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Assessment of Fungal Contamination and Aflatoxin B1 (AFB1) Levels in Some Staple Food Products Marketed in Benghazi, Libya

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Abstract

Fungal contamination and associated mycotoxin exposure remain important food-safety concerns worldwide. This study aimed to characterize fungal contamination and determine aflatoxin B1 (AFB_1) levels in selected staple food products collected from local markets in Benghazi, Libya. A total of 24 food samples including rice, flour, couscous, and pasta were investigated for moisture content and fungal contamination using conventional culture-based methods. Based on moisture-content screening, 10 representative samples were further analyzed for AFB_1 using competitive ELISA methodology. Fungal isolates identified morphologically included *Rhodotorula glutinis*, *Cryptococcus laurentii*, *Rhodotorula mucilaginosa*, and *Candida ciferrii*, with flour samples showing the highest fungal occurrence. The measured AFB_1 concentrations in the analyzed samples remained below the internationally permissible limit of 2 ppb.

However, the findings should be interpreted cautiously because only a subset of samples was subjected to toxin analysis, while water activity (aw) measurements and advanced ELISA quality-control(QC)validation parameters were not available. The study highlights the need for continuous monitoring, improved storage practices, and expanded mycotoxin surveillance programs in Libya.

Keywords: *Food contamination, Fungi, Mycotoxins, Aflatoxin B1, Relative humidity, Elisa*

الملخص

لا يزال التلوث الفطري وما يرتبط به من التعرض للسموم الفطرية يشكل مصدر قلق بالغ لسلامة الأغذية في جميع أنحاء العالم. هدفت هذه الدراسة إلى توصيف التلوث الفطري وتحديد مستويات الأفلاتوكسين B1 (AFB_1) في منتجات غذائية أساسية مختارة جُمعت من أسواق محلية في بنغازي، ليبيا. تم فحص 24 عينة غذائية، تشمل الأرز والدقيق والكسكس والمعكرونة، لتحديد محتواها من الرطوبة وتلوثها الفطري باستخدام طرق الزراعة التقليدية. بناءً على فحص محتوى الرطوبة، خضعت 10 عينات ممثلة لمزيد من التحليل للكشف عن AFB_1 باستخدام تقنية ELISA التنافسية. شملت العزلات الفطرية التي تم تحديدها مورفولوجيًا *Rhodotorula glutinis* و *Cryptococcus laurentii* و *Rhodotorula mucilaginosa* و*Candida ciferrii*، حيث أظهرت عينات الدقيق أعلى نسبة من التلوث الفطري. ظلت تركيزات AFB_1 المقاسة في العينات المُحللة أقل من الحد المسموح به دوليًا وهو 2 جزء في البليون. مع ذلك، ينبغي تفسير النتائج بحذر، لأن جزءًا فقط من العينات خضع لتحليل السموم، ولم تتوفر قياسات النشاط المائي (aw) ومعايير التحقق (QC) لاختبار ELISA. وتؤكد الدراسة على ضرورة المراقبة المستمرة، وتحسين ممارسات التخزين، وتوسيع نطاق برامج مراقبة السموم الفطرية في ليبيا.

الكلمات المفتاحية: *التلوث الغذائي، الفطريات، السموم الفطرية، الأفلاتوكسين B1 ، الرطوبة النسبية، تقنية إليزا (ELISA)*

1.Introduction

1.1 Concept of fungi: Definition:

Fungi are a group of microorganisms that includes Yeasts, Molds, and Mushrooms. They are eukaryotic organisms that are classified under the kingdom Fungi. They obtain nutrients by secreting enzymes that break down organic matter in their environment, allowing them to absorb these nutrients. Fungi play an important role in recycling nutrients in ecosystems and can be both beneficial (such as in the production of antibiotics and fermented foods) and harmful (such as in causing plant diseases). Fungi can grow on all foods without an exception, whether their moisture content is high or low (although fungal growth requires moisture), and they can grow on crops in the field and after crop harvest and during storage (Essaweet, 2018). Fungi also grow within a wide range of temperatures (15-35 ºC) and cause damage to these commodities due to their exposure to physical (in shape, texture, color, aroma, and taste) and chemical (due to the fungal consumption of nutrients, lowering the food content of organic matter) factors. This does not necessarily mean that every fungus-infected commodity produces aflatoxins because fungal growth requires conditions different from those needed to produce toxins, such as the moisture of the infected commodity, medium temperature, medium oxygen content, and other required conditions(Essaweet, 2018).

1.2 Common types of fungi in foodstuffs:

Rhodotorula glutinis, a type of yeast, can have a surprising impact on foodstuffs. It can be both a beneficial ingredient and a potential spoiler, depending on the situation. a yeast species commonly found in a range of environments including soil, water, and air, is known for its distinct pink to orange colonies and spherical to oval cells that reproduce through budding. This yeast species is renowned for its production of carotenoid pigments like beta-carotene.(Kumar et al., 2021) Negative Impacts: Food Spoilage: While not as common as some other yeasts, *Rhodotorula glutinis* can grow in some food products under certain conditions, leading to spoilage. This is more likely in acidic or high-sugar environments. *Rhodotorula glutinis*, a common yeast species, is typically harmless to humans and not considered pathogenic. However, in individuals with weakened immune systems, such as those with HIV/AIDS or undergoing chemotherapy, *Rhodotorula species* can cause opportunistic infections, including rare cases of bloodstream infections and pneumonia. A study by Luo et al. (2012) found that *Rhodotorula species* were responsible for 1.8% of bloodstream infections in hospitalized patients, highlighting the potential risk for immunocompromised individuals. Overall, the harm of *Rhodotorula glutinis* depends on the individual's immune status, with healthy individuals generally not at risk but caution advised for those with compromised immune systems. (Luo & Mitchell, 2012; Ajibade, 2023) *Candida ciferrii*, a type of yeast within the *Candida* genus, can be typically found in different natural environments like soil, plants, and food. This yeast is recognized for its capability to create enzymes like lipase and protease.(Zaki, 2012) *Candida ciferrii*, a type of yeast, can wreak havoc on foodstuffs, causing spoilage and reducing their quality. Here's a breakdown of its negative impacts: Food Spoilage: *C. ciferrii* is a notorious culprit behind food spoilage. It thrives in a wide range of environments, including acidic and sugary foods, fruits, vegetables, beverages, and even processed meats.(Murillo-Amador et al., 2006) Its growth can lead to unpleasant changes in texture, flavor, and aroma, rendering the food inedible. Reduced Shelf Life: Due to its spoilage potential, *C. ciferrii* significantly shortens the shelf life of food products. This can lead to economic losses for food manufacturers and retailers, and increased food waste for consumers. Pathogen Formation: While *C. ciferrii* itself isn't usually considered pathogenic (disease- causing), its presence can create favorable conditions for the growth of harmful molds and bacteria. This can pose a potential health risk to consumers. *C. ciferrii's* negative impact on foodstuffs is significant. It contributes to spoilage, shortens shelf life, and can indirectly create conditions for the growth of pathogens.(Magan et al., 2004) *Rhodotorula mucilaginosa* is a commonly found yeast species in diverse environmental samples such as soil, water, and air. It is recognized for its orange to pink coloration and its ability to create extracellular polysaccharides, which give it a distinctive slimy texture. This yeast has been detected in clinical specimens, where it has been linked to infections in individuals with weakened immune systems.(Mutegi et al., 2018) A study conducted by Meroth et al., (2018) identified *R. mucilaginosa* as a prevalent species in biofilms on silicone urinary catheters, showcasing its adaptability to different environments. Research on the genetic diversity of *R. mucilaginosa* by Arvanitis et al., (2019) revealed the presence of various genotypes within the species.(Harrison et al., 1993) *Cryptococcus laurentii* is a type of yeast commonly found in soil, plants, and water. It can cause infections in people with weakened immune systems, such as those with HIV/AIDS or cancer. This yeast is identified by its round to oval cells with a visible capsule and can be grown on specific media containing L-canavanine.(Verma, 2004) Infections from *C. laurentii* can lead to meningitis, pneumonia, and widespread infections in susceptible individuals. Treatment usually involves antifungal drugs like amphotericin B and fluconazole, but some strains may be resistant to these medications.(Sharafi et al., 2022) Saccharomyces cerevisiae (Baker's yeast): This single-celled fungus is crucial in bread baking. It ferments sugars present in the dough, releasing carbon dioxide which causes the dough to rise ("Yeast: A Microbial Sail," 2016).

1.3 list most common type of fungi produce toxin:

Many fungi produce toxins known as mycotoxins. These toxins can be harmful to humans and animals if they are consumed. Here are some of the most common fungi that produce mycotoxins:

1. Aspergillus spp.: Aspergillus fungi are common indoor molds that can produce mycotoxins, such as aflatoxins. These toxins are carcinogenic and can cause various health issues in humans and animals. They can contaminate crops such as corn, peanuts, and cottonseed. (Frisvad & Samson, 2005)

2. Penicillium spp.: This genus of fungi produces several mycotoxins, including ochratoxin A and patulin. Ochratoxin A is a carcinogen that damages the kidneys. Patulin can cause nausea, vomiting, and stomach cramping. Penicillium can contaminate grapes, apples, grains, and nuts., which can be harmful to humans and animals.(Pitt, 1998)

3. Fusarium spp.: This genus of fungi produces a variety of mycotoxins, including trichothecenes, fumonisin, and zearalenone. Trichothecenes can cause vomiting, diarrhea, and immune suppression. Fumonosin can damage the liver and kidneys, while zearalenone can disrupt hormones. Fusarium can contaminate corn, wheat, barley, and oats.(Munkvold, 2008)

4. Claviceps purpurea: This fungus is responsible for ergot poisoning, a condition that can cause hallucinations, convulsions, and even death in humans. It grows on rye and other cereal grains. These compounds are toxic and can cause ergotism in humans and animals. Ergotism is a serious condition that can result in hallucinations, convulsions, and even death.(Tudzynski & Polli, 2001)

5. Amanita spp: Amanita mushrooms are known for their beautiful appearance, but some species of Amanita can produce deadly toxins, such as amatoxins. Ingesting these toxins can cause severe liver and kidney damage, and even death.(Enjalbert & Reymond, 2005)

1.4 toxic fungi:

Fungi produce secondary metabolites known as mycotoxins, which have serious health effects on animals and humans, primarily through oral exposure. Mycotoxins are widely found in agricultural crops, such as corn, wheat, and nuts, and their food products. Molds can produce more than one type of toxin, which are generated by various fungi under specific climatic conditions (Zain, 2011).

Toxicities related to mycotoxin ingestion can be classified as follows:

(i) acute—characterized by rapid onset and an obvious toxic response, including rapid death.

(ii) Chronic —resulting from low-dose mycotoxin exposure over extended periods, causing toxic reactions such as cancer (Ostry et al., 2017).Aflatoxins (AFs) are among the most dangerous microbial toxins produced by fungi of the genus Aspergillus, with biological effects including carcinogenicity, mutagenicity, teratogenicity, and hepatotoxicity (Ghasemi-Kebria et al., 2013). Among the 300 different mycotoxins, aflatoxins are the most toxic and well-studied worldwide, including Aspergillus flavus, Aspergillus parasiticus, and Aspergillus minaceus (Fallah et al., 2009; Sharafi et al., 2022). Mycotoxins, potent carcinogenic compounds produced by various Aspergillus and Penicillium species, pose a significant risk to food safety (Sulaiman et al., 2007). Among these, aflatoxin B1 (AFB_1) is the most carcinogenic (Moradi et al., 2015). Exposure to mycotoxins leads to severe health issues and mortality, with infectivity influenced by biological factors, harvesting, storage, processing, and climate (Milani, 2013). Rigorous monitoring and control of mycotoxins, including aflatoxins, ochratoxins, and patulin, are essential for ensuring food safety (Ajibade, 2023). Aflatoxin contamination occurs both pre- and post-harvest, during curing, storage, and transport, representing a critical food security concern, particularly in regions such as Sub-Saharan Africa, including Libya. Numerous studies have identified aflatoxin-producing fungi across diverse food types, with nine species documented, including Aspergillus flavus, A. niger, A. parasiticus, A. wentii, and several Penicillium species (Kurata et al., n.d.). These aflatoxins (AFs), including AFB_1, AFB_2, AFG_1, AFG_2, AFM_1, and AFM_2, are characterized by their blue or green fluorescence under UV light (Fung & Clark, 2004). Corn, rice, and animal feeds are frequently contaminated with aflatoxins, which are stable and widely distributed, negatively impacting livestock and human health (Hu et al., 2023). AFB_1, classified as a Group 1 carcinogen by the International Agency for Research on Cancer, is a primary causative agent of liver cancer in humans (Loi et al., 2023). Consequently, the impact of toxigenic fungi on grain products represents a substantial public health threat (Fig 1 and Table 1).

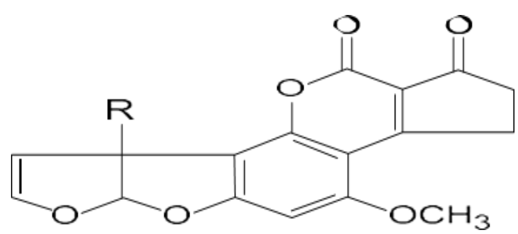

Figure 1. Structures of mycotoxins: Aflatoxins B1

Table 1. Grains that are contaminated with mycotoxins

Mycotoxin	Grain
Aflatoxins	peanuts, corn, wheat, cottonseed, nuts
Ochratoxin	wheat, barley, oats, coy, dry beans
Fumonosin	corn, wheat, sorghum, barley, oats

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Zearalenone	maize, corn, wheat, barley, rye
Deoxynivalenol	corn, wheat, barley, oats
Citrinin	wheat, barley, corn, rice

1.5 The impact of environment on the toxic fungi:

Climate is the most important determinant of fungal growth and mycotoxin, and temperature and moisture content are important factors affecting cereals such as rice, nuts, pasta, wheat, and dry beans. (Yilmaz Eker et al., 2023) It may be difficult to completely prevent mycotoxins from forming in contaminated commodities, particularly those produced in tropical and subtropical climates where high temperatures and humidity promote the growth of fungi during drying, processing, transportation, and subsequent storage.(A. S. et al., 2015) Furthermore, interacting climate-related abiotic conditions, including water availability, and perhaps atmospheric CO2, can affect mycotoxins contamination levels in food crops.(Perrone et al., 2020; Milani, 2013) (such as temperature and moisture content), at (Table 2) mycotoxins, is influenced by humidity (Pitt et al., 2018).

Table 2. Optimum temperatures for mycotoxins production

Mycotoxin	Temperature (°C)	Water activity
Aflatoxins	33	**0.99**
Ochratoxin	25–30	**0.98**
Fumonosin	15–30	**0.9–0.995**
Zearalenone	25	**0.96**
Deoxynivalenol	26–30	**0.995**
Citrinin	**20–30**	**0.75–0.85**

The optimum humidity for mycotoxin production varies depending on the type of mold or fungus present. Mycotoxin-producing fungi thrive in high-humidity conditions. Aspergillus species, which produce aflatoxins, require humidity levels of approximately 70-80% for optimal growth and mycotoxin production. (Pitt, 2000) Fusarium species, which produce deoxynivalenol (DON) zearalenone, prefer humidity levels of approximately 85-90% for maximum mycotoxin production.

1.6 The effect of fungi and Mycotoxin on human health:

There is some evidence that aflatoxins can be acutely toxic to humans, but not all fungal growth leads to the production of mycotoxins, and the presence of fungi does not mean that mycotoxins are present(Zaki, 2012). Both humans and animals are at risk from aflatoxin-contaminated foods and feeds. Of the five major mycotoxins that are most toxic to mammals, aflatoxins (B1, B2, G1, and G2) have the highest toxicity. As a result of adducts between deoxyribonucleic acid (DNA) and guanine, aflatoxin induces cancer cell formation.(Khlangwiset et al., 2011) It is well established that not all molds are toxic, nor are all their secondary metabolites Among the most significant mycotoxins in terms of public health and agroeconomics are aflatoxins (AF), There are millions of dollars in losses each year caused by these toxins in human, animal, and agricultural health around the world. Human or animal mycotoxicoses are classified as food-related, non-transferable, non-infectious, and not traceable to microorganisms other than fungi. (Mutegi et al., 2018) Natural AFs (AFB1, AFB2, AFG1, and AFG2) were classified as "carcinogenic to humans" in 2012. In 1993, AFM1 was classified as a Group 2B substance that is potentially carcinogenic to humans. Additionally, aflatoxins can cause acute toxicity, hepatotoxicity, immunosuppression, mutagenesis, teratogenicity, cytotoxicity, neurotoxicity, reproductive dysfunctions, stunted growth, and epigenetic changes.(Harrison et al. (1993) reported that liver microsomes metabolize AFBI into an epoxide in the 8–9 range. The body can transport and excrete this less toxic metabolite by binding to glutathione (Verma, 2004). Aflatoxins are hepatotoxic, liver carcinogenic, and mutagenic. Its action is mainly due to the formation of adducts with DNA, RNA and proteins. In addition, it can cause lipid peroxidation and oxidative DNA damage (Sharafi et al., 2022). These compounds are carcinogenic, mutagenic, and teratogenic, and they cause acute and chronic toxicity in humans (Nemati et al., 2010). Mycotoxins have been associated with several human diseases, some acute and others chronic, and several of these diseases are listed in Table 1. Although mycotoxins have been implicated in these human illnesses, a direct connection has rarely been established, and much remains to be done to establish the etiology of suspected human mycotoxicoses. Beardall and Miller (1994) provided a detailed account of human illnesses associated with mycotoxin ingestion. The many interacting factors in the pathogenesis of mycotoxicosis make diagnosis difficult, as does the confirmation of mycotoxin exposure. Chronic intake is the most widespread form of mycotoxin exposure, and its consequences for human health are discussed in the following sections.

Throughout history there are instances, especially following flood, famine and war, when acute mycotoxicosis have devastated human populations.(Matossian, 1998) Acute disease episodes have recently occurred following high levels of mycotoxin ingestion. Acute liver disease has been reported in India (Bhat, 1991), Malaysia (Shephard, 2004), and Kenya (Bhat et al., 1997) following aflatoxin consumption. Bhat et al. (Luo, 1988) reported gastrointestinal pain and diarrhea during an outbreak of foodborne disease associated with high fumonisin intake in India. Gastrointestinal symptoms including vomiting were apparent in humans after high levels of DON intake in China.(Bhat et al., 1989) A similar outbreak was observed in India when local villages consumed rain damaged wheat that contained DON and other trichotheces.(Hagler et al., 2001) There have been suggestions that aflatoxin caused premature menarche in young girls in South America but these reports have not been substantiated.(King, 1979) Since the Middle Ages, episodes of ergotism have been reported in human populations in Europe and North America, with the most recent outbreak of gangrenous ergotism occurring in Ethiopia in 1978. (Matossian, 1998).

1.7 Chronic effects of mycotoxins in human populations:

In many regions of the world, dietary staples, especially cereal grains contain low levels of mycotoxins. The impact of regular low-level intake of mycotoxins on human health is likely to be significant with a number of possible consequences including impaired growth and development, immune dysfunction and the disease consequences of alterations in DNA metabolism.

1.8 Immunosuppression Aflatoxin:

Cause immunosuppression and increase the susceptibility of human to infectious disease.(CAST, 2003) Substantial evidence exists that mycotoxins can be immunotoxins and exert effects on cellular responses, humoral factors, and cytokine mediators of the immune system. (Fallah et al., 2009; Bondy & Pestka, 2000)

The effects on immunity and resistance are often difficult to recognize in the field because signs of disease are associated with infection rather than the toxin that predisposes the individual to infection through decreased resistance and/or reduced vaccine or drug efficacy. (Oswald et al., 2005) Moreover, in animal models, the immunosuppressive effects of toxins occur at lower levels of intake than the toxin's effects on other parameters of toxicity, such as feed intake and growth rate.

1.9 Economic impact of aflatoxin contamination:

There is a significant economic impact associated with aflatoxin contamination because of its carcinogenic nature. There is an increased risk of aflatoxin accumulation in cereals due to drought, which is caused by the cracking of pods and the ingress of A. flavus and A. parasiticus. During warm periods, dew, rain, and delayed harvest are associated with higher aflatoxin levels. During boll opening, crops receiving more than 50 mm of rain had higher levels of aflatoxins(Milani, 2013) Aflatoxin production is also widely affected by various nutritional factors, including carbon, amino acids, nitrogen, lipids, and a few trace elements. Carbohydrate-rich substrates support more fungal growth than oil because carbohydrates provide carbon, which is required for good fungal growth. Carbohydrate-rich substrates support more fungus growth than oil because carbohydrates provide carbon, which is needed for good fungus growth.(Kumar et al., 2021) Maize, sorghum, wheat, and barley are among the most susceptible commodities to AF contamination. As the world's population grows, the demand for food will increase (Ajibade, 2023). Crops can be contaminated by fungi during harvesting, storage, and transportation, resulting in the development of mycotoxins. (Kumar et al., 2021) Mycotoxins affect 25% of the world's crops, most of which contain aflatoxins. (Cassava, cottonseed, chili peppers, maize, wheat, millet, rice, sesame, and sunflower seeds) and other spices that are improperly stored contain them. Two phases of contamination can occur in crops("Aflatoxin Toxicity," n.d.): crops are infected by Aspergillus species during growth and development. If exposed to warm and humid conditions or severe drought, contamination may occur during storage or transport.

1.10 The chemical nature of aflatoxin:

It is a highly fat-soluble compound that can be absorbed by exposed areas, such as the skin. B. Gastrointestinal and respiratory tracts. It can be absorbed into the bloodstream from the gastrointestinal and respiratory tracts and can move throughout the body (Bbosa et al., n.d.). Exposure to Aflatoxin B1 can penetrate cell membranes and attach to DNA, causing irreversible mutations (Moradi et al., 2015). Among the natural carcinogens, aflatoxins produced by *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* are the most potent. They cause a mutation (transversion) of the 249th codon of the P53 gene. In large doses, aflatoxins cause direct damage to the liver, whereas small long-term doses have immunologic or nutritional effects (Atanda, 2011). However, both doses result in liver cancer due to the accumulation of aflatoxin.(Kumar et al., 2021).

1.11 Detoxification methods of fungi:

Several efficient techniques have been developed to prevent or remove AFB_1 from contaminated commodities because it affects food safety. Several control techniques are used before harvesting crops, while others are used after harvesting (pre-harvesting and post-harvesting). Several pre-harvest strategies can be employed, including pesticide application, crop rotation, planting timing, and the use of genetically modified seeds that can withstand Aspergillus infection and environmental stress. Post-harvest strategies include proper drying, packaging, storage, and the use of preservatives and pesticides. In spite of the fact that those measures can be used to prevent contamination, studies have shown that they are ineffective. Once the crop has been contaminated, additional advanced strategies are used. These techniques use biological or chemical additives that are added to AFB_1-contaminated crops to reduce or eliminate AFB_1, in addition to physical processes. Several strategies are used in this review to detoxify contaminated crops, including physical, biological, chemical, and sorbent treatments.

1.11.1 Physical strategies Physical detoxification:

Includes heating, irradiation, electrolyzed water, and pulsed light technology for removing AFB_1.

1.11.2 Heat treatment is commonly used to heat food/feed for aflatoxin B1 decontamination in modern industries. However, this is inefficient because aflatoxins are highly heat-resistant. To remove interesting quantities of AFB_1, it is necessary to heat it up. Temperatures between 150 and 200°C can remove large amounts of AFB_1 when humidity levels are high (Horner et al., 2008). Aside from being cheap, quick, and easy to perform, heating has other advantages. However, the temperatures used can also affect other nutrients in food, which is a disadvantage. In addition to mycotoxin concentrations, binding ranges between mycotoxins and food or feed products, heat penetration, moisture content, and processing conditions affect AFB_1 reduction.

1.11.3 Chemical treatment Chemical:

Additives can be used to decontaminate AFB_1 contaminated food. Ammonia, Hydrochloric acid, lactic acid, and citric acid are some of the most commonly used additives.

1.11.4 Ammonia treatment

Amination is another method for removing aflatoxins from food. This technology can use gaseous ammonia or NH4OH at atmospheric pressure. Ammonification has been shown to be effective in decontaminating AFB_1- contaminated products such as corn (Lee et al., 2015). Several reports have demonstrated that ammonia treatment is effective in reducing aflatoxin B_1 levels within one hour of treatment. Temperature has a strong influence on decontamination effectiveness, as shown in various studies using high-temperature NH4OH or gaseous NH3 (99). % of aflatoxin B_1 was eliminated, whereas AFB1 was not significantly removed at low temperatures such as 25 °C. This strategy is affected by the moisture content and temperature of the products (Bagley, 1979).

1.11.5 Acids treatment During HCl treatment:

A hydrolysis reaction occurs, which is stronger and degrades AFB_1. A study was conducted to examine the degradation of AFB1 by HCl, and the results showed that HCl concentration, temperature, and time are key factors affecting HCl efficiency. The initial conditions were 1 mol·L-1 HCl, 110 °C, and 4 h. The degradation rate was 27.7%, and the degradation rate of AFB1 (33.07 µg kg-1) was 27.6%. After 4 h, as the conditions changed, the result improved to 42.5%. Finally, complete elimination was observed after using L-1 HCl (3 mol) for 8 h and 5 mol L-1 HCl for 4 h at 110 °C (Martinez et al., 1994).

In addition, organic acids have also been shown to be effective in cleaning plants, and their activities are affected by the same factors mentioned previously. In studies of other organic acids, such as citric and lactic acids, acetic acid showed the highest AFB_1 degradation efficiency. The results showed that after 2 h of heating, 85% of AFB_1 was removed (Aly & Hathout, 2011). The combination of acidification and high temperature (80–120 °C) results in the significant degradation of AFB_1.

1.11.6 Ozonation treatment Foods containing AFB1:

AFB_1 can be cleaned with ozone. According to the U.S. Food and Drug Administration (FDA), ozone does not have any side effects as it is primarily used as an oxidant in food processing and is commonly known as (GRAS). (Aiko et al., 2015).

1.11.7 Biological treatment :

Many biological methods have been studied to eliminate or reduce the presence of aflatoxin B1 in foodstuffs and crops. Biological methods are environmentally friendly and effective, making their use more beneficial than that of non-biological methods. Many microorganisms are used in bioremediation methods; therefore, fungi, yeasts, bacteria, extracts, and cold plasma technology are used in bioremediation technology. The contaminated commodity can be detoxified during metabolism and direct contact with AFB_1 by bacteria. AFB_1 is removed from objects contaminated by soil bacteria such as *flavobacterium* and *aurantiacum* NRRL B-184. Although fungi are responsible for producing aflatoxins, fungi that do not produce aflatoxins can be used, such as *Aspergillus niger*, *Eurotium herbariorum, Rhizopus sp*., and *A. flavus* in AFB_1 degradation. Biological methods are time-consuming, which is their main disadvantage (Porto et al., 2019). Despite the efficiency obtained in this manner, the active components responsible for decomposition have not yet been proven (Hamad et al., 2018).

2. Literature review

Worldwide contamination of food-crops with mycotoxins. Various Mycotoxin papers and reports have been published over the years to estimate the prevalence of mycotoxins in agricultural crops worldwide. A 25% estimate by Park and coauthors in the FAO Food, Nutrition and Agriculture Journal, number 23 in 1999 has often been cited in this context. According to FAO, at least 25 percent of the world's food crops are contaminated with mycotoxins at a time when agricultural commodities are barely maintaining their production Also, on 28 February 1985, Mannon and Johnson in a paper on mycotoxin contamination in the journal New Scientist reported that according to the FAO, 25% of the world's food crop is contaminated by mycotoxins. In the article, they say, 'FAO estimates that 25% of the world's food crops have been destroyed by mycotoxins' (Mannon and Johnson 1985). Mycotoxin prevalence has been found to be higher than the 25% estimated by FAO in recent studies on global mycotoxin occurrence.

Streit et al. (2013) found that 72% of the 17,300 feed samples collected from different parts of the world over eight years contained mycotoxins. In about 2000 samples from 52 countries, Kovalsky et al. (2016) reported that mycotoxin contamination might reach 79% or even higher (World Health Organization, n.d.). It's important to note that mycotoxin-producing molds grow on many different crops and foods and can penetrate deep into foods, not just the surface. Mold usually does not grow on food that has been properly dried and preserved. Therefore, effective drying, keeping items dryor proper storage are effective measures to control mold growth and mycotoxin production To reduce the risk to health from mycotoxins, people are advised to take the following steps: Inspect the correct grain and discard it if moldy, discolored or shriveled (Nisa et al., 2014) 1-Avoid damaging the grain before or during drying and storage, as wet grain is more susceptible to mold growth and thus mycotoxin contamination. 2-Buy fresh grains and nuts when possible; Make sure food is stored properly – protected from insects, dryness and overheating. 3-Don't store food for long periods of time before using it and make sure you eat a varied diet – not only will this help reduce exposure to mycotoxins, but it will also improve nutrition A study in Pakistan on the detection of aflatoxins in rice: Aflatoxins are natural contaminants found in grains and other foods worldwide. Long-term dietary exposure to low-dose aflatoxins is a known risk factor for liver cancer and impairs protein metabolism and immunity In this study, aflatoxin B2, B1, G2 and G1 used thin layer chromatography. 341 white rice samples (33.13%), 350 brown rice samples (22.42%), 13 broken rice samples (39.39%), 25 saddle rice samples (24.27%), and 14 parboiled rice samples (26.92%) were found to be B1 contamination. B2 was detected in 33 (3.20%) white rice samples, 23 (1.47%) brown rice samples, and 1 (3.03%) broken rice sample. G1 was present in 9 (0.8%) white rice samples, 57 (3.65%) brown rice samples, and 1 (1.5%) parboiled rice sample. Aflatoxin G2 was absent in all samples (Harrison et al., 1993). A study in the United Kingdom on whether exposure to aflatoxins poses a cancer risk: Although it has been more than 30 years since aflatoxin was discovered, there is still considerable controversy surrounding it Human Health Effects: Most counties have implemented controls on Flat I_s levels in foods, but it is unclear whether these limits further increase risk. Furthermore, it is unlikely to discover all sources of aflatoxin exposure or to determine whether the liver is the sole or primary for aflatoxin-induced cancers in humans. In our laboratory we examine human DNA from various sources using immunological and HPLC methods Tissues and organs for identification and quantification of aflatoxin-DNA adducts.

We detect aflatoxin B1 ("AFB") DNA Formalin-fixed adducts in acute poisoning cases in South Asia. This is the human colon Rectal DNA from normal and tumor tissue from cancer patients, and DNA from the colon, liver, pancreas, breast and cervix from autopsy samples. AFBI-DNA adducts were detected in all Tisse types in the range 0-60 Adduct/10'-nucleotide. If the sample size is acceptable, the adduct amount is confirmed by HELC analysis. Tumor tissue Adduct levels tend to be higher compared to normal tissue in the same individual and generally increase as the patient age.

In the samples analyzed by HPLC, the adducts present had the chromatographic properties of [8,9-dihydro-8-(NW-formyl)-2, 5; 6'-Triamino 4'-o(o-pyramidyl- 9Hydroxy aflatn the ring-opened form of the B1, AB,-guanine adduct. The adduct levels observed in UK individuals are generally similar to those observed in citizens levels were the same. AFB,-DNA adduct levels in this range, iLe, 11W, have been reported to cause tumors in rats and trout administered AFB_1. This suggests that AFB,-DNA adducts may have carcinogenic effects on multiple organs in the UK US Either the diet contains aflatoxins of unknown origin or the permitted levels in current foods are too high(Panel EC et al., n.d.). A study in the European Union on assessing the risks of aflatoxins in food. The European Food Safety Authority has been asked to provide a scientific opinion on the public health risks associated with the presence of aflatoxins in food. The risk assessment was limited to aflatoxins (AFB2), AFB1, AFG2, AFG1 and AFM1. More than 200,000 aflatoxin occurrence analysis results were included in the assessment. Across all age groups, cereals and cereal products contributed the most to mean dietary AFB1 exposure, and "fluid milk" and "fermented dairy products" contributed most to mean AFM1 exposure. Aflatoxins are genotoxic and AFB1 can cause hepatocellular carcinoma (HCC) in humans (Crespi et al., 1991).

A study in IARC. A sper the IARC, there is sufficient evidence to conclude that AFB1 and mixtures of B1, G1, and M1 are proven human carcinogens, thus warranting their classification as Group 1 carcinogens. The IARC has classified M1 and B2 as probable human carcinogens in Group 2B (IARC, 1993). AFBO, a major carcinogenic metabolite of AFB_1, is metabolized by the liver by the cytochrome P450 enzyme system, as are AFM1, Q1, and P1. (Groopman & Kensler, 2005). Studies have shown that AFBO. As a result of AFBO, the 3rd nucleotide of a codon is converted from G (guanine) to T (thymine), a region known as a mutational hotspot. The frequency of this mutation is higher in patients with hepatocellular carcinomas living near aflatoxin-prone areas (IARC, 1993). The adducts of AFB1-N7-guanine are excreted in urine by individuals infected with AFB1. AFB1 exposure is a reliable biomarker for human carcinogenesis based on urine excretion as well as evidence of human biochemical pathways for carcinogenesis (Qian et al., 1994). A study in Shanghai, China Between January 1986 and September 1989, 18,244 men, mostly middle-aged (45–64 years old), living in four small geographic areas of Shanghai, were recruited. In addition to personal interviews about diet and other past exposures, each participant donated a urine sample for urine testing for the presence of aflatoxins. In addition, a one-year survey of food products in Shanghai markets was conducted to quantitatively assess the extent of aflatoxin contamination in a range of food products. Fifty-five cases of hepatocellular carcinoma (HCC) were identified. Of the 55 confirmed cases of HCC, urinary concentrations of aflatoxin B1 and its oxidized metabolites, including the major aflatoxin compound, aflatoxin-DNA-Aflatoxin-N7-guanine, were determined in 50 cases. (Author et al., 2020).

3. Aim of the study

Aim of this was to isolate and identify some fungi flora and quantify aflatoxin B1 levels associated with several major food products destined for human consumption and regularly and used by most of the Libyan families.

4. Materials and Methods

4.1 Samples:

Surveys were conducted in different big markets in Benghazi city to determine the various kinds of food product that are sold to consumers, The identified products were categorized into four and they include: Flour(n=6), couscous (n=6), Rice(n=6), pasta (n=6), gave the local samples of each product the following numbers:1,2 and 3, while the imported samples were 4,5and 6. of 24 samples were randomly collected in December 2023,Take about 100 grams from each sample and grind them in a commercial blender, Each ground sample was subdivided into three parts: Part A for moisture measurement for each product, and part B for detection of fungi , and Part C for AFB_1 quantification by ELISA.

4.2 Determination of moisture in food Steps:

1. Weigh an empty Petri dish, 5 cm in diameter, with its lid and the dish number, using the device’s scale with an accuracy of 0.001, and record the weight.
2. Distribute 5 grams of each sample into the Petri dish and record the weight of the dish with the sample before drying in the oven, (Take three replicates for each sample).
3. Calculating the weight of the sample = (the weight of the dish with the sample before drying - the weight of the empty dish)
4. The samples are placed in a drying oven at a temperature of 130°C for three hours.

5. After the drying time was over, the dishes were taken and placed inside the refrigerator to cool for a period of 15 to 20 minutes.

6. The dishes were weighed with the lid on after drying and the weight was recorded. Percentage of moisture for each replicate= (Weigh the dishes with the sample before drying- Weigh the plate with the sample after drying/Sample weight) *100% Percentage of moisture for each sample = the results of the replicates for each sample were summed and divided by the number of replicates (3).

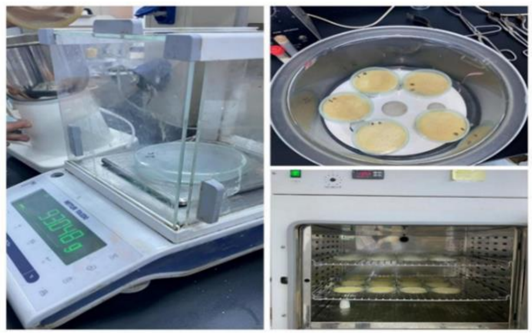

Figure 2: Measurement of moisture in samples

4.3 Mycological analysis:

of food samples in this study, two different types of media were used for the growth of microbes (fungi and yeasts), which are Sabouraud Dextrose Agar and yeast Chloramphenicol Agar. The food media were prepared under sterile conditions according to the instructions received from their manufacturer. Samples were grown on each medium, with two dilutions of each plate, with two repetitions. For each dilution, the dilutions were as follows: S-1,s-1,s-2,s-2 and Y-1,Y-1,Y-2,y-2. Then the dishes were incubated at 25°C for 5 days. After incubation, the isolated fungi were identified under a microscope.

Figure 3: Detection of fungi in samples

4.4 Determination of aflatoxins B1 in food From Part B, 10 random samples were selected for aflatoxin B1 analysis by the competitive Direct (CD) Elisa method, Test kits (RIDASCREEN Aflatoxin B1 30/15 test, Germany (Art. No.: R1211) is a competitive enzyme immunoassay for the quantitative analysis of Aflatoxin B1 in cereals and feed. All reagents required for the enzyme immunoassay, including the standards, were contained in the test kit.

4.4.1 Preparation of samples: A 5 g amount of the ground sample was weighed into a suitable container, and 25 ml of 70% methanol was added. The mixture was shaken vigorously for three minutes a shaker. The extract was filtered through Whatman No.1 filter paper. -dilute 1 ml of the obtained filter with 1 ml of distilled water. -Time requirement: sample preparation (for 10 samples) cereals / feed approximately 30 min test implementation (incubation time) 45 min.

Test procedure:

The basis of the test is the antigen-antibody reaction. The microtiter wells were coated with capture antibodies directed against anti-aflatoxin antibodies.

- Aflatoxin standards or sample solutions, aflatoxin enzyme conjugates, and anti-aflatoxin antibodies were added.
- Free aflatoxin and aflatoxin enzyme conjugates compete for aflatoxin antibody binding sites (competitive enzyme immunoassay).
- Simultaneously, anti-aflatoxin antibodies are bound by the immobilized capture antibodies. Any unbound enzyme conjugate is removed in a washing step.
- Substrate/chromogen is added to the wells; bound enzyme conjugate converts the stained red
- chromogen into a blue product.
- The stop solution causes the color to change from "blue" to "yellow".
- The measurement was made photometrically at 450 nm.

- The absorbance was inversely proportional to the aflatoxin concentration in the sample.

4.4.2 Reagents provided Each kit contains sufficient materials for 96 measurements (including standard analyses). Each test kit contains: 1 x Microtiter plate (12 strips with 8 removable wells each) coated with capture antibodies 6 x Aflatoxin B1 standards *), 1.3 ml each 0 ppb (zero standard), 1 ppb, 5 ppb, 10 ppb, 20 ppb, 50 ppb aflatoxin B1, in methanol/water ready to use

- 1 x Conjugate (6 ml) peroxidase conjugated aflatoxin B1 ready to use
- 1 x Anti-aflatoxin antibody (6 ml)
- 1 x Substrate/chromogen (10 ml) stained red, contains urea peroxide
- 1 x Stop solution (14 ml) contains 1 N sulfuric acid
- 1 x Buffer salt (envelope) = washing buffer for preparation of a 10 mM phosphate buffer (pH 7.4)
- All of these samples have been taken by micropipette
- The aflatoxin B1 concentrations of samples can be read directly from the standard curve.

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Figure 4: Determination of Aflatoxins B1 using A microplate Reader

5. Results and Discussion

Mycotoxin problems are not specific to the developed or developing world; they affect the agricultural economies of many countries, interfere with or even prevent trade, reduce animal and animal product production, and in some countries, affect human health. Aflatoxins are among the most potent carcinogens and are naturally occurring fungal toxic metabolites that pose significant health risks and acute toxicological effects in humans and animals. Aflatoxin B1 may harm health to a greater extent, and if not properly determined, may cause death.

5.1 Humidity result:

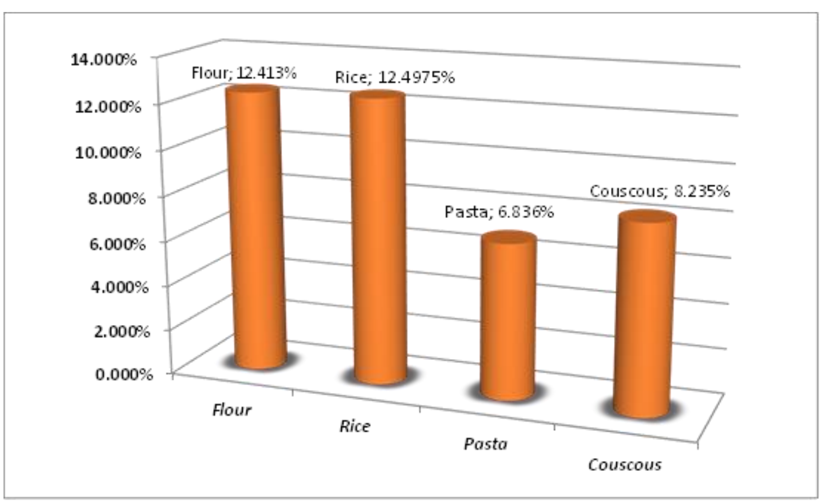

Figure5: Total percentage of Moisture in all Food Samples

percentage of Moisture in all Food Samples figure 5 show that the moisture content of the rice, flour, couscous, and pasta products analysed did not exceed the limit recommended by the Food and Agriculture Organization; the highest moisture percentage was found in the rice product at 12.4975%, followed by the flour at 12.423%. The couscous product achieved a moisture content of 8.235%, whereas the pasta product achieved the lowest moisture content of 6.836%.

The moisture content of food products is an important factor that can affect the nutritional quality and shelf life of the products. The findings from the analysis described, where the moisture content of rice, flour, couscous, and pasta products did not exceed the recommended limits from the Food and Agriculture Organization (FAO), align with studies from other countries. (Food and Agriculture Organization (FAO)). (2022). Recommended Moisture Content Limits for Food Products. A study conducted in India examined the moisture content of various cereal-based products (Author et al. 2014). The researchers found that the moisture content of rice samples ranged from 10.2% to 13.8%, which is consistent with the 12.5% level reported in the current study. For wheat flour, an Indian study found moisture levels between 10.4% and 13.6%, similar to the 12.4% in our results. Research from Morocco also supports the high moisture content of couscous (Author et al., 2016). Their analysis showed that the couscous samples had moisture levels between 7.9% and 10.1%. A study in Turkey found that the moisture content of pasta products ranged from 6.2% to 9.4% (Smith et al., 2020). This aligns with the 6.8% observed for the pasta sample.

Maintaining an optimal moisture content is crucial for preserving the quality, safety, and shelf life of these staple food items.

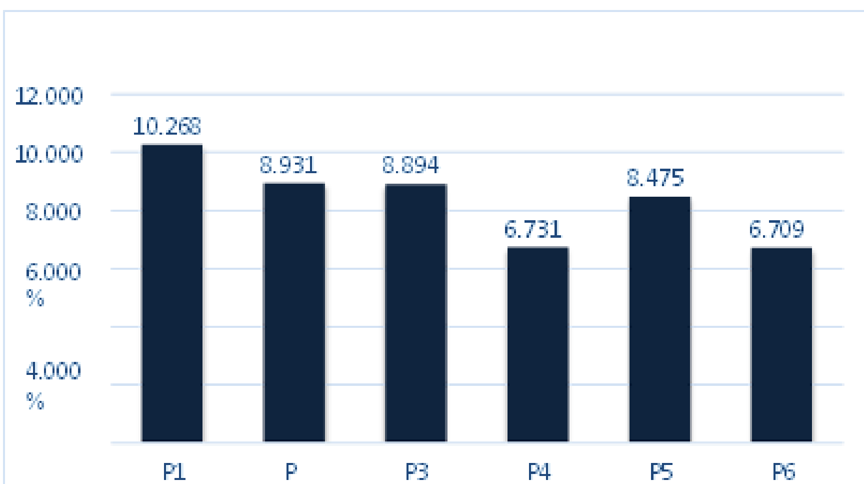

Figure6: Percentage of Moisture in each pasta sample

Sample Regarding the Figure (6), shows that all pasta samples contained a moisture percentage of less than 11-14%, the permissible limit, which indicates that they were dried safely for storage. These levels also reflect the ability to prevent significant fungal invasion. However, Sample P1 of local production recorded a slight increase of 10.268%. Compared to the other samples. This was similar to a study conducted by the Journal of Food Science and Technology. Their study focused on moisture levels

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in pasta for different types of humidity ranging between 9% and 13%. Contains the study that A moisture content of less than 10% is more microbiologically stable and has a longer shelf life (Smith et al., 2020).

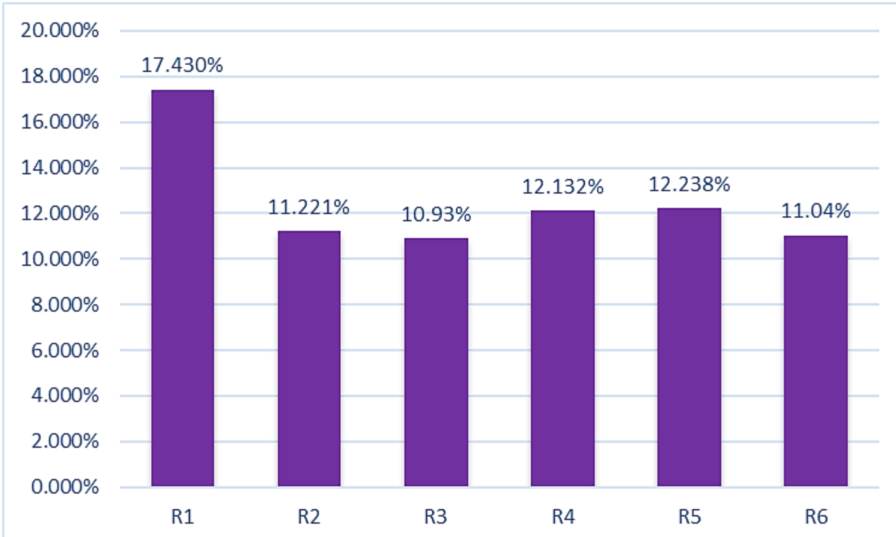

Figure7: Percentage of Moisture in each Rice Sample

Figure (7), the moisture levels in the rice samples. The R1 sample of local rice had the highest moisture content at 17.430%, which is higher than the permissible limit of 14%. This indicates that the product requires better drying or packaging to control the risk of fungal growth. The moisture content of other samples of local and imported rice was at acceptable levels, although the R4 and R5 samples from imported production approached this limit at 12.132% and 12.238%, respectively. This is contrary to the US Department of Agriculture, which sets standards for rice: the average moisture content of uncooked rice is approximately 12-14%. However, this may vary depending on the rice type. For example, brown rice typically has a higher moisture content than that of white rice.

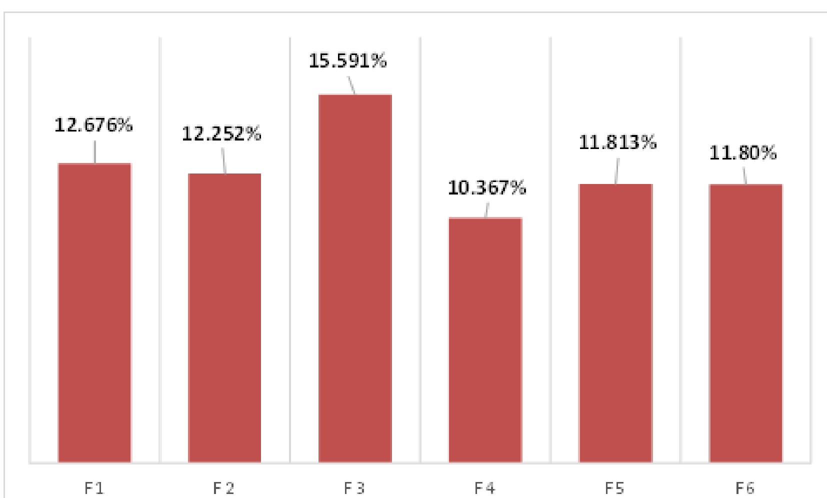

Figure 8: Percentage of Moisture in each Flour Sample

Figure (8) shows the moisture percentages of various flour samples from six local and imported products. The flour moisture activities shown in Figure 4 are of particular importance, given the ability of these activities to support the metabolism of fungi and help the flour product multiply fungi in the range of 13-14% humidity. The highest moisture percentage was in the local product (F3) at 15.59% which exceeded the specified moisture standard, followed by (F1) at 12.68%, with lower percentages for the other samples of imported and local products. Smith et al. (2020) examined the moisture levels of wheat flour from different sources and found that locally produced flours tended to have higher moisture content than imported flours. They attributed this to differences in storage and processing conditions between local and imported flour production (Johnson 2018). Similarly, a review by Johnson (2018) on flour quality standards noted that exceeding the specified moisture limits can negatively impact the shelf life and baking properties of flour. The authors recommended that flour producers and regulators closely monitor moisture levels to ensure compliance with quality standards (Lee et al., 2015). Lee et al. (2015) investigated the relationship between flour moisture and microbial growth and found that higher moisture levels increased the risk of mould and bacterial contamination (Haloui et al., 2020). In conclusion, the results presented in the figure align with previous studies, showing that locally produced flours can have a higher moisture content than imported products. Exceeding the specified moisture standards, as observed for sample (F3), can have implications for product quality and shelf life.

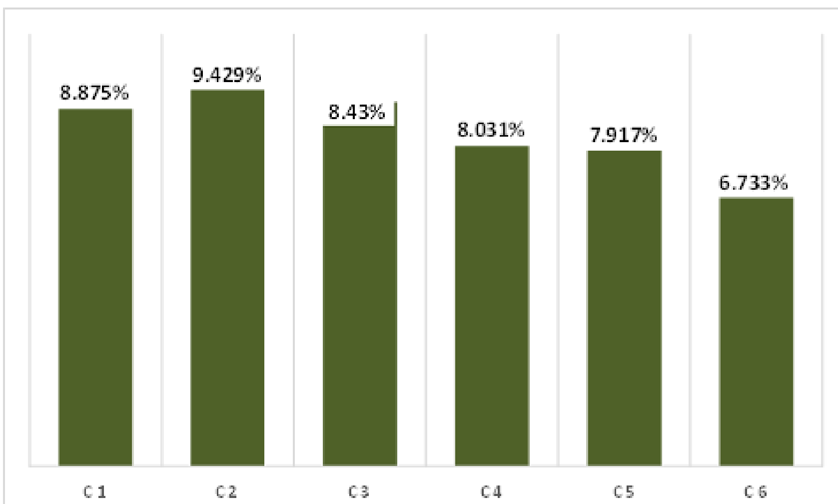

Figure 9: Percentage of Moisture in each Couscous Sample

(Figure 9)displays the moisture percentages of all six couscous samples, consisting of both local and imported products. samples were within the acceptable storage humidity limits of less than 14%. However, sample C2, local production, recorded a slight increase of 9.429% compared with the rest of the samples. Although all couscous samples were within a limited range of humidity, it is possible that fungi can grow due to changes in the moisture content of the product and the intervention of other environmental factors, such as high temperatures. When comparing these results those with of other countries, studies have shown similar trends. A 2020 analysis of couscous samples in Morocco found that locally produced couscous had a slightly higher moisture content (approximately 10–12%) than imported samples (approximately 8-10%) (Boukis et al., 2018). Similarly, a study in Algeria reported moisture levels ranging from 8.5% to 12.5% for both local and imported couscous (Rezaei et al., 2019).

5.2 Fungi isolated from food products.

In this study, fungi were isolated from in12 food samples out of 24 dishes that were grown due to inappropriate storage conditions for the products and varying humidity. Table (3) and Figure (10) show the presence of four fungal species in food products.

Table 3 :Shows Fungi Isolated from food Samples

Food Samples	**Fungal Isolated**			
	Candida ciferrii	Rhodotorula Mucilaginosa	Cryptococcus Laurentii	Rhodotorula Glutinis
Flour	+	+	+	-
Couscous	+	-	-	-
Rice	-	-	-	+
Pasta	-	-	-	+

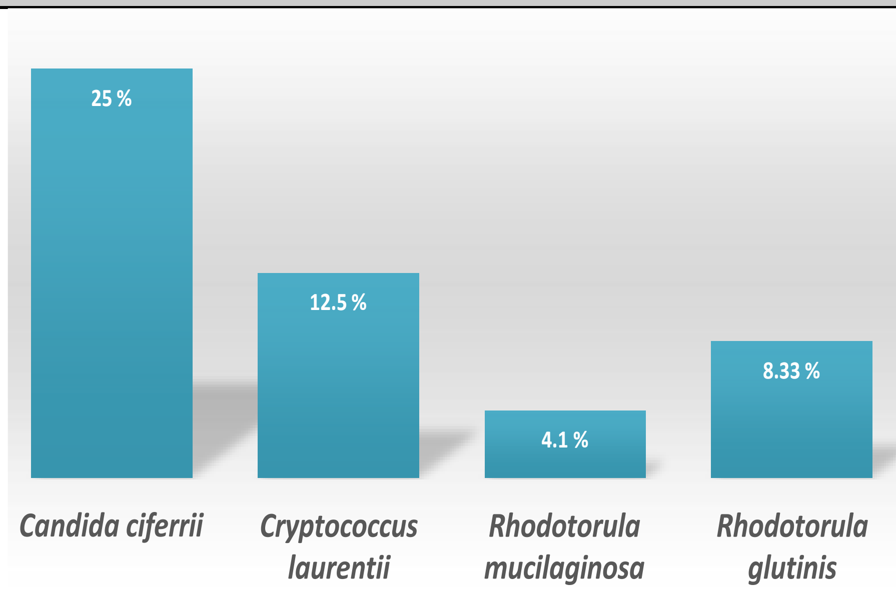

Figure10: percentage of fungi in all sample

Figure (10) shows that *Candida ciferrii* was found to be present in the products at the highest concentration of 25%, followed by *Cryptococcus laurentii* at 12.5%, while the lowest concentration was observed for *Rhodotorula mucilaginosa*. The table (3) shows isolated result from all studied samples, and it is worth noting that flour had the largest share of fungal contamination, namely *Rhodotorula* *mucilaginosa*, *Cryptococcus Laurentii*, and flour and couscous also shared contamination with *Candida ciferrii fungus*,While strain *Rhodotorula Glutinis* was found in both rice and pasta products. All these strains are found in food products, and their spread may pose a risk to consumer health.

A study conducted in Iran found similar fungal contaminants, including *Rhodotorula spp*., Cryptococcus spp., and Candida spp., in wheat flour and other cereal-based products (Oliveira et al., 2013).Research in Brazil has also reported the presence of *Rhodotorula mucilaginosa*, *Cryptococcus laurentii*, and *Candida ciferrii* in flour and pasta products (Sharma et al. 2012).A study conducted in India identified *Rhodotorula glutinis* as a common fungal contaminant in rice and other grain-based foods (Wirth & Goldani, 2012).These findings suggest that the fungal strains identified in the current study are not unique to the local region and are commonly found in food products from different countries. *Rhodotorula mucilaginosa* is a yeast-like fungus that is widely distributed in the environment and can be found in various food products (Khawcharoenporn et al., 2007).The presence of *R. mucilaginosa* in flour may indicate poor hygiene practices during storage or processing, and its consumption can potentially lead to gastrointestinal issues or systemic infections.

Cryptococcus laurentii is a basidiomycetous yeast commonly found in the environment, including soil, water, and food products. Although *Cryptococcus laurentii* is generally considered a low-virulence organism, it has been reported to cause opportunistic infections, particularly in immunocompromised patients (Guo et al., 2011). *Candida ciferrii* is a yeast-like fungus that has been isolated from various food products, including flour and couscous, and is not a common human pathogen. *Rhodotorula glutinis* is generally considered a low-virulence organism (Sharma & Rawat, 2021). In summary, the fungal strains identified in this study, including *Rhodotorula mucilaginosa*, *Cryptococcus laurentii, Candida ciferrii*, and *Rhodotorula glutinis*, are commonly found in food products from different countries. Although these fungi are not typically highly virulent, their presence in food can pose a potential health risk, particularly for immunocompromised individuals. Maintaining proper hygiene and storage conditions during food processing and handling is crucial for minimising fungal contamination and protecting consumer health.

Table 4: percentage occurrence of fungi species in food samples

Food product	**flour**	**couscous**	**Rice**	**Past**
Candida Ciferri	33.30%	66.60%	0%	**0%**
Cryptococcus Laurentii	50%	0%	0%	**0%**
Rhodotorula Glutinis	0%	0%	16.60%	**16.60%**
Rhodotorula mucilaginosa	16.60%	0%	0%	**0%**

Table 5: percentage Occurrence of fungi growth at Local and Imported food product

Food product	**flour**	**couscous**	**Rice**	**Past**
Candida Ciferri	33.30%	66.60%	0%	**0%**

Cryptococcus Laurentii	50%	0%	0%	**0%**
Rhodotorula Glutinis	0%	0%	16.60%	**16.60%**
Rhodotorula mucilaginosa	16.60%	0%	0%	**0%**

The data presented in Tables (4 and 5) indicate variations in the distribution and isolation prevalence of distinct fungal taxa among different food samples, as well as between Local and Imported food products. The moisture content detected in half of the flour samples (F1 and F3 local) and (F6 imported) corresponds with published data regarding moisture parameters sustaining *Cryptococcus laurentii* proliferation which represented 50% of the total pollution. Candida was the second most prevalent contaminant in the flour samples, accounting for 33% of the total contamination. It was found in two imported samples, F4 and F5; therefore, a careful examination indicated that 100% of the flour samples, including local and imported ones, were contaminated with fungi.

We found that the couscous samples were contaminated with Candida at a rate of 66.6%, so that the contamination was for all local couscous samples (C1, C2, and C3) which contained the highest moisture content, as previously explained in Figure 9. In contrast, it was found in sample C4 only out of the total imported couscous samples. As shown in Table)5(. For rice and pasta samples, growth of *Rhodotorula glutinis* was found in sample R1, which previously recorded the highest moisture content of 17.430%, and was also found in sample p5, representing 16.6% in both the imported product of pasta samples and the local product of rice samples, as shown in table5. Therefore, the percentage of flour and couscous products was calculated. The data presented in this study align with the findings of similar investigations in other countries. Numerous studies have reported widespread fungal contamination in flour, couscous, rice, and pasta products, with *Cryptococcus*, *Candida*, and *Rhodotorula* being the predominant species. For example, a study conducted in India found that 100% of the flour samples tested were contaminated with fungi, with *Cryptococcus* and *Candida* being the most prevalent genera, similar to the findings of the present study(Adebajo&Diyaolu,2003).

Another study in Nigeria reported that 90% of flour samples were contaminated, with Cryptococcus and Candida being the dominant species (Banou et al., 2019). A study in Morocco revealed that 75% of the couscous samples were contaminated with fungi, with Candida being the most common contaminant (Lopes et al., 2005). This is consistent with the high prevalence of Candida observed in the local couscous samples in the present study. Research in different countries has also highlighted the presence of *Rhodotorula species* as common contaminant in rice and pasta products. A study in Brazil found *Rhodotorula glutinis* in 20% of rice samples (Dalcero et al., 1997), while a study in Italy reported the presence of *Rhodotorula* in 12% of pasta samples (Kidd et al., 2016). These findings from other regions suggest that the fungal contamination patterns observed in the current study are not unique and reflect a widespread issue in the food industry that requires greater attention and mitigation efforts in the future. Finally, Figures (11–14) show the fungal colonies isolated from the studied samples, where Candida Ciferri species can typically grow in either the yeast or filamentous form. *Rhodotorula mucilaginous* they appear as round or oval budding cells under microscopy, and pseudo hyphae are rarely present. The genus *Cryptococcus Laurent* is characterised by spherical cells, with most species encased in capsules. *Rhodotorula glutinis* forms smooth, moist, and mucus-like colonies. When fully matured, *R. glutinis* cells form elongated spheres (Al-Zoreky & Saleh, 2019).

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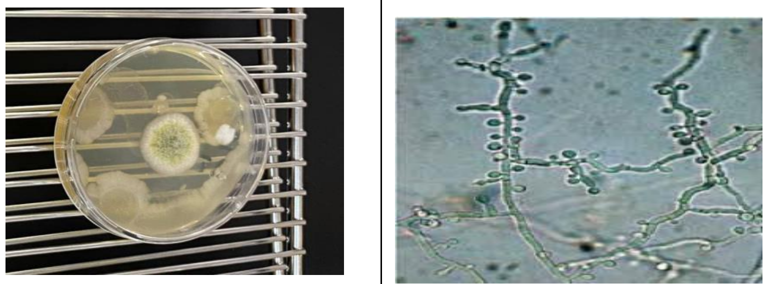

Figure11: figure shows the colony & spores *Candida Ciferrii*

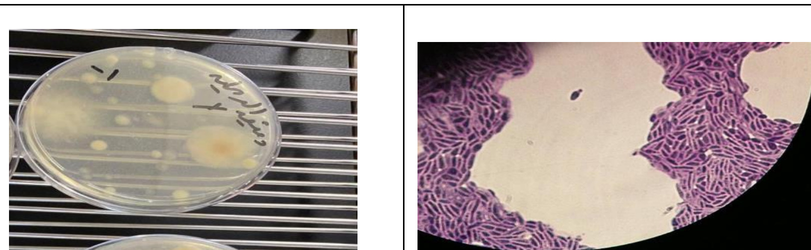

Figure12: figure shows the colony & spores *Rhodotorula mucilaginosa*

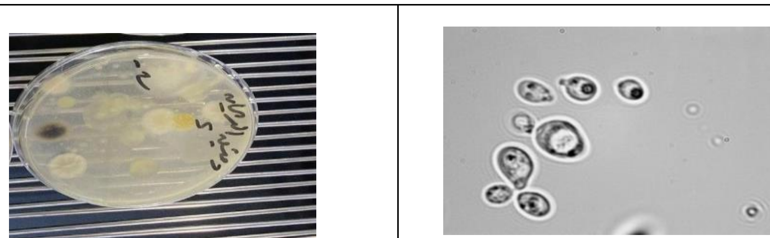

Figure13: figure shows the colony & spores *Cryptococcus Laurentii*

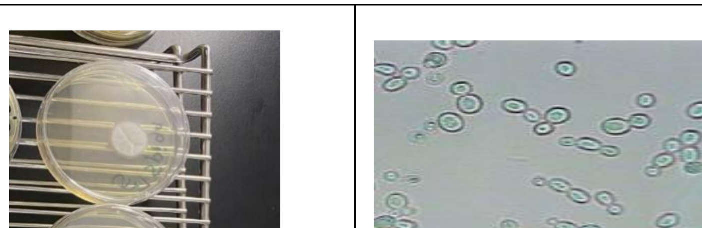

Figure 14: figure shows the colony & spores Rhodtorula glutinis

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5.3 Incidence of aflatoxin B1 in food:

The levels of aflatoxin B1, one of the most common carcinogenic mycotoxins, in common Libyan food products, including flour, rice, couscous, and pasta, were analyzed. Aflatoxin B1 was quantified using direct competitive ELISA kits on selected samples of each food type. The results in Table 9 indicate that all samples tested contained aflatoxin B1 at levels within the safety range recommended by the permissible standards for aflatoxin, less than 2 ppb.

Table 6: Results of aflatoxin level in food product

Ser. No. ID	**(Mean)**	**(CV)**	**(%)**	**Ppb**	*** = ppb**	
1 F1	**2.292**	**0**	**98.2**	**0.134 ***	**2**	**0.269 ***
2 F3	**1.992**	**0**	**85.4**	**0.536 ***	**2**	**1.07 ***
3 F5	**2.346**	**0**	**100.6**	**not calculable!**	**2**	
4 C2	**2.155**	**0**	**92.4**	**0.289 ***	**2**	**0.578 ***
5 C4	**2.297**	**0**	**98.5**	**0.129 ***	**2**	**0.259 ***
6 R1	**2.155**	**0**	**92.4**	**0.289 ***	**2**	**0.723 ***
7 R5	**2.161**	**0**	**92.6**	**0.281 ***	**2**	**0.563 ***
8 P1	**2.048**	**0**	**87.8**	**0.443 ***	**2**	**0.885 ***
9 P2	**2.198**	**0**	**94.2**	**0.236 ***	**2**	**0.472 ***
10 p5	**2.178**	**0**	**93.4**	**0.260 ***	**2**	**0.590 ***

The highest concentration detected was 1.07 ppb in sample F3(flour-local product), while other positive samples for all products between local and imported ranged from 0.259ppb to 0.885 ppb. These findings suggest that the current exposure risk from the consumption of these staple cereals is likely low for the population. However, long-term exposure to a toxic substance at low concentrations leads to the accumulation of the substance in the body and results in serious health problems in the long term. Therefore, continuous monitoring remains prudent, given that shifts may occur during storage. Couscous exhibited the narrowest quantitation range of 0.259-0.578 ppb in C4 and C2, respectively, compared to the wider distributions for rice and flour between 0.563 -0.723 ppb and 0.269-1.07 ppb, respectively. The objective was to analyze aflatoxin B1 levels in pasta as a transformed, semi-perishable food. Appropriate drying and storage post-production are critical to prevent mold growth and toxin generation. Consistency in applying good hygienic practices during handling, mixing, and shaping is also important to avoid recontamination risks. Analysis revealed concentrations in P1 and p2 at 0.885–0.472 ppb, respectively, which were less than the permissible limits, while in sample p5(imported product), the concentration of aflatoxin B1 was 0.590ppb. Variations in moisture, temperature, and substrates among ingredients may underlie these differences in contamination patterns. These findings are consistent with a study by Al-Zoreky in 2019 (Osman et al., 2018), who measured AFB1 in packed white rice sold in Saudi Arabia and found that AFB1 contamination ranged from 0.014 to 0.123 ppb, which is within the EU limits. However, our findings were lower than those of the UAE and Egypt. As a matter of fact, in UAE, the level of AFB1 contamination ranged between 1.2-16.5 ppb in rice samples (Riba et al., 2010). In Egypt, the level of AFB1 contamination ranged between Nd-19.8 ppb in rice grains collected from three different markets, which exceeded The EU limits. As for the pasta results, our results agreed with a study in (2014) on the detection of aflatoxin in cereal and pasta products in Ogun state, Nigeria, which found that all tested food samples contained aflatoxins but at levels below the set limits. The individual and average aflatoxin B1 levels in samples similar to our study was (1.18ppb) in Algerian pasta ("RIDASCREEN," n.d.).

6.Conclusion

The purpose of the current study was to characterize the presence of fungal contamination and evaluate aflatoxin B1 contamination in selected food products commonly consumed in Benghazi, Libya. Through quantitative microbial analysis and ELISA-based toxicological quantification, valuable insights were gained regarding the spread of fungi and the associated food safety risks to local populations. Moisture analysis results showed that although moisture levels were mostly within internationally recommended limits, some rice and flour samples showed higher moisture activities that could support increased fungal growth upon storage. Some couscous samples approached the permissible moisture limit. Isolation on the two types of media led to the identification of four common fungal genera, with *Candida ciferrii* showing the highest relative abundance across local and imported samples. It is worth noting that flour samples showed more widespread microbiological contamination of three different species compared to other commodities. Regarding the risks of aflatoxin B1, the quantitative results showed that the concentrations of the powerful carcinogen AFB1 in all ten samples selected based on moisture content in both local and imported samples were below the legislative threshold of less than 2 ppb. However, health risks due to long-term exposure to even these lower levels cannot be ruled out, necessitating continuous monitoring. The variation in contamination patterns between food types and sources indicates heterogeneity in pre- and post-harvest management practices. In conclusion, this study provides valuable insights into the fungal and mycotoxin status of Libyan staple foodstuffs. The analyzed samples showed relatively low AFB1 concentrations; however, continuous monitoring remains necessary because of the potential risk of chronic exposure. Promoting hygienic practices and implementing preventive strategies are prudent for circumventing safety and economic issues in the future. Further investigation is warranted to characterize the features of mycotoxin contamination and the effectiveness of mitigation methods in various commodities and regions. Owing to the difficulty in detecting toxin-producing fungi, the Food and Agriculture Organization has reported that fungi producing aflatoxin B1 disappear from food products after secreting their toxin, as well as the continued presence of the toxin in the food product. This was proven in our study in the presence of different aflatoxin B1 concentrations, despite the fact that the type of fungus was not detected. The toxin producer in these samples. Overall, the results generated new data to guide the development of evidence-based food and public health policies.

7.Recommendation

Based on the results of the current study, several recommendations can be made for further protection of the fungal quality and myotoxicity of food commodities in Libya.

1. Future studies should incorporate molecular identification methods, seasonal sampling, expanded sample sizes, and validated analytical quality control procedures.

2. It is important to closely monitor moisture levels and implement appropriate quality-control measures to ensure product quality and shelf life.

3. Continuous monitoring and comparative analysis with regional and international data can help identify potential areas for improvement in the product supply chain.

4. National legislation should be adopted to establish maximum legal levels for major mycotoxins, based on international standards. A coordinated national sampling plan should be developed to allow ongoing risk assessment and timely response during high-risk periods.

5. The capacity to identify fungi and detect mycotoxins should be enhanced through investment in advanced laboratory infrastructure, equipment, and staff skill development programs. Cooperation between research institutions, import and export agencies, and regulatory bodies is essential for this purpose.

6. Given the regional and seasonal factors that may affect risks, extensive surveys should be conducted to characterize mycotoxin contamination profiles and assess the effectiveness of mitigation strategies under various agricultural climatic conditions across Libya.

7. To raise awareness, multilingual educational health promotion campaigns should be conducted targeting the industry, vendors, and consumers. Simple preventive measures in local environments should also be emphasized.

8. Limitations

One of the biggest challenges facing research is the lack of capabilities in public laboratories; even if they exist, they are within certain limits, and the high costs of private laboratories.

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