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MASTER THESIS

GÖZDE EKİCİ

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SUPERVISOR

Assist. Prof. Dr. İSKENDER KARALTI

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ONAY

Bu tez Yeditepe Üniversitesi Lisansüstü Eğitim-Öğretim ve Sınav Yönetmeliğinin ilgili maddeleri uyarınca yukarıdaki jüri tarafından uygun görülmüş ve Enstitü Yönetim Kurulu'nun ..11../..05./..2018... tarih ve ..2018/08-15..... sayılı kararı ile onaylanmıştır.

Prof. Dr. Bayram YILMAZ
Sağlık Bilimleri Enstitüsü Müdürü

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material whisch has been accepted fort he award of any other degree except where due acknowledgement has been made in the text.

31.05.2018

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF GRAPHS

LIST OF SYMBOLS AND ABBREVATIONS

ADD	Avarage Daily Dose
AFB_1	Aflatoxin B_1
AFB_2	Aflatoxin B_2
AFG_1	Aflatoxin G_1
AFG_2	Aflatoxin G_2
DNA	Deoxyribonucleic Acid
ELISA	Enzym Linked Immuno Assay
EU	European Union
FAO	Food and Agriculture Organization
HACCP	Hazard Analysis Critical Control Points
HQ	Hazard Quotient
IU	International Unit
LD_{50}	Lethal Dose
mL	Mililiter
Nm	Nanometer
ppb	Parts Per Billion
ppm	Parts Per Million
kGy	Gamma Radiation (1 Gray = 100 Rad)
PTMDI	Provisional Maximum Tolerable Daily Intake
RNA	Ribonucleic Acid
USA	United States of America
UV	Ultraviole

WHO	World Health Organization
µL	Microliter

ABSTRACT

Ekici, G. (2018). Determination of Aflatoxin Levels and Micrufungal Flora in Unpacked Red Pepper Samples in Istanbul Province. Institute of Health Sciences, Department of Nutrition and Dietetics, M.Sc. Thesis, Istanbul.

Foodborne illnesses are among the major public health problems that are increasing continuously. Today, foodborne illnesses can be seen in developed countries, as well as in developing countries. Mycotoxins are causing long-term fatal diseases due to food poisoning from food and toxic effects. In addition, storage and drying conditions are extremely important in terms of their formation and development. Storage is the most critical stage for the formation of mycotoxins and the appropriate relative humidity and temperature conditions must be maintained during storage to ensure that the product is not contaminated during storing. Properly packaged products should be stored in a dry and cool place. It is necessary to determine the total mycotoxin values of the spices sold in the open and to evaluate them in terms of food safety. It is possible to find a solution for the contamination problem by arranging production, packaging, storage, processes in the enterprises. In our country, the maximum limits for mycotoxins have been determined in part 2 of the Turkish Food Codex, Spice Communiqué no 2013/12. In our study, 125 red pepper samples were collected periodically in July, August, September, October, November 2018 from same stores in at least 100 g bags and quantitatively examined by Enzym Linked Immuno Assay (ELISA) method in terms of aflatoxins. As a result, all of the red pepper samples examined in this study were found to contain aflatoxins. As a result, all of the red pepper samples examined in this study were found to contain aflatoxins. Of the total number of samples, 56.8% (Summer season: 50%, Autumn season: 61,3%) were above the limits stated in Turkish Food Codex (>10 ppb). As in many of the studies conducted in this topic, the number of aflatoxin averages exceeding the legal limits (average 16,7 ppb in summer and 23 ppb in autumn) in our study, supports the need to take precautions in the stages of sanitation, drying and storage in terms of mycotoxin development in spices.

Key words: Red pepper, aflatoxin, ELISA method, periodic, contamination

ÖZET

Ekici, G. (2018). İstanbul İlinde Açıkta Satılan Kırmızı Pul Biber Örneklerinde Aflatoksin Seviyelerinin ve Mikrofungus Florasının Belirlenmesi. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Beslenme ve Diyetetik Anabilim Dalı, Yüksek Lisans Tezi. İstanbul.

Besin kaynaklı hastalıklar, sürekli artış gösteren, önemli halk sağlığı sorunları arasındadır. Günümüzde, özellikle gelişmekte olan ülkelerde olduğu gibi, gelişmiş ülkelerde de besin kaynaklı hastalık vakaları görülebilmektedir. Mikotoksinler besin kaynaklı gıda zehirlenmeleri ve toksik etkileri nedeniyle uzun dönemde ölümcül olabilecek hastalıklara neden olmaktadır. Ayrıca oluşumları ve gelişimleri açısından depolama ve kurutma şartları son derece önemlidir. Depolama işlemi mikotoksin oluşumu açısından en kritik aşama olup, depolama sırasında ürünün kontamine olmaması için depolarda uygun bağıl nem ve sıcaklık koşulları kontrollü olarak sağlanmalıdır. Uygun şekilde ambalajlanmış ürünler, kuru ve serin ortamda depolanmalıdır. Açıkta satılan baharatların toplam mikotoksin değerleri belirlenerek ve gıda güvenliği yönünden değerlendirilmesi gerekmektedir. İşletmelerde üretim, paketleme, depolama, proseslerinde düzenleme yapılarak kontaminasyon sorunu için çözüm bulunması mümkün olabilmektedir. Ülkemizde Türk Gıda Kodeksi'nin 2013/12 sayılı Baharat Tebliği'nin 2. bölümünde mikotoksinler için maksimum limitler belirlenmiştir. Çalışmamızda 2018 yılında, 125 adet kırmızı pul biber örneği en az 100 gram olacak şekilde, Temmuz, Ağustos, Eylül, Ekim, Kasım aylarında periyodik olarak aynı satıcılardan toplanmış olup Enzym Linked Immuno Assay (ELISA) yöntemi ile aflatoksin varlığı açısından kantitatif olarak incelenmiştir. Sonuç olarak bu çalışmada incelenen kırmızı pul biber örneklerinin tamamında aflatoksine rastlanmıştır. Toplam örnek sayısının %56,8'inin (Yaz mevsimi: %50, Sonbahar mevsimi: %61,3) Türk Gıda Kodeksi'nde belirtilen limitlerin; >10 ppb üzerinde olduğu saptanmıştır. Bu konuda yapılmış birçok çalışma gibi bizim çalışmamızdaki örneklerin de aflatoksin ortalama üreme sayısının, belirtilen yasal limitlerin üzerinde olması (Yaz mevsiminde ortalama 16,7 ppb, Sonbahar mevsiminde ortalama 23 ppb) baharatlarla ilgili mikotoksin gelişimi açısından sanitasyon, kurutma, depolama aşamalarında önlemler almak gerektiğini destekler niteliktedir.

Anahtar Kelimeler: Kırmızı pul biber, aflatoksin, ELISA yöntemi, periyodik, kontaminasyon

I. INTRODUCTION AND PURPOSE

Aflatoxin, Trichothecene, Zearelenon, Ochratoxin, Patulin and Fumonisin are the varieties of mycotoxins. These are produced by different fungi such as *Aspergillus, Penicillium, Fusairum*. Although there are more than 300 species of natural fungi, very few are associated with the food safety. The fungi are examined under three main headings: filamentous microfungi (molds), non filamentous microfungus (yeast) and macrofungi (hatted fungi). Most important toxicological mycotoxins in agricultural areas and fertilizers; produced by *Aspergillus, Fusarium* and *Penicillium* species. Containment of cereal seeds causes acute and chronic diseases in humans due to carcinogenic, teratogenic, estrogenic, endocrine disruption and immunosuppressive effects. It is known that toxicological problems are causing serious losses in the economies of the developing countries[1,2].

Naturally occurring mycotoxins were identified by the World Health Organization (WHO) in 2002 as important sources of foodborne illness. The Food and Agriculture Organization (FAO) has reported that mycotoxins alone are estimated to contaminate approximately 25% of all agricultural products worldwide and cause significant damage to seed processors, food processors, farmers, and national economies[3,4]. Among mycotoxins, aflatoxins have great importance and there are thirteen specified aflatoxin species. Aflatoxins are toxic metabolites synthesized by *Aspergillus flavus*, *Aspergillus parasiticus*, some *Penicillium* and *Rhizopus* species. Only five of the identified aflatoxins are found in foods and are known as AFB_1, AFB_2, AFG_1,AFG_2 and AFM_1[5].

In this study, it is planned that 125 samples of red pepper flakes are obtained from the market in Istanbul, Turkey and the amount of mycotoxins will be compared. Foodborne illnesses are among the major public health problems that are increasing continuously. Today, both in developing and developed countries it is applied to the health institutions for very few of the foodborne illnes cases and only a small part of the cases can be detected at the species level. In this study it is aimed to produce the research material from red pepper flour, a spice variety, obtained from spice suppliers and at different times from the market and to compare the microfungus flora and aflatoxin values

of spices sold outdoors and evaluate them in terms of food safety regulations. It is planned to take samples during summer and autumn seasons. In July, August, September, October and November 2018, a total of 125 samples will be taken from the same stores each month as 25 samples and aflatoxin ratio of these samples will be determined. It is thought that fungal fluorosis will be in different amounts and show different effects on the aflatoxin amounts for each month and the detection is planned to be done.

II. LITERATURE REVIEW

II.1. History and Studies

The first known phenomenon of mycotoxins is "Ergotism" in Europe as a result of the consumption of rye, which is infected with *Claviceps purpurea* toxins known as "sacred fever" in the Middle Ages. Mycotoxicosis cases remained as neglected diseases until aflatoxin was discovered in the 1960's[6]. New mycotoxins have been discovered from the 1980's to the present day and species such as fumonisin have been characterized. Thus, important data on toxicology, epidemiology and exposure have been obtained by developing better detection methods and using biological indicators in other mycotoxin strains[7].

It has been reported that 34% of 140 people died due to gangrenous ergotism that erupted in Ethiopia between 1977-1978. The reason for this epidemic is that the wild oats which are sensitive to *Claviceps purpurea* were affected by the long, rainy season. In studies conducted in China and Swaziland in 1987, the role of aflatoxins in the development of liver carcinoma independent of Hepatitis B virus has been confirmed. In 2007, it was reported that the fatal hepatitis outbreak affected 400 people, 20-40% adults and 5-15% children, due to maize contaminated with aflatoxins[4].

Shortly after examinations on turkey-X disease (liver necrosis), which resulted in the death of more than 100,000 turkey in the UK in 1960, poisonings were seen in chicken and duck pups and symptoms of this disease includes bile duct hyperplasia, loss of appetite, acute hepatitis necrosis and drowsiness[8]. In the aflatoxicosis case in which one person died in Uganda, it has been determined with abdominal pain that occurs 5-30 days, and changes in the histopathology of 1.7 ppm aflatoxin-exposed liver histopathology such as centroblastic necrosis, polymorphonuclear infiltration and muscle warts[9].

In a few-week long case in India which is known to originate from maize, the exposed dose was reported to be in the range of 6.25-15.6 ppm and AFB_1 was observed in serum urine analysis. It has been reported that 106 out of 397 individuals complaining of fever, vomiting, ascites, anorexia, edema in the legs, gastrointestinal bleeding, jaundice

died[10]. In another maize-borne case in India, it has been reported that 97 out of 994 cases that have been exposed to 0.01-1.1 ppm AFB_1 for a few weeks with fever, abdominal bloating, anorexia, jaundice, tachycardia, bloody stool, gastrointestinal bleeding died. AFB_1 toxin was found in the liver autopsy and it was reported that the fat infiltration decreased and there was centrobial necrosis[11].

It has been determined that due to 3.2-12 ppm AFB_1 and 1.6-2.7 ppm AFB_2 in the aflatoxicosis case in which twelve people died in Kenya. In this case break out abdominal strain, vomiting, fever, edema, jaundice, ascitis, bloody stool and gastrointestinal bleeding symptoms were observed because of maize[12].

II.2. History of Red Pepper

Pepper is a cultivated plant of the family *Capsicum* (*Solanaceae*), which grows in a single year in temperate climates. Thanks to the flavor and color that it has gained, cultivation and consumption around the world come after tomato. The motherland of pepper, Central and South America, was first introduced from the United States to Spain in 1493, England in 1548 and other European countries in 1578. It first entered the Anatolian lands during the 16th century during the Ottoman Empire. Red pepper today is produced in all parts of the world except the Antarctic continent[13].

In the market, hot red peppers according to the plant source or country; different names such as Sandia, Chillies, Cayenne, Hontaka, Tabasco, Sport, Bell, Tomato and Cherry are also used. *Capsicum frutescens L*. and *Capsicum annum L*. are known as hot red pepper and sweet red pepper, respectively[14]. The country that produces the most pepper in the world is China with 15,000,000 tons of production value. Turkey stands after Mexico with chilli production of 2,000,000 tons. In the production of dried red pepper, India ranks first with 1.500.000 tons. Followed by China and Pakistan with production of 280,000 and 200,000 tons, respectively. Turkey, 15,000 tons of red pepper production is coming after Nepal ranks 24th.[15,16].

II.2.1. Processes Applied to Red Peppers

Although red pepper can be consumed as fresh or spice it is mostly used as spice. Today, industrial methods are widely used for the production of peppers to be consumed as spices, but some countries still produce spices from red pepper in traditional methods under natural conditions such as drying in the sun[16]. *Capsaicin*, is the compound that provides the spicy aroma to pepper. Traditionally, fresh red peppers are washed and dried after soaking, then processed into small flakes or powdered[17]. Red pepper production steps are; harvesting and transportation, washing the fruit surface, taking the seeds, drying, grinding the peppers at the desired size, and storing the product as a package[18].

II.2.2. Red Pepper Production in Turkey

In our country, red pepper farming is being carried out widely in the Southeast, Mediterranean and Marmara regions. Hot red pepper is grown in the regions of Southeastern Anatolia. In Gaziantep and Kahramanmaraş, about 80% of red pepper production is done. Since Bursa and Sakarya regions have different pepper structure and drying shapes, flake production is not possible and powdered pepper production is done. Red pepper production is usually done according to primitive conditions or traditional methods. Red peppers, which are collected by farmers, are dried on the soil and sold to the factory in contact with soil and water and open to contamination. When these conditions are taken into consideration, toxigenic mold contamination is very common in red rubbers. Studies have also reported that red pepper species of the genus *Capsicum* are among the risky products due to the formation of moldy metabolites such as aflatoxin and ochratoxin which are carcinogenic and teratogenic effects. Mold contamination of red pepper is starting on the plant and mold development and toxin formation continue during the period of time until harvest and after drying. It has been determined that aflatoxin formation has just begun with pepper. Aflatoxins in agricultural products are the most toxic mycotoxins and they are the most important microbiological problems in recent years[14,15,19].

In 1994, the presence of the aflatoxins above the regulatory limit in the red pepper which are tried to exported from Turkey to Switzerland and Germany created problems in the export, it is known that in previous years the average 2155 tons of red pepper exports while exports between 1996-1998 fell to 968 tons. Although there was an improvement in exports in the following years, it was seen that this situation was in unprocessed dry pepper and the reduction in processed red pepper continued[20].

II.3. Mycotoxins

II.3.1. Determination of Mycotoxins

The myotoxin word is derived from the word "*mykes*" (Fungus in Greek) and "*toxicum*" (Poison in Latin). Although it is known that more than 350 fungi producing mycotoxin in case of physical conditions, species of *Aspergillus*, *Penicillium* and *Fusarium*, among these species, can form mycotoxins which can cause significant health problems in humans and animals. *Fusarium*, before or after harvest, *Penicillium* and *Aspergillus* species are able to contamine the foods and feeds during and after drying and produce toxin. All mycotoxins are produced by fungal sources but all of the toxins produced by fungi can not be identified as mycotoxins. Such as antibiotics, which act on bacteria such as metabolites produced by *Penicillium* species, and phytotoxins, which have a toxic effect on plants[21].

It is known that the origin of mycotoxin-producing fungi is soil. When the physical conditions such as heat, oxygen content, humidity and time in the above-mentioned blending, storage, transport stages are compatible with fungus development, they can synthesize mycotoxin. They are produced by fungi belonging to *Aspergillus, Penicillium, Fusarium, Rhizopus, Alternaria* and *Claviceps* species[22]. There are electrochemical immunosensors designed in various formats for the safety of food and the detection of microorganisms or mycotoxins. Successfully applied to detect AFB_1 and AFM_1 in maize,

barley and milk samples, are generally used biosensors because they help to minimize possible human errors with fast analysis times, improved reproducibility with low costs. Such an automated electrochemical detection system has been applied for the detection of AFM_1 in milk samples and the system has come to the forefront with shorter analysis time and lower cost when compared with the results obtained by High Performance Liquid Chromatography (HPLC) method[23].

II.3.2. Prevention of Mycotoxin Occurrence

Control of toxin formation to prevent and control mycotoxin exposure requires some basic strategies before and after harvest. It is also important to include good agricultural practices such as pre-harvest prevention, use of healthy seeds and proper irrigation, as well as the destruction of previous remains[24].

The main factors affecting mycotoxin formation can be classified as; physical, chemical and biological. The amount of moisture in the surrounding or foodstuffs is related to the physical factors. The composition of air and the natural environment of the substrate are chemical factors. Factors related to host species are biological factors. Suitable temperature ranges for aflatoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* are 25-35 ^{o}C, 20-25 ^{o}C for Patulin synthesized by *Penicillium expansum* and 25-30 ^{o}C for rubratoxin synthesized by *Penicillium rubrum*. For mycotoxin-producing molds, the chemical composition of the environment they feed is very important. The composition of the food moisture content, acidity and pH value determines the chemical prpperties of the environment. Biological factors include the ability of toxins to be toxic or not, the ability to synthesize toxins if toxigenic, the competition with other mold-synthesizing species in the same environment, and microbial detoxification[21].

There are studies reported to have protective effects of different vitamins against the toxic effects of mycotoxins. Studies have been carried out to demonstrate the

protective effects of vitamin C and vitamin E against many natural or synthetic carcinogenesis agents. Vitamin A is an oil-soluble, powerful radical scavenger and an antioxidant molecule. The efficacy of vitamin A on the toxicity and activity of AFB_1 has not been fully elucidated. In a study of the protective effect of vitamin A on haemolysis induced by erythrocytes in AFB_1, it was determined that the highest protective effect was at 55% at 1000 IU/mL concentration[25]. However, in another study, when the pups of the control group were immunologically assessed with 300 ppb AFB_1 plus A and E vitamins, the lymphocyte count was significantly reduced in the AFB_1 group and morphological examination of the thymus, liver and lymph nodes revealed no evidence of damage to the A and E vitamins it has been reported[26].

Also in another study, it was reported that when β-carotene and lycopene were administered to human hepatocyte cells against AFB_1 toxicity, AFB_1-mediated mitochondrial-related damage, intercellular disturbance of cellular function and deterioration of intercellular functions, and antioxidants play a protective role[27]. AFB_1 is known to have a deleterious effect on the histological structure of the kidney. The study on curcumin which has antioxidant, antiinflammatory and antiapoptotic effects, proves that curcumin has a protective effect against the harmful effect of aflatoxicosis on the kidney[28,29].

In the case of aflatoxin production, fungal contamination and toxin production may occur prior to haystack and may continue to increase during moist conditions after harvest. Contamination in the field is usually caused by insect damage and lack of water. However, storage practices can also affect the fungal growth and aflatoxin development after harvest. It has been proven that the drying of grains on proper conditions, the good availability of storage during storage, the removal of molds and the use of pesticides are effective in reducing aflatoxins in the postharvest stage[30].

II.4. Aflatoxins

II.4.1. Contaminated Foods with Aflatoxins

Aflatoxins are the most important mycotoxins in terms of toxicity and human effects. There are two large species of *Aspergillus* that produce aflatoxin. These*; A. flavus* producing only aflatoxin B, *A. parasiticus* producing both aflatoxin B and G type 2 ones. There are four main aflatoxins, B_1, B_2, G_1 and G_2. 2 are missing double bonds in the structural isomer when compared to type 1 ones. The four food-related aflatoxins are named blue (Blue-B) or green (Green-G) under ultraviolet (UV) light and are based on their chromatographic mobility. The aflatoxins, known as M_1 and M_2, are not associated with cereal products, but are found in the milk and milk products of cereal-consuming mammals contaminated with AFB_1 and AFB_2[24,31].

In 2003, a study was conducted to examine aflatoxin contamination in maize, peeled groundnut, rice, walnut (peeled and unsealed) and pine nuts samples from eight different regions in China. As a result of the study, maize was found to be contaminated with aflatoxins not only in high contamination ratio (70.27%) and high pollutant content (Highest: 1098.36 μg / kg) but also in large geographical areas. The contaminated samples were detected in all areas where samples were taken and the contamination rates varied between 40% and 100%. The contamination rate of aflatoxins in ground nut was 23.08% which is at a level that cannot be underestimated. It is reported that AFB_1, is the most food contaminating aflatoxin type which is followed by AFB_2, AFG_1 and AFG_2[32]. Recent studies have also shown contamination of aflatoxins on medicinal plants. Rizzo et al.[33] reported that AFB_1 and AFB_2, which ranged from 10 to 2000 μg/kg in 152 medicinal plants. It has been argued that the plants that are contaminated with aflatoxins should be banned because the medical plants are imported as raw materials. Stricter guidelines and strategic measures have to be taken with regard to medical plants, since packaging and distribution phases may increase the possibility of contamination with aflatoxins[34].

AFB_1 has been widely observed in 48 basmati rice samples taken from different locations in a study conducted in Pakistan (2016). Approximately 56% of the rice samples were contaminated with AFB_1 and 33% were contaminated with AFB_2. It was observed that the amount of aflatoxin in 64.3% of the contaminant rice containing AFB_1 are lower than the maximum tolerable limit of 20 ppb, and 35.7% was detected as higher. Also it was reported 87.5% of contaminant rice containing AFB_2 was above the maximum

tolerable limit[35]. It has been reported that the basmati rice, mostly found in India and Pakistan and the black and red rice growing in Thailand are contaminated with aflatoxins [36]. In a Thai medicinal plant survey (2004), it was determined that aflatoxins analyzed in 5 of 18 samples (18%) were between 1.7-14.3 ng/g and AFB_1 and AFB_2 were the most common aflatoxins for tested samples. It has been reported that all samples with contaminates are generally taken from the northern and northeastern regions of Thailand, which are usually moist area[37].

It has been found that there is a positive correlation between consumption of aflatoxin-contaminated foods and the incidence of cancer in developing countries. AFM_1 is thought to be related to the casein fraction in the milk. Milk products, such as cream cheese and butter, were found to contain less AFM_1 than the milk they produced. The white cheese contains 3-5 times more AFM_1 than the AFM_1 in the milk produced. It has been reported that AFM_1 can be also found in animal tissues and eggs as well as in cheese and milk, eggs and meat may be contaminated in this way[29]. Globally, about 5-10% of agricultural products are deteriorated due to fungi, and 25% of mycotoxins are inadequate for human and animal use due to mycotoxin contamination. Fruits and vegetables are located in the group of rapidly deteriorating foods and red peppers are found in this food group[38].

II.4.2. Pathogenic Effects of Aflatoxins

Aflatoxins are acute toxic, immunosuppressive, mutagenic, teratogenic and one of the strongest known carcinogenic compounds. The target organ for toxicity and carcinogenicity is the liver. The International Agency for Research on Cancer (IARC) has come to the conclusion that sufficient evidence is available when carcinogenicity is assessed in humans, taking into account all relevant information on aflatoxins. Oral acute LD_{50} for mice is around 7.2 mg/kg, reported to be less toxic than strychnine[39,40].

Acute exposure to aflatoxin at high levels can lead to fatal liver damage, and such cases occur periodically in Africa in particular. Of the reported acute aflatoxicosis outbreaks, the most severe occurred in Kenya (2004) and 125 of 317 cases with acute liver failure were fatal. The maize samples in the affected area were found to be contaminating with 4400 ppb aflatoxin[30]. Analytical measurement of nutrient pollution and nutrient intake parameters can be difficult. In addition, toxicokinetic and toxicodinamia related to toxin ingestion have to be taken into account. For these reasons, great efforts have been made to develop biological markers for aflatoxins and fumonisins. For example; For AFB_1, the urinary biomarkers AF-N7-Guanine and AFM_1 reflect shorter-term exposures, while peripheral blood AF-Albumin biomarkers are considered chronic long-term (several months) exposure. The application of these biomarkers has been linked to the link between AF exposure and liver cancer development[41].

Conditions that increase the likelihood of acute aflatoxicosis in humans are due to the limitations of food use, the conditions that cause fungal growth in plants, and the lack or absence of regulatory systems for aflatoxin monitoring and control. Aflatoxin-related diseases in humans; age, gender, nutritional status and/or exposure to other active substances such as viral hepatitis (especially HBV), parasitic infestation[42]. It is known that AFB_1 is above the cancer threshold and has a reactive metabolite that can interact directly with DNA. The IARC and the United States Toxicology Program (NTD) for carcinogenic substances are two establishments that provide evidence weight (WOE) assessments. WOE is a term that expresses the power of evidence, agents can cause cancer in humans. The IARC classified the agents in terms of carcinogenicity in humans, taking WOE into account (Table II.1)[43].

Table II.1: IARC classification in terms of carcinogenicity for humans

Category	**Importance**
Group 1	Carcinogen for humans
Group 2A	May be carcinogenic to humans
Group 2B	Possible carcinogenesis for humans
Group 3	Not classified for human carcinogenicity
Group 4	Probably not carcinogenic for humans

If the risk factor (HQ = Hazard Quotient) of the agents that can cause cancer is higher >1, an individual or population may be at risk of a health hazard because of exposure to dangerous agents. If the risk factor lower <1, it is unlikely that an individual or population will be exposed to a health risk based on the exposure levels available for the agent. The hazard quotient (HQ) is found by dividing the Average Daily Dose by the temporary maximum tolerable daily intake (PMTDI = Provisional Maximum Tolerable Daily Intake). The safety factor assumes that people are ten times more sensitive than the most sensitive animal tested for a given toxin, and that the most sensitive person is ten times more sensitive than the least sensitive person[43].

$$HQ=ADD/PMTDI$$

In addition to the IARC classification, the Department of Health and Human Rights of the United States publishes a report on carcinogens (Report on Carcinogens - RoC), a science-based document every two years. The RoC lists only the items that a significant number of people in the United States are exposed to and divides into two categories;

1. Known as carcinogens for humans: contains the substances that indicate that there is a causal relationship when there is sufficient evidence of exposure between the agent or exposure to cancer and cancer. In the 13th edition of RoC, there are 56 articles in this category.

2. Expected to be carcinogenic to humans: Contains the substances that are causally related to substances that are limited to humans but contain sufficient evidence of cancer in animals. In RoC's 13th edition, 187 items were listed in this category[39].

One of the other important effects of aflatoxins is the anticoagulation of the blood, probably due to the ability of AFB_1 to inhibit the synthesis of prothrombin synthesis and coagulation mechanism-related factors. It is also known that this mycotoxin inhibits ribonucleic acid (RNA) polymerase and protein synthesis. It has also been suggested that oral administration of aflatoxin in childhood cirrhosis, viral diseases and hereditary factors may be etiologic agents. There are some evidences that cirrhosis develops as a result of exposing the child to the aflatoxin pumped by the child and consuming nutrients such as unrefined peanut oil[29]. The highest toxic concentration is found in the liver after the absorption in the gastrointestinal tract is complete. After reaching the liver AFB_1 is separated by microsomal enzymes into different metabolites by hydroxylation, hydration, dimethylation and epoxidation (Figure II.1). AFB_1 chemically binds to DNA and causes structural DNA changes as a result of genomic mutation. The presence and activity of glutathione is a toxic effect of AFB_1. In addition, drug use and food components play an important role in the activation or inhibition of AFB_1 toxicity[29,44].

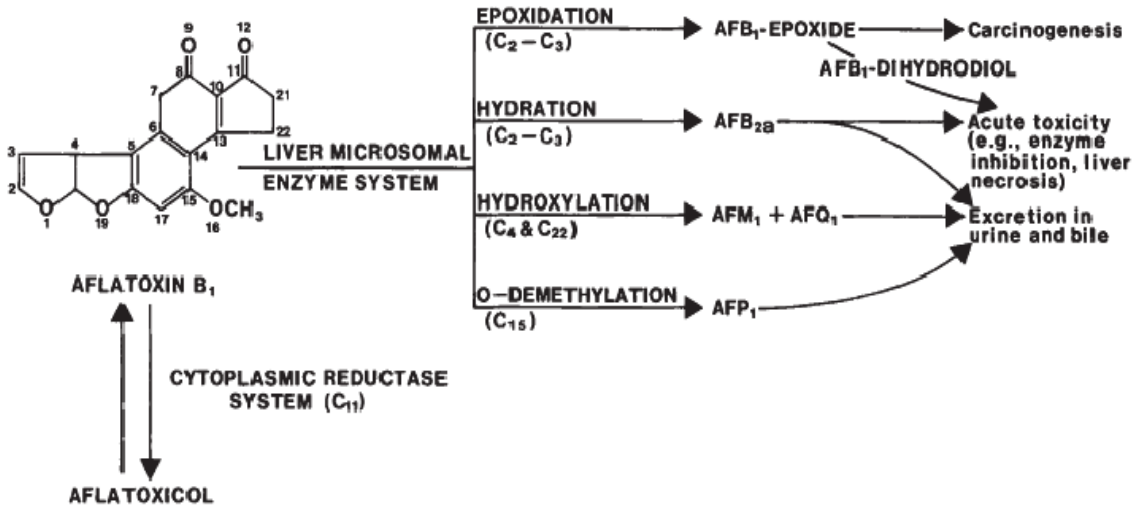


Figure II.1: Metabolism of AFB_1 in the liver

II.4.3. Methods Used to Prevent Aflatoxins

Some physical, chemical and biological methods have been developed for use in the prevention of mycotoxins. It is known that aflatoxin or other mycotoxins can only partially be eliminated with heat treatments which is among the physical methods, such as boiling in water or autoclaving. Pure aflatoxins are generally stable up to 250 ^{o}C, which is the melting point. The reason for the loss of aflatoxin in the heat treated contaminated products is most likely due to pH, humidity and environmental factors. The reason why this method can not be practically used is that aflatoxins must be resistant to high temperatures and lose the nutritional value of the product in these heat treatments. For example; there are studies of AFM_1 in the milk not resistant to changing temperatures during processing such as pasteurization and storage, but a method has not yet been developed that will remove AFM_1 without altering its components. Many mycotoxins have been reported to be stable against different heat treatments. For example; it was observed that aflatoxin level could be reduced by 45-85% with roasting in ground nuts, but there was no reducing effect on fumonisin and zearalenon levels[45,46].

Studies on the effect of extrusion (molding under pressure of food and feed, shaping) process which is commonly used in food sector to reduce mycotoxins are being carried out. The decrease in the concentration of mycotoxin during this process varies with the conditions such as the extrusion temperature, the compression speed, the amount of moisture in the process. Another application among physical methods is radiation application. Radiation irradiation for the destruction of mycotoxins was first reported at the end of the 1950s. The first known work was done in 1971 with gamma irradiation against *A. flavus*. 1.6-2.4 kGy radiation was irradiated into the tube containing *A. flavus* and the viability decreased by 30%. A study has been carried out to ensure the destruction of AFB_1 in maize, wheat and soybeans by radiation irradiation. 20 kGy gamma radiation did not affect AFB_1. In another study, it was reported that naturally occurring contaminated peanuts were exposed to 25 kGy gamma radiation, but no destruction of aflatoxins was observed. Physical methods include methods such as mechanical processes, densitometric separation and beam application[25,46].

Chemical methods include application of ammonia, application of ozone and use of adsorbents. During ammonia application; ammoxidated feeds were given to puppies

and necrosis cases were investigated when examined at the cellular level and it was seen that there was a decrease in the findings of aflatoxicosis. When these results were observed in turkey, rat and rats, AFM_1 was detected in milk in a study performed on cows. There are studies regarding the reduction of aflatoxins in cotton seed pods and ground pods in relation to ozone application. It has been reported that 91% of total aflatoxin is reduced from 214 ppb to 20 ppb in the cottonseed cube, and 78% of the aflatoxin is lost in 1 hour in the groundnut cube and the amount is decreased from 82 ppb to 18 ppb. The adsorbents, which are a chemical method, are compounds which form compounds with mycotoxins thanks to their binding properties and allow them to go out without being absorbed in the intestines[47].

Ethanol, a physiochemical inhibitor used to prevent mycotoxins, also plays an important role. Ethanol has been used as a fungicide for a long time and is commercially used in many products such as food stains, alcoholic beverages, perfumes and food additives. It has also been suggested that baked goods such as bread and pasta extend the shelf life of moldy an additional barrier in suppressing fungal growth in food products and may be an alternative way in food preservation[38]. In biological methods, pure bacterial and fungal cultures, which can detoxify mycotoxins, can be isolated from microbial populations. It has been reported that the harmful effects of aflatoxin and other mycotoxins can be reduced or prevented by the obtained pure cultures. In addition to the bacteria, studies with some yeast strains showed positive results. It has been observed that Saccharomyces cerevisiae, alive yeast cell, is effective in preventing the aflatoxin-related side effects in poultry. Another approach to inhibit aflatoxin contamination is based on the development of non-toxigenic strains of *A. flavus* and *A. parasiticus* and the slowing down or preventing of the replication of toxic *Aspergillus* strains. Probiotic and lactic acid bacteria can bind mutagenic and carcinogenic substances. Strains of lactic acid bacteria from different origins can be used as starter culture to reduce or eliminate AFM_1. It has been proposed that studies on yogurt may be a potential application to reduce AFM_1 to safe levels[45,47,48]. Detoxification methods should inactivate, shred, or remove toxin, leave no new toxic substances in the medium and protect the nutritional value of the product. Ideal mycotoxin removal methods should ensure breaking down of the main compounds to carbon dioxide and water levels[49].

II.4.4. Legal Arrangements for Aflatoxins

According to FAO, the maximum mycotoxin limits that certain countries have identified for all food products and spices are; The European Union (EU) for AFB_1 rate in all the spices 5 μg/kg total aflatoxins rate of 10 μg/kg, United States of America (USA) for total aflatoxin rate for all food products other than milk 20 μg/kg, while for Turkey, just as in the EU it was determined that AFB_1 ratio should be 5 μg/kg and total aflatoxin ratio should be 10 μg/kg in all spices[50].

International regulations on mycotoxins have been introduced for food or feeds. More specifically, the maximum content of AFB_1, AFB_2, $AFBG_1$ and AFG_2 for Brazilian peanuts and pistachios is 20 ppb maximum, the ratio of AFB_1 for garlic powder, ginger and ginger is 5 ppb maximum it has been reported that the ratio of AFB_2, AFG_1, AFG_2 for seasonings should be maximum 10 ppb, ground nuts, hazelnuts, dried fruits for direct consumption, 2 ppb for cereals and their products, AFM_1 ratio for milk should be maximum 0.05 ppb. According to the Codex Alimentarius Commission it is recommended that AFB_1, AFB_2, AFBG1 and AFG_2 ratios of maximum 15 ppb and maximum recommended 0.5 ppb of milk for later processed ground nuts[51].

According to Turkish Food Codex Spice Communique (Communique No: 2013/12); Red pepper: plants that enter the genus *Capsicum* (*Solanaceae*) express the dried milled state after the stem has been taken according to the technique of fully ripe fruit and the legal limits are given in Table II.2[52].

Table II.2: Turkish Food Codex Contaminants Limit

Food		Maximum Limit (µg/kg)		
2.1.	**AFLATOXIN**	**B_1**	**$B_1+B_2+G_1+G_2$**	**M_1**
2.1.13.	For the following types of spice; Red pepper (*Capsicum* spp.) (including their dried fruits, whole and ground) Black pepper (Piper spp.) (including their fruity, white pepper and black pepper) Indo-Chinese/Muscat(Myristica fragrans) Ginger (*Zingiber officinale)* Turmeric (*Curcuma longa)* Mixture spices containing one or several	5,0	10,0	(-)

II.4.5. Aflatoxin Occurrence in Red Peppers

Pepper is very susceptible to mycotoxin contamination due to environmental conditions and processing techniques. Red pepper can only be infected with toxic fungi found in the field during the production of the plant, either during drying or in storage or storage media. Failure to adequately clean new harvested red pepper capsules and use of inappropriate drying techniques will most likely increase the risk of fungal contamination and mycotoxin production. It is the most common method used to spread the product over an open area so that the product is exposed to the sun while different drying methods are available. In a study conducted, it was observed that mechanically dried powder peppers had slightly lower mycotoxin levels in sun dried powder peppers, but this difference was not statistically significant[17].

The formation and contamination of aflatoxin in the red peppers varies depending on the year, geographically and climatically, where the peppers are planted. Mycotoxins, and thus aflatoxins, can also arise not only during the growth of the plant but also during outdoor drying and storage during harvesting. Many factors can affect the aflatoxin, formation and *Aspergillus* growth, such as temperature, humidity, drying speed, mechanical damage to the water, heating, the atmospheric pressure of the reservoir, the amount of carbon dioxide (CO_2) and oxygen (O_2), chemical applications and the natural structure of the product. Red pepper dried by natural means due to hand harvesting as well as less or no drying facilities are contributing to aflatoxin contamination. Studies conducted so far have been confronted with aflatoxin contamination in most countries[18].

Mold and yeast can be found in all kinds of spices, but mycotoxin contamination is more frequent and are generally regarded as deterioration organisms in spices. Spices are known to be an important source of mycotoxin contamination in meat products and meat group foods. Presence of mycotoxin is important for these foods because it can lead to food poisoning or deterioration of food products due to the fact that the fungus toxins are still active following cooking[53].

III. MATERIAL AND METHODS

III.1. Material

125 non-packaged red pepper samples are which bought as 25 samples during 5 months (July, August, September, October, November 2018) from the same stores in Istanbul, were studied. Samples were collected as at least 100 g, and were stored in 4 ºC and in original package until analysis.

III.1.1. Aflatoxin Standard

- Romer Labs AgraQuant Aflatoxin (1-20 ppb) Test Kit, 110210-1702, USA

III.1.2. Devices

- ELISA washer (ELx50 259195, Biotek, USA)
- ELISA detector (DTX880, 262502226, Beckman Coulter, USA)
- Autoclave (MLS-3770, Sanyo, Japan)
- Inoculation cabinet (LaminAir 1.8, Holten, India)
- Binocular Light Microscope (E1, WF 10x/18, 13391000, Leica, Germany)
- Incubation shaker (GFL 3031, Germany)
- Precision Scale (AP250D, 1125400585, USA)
- Vortex (MS1, IKA, 012560, USA)

III.1.3. Equipments

- Glasswares (50, 100, 500 mL)
- Paraffin tape (IL, 60631, Chicago)
- Beaker (100 mL)
- Funnels
- Volumetric pipets (5 mL)
- Micro pipets (100, 200 µL)
- Filter paper (Whatman No:4, China)

- Eppendorf tubes
- Swab

III.5. Methods

III.5.1. Preparation of the Petri Dish

65 g of Sabouraud 4% Dextrose Agar (1.05438.0500, Merck, Germany) used for microbiology was weighted (AP210, Ohaus Analytical Plus, USA) and added to1 liter of distilled water in a bottle whose cover was covered with gauze patch. It was sterilized in autoclave (MLS-3770, Sanyo, Japan) at 121 ºC for about 15 minutes and then was allowed to stand outside for cooling. It was poured into petri dishes and waited to solidify for approximately 20 minutes. In the oculation cabinet with the help of swab, samples were taken and planted to two different points of the same petri dish. The date and the sample number/shop number (eg 1/1 = 1st party/1st shop) were written on the sowed petri dishes. Petri dishes were kept in the cabinet at room temperature.

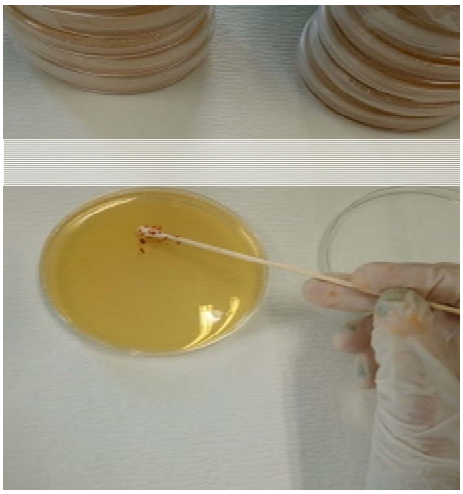


Figure III.1: Sowing of Petri Dish

Figure III.2: Sabouraud Dextrose Agar

III.5.2. Preparation

The medium to be prepared is taken into the inoculation cabinet (LaminAir 1.8, Holten, India) and two drops of lactophenol-cotton blue solution were dropped side by side on the slide. Swaps were taken from the seeds of the cultured media, which were not dense with the substance, and mixed on the lactophenol-cotton blue solution on the slide. This process was completed by gently pressing down on the coverslip at 45°, taking care not to form air bubbles. The slides were stored at room temperature, put into the storage vessel respectively. Prepared media were also re-taped to maintain air temperature at room temperature (Figure III.3).

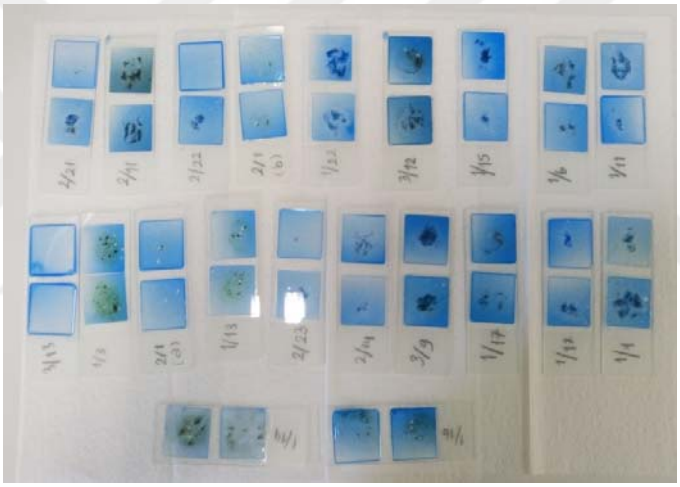


Figure III.3: Preparation process of slades

III.5.3. Preparation of Extraction Solution

20 g red pepper samples were taken into a 100 mL volumetric flask. 70 mL of methanol (106007.2500, Merck, USA) / 30 mL of distilled water was added and the flask was covered with paraffin tape (IL 60631, Parafilm, Chicago). The resulting mixture was shaken for 10 minutes on an incubation shaker (GFL 3031, Germany). After agitation, the mixture is left for 10 minutes and filtered on filter paper (Whatman No: 4, China).

The filtered mixture from the filter paper was pipetted into 2 mL eppendorf tubes and kept at -20 °C until the day of operation (Figure III.4).

Figure III.4: Extract filtration process

III.5.4. Standard Kit Preparation and Calculation of Aflatoxin Levels

All components to be processed are brought to room temperature. Sample extracts were stored for 3-5 seconds on a vortex instrument (MS1, IKA, 012560, USA). Enzym Linked Immuno Assay (ELISA) Test kit (Romer Labs AgraQuant Aflatoxin (1-20 ppb), 110210-1702, USA) was used and 200 µL of conjugate was added to the green beaker dilution wells. To the same wells, 100 µL of the sample extract, which had been previously vortexed, was added by pipetting 3 times and draining. It was taken from the mixtures in the green-edge dilution wells as 100 µL with the help of a multichannel pipette and transferred to the test wells. Incubated for 15 minutes at room temperature (Figure III.5).

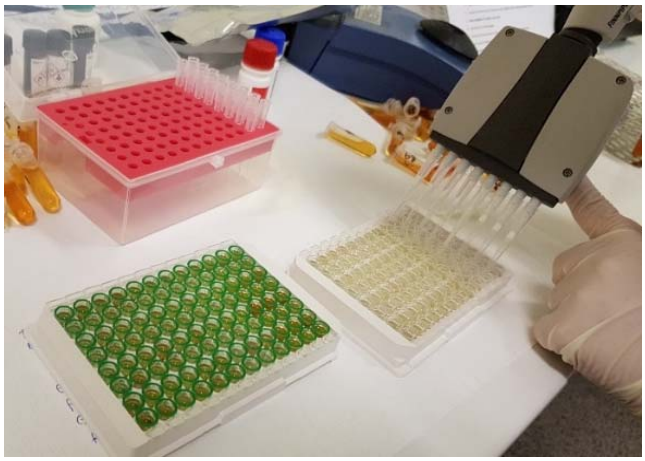


Figure III.5: Dilution wells process in ELISA

The wells were washed 5 times with an ELISA washing device (ELx50 259195, Biotek, USA). As the next process; Each well was incubated at room temperature for 5 minutes with the addition of 100 µL of substrate solution. Plates were kept indoors during incubation and daylight contact was avoided directly to avoid substrate activity (Figure III.6).

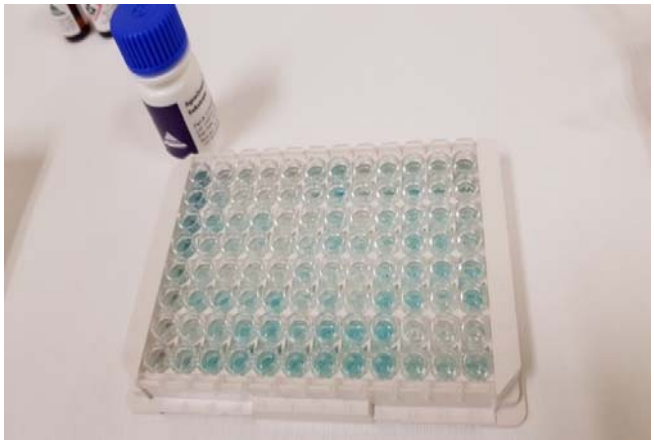


Figure III.6: Substrat solution addition

At the end of the incubation, 100 µL stop solution was added to each well. The resulting color density was measured at a wavelength of 450 nm. The absorbance values obtained are transferred to the excel calculation table. The total concentration of aflatoxin in the samples was automatically calculated by means of the calibration curve generated by the absorbance values obtained from the standards (Figure III.7).

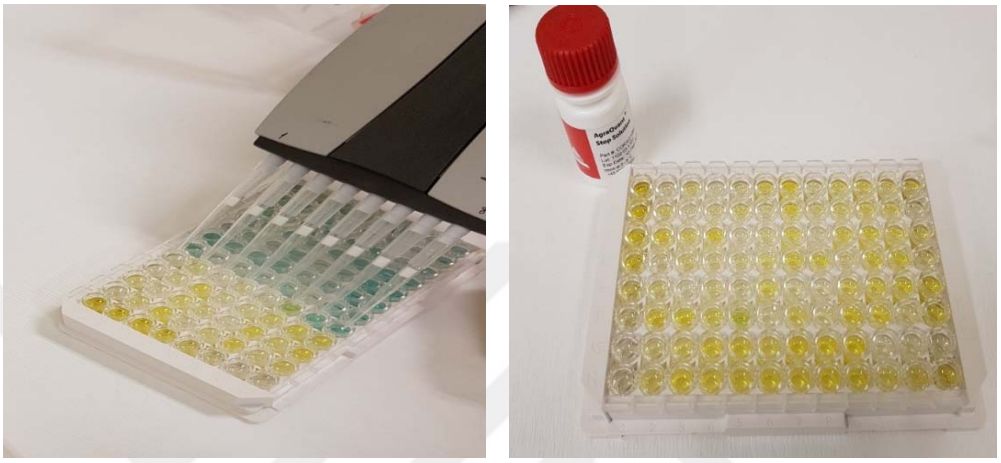
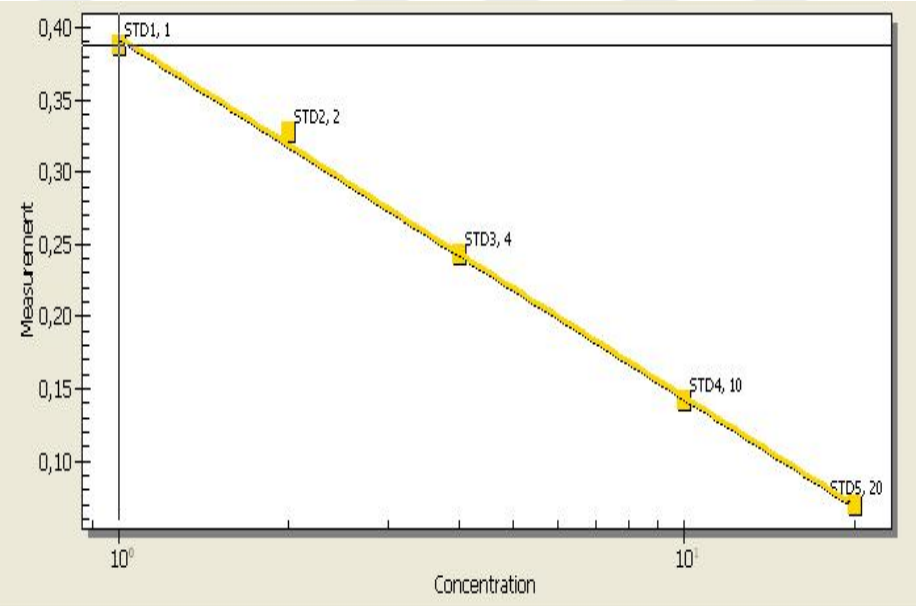


Figure III.7: Stop solution addition

IV. RESULTS

In this study, samples of red pepper sold in Istanbul between July and November with 1 month periods, were examined in terms of aflatoxin presence. Calibration curve (Graph IV.1) was obtained according to the absorbance values of the standards was used to determine aflatoxin levels in this study. The aflatoxin concentrations were evaluated as ng/kg according to the form obtained from the calibration curve, taking into account the absorbance values read at 450 nm with 620 nm reference filter of the red pepper samples examined.



Graph IV.1: Standard curve analyses

Aflatoxin values were given for all samples taken. It has been found that the values are lower in the summer months, and the values increase as the autumn season approaches. The first 25 samples are the samples of July, the samples 25-50 are samples of August. Examples 50-75 are samples of September, samples 75-100 are samples of October, samples 100-125 are samples of November. The highest aflatoxin was 52,62 ppb in July and 49.24 ppb in August. In autumn season, the highest rate of reproduction in September was measured as 69,64 ppb, 131,84 ppb in October and 76,45 ppb in November (Table IV.1).

Table IV.1: Aflatoxin values in samples

Sample No	July Aflatoxin Value (ppb)	Sample No	August Aflatoxin Value (ppb)	Sample No	September Aflatoxin Value (ppb)	Sample No	October Aflatoxin Value (ppb)	Sample No	November Aflatoxin Value (ppb)
1	52,62	**26**	45,17	**51**	36,79	**76**	49,51	**101**	13,75
2	9,90	**27**	17,17	**52**	8,07	**77**	35,93	**102**	67,00
3	35,36	**28**	6,63	**53**	6,34	**78**	41,55	**103**	29,04
4	21,35	**29**	49,24	**54**	4,70	**79**	41,55	**104**	40,77
5	5,36	**30**	5,81	**55**	4,29	**80**	8,50	**105**	15,26
6	2,35	**31**	5,08	**56**	4,24	**81**	4,29	**106**	14,79
7	21,99	**32**	6,77	**57**	4,43	**82**	5,79	**107**	25,96
8	7,38	**33**	7,14	**58**	8,60	**83**	5,28	**108**	25,21
9	5,62	**34**	40,13	**59**	3,75	**84**	4,94	**109**	76,45
10	9,68	**35**	13,91	**60**	30,72	**85**	5,51	**110**	23,86
11	5,84	**36**	20,66	**61**	8,28	**86**	4,08	**111**	13,75
12	12,13	**37**	17,36	**62**	19,54	**87**	4,26	**112**	21,50
13	41,30	**38**	9,66	**63**	4,10	**88**	8,12	**113**	19,49
14	16,96	**39**	6,19	**64**	4,65	**89**	7,25	**114**	19,76
15	9,76	**40**	5,92	**65**	22,19	**90**	6,55	**115**	66,64
16	24,49	**41**	26,05	**66**	4,72	**91**	63,28	**116**	21,63
17	4,92	**42**	4,87	**67**	69,64	**92**	116,88	**117**	19,36
18	13,57	**43**	5,19	**68**	16,53	**93**	131,84	**118**	20,29
19	10,71	**44**	15,23	**69**	10,77	**94**	15,28	**119**	17,24
20	7,73	**45**	14,61	**70**	4,92	**95**	19,65	**120**	24,96
21	8,43	**46**	54,02	**71**	4,78	**96**	14,22	**121**	18,98
22	38,56	**47**	16,86	**72**	7,12	**97**	18,95	**122**	16,20
23	4,05	**48**	28,02	**73**	3,29	**98**	21,85	**123**	14,58
24	8,89	**49**	6,50	**74**	3,33	**99**	14,30	**124**	60,85
25	3,72	**50**	24,11	**75**	2,53	**100**	16,96	**125**	74,78
	15,3068		**18,092**		**11,9328**		**26,6528**		**30,484**

Table IV.2: Growth of microfungus according to months

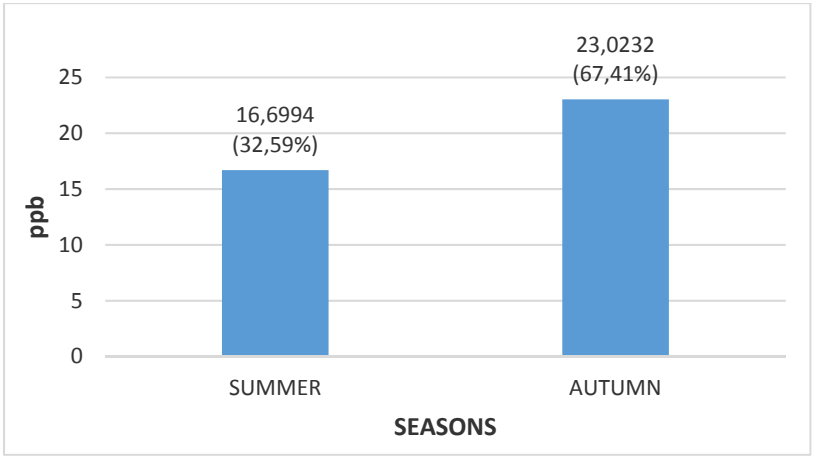
SHOP /MONTHS	**JULY** (1)	**AUGUST**(2)	**SEPTEMBER**(3)	**OCTOBER**(4)	**NOVEMBER**(5)
1	***Mucor***	***A. flavus*** ***A. fumigatus***	No growth	No growth	No growth
2	No growth	No growth	No growth	No growth	No growth
3	***A.fumigatus***	No growth	No growth	No growth	No growth
4	No growth	No growth	No growth	***Aspergillus*** **spp.**	***Rhizomucor*** **spp.**
5	No growth	No growth	No growth	No growth	No growth
6	***Rhizopus***	No growth	No growth	No growth	***Penicillium spp.***
7	No growth	No growth	No growth	No growth	No growth
8	No growth	No growth	No growth	No growth	No growth
9	No growth	No growth	***Mucor***	No growth	No growth
10	***A. flavus***	No growth	No growth	No growth	No growth
11	***Mucor***	***Rhizopus***	No growth	No growth	No growth
12	***Mucor***	No growth	***Rhizopus*** ***A. niger***	No growth	No growth
13	***A.fumigatus***	No growth	No growth	No growth	No growth
14	***A. niger***	No growth	No growth	No growth	No growth
15	***Rhizopus***	No growth	No growth	No growth	No growth
16	***A. niger***	No growth	No growth	No growth	No growth
17	***Rhizopus***	No growth	No growth	No growth	***Penicillium*** **spp.**
18	No growth	No growth	No growth	No growth	No growth
19	No growth	No growth	No growth	No growth	No growth
20	No growth	No growth	No growth	No growth	No growth
21	No growth	***Mucor***	No growth	No growth	No growth
22	***Rhizopus***	No growth	No growth	No growth	No growth
23	No growth	***Rhizopus***	No growth	No growth	No growth
24	***A. flavus***	No growth	No growth	No growth	No growth
25	No growth	No growth	No growth	***Trichoderma*** **spp.**	No growth
TOTAL COUNT	**13**	**5**	**3**	**2**	**3**

Fungus species breeding in July; *Mucor, A. fumigatus, Rhizopus, A. flavus* and *A. niger*. In August; *A. flavus, A. fumigatus, Rhizopus* and *Mucor*. In September, *Mucor, Rhizopus* spp. and *A. niger*. *Aspergillus* spp. and *Trichoderma* spp. the species. In November, *Penicillium* spp. *and Rhizomucor* spp. (Table IV.2). In this study species determination is made but it is suggested to make that determination on moleculer level.

Table IV.3: Average of aflatoxin values according to season and percentage of contaminated limit

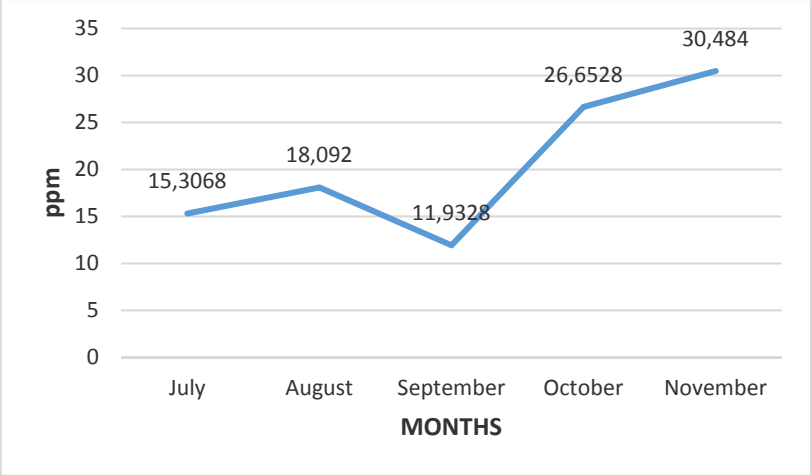
Season	**Sample Number**	**Minimum** (ppb)	**Maximum** (ppb)	**Average** (ppb)	**>10 ppb (n)**	**> 10 ppb (%)**
Summer	50	2,35	54,02	16,7	25	50
Autumn	75	2,53	131,84	23	46	61.3
Total	125	2,35	131,84	**19,85**	71	56,8

It was observed that the minimum aflatoxin level (2.53 ppb) in the Autumn season, and about 2.35 ppb in the Summer season. However, it was determined that the maximum aflatoxin amount (131,84 ppb) observed in the Autumn season was about three times higher than the highest aflatoxin level (54,02) observed in the Summer season. It was also observed that the average total aflatoxin value was 16,7 ppb in Summer and 23 ppb in autumn. In the Summer months, the rate of reproduction above 10 ppb was 50%. The Autumn, the rate of reproduction above 10 ppb was 61,3 (Table IV.3).



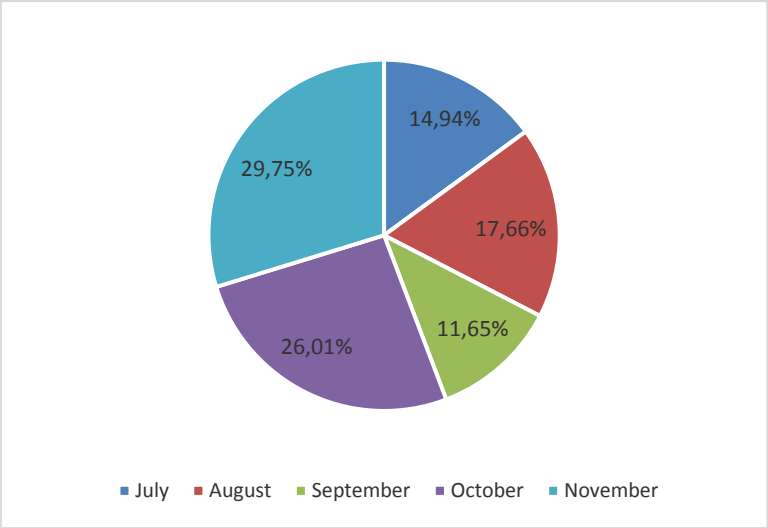
Graph IV.2: Average of aflatoxin levels according to seasons

In July and August, on average 16,7 ppb aflatoxin production was observed and in September, October and November, it was observed that there was a higher growth rate, which is 23,02 ppb. Aflatoxin levels were found to be closer to the acceptable limit in summer months compared to the average of autumn months. However, it was determined that the average of both seasons was not within acceptable limits (Graph IV.2).



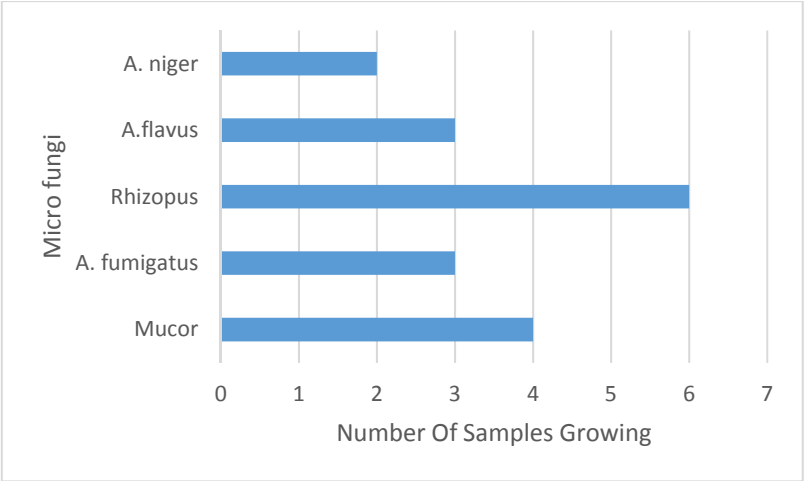
Graph IV.3: Average of aflatoxin levels according to months

The rate of aflatoxin was detected as 15,30 ppb in July and was 18,09 ppb in August. It was observed that the rate of aflatoxin was 11.93 ppb in September and it was the lowest level of growth. The average aflatoxin level was measured as 26.65 ppb in October, which then rose to the highest rate of 30.48 ppb in November (Graph IV.3).



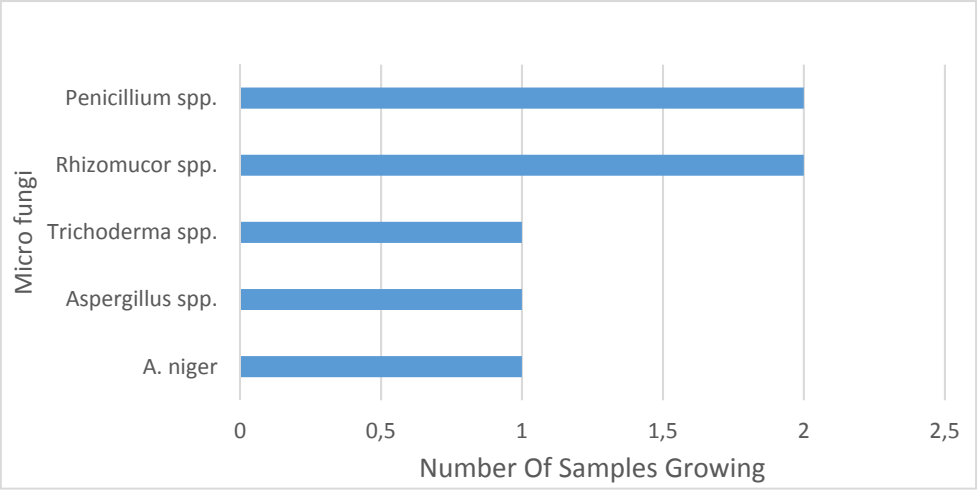
Graph IV.4: Distribution of monthly microbial growth

Out of 125 samples, aflatoxin contaminations were found in 11,65% in September, 14,94% in July, 17,66% in August and 26,01% in October. The highest rate of reproduction was found in November with 29.75% (Graph IV.4).



Graph IV.5: Number of growing samples in Summer

Of the 50 samples collected in two months, 6 (12%) *Rhizopus* spp. production was observed. 4 (8%) *Mucor*, 3 (6%) *A. flavus* and *A. fumigatus* and 2 (4%) *A. niger* were observed. *Rhizopus* spp. has the highest growth rate . *Aspergillus* spp., *Trichoderma* spp., *Rhizomucor* spp. and *Penicillium* spp. were not observed in the summer months which were seen at autumn months. (Graph IV.5).



Graph IV.6: Number of growing samples in Autumn

Of the 75 samples collected in 3 months, 2 (2.67%) *Penicillium* spp. and 2 (2.67%) *Rhizomucor* spp. species. 1 (1.33%) *Trichoderma* spp., 1 (1.33%) *Aspergillus* spp. and 1 (1.33%) *A. niger* were observed. *A. flavus, A. fumigatus* and *Rhizopus* spp. species were not found in Autumn (Graph IV.6).

V. DISCUSSION AND CONCLUSION

Spices are widely used in the world and in our country, especially red pepper is a spice variety which is used in the production phase of the dishes or in addition to appeal to the taste of many people in our society. Widely used, the determination of aflatoxin levels that can be found in spices against the adverse effects of mycotoxins is of great importance in terms of public health. All of the red pepper samples examined in this study were found to be aflatoxin. Of the total number of samples, 56.8% (Summer season: 50%, Autumn season: 61,3%) was above the limits stated in Turkish Food Codex (>10 ppb) (Table IV.3).

In our study it is thought that the maximum amount of aflatoxin observed in the Autumn season (131,84 ppb) is about three times higher than the highest aflatoxin value (54,02 ppb) observed in the Summer season, which might be because of product storage conditions (Table IV.3). It is also possible that in this season when the temperature drops, from the production point, the product which is sold may have been exposed to rain or high humidity by being produced under primitive conditions such as drying in the ground. 5 µg/kg for AFB_1, 10 µg/kg for AFB_1, AFB_2, AFG_1 and AFG_2 have been determined for spices according to the Turkish Food Codex, Communiqué on Spices (Communiqué No: 2013/12). However, in our study, it was observed that the average aflatoxin value was 16,7 ppb in Summer and 23 ppb in Autumn (Graph IV.2).

In the table (Table IV.1), where aflatoxin growth values were determined for all samples taken, aflotoxin growth was found to be lower in the summer months and increased as the autumn season approached. In our study, some low levels of aflatoxin were detected in some samples, eg, 2.35 ppb in sample 6, and some very high levels of aflatoxin were detected, for example in sample 93, 131,84 ppb of aflatoxin was encountered. In this example, it is considered that, even if there is no fungus as a result of sowing, contamination due to environmental conditions may have developed beforehand or that the sample is not homogeneous[54].

In a study, the effect of pepper production zone on the formation of micoflora and aflatoxin in red pepper in Adana, Gaziantep, Şanlıurfa and Kahramanmaraş is investigated and it was found that as the relative humidity increases, the contamination

rate increases and mold development is visible[14]. In our work, it has been observed that, as the humidity increases from summer to autumn season, the reproduction of *A. flavus, A. fumigatus, Rhizopus* spp. reproduction of species has stopped. It has been determined that in summer seasons when the humidity is relatively low, It has been determined that *Trichoderma* spp*., Rhizomucor* spp*., Penicillium* spp. re-production did not occur. This situation explains that *A. flavus, A. fumigatus* and *Rhizopus* spp. type fungi are more suitable for reprecipitation at low moisture contents, and *Trichoderma* spp., *Rhizomucor* spp., *Penicillium* spp. type fungi are more suitable for high humidity.

In another study, 20.6% and 27.2% of the 180 red pepper samples were above legal limits for aflatoxins and AFB_1, respectively[18]. In our study, 56.8% of 125 red pepper samples were above the legal limits for aflatoxins (>10 ppb) (Table IV.3). Again, the highest aflatoxin level was observed in October and the lowest aflatoxin level was found in August. They reported that the red peppers increased aflatoxin and AFB_1 ratios are as a result of being kept in crop fields until October. In our study, the lowest rate of aflatoxin was reported in September, with the highest rates of aflatoxin being similar in October and November (Graph IV.4). The reason for this is the increase in humidity in our country during the Autumn months. The fall in aflatoxin levels in September can be explained by the fact that the monthly product circulation is high, depending on the number of tourists the region is selling.

In a study conducted on various spices, the total number of bacteria and molds were determined. *A. glaucus, A. flavus, A. nidulans, A. niger* and *A. vericolor* species, which are the dominant species of *Aspergillus* that develop in red pepper. In addition, *Mucor pusillus, Penicillium* spp. and *Rhizopus* spp. molds were also in the microflora[14]. In this study we have done; the most dominant species in total *Rhizopus* spp. (12%). *Mucor* (8%), *A. flavus* (6%), *A. fumigatus* (6%) and *A. niger* (5,33%), *Penicillium* spp. (2.67%), *Rhizomucor* spp. (2.67%), *Aspergillus* spp. (1.33%), *Trichoderma* spp. (1.33%), *Penicillium* spp. (2.67%) and *Rhizomucor* spp. (2,67%) were encountered (Graph IV.5, Graph IV.6).

In a study conducted in a hospital environment, it was reported that the number of fungal spores in the air and the seasonal conditions were very effective on fungal concentration. In particular, they found that a very high number of fungi were isolated during the summer season, followed by autumn and winter seasons, respectively. Many

studies in our country have indicated that the same seasonal sequence is observed and that the optimal temperature for fungal growth is 20-24 ºC and the ideal humidity is 50%[55]. In this study, we observed that the highest fungus-isolated season (total 18 breeds in 2 months) was observed to be summer, while in the autumn season a total of 8 breeds were observed in three months (Table IV.2).

Among these molds that can be seen with bare eye, *Aspergillus* spp. *and A. flavus, A. fumigatus* are known to produce aflatoxin. *Aspergillus* species have become one of the most important airborne pathogens in developed countries, playing a role in deadly invasive pulmonary aspergillosis. People with impaired immunity have been reported to have a >50% mortality and >100% mortality in bone marrow transplant patients[56].

Aflatoxins are known to be acute toxic, immunosuppressive, mutagenic, teratogenic and the most powerful carcinogenic compounds known. The target organ for toxicity and carcinogenicity is the liver. Taking all the information about aflatoxins into account, IARC has made a definite decision that this mycotoxin variety is carcinogenic in humans[41]. For this reason, more strict controls and sanctions are required for aflatoxins, which become a threat for health. It is a fact that the humidity rate in our country and the red pepper production areas are very important issues to be considered. There is a need for a control chain that needs to be set up to control the production areas of the crop, planting, harvesting, drying and storage stages until reaching the consumer.

The maker has examined three different drying methods such as outdoor drying, oven drying and microwave drying, and reported that microwave drying was the best method for microorganism load, but they observed that they could not obtain good quality products due to the deterioration in color. Drying in the oven, which is the only method in which the load of the microorganism is within acceptable range and the product color is not deteriorated, is the most appropriate method[57]. For our country, red pepper, which has an important share in exporting, should be replaced with modern drying technologies instead of drying in primitive conditions. Both the manufacturer and the dealer should be careful to store in suitable storage and humidity conditions. Attention should be paid to the traceability of mycotoxins in terms of human health and the country's economy, and the prohibition of food and feed consumption exceeding legal limits in this context.

As a result, since the mycotoxins are widely available in the soil (soil and air) and the toxins they produce are very resistant, precautions of land handling and harvesting are very important measures and should be accomplished according to the HACCP rules. Careful handling of storage conditions at every stage and serious microbial control of the product is required, as well as serious sanctions regarding legal limits.

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Program	Level
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Nutrition Data Systems (BEBIS)	Advanced