An – Najah National University
Faculty of graduate studies

The Presence Of Aflatoxins In Medicinal Plants Used As Natural Remedies And In Spices

By
Wafa Adel Mahmoud Al - Mosleh

Supervisor
Dr. Mohammed Musmar

Co – supervisor
Dr. Samar Ghazal

Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biological Sciences, Faculty of Graduate Studies, at An – Najah National University, Nablus, Palestine.

2002

The Presence Of Aflatoxins In Medicinal Plants Used As Natural Remedies And In Spices

By

Wafa Adel Mahmoud Al –Mosleh

This Thesis was Successfully Defended and Approved on 28th March 2002.

By

Committee Members	Signature
1- Dr. Muhammed Musmar (Supervisor).	.....
2- Dr. Samar Ghazal (Co- supervisor).	.....
3- Dr. Awni Abu- Hijleh (Internal Examiner).	.....
4- Dr. Ziad Al- Shakhshir (External Examiner).	.....

DEDICATION

TO
MY DEAR PARENTS, HUSBAND, BROTHERS, SISTER

SONS, DAUGHTERS FOR THEIR ENCOURAGEMENT

WITH LOVE AND RESPECT

ACKNOWLEDGMENTS

I would like to express my sincere special thanks and gratitude to my supervisor, Dr. Mohammed J. Musmar for his supervision, encouragement, guidance and help throughout this study.

I would like also to express my sincere gratitude to my co – supervisor, Dr Samar Ghazal for her valuable comments and time in reviewing this manuscript.

Special thanks to Dr. Nidal Za'ter and Dr. Awni Abu - Hijleh for their help

Also special thanks for my colleagues Anhar Al– Assali and Nisreen Aslan for their help and encouragement.

Page

TAPLE OF CONTENTS

COMMITTEE DECISION	I
DEDICATION	II
ACKNOWLEDGMENT	III
TABLE OF CONTENTS	IV
LIST OF TABLES	V
LIST OF FIGURES	VI
ABSTRACT IN ENGLISH	VII
CHAPTER ONE: GENERAL INTRODUCTION	
1.1 Medicinal plants	1
1.2 Mycotoxins	1
1.3 Aflatoxins	2
1.3.1 Aflatoxins formation	5
1.3.2 Pathological effect of aflatoxins on humans	9
1.3.3 Example of humans affected by aflatoxins	12
1.3.4 Occurrence	13
1.3.5 Inactivation	13
1.3.6 Impact of aflatoxins on economy	15
1.4 Methods for analysis of aflatoxins in foods and feeds	15
1.5 Objective	16
CHAPTER TWO: MATERIALS AND METHODS	
2.1 Equipments and reagents	18
2.2 Sampling	18
2.3 Preparation of solutions	18
2.4 Recommended analysis method	19
2.4.1 Sample extraction and column separation for the samples that expected to have aflatoxins concentration between 0 and 300 ppb per 0.2 gram sample.	19
2.4.2 Sample extraction and column separation for the samples that expected to have aflatoxins concentration between 0 and 50 ppb per 1.0 gram .	20
2.4.3 Sample extraction and column separation of aflatoxins from the following matrix samples ; coffee, mahaleb, massala, carob, maize, sumac and cloves.	22
2.4.4 Sample extraction and column separation for the samples that expected to have aflatoxins concentration between 0 and 50 ppb per 1.0 gram sample in nuts , almonds and coconuts.	23

2.4.5 Sample extraction and column separation for the samples that expected to have aflatoxins found in soy sauce. 24
2.4.6 Sample extraction and column separation for nutmeg sample that expected to have aflatoxins concentration between 0 and 300 ppb per 0.2 gram sample. 25
2.5 Fluorometeric meaurements 26
29

CHAPTER THREE: RESULTS AND DISCUSSION
CONCLUSION 38
REFERENCES 39
ABSTRACT IN ARABIC 46

LIST OF TABLES

Table	Page
1 .1 Food and Drug Administration (FDA) tolerance levels for total aflatoxin.	11
1 .2 Mycotoxin regulation for foods in some Southern African countries.	12
3 .1 Description of local seed samples tested.	29
3 .2 Description of imported seed samples tested.	30
3 .3 Description of local high fibre samples tested.	31
3 .4 Description of imported high fibre samples tested.	32
3 .5 Description of fruit and sauce samples tested.	33
3 .6 Recent data on the occurrence of aflatoxins in various foods.	35

LIST OF FIGURES

	Page
1.1 Chemical structure of most frequently occurring forms of aflatoxin	4
1.2 Metabolism of aflatoxin B1	7
1.3 The pathway for metabolism and elimination for aflatoxin B1	8
2.1 Schematic representation of aflatest a ffinity column assay	17

Chapter One

Introduction

THE PRESENCE OF AFLATOXINS IN MEDICINAL PLANTS USED AS NATURAL REMEDIES AND IN SPICES

Wafa Adel Mahmoud Al mosleh

Supervisor
Dr. Mohammed J. Musmar

Co - Supervisor
Dr. Samar Ghazal

ABSTRACT

Fifty samples of commonly used remedies and spices were collected randomly from different well known stores at Nablus municipality in a three months period. The collected specimens were evaluated for aflatoxins using VICAM AFLATEST.

The results showed that fourty – eight samples contained the aflatoxins. Thirteen samples were found to contain over 20 ppb, and thus exceeding most permitted international standard limits. Liquorice and sumac contained the highest amounts of aflatoxins 76 ppb. Five samples of famous brand tea samples were analysed. All samples were contaminated with aflatoxins, ranging from 20 – 69 ppb. Commonly used spices like anise, chamomille and nutmeg contained also aflatoxins in the range 23.5 – 36 ppb. Aflatoxins are known to cause liver cancer. The finding that tea and spices were rich in aflatoxins is very alarming specially for children and pregnant women.

Introduction

1.1 Medicinal Plants

Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. The use of traditional medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed. Developing countries, in recent times, are turning to the use of traditional medicinal systems that involve the use of herbal drugs and remedies [1].

Contamination of herbal medicine raw materials with aflatoxins can cause potential carcinogenic effects if taken or consumed even in small amounts. The medicinal plant raw materials carry a great number of molds, often from the soil. Non - professional practices of harvesting, handling and production, cause additional contamination [2]. Economical value of the medicinal plant exportation, as well as frequent use of some herbal preparations in infant, elderly and even normal persons evoked to carry out this research.

1.2 Mycotoxins

Mycotoxins are a mixed bag of acute toxins, secondary metabolites of molds that can be produced on a wide range of commodities and under adverse range of situations due to their high

stability to heat treatment, the presence of mycotoxins in food and feed is potentially hazardous to the health of both humans and animals. They also have a significant impact on economics, by causing losses in farm animals, or by rendering commodities unacceptable in international trade because they do not conform with existing regulations [3]. Mycotoxins, a term derived from (myco) meaning fungus or mold and (toxin) meaning poison, they are usually associated with temperate regions.

These molds can propagate in agricultural plants whenever environmental conditions are favorable. Mycotoxins may remain in food and feed long after the fungus that produced them has expired. Therefore toxins can be present at potentially dangerous levels in products that are not visibly moldy. Diseases caused by mycotoxins in humans and animals are called mycoses [4].

1.3 Aflatoxins

Aflatoxins are secondary metabolites produced by species of *Aspergillus*, especially *Aspergillus flavus* and *Aspergillus parasiticus*, which are found worldwide in air and soil. The discovery of aflatoxin dates back to the year 1960 following the severe out break of the turkey X disease in the UK, which resulted in the deathes of more than 100000

turkeys. The cause was attributed to a feed, containing *Aspergillus flavus* [5,6]. The name aflatoxin, has been formed from the following combination : the first letter, "A" for the genus *Aspergillus,* the next set of the three letters "FLA"for the species *flavus,* and the known "toxin" meaning poison [42]. Aflatoxins are powerful tasteless, odorless and colorless, they are mutagenic, carcinogenic, teratogenic and acutely toxic to most animals and humans, they can cause humans and animals to lose their appetite or cause death. Aflatoxins also inhibit the body's immune system and reduce the effectiveness of vaccines [7,17].The four main aflatoxins are aflatoxin B1,B2,G1 and G2. *Aspergillus flavus* only produces aflatoxins B1 and B2, but *Aspergillus parasiticus* produces these same metabolites along with aflatoxins G1 and G2. When aflatoxin B1 is ingested some transformation occur and secondary aflatoxin M1 and M2 having same acute toxicity as B1 are produced [8,9,19].

The molecular structures of the four most frequently occurring aflatoxins B1,B2,G1 and G2 are shown in figure. 1, along with two common metabolic by products, M1 and M2, which are secreated in the milk of lactating animals that have consumed feed contaminated with aflatoxin [10].

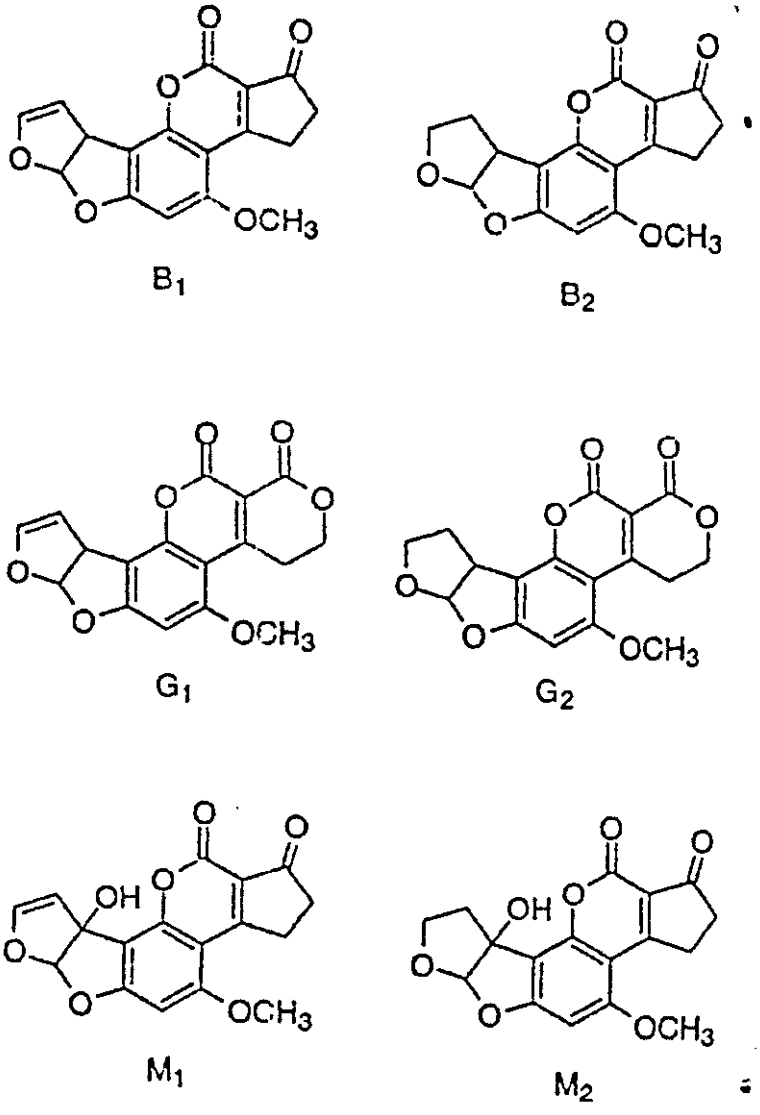

Figure 1.1 Chemical structure of most frequently occurring forms of aflatoxin.

1.3.1 Aflatoxins formation

The first step in the biosynthetic pathway of aflatoxin is the condensing of acetate units to form norsolorinic acid. Sixteen enzymes have been estimated to be involved in the bioconversion of norsolorinic acid to aflatoxins [11].

Versicolorin A is of importance because it is the first molecule in the aflatoxin B1 (AFB1) pathway that contains a double bond. This double bond is the target for highly reactive epoxide resulting in activation and adduct formation with DNA. In contrast, aflatoxin B2, which does not have double bond is hundreds of times less carcinogenic [12]. AFB1 alone is not carcinogenic, but is metabolized by the body to produce an ultimate carcinogenic metabolite, AFB1 is oxidized by human cytochrome P450 enzymes to several products. Only one of these , the (AFB1 – 8,9 – epoxide) appears to be mutagenic and the others are detoxication products. P450 3A4, which can both activate and detoxicate AFB1, is found in the liver and small intestine. AFB1-8,9 epoxide, is formed by oxidation of the 8,9 vinyl ether bond. When the AFB1molecule has been transported across the plasma membrane, it is activated by microsomal mixed - function monoxygenase which then reacts with the N – 7 atom of B – DNA guanine this reaction is not random, but preferentially occurs in certain G-C base – pair clusters[13],

inducing almost exclusively G-T substitutions. Abnormalities of the structure and expression of a putative tumor – suppresser gene p^{53}, are frequent in hepatocellular carcinoma. Some investigators have therefore suggested that p^{53} mutations caused by aflatxin B1.

Conjugation of aflatxin B- 8,8 epoxide, a phase II metabolic reaction is an important detoxification route for the reactive epoxside. Although aflatxin B- 8,9 epoxide can be hydrolysed to the diol by epoxide hydrolase, the doil product is toxic, since it reacts readily with proteins by Schiff-base formation or binds to DNA. Gluathione conjugation prevents the toxicity of both the epoxide and its hydrolysis product. This conjugation reaction is catalysed by cytosolic glutathione S-transferases. The aflatoxin – glutathione conjugate is subsequently excreted from the hepatocyte into bile as a major biliary metabolite [13,29,39]. Figure (1.2) shows metabolism of aflatoxin B1and figure1.3 shows the pathway for metabolism and elimination for aflatoxin B1.

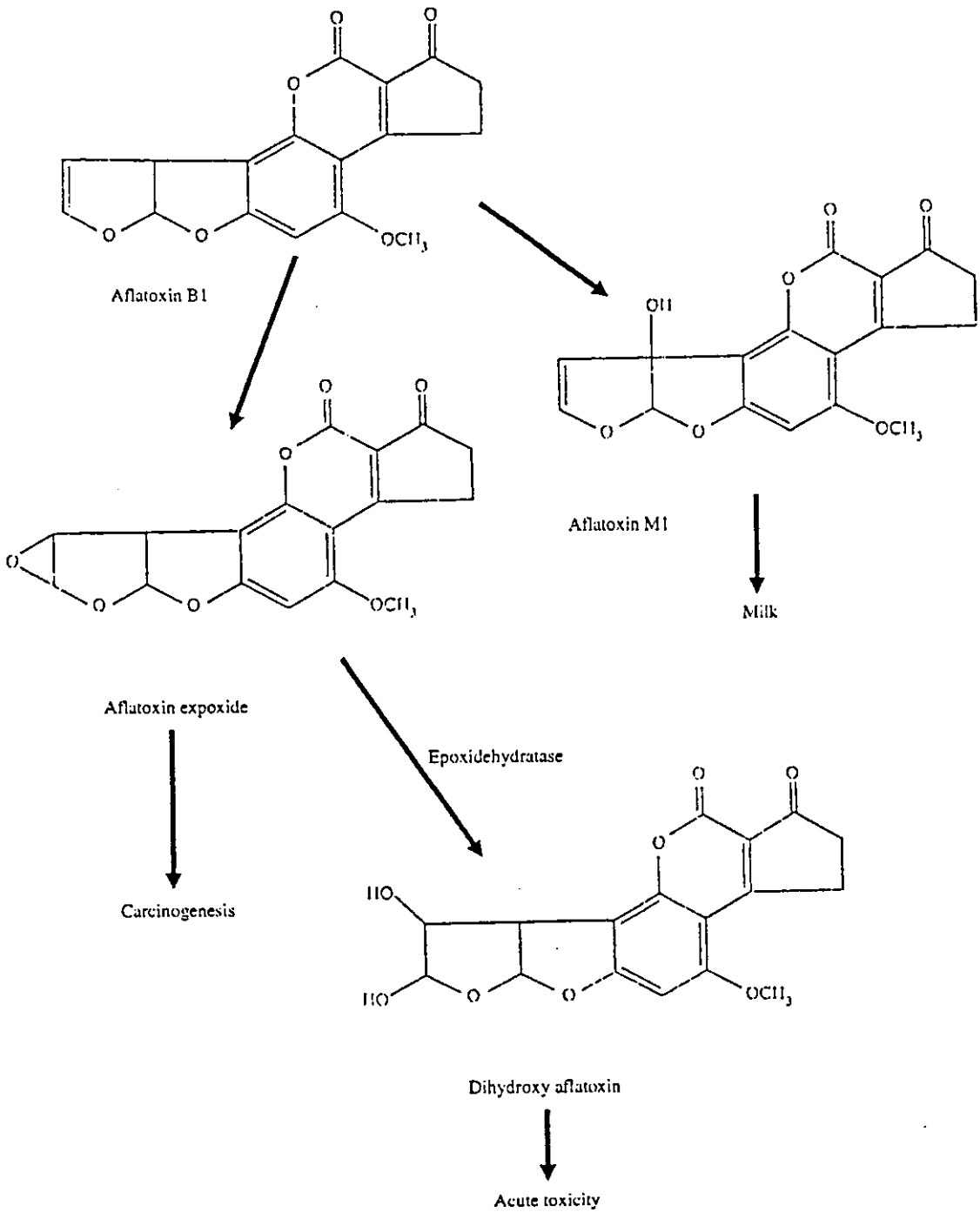

Figure 1.2 metabolism of aflatoxin B_1

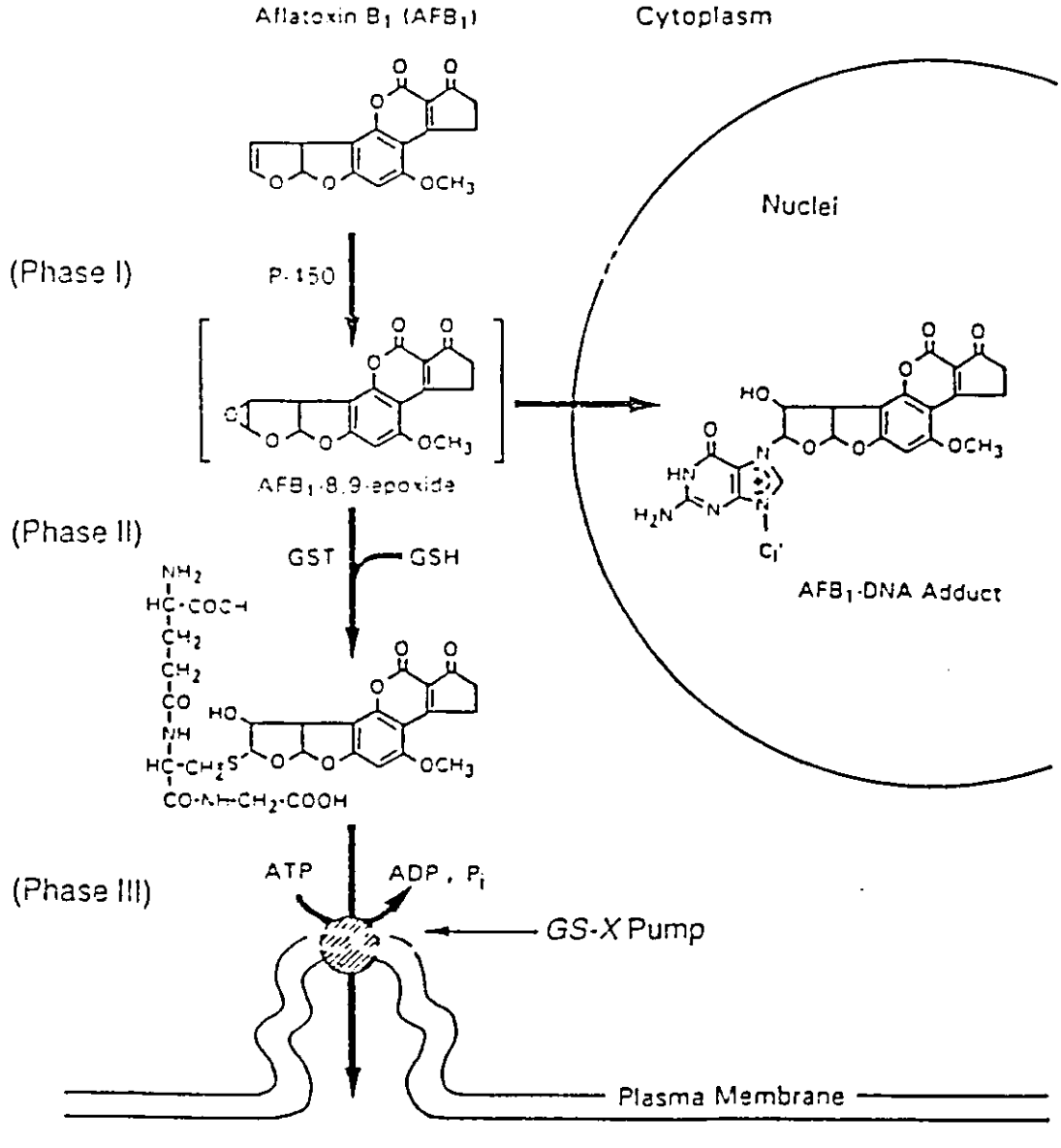

Figure 1.3 shows the pathway for metabolism and elimination for aflatoxin B1

1.3.2 Pathological effects of aflatoxins on humans

Humans are exposed to aflatoxins by ingesting food contaminated with products of fungal growth. It is difficult to prevent fungal growth in food. Aflatoxins bind to DNA disrupting genetic coding thus promoting tumorgenicity. The incidence of liver cancer in Africa shows a distinctive geographical distribution similar to that of aflatoxin contamination of food. Evidence of acute aflatoxicosis have been found in humans , in third world countries like Taiwan and many others. Symptoms include vomiting, pulmonary edema, abdominal pain, coma, convulsion and death.

The conditions that increase the likelihood of this disease Include limited availability of food, environmental conditions that favor fungal development in crops and lack of regularity systems for aflatoxin monitoring and control. Because aflatoxins are potent carcinogens, there has been an increase concern or long–term exposure to low levels of these mycotoxins on humans. Ingestion of large amount of toxins in short period of time causes acute toxicity leading to death while small doses in prolonged length of time will result in chronic effects [14, 15, 16, 33,22].

The result of epidemiological studies carried out in Kenya, and Thailand shows a significant correlation between the incidence of liver cancer and exposure to aflatoxins . As a result of these and other studies the International Agency for Research on cancer have recently stated that there is now sufficient evidence to classify aflatoxins as human carcinogens [26,27,28,29].

Studies in several African countries showed a correlation between the logarithm of aflatoxins intake and the occurance of primary human liver cancer. Aflatoxin B1 is cosnidered to be the most potent liver carcinogen known. The metabolite of aflatoxin B1 (aflatoxin M1) was less potent than aflatoxin B1, but also considered carcinogens [18].

Aflatoxin possess a significant hazard to humans. It's presence in food and feed should be restricted to a minimal level. Food and Drug Administration (FDA) of the USA, proposed a tolerance level of 30 μg/kg of total aflatoxins (B1+B2+G1+G2). Then the level was lowered to 20 μg/kg. The current tolerance levels established by the (FDA) for food and feed are listed in (Table 1.1) [10,20,21,34,35].

Table 1.1 Food and Drug Administration (FDA) tolerance levels for total aflatoxin [36]

Item	Tolerance level (μg/kg)
Food for human consumption	20
Feed for cattle and poultry	300
Feed for swine	200
Feed for breeding livestock	100
Milk	0.5

μg/kg: ppb

Different countries have different regulations for aflatoxin. Industrialized countries usually set lower tolerance levels than the developing countries, where most of the susceptible commodities are produced. For example, the tolerance level for aflatoxin in foods is 5 μg/kg in Sweden, and is 30 μg/kg in Brazil [23,32]. (Table 1.2) shows maximum permitted level of mycotoxins for food in some Southern African countries[15].

Table 1.2 Mycotoxin regulations for foods in some Southern African Countries[15]

Country	Authority institution	Food item	Maximum permissible level (ppb)	Mycotoxin
Zimbabwe	Ministry of Agriculture	Peanut	20	AFB1
		All foods	20	AFB1
Nigeria	Food and Drug Administration	Food stuffs	50	Aflatoxin
		Fluid milk	1	Aflatoxin
		Infant foods	0	Aflatoxin

1.3.3 Examples of humans affected by aflatoxins

Example of humans affected is the intake of aflatoxins in Sierra leone. The infants were exposed to aflatoxins through the weaning of food or diet of the family or through breast milk which is highly contaminated . Another example on humans affected by aflatoxin, when an out break of aflatoxicosis in India was linked to moldy corn containing aflatoxin, killing more than 100 persons. Aflatoxin has been found in the tissues of children suffering from Reyes Syndrome in the orient and in colon cancer lesions [24,25].

1.3.4 Occurrence

The incidence of aflatoxins in food and feeds is relatively high in tropical and subtropical regions, where weather provides optimal conditions for the growth of the molds. The growth of *A.flavus* and production of aflatoxin in natural substrates are influenced by number of factors. High temperature, long drought conditions and high activity of insects influence pre-harvest of aflatoxin on peanuts and corn Post-harvest contamination can occur if crop drying is delayed. Aflatoxins are detected occasionally in nuts, almonds, spices and variety of other foods and feeds [30,31,32].

1.3.5 Inactivation

The awarness of aflatoxin as a potent source of health hazards to both man and farm animals, efforts has been made to completely eliminate the toxin or reduce its content in foods and feed stuffs to significantly lower levels. Pre–harvest contamination can be reduced by appropriate cultural practices that limit the growth of aflatoxigenic fungi. Post–harvest contamination can be minimized by application of proper drying, storage procedures. Methods have been investigated to inactivate aflatoxins, these methods aim at either the removal of the toxin or destroying it in the food. They can be classified into chemical, biological and physical methods.

A large number of chemicals can react with aflatoxins and convert them to less toxic and mutagenic compounds. These chemicals include acids, bases, oxidizing agents, bisulphites and gases. However, most of the chemical processes that have been investigated are impractical (carried out under drastic conditions of temperature and pressure), unsafe (form toxic residues) and unfavourable (degrade the nutritional, sensory and functional properties of the product). Currently, ammoniation and treatment with sodium bisulphite are the major industrial processes widely used to inactivate peanut meal, maize and cottonseed destined for animal feeding.

Many microorganisms including bacteria and acid-producing molds can metabolize and inactivate aflatoxins, with *Flavobacterium aurantiacum* as the most active organism. It was postulated that the inactivation was a result of acid production and subsequent conversion of AFB1 to AFB2a, which is 1000 times less mutagenic than the parent toxin.

Inactivation by physical methods involves extraction with solvents, adsorption, inactivation by heat and irradiation [36].

1.3.6 Impact of aflatoxins on economy

Aflatoxin can affect economy through animals because animals can eat the crops contaminated with aflatoxins and get sick, therefore reducing farming production. The Food and Agricultural Organization (FAO) estimates that 25% of the worlds food crops are affected by mycotoxins, of which the most notorious are aflatoxins, other adverse economic effects of aflatoxins include lower yields of food and fibre crops[21,38].

1.4 Methods for analysis of aflatoxins in food and feed

There are currently several different accepted measurement techniques available for determining aflatoxin levels. In general most measurement techniques require three steps : extraction to remove the aflatoxin from complex mixtures of materials in which it is found; purification to remove interference and finally detection and quantification. The more traditional techniques used to purify aflatoxins before presenting it to a detector for quantification utilize a form of chromatography, a main stay of analytical chemistry. Examples include, thin layer chromatography (TLC), gas chromatography (GC), and high performance liquid chromatography (HPLC).

Some more modern methods of aflatoxin extraction, purification relay on immunochemical techniques. These techniques utilize aflatoxin-specific antibodies to efficiently extract and purify aflatoxin from complex mixtures. There are a number of immunochemical based assays, that are used for detecting aflatoxin radioimmunoassay (RIA), enzyme–linked immunosorbent assay (ELISA), and immunoaffinity column assay (ICA) [10,38].

1.5 Objective

Since no previous studies on aflatoxin levels on medicinal plants were carried out in Palestine, the current purpose of the study was to investigate the possible contamination of several medicinal plants, and spices with these dangerous substances.

Chapter Two

Material and Methods

Methodology

The method which is carried out in this research based on the immunoaffinity column assay (ICA). The test is called aflatest which is a quantitative method, with no need to use toxic solvents, easy to perform and highly accurate aflatoxin analysis can prevent contamination [43]. The principle of separation technique of aflatoxins is based on the separation of aflatoxins from the sample solution using aflatest immunoaffinity column. The separation is a result of an interaction between aflatoxin molecule and a specific type of monoclonal antibody attached chemically to solid support surface and protected in a special solution during storage, as shown in figure 2.1.

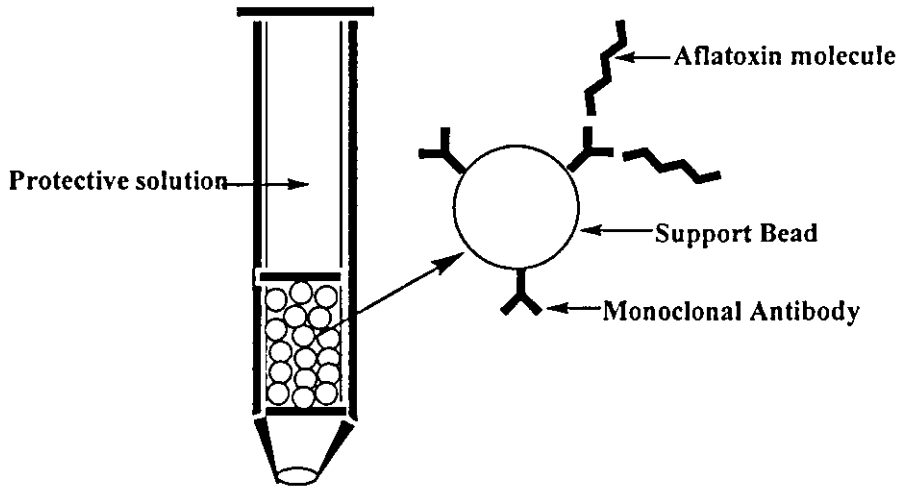

Figure 2.1 : Schematic representations of aflatest affinity column assay.

Experimental :

2.1 Equipments and reagents;

All aflatest measurements were carried out using fluorometer, (model 2-K compliant VICAM). Aflatoxins were separated by using aflatest-P-columns, VICAM fluted filter paper, micro fiber filter (1.5mm, 11cm) and aflatest developer of 0.03% bromine solution, which are supplied by VICAM for Science Technology (Somerville, MA, USA). All aflatest solutions were prepared from methanol and water as HPLC grade purechased from Froturam. Tween 20 solution (15%) was obtained as an analytical grade reagent. All used glassware were washed well with detergents, then with a solution of methanol and water (10:90).

2.2 Sampling

Different fifty medicinal plant samples were collected randomly from Nablus markets.

2.3 Preparation of solutions

A freshly prepared aflatest developer solution is prepared by diluting 5.0 ml of concentrated aflatest developer solution to 50.0 ml using HPLC grad water, which was kept in a tightly capped bottle

when it was not used. this aflatest solution was not used after 8 hours from the time of its preparation.

2.4 Recommended analysis method

The determination of aflatoxins in real plant samples has to follow many steps of sample extraction, separation by column chromotography and spectroscopy determination by fluorometer. There are some differences in the details in preforming the first two steps of sample extraction and column separation, which depend on the type and the part of the plant sample, also on the expected concentration level of the aflatoxin in the sample. While the final step in analysis by fluorometer is the same for all samples regardless to the nature of the plant sample.

2.4.1 Sample extraction and column separation for the samples that are expected to have aflatoxins concentration between 0 and 300 ppb per 0.2 gram sample, found in the following samples; tea, chamomille, garlic, roses, laurel, galangal, margoram, qadam assad, ginger, sage, thyme liquorices and cardamom are performed as follows;

50.0 g sample and 10 g sodium chloride salt were placed in blender jar. and 200 ml of a mixture of methanol and water (80:20)were added to it .The mixture was blended at high speed for one minute. The extract was poured from the blender jar into fluted filter paper, and collecting the filtrate in a clean vessel. 10.0 ml of this extract was

diluted up to 50.0 ml using water, and mixed well. Finally, the diluted extract was filtered through microfiber glass filter directly into the barrel of a glass syringe to measure exactly 4.0 ml.

The aflatoxins were separated from the sample matrix using column chromatography; by passing 4.0 ml (equivalent 4.0 ml per 0.2 g sample) of the extracted sample, were passed and collected in the glass syringe, through aflatest-P-affinity column at a rate of about 1-2 drops per second until air comes through the column. Then the loaded column was washed by passing 5.0 ml of water through the column at a rate of about 2 drops per second. By repeating the washing one more time by another 5.0 ml of water, until air comes through the column. Finally, the aflatoxins in the affinity column were eluted by passing 1.0 ml of HPLC grade methanol at a rate of 1-2 drops per second. Then all of the eluate of 1.0 ml sample was collected in a glass cuvette.

2.4.2 Sample extraction and column separation for the samples that are expected to have aflatoxins concentration between 0 and 50 ppb per 1.0 gram sample, found in the following samples: chilli pepper, caraway, fennel, cumin, anise, rice seasonings, leroal bean, grains, dill seeds, spinach, chinese seasoning, parsely, daket mamoul, melon seeds, fenugreek and white pepper, are performed as follows;

25.0 g sample and 5 g sodium chloride salt were placed in blender jar and 200 ml of a mixture of methanol and water (80:20) were added. The mixture was blended at high speed for one minute. The

extract was poured from the blender jar into fluted filter paper, and collecting the filtrate in a clean vessel. Immediately, diluting 10.0 ml of this extract up to 50.0 ml by water, and mixed well. Finally, filter the diluted extract through microfiber glass filter directly into the barrel of a glass syringe to measure exactly 8.0 ml.

The aflatoxins were separated from the sample matrix using column chromatography; by passing 8.0 ml (equivalent 8.0 ml per 0.2 g sample) of the extracted sample, collected in the glass syringe, through aflatest-P-affinity column at a rate of about 1-2 drops per second until air comes through the column. Then the loaded column was washed by passing 10.0 ml of water through the column at a rate of about 2 drops per second. By repeating the washing one more time by another 10.0 ml of water, until air comes through the column. Finally, the aflatoxins in the affinity column was eluted by passing 1.0 ml of HPLC grade methanol at a rate of 1-2 drops per second. Then the eluate of 1.0 ml samplewas collected in a glass cuvette.

Note: For chillipepper and white pepper, sample extraction was done in 100 ml of methanol and water mixture instead of 200 ml. Also,4.0 ml of filter extract was passed through the column instead of 8.0 ml.

2.4.3 Sample extraction and column separation of aflatoxins From the following matrix samples; coffee, mahaleb, massala, carob, maize, sumac and cloves, are performed as follows;

50.0 g sample and 5 g sodium chloride salt were placed in blender jar, 100 ml of a mixture of methanol and water (80:20) were added . The mixture was blended at high speed for one minute. The extract was poured from the blender jar into fluted filter paper, and collecting the filtrate in a clean vessel. 10.0 ml of this extract was diluted up to 30.0 ml by water, and mixed well. The diluted extract was filtered through microfiber glass filter directly into the barrel of a glass syringe to measure exactly 4.0 ml.

The aflatoxins were separated from the sample matrix using column chromatography; by passing all the 1.0 ml (equivalent 1.0 ml per 0.167 g sample) of the extracted sample, collected in the glass syringe, through aflatest-P- affinity column at a rate of about 1-2 drops per second until air comes through the column. Then the loaded column was washed by passing 1.0 ml of water through the column at a rate of about 2 drops per second. By repeating the washing one more time by another 1.0 ml water, until air comes through the column. Finally, the aflatoxins in the affinity column were eluted by passing 1.0 ml of HPLC grade methanol at a rate of 1-2 drops per

second. Then the eluate of 1.0 ml sample was collected in a glass cuvette.

2.4.4 Sample extraction and column separation for the samples that are expected to have aflatoxins concentration between 0 and 50 ppb per 1.0 gram sample, found in the following samples; nuts, almonds and coconuts, are performed as follows;

25.0 g sample and 5 g sodium chloride salt were placed in blender jar, 125 ml of a mixture of methanol and water (60:40) were added . The mixture was blended at high speed for one minute, the extract from the blender jar was poured into fluted filter paper, the filtrate was collected in a clean vessel. Immediately, dilute 20.0 ml of this extract was diluted up to 40.0 ml by water, and mixed well. The diluted extract was filtered through microfiber glass filter directly into the barrel of a glass syringe to measure exactly 10.0 ml.

The aflatoxins were separated from the sample matrix using column chromatography ;by passing all the 10.0 ml (equivalent 10.0 ml per 1.0 sample) of the extracted sample, collected in the glass syringe, through aflatest-P-affinity column at a rate of about 1-2 drops per second until air comes through the column. The loaded column was washed by passing 10.0 ml of water through the column at a rate of about 2 drops per second. By repeating the washing one more time by

another 10.0 ml of water, until air comes through the column. The aflatoxins were eluted in the affinity column by passing 1.0 ml of HPLC grade methanol at a rate of 1-2 drops per second. The eluate of 1.0 ml sample was collected in a glass cuvette.

2.4.5 Sample extraction and column separation for the samples that are expected to have aflatoxins concentration between 0 and 300 ppb per 0.2 gram sample, found in soy sauce, are performed as follows;

20.0 g sample and 2 g sodium chloride salt were placed in blender jar, 80 ml of pure methanol (100%) was added .Tthe mixture was blended at high speed for one minute. The extract from the blender jar was poured into fluted filter paper, the filtrate was collected in a clean vessel. 10.0 ml of this extract was diluted up to 50.0 ml by water, and mixed well. Finally, the diluted extract was filtered through microfiber glass filter directly into the barrel of a glass syringe to measure exactly 4.0 ml.

The aflatoxins were separated from the sample matrix using column chromatography; by passing all the 4.0 ml (equivalent 4.0 ml per 0.2 g sample) of the extracted sample, collected in the glass syringe, through aflatest-P-affinity column at a rate of about 1-2 drops per second until air comes through the column. The loaded column was washed by passing 10.0 ml of methanol and water mixture (20:80)

through the column at a rate of about 2 drops per second. By repeating the washing one more time by another 10.0 ml of the methanol and water mixture, until air comes through the column. Finally, the aflatoxins were eluted in the affinity column by passing 1.0 ml of HPLC grade methanol at a rate of 1-2 drops per second ,then the eluate of 1.0 ml sample was collected in a glass cuvette.

2.4.6 Sample extraction and column separation for nutmeg sample that is expected to have aflatoxins concentration between 0 and 300 ppb per 0.2 gram sample, are performed as follows;

25.0 g sample and 5 g sodium chloride salt were placed in blender jar, 100 ml of a mixture of methanol and water (80:20) were added . The mixture was blended at high speed for one minute. The extract from the blender jar was poured into fluted filter paper, and collecting the filtrate in a clean vessel. 5.0 ml of this extract was diluted up to 25.0 ml by Tween-20 solution (15%), and mixed well. Then, the diluted extract was filtered through microfiber glass filter directly into the barrel of a glass syringe to measure exactly 4.0 ml.

Note that Tween 20 was used as a surfactant to reduce nonspecific binding of other substances could be present at high level beside aflatoxins in this kind of sample of nutmeg with the

immunoaffinity column, which causes a potential interference during the separation.

The aflatoxins were separated from the sample matrix using column chromatography; by passing all the 4.0 ml (equivalent 4.0 ml per 0.2 g sample).of the extracted sample collected in the glass syringe, through aflatest-P-affinity column at a rate of about 1-2 drops per second until air comes through the column. Then the loaded column was washed by passing 10.0 ml of water through the column at a rate of about 2 drops per second. The washing was repeated one more time by another 10.0 ml of water, until air comes through the column. Finally, the aflatoxins in the affinity column was eluted by passing 1.0 ml of HPLC grade methanol at a rate of 1-2 drops per second 1.0 ml of the eluate was collected in a glass cuvette.

2.5 **Fluorometeric measurements :**

Specterofluorometer is used as fluorometric measurment to detect and measure the concentration of aflatoxins. It is based on the measurement of fluorescence " photoluminescence" comes out from the excited chemical molecule as a result of the absorption of radiant energy, which is called photoexcitation.

The intensity of emitted fluorescence is directly proportional to the concentration of excited chemical molecules. It is well known that chemical molecule which is weakly absorbing excitation energy and does not emit enough fluorescence intensity, usually a chemical method is applied to treat this chemical molecule and so introduce to it anew chemical functional group, so that it becomes highly fluorescent product. Using this method makes this technique applicable for trace quantities measurement. Since chemical molecule of aflatoxins does not have high fluorescence intensity, so it is difficult to detect aflatoxins at trace quantities. In order to overcome this limitation, a new chemical functional group is added chemically to this molecule to convert it to highly fluorescence molecule. Since chemical attachment of halogen group to aromatic hydrocarbon molecule makes it highly fluorescence, therefore, the reaction of bromine with aflatoxins converts the aflatoxins molecules to a highly fluorescence molecule.

Overall , the main advantage of fluorometric analysis over others spectrometry methods is due to its high sensitivity to very low concentration down to 10^{-9} M. Which makes fluorometric technique very useful and applicable for trace measurement of aflatoxins.

The detection of aflatoxins concentration by this used VICAM-fluorometer is designed to readout directly the concentration of aflatoxins in the solid sample in ppb. Since the concentration of aflatoxins in the final tested solution is not related to a constituent amount of the solid samples and changed according to the equivalent weight of solid sample in origin constant volume, therefore, a calibration of the fluorometer is required before each testing to different consistent samples.

The calibration is done by using standard reference samples, in which the digital display readout should be adjusted to a desired value for each standard reference sample according to the equivalent weight of the tested solid sample, the steps of calibration is explained in details by VICAM operator's manual.

After column separation to aflatoxins in each of the samples, the fluorometeric measurements to aflatoxins concentration was done using the same procedure for all samples. In which, the collected eluate of 1.0 ml of aflatoxin solution in methanol in glass cuvette was mixed with 1.0 ml of aflatoxin developer. Then, after 60 seconds the cuvette was placed in a calibrated fluorometer. The concentration of aflatoxin was found directly from the reading of the calibrated fluorometer.

Chapter Three

Results and Discussion

Result and Discussion

Fifty medicinal plant samples were examined. Contamination of the examined samples with aflatoxin is shown in the following tables .

Table 3.1 Description of local plant seed samples tested.

English name	Scientific name	Part of plant used	No of samples	Concentration of aflatoxin (ppb)
Fennel	*Foeniculum vulgare*	Dry seeds	2	1.31 , 0.99
Spinach	*Spinacea - oleracea*	Dry seeds	1	0.65
Maize	*Zeamays*	Dry seeds	1	0.51
Fenugreek	*Trigonella foenum graecum*	Dry seeds	1	0.65
Garlic	*Allium sativum*	Dry seeds	1	0.11
Anise	*Pimtinella anisum*	Dry seeds	1	0.31
Caraway	*Carum carvi*	Dry seeds	1	1.20
Parsely	*Petroselinum sativum*	Dry seeds	1	0.00
Melon seeds	*Cucumis melo var*	Dry seeds	1	0.07
Grains (wheat)	*Triticum sativum*	Miller dry seeds	1	0.71
Leroal bean	*Vicia faba*	Dry seeds	1	0.77

Table (3.2) Description of imported seed samples tested

English name	Scientific name	Part of plant tested	No of samples	Concentration of aflatoxin (ppb)
Coffee	*Coffee arabica*	Seeds	1	0.32
Walnut	*Juglans regia*	Dry seeds	1	2.71
Mahaleb	*Cerasus mahaleb*	Dry seeds	1	0.13
Cloves	*Eugenia maricima*	Dry seeds	1	0.00
Chinese seasonings		Mixed dry seeds	1	2.40
Rice seasonings		Mixed dry seeds	1	0.35
Daket mamoul		Mixed dry seeds	1	2.51
Massala		Mixed dry seeds	1	0.38
Nutmeg	*Myristica fragrans*	Dry seeds	1	0.36
White pepper	*Piper album*	Dry seeds	1	1.20
Almond	*Amygdalus communis*	Dry seeds	1	0.56
Cumin	*Cuminum cyminum*	Dry seeds	1	1.83
Walnut	*Inglans regia*	Dry seeds	1	6.54
Cardamom	*Elettaria cardamomum*	Dry seeds	1	1.82
Sumac	*Rhus cotinus*	Dry seeds	١	0.٧٦

Table (3.3) Describtion of local high fiber plant samples tested.

English name	Scientific name	Part of plant tested	No of samples	Concentration of aflatoxin (ppb)
Roses	*Rosa caninal*	Dry flowers	1	9.60
Sage	*Salvia officinalis*	Dry leaves	1	4.22
Chamomille	*Matricaria chamomilla*	Dry flowers	2	11.0 36.0
Qadam Assad	*Alchemilla vulgars*	Leaves & stem	1	3.41
Thyme	*Thymus vulgaris*	Dry leaves	1	3.23

Table (3.4) Description of imported high fiber plant samples.

English name	Scientific name	Part of plant used	No of samples	Concentration of aflatoxin (ppb)
Ginger	*Zingiber officinale*	Rhizome	1	7.50
Dill seeds	*Anethum graveolens*	Stems	1	0.41
Majorana	*Magorana hortensis*	Dry leaves	1	0.25
Liquorice	*Glycyrrhiza glabra*	Roots	2	0.76 0.70
Tea brand I	*Thea sinesis*	Dry leaves	1	0.46
Tea brand II	*Thea sinesis*	Dry leaves	1	0.25
Tea brand III	*Thea sinesis*	Dry leaves	1	0.32
Tea brand IV	*Thea sinesis*	Dry leaves	1	0.69
Tea brand V	*Thea sinesis*	Dry leaves	1	0.55
Galangal	*Kaempseria–galangal*	Dry stem	1	4.90
Laurel	*Laurs nobilis*	Dry leaves	2	0.10 0.20

Table (3.5) Description of fruit and sauce plant samples.

English name	Scientific name	Part of plant used	No of samples	Concentration of aflatoxin (ppb)
Carob	*Ceratonia siliqua*	Dry fruit	1	0.42
Coconut	*Cocos nucifera*	Fruits	1	5.00
Chilli pepper	*Capsicum annum*	Dry fruits	1	6.51
Soy sauce	*Glycin soya*	Sauce	1	0.11

As seen from the tables the concentration of aflatoxin in medicinal plants ranged from 0 to 76 ppb (part per billion). In twelve medicinal plant and spices samples the quantity of aflatoxin was higher than 20 ppb, thus exceeds the permitted levels for human consumption. The highest incidence of aflatoxin contamination was detected in liquorice and sumac. Both liquorice root powder and sumac seed powder contained 76 ppb,a second liquorice sample contained 70 ppb .Five brand of tea samples were tested. The five samples were contaminated with aflatoxin, at the levels between 25 to 69 ppb. Also spices showed high incidence of aflatoxin contamination as nutmeg and rice seasonings at levels of 36 ppb and 35 ppb respectively, while two chamomille samples, one with concentration of 36 ppb and the other

sample contain 11ppb. One anise sample was found contaminated with aflatoxin at level of 31 ppb.

Commodities with a lower risk of aflatoxin contamination include soysause, two laurel samples, mahaleb, rosses, ginger coconut, sage, qadam assad , chillipepper, walnuts galangal,and thyme, were contaminated by aflatoxin at lower levels between 3.2 – 20 ppb .

Twenty - one samples were found to contain lower residues of aflatoxin with levels ranging from 0.07- 2.50 ppb, these samples are Chinese seasonings, marjoram, dill seeds, daket mamoul, massala, white pepper, melon seeds, grains (wheat) almond, cumin, leroal bean, cardomom, fennel, coffee, spinach, maize, fenugreek, garlic and caraway.

Finally cloves and parsely seeds were found to be free from aflatoxin at level of Zero ppb.

Many researchers studied the problem of medicinal plants contaminated with mycotoxins. For example, Roy et al examined the incidence of aflatoxin on some medicinal plants obtained from India. Out of fifteen samples analyzed, fourteen gave positive results {45}. In

a different study Aziz found that chamomille, fennel, and ginger were contaminated with aflatoxin B1, at a levels of 145µg/kg, 160µg/kg, 10µg/kg {46}.

Rani and Singh (1990) found that fennel and cumin were contaminated with aflatoxin B1 at levels of 3000 ppb and 1580 ppb {47}. (Table 3.6) shows some recent data on the occurrence of aflatoxins in foods in various countries.

Table 3.6 Recent data on the occurrence of aflatoxins in various foods

Commodity	Country	No. of sample	Range µg/kg
Almonds	USA	44	Trace –372
Soybeans	Argentina	94	1 – 36*
Wheat	Uruguay	123	2 – 20
Nutmeg	Japan	67	0.2 – 16.6
Chillies	Pakistan	176	1 –79.9*

* Determination of aflatoxin B1 only.

Although the relatives risk of adverse health effects from aflatoxins due solely to spice consumption seems small when compared with other food products, the number of foods containing spices is

increasing and also the intake of these kinds of foods is daily increasing [3].

In this study the differences in the level of concentration of aflatoxin, result from analysis of various plants, originating from countries of specific climate conditions. Mycotoxins are produced by fungi on plants in the field before harvest or later after harvest during long storage under favourable conditions [46]. The vegetable drugs which are harvested and preserved in unsuitable conditions may be infected by fungi and contaminated with mycotoxins being injurious to human health[55].

Contamination of medicinal plants and spices with aflatoxins is a special hazard. Since aflatoxins is extremely thermo stable, as shown in many studies [49,50,51,52]. In addition aflatoxins are thermo stable compounds and they stand up to the boiling point of water. The absorption of the toxins from the intestine is almost complete, then the mycotoxins are activated by metabolic enzymes to the epoxide which is able to alkylate the DNA material. By this way very small quantities of aflatoxin could be accumulate in the human system. It is known that aflatoxins accumulates in the liver [5,53,54]. Therefore, it is essential to avoid contaminated food such as plants or dairy products. Plant

material used as spices and condiments or used in falk medicine, should be carefully stored and the growth of naturally found toxic fungi should be inhibited.

Finally, based on this investigation, it may be concluded that the contamination of herbal drugs and spices with aflatoxin is alarming, and such products need thorough inspection before being channeled to the drug and food industries.

Conclusion

1 – This study showed that aflatoxins are common pollutants in several food products.

2 – Because of the limitations of the budget we were not able to increase our study to other food products specially milk and baby food.

3 – Our research is also important to veterinary medicine as cattle food may be contaminated with aflatoxins and therefore dairy products.

4 – We need to draw attention that natural products are not always safe as many people believe. Therefore, we need to evaluate all products used in folk medicine for aflatoxins. Some natural products are even used for infants as anticolic drugs.

5- Because of alarming results, we like to draw the attention of Health Ministry & Standard Palestinian Institution for these results.

References

1-Unesco (1996). Culture and health, Orientation text – world Decade for cultural Development 1988 – 1997, Document CL/DE/PRO- 1996 , Paris, France,129.

2-Schilcher, H., Peters, H., &Wank, H. (1987). Pestiszide & Schmermretalle in Arzeipmanzen & Arzneiplanzen Zubereitungen. Pharm. Ind., 49,202 -211.

3-Pittet, A. (1998). Natural occurrence of mycotoxins in foods – an up dated review. Revue De Medicine Veterinary, 149,6,479-492.

4- http://exp.interspeed.net/flair/ffe16395.htm

5-Sargeant, K.,Sheridan, A., O" Kelly, J. and Carnaghan, R.B.A. (1961). Toxicity associated with certain samples of groundnuts nature, 192, 1095 – 1097.

6- http:// www.fao.org/docrep/x0036E/x0036E .

7-Phillips, Douglas, (1980). Aflatoxins in Almonds, USDA,Science & Education Administration, Agricultural Reviews & Manuals, November.

8-Mclean, M. & Dutton, MF. (1995). Cellular interactions & metabolism of Aflatoxin: An update. Pharmacology & Therapeutics 65: 163 –192 .

9-Coker, B.D. (1997). Aflatoxin: past, present & future. Trop Sci. 21 (3): 143 .

10-Eaton, D. L., Groopman J. D. (EDs), (1994). The Toxicology of Aflatoxins. Academic Press, New York, 521 – 523.

11-Jiujiang, Y., Chang, P., & Jeffrey W (1995) . Comparative mapping of aflatoxin pathway gene clusters in *Aspergillus parasiticus & A. flavus*. Applied & Environmental Microbiology. 61: 2365 – 3271.

12-Trail, F., Mahant, N., & Linz, J. (1995) . Molecular biology of aflatoxin biosynthesis: Aflatoxins: Human & animal health; economic impact. Micribiology. 141: 755 – 765.

13- Devero, A, (1999) . The Effects on Human & Animal Health. Senior Seminar Presentation: BIOL 4900 .

14- Mori, T., Matsumura, M. & Yamada, K. (1998). Systemic aspergillosis caused by aflatoxin – producing strain of Aspergillus flavus. Journal of Medicinal & Veterinary Mycology. 36: 107 – 112.

15- Sibanda, L., Marovatsanga, L, T, & Pestka, J. J. (1997). Review of mycotoxin work in sub – Saharan Africa .

16- Sydenham, E. W., Thiel, P.G., Marsasas, W.F.O., Sherpard, G.S., Van Schalkwyk, D.J. & Koch K.R. (1990). Natural occurance of some Fusarium mycotoxin corn from law & high oesophageal cancer prevalence areas of the Transkei, southern Africa. Journal of Agriculture & Food Chemistry 38 (10). 1900 -1903.

17- http://www.Noble.Org/ag/pests/Aflatoxin/poisoning.htm .

18- http://www.fao.org/docrep/X0036E/X0036E08.htm .

19- Klick, M.A. & Pitt, J.I. (1988). Differentiation of Aspergillus flavus from A. parasiticus & other closely related species. Trans. Br. Mycol. Soc. 91: 99 – 108 .

20- Edlefsen, M., & Brewer, M.S. (1996). Aflatoxin.

21http://www.ansci.cornell.edu/plants/toxicagents/aflatoxin/aflatoxin.htmocurrence .

22-Adhikari, M., Ramjee, G. and Berjak, P. (1994). Aflatoxin, Kwashiorkor, and morbidity. Nat. Toxin, 2, 1 – 3.

23- Sabino, M., Zorzetto, M. A. P., Pedroso, M. O. & Milanez, T.Vv. (1989). Incidence of aflatoxins in peanuts and peanut products consumed in Sao Paulo, between 1980 and 1987. Rev. Inst. Adolfo L UTZ, 49,41–44.

24- Jonsyn, F. (1999). Intake of aflatoxins & orchratoxins by infants in Sierra Leon: Possible effects on the general health of these children. Journal of Nutritional & Environmental Medicine. 9: 15 – 23.

25- http://www.Aces.edu/department/ipm/mm.htm .

26- Anon. In: LARK Monographs on the Evaluation of carcinogenic Risks of Humans. (1987). supplement 7. International Agency for Research on Cancer, Lyon, France, 83-85 .

27- Peers, F.G. & Linsell, C.A., (1973). Br. J. Cancer. 27: 473 – 484.

28- Shank, R.C. wogan, G. N., Gibson, J. B. & Nondasuta, A. , (1972) . Dietary aflatoxins and human Liver cancer II. Aflatoxins in market foods and foodstuffs of Thailand and hong kong . Food Cosmet . Toxicol., 10, 61 – 69 .

29-Degan, G . H. and Neumann . H. G. (1978) . Gnem. Biol . Interact. 22. 239-255 .

30- Viquez, O. M., Castell Perez, M.E., Shelby, R.A. & Brown, G. (1994). Aflatoxin contamination in corn samples due to environmental conditions, aflatoxin – producing strains, & nutrients in grain grow in costa Rica.J. Assoc. off. Anal. Chem., 54,533 – 539 .

31- Sanders, T.H., cole, R.J., Blankenship, P.D. & Dorner, J. W. (1993) . Aflatoxin contamination of peanuts from plants drought stressed in pod or root zones. Peanut sci., 20,5 –8 .

32-

http://www.abc.cornell.Edu/plants/toxiagents/aflatoxin/aflatoxin.htm.

33- Castegnaro, M., & Mcgregor, D. (1998). Carcinogenic risk assessment of mycotoxins. Revue De Medicine Veterinary, 149.6, 671 – 678.

34- FDA (1977). FDA Proposed guidelines for aflatoxin: Cereal Foods World, 22, 532 -533, 541.

35- Park, D.L. & Njapau, H. (1989). Contamination issues & padding. J. AM. Oil Chem. Soc., 66, 1402 – 1405.

36- Park, D.L. & Liang, B. (1993). Perspectives on aflatoxin control for human food & animal feed. Trends Food Sci. Technol., 4, 334 – 342 .

37- Horn, B.W., Green, R.L. & Dorner, J.W. (1995) . Effect of corn & peanut cultivation on soil population of *Aspergillus flavus & A. parasiticus* in Southeastern Geargia. Applied & Environmental Microbiology. 61: 2472 – 2475.

38- Chu, F.S., (1990). Immunoassays for mycotoxins: Current state of art, commercial & epidemiological applications. Vet. Hum. Toxicol. (supp) 32, 42 – 50.

39- Johnson, W.W., and Guengerich, F.P. (1997). Proc. Natl. Acad. Sci. USA 94, 6121 – 6125. Reaction of aflatoxin B1 – 8,9 – exo – Epoxide with DNA : Kinetics of covalent binding and DNA – induced hydrolysis.

40- Bash G., & Rae, I.D. (1989). The structure & chemistry of aflatoxins, In aflatoxin,ed.L.A.Goldblatt. Academic press, New York, PP. 55 – 75 .

41-Davis,N.D. & Diener, V.L. (1979). Mycotoxins. In food & Beverage Mycology, ed. L.R. Beuhat. AVI publishing company, West Port, CT, PP. 397 – 444.

42-Ellis, W.O., Smith, J.P., Simpson, B. K. & Oldham, J.H. (1991). Aflatoxin in food: occurrence, biosynthesis, effects,on organisms,

detection, & methods of control. Crit. Rev. Food Sci. Nutr., 30, 403 – 439.

43- VICAM, L.P. EMAIL: vicam @ vicam.com.

44- http://www.sok.co.jp/shodex/english/da0401.htm.

45- Roy AK, Sinha KK, Chourasia HK. (1988). Aflatoxin contaminationof some common drug plants. Environ Microbiol, 54:842-843.

46- Aziz, N.H., Youssef, Y.A., EL – Fouly, M.Z.,& L.A.Moussal. (1998). Contamination of some common medicinal plant samples & spices by fungi & their mycotoxins .

47- Rani, N. and Sinagh. (1990). Aflatoxin contamination of some umbelliferous spices of human use. Int. sym. And Workshop on food cont. Mycotoxins and phycotoxins, November 4 – 15, 1990, Cairo, Egypt, Abst. Book, 79 – 80.

48- Greek, B. (1985). Toxins, particularly mycotoxin in feed. Deutsche. Tierarztl. Wochenschr. 92 : 215 – 218.

49- Feuell AJ. Aflatoxin in groundnuts, Part a : Problems of detoxification. Trop Sci. 1966; 8 : 61 – 70.

50- Fishbach H, Campbell AD. (1965) . Note on detoxification of aflatoxins. J A ssoc Offic Agr Chem, 48 :28.

51- Haberle V, Balenovic J, Briski B. (1978) . Aflatoxin control content of imported peanuts, coffee, barely, wheat and kernel. Hrana I ishrana, (9– 10): 451 – 460.

52- Sutic M-, Pantovic D.(1976). Effect of certain ecological factors on aflatoxin production in food cultures, III. Bled :Knogres mikrobiologa jugoslavije, 688 – 689.

53- Durak ovic S, Garlic J, Pajnovic P.(1989). Toxic and cancer metabolites of moulds in food and fodder. Hrana I ishrana. 30 (2): 71 – 100.

54- Halt. M. (1998) . Moulds and Mycotoxins in herp tea and medicinal plants., Faculty of food technology, osijek, croatia

55- Hendry KM. cole Ec., (1993). Journal of Toxicology 38 (2).

56- Maurice, O. Moss.,Mycotoxins. (1996). Mycot. Res. 100 (5) : 513 – 523 .

بسم الله الرحمن الرحيم

وجود مركبات الأفلاتوكسين في النباتات الطبية الشعبية المستعملة في الأدوية الشعبية وفي البهارات.

وفاء عادل محمود المصلح

إشراف

د. محمد جواد سمار

د. سمر غزال

الملخص

تم جمع خمسون عينة من النباتات الطبية الشعبية حيث جمعت النباتات بطريقة عشوائية من محلات العطارة في مدينة نابلس خلال فترة 3 أشهر ثم فحصت العينات لمعرفة تركيز سموم الافلاتوكسين المسببة لمرض سرطان الكبد.

تم فحص العينات بواسطة جهاز في كام أفلاتست (VICAM AFLA TEST)، تبين بالفحص أن 48 عينة كانت تحتوي على سموم الافلاتوكسين منها 13 عينة احتوت على تراكيز أعلى من 20 نانو غرام / غرام (ppb) وهي كمية كبيرة وغير مقبولة بالنسبة لبعض المعايير والمواصفات العالمية. كان أعلى تركيز في نبتة السوس والسماق إذ احتوت كل واحدة منها على 76 نانو غرام / غرام، تلاها الشاي إذ تبين أن الشاي احتوى على 25 ،32 ، 46،55 ، 69 ، نانو غرام / غرام. أما اليانسون، جوزة الطيب، توابل الأرز، البابونج، ورق الغار، دقة المعمول، فقد وجد أن التركيز فيها قد تراوح بين 20–36 نانو غرام /غرام وهذه الكميات أيضاً غير مقبولة لبعض المعايير والمواصفات العالمية، في حين كانت بقية العينات خالية تقريباً من مركبات الأفلاتوكسين، إلا أنه لا يوجد تركيز مسموح به من هذه المواد نظراً لخطورتها وتراكمها في الجسم. من الضروري إظهار أن مادة الشاي في بلدنا تستعمل بكثرة ويتعرض الأطفال من جميع الأعمار والسيدات والحوامل باستمرار لمواد تسبب السرطان.