An-Najah National University

Faculty of Graduate Studies

AFLATOXIN B1 LEVELS IN CORN (*ZEA MAYS*) AND SOYBEAN (*GLYCCINEMAX L.MERR.*)-BASED FEED IN NORTHERN PALESTINE

By

Elham Hamdan

Supervisors

Prof. Jamal Abo Omar

Dr. Ibrahim Alzuheir

This Thesis is Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Animal Production, Faculty of Graduate Studies, An-Najah National University, Nablus - Palestine.

2023

AFLATOXIN B1 LEVELS IN CORN (*ZEA MAYS*) AND SOYBEAN (*GLYCCINEMAX L.MERR.*)-BASED FEED IN NORTHERN PALESTINE

By

Elham Hamdan

This was Defended Successfully on 20/12/2023 and approved by

Prof. Jamal Abo Omar	
Supervisor	Signature
Dr.Ibrahim Al-Zuhair	
Co supervisor	Signature
Dr. Ayed Salama	
External Examiner	Signature
Prof. Raed Alkowni	
Internal Examiner	Signature

Dedication

All the words do not dictate to describe the extent of the effort, love and support that have been done to me to reach this day. Thank you from the depth of my heart my dearest father, and my dear mother.

I must thank my family for their love, support, and help throughout my life. Thank you for giving me strength, throughout this study, for your encouragement and support.

.

Acknowledgments

This work is dedicated, first and foremost, to the memory of my beloved mother, whose passing left an irreplaceable void in my life but whose love, values, and unwavering faith in me continue to be a guiding force. Her kindness, resilience, and wisdom shaped the person I am today, and I carry her spirit with me in every step of this journey. I am forever grateful for the foundation she provided and the strength she instilled in me, which has been my solace and motivation in pursuing this work.

I also dedicate this thesis to my family, whose steadfast support and understanding have been invaluable, especially through the most challenging moments of this journey. Their encouragement has been my anchor, and their sacrifices have allowed me to reach this milestone.

My deepest gratitude extends to my supervisors, Prof. Jamal Abo Omar and Dr. Ibrahim Alzuheir, for their exceptional mentorship, patience, and belief in my potential. Their guidance and insights have enriched my understanding and have been instrumental in shaping this research. I am grateful to have had the privilege of learning under their guidance.

Lastly, I dedicate this work to An-Najah National University, an institution that has not only provided the academic environment and resources necessary for my growth but has also fostered a community of learning, innovation, and support. This university has been a nurturing ground for my ambitions, and I am honored to be part of its legacy. Thank you all for your irreplaceable roles in this journey.

Declaration

I, the undersigned, declare that I submitted the thesis entitled:

AFLATOXIN B1 LEVELS IN CORN (*ZEA MAYS*) AND SOYBEAN (*GLYCCINEMAX L.MERR.*)-BASED FEED IN NORTHERN PALESTINE

I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: **Elham Hamdan**

Signature:

Date: **20/12/2023**

List of Contents

List of Tables

List of Figures

List of Appendices

AFLATOXIN B1 LEVELS IN CORN (*ZEA MAYS*) AND SOYBEAN (*GLYCCINEMAX L.MERR*.)-BASED FEED IN NORTHERN PALESTINE

By

Elham Hamdan

Supervisors

Prof. Jamal abo Omar

Dr. Ibrahim Alzuheir

Abstract

Aflatoxin, a by-product of *Aspergillus* fungi metabolism, poses serious health risks, as highlighted by numerous studies. This study aims to investigate the presence of aflatoxin B1 in two key crops—corn and soybean—which are widely used as primary raw materials in poultry feed concentrates. Both ingredients are imported and undergo processing in local factories. A total of 42 samples of corn and soybean were collected in October 2021 from silos at feed processing factories in the districts of Jenin, Nablus, and Tulkarm. The samples were stored in cool, dark containers and subsequently analyzed for aflatoxin B1 concentration using the RIDASCREEN® Aflatoxin B1 30/15 procedure.

The results revealed the presence of aflatoxin B1 in 80%, 100%, and 100% of corn samples from Jenin, Nablus, and Tulkarm, respectively. In soybean samples, aflatoxin B1 was detected in 60%, 85.7%, and 100% of samples from Jenin, Nablus, and Tulkarm, respectively. The overall average concentration of AFB1 in corn was 0.69 µg/kg, with district averages of 0.64 µg/kg in Jenin, 0.604 µg/kg in Nablus, and 0.80 µg/kg in Tulkarm. These levels fall within acceptable limits as defined by relevant standards. Significant differences in aflatoxin concentrations between the districts were observed for both crops. For soybean, the overall average concentration of AFB1 was 0.66 µg/kg, with district averages of 0.78 µg/kg in Jenin, 0.61 µg/kg in Nablus, and 0.65 µg/kg in Tulkarm. All results were below the maximum allowable limits set by European and US FDA guidelines, which specify a threshold of 20 ppb (20 µg/kg).

The findings suggest no significant differences in aflatoxin concentrations between corn and soybean. Continuous monitoring of aflatoxin levels is essential, particularly in

relation to storage conditions. Additionally, further research is recommended to assess aflatoxin levels in processed feed materials to ensure ongoing safety and compliance with standards.

Keywords: Aflatoxin B1, Corn, Soybean, Aspergillus, Fungi.

Chapter One
Introduction

1.1 Introduction (FAO)

Food is the basic required condition of life as a source of energy protein, etc. The global demand for food is increasing with the continuous increase in world population growth, accompanied by the continuous world population growth, the enhancement of livelihood levels and the economic status are pressing on obtaining sufficient food quantities with high quality. Food quality and safety have importance the same as sufficient supply. Quality has a great effect on human health (WHO a, 2022). If food is polluted or contaminated it is forming a huge health risk. This kind of unsafe food threat not only for human health but also it causes economic losses and hinders development. Meat and other animal-origin products are forming an important portion of the human daily diet. According to the Food and Agriculture Organization (FAO), cereals and grains are providing more than 50% of energy and 50% of protein, while animal products supply around 17% of energy and 32% of protein (Bender, 1998). Bender (1998) summarized the importance of animal-origin food in the human diet in developing countries by the content of protein and fats, Iron, Zinc, vitamin B and vitamin A.

Meat consumption could be divided into two categories, quality consumption, and sufficient quantity. Quality consumption is more concern in developed countries, while in developing countries the concern is more on the quantity to obtain the insufficient energy supply. Meat consumption in industrial countries which are developed or rich is three to four times that in poor countries (Delgado et al, 1998). However, as meat and animal-origin foods are a part of our daily food, the safety and health of these sources have an important effect on human health. Therefore, any contaminated or polluted food used in animal feeding may affect animal health causing either health risks to humans or economical losses. The most causing source of pollution is coming from Fungi. Devegowda et al (2019) reported that fungi produce Mycotoxins which is the result of the fungi metabolism, and this mycotoxin is poisonous to humans and animals. The produced mycotoxin is either in the field or in transportation and storage. The Food and Agriculture Organization of the United Nations (FAO) estimated that more than 25% of the world's grains are contaminated with mycotoxins as reported by (Eskola et al, 2019). This

estimation was confirmed by Eskola et al, (2019). While Council for Agricultural Science and Technology in the United States (CAST, 2003) estimated the annual economic losses caused by mycotoxins by 1.5 billion $. Among the mycotoxins, Aflatoxin B1 is the most studied as reported by Ezekiel et al, (2012). Aflatoxin was discovered in the 1960s after it caused the death of 100,000 turkeys (Kotinagu et al, 2015). This aflatoxin is carcinogenic to both humans and animals (McCullough et al. 2019).

In Palestine, the agricultural sector plays an important role in the Palestinian economy. In addition, due to its political status, agriculture becomes a crucial sector for Palestinian life. Agriculture is the sector to absorb the working power and. food source for the citizens to accomplish food security. Food security status in Palestine shows that around 25% of the Palestinians are suffering from inadequate food supply (MoA, 2019), however, the document on national food security policy shows that 7.4% of the children below five years are suffering from a shortage in food and that Palestinian have malnutrition. In addition, 23.6% of the household have poor or borderline food consumption (MoA, 2019). This 23.6% have not only low food consumption but also consume less important food items like meat and dairy (FAO – WFP, 2016).

The animal production sector in Palestine is very important to supply protein and fat(reference). The Palestinian agricultural strategy of 2017 – 2022 (MoA, 2016) estimated the livestock population raised in the Palestinian areas as follows: 2058 camels 33,980 dairy cows, 730894 sheep, and 215335 goats. The poultry sector is estimated as 32.5 million broilers, of which (994.6 thousand mothers), 1.8 million laying hens, 546.4 thousand turkeys, 19690 rabbits." (MoA, 2016). Most of the animal raising costs are coming from the feeding costs where it was estimated that feeding costs are 75-85% (MoA, 2016). At the same time, the majority of the food supplied to animals (despite being concentrated or filling) is imported, where it is estimated to be around 85 – 90% (MoA, 2016). Imported animal feeding materials are subjected to be contaminated with the widespread fungi, including *Aspergillus* spp, the source of aflatoxins (reference). Fungi favor humid warm conditions that are available in the storage of grains. In these conditions, the stored grains are subjected to growth and contamination with aflatoxin as fungi by-products. This is a health risk for animals and through it to humans or may cause severe losses.

In Palestine, information on aflatoxin in food is limited, there are two studies on the subject the study carried out by Ahmad et al (2022) regarding the contamination in some human food items. This study aimed to investigate and quantify the AFB1 concentrations at factories of animal feeding. These factories are importing grains; mainly corn and soybean from Israel. Corn and soybean are transported and stored, which, subjects them to be contaminated by aflatoxins. During processing, the grains are subjected to grinding and mixing, and packed for distribution in the local market under this condition no removal of the toxins and the animal would be subjected to huge health risks, therefore, the food could affect human health.

In recent years, the global agricultural industry has faced increasing challenges related to food safety and security, particularly in developing regions like Palestine. The contamination of staple crops by mycotoxins, such as aflatoxin B1, poses a significant threat to both human and animal health. As a by-product of fungi like *Aspergillus flavus* and *Aspergillus parasiticus*, aflatoxins can contaminate crops during cultivation, harvesting, or storage. In the context of Palestine, where the agricultural sector plays a crucial role in the economy and food security, the impact of such contamination can be profound. This issue is compounded by climate conditions that may favor fungal growth, leading to higher risks of contamination. Thus, understanding the prevalence of aflatoxins in essential animal feed ingredients, such as corn and soybean, is vital for protecting public health and supporting sustainable agricultural practices.

1.2 Study hypothesis

The hypothesis of the study is:

- The initial hypothesis is: The level of aflatoxin levels in animal manufactured feeds with corn and soybean manufactured feeds doesn’t exceed the allowed upper maximum level.
- The alternative hypothesis is: The level of aflatoxin levels in animal manufactured feeds with corn and soybean manufactured feeds exceeds the allowed upper maximum level.

1.3 Study Objectives

The main objective of this study is to investigate the level of AFB1 contamination of animal feeds in three Palestinian districts; Nablus, Jenin, and Tulkarm, as an indicator of food contamination.

Chapter Two

Literature review

2.1 Food safety and security

The continuous increase in food demand is a normal result of global population growth. However, food is the basic source of energy required for human life. A sufficient supply of food is the basic key to the development sustainability of any community (WHO, 2019). In addition, the safe food supply supports the economy on both local and international scales. However, there are wide gaps in food supply between developed and rich countries compared to the developing and poor countries as reported (Maurice, 2013). According to (FAO, 2018) more than 821 million humans are hungry. Billions, mainly in developing countries may face a shortage of water or food insecurity (Filho et al, 2021). International Food Policy Research Institute (2022) defined food security according to United Nations' Committee on World Food Security as the state in which all people, at all times, have physical, social, and economic access to enough, safe, and nourishing food that satisfies their dietary needs and food preferences for an active and healthy life. On the opposite, the insecurity of food could be explained as a restricted or unclear capacity to get the essential palatable foods in socially acceptable ways, or limited or uncertain availability of nutrient-dense, safe foods (Bickel et al, 2000).

Considerable differences are found on regional levels and also between rural and urban areas as reported by the World Food Program (WFP, 2009). This issue WFP (2009) report stated that more likely; persons in rural regions live under the poverty line four times compared to those who live in urban areas. These differences are reflected in the kind of food and nutritional values. The people under-poverty line consume less protein than those in rich countries (FAO et al, 2013). Not only poverty but in addition climate change and climate extremes affect the availability and accessibility to food mainly cereals and meat (Elbehri et al, 2017). Food security, therefore, is a term considering the availability of food. However, the Global Food Security Index is defining both food quantity and quality (Mahmoud, 2019). The absence of sufficient and safe food cause food insecurity as reported by Asan-Ghomi et al, (2012).

The major supply of protein is the food of animal origin, which is known as a source of protein from early ages as mentioned by Delgado et al, (1998). Bender (1998) reported

that meat is the major supplier of Iron, Zinc, vitamin A and vitamin B. In developed countries, people have a variety of food sources. While in developing countries meat plays an important role in the human diet (Bender, 1998). This nutritional richness makes meat play an important role in any balanced diet (Pereira and Vicente, 2013). Fats are a major concern when talking about meat consumption (Pereira and Vicente, 2013). Fats are very rich in energy. In developing countries, many diets are not including fats, this subject human to energy intake shortage (Bender, 1998). Makkar and Beever, (2013) reported that the global contribution of animal products to total energy consumption is ranging from year to year due to many different reasons. However, the global average was 17% in 2005 – 2007 (Makkar and Beever, 2013). But at the same time this contribution was less than 5% in 18 countries (Makkar and Beever, 2013).

The consumption of organic meat products is spreading widely around the world as sustainable food practice (Nguyen et al, 2019). According to Delgado et al, (2001) the transition in nutritional behavior in the 20th century made livestock the basic and primary source of protein. For those people who spend high levels of energy (as athletes or hand workers), food containing high levels of protein and energy is essential in their diets (Barybina et al, 2019). It is estimated that meat and other diary production need to be doubled to meet the demand in 2050 (Ozturk and Hamaker 2023). To meet the growing demand for meat livestock production is to be expanded (Bender, 1998). The production of meat and other dairy products (such as milk) forms pressure on the environment (Bruinsma, 2009). This is due to the need to transform large quantities of grains into the meat (de Boer et al, 2017). At the same time, the production of grain requires land and water. Aflatoxin contamination in animal feed is a pressing concern worldwide, especially due to its implications for animal and human health.

Aflatoxin contamination specifically raises concerns for food safety due to its resilience in various processing methods. Even after typical food processing practices like cooking, roasting, or milling, aflatoxins can remain stable and continue to pose health risks. This persistence underscores the challenge of controlling aflatoxins across the food production chain, from cultivation to consumption (Adeyeye, 2016). Additionally, food insecurity exacerbates the issue, as populations facing limited food access may consume contaminated foods out of necessity. In such contexts, implementing safety controls and rigorous monitoring for aflatoxins is essential to prevent adverse health outcomes.

Investments in local testing facilities and education about safe storage and handling practices are critical steps for regions like Palestine, where aflatoxin contamination in staples such as corn and soybeans could undermine food security efforts and public health objectives.

In Palestine, meat consumption is affected by the economic status of the people. In 2007 it was estimated that the annual per capita meat consumption is around 46.8 kg/ year and 2.4 kg/ year of fish, while the family's expenditure is around 10% on meat (PCBS, 2008). While in 2017 it decreased to 44.8 kg/ year (PCBS, 2018).

2.2 Food and feed contamination of mycotoxins

According to the World Health Organization (WHO) contaminated food causes 420,000 death annually, and unsafe food causes more than 200 kinds of diseases in humans. Where it is estimated that 10% of people around the world have diseases from eating contaminated food (WHO a, 2022). Food contamination could be by bacteria, viruses, or mycotoxins (WHO b, 2022). In the same manner, unsafe food is known since the historical ages, even though, safe food is a basic need for humans, and it is required to guarantee food safety through the food processing chain (Kamboj et al, 2020). Consumers are concerned about food safety and contamination of all sources. According to (WOAH, 2008), safe food is required for animals as they are a part of human food. Fenton and Van Saun (2018) published that both the safety and quality of feed grains and fodders are crucial to eliminate and avoid health hazards and guarantee herd health, in addition, to minimizing the losses in production.

Foods for humans and animals that are made from fungus-contaminated agricultural products pose a serious risk to human and animal health. Under ideal temperature and humidity circumstances, several types of toxigenic fungus can thrive during crop growth, after harvesting, during crop storage, and during the processing of food or feed products (Richard, 2007). According to Nnamani et.al, (2021), mycotoxins may serve as an insecticide or combat the plant's defense mechanism against the fungus in order to fulfill their ecological development functions in nature. The exact roles of mycotoxins are not well understood until now, and it is still yet unknown. Mycotoxins are innocuous by nature and are not precepted or recognized as disturbance causing materials by the human or animal immune systems (Nnamani et al, 2021). Adeyeye (2016) connected the food

security to mycotoxin contaminated food as he reported that, the goal of food safety is to reduce the amount of acute and chronic dangers that could endanger the health individuals who consume it. Food products that contain mycotoxins are completely unsafe for individuals to eat, which eventually causes post-harvest loss and compromises food security. The spread of various fungus in agricultural products results in reduced yield and ensuing financial losses (Adeyeye, 2016)

Farm animals are subjected to toxic mycotoxins through feedings (Alvarado et al, 2017). Cattle are exposed to these toxic mycotoxins through the corn (grain; silage, and corn processing by-products) as reported by Fenton and Van Saun (2018). The animal's contaminated feeds and food are exposing them to mycotoxins – mainly aflatoxins (Chase et al, 2013). Animal health diseases and problems related to mycotoxins are resulting from long contact (Alvarado et al, 2017). The European Food Safety Authority assessed the risk of aflatoxins to health. In the assessment, Schrenk et al (2020) found that the main contributor to dietary chronic exposure risk is grain and grain – based products. In their review Kamboj et al, (2020) present the sources of contamination with:

- External raw food contamination (the heavy metals, the pesticide residues… etc.).
- Contamination during food transport (vehicle gasses, or fungal residues, or microbial activities).
- Contamination is caused by cleaning processes (Chemicals or disinfectant cleaners).
- Contamination due to heating steps (due to heating some chemicals are formed).
- Food packaging (direct or indirect contact with packaging material).
- Contamination during food storage (breakdown of packing material during storage, material changes).

Contamination during the transportation is very important since it affects food safety (reference). The contamination of food starts before harvest as indicated by the WHO (2022). In Palestine, the risk of mycotoxin contamination in staple crops is particularly high due to climatic factors and storage conditions that facilitate fungal growth. Aflatoxin B1, one of the most toxic mycotoxins, poses significant health threats, particularly in regions with limited regulatory and monitoring frameworks. This issue is exacerbated by economic constraints, which often limit access to proper storage facilities and resources for fungal contamination management. Recent studies emphasize the necessity of implementing aflatoxin control measures, including the development of regional testing

facilities and educating local farmers on safe storage and handling practices. By enhancing local regulatory systems, Palestine can not only mitigate the risks associated with mycotoxin exposure but also improve public health outcomes and agricultural sustainability.

2.3 Mycotoxins and the effect of aflatoxins

World health organization (2022) mentions that: "mycotoxins, which are toxic compounds that are naturally produced by certain types of fungus". Guchi (2015) explains mycotoxins as secondary metabolites products that naturally result from filamentous fungi metabolism, which are low molecular weight.

Ajmal et, al, (2022) reported that over half of the world's population, or over 4.5 billion individuals, are thought to be at risk from chronic AF exposure, with many of them living in underdeveloped nations. The number of people afflicted may grow as a result of the current coronavirus pandemic, supply chain difficulties, and higher intake of AF-contaminated foods anticipated as a result of political unrest (COVID-19)

Council for Agricultural Science and Technology in the United States CAST (2003) indicated that three types of fungi are the main producers of the mycotoxins they published:" Most of the mycotoxins that are considered to be important are produced primarily by three genera of fungi, namely, *Aspergillus*, *Penicillium*, and *Fusarium*. *Claviceps* and *Stachybotrys* also are important producers of mycotoxins" (CAST, 2003).

Among the mycotoxins; aflatoxins were the widest spread and the most studied group (Devegowda et al, 2019). A byproduct of the aspergillus species, they prevail in warm and humid climatic conditions. Aflatoxins are biochemical substances produced by *aspergillus* (Guchi, 2015).

Diener et al., (1987) reported the variations between the main two species of aspergillus that produce different mycotoxins. They mentioned that A. *flavus* seems to be suited to the aerial and foliar environment, as seen by its dominance in cottonseed, tree nuts, and peanuts, while *A. parasiticus* looks like it's adapted to a soil environment. Only *A. flavus* and *A. parasiticus* are capable of producing aflatoxins. *A. parasiticus* isolates generate aflatoxin GI (AFGI), aflatoxin G2 (AFG2), aflatoxin Ml (AFM1), as well as AFBI and AFB2. In contrast, *A. flavus* normally produces the mycotoxins as aflatoxin BI (AFBI) as

well as aflatoxin B2 (AFB2). AFGj and AFG2 are typically found in tests of peanuts, however only AFBI and AFB2 are found in almost 90% of the infected com samples tested for aflatoxin.

Aflatoxin was discovered in the 1960s after causing the death of 100,000 turkeys in England as reported by Kotinagu et al, (2015). This is confirmed by NNAMANI et ,al, (2021), who reported that, the term "aflatoxins" was initially used in the United Kingdom in the early 1960s to characterize the toxins linked to tainted animal feed known as peanut and the lost turkey in England. It is likely that agricultural products like cereals, grains, nuts, and dried fruits harbor aflatoxins (NNAMANI et al, 2021). Aflatoxins are toxic compounds that could exist in human or animal food under certain circumstances (Chase et al, 2013). These aflatoxins are carcinogenic to both humans and animals (Kotinagu et al, 2015). Aflatoxins were classified into aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), and aflatoxin M1 (AFM1) (the chemical composition of the different mycotoxins is shown in figure 1). Aflatoxin B1 (AFB1) is the most prevalent of these aflatoxins. The other aflatoxins are not present when AFB1 is absent (Schrenk et al 2020).

Mycotoxins, mainly aflatoxin are caused by many species of aspergillus fungi, but the main aflatoxin producing fungus species are both by *Aspergillus flavus* and *Aspergillus parasiticus* (Lopez-Garcia et al, 1999). The contamination of food and animal feeding staff is a major nutritional issue that is globally spread, and known mainly as a post-harvest problem (Chakraborty et al., 2021). It is health risking and causing cancers and other diseases to human and animal (Spencer Smith et al, 2019). NNAMANI et al (2021) confirm this as they published that aflatoxin poses a major risk to human and animal health since it can build up via the food chain.

In their review, Bennett and Klich (2003) reported the different types of mycotoxins and the fungus species producing these mycotoxins on different crops (Table 1) summarize these mycotoxins.

Table 1

Different fungi species, their mycotoxin type, and crops

Fungus species	Crops	Mycotoxins
Aspergillus flavus	Corn, groundnut, oilseed, cottonseed	Aflatoxin (carcinogenic to human)
Aspergillus parasiticus	Corn, groundnut, oilseed, cottonseed	
Aspergillus nomius	Corn, groundnut, oilseed, cottonseed	
Fusarium oxysporum	Wheat, Barley, Corn	Fumonisins (Hepatotoxic and Nephrotoxic)
Fusarium sp.	Wheat, Barley, Corn	T-2 Toxin
Penicillium verrucosum	Wheat, Barley, Corn	Ochratoxin (Potentially carcinogenic)

Sources: Bennett and Klich, 2003

According to LOPEZ-GARCIA et al. (1999), mycotoxin-contaminated food and feeds present a special challenge regarding food and feed safety because they are unpredictable and inescapable. long-term exposure to contaminated food with aflatoxin causes immunodeficiency and interferes with children's metabolism of micronutrients. Aflatoxin is also highly prevalent in staple foods and in places where there are no regulations or laws that govern food safety (Wu et al.2014; Jallow et al, 2021).

The risk of aflatoxins to human health was evaluated by the European Food Safety Authority. Grain and grain-based products were identified as the primary contributor to the risk of dietary chronic exposure as reported in the assessment published by Schrenk et al (2020).

Aflatoxins cause liver cancer as published by FAO (2018). Yiannikouris et al, (2021) mentioned that aflatoxins can affect animals' health and performance when eating contaminated foods. Animals afflicted by mycotoxins may exhibit symptoms such as digestive problems, decreased feed consumption, poor thrift, impaired immunity, impaired reproduction, and a look of malnutrition, according to Quinn et al. (2002) who

reported the syndromes of mycotoxins on animals. They are linked to the ingestion of contaminated feeds and forages but are not spread from animal to animal (Quinn et al, 2002). However, mycotoxins resist decomposition through the digestive tract, even in ruminants, therefore, they may exist in meat, milk, or any other dairy products (Attia and Harisa, 2016).

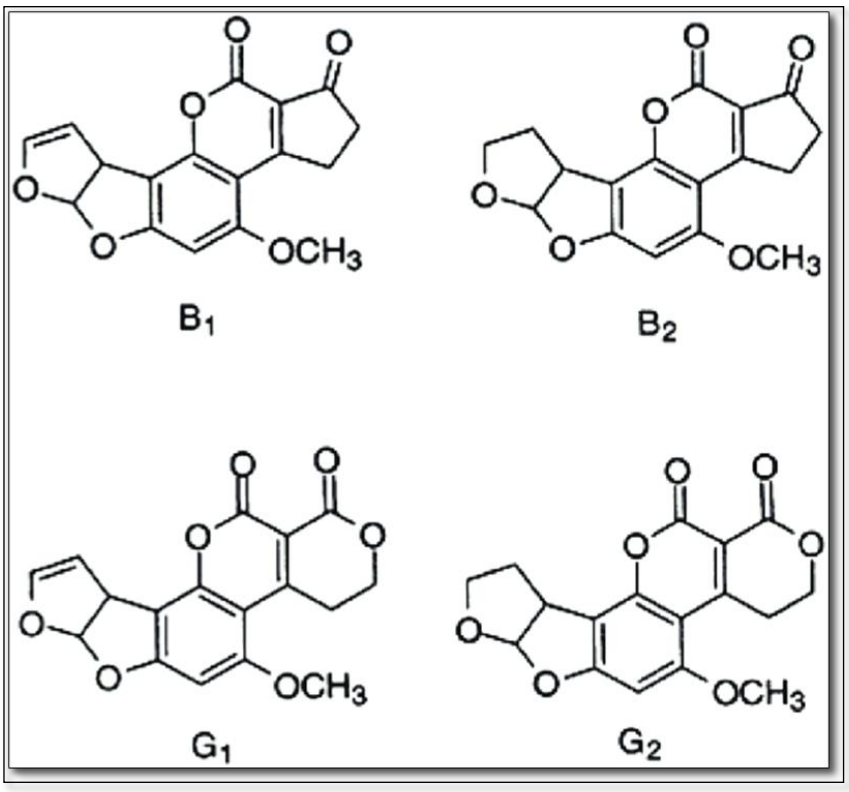
High humidity and moderate temperature under aerobic conditions are the fungi's optimal growth conditions (Larousse et al., 2017). Diener et al., (1987) reported that for aspergillus the ideal temperature range for fungal growth is 25 up to 28 degrees Celsius. The relative humidity levels are in the range of 65% up to 80%. In instance, Aspergillus flavus can only flourish in environments where the relative humidity is higher than 85 percent. Or the grain content of moisture exceeds 16 % (Ongoma, 2013). However, there are several variables involved in the incredibly intricate relationship between mycotoxins and climate change.

Medina and Megan (2014) established a model that incorporates the relative expression of ten metabolic genes in the process, growth, and synthesis of aflatoxin B1 (AFB1) has been constructed and validated under circumstances of increased temperature and water stress. For the first time, the effects of the interacting circumstances of air humidity, temperature, raised carbon dioxide. (Interaction of temperature and the surrounding environment have little influence on growth, but they have an important effect on CO2 existing levels). This shows that while such interacting genes are involved in aflatoxin biosynthesis, they may also strongly increase the production of AFB1 (Medina and Megan, 2014).

The existence of such conditions motivates the germination and growth of fungi spores. If the spores pass through biotic and abiotic stresses, they can directly release mycotoxins as secondary metabolites to stored components or plants (CAST,2003). This is confirmed by FAO (2018) which published that the main aflatoxin-producing fungi are aspergillus. Among these, *Aspergillus flavus* and *Aspergillus parasiticus*, are the major fungi species that produce aflatoxins. These fungi prefer to grow in warm and humid regions of the world. Crops contamination could occur during different sites, as the growth in the field, or during the harvest, transportation and storage.

Figure 1

The chemical composition of four kinds of aflatoxins



Source: Attia and Harisa, 2016

Liu and wu, (2010) reported that aflatoxin B1 is the most harmful aflatoxin of the four types of aflatoxin. It is the most toxic and potentially carcinogenic that is correlated to liver cancer in many species.

Aflatoxin damages the liver, particularly when combined with hepatitis B infection, where it causes 5-28% of instances of hepatocellular carcinoma worldwide as reported by Liu and Wu, (2010). According to Attia (2010), aflatoxin B1 is an indirect carcinogen. The liver metabolizes aflatoxin. This metabolism process by liver enzymes produces “B1-Exo-8,9-epoxide. The final process causes DNA mutations and genomic instability (Attia, 2010). This is confirmed by IARC (2012), which published that the aflatoxin effect in all previous assessments is liver cancer. Aflatoxin passes first through metabolism in the liver, and through this process, toxic metabolites are formed. AFB1, AFB2, and AFG1 cause mutations and may induce damage to DNA. While AFB1 may cause point mutations in cells in addition to the genetic instability for mammal (IARC, 2012).

In comparison to poultry, which is vulnerable to AFB1, ruminants are more resistant to aflatoxins and other mycotoxins (Monson et al 2015). According to Oğuz et al. (2000), AFB1 can cause hepatic toxicity, teratogenicity, or carcinogenicity in addition to other hematological issues.

Aflatoxins, particularly AFB1, pose substantial health risks due to their high toxicity and carcinogenic properties. Aflatoxin B1, produced by Aspergillus species, is especially prevalent in warm, humid climates, where it contaminates staple crops such as corn, peanuts, and tree nuts. Once ingested, AFB1 can lead to severe liver damage, particularly in individuals with pre-existing liver conditions. Research has highlighted the need for comprehensive aflatoxin control strategies, emphasizing rigorous testing and prevention protocols to limit aflatoxin contamination across all levels of the food chain. The European Food Safety Authority underscores the importance of monitoring dietary exposure, especially in regions where consumption of contaminated grains is high.

2.4 Economic impact of aflatoxins

The effect of aflatoxins goes beyond the health risks. It causes severe economic losses around the world. Bouhet and Oswald (2005) published that aflatoxin is causing losses of millions of dollars around the world as agriculture products losses. CAST (2003) estimated a loss of 1.5 billion $, among them 932 million US$ in the United States. The economic losses of African agricultural exports to Europe are reduced by 64% causing losses estimated at 670 million US$ annually (Bankole and Adebanjo, 2003).

Diener et al., (1987) reported that Aflatoxin contamination before harvest seems to be common in the US, mostly in cottonseed, peanuts, and corn, however it can affect any crop that hasn't been fully dried after harvest. They reported that A review of A. flavus's preharvest infestation of peanuts and the ensuing aflatoxin contamination which has been published found that, the Southeast of United States, had significant economic losses in cattle and peanut and corn crops in 1972, 1977, 1980, and in some places in 1978, 1984, and 1985, which highlighted the significance of aflatoxin contamination.

Climate changes favor the deterioration of crops. Under rainy-warm climates the growth of aspergillus is motivated, and the mycotoxins production increase as reported by Stathers (2013).

Certain environmental conditions drive the formation of mycotoxins; however, the degree of contamination varies depending on the area, agricultural practices, and the vulnerability of commodities to fungal penetration during storage and processing periods. Accordingly, fungi that cause poisons in food are divided into two categories: field fungus and storage fungi, depending on the conditions in which they must flourish ecologically (Adeyeye, 2016).

Environmental factors affect the occurrence of aflatoxins, for that, the contamination extent will vary by location and agricultural behavior of the farmers (Algul and kara, 2014). 'Elevated temperatures and the effects of drought are the main causes of elevated aflatoxins. Corn and A. flavus are directly impacted by the two climate conditions. According to Cotty and Jamie-Garcia (2007), A. flavus grows, conidiates, and disperses due to extreme temperatures and dry circumstances, which hinders the development and growth of corn (Ongoma, 2013).

The drought which is growing as a result of climate change, made the plants to be more susceptible to fungal mycotoxins as stated earlier (CAST, 2003). As a result, increasing aflatoxins contamination reduces the produced quantity as a result of fungal growth, in addition to the reduced crop quality. At the same time, it decreases livestock productivity (Gbashi et al, 2018). The adaptation of fungal species that produce mycotoxins to the new climatic changes may produce a new status with a more aggressive fungal invasion of the crops, which causes (Medina et al., 2016) increased contamination with mycotoxins or the worst; the production of new mycotoxins (Medina et al., 2017).

Megan (2017) stated that, in growing countries such as African countries and developing Asian countries, this new situation is expected to pose higher significant impacts on crop production. This status is forming major risks to food security in these countries in the form of low nutritional quality attached to the crop quality and quantity losses in these low and medium-developed countries (Magan, 2017).

Globally About 35% of world food and animal feed products are expected to be contaminated with mycotoxins as estimated by Rahmani et al, (2009). While Eskola et al, (2019) estimated this percentage of contaminated food by 25%. However, it was estimated that annually 1 billion tons of food are lost or wasted due to contamination (Rahmani et al, 2009). The trade in Africa is severely affected by aflatoxins, as a result of the low-value products presented for sale, which reduces the prices of agricultural products at all trading levels as reported by Gbashi et al (2018). This confirms the results of Marechera and Ndwiga (2015) who reported that more than 2.3 million corn bags were found not suitable for trading in the period 2004 – 2006 due to aflatoxicosis, while corn prices dropped down by 50% in Kenya in 2009 as a result to aflatoxin alert (Marechera and Ndwiga, 2015).

In addition, economic losses in all steps of the chain of food production are expected to occur. Farmers; distributers, crop processors, and consumer is to face losses due to contamination with mycotoxins (Alvarado et al, 2017). Ajmal et al, (2022) confirmed this as they reported the economic losses in Pakistan. They reported that the Elevated levels of aflatoxins in frequently used goods made in Pakistan, are an important concern for food safety, presenting grave health hazards to the populace. Aflatoxin poisoning also limits the amount of these goods that can be exported, which results in financial losses (Ajmal et al, 2022).

Among the direct losses is the increase in veterinary costs and reduced livestock production (Alvarado et al, 2017). Gallo et al (2015) noticed that in processed animal feed if an ingredient is contaminated the completely feed batch could be contaminated. This is confirmed by Kovalskv et al, (2016), who reported that the spoilage could exceed the batch to reach the whole shipment if a feedstuff iscontaminated. Furthermore, this could result in spoilage of other shipments as the transporting method could be described as fungi contaminating sources (Kovalsky et al, 2016). This deterioration influence has repercussions on the global trade of food material and the exchange of animal processed feed and animal feed components as reported by Kovalsky et al, (2016).

In Palestine, the researches on aflatoxin's existence in food and feedstuff are very limited. According to Ahmad (2020) this topic is new in the field of research in Palestine, where

he stated this by reporting that:" This is the first study in Palestine on account of AFB1 in these food staff" (Ahmad, 2020).

The economic repercussions of aflatoxin contamination extend beyond the agricultural sector, affecting entire economies, especially in developing regions. Contaminated crops result in reduced market value, limiting trade opportunities and discouraging investments in agriculture. For example, losses in African agricultural exports to Europe due to aflatoxin contamination are estimated to reach $670 million annually. Furthermore, the costs incurred by livestock farmers increase due to contaminated feed, which reduces animal productivity and necessitates higher veterinary expenses. Climate change exacerbates these economic impacts, as rising temperatures and variable rainfall promote aflatoxin-producing fungi, leading to increased contamination and reduced crop yield. Such climate-induced losses may result in even greater economic instability in vulnerable regions, underscoring the importance of aflatoxin management and regulatory policies to safeguard both health and economic growth.

2.5 Main component of animal feed

In ruminants, grain and cereals are not essential part of feed in all cases as reported by Makkar and Beever (2013), who mentioned that remenant can produce without including grain and cereals in the feeding diet, therefore these components could be dispensed and substituted with other components, while, at the same time, the efficiency of animal production increases highly when the grain is included in the diets (Makkar and Beever, 2013).

This increase in production and enhanced efficiency raises the net review for the farmers and makes livestock farms more profitable (Makkar and Beever, 2013). However, the feeding costs are forming almost two – thirds of the production costs (Guerre, 2016). Under the The amount of nutrients required for livestock is known after the report of US National Research Center (NRC) first report regarding swine (Guerre,2016). Since those regular reports for the upgrade are published. The most spread recommendations around the world to define the nutrient requirements of poultry are those released by (NRC) 1994 report (NRC, 1994). But at the same time, there is a need for the upgrade of these reports as reported by (Applegate and Angel, 2014). Indeed in different countries, many tables are showing the composition of the ratio and the nutrient values of the feed materials,

which could be used to upgrade and complete the (NRC) recommendations (Kyntäjä et al, 2014). As published by Ravindran (2013) the feeding requirement which is based on nutritional requirements varies according to age; stage of production; and animal species. These factors define the ration composition (Ravindran, 2013). The main component of the ration is the energy source materials, in the specific growth stage of ducks and geese it can raise up to 99% (Arroyo et al, 2016). The main cereal crop used in animal feed is corn. It is used mainly as a source of energy (Guerre, 2016). Corn is forming 50 – 80% of the animal diet in the USA as well as in Europe (Ravindran, 2013). While in Africa and Asia the percentage is less, since low corn is produced (Ravindran, 2013), and the price of imported corn is high for the farmers. Therefore, corn is forming a lower percentage in the diet as it is imported, as published by (ITC, 2017). Around the world, the major producers of corn are USA and Brazil, while the major importers are Japan and Mexico (ITC, 2017).

During the transportation of corn, the humidity plays a direct factor in the possibility of contamination with mycotoxins, where it may favor the increment of aflatoxin concentration in the grains or feed products (Kana et al, 2013). As mentioned, the percentage of corn in the diet is low in Africa and Asia. Therefore, alternatives of corn are used and corn is not widely used. Pearl millet is an alternative that is widely known as an energy supplier source for poultry, especially in areas with hot temperatures with drought as in India (Batonon et al, 2015). During the production process, there is the by-product as: rice bran; wheat feed, corn gluten…etc. The use of these by-products is increasing in feeding animals (Hazzledine et al, 2011). Usually, these compounds are rich in fibers with energy content, therefore it is rarely used in poultry feeding, even though it is used in some rations with percentages reaching 10 – 15% (Stein and Shurson, 2009).

True lipids and oils are very rich in fats which are concentrated energy forms (9 kcal/g in general), but due to the very high cost of using fats and oils as an energy source in livestock diets, in addition to the rancidity risks usage is limited to 2-5% only (Baião and Lara, 2005).

In response to high costs and sustainability concerns, alternative feed ingredients are being increasingly adopted. For instance, sorghum and millet serve as cost-effective substitutes for corn, particularly in hot, arid regions where corn production is limited.

These grains provide a reliable source of energy and are drought-tolerant, making them valuable for areas like Palestine, where water scarcity affects crop choices. Similarly, by-products from the milling industry, such as rice bran and wheat feed, are used as supplementary components. Though rich in fiber, these by-products are generally limited in poultry diets due to lower digestibility and energy value compared to grains like corn

2.5.1 Protein supplier grains

The second main component of animal feeding diets is the grain which supplies protein as soybean. These grains are the second component (second largest) of these diets (Wu, 2014). It is used to supply amino acids to animals, and the supplementation of amino acids is a common practice in the animal feed process. Both essential amino acids and non-essential amino acids are supplied together. This is to avoid the convert of basic essential amino acids into non – essential amino acids to substitute the shortage if the non-essential acid is not supplied in the diet. To prevent this conversion both types of amino acids are supplied during the feed processing stage (Wu, 2014).

Soybeans are among the most significant crops grown worldwide is soybeans, a kind of legume with East Asian origins. Soybeans are essential to the world's food supply since they produce about half of the protein and vegetable oil produced globally. Vegetarians and vegans frequently utilize soybeans in place of animal protein sources due to their high protein level. Furthermore, it is commonly known that soybeans, their byproducts, and processed byproducts are abundant suppliers of phenolic compounds, particularly isoflavones (Calori-Domingues et al, 2018).

Soybean meal is the widest used grain as a source of protein mainly in poultry diets (Beski et al, 2015). Soybean meal is high in protein content, where it contains 40- 50% of its weight as crude protein. This percentage is affected by the removed hulls and the procedure of oil extraction (Beski et al, 2015). This is supported by the findings of Hulshof et al. (2016), who reported that despite the presence of anti-nutritional elements in soybean meal soybean is still used up to 30% in the complement of diets that is cereal-based (Hulshof et al, 2016).

Rapeseed, sunflower, and canola meals are further oil extraction process byproducts. These byproducts can be included in animal diets as a source of protein as indicated by

Choi et al, (2015). Considering its nutritional value, rapeseed has a lower nutritional value than soybean. This is due to the lower protein content of rapeseed, and the fiber content is higher, in addition to the fact that rapeseed contains anti-nutrients (Lomascolo et al, 2012). If rapeseeds are used in the animal diet to supply protein as an alternative to soybean it should be less than 10% of the diet as suggested by (Kaminska, 2003), or the proteins content should be substituted from other source.

Mejicanos et al, (2016) suggested that, canola could be used in animal feeding diet processing, where it is another grain with good protein content, therefore, this crop is possibly used in animal feeding as a protein source in the diet. Canola was developed during the 70s of the last centuries, during a plant cross-breeding process which was conducted aiming to remove glucosinolates in addition to removing erucic acid from rapeseed (Mejicanos et al, 2016). However, animal performance is affected if canola substitute more than 50% of soybean in the diet (Mejicanos et al, 2016).

To meet the protein requirements, protein from animal sources like fish meal is possibly to be used to balance the protein content. This is especially in young animals feeding diets, where the animal protein-building processes in this stage require high quantities of amino acids. However, due to the high costs of this protein supplier, the rate of use of this source is limited and low (Ravindran, 2013). As the major component of animal diets are coming from grain, which is subjected to the risk of contamination with aflatoxins and other mycotoxins. Table (2) shows the normal percentage of most used grains in poultry diets. The possible risk of contamination with mycotoxins attached to each component is presented in table (3).

Table 2

Grains as a component of poultry diets

Grain crops	% in Diet
Millet, Corn, Sorghum	0–60%
Wheat, barely	up to 30%
Oats	0–90
Rice	less than 30%
Cereal by-products	0–30 %
Soybean meal	0–40 %

Source: Guerre 2016

Table 3

Risk of mycotoxin contamination of diets component

Raw Material	Mycotoxins [n]
Cereals: Wheat, Barley, Oats	Usually, weak contamination with aflatoxins
Rice	The contamination risk with aflatoxins is high.
Corn	High risk of aflatoxin contamination
sorghum Millet	Similar to corn with a lower rate of contamination
Lipids as energy sources such as animal fats or Oil	Usually very low risk.
Soybean	Low to medium risk of contamination.
Rapeseed, Sunflower meal, and canola meals	Indirect evidence for the presence of Aflatoxins
Cotton seed meal	High level of Aflatoxins
Animal protein	Rare to very low contamination.

Sources: Adapted from Guerre, 2016

In addition to soybean, several alternative protein sources are utilized to balance the amino acid profile in animal feed. Rapeseed, sunflower, and canola meals, by-products of the oil extraction process, serve as viable options for supplying protein when soybeans are limited or costly. Rapeseed meal, though lower in protein than soybean, is used in certain regions, albeit with limitations due to its higher fiber content and presence of anti-nutritional factors. Research suggests that while rapeseed can replace soybean partially, it is generally advised to keep its inclusion rate below 10% of the diet to avoid adverse effects on animal growth and feed efficiency

2.6 Regulations on the maximum level of aflatoxins

The global consideration of the maximum levels in agricultural products is taking into account the resistant nature of mycotoxins to the main food processing practices like milling (Adeola & Kong, 2014).

In many countries around the world, there are regulations restricting the maximum levels of mycotoxins concentrations in the food or feed raw and processed materials. At the same time, there are no complete regulations for all mycotoxins (Alvarado et al, 2017). The most regulated mycotoxins are Aflatoxins and some other types of mycotoxins.

Those types have demonstrated health risks and toxic effects on both humans and animals (Alvarado et al, 2017). These regulations consider many factors when defining the maximum limits of the allowed concentration level of mycotoxins in food and feed. Among but not all of the considered factors are: the nature of the agricultural goods and products; the type of use; the age and growth period of the livestock that is subjected to consume the processed feeds (Alvarado et al, 2017).

Different countries around the world adopt national standards regarding the acceptable levels of concentrations of different contaminants. Part of these acceptable levels is the concentration of aflatoxins in human food or animal feed (or in feeding components). The regulations are mainly relying on American or European regulations. The major differences are in the type of classifications whereas in the US guidelines the classification is based on the animal's kind and age. While in European regulations the classification considers the feeding materials together with the animal kinds. In the Food and Drug Administration, the upper limit of the allowed concentration of aflatoxins in most of the used grains is 20 parts per billion. Table (4) gives an example of the guidelines of both the American and European regulations regarding aflatoxin. In this example, the difference is found mainly in the allowed concentration for dairy animals, and small animals.

Table 4

Example of the maximum allowed levels of aflatoxin concentrations in EU and US

Grain	Animals	Aflatoxin Level	
		(Parts per billion)	
		US	European
Corn, Peanut	Diary animals	20	5
	Animals not listed or unknown uses	20	Not listed
	cattle, sheep, goats.	not listed	20
	immature animals	20 ppb	Not listed
	Calves and lambs		10

Sources: adapted from Alvarado et al, 2017

As shown, the maximum levels (MLs) recommended for aflatoxin B1 (AFB1) concentration in corn is 20 (μg kg−1) for corn, however, the MLs are different according to the factors mentioned earlier. For example, EFISC (2015) explains the maximum levels of corn as the following:

- Corn passes through treatment (either physical or sorting) before human direct consumption or before being an ingredient in foods. The maximum allowed level of aflatoxin B1 concentration is 5 (μg/kg). At the same time, the sum of aflatoxins (B1+ B2+ G1 + G2) is less than 10 (μg/kg).
- If the concentration of Aflatoxin B1 is between 2 – 5 (μg/kg) and the sum of other aflatoxins is 4 – 10 (μg/kg) in a shipment of corn. The shipment must be labeled to show the existing concentrations and showing the possible risks.

Before the production of foodstuff that contains corn, the aflatoxin B1 concentration must be reduced to less than 2 (μg/kg) and the sum of aflatoxins is less than 4 (μg/kg). (for instance, the reduction could be achieved by mixing with uncontaminated corn). Maximum limits for aflatoxin B1 in food materials are specified at 2,0 ppb, in the same time the total of aflatoxin B1+B2+G1+G2 for any food or feedstuff for corn and any product that contains corn is 4,0 ppb. While for animal feeding the maximum level of aflatoxin concentration 20,0 ppb, for diary and young animals, the maximum concentration level is 5,0 ppb. This includes the goods that processed corn is an ingredient in it (EFISC, 2015). In this study, Blonski, Wojciech, David S. Kotlyar, and Kimberly A. Forde, (2010) The most specific and sensitive analytical method for this purpose is liquid chromatography-tandem mass spectrometry, using stable isotope-labeled isotopes of AFB1–FapyGua and AFB1–N7–Gua as internal standards. As internal standards, cis-AFB1-FapyGua-15N5, trans-AFB1-FapyGua-15N5, and AFB1-N7-Gua-15N5 have all been synthesized and effectively employed. Nevertheless, neither commercial nor scholarly sources are currently offering these criteria. As a result, it is still difficult to collect quantitative genomic data on AFB1-induced DNA damage in animal and human models (Medina et al., 2014).

This study examines the data that is currently available on the possible effects of important environmental parameters and how they interact with Aspergillus flavus's molecular ecology, growth, and production of aflatoxin in maize grain in vitro. Recent research on the effects of temperature and water activity on the biosynthesis of aflatoxin

and the formation of phenotypic aflatoxin is reviewed. These have demonstrated a direct correlation between aflatoxin B1 synthesis and the relative expression of important structural and regulatory genes under various environmental circumstances. A model that incorporates the relative expression of ten biosynthetic genes in the pathway, growth, and the formation of aflatoxin B1 (AFB1) has been constructed and validated under conditions of increasing temperature and water stress. The result for the first time, the effects of the interacting conditions of aw x temperature x high CO2 (2x and 3x current levels) are outlined. This shows that although these interacting environmental factors have little influence on growth, they can greatly enhance the production of AFB1 and have a major impact on the expression of the genes involved in the biosynthesis of aflatoxin (structural aflD and regulatory aflR genes). The combined effect of these three abiotic factors affects the generation of mycotoxin, even though each factor alone has some effect. This method offers the data required to forecast the actual effects of climate change on mycotoxigenic fungus (Calori-Domingueset et al., 2018).

Four states in Brazil provided samples from commercial lots used in this investigation. An HPLC-FLD analysis was performed to determine the distribution of mycotoxins in soybean fractions based on their commercial grading system: whole kernels (WK), split, broken, and crushed kernels (SBCK), damaged kernels (DK), heat damaged and burned kernels (HDBK), moldy kernels (MK), greenish kernels (GK), and foreign material + impurities (FMI). In 43.3 and 80% of cases, respectively, AFB1 and ZEN tested positive. MK had the highest incidence of AFB1 (50%), followed by HDBK (30.4%) and FMI (26.0%). The incidence of ZEA varied between 69% (SBCK) and 100% (HDBK). Additionally, co-occurrence (53.3%) in a minimum of one fraction was noted. Brazil is the second-largest soybean producer in the world.

Aflatoxin regulations vary across regions, reflecting differences in local agricultural practices, consumption levels, and environmental conditions. In the European Union, for example, the maximum level for aflatoxin B1 in grains intended for human consumption is set at 2 μg/kg, with a combined total for aflatoxins (B1, B2, G1, and G2) at 4 μg/kg, while higher limits are applied to feed materials. In contrast, the U.S. Food and Drug Administration permits up to 20 parts per billion (ppb) of aflatoxins in most feed grains, with a stricter limit of 5 ppb for dairy products to prevent transmission into milk. These regulatory frameworks highlight the emphasis on protecting vulnerable populations, such

as young children and dairy consumers. However, developing countries often face challenges in enforcing aflatoxin limits due to limited resources and infrastructure, resulting in higher exposure risks for local populations.

2.7 Impacts of Climate Change on Mycotoxin Contamination

The relationship between climate change and the prevalence of mycotoxin contamination in crops has garnered increasing attention in recent years. Rising global temperatures, along with fluctuations in humidity and rainfall patterns, have created more favorable conditions for fungal growth, particularly for Aspergillus species that produce aflatoxins. Studies have shown that extreme weather events, such as droughts followed by intense rainfall, can stress crops and make them more susceptible to fungal colonization and mycotoxin production (Medina et al., 2014). In regions like Palestine, where agricultural production is critical for food security, these environmental changes can have serious implications for both crop yield and quality.

Research indicates that aflatoxins, particularly aflatoxin B1, are more likely to contaminate crops in warmer climates. The shift in global climate conditions has led to higher incidences of aflatoxins in areas that were previously less affected, thereby broadening the geographical scope of contamination risks (Wu et al., 2011). This highlights the importance of monitoring and regulating aflatoxin levels in agricultural products, especially in regions like Palestine, where the agricultural sector is highly vulnerable to climate-related disruptions. Understanding the complex interaction between environmental factors and mycotoxin production is crucial for developing strategies to mitigate the risks associated with aflatoxin contamination in food and feed.

The influence of climate change on mycotoxin contamination has gained critical attention as global temperatures and carbon dioxide levels continue to rise. Studies have shown that elevated temperatures and increased drought frequency create conditions that favor the growth of aflatoxin-producing fungi, particularly Aspergillus flavus. Research indicates that extreme weather, such as prolonged droughts followed by heavy rains, can weaken crops, making them more susceptible to fungal infection and mycotoxin production. Aflatoxin B1 contamination is particularly concerning, as warmer and more humid environments accelerate its biosynthesis, amplifying health risks associated with contaminated food and feed. In regions like Palestine, where agriculture is integral to food

security, these climate-induced risks necessitate proactive monitoring and mitigation strategies to ensure food safety and reduce potential economic impacts on the agricultural sector.

Chapter Three
Methodology

3.1 Study area and samples collection

A cross-sectional study was conducted in Jenin, Nablus, Tulkarm districts. The study targeted measuring the aflatoxin B1 concentration in both corn and soybean in the siloes of animal concentrate feed processing factories in the three districts. The study included 5 factories in Jenin, 7 factories in Nablus, and 8 factories in Tulkarm.

Due to its contribution to the Gross domestic product (GDP) and the employment opportunities it offers to the labor force, the agricultural sector is one of the most significant parts of the Palestinian national economy. Because it provides meat and milk and accounts for 39.1% and 45% of agricultural revenue, respectively, livestock is a vital part of the agricultural industry. Small ruminants are regarded as one of the most significant parts of the livestock sector when it comes to the overall contribution of the agricultural sector to the national income, as sheep and goats are crucial to animal production and serve as its backbone and main nerve because of Their social and economic significance to Palestinian farmers, keep in mind that there are 25,311 cattle, 177,401 goats, and 541,299 sheep in Palestine.

Two composite samples of 1 kg from each source were collected from each factory. The first sample was corn, while the second was soybean. The sample were collected from different locations in the feed silos, then the grains were mixed well, and the composite sample was packed and stored in cold dark container and transferred to the lab for analysis.

Figure 2

The geographical location of the study implementation within the red circle: Nablus, Jenin, Tulkarm

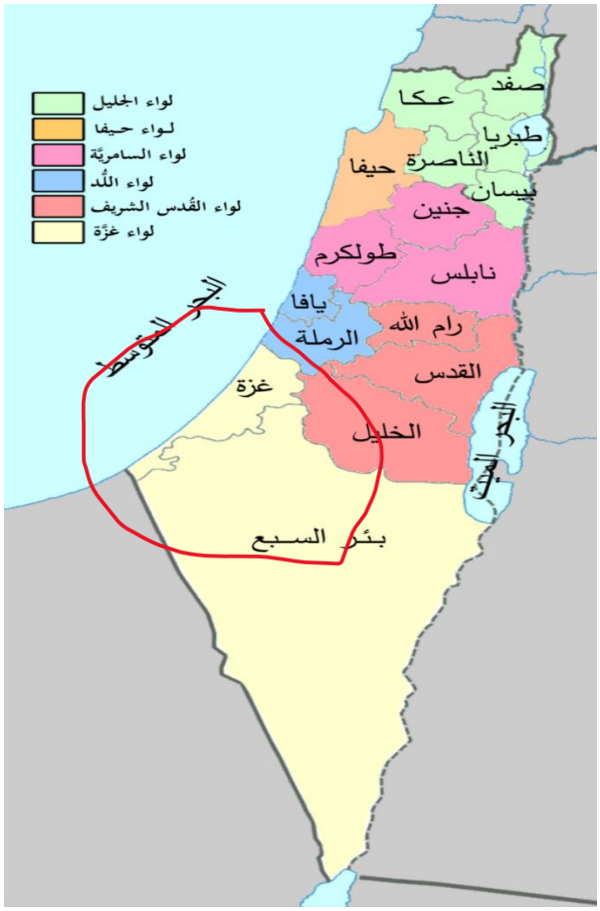


Figure 3

Collecting three corn and beans expected from the feed silos

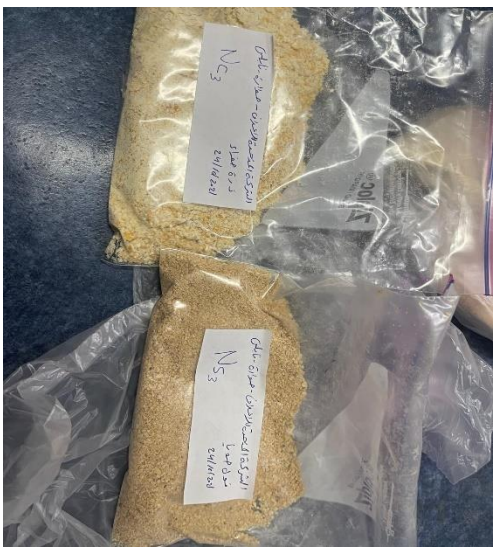
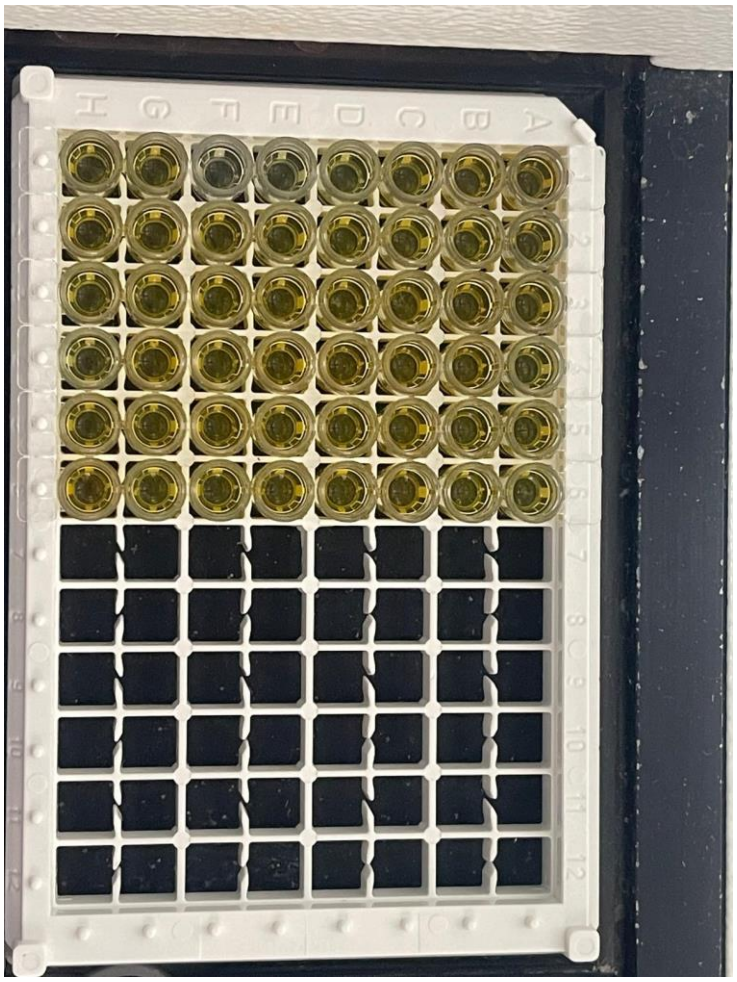


Figure 4

Determination of aflatoxin B1 concentration in samples



3.2 Enzyme-linked immunosorbent assay (ELISA)

Quantitative AFB1 in the samples was analyzed using competitive direct enzyme-linked immunosorbent assay (ELISA) of commercial ki RIDASCREEN® Aflatoxin B1 30/15 kits according to the manufacturer's instructions. Samples were dried and brought to room temperature and grinded. Five gm of the sample powder was mixed with 25 ml methanol (70%). The mixture is strongly shaken for three minutes. Then it was extracted using filter paper (Whatman No. 1 filter paper). The extract was diluted with 1 ml of the filtered extract and 1 ml of distilled water (1:1 ratio). In each ELISA plate, 50 µl of the diluted extract and 50 µl of the supplied conjugate were placed in each well. Following this, 50 µl of antibody was added to each well and mixed slowly by shaking the plate and incubating for 30 minutes at room temperature. The extract was poured out of the wells, the micro-wells holder was tapped upside down against absorbent paper vigorously to

ensure the complete removal of extract from the wells. The wells were washed with 250 µl of the washing solution two times, then 100 µl of substrate/chromogen is added to each well. Mixed gently by shaking the plate manually. 100 µl of stop solution is added to each well and incubated for 15 min. In order to measure the absorbance, the spectrophotometer is used at 450 nm. The measured optical density and the concentration of aflatoxin B1 has a reverse proportion.

Figure 5

Preparation Sample of kits through ELISA

Figure 6

Sample inspection process

3.3 Relative humidity data

The data on relative humidity in the three districts were collected from the department of climate change monitoring in ministry of agriculture, and the Palestinian meteorological department. Both sources have similar data, for that in the calculations the study used the data of meteorological department as cited in the references.

The data is for the outside air relative humidity, which reflect the conditions in the siloes surrounding environment, however as the siloes are not sealed, and the air is entering the siloes from outside, it is assumed that the air relative humidity inside the siloes is similar to the outside humidity.

3.4 Statistical analysis

Standards with aflatoxin concentrations of zero; 1; 5; 10; 20;50 µg/kg were prepared, and measured for the generation of the standard curve. The Optical Density (O.D.) was divided with the O.D. of zero concentration standard to obtain the absorbance percentage: The optical density (OD) values of the standards and the samples were normalized to the

mean OD value of the zero standards. The percentages of the absorbance values that were obtained for the standards and the samples were plotted on semi-logarithmic graph paper, against the concentration of the AFB1 standard in µg/kg (Figure 1). The Analysis of Variance (ANOVA) was used for the comparison of the average of AFB1 (µg/L) in the different foodstuffs and the cities. The results were considered statistically significant at $p < 0.05$. Absorbance standard (or sample)/ absorbance zero standard x 100 = % absorbance.

On semilogarithmic graph paper, the results of the standards % of absorbance (the results of computation) versus aflatoxin concentration were displayed. From the results of standard curve, the concentration of aflatoxin B1 present in the samples was identified and tabulated. The measured concentration of aflatoxin in both corn and soybean was statistically analyzed for the differences between groups (different districts) and inside the same group.

Chapter Four

Results

To guarantee the validity of results calibration curve was established.

Figure 7

The calibration curve of 0;1;5;10;20;50 µg/kg aflatoxin to the normalized % of absorbanc

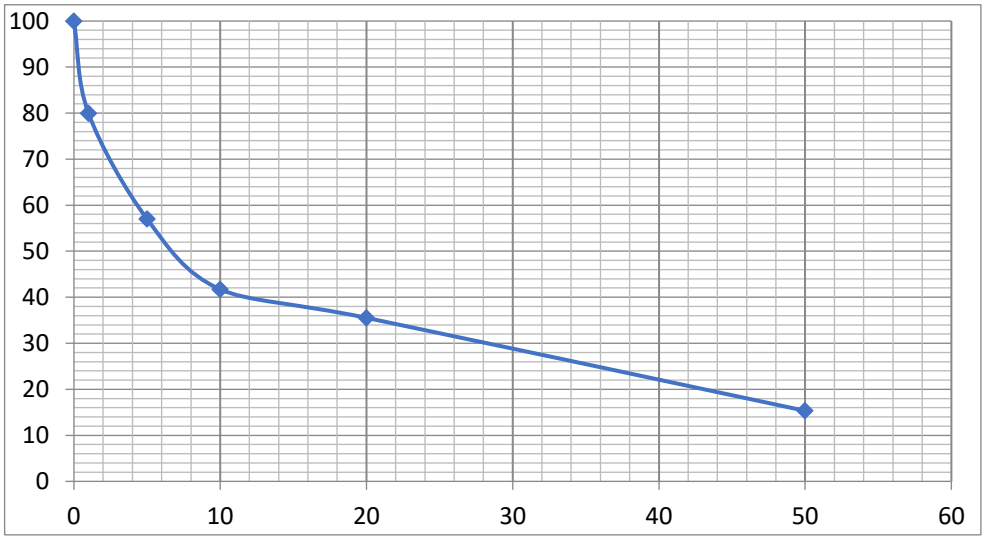


Figure 7 shows the calibration of standards of fixed aflatoxin concentration versus their optical density absorbance after normalization to the zero standard (O.D.) according to the following formula:

$$\% \text{ absorbance} = \frac{\text{Standard O.D.}}{\text{Zero O.D}} \qquad 1$$

As the calibration curve was set, the concentration of aflatoxin B1 was determined directly from the curve.

4.1 Corn results

The results of aflatoxin concentrations in corn in the different districts are shown in (table 5). The results showed evidences of aflatoxin contamination in corn crop in the samples collected from the three districts.

The results showed that, aflatoxin concentration in Nablus district was ranging from 0.05 µg/kg – 0.6 µg/kg with an average equal to 0.336 µg/kg. While the concentration of aflatoxin in the samples collected from Jenin district was ranging from 0 – 1 µg/kg with

an average of 0.36 µg/kg. In the same times results of aflatoxin concentration in the samples collected from Tulkarm have the range of 0.2 – 2.8 µg/kg, with an average of 0.688 µg/kg with 95% confidence interval, and standard deviation ranging from 0.201 – 0.89 µg/kg with an average standard deviation of 0.604 µg/kg. The results of statistical analysis of the results, and the measured aflatoxin concentrations are presented in (Table 5 and 6) and (Fig. 7 and Fig 8).

Table 5

Aflatoxin concentration in corn samples in Jenin, Nablus, and Tulkarm districts (µg/kg)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Nablus	7	0.3357	0.20148	0.07615	0.1494	0.5221
Jenin	5	0.36	0.38471	0.17205	-.1177-	0.8377
Tulkarm	8	0.6875	0.89032	0.31478	-.0568-	1.4318
Total	20	0.4825	0.60465	0.1352	0.1995	0.7655

Table 6

Aflatoxin concentration in corn samples in Jenin, Nablus, and Tulkarm districts (µg/kg)

	Nablus			Jenin			Tulkarm		
Sample	OD	%	conc	OD	%	conc	OD	%	Conc
1	2.095	84	0.6	2.5	100	0	2.141	85	0.6
2	2.266	90	0.3	2.07	83	0.4	1.697	68	2.8
3	2.364	94	0.2	1.995	80	1	2.415	96	0.2
4	2.163	86	0.5	2.38	95	0.2	2.318	93	0.2
5	2.45	98	0.05	2.352	94	0.2	2.213	88	0.4
6	2.144	86	0.5				2.393	96	0.2
7	2.336	93	0.2				2.417	96	0.2
8							2.038	81	0.9

1. (OD): Optical Density reading.
2. (%): the relative percentage (OD of the sample/ OD of the zero-concentration reading).
3. Conc: the calculated aflatoxin concentration from the calibration curve.

The analysis of aflatoxin B1 concentration in corn samples from Jenin, Nablus, and Tulkarm highlighted significant contamination across all districts, with varying levels that reflect regional storage and climatic conditions. In Tulkarm, the concentration levels were notably higher, averaging 0.69 µg/kg, compared to 0.34 µg/kg in Nablus and 0.36 µg/kg in Jenin. This trend may be associated with the higher humidity levels in Tulkarm during the summer months, which can enhance fungal growth and aflatoxin production. Statistical analysis confirmed that while there were no significant differences in contamination levels between districts at a 95% confidence level, Tulkarm's mean values were consistently higher, indicating a potential hotspot for aflatoxin contamination. These findings underscore the importance of climate-controlled storage and regular monitoring, especially during the humid months, to minimize aflatoxin risks in corn.

Figure 8

The average of aflatoxin concentration in corn in Jenin, Nablus, and Tulkarm districts (µg/kg)

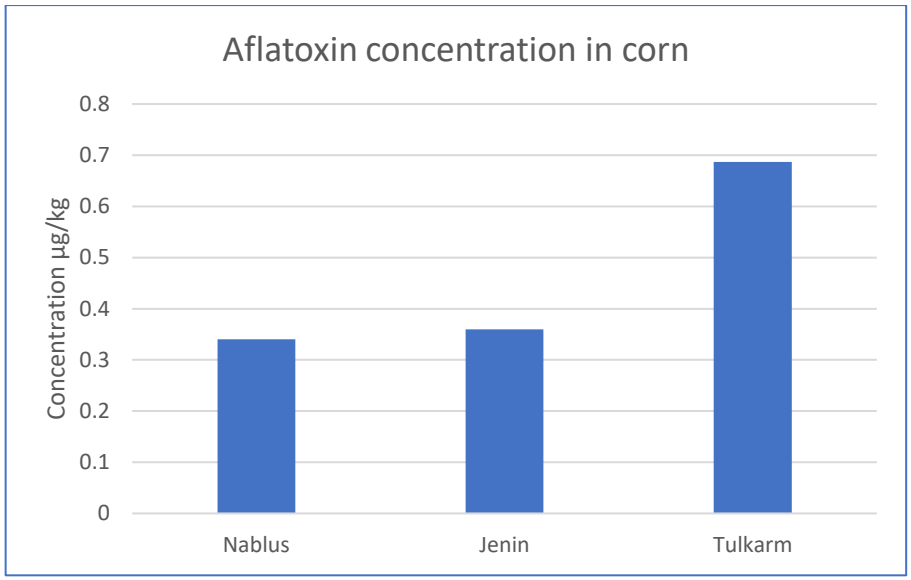
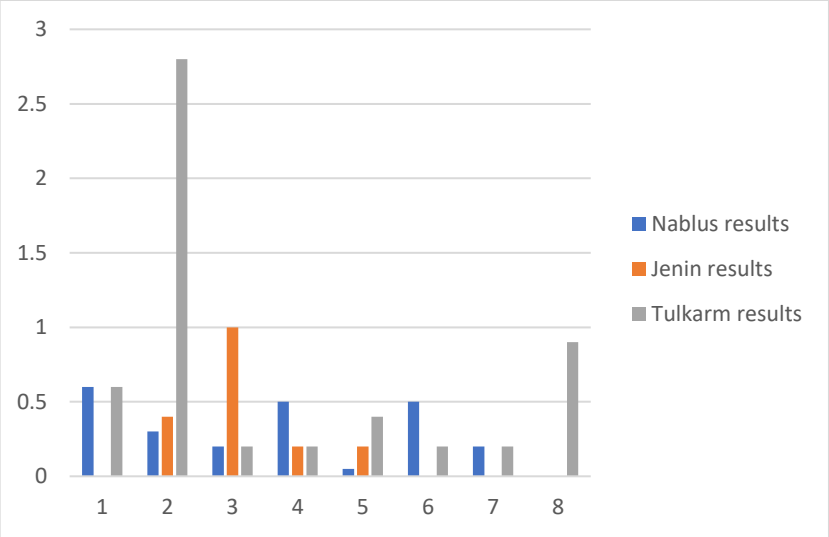


Figure 9

The results of measured aflatoxin B1 concentration in corn (µg/kg), in Nablus, Jenin, and Tulkarm districts



As it is shown in the figure, the district overall value of the average concentration of aflatoxin B1 differs from district to other, where in both Jenin and Tulkarm the value of the overall average was close, compared to Nablus. In the same time, The results of statistical analysis of corn samples show that there is no significant differences in concentration between the three districts at confidence level of 95% ($\alpha = 0.05$) as presented in table 4.3.

Table 7

ANOVA results of aflatoxin B1 concentration in corn samples of Nablus, Jenin, and Tulkarm districts

ANOVA results of aflatoxin B1 concentration in the samples of the districts

µg/kg

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.562	2	.281	.748	.488
Within Groups	6.384	17	.376		
Total	6.946	19			

4.2 Soybean results

The results of samples analysis for aflatoxin B1 concentration in soybean samples in Jenin, Nablus, and Tulkarm reveal that contamination with aflatoxin was found in the samples collected from the three districts with variations in the concentrations as presented in (Table 8), but at the same time these variations are not significant as the statistical analysis reveals (Table 9.). As the results show, the standard deviation was ranging from 0.33 – 1.01 µg/kg with a total standard deviation of 0.542 µg/kg for the three districts at confidence level of 95%.

The comparison of aflatoxin B1 concentrations in corn and soybean samples across Jenin, Nablus, and Tulkarm revealed regional differences that underscore the impact of local environmental and storage conditions. Tulkarm consistently exhibited higher aflatoxin levels in both crops, averaging 0.69 µg/kg in corn and 0.65 µg/kg in soybean, possibly due to its higher humidity during storage seasons, which fosters fungal growth. Although these variations were not statistically significant, they indicate a trend of increased contamination in high-humidity districts. Conversely, Nablus showed the lowest aflatoxin concentrations in soybean samples, with an average of 0.35 µg/kg, suggesting that its relatively lower humidity may inhibit fungal growth and aflatoxin production.

Table 8

Aflatoxin concentration in soybean of the three districts (µg/kg)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Nablus	7	.3514	.36476	.13787	.0141	.6888
Jenin	5	.6400	1.01390	.45343	-.6189-	1.8989
Tulkarm	10	.4150	.33170	.10489	.1777	.6523
Total	22	.4459	.54164	.11548	.2058	.6861

Table 9

The measured aflatoxin concentration (µg/kg) in soybean in Jenin, Nablus, and Tulkarm

Sample	Nablus			Jenin			Tulkarm		
	OD	%	conc	OD	%	conc	OD	%	Conc
1	2.111	84	0.6	1.751	70	2.4	2.013	80	1
2	2.151	86	0.5	2.47	99	0	1.993	80	1
3	2.304	92	0.2	2.504	100	0	2.267	90	0.3
4	2.499	100	0	2.375	95	0.2	2.156	86	0.5
5	2.002	80	1	2.103	84	0.6	2.308	92	0.2
6	2.44	97	0.08				2.464	98	0.05
7	2.427	97	0.08				2.417	96	0.2
8							2.251	90	0.3
9							2.182	87	0.4
10							2.346	94	0.2

- (OD): Optical Density reading.
- (%): the relative percentage (OD of the sample/ OD of the zero-concentration reading).
- Conc: the calculated aflatoxin concentration from the calibration curve.

Table 10

The results of statistical analysis (ANOVA) for aflatoxin concentration (µg/kg) in soybean samples in Jenin, Nablus, and Tulkarm districts

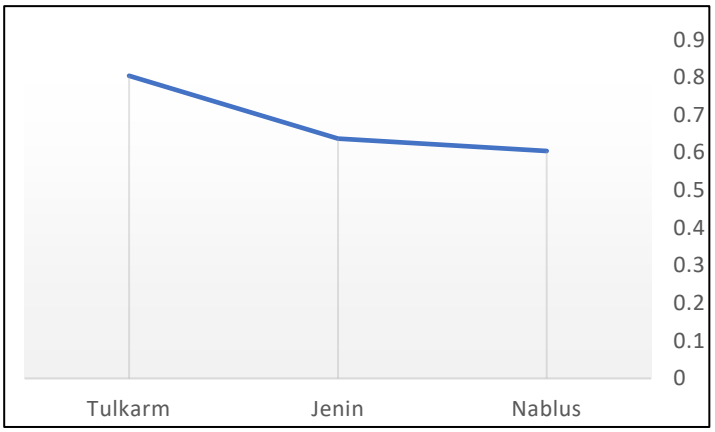
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.260	2	.130	.419	.663
Within Groups	5.901	19	.311		
Total	6.161	21			

The ANOVA results presented in (Table 10) indicate no statistically significant differences in aflatoxin B1 concentrations among soybean samples from Jenin, Nablus, and Tulkarm. Despite some variation in mean concentrations—0.64 µg/kg in Jenin, 0.35 µg/kg in Nablus, and 0.42 µg/kg in Tulkarm—the differences were not significant at the 95% confidence level ($p = 0.663$). These findings suggest that while environmental factors like humidity and storage practices can influence aflatoxin levels, the source of

the soybean, which is imported from similar suppliers, might contribute to the consistency in contamination levels across districts.

Figure 10

The districts average value of aflatoxin B1 concentration (µg/kg) in soybean samples



In Jenin the concentration range was zero – 2.4 µg/kg with an average of 0.64 µg/kg, while in Nablus the range was zero – 1 µg/kg with an average equal to 0.35 µg/kg. While in Tulkarm it was ranging from 0.05 – 1 µg/kg with 0.42 µg/kg as an average. As shown in (Figure 10)

4.3 Comparison of the districts

Aflatoxin was found in the samples of corn and soybean collected from the three districts with different concentrations.

The minimum concentration of aflatoxin B1 in the samples was zero µg/kg, found in a sample belongs to Jenin district samples, while the maximum aflatoxin B1 concentration was 2.0 µg/kg in one of Tulkarm district samples. Results of aflatoxin in corn for the three districts are shown in (Fig. 11, Appendix b). The comparative analysis of aflatoxin B1 concentrations across Jenin, Nablus, and Tulkarm in both corn and soybean samples reveals distinct regional patterns linked to environmental conditions and storage practices.

Tulkarm displayed the highest average aflatoxin levels in corn, likely due to higher summer humidity levels, which create favorable conditions for fungal growth. Conversely, Nablus exhibited the lowest contamination, especially in soybean samples, where concentrations ranged from 0 to 1 µg/kg, suggesting that lower relative humidity

may inhibit aflatoxin production (Fig.12, Appendix b). This district-wise comparison aligns with previous studies showing that regions with varied microclimates and storage conditions experience different contamination risks. The overall contamination levels, while within safety limits, underscore the importance of tailored monitoring strategies based on local climate patterns to mitigate aflatoxin risks effectively. As the results show, the differences in the aflatoxin concentration in corn samples are not significant, even though, the mean differences are not statistically significant, but at the same time, the highest mean differences are between Nablus and Tulkarm, therefore, the highest concentration is found in Tulkarm, Jenin, and Nablus consequently as shown in table 11, (appendix a).

In soybean samples, aflatoxin was found in the three districts with different concentrations. The comparison between aflatoxin concentrations in the three districts is shown in (Table 12 and Fig. 12). The results were ranging from a minimum of zero in samples in Nablus and Jenin, to a maximum of 2.4 µg/kg in Jenin. From the comparison between districts result it is clear that there is no significant differences between the three districts, while it show that the mean differences are higher between Nablus and Jenin compared to Nablus and Tulkarm. This shows that the highest district average is existing in Jenin, as the means plot of aflatoxin B1 concentration in soybean shows (Fig. 12, appendix b).

The average concentration of both corn and soybean according to the districts showed that levels of aflatoxin in corn are lower than soyabean in both Jenin and Nablus, while in Tulkarm the aflatoxin average concentration in soyabean is higher than corn. However, when comparing the results of each crop inside the district (Table,12 appendix), the result of means comparison shows that there are no significant differences in aflatoxin B1 concentrations between corn and soybean in the same district.

4.3.1 Relative humidity data

The data on relative humidity in the three districts (Fig.13) revealed that during the winter period mid-October – mid April, Jenin has higher relative humidity than Tulkarm, while during the hot periods in the summer the relative humidity in Tulkarm is higher. In Nablus the relative humidity is the lowest in the three districts (Table 13, Appendix a).

Chapter Five

Discussion

Contamination of animal and human feed with aflatoxin B1 represents a significant challenge in nutrition and food safety, posing serious health risks and economic burdens. Regular monitoring of mycotoxins like aflatoxin B1 in feed sources—whether directly consumed by humans or indirectly through animal products—is essential for maintaining food safety standards. In this study, the analysis of corn and soybean samples from three Palestinian districts confirmed the presence of aflatoxin B1 contamination at varying levels. The findings revealed that not all samples were contaminated; however, contamination rates ranged from 60% to 100%, depending on crop type and district. This variability underscores the influence of both crop type and local environmental conditions on contamination levels. Factors such as humidity and storage practices in each district likely contribute to these differences, suggesting a need for targeted interventions and better storage practices to minimize aflatoxin contamination in animal feed.

5.1 Corn

The results showed that aflatoxin B1 was existing in all samples (except one sample from Jenin), in other words its existence in the corn samples was 100% in the samples of Nablus, 100% of Tulkarm, and 80% of samples of Jenin. The level of existence is differing not only in the number of samples, but in addition in the concentrations. However, the results of statistical analysis of aflatoxin concentration in corn samples from different districts revealed that the concentration differences are not significant at 95% confidence level. In addition to this existence in the samples, the concentration of aflatoxin in corn samples is still in the accepted levels and below the maximum levels according to both the European and the American guidelines.

Aflatoxin B1 concentration in some of these samples exceed 2 µg/kg, therefore, it should be The results show an overall average of aflatoxin B1 concentration in corn samples of the three districts is 0.482 µg/kg, and ranged from 0.34 µg/kg in Nablus to 0.69 µg/kg in Tulkarm. This low concentration indicated a relatively low contamination. These low concentrations of aflatoxin in the samples, reflect new contaminations, and therefore, the concentration is subject to increase with time, if the environmental conditions in the silos are appropriate to fungal growth, as the case in the three districts. In addition, the results

agreed with the results of Abdallah et al (2016); Mngqawa et al (2016), and in the same time, the results were similar to the results published by (Diedhiou et al, (2010) where, aflatoxin concentration was below 1 µg/kg. At the same time, the results of this study were much lower than those of (El-Shanshoury et al, 2014) who found a concentration of 4400 µg/kg. Falade et al, (2022) reported concentration of aflatoxin as (in hundreds) in samples from different African countries. The high differences between aflatoxin concentration in the study area, and those in Africa could be referred.

5.2 Soybean

Most of the tested samples were contaminated with aflatoxin B1. In Jenin aflatoxin existed in 60% of the samples, while it existed in 85.7% of the samples in Nablus, and 100% of the samples in Tulkarm.

As shown in the results the average levels of Jenin district were the highest (0.64 µg/kg), while the lowest concentration (0.35 µg/kg) was in Nablus district, but in the same time, the variations between the three districts has no significant differences, or in other words the concentration of aflatoxin B1, is close in the three districts. This could be referred to the source of soybean, where the processing factories import the seeds from the same origin, while the microclimate conditions and the storing conditions reflect the low variations in the local sites.

These results are still below the maximum allowed level as indicated by the European union regulations which sets the maximum allowed concentration in soybean and nuts to 4 µg/kg (FAO, 2004). These samples are located in the labeling category; therefore, the existing levels should be labeled according to (EFISC, 2015). The maximum level was detected in Jenin samples (2.4 µg/kg). The results agreed with the findings of (Adedeji et al, 2017; Abia et al, 2017). As the results show, the level of contamination in soybean in the samples didn’t significantly vary from those in corn results in the three districts.

5.3 Comparison among districts

The results of statistical analysis show that there are no significant differences between the three districts for content of aflatoxin B1 in corn samples. The highest average concentration of aflatoxin in corn was found in Tulkarm district (0.6875 µg/kg).

The results showed significant differences in the average concentration of aflatoxin in soybean according to the district. The highest concentration of aflatoxin was in Jenin district. This could be explained by the differences in relative humidity as reported by (CAST, 2003; FAO,2018). Jenin has the highest relative humidity (RH) according to the long-term data of climatic parameters (PMD, 2023) among the three districts. At the same time, the rate of existence in Jenin was lower than Tulkarm. In Jenin aflatoxin B1 was detected in 86% of the samples, while it was in 100% of the samples of Tulkarm. The results of corn, showed average concentration of aflatoxin in Tulkarm higher than Jenin. These results indicated that aflatoxin content was influenced by the importing date rather than the climatic conditions, the corn is harvested in late summer, where the where the relative humidity in Jenin is higher than Tulkarm, in the same time, the crop is imported in this period. These factors indicate that the contamination is related to the source and importing date. This agrees with the findings of Niyibituronsa et al, (2018) who found aflatoxin concentration in soybean less than 1 µ g/kg. Niyibituronsa et al (2018) reported that soybean is not favorable media for the production of aflatoxin. This confirms the earlier findings of (Alvarado et al, 2017) what they found? However, the results of this study showed that aflatoxin concentration in soybean was higher than that in corn under the same factories except in Nablus district, but the difference is not significant at 95% confidence level.

Concentration results were not in contradiction. It could be explained by knowing that both corn and soybean are imported by the processing factories in the three districts from Israeli sources, therefore, the original contamination level is the limiting factor for aflatoxin presence. While storing conditions, and local climatic conditions could be considered as the effecting factor during the next stage, as the fungal requirements of climatic conditions.

The results indicated that the contamination started before the arrival to the storing sites, where the samples were collected in a period close to the importing period. This is

supported by the fact that aflatoxin is a by – product of aspergillus growth, therefore the presence of aflatoxin in newly imported samples indicates a prior contamination of aspergillus, which produced aflatoxin on the crops.

In the three districts both corn and soybean are imported from the same source Israel. However, it is known that Israel is not a producing country i.e. the crops are imported from a third country, and distributed. This explains that the average of the three district was similar with some variations. Therefore, the existence is an indicator of the contamination from the source, while the variations which are not significant are coming from the microclimate differences in storing conditions.

The animal feed factories in the three districts make the rations (livestock feeds) by grinding, mixing the imported crops with a specific percentage depending on the use. This means that the storing period is short, and the existence of aflatoxin in the raw material if present then it will result in having contaminated animal feeds.

The results revealed that the measured aflatoxin concentration in the samples, was below the maximum accepted levels according to USA and EU regulations (Alvarado et al, 2017). These concentrations may increase during the storing period, because aflatoxin and other mycotoxins are produced by fungi. For that aflatoxin is considered as an indicator on the existence of the fungi. In the three districts, the climatic conditions are favorable for the fungi growth (Pratiwi et al, 2015). Therefore, with polluted crops from the source, the concentrations will increase. The rate of increase is affected by the storing period.

The findings of this study highlight the significant presence of aflatoxin B1 in corn and soybean samples across the three districts in Palestine, reflecting both local environmental conditions and the broader challenge of mycotoxin contamination in agricultural production. The variability in aflatoxin concentrations observed between Jenin, Nablus, and Tulkarm can be attributed to factors such as differences in storage conditions, humidity, and temperature, which are known to influence fungal growth and mycotoxin production. These results are consistent with previous studies that have shown how climatic factors, particularly in regions with warm and humid climates, exacerbate the risk of aflatoxin contamination (Wu et al., 2011). Moreover, the presence of aflatoxins in animal feed, even at permissible levels, raises concerns about the potential cumulative

effects on livestock health and the potential for aflatoxin residues to enter the human food chain. This emphasizes the need for stringent monitoring and control measures to mitigate the risks associated with aflatoxin contamination, especially in regions like Palestine where food security is already a critical issue.

Chapter Six

Conclusions and Recommendations

6.1 Conclusions

The study confirmed that both corn and soybean in the three targeted districts (Jenin, Nablus, and Tulkarm) were contaminated with aflatoxin B1. Although the majority of the contamination levels were within the permissible limits, two samples—one corn sample from Tulkarm (2.8 µg/kg) and one soybean sample from Jenin (2.4 µg/kg)—exceeded the maximum allowed aflatoxin B1 levels, set at 2 µg/kg for corn and soybean by EFISC (2015). Additionally, two other samples were within the "should be labeled" category, which implies a potential risk if not monitored properly. These findings indicate that while most samples met safety standards, there are still outliers that pose a threat to public health and livestock.

The variability in aflatoxin levels across districts underscores the influence of environmental factors, such as storage conditions and humidity, on fungal growth and toxin production. This calls for stricter oversight of storage practices and greater awareness among feed manufacturers about the risks associated with improper handling and storage of feed ingredients.

6.2 Recommendations

- Given the significant health risks posed by aflatoxin contamination, it is crucial to establish a national monitoring and regulation program that focuses on both human food and animal feed production. This program should cover not only feed factories but also extend to monitoring animal products, ensuring that contamination risks are minimized across the entire food supply chain.
- As part of the nation's phytosanitary measures, random testing of imported animal feed products should be conducted regularly. This will help identify and prevent the entry of contaminated materials into the Palestinian market, particularly since a significant proportion of animal feed ingredients are imported.
- Further research is recommended to investigate the environmental and operational factors that influence mycotoxin contamination in local conditions. Studies on climate impacts, storage techniques, and fungal growth patterns will help provide a more comprehensive understanding of how to prevent aflatoxin contamination in the future, improving both public health and the productivity of the agricultural sector.

References

Aasan-Ghomi M, Mirmiran. P, Amiri. Z, Asghari. G, Sadeghian. S, and Sarbazi. N, 2012, The Association of Food Security and Dietary Variety in subjects aged over 40 in district 13 of Tehran, Iranian J Endocrinol Metab, 2012;14(4):360–7.

Abdallah. M, Krska. R, and Sulyok. M, 2016, Mycotoxin contamination in sugarcane grass and juice: First report on detection of multiple mycotoxins and exposure assessment for aflatoxins B_1 and G_1 in humans, Toxins, 8(11). https://doi.org/10.3390/toxins8110343

Abia. W, Warth. B, Ezekiel. C, Sarkanj. B, Turner. P, Marko. D, Krska. R, and Sulyok. M, 2017, Uncommon toxic microbial metabolite patterns in traditionally home-processed corn dish (fufu) consumed in rural Cameroon, Food and Chemical Toxicology, 107(Part A): 10 – 19, https://doi.org/10.1016/j.fct.2017.06.011.

Adedeji. B., Ezeokoli. O, Ezekiel. C, Obadina. A, Somorin. Y, Sulyok. M, Adeleke. R, Warth. B, Nwangburuka. C, Omemu. A, Oyewole. O, and Krska. R, 2017, Bacterial species and mycotoxin contamination associated with locust bean, melon and their fermented products in south-western Nigeria, International Journal of Food Microbiology, 258: 73–80, https://doi.org/10.1016/j.ijfoodmicro.2017.07.014.

Adeola O, and Kong. C, 2014, Energy value of distillers dried grains with solubles and oilseed meals for pig, Journal of Animal Science, 2014;92(1):164-170. https://doi.org/10.2527/jas.2013-6662.

Adeyeye, S. A. O. (2016). Fungal mycotoxins in foods: A review. *Cogent Food and Agriculture*, *2*(1), 1–11.https://doi.org/10.1080/23311932.2016.1213127

Ahmad, B., Alzuheir, I., & Abo Omar, J. (2022). Aflatoxin B 1 contamination of wheat flour, coffee, and pistachios consumed in Northern Palestine. International Food Research Journal, 29(1).https://doi.org/10.47836/ifrj.29.1.05

Ahmad. B, 2020, Aflatoxin B1 Levels in Wheat Flour, Coffee and Pistachios, master thesis, An-Najah National University, Nablus, Palestine.

Ajmal, M., Bedale, W., Akram, A., Yu, J. H. 2022. Comprehensive Review of Aflatoxin Contamination, Impact on Health and Food Security, and Management Strategies in Pakistan. In *Toxins* 14, (12). https://doi.org/10.3390/toxins14120845

Algul. I, and Kara. D, 2014, Determination and chemometric evaluation of total aflatoxin, aflatoxinB1, ochratoxinA and heavy metals content in corn flours from Turkey, Food Chemistry. 157:70–6. https://doi.org/10.1016/j.foodchem.2014.02.004

Alvarado. A. M, Zamora-Sanabria. R, and Granados-Chinchilla. F, 2017, A Focus on Aflatoxins in Feedstuffs: Levels of Contamination, Prevalence, Control Strategies, and Impacts on Animal Health. Aflatoxin-Control, Analysis, Detection and Health Risks. https://doi.org/10.5772/intechopen.69468

Applegate. T. J, and Angel, R, 2014, Nutrient requirements of poultry publication: History and need for an update, The Journal of Applied Poultry Research; 23(3): 567–575. https://doi.org/10.3382/japr.2014-00980

Arroyo. J, Dubois. J.P, Lavigne. F, Brachet., M, and Fortun-Lamothe. L, 2016, Effects of replacing corn with sorghum on the performance of overfed mule ducks, Poult, Sci 2016, 95, 1304–1311. https://doi.org/10.3382/ps/pew072

Attia SM, 2010, Deleterious effects of reactive metabolites, Oxidative Medicine and Cellular Longevity. 3(4): 238–53. https://doi.org/10.4161/oxim.3.4.13246

Attia, S. M., & Harisa, G. I. (2016). Risks of Environmental Genotoxicants. In Environmental Health Risk - Hazardous Factors to Living Species. https://doi.org/10.5772/62454.

Baião. N.C, and Lara. L.J.C, 2005, Oil and fat in broiler nutrition, Rev, Bra, Ciênc, Avícola 2005, 7: 129–141. https://doi.org/10.1590/S1516-635X2005000300001

Banhazi. T, Babinszky. L, Halas. V, and Tscharke. M, 2012, Precision livestock farming: precision feeding technologies and sustainable livestock production, Int, J, Agric, Biol, Eng, 5 (4), 54-61. http://dx.doi.org/10.3965/j.ijabe.20120504.006

Bankole. SA, and Adebanjo. A, 2003, Mycotoxins in food in West Africa: Current situation and possibilities of controlling it, African Journal of Biotechnology, 2003;2(9):254-263. https://doi.org/10.5897/AJB2003.000-1053

Barybina. L, Beloysova. E, Voblikova. T, Statsenko. E, Amanova. S, Borisenko. A, Nagdalian. A, Simonov. A, and Ziruk. I, 2019, multicomponent meat products for sports nutrition, Journal of Hygienic Engineering and Design.

Batonon-Alavo. D.I, Umar Faruk. M, Lescoat. P, Weber. G.M, and Bastianelli. D, 2015, Inclusion of sorghum, millet and cottonseed meal in broiler diets: A meta-analysis of effects on performance, Animal 2015 (9): 1120–1130. https://doi.org/10.1017/S1751731115000282

Bender. A, 1998, Meat and meat products in human nutrition in developing countries, FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO), FOOD AND NUTRITION PAPER 53. https://www.fao.org/4/t0562e/T0562E00.htm

Bennett, J. W., & Klich, M. (2003). Mycotoxins. *ClinicalMicrobiology Reviews*, *16*(3), 497–516. https://doi.org/10.1128/CMR.16.3.497–516.2003.

Beski. S.S.M, Swick. R.A, and Iji. P, 2015, Specialized protein products in broiler chicken nutrition: A review, Anim, Nutr 2015, 1, 47–53. https://doi.org/10.1016/j.aninu.2015.05.005

Bickel. G, Nord. M, Price. C, Hamilton. W, and Cook. J, 2000. Guide to Measuring Household Food Security, United States Department of Agriculture, Measuring Food Security in the United States. http://dx.doi.org/10.22004/ag.econ.337157

Black. R. E, Victora. C. G, Walker.S.P, 2013, Maternal and child under nutrition and over weight in low-income and middle-income countries .Lancet382, 427–451. https://doi.org/10.1016/s0140-6736(13)60937-x

Bouhet. S, and Oswald. I. P, 2005, The effects of mycotoxins, fungal food contaminants, on the intestinal epithelial cell-derived innate immune response, Veterinary

Immunology and Immunopathology; 108(1-2): 199–209. https://doi.org/10.1016/j.vetimm.2005.08.010

Bruinsma. J, 2009, The Resource Outlook to 2050-By how much do land, water use and crop yields need to increase by 2050, In: How to Feed the World in 2050, pp. 24e26. https://doi.org/10.1016/B978-0-323-10199-8.00006-2

Calori-Domingues, M. A., Iwahashi, P. M. R., Ponce, G. H., Gloria, E. M. da, Dias, C. T. dos S., Button, D. C., & De Camargo, A. C. (2018). Aflatoxin B1 and zearalenone in soybeans: occurrence and distribution in whole and defective kernels. Food Additives and Contaminants: Part B Surveillance, 11(4), 273–280. https://doi.org/10.1080/19393210.2018.1502818

CAST, 2003, Mycotoxins: Risks in Plant, Animal, and Human Systems, Council for Agricultural Science and Technology: Ames, IA, USA.

Chakraborty, S. K., Mahanti, N. K., Mansuri, S. M., Tripathi, M. K., Kotwaliwale, N., and Jayas, D. S. (2021). Non-destructive classification and prediction of aflatoxin-B1 concentration in corn kernels using Vis–NIR (400–1000 nm) hyperspectral imaging. *Journal of Food Science and Technology*, *58*(2), 437–450. https://doi.org/10.1007/s13197-020-04552-w

Chase. L, Brown. D, Bergstrom. G, and Murphy. S, 2013, flatoxin M1 in Milk, Dairy Nutrition Fact Sheet, cooperative extension, Cornell University. https://doi.org/10.1016/j.tifs.2015.08.005

Choi. H.B, Jeong. J.H, Kim. D.H, Lee. Y, Kwon., H, and Kim. Y.Y, 2015, Influence of Rapeseed Meal on Growth Performance, Blood Profiles, Nutrient Digestibility and Economic Benefit of Growing-finishing Pigs, Asian-Australas, J, Anim, Sci. 28:1345–1353. https://doi.org/10.5713%2Fajas.14.0802

Cotty, P. J., and Jaime-Garcia, R. (2007). Influences of climate on aflatoxin producing fungi and aflatoxin contamination. *International Journal of Food Microbiology*, *119*(1–2), 109–115. https://doi.org/10.1016/j.ijfoodmicro.2007.07.060

de Boer. J, Schösler. H, and Aiking. H, 2017, Towards a reduced meat diet: mindset and motivation of young vegetarians, low, medium and high meat eaters, Appetite; 113:387–397. https://doi.org/10.1016/j.appet.2017.03.007

Delgado, C. L., Courbois, C. B., Rosegrant, M. W. 1998. Global food demand and the contribution of livestock as we enter the new millennium. BSAP Occasional Publication, 21, 27–42. https://doi.org/10.1017/S0263967X00032043

Delgado. C, Rosegrant. M, Steinfeld. H, Ehui. S, and Courbois. C, 2001, Livestock to 2020: the next food revolution, Outlook on Agriculture. http://dx.doi.org/10.5367/000000001101293427

Devegowda .G , Sridhar. V, and Pankaj L Sherasia, 2019, Aflatoxin: Prevalence and Control in Diary Feed and Milk, Booklet on aflatoxin, National Diary Development Board, India.

Diedhiou. P, Bandyopadhyay. R, Atehnkeng. J, and Ojiambo. P, 2010, Aspergillus Colonization and Aflatoxin Contamination of Corn and Sesame Kernels in Two Agro-ecological Zones in Senegal, Journal of Phytopathology, 159(4): 268–275. https://doi.org/10.1111/j.1439-0434.2010.01761.x

Diener, U. L., Cole, R. J., Sanders, T. H., Payne, G. A., Lee, L. S., & Klich, M. A. (1987). Epidemiology of Aflatoxin Formation by Aspergillus Flavus*. In Annual Review of Phytopathology. 25, (1): 249–270. https://doi.org/10.1146/annurev.py.25.090187.001341

Elbehri, A., Challinor, A., Angelsen, A., Hess, T., Ouled Belgacem, A., & Clark, H. (2017). FAO-IPCC Expert Meeting on Climate Change, Land Use and Food Security: Final Meeting Report; January 23–25, 2017. Rome, FAO and IPCC.

El-Shanshoury, A., El-Sabbagh, S., Emara, H., & Saba, H. (2014). Occurrence of moulds, toxicogenic capability of Aspergillus flavus and levels of aflatoxins in corn, wheat, rice and peanut from markets in central Delta provinces, Egypt. *International Journal of Current Microbiology and Applied Sciences*, *3*(3), 852–865. https://doi.org/10.18697/ajfand.125.23920

Eskola, M., Kos, G., Elliott, C. T., Hajšlová, J., Mayar, S., & Krska, R. (2019). Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited "FAO estimate" of 25%. *Critical Reviews in Food Science and Nutrition*, 1–17. https://doi.org/10.1080/10408398.2019.1658570

European Feed Ingredients Safety Code (EFISC). (2015). Code of good practice for the monitoring of Aflatoxin B1 in corn and corn co-products (feed materials), Version 1.1. Retrieved from EFISC.

Ezekiel, C. N., Sulyok, M., Warth, B., Odebode, A. C., & Krska, R. (2012). Natural occurrence of mycotoxins in peanut cake from Nigeria. *Food Control*, *27*(2), 338–342. https://doi.org/10.1016/j.foodcont.2012.04.010

Falade, T. D. O., Neya, A., Bonkoungou, S., Dagno, K., Basso, A., Senghor, A. L., Atehnkeng, J., Ortega-Beltran, A., & Bandyopadhyay, R. (2022). Aflatoxin contamination of corn, groundnut, and sorghum grown in Burkina Faso, Mali, and Niger and aflatoxin exposure assessment. *Toxins*, *14*(10). DOI: 10.3390/toxins14100700.

FAO – Food and Agriculture Organization of the UN and WFP – World Food Programme. (2016). Socio-Economic & Food Security Survey 2014. FAO, WFP, PCBS. Ramallah, May 2016. Downloadable at FAO.

FAO – Food and Agriculture Organization of the UN. (2004). Worldwide regulations for mycotoxins in food and feed in 2003. *FAO Food and Nutrition Paper 81*. Rome 2004.

FAO, IFAD, and WFP. (2013). The State of Food Insecurity in the World 2013: The multiple dimensions of food security. Rome, FAO.

Fenton, G., & Van Saun, R. (2018). Animal Feed Safety Practices to Prevent Aflatoxin in Milk. Pennsylvania State Extension, College of Agricultural Sciences, The Pennsylvania State University.

Filho, W., Azeiteiro, U., Balogun, A., Setti, A., Mucova, Ayal, D., Totin, E., Lydia, A., Kalaba, F., Oguge, N. (2021). The influence of ecosystems services depletion to

climate change adaptation efforts in Africa. Science of The Total Environment, 779, 146414. https://doi.org/10.1016/j.scitotenv.2021.146414

FAO (2018). *The State of Food Security and Nutrition in the World Building Climate Resilience for Food Security and Nutrition.*

Gallo, A., Giuberti, G., Frisvad, J.C., Bertuzzi, T., & Nielsen, K.F. (2015). Review on mycotoxin issues in ruminants: Occurrence in forages, effects of mycotoxin ingestion on health status and animal performance, and practical strategies to counteract their negative effects. *Toxins*, *7*(8), 3057-3111. https://doi.org/10.3390/toxins7083057

Gbashi, S., Madala, N.E., De Saeger, S., De Boevre, M., Adekoya, I., Adebo, O.A., & Njobeh, P.B. (2018). The Socio-Economic Impact of Mycotoxin Contamination in Africa. *In Njobeh, P.B., & Stepman, F. (Eds.), Mycotoxins Impact and Management Strategies*, IntechOpen. DOI: 10.5772/intechopen.79328

Guchi, E. (2015). Implication of aflatoxin contamination in agricultural products. *American Journal of Food and Nutrition*, *3*(1), 12-20. http://dx.doi.org/10.12691/ajfn-3-1-3

Guerre, P. (2016). Worldwide mycotoxins exposure in pig and poultry feed formulations. *Toxins, 8*(12), 350. https://doi.org/10.3390/toxins8120350

Hazzledine, M., Pine, A., Mackinson, I., Ratcliffe, J., Salmon, L., Levels, R., & Staffs, W. (2011). Estimating Displacement Ratios of Wheat DDGS in Animal Feed Rations in Great Britain. DOI: 10.1016/j.appet.2017.03.007

Hulshof, T.G., van der Poel, A.F.B., Hendriks, W.H., & Bikker, P. (2016). Processing of soybean meal and 00-rapeseed meal reduces protein digestibility and pig growth performance but does not affect nitrogen solubilization along the small intestine. *Journal of Animal Science, 94*, 2403-2414. https://doi.org/10.2527/jas.2015-0013

IARC (International Agency for Research on Cancer) (2012). Aflatoxins. *Chemical Agents and Related Occupations, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 100F*, 225-248.

International Trade Centre (ITC) (2017). *Trade Map—Trade Statistics for International Business Development*.

Jallow, A., Xie, H., Tang, X., Qi, Z., & Li, P. (2021). Worldwide aflatoxin contamination of agricultural products and foods: From occurrence to control. *Comprehensive Reviews in Food Science and Food Safety, 20*(3), 2332-2381. https://doi.org/10.1111/1541-4337.12746

Kamboj, S., Gupta, N., Bandral, J.D., Gandotra, G., & Anjum, N. (2020). Food safety and hygiene: A review. https://doi.org/10.22271/chemi.2020.v8.i2f.8794.

Kaminska, B.Z. (2003). Substitution of soyabean meal with "00" rapeseed meal or its high-protein fraction in the nutrition of hens laying brown-shelled eggs. *Journal of Animal Feed Science, 12*, 111-119.

Kana, J.R., Gnonlonfin, B.G.J., Harvey, J., Wainaina, J., Wanjuki, I., Skilton, R.A., & Teguia, A. (2013). Assessment of aflatoxin contamination of corn, peanut meal, and poultry feed mixtures from different agroecological zones in Cameroon. *Toxins, 5*(4), 884-894. https://doi.org/10.3390/toxins5050884

Kotinagu, K., Mohanamba, T., & Kumari, L.R. (2015). Assessment of aflatoxin B1 in livestock feed and feed ingredients by high-performance thin layer chromatography. *Veterinary World, 8*(12), 1396-1399. DOI: 10.14202/vetworld.2015.1396-1399

Kovalsky, P., Kos, G., Nährer, K., Schwab, C., & Jenkins, T. (2016). Co-occurrence of regulated, masked, and emerging mycotoxins and secondary metabolites in finished feed. *Toxins, 8*(12), 363. https://doi.org/10.3390%2Ftoxins8120363

Kyntäjä, S., Partanen, K., Siljander-Rasi, H., & Jalava, T. (2014). Tables of Composition and Nutritional Values of Organically Produced Feed Materials for Pigs and Poultry. http://urn.fi/URN:ISBN:978-952-487-571-4

Ozturk, O. K., & Hamaker, B. R. (2023). Texturization of plant protein-based meat alternatives: Processing, base proteins, and other constructional ingredients. In

Future Foods (Vol. 8, p. 100248). Elsevier BV. https://doi.org/10.1016/j.fufo.2023.100248

Lammers, P.J., Stender, D.R., & Honeyman, M.S. (2007). *Feed Budgets*.

Blonski, W., Kotlyar, D. S., & Forde, K. A. (2010). Non-viral causes of hepatocellular carcinoma. *World Journal of Gastroenterology*, 16(29), 3603. https://doi.org/10.3748/wjg.v16.i29.3603.

Calori-Domingues, M. A., Iwahashi, P. M. R., Ponce, G. H., Gloria, E. M. da, Dias, C. T. dos S., Button, D. C., & De Camargo, A. C. (2018). Aflatoxin B1 and zearalenone in soybeans: occurrence and distribution in whole and defective kernels. *Food Additives & Contaminants: Part B*, 11(4), 273–280. https://doi.org/10.1080/19393210.2018.1502818.

Larousse, M., Rancurel, C., Syska, C., Palero, F., Etienne, C., Industri, B., Nesme, X., Bardin, M., & Galiana, E. (2017). Tomato root microbiota and *Phytophthora parasitica*-associated disease. *Microbiome*, 5(1), 1–11. https://doi.org/10.1186/s40168-017-0273-7.

Liu, Y., & Wu, F. (2010). Global burden of aflatoxin-induced hepatocellular carcinoma: A risk assessment. *Environmental Health Perspectives*, 118(6), 818–824. https://doi.org/10.1289/ehp.0901388.

Lomascolo, A., Uzan-Boukhris, E., Sigoillot, J.-C., & Fine, F. (2012). Rapeseed and sunflower meal: A review on biotechnology status and challenges. *Applied Microbiology and Biotechnology*, 95(5), 1105–1114. https://doi.org/10.1007/s00253-012-4250-6.

Lopez-Garcia, R., Park, D. L., & Phillips, T. D. (1999). Integrated mycotoxin management systems. *Food Nutrition and Agriculture*, 38–48. FAO. http://www.fao.org/docrep/X2100T/x2100t07.htm

Magan, N., & Medina, A. (2016). Mycotoxins, food security and climate change: Do we know enough?

https://microbiologysociety.org/publication/past-issues/fungal-diseases/article/mycotoxins-food-security-and-climate-change-do-we-know-enough-fungal-diseases.html.

Mahmoud, B. (2019). Food Quality and Safety in the Global Food Security Index. *Feed the Future*, https://www.agrilinks.org/post/food-quality-and-safety-global-food-security-index-0

Makkar, H. P. S., & Beever, D. (2013). Optimization of feed use efficiency in ruminant production systems. *FAO Animal Production and Health Proceedings No. 16*, Rome: FAO and Asian-Australasian Association of Animal Production Societies.

Marechera, G., Ndwiga, J., Marechera, G., & Ndwiga, J. (2015). Estimation of the Potential Adoption of Aflasafe Among Smallholder Maize Farmers in Lower Eastern Kenya. Unknown. https://doi.org/10.22004/AG.ECON.200589 .

Maurice, J. (2013). New goals in sight to reduce poverty and hunger. *The Lancet*, 382(9890), 383–384. https://doi.org/10.1016/s0140-6736(13)61642-6.

McCullough, A. K., & Lloyd, R. S. (2019). Mechanisms underlying aflatoxin-associated mutagenesis – Implications in carcinogenesis. *DNA Repair*, 77(December 2018), 76–86. https://doi.org/10.1016/j.dnarep.2019.03.004.

Medina, A., Rodriguez, A., & Magan, N. (2014). Effect of climate change on *Aspergillus flavus* and aflatoxin B1 production. *Frontiers in Microbiology*, 5(JULY), 1–7. https://doi.org/10.3389/fmicb.2014.00348.

Medina, A., Akbar, A., Baazeem, A., Rodriguez, A., & Magan, N. (2017). Climate change, food security and mycotoxins: Do we know enough? *Fungal Biology Reviews*, 31(3), 143–154. https://doi.org/10.1016/j.fbr.2017.04.002.

Mejicanos, G., Sanjayan, N., Kim, I. H., & Nyachoti, C. M. (2016). Recent advances in canola meal utilization in swine nutrition. *Journal of Animal Science and Technology*, 58, 1–13. https://doi.org/10.1186/s40781-016-0085-5.

Ministry of Agriculture (MoA). (2019). *National Food and Nutrition Security Policy 2019 – 2030*. State of Palestine.

Ministry of Agriculture (MoA). (2016). *National Agricultural Sector Strategy (2017 2022): Resilience and Sustainable Development*. Ramallah, Palestine.

Missouri Department of Agriculture (MDA). (2022). *U.S. Food and Drug Administration Guidelines for Aflatoxin Levels*. https://agriculture.mo.gov/plants/feed/aflatoxin.php.

Mngqawa, P., Shephard, G. S., Green, I. R., Ngobeni, S. H., Rijk, T. C. D., & Katerere, D. R. (2016). Mycotoxin contamination of home-grown corn in rural northern South Africa (Limpopo and Mpumalanga Provinces). *Food Additives and Contaminants: Surveillance*, 9(1), 38–45. https://doi.org/10.1080/19393210.2015.1121928.

Monson, M. S., Coulombe, R. A., & Reed, K. M. (2015). Aflatoxicosis: Lessons from toxicity and responses to aflatoxin B1 in poultry. *Agriculture*, 5, 742–777. https://doi.org/10.3390/agriculture5030742.

National Cancer Institute (NIH). (2022). Aflatoxins. *Cancer – Causing Substances*. https://www.cancer.gov/about-cancer/causes-prevention/risk/substances/aflatoxins.

Nguyen, H. V., Nguyen, N., Nguyen, B. K., Lobo, A., & Vu, P. A. (2019). Organic food purchases in an emerging market: The influence of consumers' personal factors and green marketing practices of food stores. *International Journal of Environmental Research and Public Health*, 16, 1037. https://doi.org/10.3390/ijerph16061037.

Niyibituronsa, M., Onyango, A., Gaidashova, S., Imathiu, S., Uwizerwa, M., Wanjuki, I., Nganga, F., Muhutu, J., Birungi, J., Ghimire, S., Raes, K., De Boevre, M., De Saeger, S., Harvey, J. (2018). Evaluation of mycotoxin content in soybean (*Glycine max L.*) grown in Rwanda. *African Journal of Food, Agriculture, Nutrition, and Development*, 18(3). https://doi.org/10.18697/ajfand.83.17710.

Nnamani, G. C., Gana, A. S., Shaahu, A., Msaakpa, T. S., Lemibe, P. C., Nnamani, M. A., Nwanyanwu, E. O. (2021). Effects of aflatoxin on soybean. *Journal of Plant Development*, 161–168. https://doi.org/10.47743/jpd.2021.28.1.855.

Oğuz, H., Keçeci, T., Birdane, Y. O., Onder, F., Kurtoğlu, V. (2000). Effect of clinoptilolite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis. *Research in Veterinary Science*, 69(1), 89–93. https://doi.org/10.1053/rvsc.2000.0395.

Ongoma, V. (2013). A review of the effects of changing climate on aflatoxin contamination of peanuts in Kenya. *International Journal of Humanities and Social Science*, 3(16), 85–90. http://www.ijhssnet.com/journals/Vol_3_No_16_Special_Issue_August_2013/9.pdf.

Oyelami, O. A., Maxwell, S. M., Adelusola, K. A., Aladekoma, T. A. (2003). Aflatoxins in the lungs of children with kwashiorkor and children with miscellaneous diseases in Nigeria. *Journal of Toxicology and Environmental Health Part A*, 66(20), 2117–2123. https://doi.org/10.1080/15287390306460.

Pal, M., Gowda, C. K., Viagasini, R. (2017). Mycotoxins in poultry and poultry products: A review. *International Journal of Livestock Research*, 7(12), 1–11. https://doi.org/10.5455/ijlr.20170707074159.

Partnership for Aflatoxin Control in Africa (PACA). (2020). *Africa Aflatoxin Information Management System (Africa AIMS) Report*. https://aflatoxinpartnership.org

Palestinian Central Bureau of Statistics (PCBS). (2008). *Levels of living in the Palestinian Territory, Final Report (January 2007 - January 2008)*. Ramallah-Palestine. https://www.pcbs.gov.ps/Downloads/book1474.pdf

Palestinian Central Bureau of Statistics (PCBS). (2018). *Main Findings of Living Standards in Palestine (Expenditure, Consumption and Poverty)*. Ramallah–Palestine. https://www.pcbs.gov.ps/Downloads/book2368.pdf?date=7_5_2018

Pereira, P. M., & Vicente, A. F. (2013). Meat nutritional composition and nutritive role in the human diet. *Meat Science, 93*(3), 586–592. https://pubmed.ncbi.nlm.nih.gov/23273468/

Palestinian Meteorological Department (PMD). (2023). *Climatic averages*. Data retrieved on 1/6/2023. https://www.pmd.ps/en/climatic-averages

Pratiwi, C., Rahayu, W., Lioe, H., Herawati, D., Broto, W., & Ambarwati. (2015). The effect of temperature and relative humidity for *Aspergillus flavus* BIO 2237 growth and aflatoxin production on soybeans. *International Food Research Journal*, 22, 82–87.

Spencer Smith, J., Paul Williams, W., & Windham, G. L. (2019). Aflatoxin in corn: A review of the early literature from "moldy-corn toxicosis" to the genetics of aflatoxin accumulation resistance. *Mycotoxin Research*, *35*(2), 111–128. https://doi.org/10.1007/s12550-018-00340-w

Stathers, T., Lamboll, R., & Mvumi, B. M. (2013). Postharvest agriculture in changing climates: Its importance to African smallholder farmers. *Food Security*, *5*(3), 361–392.

Stein, H. H., & Shurson, G. C. (2009). Board-invited review: The use and application of distillers dried grains with solubles in swine diets. *Journal of Animal Science*, *87*(4), 1292–1303. https://doi.org/10.2527/jas.2008-1290

WFP. (2009). *Comprehensive Food Security and Vulnerability Analysis (CFSVA), Ghana*.

World Health Organization (WHO). (2019). *Food safety*. https://www.who.int/health-topics/food-safety

World Health Organization (WHO)a. (2022). *Food safety: Key facts*. Retrieved 5/5/2022. https://www.who.int/news-room/fact-sheets/detail/food-safety

World Health Organization (WHO)b. (2022). *Mycotoxins: Key facts*. Retrieved 5/5/2022. https://www.who.int/news-room/fact-sheets/detail/mycotoxins

World Organization for Animal Health (WOAH). (2008). *Animal production food safety*. Bulletin no. 2008–1. https://www.oie.int/app/uploads/2021/03/bull-2008-1-eng.pdf

Worldmeter. (2022). *World population by year*. Dadax Data Company. https://www.worldometers.info/world-population/world-population-by-year/

Wu, F., Groopman, J. D., & Pestka, J. J. (2014). Public health impacts of foodborne mycotoxins. *Annual Review of Food Science and Technology, 5*(1), 351–372. https://doi.org/10.1146/annurev-food-030713-092431

Wu, G. (2014). Dietary requirements of synthesizable amino acids by animals: A paradigm shift in protein nutrition. *Journal of Animal Science and Biotechnology, 5*(34). https://doi.org/10.1186/2049-1891-5-34

Yiannikouris, A., Apajalahti, J., Siikanen, O., Dillon, G. P., & Moran, C. A. (2021). *Saccharomyces cerevisiae* cell wall-based adsorbent reduces aflatoxin B1 absorption in rats. *Toxins, 13*(3), 209. https://doi.org/10.3390/toxins13030209

Quinn, P. J., Markey, B. K., Carter, M. E., Donnelly, W., & Leonard, F. C. (2002). *Mycotoxins and mycotoxicosis*. In Veterinary Microbiology and Microbial Diseases (pp. 229-266). Blackwell Science Ltd.

Rahmani, A., Jinap, S., & Soleimany, F. (2009). Qualitative and quantitative analysis of mycotoxins. *Comprehensive Reviews in Food Science and Food Safety*, 8, 202–251.

Ravindran, V. (2013). Poultry feed availability and nutrition in developing countries: Main ingredients used in poultry feed formulations. In *Poultry Development Review*. FAO, Rome, Italy. https://www.fao.org/docrep/019/i3531e/i3531e.pdf

Richard, J. L. (2007). Some major mycotoxins and their mycotoxicoses—An overview. In International Journal of Food Microbiology. 119, 1–2: 3–10. https://doi.org/10.1016/j.ijfoodmicro.2007.07.019

EFSA Panel on Contaminants in the Food Chain (CONTAM), Schrenk, D., Bignami, M., Bodin, L., Chipman, J. K., del Mazo, J., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L. (Ron), Leblanc, J., Nebbia, C. S., Nielsen, E., Ntzani, E., Petersen, A., Sand, S., Schwerdtle, T., Vleminckx, C., Marko, D., Oswald, I. P., … Wallace,

H. (2020). Risk assessment of aflatoxins in food [JB]. EFSA Journal, 18(3). https://doi.org/10.2903/j.efsa.2020.6040

Appendices

Appendix A

Tables

Table 11

Comparison between aflatoxin B1 concentration in corn samples in Nablus, Jenin, and Tulkarm

Dependent Variable: AFB1 concentration (µg/kg) LSD						
(I) City	(J) City	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Nablus	Jenin	-.02429-	.35883	.947	-.7814-	.7328
	Tulkarm	-.35179-	.31716	.283	-1.0209-	.3174
Jenin	Nablus	.02429	.35883	.947	-.7328-	.7814
	Tulkarm	-.32750-	.34936	.362	-1.0646-	.4096

Table 12

Comparison between aflatoxin B1 concentration in soybean samples in Nablus, Jenin, and Tulkarm

Dependent Variable: AFB1 concentration (µg/kg) LSD						
(I) City	(J) City	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Nablus	Jenin	-.28857-	.32631	.388	-.9715-	.3944
	Tulkarm	-.06357-	.27463	.819	-.6384-	.5112
Jenin	Nablus	.28857	.32631	.388	-.3944-	.9715
	Tulkarm	.22500	.30523	.470	-.4139-	.8639

Table 13

Means comparison of corn and soybean for aflatoxin B1 concentration in Nblus, Jenin, and Tulkarm

District	Levene's Test for Equality of Variances	
	F	Sig.
Nablus	3.253	0.096
Jenin	2.011	.194
Tulkarm	2.419	.139

Appendix B

Figures

Figure 11

Results of mean plot for district averages Aflatoxin concentration in corn

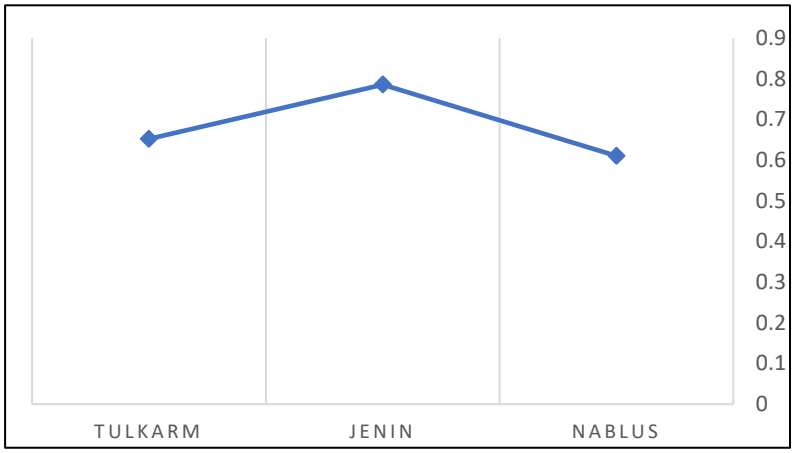


Figure 12

District averages results of Aflatoxin concentration in soybean

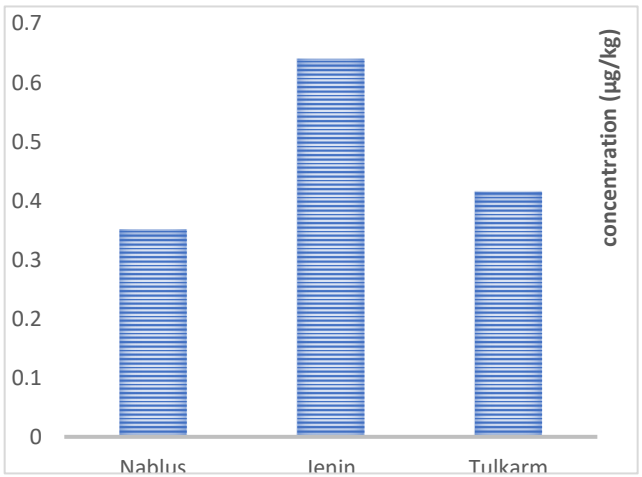
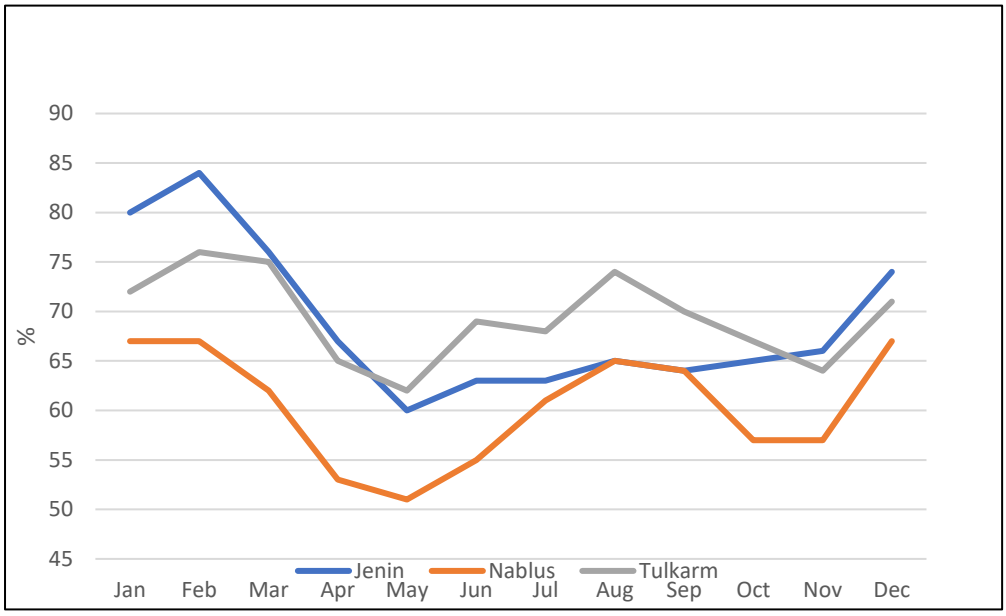


Figure 13

Long term relative humidity in Nablus, Jenin, and Nablus districts



جـامعـــة النجاح الوطنية

كليـــة الدراســـات العليـــا

مستويات الافلاتوكسين B1 في الأعلاف المعتمدة على الذرة وفول الصويا في شمال فلسطين

إعداد

إلهام حمدان

إشراف

أ.د. جمال أبوعمر

د. إبراهيم الزهير

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في الإنتاج الحيواني، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس– فلسطين.

2023

مستويات الافلاتوكسين B1 في الأعلاف المعتمدة على الذرة وفول الصويا في شمال فلسطين

إعداد

إلهام حمدان

إشراف

أ.د. جمال أبوعمر

د. إبراهيم الزهير

الملخص

الأفلاتوكسين B1 هو ناتج ثانوي لعملية التمثيل الغذائي لفطريات Aspergillus، وله تأثيرات صحية خطيرة على صحة الحيوانات، كما أثبتت ذلك العديد من الدراسات. تهدف هذه الدراسة إلى تحليل تركيز الأفلاتوكسين B1 في محصولين أساسيين، وهما الذرة وفول الصويا، اللذان يُستخدمان كمواد خام في تصنيع الأعلاف المركزة لحيوانات المزرعة. يتم استيراد كلا المكونين من الخارج ويتم تصنيعهما في المصانع المحلية.

في هذه الدراسة، تم جمع 42 عينة من الذرة وفول الصويا خلال شهر أكتوبر 2021 من صوامع مصانع الأعلاف في محافظات جنين، نابلس، وطولكرم. تم تخزين العينات في عبوات مظلمة وباردة قبل إرسالها للتحليل المخبري لقياس تركيز الأفلاتوكسين B1 باستخدام إجراء RIDASCREEN® Aflatoxin B1 30/15. أظهرت النتائج وجود الأفلاتوكسين B1 في 80%، 100%، و100% من العينات المأخوذة من جنين، نابلس، وطولكرم على التوالي. وفي فول الصويا، وُجد أن نسبة الأفلاتوكسين B1 كانت 60%، 85.7%، و100% في عينات جنين، نابلس، وطولكرم على التوالي.

بلغ متوسط تركيز الأفلاتوكسين B1 في عينات الذرة 0.69 ميكروغرام/كيلوغرام، بمتوسط 0.64 ميكروغرام/كيلوغرام في جنين، 0.604 ميكروغرام/كيلوغرام في نابلس، و0.80 ميكروغرام/كيلوغرام في طولكرم. وفي عينات فول الصويا، كان المتوسط العام لتركيز الأفلاتوكسين B1 هو 0.66

ميكروغرام/كيلوغرام، حيث بلغ 0.78 ميكروغرام/كيلوغرام في جنين، 0.61 ميكروغرام/كيلوغرام في نابلس، و0.65 ميكروغرام/كيلوغرام في طولكرم. تقع هذه القيم ضمن الحدود المسموح بها وفقاً للمبادئ التوجيهية الأوروبية والأمريكية (FDA)، التي تحدد الحد الأقصى المسموح به بـ 20 جزء في البليون (20 ميكروغرام/كيلوغرام).

أظهرت النتائج أن الذرة تعتبر بيئة أكثر ملاءمة لنمو الأفلاتوكسين مقارنة بفول الصويا، مما يبرز الحاجة إلى مراقبة ظروف التخزين بدقة لضمان عدم تطور الفطريات وإنتاج الأفلاتوكسين. كما أن التحقق المستمر من تراكيز الأفلاتوكسين في الأعلاف المعالجة يعد أمرًا حيويًا للحفاظ على جودة الأعلاف وسلامة الحيوانات.

تؤكد هذه الدراسة على الحاجة الملحة لتبني استراتيجيات رقابية مشددة لضمان سلامة الأعلاف وتجنب المخاطر المرتبطة بالأفلاتوكسين. من المقترح إنشاء برامج مراقبة وطنية تشمل الفحص الدوري للأعلاف المستوردة والمحلية، مع التركيز على تحسين ظروف التخزين مثل التحكم في الرطوبة ودرجات الحرارة في الصوامع. بالإضافة إلى ذلك، يجب تعزيز الوعي لدى المصنعين والمزارعين حول كيفية التعامل مع الأعلاف بطريقة تقلل من أخطار التلوث بالأفلاتوكسين، مما يضمن سلامة الحيوانات ومنع انتقال هذه السموم إلى الإنسان من خلال المنتجات الحيوانية.

الكلمات المفتاحية: B1 الأفلاتوكسين، الذرة، فول الصويا، الفطريات، الرشاشيات.