar mikotoksin tespiti için kullanıldı. 88 örneğin 6'sı (% 6.8) aflatoksin için pozitifken, 86'sı okratoksin için, 15'i (% 17.4) pozitifti. Örneklenen yiyeceklerin çoğunda aflatoksinden daha fazla saptanabilir okratoksin vardı. Çay ve kahve aflatoksin için pozitif değerlere sahipken, cips, baharat, fındık, çay ve tahıl okratoksin için pozitif değerlere sahipti. Örneklenen gıdaların çoğu, Avrupa Komisyonu tarafından önerilen tolere edilebilir sınır dahilinde aflatoksin ve okratoksin insidansına sahipti. Bu araştırmanın sonuçları, gıdaların mikotoksin kontaminasyonu, halk sağlığı üzerindeki etkileri ve ekonomikkayıplar hakkındaki bilincimizi uyandırmalıdır. Çiftçiler, tüketiciler ve düzenleyici kurumlar, gıda kontaminasyonundan kaçınmak ve uygun hasat ve depolama prosedürlerine uymak için katı önlemler almaya teşvik edilmelidir.

Anahtar Kelimeler: Aflatoksin, Mantarlar, ELISA, Okratoksin, Irak

ABSTRACT

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Fungi are eukaryotic organisms, the moulds, mushrooms and yeasts. A large number of fungi cause plant diseases. Plant diseases lead to decrease in crop yield and quality. Fungi produce secondary metabolites known as mycotoxins. The ingestion of harmful mycotoxins by animals and human beings has great public health implications. This study, therefore, investigated the incidence of aflatoxins and ochratoxins in various types of commonly consumed foods using Enzyme Linked Immunosorbent Assay (ELISA). One hundred and seventy-four (174) different food samples were obtained from local markets and supermarkets in Sulaymaniah, Iraq. According to the manufacturer's instructions, the food samples were prepared for mycotoxin extraction. The extracts were used for mycotoxin detection. Of the 88 samples, 6 (6.8%) were positive for aflatoxin while of the 86 samples for ochratoxin, 15(17.4%) were positive. More of the foods sampled had more detectable levels of ochratoxin than aflatoxin. Tea and coffee had positive values for aflatoxin while chips, spices, nut, tea and cereal had positive values for ochratoxin. Most of the sampled foods had aflatoxin and ochratoxin incidence within the tolerable limit recommended by European Commission. The results of this research should awake our consciousness about mycotoxins contamination of foods, its public health implications and economic losses. Farmers, consumers and regulatory agencies should be encouraged to observe strict measures to avoid food contamination and adhere to proper harvest and storage procedures.

Key Words: Aflatoxin, Fungi, ELISA, Ochratoxin, Iraq

CHAPTER ONE

1. INTRODUCTION

1.1. Mycotoxins

Mycology is a medical science treat with fungi and fungi are an enormous groups of mould and yeasts, it is contained approximately around 100,000 species of fungi have been defined until nowadays. In addition, they are quite varied in habitats. Most fungi, however, are earthen. A large number of fungi are parasites of plants which cause many of the crop plant's economically important diseases. Decreasing of yields and worth happen in plants mostly with enormous economic losses due to fungal infection. As well as, Infection of cereals by unsafe subordinate fungal metabolites which they are known as mycotoxin. The ingestion of grains polluted with that mycotoxin by animals and humans cause major public health consequences. There are three main type of routes to contact with mycotoxins. (i) The oral route is the main rout throught the eating of food and vegetables. (II) The dermal is other routes of contact via touching mycotoxin. (iii) The inhalation, is by using parts of fungi or their mycotoxin via respiratory. (Bhat and Vasanthi, 2003)

The expression of mycotoxin through word is comes from the Greek term "mycos" meaning mold, in addition, from the Latin word "toxicum" meaning venom and/or poison. Mycotoxins are minor fungal metabolites that have low molecular weighing In addition they are venomous in human. Mycotoxins are subaltern metabolites the reason turned most probability to not require for promoting fungal development in addition they were essentially creation the essential of major metabolic procedures. Moreover the fungal mycotoxins mostly produce and released out through mounting specific colonies of estimated period of sporulation development (Calvo, et al., 2002), whatever mycotoxin roles until nowadays are not understood completely through without and killing living thing, also they are believed to define the mould as it is

serve as a defines mechanism. In generally speaking, the production of a specific secondary metabolite in addition to using of treatment as an antibiotics is typically limited to reduce the total number of fungal species to unique to organisms strains (Smith and Moss, 1985).

The rates of toxin produce by fungi known as mycotoxins that inflict adverse effects on human healthy vary widely between toxins and the human immune system. In order to understand the deleterious effects of mycotoxins and it is effect on human health, two concepts are needed: (i) Severe toxicity: this is due to the rapid start of an opposing effect from a single contact. (ii) Long-lasting toxicity: is the sluggish and/or intermittent opposing effect arising from multiple long-term exposures. Whatever the mycotoxins have toxic which have ability of sever and long lasting, together or separately, relay on the type and dosage of toxin moreover the descriptor of severe toxicity and it is the most commonly found mycotoxins in food are containing less toxigenicity than malignant of toxin that produce by bacteria known as botulism. whatever it is consider as a lifelong within body that is particular concern the reason turned to some of mycotoxins swallowed in small amount of consuming food consumption over a long period of time they are considered one of toxic that play role in carcinogenic and have an effect on the human immune response (Moss, 1985).

There are more than 300 mycotoxins, produced by about 350 species of different type of fungi which have already been described (Betina, 1989). Today rate noted to be raised by the time (Bennet, J.W. and Klich, M., 2003) estimated approximately 400 mycotoxins but there was not reliably to determine the accurate quantity number. Nevertheless, all fungi do not produce mycotoxins in addition some of them produce only one type of mycotoxin among the toxigenic fungal species, while others produce more than one, various kind of fungal species can also produce a specific type of mycotoxin (Boutrif and Bessey, 2001). The most probability hypothesis is that almost all fungal metabolites would be harmful if examined, moreover a nourishments prone to mould may potentially polluted underneath the suitable condition for fungi

(Pohland, 1993). Hence, if the pathogen suspected associated with a mycotoxigenic fungus information must be gathered by tracking not only the host, in addition to disease and climate also toxins must be accumulate (Battilani, et al., 2003).

Fungal toxic like(ochratoxins and aflatoxins) are associate with the major mycotoxins which are also associated with human intoxication (Peraica and Dominjan, 2001). The Population division of the United Nation (2017) estimated that by 2050 a global population will be reach approximately to 9.8 billion. Some scientists have argued that only around 10 billion people in earth's can resources support. If this situation were possible a rapid lessening of resources may result in incomplete availability of water and food. (Moretti, et al., 2017). This community of fungi can reduce the amount of agricultural products by destroying and/or eliminate commodities of different food, and it is during secreting mycotoxins that can be cancerous. Producing mycotoxin by fungi is a serious concern because for detection mycotoxins need several analytical facilities and it is expensive kits. The mycotoxigenic fungi and it is production of toxin are a great concern universal, inclusively the Asia-Pacific area because of the storage conditions that facilitate mycotoxigenic fungi growth and increase being there (Anukul, et al., 2013). For example the rice is primarily grown and consumed by people in wold wide especially in Asia it is highly susceptible during storage to mycotoxigenic fungi and mycotoxin contamination this due to geographic area. (Gummert, et al., 2009)

The most prone to problems with mycotoxigenic fungi and mycotoxins are developing in many countries especially small-holder agriculturalists in post-harvest operations. Moreover mycotoxigenic fungi that produce toxin significantly participate in the decreasing in both quality and quantity of the agricultural product. For example, the rice contamination with mycotoxigenic Fusarium fijikuroi has been reported many time in addition it cause bakanae disease stunting and elongation. Also there is common and essential harvests (rice and maize) are mycotoxigenic fungi. The maximum of the F. fijikuroi isolates from some product as rice are potential producers

of fumonisin. F.verticillioides isolates that cause corn root, stalk, and ear rots (Cruz, et al., 2013).

1.2. Mycotoxigenic Fungi

There are many mycotoxigenic fungi and their products are isolated from different crop commodities and others. Such as mycotoxigenic fungi are widely attacked different type of food in addition to grown in changed areas. There are group of fungi that consider pertaining mycotoxin widely such as (*Aspergillus, Fusarium sp*, and there is single species of *Penicillium*) are the most largest group(Balendres, et al., 2019).

1.2.1. *Aspergillus species*

There are two type of fungi until year 2000 which are (*A.flavus* & *A.niger*) concider to be pertaining of toxin and it is recognized as risk factor until the year 2000, as the mycotoxigenic *Aspergillus* species, commonly associated with maize grains and groundnuts; nevertheless, coffee there is many research found five new different type of *Aspergillus* species capable of producing pathogenic fungal mycotoxins which are (*A.carbonius, A. japonicus, A. ochraceous, A.niger, A. westerdijkiae*), also OTA was detected for the first time which are very Similarly to (Alvindia, D.G. & de Guzman, M.F., 2016; Barcelo, J. M., & Barcelo, R. C., 2018). In addition to finding *A.niger* and *A.ochraceus* in the Cordillera Administrative Region (CAR) and the co-related between OTA in Arabica and Robusta. *A.niger* also was often isolated in an experiment in different type of food and/or product (Alvinda and Acda, 2010). seven species of Aspergillus discoveries in these new experiment and result in finding that are taint great crop commodities, whatever there was no new detection of mycotoxin, contamination with production of *A.flavus* toxin and it was the first verification in both fresh and dried food together (Sales et al., 2005), whatever it thought that the drying product will suspect to decrease in pertaining toxigenic product by fungi. The

amount of *A.flavus* colony forming components containing was decreased. It was also noticed that *A.flavus* was protected in different product (Sales and Yoshizawa, 2006). The all rice products have been found to be tainted with *A.flavus* and *A.parasiticus*, in some research design. In polished and brown rice, a high occurrence (100 percent) of *A.flavus* was declared. *A.parasiticus* isolates were found in coconut field soil samples and experiment found that all four types of aflatoxins were produced (Hussein, et al., 2019).

1.2.2. *Fusarium Species*

Four species of Fusarium mycotoxigenic most probability are described in different research procedure, *F. moniliforme* and *F.proliferatum* contaminate corn grits. From Laguna and Nueva Ecija foremost report of mycotoxigenic that produce by *F.fujikuroi* (Cruz, et al., 2013). Out of total number (32) of *F.fujikuori* recognized (13) of them as a strains of fumonisin B1 (FB1) producers whatever the *F.verticillioides* species are the most frequently reported as it is mycotoxigenic in addition to *F.species* also isolated in maize and is the predominant (Balendres, et al., 2019). There was many experimental study deal with presence of *F.verticillioides* as a high rate also found in the experimental research in kernels of maize. There are many experimental design deal in their study that the *F.species* associated with corn ear rot discovered the maximum species belong to *F.verticillioides* which are associated with mycotoxigenic toxin (Pascual, et al., 2016) &(Hussein, et al., 2017)

1.2.3. *Penicillium Species*

There are twelve type of *Penicillium sp.* were isolated from different type of coffee beans. The mycological fungal species isolated until now were *P. implicatum, P. montanense, P. purpurogenum, P. variabile, P. verruculosum, P. citrinum, P. corylophylum, P. decumbens, P. pelutatum, P. oxalicum, P. waksmanii,* and *P.digitatum,* Moreover from these twelve only just one of them *P. verruculosum,* was

determined by high-performance liquid chromatography method during evaluation the result they discover there was OTA produce inside the media. (Alvindia, D.G. & de Guzman, M.F., 2016)

1.3. Influence Factors that Incidence Mycotoxigenic and Mycotoxins Fungi

When there are some group of issues behind of the occurrence of fungi and it is relate to mycotoxins in the food by fungi, there are also multiple approaches are used for some classify these factors as both extrinsic and intrinsic type, while the others categorize as environmental in addition to their storage condition reasons (D'Mello, J. P. F., & MacDonald, A. M. C., 1997; Zain, M. E., 2011). Regardless of the categorization process (Lacey, 1986) in his experiment recognised the main rudiments included or pertaining in claiming that the type of quantity of mycotoxin produced is most probability recognized by substrate relate to fungi product in addition to ecological influences of fungi(Lacey, 1986).

1.4. Systems of Farming and Agricultural Techniques

In various reports and many of research project farming systems have been shown to stimulate the growth of moulds in farm area and it is products for instance more *A.flavus.* Infested crops and others were type of groundnut experimentally planted in earlier year following resulting with more aflatoxin than crops, which were grown before. This means mycotoxigenic production and it is growth of the fungus have been more affected by crop rotation. It has also been shown that soil previously treated with fungicides has reduced the rate of *A.flavus* in groundnut and other suspected food and others that fungi may affect it and result in producing mycotoxins (Makun, et al., 2009).

1.5. Pre-harvest Conditions

Genetic composition, dryness, types of soil, plant population, fertilization rates in addition to the degree of action and activity refer to insect are consider to be a significant in and insect action are important in assessing the pre-harvest pollution (Cole R. J., et al., 1995).

1.6. Harvesting Time

The initial stage in the output chains is Harvest in addition the humidity is the one of the most significant factor for the crop safety and management. It also characters a change from issues produced by specific pathogenic fungi such as *Fusarium* to stimulate complications and it is produced by storing fungi such as *P. verrucosum*. but in normal grains are continuously harvested at a moisture level after a period of dry environment and rained weather, therefore drying early is not important or not required whatever always it is not possible to occur to be risk factor it is unsuitable harvesting time. The physical check-up of the grain for signs and it is risk factor for causing of disease. Early harvesting minimizes crop fungal poison and it is toxin on their field and harvested product several complications. While most farmers are taken sufficiently and aware of what is need for early harvesting, there are many reason force farmers to harvest at the wrong time like cash requirements insufficient storage space these are also consider as a factors whatever also changeable rodents and other creatures condition of weather, labour constraints(Bankole and Adebanjo, 2003).

According to experimental which conduct by (Kaaya, et al., 2006), the duration lasted for 4 weeks aflatoxin levels was noted to be increase in quantity about four field than the first time (4 time) in addition during the third week more than 7 times noted. If crops are harvested early and we want to minimize it and planning to stop fungal growth and it is effecting, they need to be dried to safe points this is due to reasoned in early harvesting and threshing of groundnuts.(Rachaputi, et al., 2002).

1.7. Pest Attacks

Main causes of cereal spoilage and loss, the insects cause grain and seed invasion contributes to decrease level in agricultural products (price), grade and market value. In most cases these crops are made hazardous for human. Therefore, the level of insect damage defines the scope of mycotoxin this is most probability turned to primarily unsuitable storage and it is environments state. Grain infestation with plague is a good predictor of contamination with fungi known as fusarium mycotoxin. Insect transfers spores of creating champignons plant from the outsides toward inside of stem or seed which may resulting in makes infectious injuries through the nourishing habits(Avantaggio, et al., 2002).

1.8. Post-harvest Processing

Processing of the first harvest which known as post-harvest stage is contributes and influence with the primary handling such as friction typically this will require steps to dry, store and transport. It can result in difficult to move food resources after harvest, going through a variety of intermediaries such as traders and intermediate processors found in different geographic areas. Whatever the goodness will usually to be processed in farmhouse especially when handle or buffered for short while beforehand being moved straight to computer it. If the harvested wet, such as grains and kept in storage cabin for long or short time before inward in aim to computers it or using it possibly will following permit over the hands of 3rd drying amenities. The goodness can always become vulnerable to fungal pollution and risk factors of toxic exposure and the development of mycotoxin unless the storage circumstances are severely monitored(Makun, et al., 2009).

1.9. Drying Condition and its Duration

There are an essential point should be mention that dry farm produce quickly to low levels of wetness. This provides less positive circumstances for growth of fungi, development, and attack of insects. Drying assistances to maintain longer goods (Lanyasunya, et al., 2005). In order to reduce mycotoxin contamination both of Ayodele & Edema in 2010 organized idea nouns as Critical Control Points (CCP) in development of dried yam chips and found that the drying stage was a CCP. If field harvested maize is stored with great wetness content, infection with aflatoxin may increase 10 times over a 3-day span. The overall commendation to reduce moisture levels to 10–13 percent is that harvested crops should be dehydrated as speedily as possible; it is quite difficult to achieve this by sun-drying under the great wetness circumstances. It is also not always finished while drying in the dry season until grains are put into stores, and goods can be easily tainted with aflatoxins. Low moisture content should be preserved during storage, transportation and marketing by preventing leakage of the tops and concentration due to poor ventilation. (Mestre, et al., 2004)

1.10. Factors of Storage

Insufficient storage or poor handling of harvested crops can cause mycotoxin impurity of foods or feeds. By sufficiently drying up to less than 10% moisture and avoiding insect activity In order to keep storage quality, it is important to prevent biological activity, which can rise moisture content through inhalation condensation, low temperatures and inert atmospheres(Lanyasunya, et al., 2005; Turner, et al., 2005).

1.11. Sanitation

To reduce on-field infection and product infestation basic sanitary consider as a removing following of destroying in previous harvest debris are necessary. Aflatoxin levels may decreased by 40-80 percent when sorting bodily damaged and diseased grains using complete product colorations, abnormal shapes and size.(Turner, et al., 2005).

1.12. Types and Properties of Substrates

Agricultural crops are used in the development of certain different type of production in addition to moulds, the maize enables to development of aflatoxins as well as fumonisins creating moulds overhead others, and peanuts is a good substrate for infection with aflatoxins. Other food products confirmed to be tainted with mycotoxin were dehydrated yams and plants stored. It appears that peanuts, cereal grains, cottons and some forage are usually infected with mycotoxins.(Bankole and Adebanjo, 2003).

1.13. Poor Awareness

The key factor that is responsible for its high occurrence of farm producing pollution with mycotoxin is lack of awareness. Some farmers doesn't have enough knowledgment about safety and it is storing of different type of food, some thru almost non experience of the toxigenic mould insinuations, the powdery element could be easily cleaned and/or washed with rainwater beforehand eating or processing food for consumption without associated risks it is assumption of the investors (Hussein, et al., 2019).

1.14. Occurrence and Distribution of Mycotoxins

Mycotoxins are found in various items such as feeds for cattle, cereal crops, leguminous plants and foods for livestock. Aflatoxins can be present in all cereal crops. Serious farming practices and decreased genetic diversity in cereal crops are likely to contribute to increased pre-harvest infections of aflatoxin-producing commodities with fungi (Brown and Payne, 1998). Both temperate and tropical regions, pre-harvest contamination of crops with aflatoxins occurs. The seeds are the most susceptible to fungal invasion and aflatoxin production in growing-stressed plants. Dryness, insect damage and timing of irrigation are the most commonly recognized plant stressors. Post-harvest pollution happens all over the world as

situations for the growth of aflatoxigenic fungi exist in the storage unit. Insects disperse aflatoxigenic fungi spores to plants and insect damage zones are colonized by the fungi. The corn flower and silk may be entry portals for Aspergillus species. (Diener, et al., 1987).

Cotton seed in animal nourishments may be a source of aflatoxins. Pest infestation, moisture, irrigation or rain timing, relative around the bolls, factors affect seeds of cotton infection with aflatoxins in pre-harvest are cotton variety and maturity stage.(Lillehoj, et al., 1987). If the rate level of moisture is bigger than (7–8) percent growth of aflatoxigenic fungi in stored cottons will happen. Cottonseed lipids and proteins facilitate the growth of aflatoxins. The most abundant in the seeds are aflatoxins(Mellon, et al., 2000). Aflatoxigenic fungi grow in stored peanuts if moisture exceeds (8) pecencet and ambient temperature exceeds (25°C), When peanuts are drought and stressed they have been reduced fungal-producing aflatoxin resistance to infection(Wotton and Strange, 1987). Increased phytoalexin released by the infected peanut seed inhibited *A.flavus* development in spite of that the levels of aflatoxin continue to rise for an extra day. The moisture availability can set determination of phytoalexin production, and the production of aflatoxin in drought and stressed peanut kernels. By using those for alcohol production, maize and other high starch commodities polluted with aflatoxins can be saved. The fermentation process will not destroy aflatoxins. As a result of starch loss, the concentration of aflatoxins in the sillage is increased equalled to aflatoxins in the feedstock by depending On a dry matter basis. The syrup part (solubles of the distillers) contain around (40) percent of aflatoxins but solids part contain (60) percent. Corn-based human feed frequently contains fumonisins in several countries Commercial from retail outlets(Pittet, et al., 1992).

The pollution level of mycotoxins in leguminous crops varies geographically and the main source of mycotoxins is peanut. *A.flavus* and *A.niger* primarily infect groundnut seeds. The main source of mycotoxins are cereal crops such as `wheat, sorghum and

barley. Deoxynivalenol existed in sorghum, wheat and barley with a cumulative incidence of (48.8) percent of (84) reported samples, resulting in identification of aflatoxin and ochratoxin in barley, sorghum and wheat. Wide-spread storage of underground sorghum grain causes pollutant due to leading to great levels of seed moisture midst these cereal crops, sorghum is the main source of mycotoxin. Milk is an animal product and it is the source of human infection with aflatoxin. (Gebreselassie, et al., 2014)

1.15. Types of Mycotoxins

The devastating risks related with the ingesting of mould-contaminated products were clearly indicated in previous reports. Some of the Egyptian tombs have been held responsible for the mysterious deaths of several archeologists, found to contain ochratoxin A. Historically the ergotism is the longest known mycotoxicosis, it was considered that a pilgrimage to St. Anthony's the name obtained from the intense burning sensation experienced by affected people would bring relief to the head. Several of these epidemics happened between the (8-16) of centuries, and the attributed to poor dietary conditions was possible reason, especially the eating of tainted flour from ergots. People affected by ergotism were exposed to lysergic acid diethylamide (LSD), when a hallucinogen made from ergot-contaminated wheat during the baking of bread. In 1954, a large number of people were victims of ergotism, France scientist reported the first recognized acute intoxication. Also it was between.(1977 - 1978) when Ethiopia during work saw the last registered major gangrenous ergotism outbreak affecting nearly (140) people which (34 percent) of them was died because of it. The long wet season cause this outbreak because that favoured weather help the growth of Claviceps purpurea which is susceptible wild oats. In 1966, after ingestion of pure aflatoxin B1, a case of attempted suicide was recorded from the United States(Willis, et al., 1980).

There are over 100,000 young turkeys died in the United Kingdom during the years around 1960s as a result of aflatoxicosis with affecting thousands of other animals and humans. The (moldy sugarcane poisoning) happened between (1972 and 1988) it is a food poisoning outbreaks an overall of 884 people were affected and identified as (MSP) which it is caused by an *Arthrinium species* (3- nitropropionic acid). Together with people have been educated and obtainable of data, however, a universal association wants to make a connection between historical mycotoxin with the newly detected one because they are important in the future (Balendres, et al., 2019).

1.15.1. Aflatoxin

Aflatoxins formed by some specific fungal species which produce real risky an essential metabolites to human and many healthy complication (Brown and Payne, 1998). These types of fungi typically taint cereals and cereal product eg: corn, wheat, walnut, peanuts, cotton, tree nuts.(Severns, et al.,2003), this aflatoxin has several complications and can cause serious threats to human and animal health for example (teratogenicity, hepatotoxicity, immunotoxicity). The main aflatoxins involvement are(B1, B2, G1, and G2) moreover it can infect the human via respiratory tract, mucous surface area in addition to cutaneous pathways, causing the inflammatory response to over activate. The safety of food and it is storage consider one of major challenges issues in the worldwide at the moment; thus, a number of studies have been carried out to explore ways of addressing customer concerns about different aspects of food safety(Nielsen, et al., 2009). The U.S. Food and Drug Administration (USFDA) has been limiting the amount of mycotoxins allowed in food products since 1985. The U.S. Grain and Plant Inspection Service (GPIS) has established a grain-based mycotoxin inspection service laboratory. In addition, many toxins found in agricultural products have been recognized by Food and Agricultural Organization (FAO) in addition of World Health Organization(WHO). Normal processes of cooking cannot destroy mycotoxins when found in food. While there are numerous modern improvements nowadays toward the food dispensation have been

industrialised to keep it safe and healthy keeping foods such as Hazard Analysis and Critical Control Points(HACCP) moreover the Good Manufacturing Practices(GMP) (Maldonado-Simian, et al., 2014).

1.15.2. Outbreaks Due To Aflatoxins

A big hepatitis eruption in India especially involved two city named "Gujrat and Rajasthan the cause turned to presence of aflatoxin in 1974, there was approximately 106 deaths have been estimated as a result (Krishnamachari, et al., 1975), this eruption has been continue for two months and this narrowed to family persons because there food consumption, the maize was subsequently long-established to cover fungal aflatoxin moreover initial examination definite that there was A.flavus was consumed (Krishamachari, et al., 1975; Bhat and Krishnamachari., 1978). There was additional fungal aflatoxin eruption touching people and it is described in Northwest of India also in 1974, (Tandon, B.N., et al., 1977; Bhatt, R. V., and Krishnamachari, K. A., 1978; Reddy, B. N., and Raghavender, C. N., 2007). Worldwide several eruptions of aflatoxicosis have been detailed since in 2004 also there was some report mention it which this leading to 200 deaths with about 500 severe disease according to organization (Centres for Disease Control and Prevention (CDCP), (2004); Azziz-Baumgartner, E., et al., 2005).

1.15.3. Aflatoxin Producing Fungi

The group of fungi known as A.flavus, A.parasiticus, A.nominus are the main sources of aflatoxins (Kurtzman, et al., 1987). While some other Aspergillus species as well as have ability to produce this toxin like Emericella spp, (Reiter, E., et al., 2009). Until now there are over 20 recognized aflatoxins reported, but commonly there are top four which include aflatoxin(AFB, AFB2, AFG1, AFG2) (Inan, F., et al.,2007). The Aflatoxin AFM1 and AFM2 are derived from the hydroxylated fungal metabolites of both type of AFB1 and AFB2 (Giray,B., et al., 2007; Hussain, I., and Anwar, J., 2008).

1.15.3.1. *Aspergillus spp*.

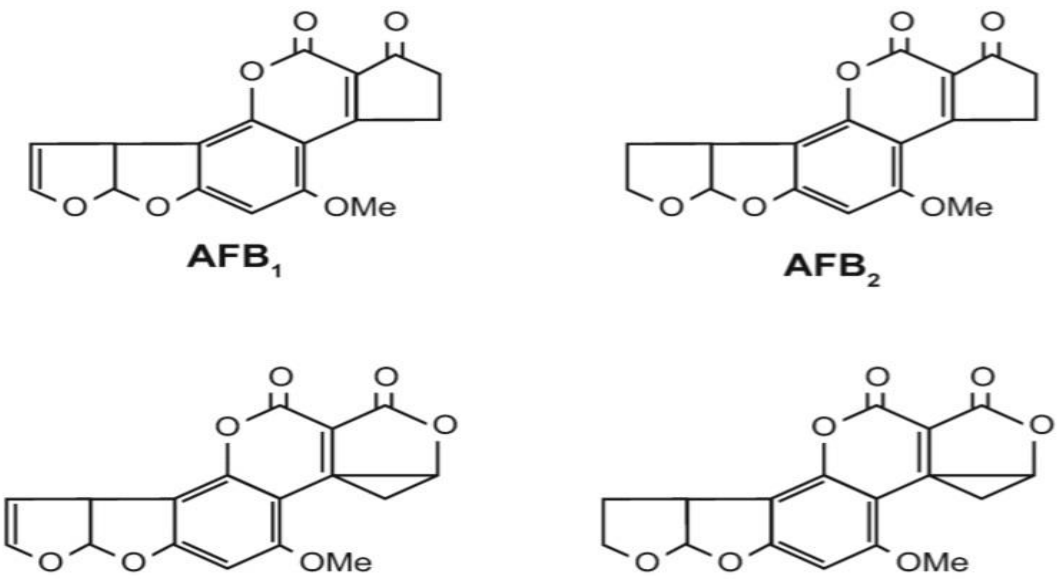
The group of fungal microorganism wich noun as A. species are belong to mycology branch and it is spread worldwide and are of industrial importance (*A. niger*) was given typically documented as harmless GRAS ranked by USFDA (Schuster, E., et al., 2002). In addition they have bad effects on (peanut, grape, corn, garlic, onion, coffee, and other fruit with vegetable) diseases (Lorbeer, et al., 2000; Waller, J. M., et al., 2007; Rooney-Latham, S., et al., 2008). In addition to *A.niger* produces mycotoxins in different food such as ochratoxins and fumonisins. (Astoreca, et al., 2007; Mogensen, et al., 2009). The method known as green fluorescent protein is a molecular markers using for examination of Interactions between plant one and pathogen one were (GFP) detected from *Aequorea victoria*. (Prasher, D. C., et al., 1992). In *Undifilum oxytropis* (Mukherjee, et al., 2010), in addition the *F.equiseti* and *Muscodor albus.*(Ezra, D., et al., 2010; Macia-Vicente, J. G., et al., 2009), the GFP gene was successfully inserted and were used to study protein expression and mycotoxin production in the period of processing, storage and cultivation *A. flavus* and *A. parasiticus* which infects a large number of crops in world wide. Whatever the *A.flavus* are leading in treenuts, corn, and cottonseed, but the *A. parasiticus* in peanuts, predominates. *A. flavus* produce at temperatures between (12 and 48^{o}) and it is made up of mycelium, conidia or sclerotia (Hedayati, M. T., et al., 2007), the *A. flavus* yields AFBI and AFB2 however the *A. parasiticus* separates produce (AFGI, AFG2, AFBI, AFB2) (Lee, et al., 1986).

1.15.4. Chemistry of Aflatoxins

The aflatoxins (AFTs) are difuranocoumarin derivatives in which one side of the coumarin nucleus attached to the bifuran group the a pentanone disc is linked to other sideways in the situation of AFTs and AFTs-B sequence or the AFTs-G series attached to the six member lactone ring(Bennet, J.W. and Klich, M., 2003; Nakai, et al., 2008). The aspergillus production affect by factors as physical, biological, and

chemical factors to production toxin. The fungi named *A.flavus* produced AFT-B1, and AFT-B2 type whereas AFT-G1 and AFT-G2 in conjunction with AFT-B1 and AFT-B2 and it is formed by *A.parasiticus* (Bennet, J.W. and Klich, M., 2003). The four main naturally produced aflatoxins are AFT-B1, AFT-B2, AFT-G1, AFT-G2 (Pitt, 2000). During several metabolisms procedures in addition articulated with animal foods AFTsM1 and AFTs-M2 are derived from aflatoxin B types (Weidenborner, M., 2001; Wolf-Hall, C., 2010) The AFT-B1 are enormously cause carcinogenic in addition to heat resistant to different intensity of temperatures (Sirot, et al., 2013).

Figure 1.Structure of Major Aflatoxins.



1.15.5. Aflatoxin Biosynthesis and Metabolism

There is 18 enzymatic steps are involve in the biosynthetic pathway of aflatoxins change from acetyl-CoA and there is about or nearly 25 type of genes encoding the enzymes and it is participate controlling passageways were copied categorised (Yu, J., et al., 2002; Yabe, K., and Nakajima, H., 2004). These gene are consists of the 70 kb of genome in addition to controlled by supervisory gene aflR.(Yabe, K., and

Nakajima, H., 2004; Yu, J., et al., 2004; Price, M. S., et al., 2006). However, Hydroxyversicolorone(HVN) is transformed by a cytosol monooxygenase into versiconal hemiacetal acetate (VHA), where NADPH is a co-factor. The moxY gene had made encode on Monooxygenase, that catalyzes HVN's conversion to VHA and HVN and p (VONE) in the non-appearance of the moxY gene accumulation happens. There are different genes are complicated in synthesis and product of aflatoxin precursors. Sterigmatocystine (ST) dihydrosterigmatocystin (DHST) are these forerunners in addition to the nor-1 gene for aflatoxin biosynthesis was first cloned in A.parasiticus(Wen, et al., 2005).

1.16. Aflatoxins and Health Effects

1.16.1 Role in Cancer Development

The most dangerous naturally occurring carcinogens well-known are AFB1, AFG1 and AFM1, and the most hepatocarcinogenic compound is AFB1. The AFB1 induces various cancers in humans and animals of the liver and other organs (Kitya, et al., 2009; WHO, 2008). The cancer inducing ability of aflatoxins is in its capacity to produce altered forms of DNA adducts. Hepatocellular carcinoma (HCC) and/or liver malignance is the major illness associated with the ingestion of aflatoxin it causes cancer ranked third in the world (WHO, 2008).In most countries, the prevalence of liver cancer has been consistently higher for men than in women with a sex ratio of 2 to 3 (WHO, 2000; Kirk, et al., 2006) estimated that 83% of deaths due to cancerous happened most probability in East Asia and sub-Saharan Africa area and it is one of the most commonly diagnosed cancers in the world. In South-East Asia and sub-Saharan Africa, hepatitis (HBV and HCV) viruses and dietary exposure to aflatoxins are the major risk factors for chronic infection (Wild and Montesano, 2009). In human HCC, Aflatoxin B1, is the most abundant and active, is related to a particular AGG to AGT amino acid transversion change at codon 249 of the p53 gene, thus as long as mechanistic evidence for a causative of the relationship between disease and exposure

(Groopman, et al., 2008; Liu and Wu, 2010). In humans, there is association between the incidence of cancer and the dietary aflatoxin content showed by studies in the USA, Southeast Asia, Africa and other western countries with a high occurrence of hepatocellular carcinoma(Thraser, 2012).

1.16.2 Role of Aflatoxins in Hepatic Injury as well as other Body Parts

It has been documented that aflatoxins cause cirrhosis of the liver and kidney, cholangiocellular cancer, lung adenomas, liver cancer in animal (Thraser, 2102; USAID, 2012), in addition to liver damage can be either severe or long-lasting, due to a number of toxic substances and including aflatoxins, chemicals and medications, trauma and infectious agents (Barret, 2005; Bommakanti and Waliyar, 2012). The drop in total protein levels indicates the toxic effect of AFB1 on the liver forming adducts with proteins and (DNA, RNA) it means lack of liver protein synthesis is an indication that aflatoxins impair protein biosynthesis, the endoplasmic reticulum degranulation will happen Because the inhibition of DNA-dependent RNA polymerase activity RNA synthesis. Acute liver injury caused by aflatoxin causes an increase in serum enzymes including lactate dehydrogenase, glutamate dehyrogenase, aspartate aminotransferase, gamma-glutamyltransferase, alkaline phosphatase and bilirubin, also other commonly used liver enzymes are gamma-glutamyltransferase (GGT), gamma-glutamyltranspeptidase and alkaline phosphatase (ALP), (GGTP), which show impairment to system of biliary, whichever outside the and/ or inside of the liver (WHO, 2008). The Aflatoxin B1 has been reported to cause pale hepatic discoloration and hepatic and kidney enlargement, liver parenchyma congestion, hepatocyte necrosis, fatty hepatocyte changes or cytoplasmic vaculation, and create new bile adduct. In aflatoxin-fed broiler chicks, mononuclear and heterophilic celal infiltration was reported(Hussain, et al., 2008).

1.16.3 Role in Immunodeficiency

Ingestion of food which have been polluted with aflatoxin for long-term by persons and animals universality has been reported to cause immunosuppression. (USAID., 2012). In humans, aflatoxins affect cellular and humoral immune responses where they change immunological parameters in participants with high levels of AFB1 resulting in damage of cellular immunity and these transfer to reduction of host resistance to infections (Sahoo, et al., 1996). Especially in cell-mediated responses exposure to aflatoxin cause immune suppression. The persistent exposures to aflatoxin decrease phagocytic activity of phagocytes and delay responses to bird hypersensitivity. This decreases the bone marrow and the number of red and also white blood cells, the number of macrophages and the phagocytic function of the cells. It also depresses the functions of splenic lymphocytes in mice that are dependent on T-cells. Aflatoxins in particular AFB1 also influence the natural killer cell function of peripheral blood lymphocytes. A decrease in peripheral blood leukocyte immunophenotypes, proliferative response of CD4 + T cells, cytokine profiles of CD4 + T and CD8 + T cells, and phagocytic monocyte activity have been documented. In developing countries, children appear to be exposed to aflatoxin naturally through their diet at levels that suppressed the immune system. The proportion of childhood growth stunting was generally directly proportional to the population living below the national poverty line and was inversely correlated with the per capita domestic product gross. In regions such as Southeast Asia and Sub-Saharan Africa, where exposure to aflatoxin by exhaustion polluted nourishment is mutual, childhood stunting is similar to liver cancer(WHO, 2008; USAID, 2012).

1.16.4 Role in Fertility

As we know toxin produce by fungi produce many complication to human the fertility complication is one of them, accrediting of higher concentrations of aflatoxins in the semen of infertile people in humans exposed to chronic aflatoxin-contaminated foods

(Gupta, 2011). Low childbirth weight is also associated with aflatoxin. Also it may cause jaundice in babies also cause harmful effects in newborns when presence of AFM in breast milk in the mother. In many of research experiment prove that aflatoxins have a role in infertility, sperms, sterility, and affect hormone activity also it can affect sex reproductive skills and can disrupt male and female reproductive system after ingestion of infected foods it have been shown by experimental results. (Hasanzadeh and Rezazadeh, 2012)

1.16.5 Teratogenic Effects

As enlarged embryo liver and eye sockets, the teratogenic effects of aflatoxins were described. Also affects the poultry, aflatoxins reduced egg yield, volume of semen, spermatocrit, tested weight and plasma testosterone. (Clarke, et al., 1987).

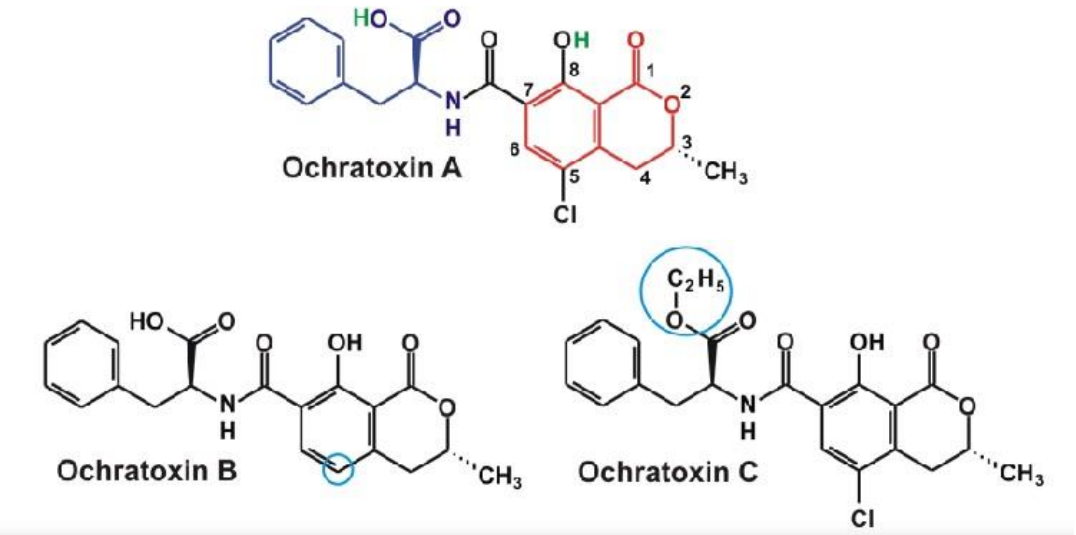
1.17. Ochratoxins

1.17.1. Chemistry of Ochratoxin

The *Aspergillus* and *Penicillium* are two main genera of fungi that produce Ochratoxins. Chemically, by peptide bond 1-phenylalanine joined to a moiety of dihydroisocumarins also organic acids in ochratoxins are described as weak(O'Brien, E. and Dietrich, D.R., 2005). Also 3 structural types of ochratoxin A, B with C a small difference between them however, the most toxic and chlorinated one is ochratoxin A, and OTB have been taken the Second level (chloride substitution for a hydrogen atom in isocumarin moiety), it has a smaller size in toxigenicity, and OTC, or ethyl OTA, they have small or no possibility in toxigenicity(Van der Merwe, K.J., et al.,1965; Li, et al., 1997), Sometimes OTA and OTB are occure in mouldy normally which ochratoxin β, methyl and ethyl esters are dechlorinated analogue of a ochratoxin α- the OTA isocoumarin nucleus also several analogue of amino acids are synthesized. Hydrolysis products of OTA and OTB are Ochratoxin α and β. and are

non-toxic due to the lack of the phenylalanine molecule`. (Moss, 1996; Xiao, et al., 1995).

Figure 2. Structure of Major Ochratoxins



1.17.2. Ochratoxin Producing Fungi

The *Aspergillus ochraceus* was the first mould which Ochratoxin A isolated from and it is the most toxic among other types, also its name derived from this species. while, reported show that other genera could produce this toxin, and the OTA is present commonly in adequate and at great levels in nutrition and beverages in some countries to cause concern for human safety`. Van der Merwe, K.J., et al., 1965)

1.17.3. Ochratoxin Biosynthesis and Metabolism

Protein binding in any given species is potentially the determining influence factor in causal of the OTA. The OTA has a very high affinity in the blood for serum albumin and other macromolecules it is declare by some studies (Galtier, P., et al., 1981; Hagelberg, S., et al, 1989). This association with albumin in human blood serum and it has been suggested to result of production of a moveable ochratoxin, this may lead

to secreting slowly in addition to reduced bioavailable over lengthy phases of time, also prolong the removal of OTA in body(O'Brien, E. and Dietrich, D.R., 2005). Inactively and actively Ochratoxin A (OTA) is processed in the kidneys throughout the gastrointestinal tract (Xiao, H., et al, 1995). The maximum concentricity of OTA have been establish the blood and are distributed in decreasing order in the tissue of the kidney, liver, muscle and adipose (Gareis and Scheuer, 2000). Mainly this toxin may visible also in urine and faeces in a lesser degree, like in bile and milk, have been found ochratoxin α or OTA. Intravenously injected OTA has a longer half-life of orally consumed and experimentally OTA undergoes a first-pass hepatic elimination, and extracted before getting in blood system by bile. The OTA can removed through a half-life after intravenous administration in rats in 3 days, and half-life in pigs its between 3-5 days(Galtier, P., et al. 1981) however in monkeys between 19-21 days the period of half-life time. (Hagelberg, S., et al., 1989; Stander, M.A., et al., 2001).

The (Studer-Rohr, I., 1995) the human blood serum which contain OTA's duration of half-life was 35 days after oral intake. It would still have a measurable serum level in humans 280 days after a single take-up. The chemical structure of ochratoxin A is C20H18ClNO6 also OTA have 403.82 daltons molecular weight, is a substituted isocoumarin phenylalanyl derivative. It is classified as L-phenylalanine N [5-chloro-3, 4-dihydro-8-hydroxy-3-methyl-1-oxo-1H2-benzopyran-7-yl] carbonyl (R) (C.A. No. 303-47-9) in the Chemical Abstracts index. The amino acid phenylalanine (Phe) is structurally similar to OTA. This is why it has an inhibitory effect on a number of enzymes that use Phe as a substrate, particularly Phe-tRNA synthetase, which can result in protein synthesis inhibition. OTA can also induce lipid peroxidation for the same purpose. Ochratoxin A is solvable in organic alkaline solution and it's a crystalline, colourless compound. It crystallizes from benzene to give a 90 ^{o}C liquid melt containing one benzene molecule. To bounce a material melting at 168 ^{o}C this could helpful in order to removed further down vacuum at 120 ^{o}C. Later it crystallizes from xylene in a pure form. OTA shows blue fluorescence under UV light due to its optical activity, but with changing in pH and solvent polarity the spectrum of

ultraviolet will change. The average emission of fluorescence in 96% ethanol is 467 nm and is 428 nm in absolute ethanol(Scott, 1994).

1.17.4. Ochratoxin and Health Effects

Frequently only one mycotoxin can cause toxic effects of more than one form moreover the kidney is the target organ of OTA toxicity in all tested mammalian species, in which acute and chronic exposure can cause lesions. Based on many reason animals may display variable susceptibility to OTA for example physiological factors like (diet, sex, age) genetic factors like (strain, race and species) and many diseases and climatic circumstances, and management as environmental factors. The lethal dose (LD) 50 is one way of measuring a compound's potential for short-term poisoning (acute toxicity). Also it is the sum of a substance supplied all at once which resulting in the 50% of death animalsin toxicity studies, however, `OTA LD50 standards differ significantly between animals, going from an oral LD50 of 0.20 mg kg-1 in dogs and 1 mg kg-1 in pigs to more than 30 mg kg-1 in rats. The standards of LD 50 there are also powerfully affected by the ways of administration (intubation, mouth taken, intraperitoneal injection, intravenous or) toxin solvents, other mycotoxins and diet composition (Harwig, et al., 1983)

1.17.5. Role in Cancer Development

There are many report mention that OTA have role in renal tumors in rats and mice were developed by oral administration of OTA (Boorman, 1989). In addition to liver tumors in together sexes exist in mice (Kuiper-Goodman, T. and Scott, P. M., 1989). In other mammalian species, Nephrotoxic effects have also been shown. However in the primary 1970s in country of Denmark reported a great occurrence of nephritis in domestic pigs have been observed (Krogh, 1972), involvement of OTA in feed samples associated with the use of mouldy rye cause disease is now known as Danish

porcine nephropathy, also assuming correctly that ochratoxin a kidney toxin in humans, in certain kidney failure rates have been shown to be high, and the consumption of pig meat that having too much rate of OTA was a possible cause (Krogh, 1972).

1.17.6. Role in Genotoxicity or Mutagenesis

The toxic produce by fungi which know as OTA has not been considered genotoxic for a long time however Mutagenic or genotoxic chemicals can cause DNA damage (Creppy, et al. 1985), showed that after high doses of OTA were injected the OTA may lead to breaks DNA in mice spleen, kidney in addition liver after high doses of OTA were injected. In addition, after oral application of OTA to mice. (Pfohl-Leszkowicz, et al., 1991) recognized many DNA adducts. When these toxin rich to animal cells the main target organ will be ureter because of the high sensitively of it is DNA to changes this topic received significant reinforcement. Whether OTA disrupt DNA by responds straight with DNA nucleic acids, however, there is still some disagreement (Föllmann, W., et al., 1995; Dörrenhaus, A. and Föllman, W., 1997).

1.17.7. Role in Teratogenesis

In one of the experimental study mention that Ochratoxin A is a powerful rodent teratogen (Hayes, A.W., et al., 1974; Brown, M.H., et al., 1976; Gilani, S.H., et al., 1978; Shreeve, B.J., et al., 1977). OTA causes rodents to have birth defects also OTA is transmitted by lactation to infants crosses the placenta. (Hallen, et al., 1998). OTA has also considered a compound with neurotoxicity and the main target in the foetus is the development of the central nervous system. OTA make DNA adducts are also produced in the progeny's liver, kidney and other tissues. Also the OTA's mediated teratogenesis mechanism is not yet understood whatever it tends to directly produce complication toward to both of (progenitor and embryo). In the scientific literature, therefore, there is ample experimental evidence to describe the OTA as one of the

causative of teratogen effect moreover to immune system complication, (Pfohl-Leszkowicz, A., et al., 1993; Petkova-Bocharova, T., et al., 1998)

1.17.8. Role in Immunodeficiency

Ochratoxin A in some of the mammalian species consider and one of the complication toward to mammalian immune defects, in addition there are number of factors that affect the type of immunosuppression knowledgeable, including the involved species and administration of route, the tested doses and the degree of effective were evaluated by the approaches (O'Brien, E. and Dietrich, D.R., 2005). Upon exposure to adult life, postnatal and prenatal, ochratoxin A (OTA) induces immunosuppression. Such results are decreased markers of phagocytosis and lymphocytes(Müller, G., et al, 1999). Studies of the influence of ochratoxin A on immune and defence reactions in weaners. *Mycoses*, *42*(7-8), 495-505.) Delayed immunization reply and there is some not toward increased of bacterial susceptibility (Stoev, et al., 2000). In addition if there are OTA in vitro adversely affects and produce complication to untreated human lymphocyte populations and subpopulations (Lea, et al., 1989).

1.18. Significance of Study

Globally, natural products are generally claimed to be safe. Contamination of human or animal food by natural toxins, however, may lead to multiple disease outbreaks. Because of their global distribution, mycotoxins are more important. Some of the colonizing fungi are able to produce toxins that can have harmful effects on humans or animals that have consumed the contaminated products. Food safety and demand for high-quality foods are increasingly worried, and frequent occurrence of mycotoxins in food would undoubtedly have a negative impact on individuals economic and health status. Therefore, this research will add to the existing literature on the frequency and health effects of mycotoxins in food.

1.2. OBJECTIVES

Specific goals of study's are:

i. To determining the levels of aflatoxin in certain food types

ii. To determine ochratoxin levels in certain food types

iii. Determine the levels of aflatoxin and ochratoxin in different types of commonly consumed foods.

CHAPTER TWO

2. MATERIALS AND METHODS

2.1. Study Area

Samples were obtained in Sulaymaniyah Iraq from various supermarkets and local markets.

2.2. Sample Size

A total of 174 food samples from various locations were obtained. 88 samples were tested for aflatoxin identification and 86 samples were analyzed for ochratoxin.

2.3. Sample Collection

Different food samples were collected from different food sellers, they were taken to the laboratory. The samples were prepared for mycotoxin extraction and the extracts were used to detect mycotoxin presence. The Enzyme Linked Immunosorbent Assay (ELISA) kits were obtained from America (Veratox for aflatoxin and ochratoxin quantitative test USDA GIPSA 2015 070).

2.4. Detectionof Aflatoxin

The following food items were used for the detection of aflatoxin: Chips,14; Biscuits,10; Spices, 6; Nut, 14; Tea, 4; Coffee, 3; Cereal, 25; Starch, 5; Spaghetti, 4; Seed, 2; and Dried fruit, 1.

2.4.1. Assay Principles

The ELISA method were perform in this experimental study the firm Kit name was (Veratox) which is specific for detection amount of aflatoxin in foods were used in order to detect amount of toxin in daily consuming food. This method permit occurate concentration in parts per billion (ppb). Controls and samples contain free aflatoxin that permit to react or combine with conjugate reagent with antibody. Substrate will be added after wash step, producing blue colour after reacting with the bound conjugate. Less aflaoxin gives more bluecolor. Using microwell reader to produce optical densities (OD) to calculate the exact concentration of aflatoxin. The ODs of the samples and controls finally compare in order to evaluate the positive samples with negative ones by using standard curve plot referred to the kit.

2.4.2. Sample Preparation and Extraction

The examples to be established were collected aseptically from the food vendors. The samples were ground and stored at 2-8^{o}C until analysed

Figure 3.1 Sample preparation

`For each sample to be analyzed, methanol solution (70%) was prepared by combining 7 unit by 3 unit of methanol mixing with distilled water, the test sample was ground to fine particles (fine instant coffee size) so that at least 75% of the sample passed through a 20 mesh sieve. The solution of 5 grams of ground sample was shook actively for 3 minutes in 25mL of 70 percent methanol, by adding 5 ml through Whatman No.1 filter other extracts were collected as a samples.`

Figure 3.2 Filtered Samples

2.4.3. Test Procedure

The kit used to performing test should be gain room temperature (18-30^{o}C) before performing starting test procedure nearly 2 hours before using it, following performing each test the sample red-marked well and 4 red-marked wells for controls were removed. They were put in the holder of the well. The same number of wells covered with antibodies were removed for the test. "1" was numbered. The strips on one side following using wells holder on order to mark it. Before use, each reagent was combined with swirling. 100uL of the blue-labelled bottle conjugate was well placed in each red-labelled mix, following transferring 100uL samples to red-marked wells using a new pipette tip for each such as:

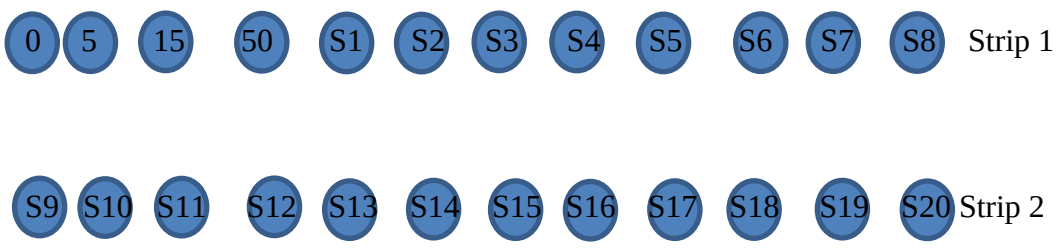


Figure 3.3 Well holder

By pipetting it up and down 3 times, the liquid in the wells was mixed using a 12-channel pipettor. 100uL has been added to the wells coated with antibodies. The red-marked mixing wells were discarded. For 2 minutes, the timer was set. The wells were mixed by moving the microwell holder back and forth on a flat surface for the first 10-20 seconds at room temperature incubations. It was taken care not to spray reagents from the wells. The antibody well contents were shaken out and purified water filled the wells and drained out. This step was repeated five times. The wells were turned upside down and rubbed on a paper towel until the remainder of the water was drained. Some volume of substrates were poured into the green-labelled reagent boat from the green-labelled container. Following pipetting the substratum 100uL was primed to wells, later on waiting for 3 min necessary after mixed for the first 10-20 seconds. The rest of the substratum was discarded and water rinsed the reagent boat. The reagent is added which noun as Red stop solution into the red-labelled reagent boat from the red-labelled container. The excess substratum was expelled from the 12-channel pipettor, the tips were primed, and pipetted and dropped into each well 100uL red stop. It was mixed on a flat surface by sliding back and forth. The tips were removed. The microwave bottom was wiped with dry cloth and read using a 650 nm filter in a microwell reader. Air bubbles were removed because the analytical findings could be affected. Results must be read within approximant 20 min.

2.4.4. Detecting positive Sample

According to the manufacturer's instruction, the values of aflatoxin above (20) ppb were considered as positive sample.

2.5. Detection of Ochratoxin

The following food items were used for the detection of ochratoxin: Chips, 10; Biscuits, 9; Spices, 13; Nut, 13; Tea, 4; Coffee, 2; Cereal, 24; Starch, 4; Spaghetti, 2; Seed, 3; and Dried fruit, 2.

2.5.1. Assay Principles

Ochratoxin veratox is a competitive direct immunosorbent enzyme-linked assay (CD-ELISA). It allows the user to obtain exact parts per billion (ppb) concentrations. In the samples and tests, free ochratoxin is allowable to participate and react with conjugate. The next step perform by washing wells, later on substratum is added, the aim of adding substratum are means less ochratoxin when reacting with the bound conjugate to produce blue colour. The test is read to obtain optical densities (ODs) in a microwell screen. The result was obtain by the procedure calculated on specific standard plot refer to the kit in order to decided wich one of thes test consider to be pertaining toxins and wich one are free of mycotoxygen control.

2.5.2. Sample Preparation and Extraction

The samples to be tested were collected aseptically, ground and stored at 2-8^{o}C until analysed. Methanol solution (50 percent) was prepared for each sample to be tested by mixing 1 part ACS grade methanol with 1 part distilled water. By mixing 7 parts of ACS grade methanol with 3 parts of distilled water, 70 percent of methanol was prepared and used for samples of wheat, barley, oats and rice. This method enhanced ochratoxin's optimal recovery. Through sample was ground to fine particles (fine instant coffee size) so that at least 75% of the ground content passed through a 20 mesh sieve. To 40 mL of 50 percent methanol and water, 10 grams of ground sample were added and shaken actively for 5 minutes. The extracts were routed through a Whatman No.1 filter, pouring around 5mL.The filtrates were collected as the sample.

2.6. Test Procedure

All the reagents were allowed to warm to room temperature (18-30^{o}C) 2 hours before use. For each sample, red-marked mixing well was replaced with 5 red-marked wells with controls to be checked for each sample. They were put in the holder of the well.

The same number of wells filled with antibodies have been removed for the examination. "1" was numbered on one end of the strip and the strip was put in the well holder with the marked left end. Before use, each reagent was combined by swirling the bottle of the reagent. 100uL of the blue-labelled bottle conjugate was well put in red-labelled, following pipette for each one 100uL of controls, later on transferred to the red-marked wells in the form of:

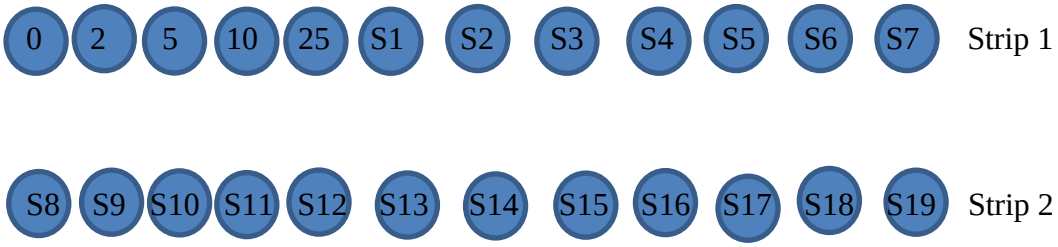


Figure 3.4. Well holder

The wells was mixed 3 times using a 12-channel pipettor. 100uL has been added to the wells coated with antibodies. The set-up was mixed for 10-20 seconds by sliding back and forth the microwell holder on a flat surface. It took care not to sprinkle the reagents from the wells. The set-up has been incubated for 10 minutes at room temperature. The red-marked mixing wells were discarded. The antibody wells contents were shaken out. The later on wells are washed by distilled water for 5 time, the wells are rubbed by towel of paper until the remainder of the water was drained. Some volume of substrates were poured into the green-labelled reagent boat from the green-labelled bottle. Using new tips on the 12-channel pipettor, the substrate's 100uL was primed and piped into the wells. This was blended for 10-20 seconds by sliding back and forth on a flat surface. For 10 minutes, the mixture was incubated. The rest of the substrate was discarded and water rinsed the reagent boat. Red-stop solution was poured from Red Stop solution into red-labelled reagent boat. Excess substrate was expelled from the pipettor of 12 channels, its tips were primed` and 100uL red stop dropped into each well. This was mixed on a flat surface by sliding back and forth. The 12-channel pipettor tips have been removed. The microwell bottom was

cleaned with a dry cloth and read using a 650 nm filter in a microwell scanner. Air bubbles were removed because the analytical findings could be affected. Results were read in 20 minutes after the Red-stop was applied.

2.7. Detecting positive Sample

According to the manufacturer's instruction, the values of ochratoxin above(10) ppb were considered as positive sample

2.8. Statistical Analysis

Fore data analysis SPSS software were used in addition, because of the data pertaining non-parametric and there are all parameter contain quantitative (continues) data therefor for all type of food Frequency test option, in addition to compare mean test perform in order to obtain (minimum, maximum, mean, standard deviation). Was the best option for comparison our result to standard of KIT Veratox (control group) and evaluate our result to be positive and negative.

CHAPTER THREE

3. RESULTS

Table 3.1 Shows the level of aflatoxin in the different foods sampled. Tea had the highest number of samples with aflatoxin 4(100%) and spices had the lowest level of aflatoxin 1(16.7%). The overall prevalence of aflatoxin in the total samples was 6(6.8%).

The samples of this study were for Cereal(Rice, Black eyed bean, Mung beans, White beans, Chichpeas, Qarakharman (immature wheat burned), Oat, Barly, Wheat, Bulgur wheat, Lentil, Indomie, Soyabeans, Coconut shredded dry). Chips (Chips, Pringles). Nuts (Almond, Sunflower seed, Pistachio, Peanut, Cashew, Walnut, Hazelnut). Biscuit, Cake and Flour (Biscuit, Popcake, Flouer). Spices (different types of Spices, Chilli pepper, cinnamon). Starch and corn (Starch, Corn). Tea (different black tea). Seeds (Flaxplant, Fennel seed). Dried fruit (Apricot).

The positive sample of this study according to the kit were all Black tea samples and one of Coffee samples.

All sample and their results in parts per billion are shown in (Appendix 2).

Table 3.1: Incidence of Aflatoxin in Different Food Samples

Types of samples	N	+ ve	Incidence (%)	0.00 -19	20-29	30-49	50-100	>100	Minimum	Maximum	Mean	STD
Chips	14	0	0	0	0	0	0	0	0.000	0.000	0.000	0.000
Biscuit	10	0	0	3	0	0	0	0	0.211	0.278	0.060	0.102
Spices	6	1	16.7	3	0	0	1	0	1.155	84.911	17.926	33.208
Nut	14	0	0	3	0	0	0	0	0.007	0.496	0.055	0.144
Tea	4	4	100	0	0	1	3	0	37.188	69.979	54.584	13.750
Coffee	3	1	33	2	0	0	0	1	4.361	146.961	53.778	80.748
Cereal	25	0	0	7	0	0	0	0	0.004	1.662	0.094	0.334
Starch	5	0	0	2	0	0	0	0	0.170	1.767	0.388	0.774
Spaghetti	4	0	0	2	0	0	0	0	0.000	0.177	0.044	0.088
Seed	2	0	0	2	0	0	0	0	0.069	2.618	1.343	1.802
Dried fruit	1	0	0	1	0	0	0	0	6.445	6.445	6.445	

Table 3.2 shows the level of Ochratoxin in the different foods sampled. Tea had the highest level of Ochratoxin 4(100%), followed by dried food 1(50%) and cereal had the least level of ochratoxin 1(4.2%). The overall prevalence of Ochratoxin in the total samples was 15(17.4%). The samples of this study were for Cereal (Rice, Black eyed bean, Mung beans, White beans, Chichpeas, Qarakharman (immature wheat burned), Oat, Barly, Wheat, Bulgur wheat, Lentil, Indomie, Soyabeans).

Chips(Chips, Pringles). Nuts(Almond, Sunflower seed, Pistachio, Peanut, Cashew, Walnut, Hazelnut). Biscuit, Cake and Flour (Biscuit, Popcake, Flouer). Spices(different types of Spices, Chilli pepper, cinnamon, Blackpapper). Starch and corn (Starch,Corn). Tea (different black tea). Seeds (Flaxplant, Fennel seed). Dried fruit(Apricot, Lime tree). The positive sample of this study according to the kit were all Black tea samples and 1 of each Cereal and Dried Fruits, 3 of Nuts, 4 of spice samples

All sample and their results in parts per billion are shown in (Appendix 3).

Table 3.2: Incidence of Ochratoxin in the Different Food Samples.

Types of samples	N	+ ve	Incidence (%)	0.01 -9	10-29	30-49	50-100	>100	Maximum	Minimum	Mean	SD
Chips	10	2	20	8	2	0	0	0	21.554	0.03	4.751	7.144
Biscuit	9	0	0	8	0	0	0	0	3.89	0.07	0.689	1.259
Spices	13	4	30.7	0	3	0	0	1	202.25	1.05	23.34	54.98
Nut	13	3	23	10	2	1	0	0	40.05	0.05	7.168	12.87
Tea	4	4	100	0	3	0	1	0	66.694	18.591	36.465	21.745
coffee	2	0	0	2	0	0	0	0	2.24	0.41	1.320	1.293
Cereal	24	1	4.1	11	0	0	1	0	78.40	0.12	4.587	15.890
Starch	4	0	0	1	0	0	0	0	9.15	0.000	2.287	4.574
Spaghetti	2	0	0	0	0	0	0	0	0.000	0.000	0.000	0.000
Seed	3	0	0	2	0	0	0	0	0.95	0.67	0.540	0.487
Dried fuit	2	1	50	0	1	0	0	0	60.920	1.915	31.417	41.722

CHAPTER FOUR

4. DISCUSSION

Mycotoxins are some fungi's highly toxic secondary metabolites. *Aspergillus, Penicillium* and *Fusarium* are among the genera of mycotoxigenic fungi. Mycotoxin identification relies on accurate and reliable methods. Typical analytical methods require different techniques of separation and detection, all of which require an adequate phase of sampling. The methods focused on immunology are preferable because they reduce the time and money needed to evaluate natural toxicants (Baydar, et al., 2005).

ELISA is a highly sensitive and precise technique that can also be used to examine a number of samples. Because of the obtainability of test kits for almost all specific mycotoxins, they are usually used for routine measurement of mycotoxins. It is an algorithm that is commonly used, based on specific antibodies. This requires antigen and antibody reaction in wells of micro-plate. All the samples were tested in this research using ELISA Veratox kits for quantitative aflatoxin and ochratoxin determination. This is the first study to detect aflatoxin and ochratoxin in commonly consumed foods in our country,Iraq.

A total of 174 samples were collected in order to examine and screen by using ELISA kits (88 for aflatoxin and 86 for ochratoxin). As shown in Table 1, six (6) samples contained aflatoxin. Tea had 4 positive tests, 4(100%), 1(33%) coffee and 1(16.7%) spices. Such findings are in line with (Abdel-Hafez and El-Maghraby, 1992) tests. Of the three samples among coffee, one was positive for aflatoxin. However, 2 samples had aflatoxin level between 0.00-19ppb while 1 has aflatoxin level greater than 100ppb.Three (3) samples each of Biscuit, Spices and Nut had aflatoxin concentration between 0.00-19ppb. Cereal had 7 samples within the level of 0-00-19ppb. Two (2) samples each of starch, spaghetti and seed had aflatoxin level between 0.00-19ppb. Dried fruit had 1 and chips had none.

However, the detection of total aflatoxin in 27 chips was reported by (Catak, et al., 2020) using HPLC and post-column UV derivation system in Turkey. They said that at detectable levels 17 of their samples had aflatoxins and 10 did not. According to the Turkish Food Codex, gross aflatoxin levels were contained in 3 chips above the maximum levels. (Amir, et al., 2014) in Iran reported multiple studies having low mean concentration in spices but high incidence of aflatoxin. They measured AFB1 contamination in spices such as black pepper, chili powder and whole black pepper sold in Iran and India markets and found that they were all polluted with AFB1. Most of the foods sampled in our study had aflatoxin levels within the tolerable limits.

Our research project result was found supported by previous studies on the amount of contamination of aflatoxins in cereals.(Lutfullah and Hussain, 2012) from Pakistan reported an average of 4.6 and 10.4ug / kg for 20 rice and 15 corn samples analyzed for aflatoxins. A study in France reported higher levels of aflatoxin. The variability may arise from variations in the origin and harvest year of the raw materials that may influence the mycotoxin content (Kuiper-Goodman, 1999). It has also been documented that inappropriate storage may result in the contamination of mycotoxins in cereals (Reddy, et al., 2009). Therefore, the explanation for the positive samples in this study may be due to the fact that we import most of our food from other countries and they have been in storage or transportation problems for a longer time. (Abdallah, et al., 2019) reported that AFB1 was present in Egyptian maize at levels above national and international limits.

There are 86 samples and around 15 (17.4%) were found to contain ochratoxin as shown in Table 2. Tea had four (4) positive samples for ochratoxin 4 (100%), dried fruit 1(50%), spices 4 (30.7%), Nut 3 (23%), Chips 2(20%) and Cereal 1 (4.1%). Two (2) samples each of Coffee and seed had ochratoxin level within 0.01-9 ppb. Eight (8) samples each of Chips and Biscuit had ochratoxin level between 0.01-9 ppb. Nut had 10 samples between 0.01-9ppb while cereal had 11 samples within the range 0.01 -9 ppb. Tea and spices had 3 samples each between 10-29 ppb and Chips and nut had 2 samples each within the level of 10-29 ppb. Dried fruit had 1 sample within this range. In Hungary, (Fazekas, et al., 2005) reported detection of OTA in 32 out of 70

ground red pepper samples (45.7%). Of the other spices tested, only one single sample contained OTA. These results were consistent with our findings where ochratoxin had a positive value for chili pepper. (Abdallah, et al., 2019) said OTA in Egypt is not a frequent contaminant of food and feed. According to them, this is supported by a recent survey from Upper Egypt, where animal feed and maize screened a wide range of fungal metabolites. The majority of the foods sampled contain ochratoxin within the tolerable limit (Hussein, et al., 2019).

Ochratoxin is a highly concentrated contaminant in food and feed and is usually found in all cereals and cereal goods, as well as in chocolate, cocoa, spices, nuts, wine and beer. A European report estimated 44 percent of cereals, 10 percent of wine, 9 percent of coffee contributions to total human exposure to ochratoxin. 7% of milk, 5% of chocolate, 4% of dried fruit, 3% of meat, 3% of spices and 15% of others(Heussner and Bingle, 2015). The mean levels of ochratoxin in European foods are relatively low, and there may be high concentrations in individual lots. There may be even higher levels of contamination in other countries where food screening is rare and obsolete storage and transport practices are still in use. According to the European legislation on aflatoxin, the total limits for aflatoxin were set at 4ppb. From this analysis, only cereals had an aflatoxin level of 7 ppb; the other samples had levels below 4 ppb. This result agrees with the findings by (Baydar, et al., 2005).

From their study, (Shamma, et al., 2014) reported similar results. They said that in maize and maize products there was a high frequency of aflatoxins. Tea fungal contamination may occur at any stage of tea production. This is because the warm and humid environment which favours the production of tea also favours fungal growth.

Our finding also agrees with (Malir, et al., 2014), who in black tea from the Czech Republic reported an extremely high concentration of ochratoxin. All black tea examined from Portugal were hundreds of ppb infected with fumonisins (Martins, et al., 2001). ELISA's study of Spanish white and red tea samples showed very high levels of aflatoxins, ochratoxin A and other mycotoxins, according to (Santos, et al. 2009).

The cumulative incidence of aflatoxin resuls was (6.8 %), in addition our experimental result for ochratoxin was (17.4 %) obtained in this study and there is clearly we can noted a high level of both toxin was found in different food, moreover the important thing should awaken our understanding of the need to both assess and evaluate mycotoxin levels in common foods.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1. CONCLUSIONS

There are no doubt that this dissertation findings has direct relevant with human health. Fungi found everywhere in the world, however occurrence of it depend mostly on temperature and moisture therefor different place have dissimilar amount of fungi. The fact is that all types of fungi produce amount of harmful secondary metabolites which called mycotoxin. For example Aflatoxin and ochratoxin are types of mycotoxin both of them can be find in our dayly food like: rice, beans, wheat, flour, wine, beer, all natural dry fruits, meat, and milk. So, eating these product which polluted with such mycotoxin surly affect individuals heath. For instance aflatoxin cause liver cancer and ochratoxin cause impair of kidney if they consume fore along time if it was in small amount, or some time they have sever affect if they consume in large amount might cause death. Therefore, the result and what finding in this research should be helpful for regulatory agencies, disseminated to local farmers to practice strict measures, the farmers should also have good storage conditions and practice. Also Public enlightenment needed to be conducted to improve awareness about mycotoxins across different sectors. Conduct of epidemiological studies to determine acute and chronic health effects of mycotoxin exposure. Better description of the prevalence and level of mycotoxin exposure in the regions of the world most affected. This will serve as the basis for prioritising control and estimating risks. Development of accurate and applicable mycotoxin testing kits, sampling equipment for use in low-income countries. This will permit rapid assessment of mucotoxin in staple foods. There is the need to enhance the capacity to perform mycotoxin analysis in order to manage emergency situations and promote public health.

5.2. RECOMMENDATIONS

1. Farmers should have good storage conditions and farming practice.
2. It is necessary to conduct public enlightenment to increase awareness of mycotoxins across different sectors.
3. Epidemiological studies should be performed to evaluate the acute and chronic health effects of exposure to mycotoxin.
4. The commonly consumed foods should be routinely detected for mycotoxin contaminations.
5. To create awareness about the occurrence of mycotoxins in food and their related health risks
6. To recommend measures to be taken to avoid consumption of mycotoxins in foods.

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APPENDEX Appendex A

Cereal	Final R	chips	Final R	Nut	Final R	biscuit,cak,flour	Final R	spices	Final R	Staech,corn	Final R
rice	0.000	Chips	0.000	Almond	0.000	Biscuit	0.000	spices	0.000	corn	0.000
rice	0.000	Chips	0.000	Sunflower	0.000	Biscuit	0.000	spices	10.5	corn	0.000
rice	0.000	Chips	0.000	Pistachio	0.000	Biscuit	0.000	spices	0.000	Starch	1.767
rice	0.000	Chipse	0.000	Almond	0.000	Popcake	0.000	pepper	1.155	corn,	0.000
rice	0.000	Chips	0.000	Almond	0.000	Biscuit	0.000	Cinnamon	84.911	Corn	0.170
Black eyed beans	0.000	Chips	0.000	Pea nut	0.000	Biscuit	0.000	spices	10.988		
Mung bean	0.000	Chips	0.000	Cashew	0.000	Biscuit	0.000				
White bean	0.120	Pringles	0.000	peanuts	0.000	Biscuit	0.112				
Chichpea	0.000	Chips	0.000	Walnut	0.000	Biscuit	0.203				
White beans	0.000	Chips	0.000	Sun flower seed	0.000	Flour	0.278				
White beans	0.000	Chips	0.000	Sun flower seed	0.000						
wheat burned	0.027	Chips	0.000	Hazelnut	0.007	Tea	Final R	spagetti	Final R	coffe	Final R
Oat	0.004	Chips	0.000	Walnut	0.496	tea	69.979	spaghetti	0.000	coffee	146.961
Barley	0.000	Chips	0.000	walnut	0.262	tea	52.009	spaghetti	0.000	coffee	4.361
Wheat	0.000					tea	59.163	spaghetti	0.000	Nescafe	10.013
Chick peas	0.000					tea	37.188	spaghetti	0.177		
Bulgur wheat	0.000	Seed	Final R	Dried fruit	Final R						
White Oats	0.000	Flax plants	0.069	Apricot dried	6.445						
Lentil	0.335	Fennel seed	2.618								
Indomie	0.000										
Indomie	0.000										
chick peas	0.061										
Soya bean	0.000										
Soya protein	1.662										
Coconut dry	0.145										

Appendex B

Cereal	Final Result	Spices	Final Result	NUT	Final Result	chips	Final Result	flour,biscuit	Final Result
rice	0.000	spicies	6.866	pistachio	0.821	chips	0.907	Biscuit	3.893
rice	0.000	cinnamo	11.812	Almond	2.075	chips	0.000	Biscuit	0.000
rice	0.941	Red pepper	4.421	Cashew	3.418	Pringles	3.704	Biscuit	0.000
rice	1.099	spicies	1.923	Pea nut	14.683	chips	0.000	Biscuit	1.040
rice	9.556	spicies	1.054	Walnut	1.530	corn chips	11.099	Biscuit	0.074
rice	0.000	spicies	28.911	almond	0.000	chips	0.595	Biscuit	0.000
Brown rice	0.000	cumin spices	1.371	Walnut	40.046	chips	21.554	Biscuit	0.617
Chick-pea	0.000	spicies	202.245	Walnut	28.596	chips	0.562	Flour	0.578
Indo mie	0.000	spicies	1.652	Almond	0.201	chips	9.068	Pop cake	0.000
Bulgurt	2.306	spicies	37.218	Pea nut	0.403	Cipso	0.026		
wheat burned	4.381	spicies	1.700	sun flower	0.968				
White oate	4.381	spicies	0.000	Hazel nut	0.050				
Indo mie	0.000	Black pepper	4.303	Sun flower	0.403				
Barely	3.169								
Oats	0.299	TEA	Final Result	starch,corn	Final Result	seed	Final Result		
Chick pea	0.000	tea	66.694	Corn	0.000	Sesame seed	0.948		
Lentil	0.000	tea	18.591	Corn	0.000	Flax	0.000		
Chick pea	0.000	tea	22.906	Corn	0.000	Fenugreek	0.674		
Black eyed beans	1.255	tea	37.670	Corn	9.148				
White bean	0.117								
White beans	4.188	coffee	Final Result	dried fruit	Final Result	spagetti	Final Result		
Mung beans	0.000	coffee	2.235	Apricot	1.915	spaggetti	0.000		
chick pea	78.396	coffee	0.406	Lime tree	60.92	spaggetti	0.000		
Soya protein	0.000								

CURRICLUM VITAE

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Education level

	Name of the institution	**Year**
Post graduate /speciliazation	**Medical & clinical microbiology Near East University**	
Under graduate	**Biology /Salahadin university**	**1997**
High school	**Shireen secondary school**	**1993**

Job experience

Duty	**institution**	**Duration**
Teacher	**Khurmal secondary school**	**1998-2001**
Teacher	**Shiren secondary school**	**2001-2006**
Teacher	**Institution of computer**	**2006-2011**
Employee	**Ministry of higher education**	**2001-2013**
Assist-researcher	**Kurdistan Institution for Strategic Studies and Scientific Research**	**2013- now**

Foreign Language

Languages	**Reading**	**Speaking**	**Writing**
Arabic	**Very good**	**Very good**	**Very good**
English	**Good**	**Good**	**Good**

Computer knowledge

Program	**User proficiency**
All seven modules in Microsoft office	**Very good**
Specialized in web design	**Very good**
Photshope	**Good**
Flash software	**Good**