

**STUDIES ON CONTAMINATION LEVEL OF AFLATOXINS IN  
CEREALS, DRIED FRUITS/NUTS AND APPLICATION OF  
METHOD FOR DECONTAMINATION**



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*Submitted To*

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*In fulfillment for the degree*

*Of*

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**By**

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**THE CENTER OF BIOTECHNOLOGY AND MICROBIOLOGY  
UNIVERSITY OF PESHAWAR**

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**This Dissertation is submitted**

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# DEDICATION

## *OF RESEARCH*

To My Respected and Compassionate Parents

To My Brother and Sisters

To My Wife

&

To Everyone Who Supported Me



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## SUMMARY

In this study survey was conducted to determine the incidence of total aflatoxins in some dried fruits, nuts, cereals and beans. Samples of dried fruits/nuts (180) and cereals/beans (160) were analyzed for the detection of total AFs by high pressure liquid chromatography. The contamination level (%) of total AFs was observed as in; dried apricot (20 %), dates (10 %), dried figs (50 %), dried mulberries (26 %) and raisins (20 %), apricot kernels (26 %), almonds without shell (30 %), walnuts with shell (40 %), walnuts without shell (70 %), peanut with shell (40 %), peanuts without shell (50 %), pistachios with shell (20 %), pistachios without shell (50 %) and pine nuts with shell (20 %). The contamination level (%) of total AFs was also observed as in; rice (25 %), rice broken (15 %), wheat (20 %), maize (40 %) barley (20 %) and sorghum (30 %), red kidney beans (20 %), split peas (27 %), chick pea (10 %), cow pea (20 %), and soybean (15 %).

The studies on decontamination by different methods / techniques were also evaluated to decontaminate the mycotoxin (aflatoxins) contaminated agricultural commodities. In biological decontamination, the anti-fungal activity of some chemicals, herbal compounds/spices and plants at different concentrations were evaluated against the toxin producing *Aspergillus flavus* and *Aspergillus parasiticus* growth inhibition and aflatoxin production. Among the chemicals screened, benzoic acid (0.1 – 0.5 %), propionic acid (0.1 – 0.5 %) and copper sulphate (0.2 – 0.5 %) showed complete inhibition of *Aspergillus flavus* growth. Sodium propionate and potassium metabisulphite showed high, while ammonia, urea and citric acid were moderate in inhibiting fungal growth. In case of the herbal compounds/spices, clove (0.5 %) and clove oil (0.5 %) showed complete inhibition. Clove oil, cinnamon,



coriander and neem oil represented high, while kalonji and neem oil showed moderate antifungal activity. Among the plants garlic and onion showed complete inhibition, while ginger, neem and lemon grass had high antifungal activity.

On the basis of reduction in aflatoxins production in maize used as substrate, the effect of different concentrations of chemicals, herbal compounds/spices and plants on the production of aflatoxins by *Aspergillus flavus* is presented. All the selected chemicals showed reduction ( $P<0.01$ ) in production of aflatoxins. Among the chemicals evaluated, benzoic acid, copper sulphate and propionic acid (0.2 - 0.5 %) showed complete reduction in production of aflatoxin ( $P<0.01$ ), while high reduction was observed for sodium propionate, potassium metabisulphite and urea. In herbal compounds/spices, clove and clove oil at 0.5% completely inhibited ( $P<0.01$ ) aflatoxin production, followed by neem oil, cinnamon and coriander. In plants, garlic and onion (0.5 %) showed complete reduction of aflatoxin synthesis, followed by ginger, lemon grass and neem.

All the samples were also screened against *Aspergillus parasiticus* growth. Among the chemicals screened, benzoic acid (0.2 - 0.5 %), copper sulphate (0.1 - 0.5 %) and propionic acid (0.2 - 0.5%) showed complete inhibition of growth, while benzoic acid, potassium metabisulphite, sodium propionate and urea showed high, whereas ammonia, citric acid and sodium propionate showed moderate anti fungal activities. In herbal compounds/spices, clove and clove oil (0.5%) showed complete inhibition of growth. Cinnamon, coriander and neem oil showed high antifungal activity. Among the plants, garlic and onion (0.5 %) showed complete inhibition of growth, while ginger, garlic, neem, lemon grass and onion had high, mint had moderate, while carrot and sour orange peel had poor antifungal effect.



On the basis of reduction in aflatoxins production in maize used as substrate, the effect of different concentrations of chemicals, herbal compounds/spices and plants on the production of aflatoxins by *Aspergillus parasiticus* is presented. Among the chemicals tested, copper sulphate, benzoic acid and propionic acid showed complete reduction in production of aflatoxin ( $P < 0.01$ ), while high reduction was observed for potassium metabisulphite, sodium propionate and urea. In herbal compounds/spices, clove and clove oil exhibited best antifungal activity followed by neem oil, cinnamon and coriander. Among the plants tested, garlic and onion exhibited best antifungal activities followed by ginger, lemon grass and neem.

In case of the physical decontamination, the reduction in contamination level of AFB<sub>1</sub> in different wheat varieties were studied by applying oven heating and washing. Heating in oven reduced the concentration of AFB<sub>1</sub> more effectively as compared to washing. The wet wheat showed more reduction in the concentration of aflatoxin B<sub>1</sub> by heating than the dried wheat. Moreover, different cooking methods were applied for rice to evaluate the level of reduction in AFB<sub>1</sub> and OTA. The cooking of rice in an excess of water showed maximum reduction on the levels of AFB<sub>1</sub> (89.1 %) and OTA (86 %) in samples of basmati rice contaminated artificially, than normal cooking (84.0 % for AFB<sub>1</sub> and 83.0 % for OTA) and microwave oven cooking (72.5 % for AFB<sub>1</sub> and 82.4 % for OTA). The samples artificially contaminated with both AFB<sub>1</sub> and OTA, the highest reductions were also observed for rice cooked in excess water (87.5 & 83.0 %) than normal cooking (82.5 & 76.6 %), and microwave oven cooking (77.6 & 75.9 %). Overall, it is clear that no single method/technique will be suitable for all foods. The effectiveness of a method depends on the nature of the food, as well as the type of mycotoxin and its concentration level.

# CHAPTER – I

## INTRODUCTION



## 1. INTRODUCTION

### 1.1. Mycotoxins

A mycotoxin (from Greek (mykes, mukos) “fungus” and Latin (toxicum) “poison”) is a toxic secondary metabolite produced by organisms of the fungus kingdom, commonly known as molds (Turner et al., 2009; Richard, 2007). The term ‘mycotoxin’ is usually reserved for the toxic chemical products produced by fungi that readily colonize crops (Turner et al., 2009). One mold species may produce many different mycotoxins and/or the same mycotoxin as another species (Robbins et al., 2000). Most fungi are aerobic (use oxygen) and are found almost everywhere in extremely small quantities due to the minute size of their spores. They consume organic matter wherever humidity and temperature are sufficient. Where conditions are right, fungi proliferate into colonies and mycotoxin levels become high (Fox and Howlett, 2008).

Mycotoxins are not only hard to define; they are also challenging to be classified, due to their diverse chemical structures, biosynthetic origins, biological effects and production by a number of different fungal species. Classification schemes of these mycotoxins tend to reflect the background and area of specialization of the person doing the categorization. Clinicians often determine them by prefixing with the name of the organ they affect. Thus, mycotoxins can be classified as hepatotoxins, nephrotoxins, neurotoxins, immunotoxins, and so forth. Cell biologists put them into generic groups such as teratogens, mutagens, carcinogens, and allergens. Organic chemists have attempted to classify them by their chemical structures e.g., lactones, coumarins, biochemists according to their biosynthetic origins have named them as polyketides, amino acid-derivatives, physicians by the illnesses they cause e.g., St. Anthony's fire, stachybotryo-toxicosis, and mycologists by the fungi that produce them e.g. *Aspergillus* toxins, *Penicillium* toxins (Bennett and Klich, 2003). The filamentous fungus produces



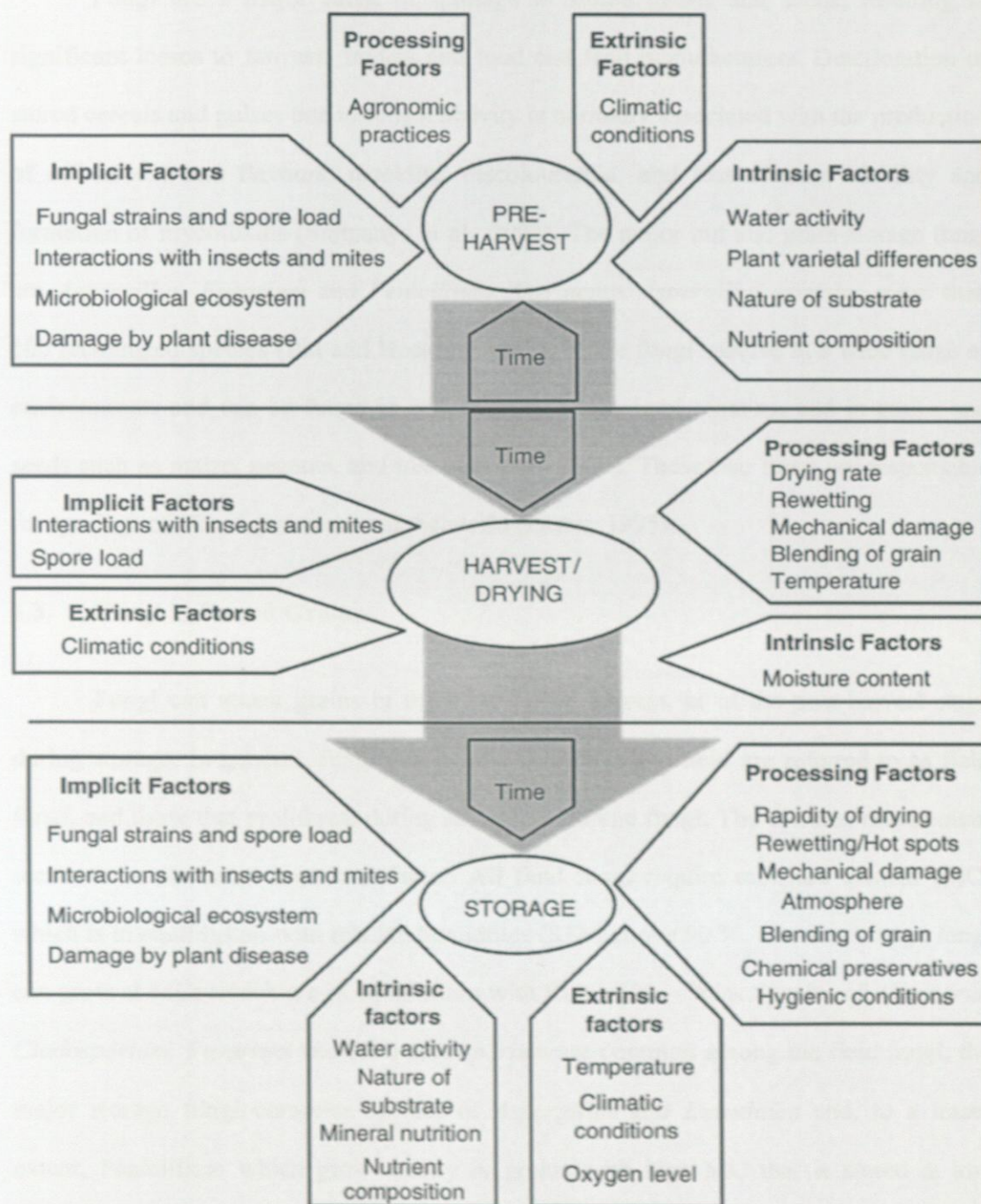
mycotoxins which are the secondary metabolites that can cause disease in animals as well as in human. These can be ingested, inhaled and absorbed through the skin (Frisvad, et al., 2007). Mycotoxins are produced by *Aspergillus*, *Fusarium* and *Penicillium*, which can attack many crops under the favorable conditions of humidity and temperature in the field may grow during storage on the foods. The intake of mycotoxins produces hazardous diseases in animals as well as in humans, the phenomenon is known as mycotoxicoses (Berry, 1998; Smith, et al., 1995).

It has been reported that mycotoxins are the metabolites which are mainly originates from the species *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria*. More than 100 moulds produces about 400 hazardous secondary metabolites, and it has been found by Food and Agriculture Organization that, 25% of the agricultural commodities in the world showed mycotoxins contamination (Bulent et al., 2006). Primarily, mycotoxins may occur in the spore as well as in the mycelium of these toxics moulds (D' Mello, 1997).

The synthesis of these metabolites occurred at the end of the growth exponential phase, while no biological significance have be appeared relating to competitiveness or development of the mould growth (D' Mello, 1997; Deacon, 2001; Hussein and Brasel, 2001). Species in limited numbers can produce some mycotoxins as all the moulds are not pathogenic, while several genera may synthesize other mycotoxins relatively in large quantities (D' Mello, 1997; Deacon, 2001). The production of toxins depends on the surrounding intrinsic and extrinsic environments and the toxins vary greatly in their severity, depending on the organism infected and its susceptibility, metabolism, and defense mechanisms (Hussein and Brasel, 2001). Spoilage of stored grain by fungi is determined by a range of factors which can be classified into four main groups including (a) intrinsic nutritional factors, (b) extrinsic factors (c) processing factors and (d) implicit



microbial factors (Magan and Aldred, 2007). The factors which affect fungal colonization of stored grain is summarized [Fig 1.1].



**Fig1.1:** The interaction between intrinsic and extrinsic factors in the food chain which influences mould spoilage and mycotoxin production in stored commodities



## 1.2. Storage Mycoflora

Fungi are a major cause of spoilage in stored grains and seeds, resulting in significant losses to farmers, traders and food and feed manufacturers. Deterioration of stored cereals and pulses due to fungal activity is normally associated with the production of off-colours and flavours, rancidity, discolouration, and loss of seed viability and formation of mycotoxins (Simpanya et al., 2001). The major nut and grain storage fungi are *Aspergillus*, *Fusarium* and *Penicillium*. The genus *Aspergillus* contains more than 100 recognized species (Pitt and Hocking, 1997). These fungi survive in a wide range of environments and can be found in soil, in plant and animal remains, and in grains and seeds such as maize, peanuts, and tree nuts (Pitt, 2000). These two fungi are responsible for spoilage of stored grains around the world (Paster, 1995).

## 1.3. Fungi in Stored Grains

Fungi can attack grains in the field before harvest, or at the post-harvest stage during storage. In general, fungi that invade kernels in the field are referred to as field fungi, and those that proliferate during storage as storage fungi. The division requirement seems to be the most important factor. All field fungi require moisture content (MC) which is in equilibrium with relative humidities (RHs) above 90 %, whereas storage fungi can grow at MCs which are in equilibrium with RHs of 70 – 90%. Species of *Alternaria*, *Cladosporium*, *Fusarium* and *Helminthosporium* are common among the field fungi; the major storage fungi comprise species of *Aspergillus* and *Eurothium* and, to a lesser extent, *Penicillium* which grow mainly in grains with high MC that is stored at low temperatures. The abiotic factors are: dockage; intergranular air; water vapour; temperature; and the storage structure. All the components interact and affect each other to cause or to prevent damage to stored grains. Parameters affecting growth of storage



fungi are: water activity ( $a_w$ ); temperature; grain condition (e.g. soundness, the state of the covering tissue, presence of mechanical or insect damage); nutrient composition in the grain and its availability; the gas composition of the intergranular air; competition between the species present (microbial interactions); and inhibiting materials (types and quantity).

According to their moisture requirement, the storage fungi are divided into three main groups: hydrophytes, which grow at an RH of 90 - 100 % (bacteria and many yeasts); mesophytes, with an RH range of 80 - 90 % (*A. candidus*, *A. flavus*); and xerophytes, which have a lower RH limit of 70 % (members of the *Eurothium* group and *A. restrictus*). When  $a_w$  at a given spot in the grain bulk starts to rise, the xerophilic species will be the first to develop. As a result of their metabolic activity, the moisture increases first at that spot and later in other parts of the bulk also (as a result of moisture migration), thus creating favourable conditions for the appearance of mesophytes and hydrophytes. Therefore, the safest means of preventing fungal growth on grain is to reduce  $a_w$  to below 0.70 equilibrium Relative humidity and to maintain the level throughout the storage period. It is worth mentioning that, numerically, ERH and  $a_w$  are the same. However,  $a_w$  is a commonly used measure of the water available for microbial activity.

According to the temperature needed for the storage fungi to proliferate, they are generally also classified into three main groups: psychrophiles, mesophiles and thermophiles. The temperature requirements (minimum, optimum and maximum, in °C) for these groups are: psychrophiles: -5 to 0, 10-20, 25-30; mesophiles: 5-10, 20-40, 40-45; thermophiles: 25-40, 50-60, 70-80. Of all the conditions known to affect fungal growth or composition, the effects of  $a_w$ , temperature and intergranular air composition are regarded as the most crucial. Note, however, that all the factors interact, and that the range of each



of these parameters is closely related to the values of the other parameters involved in growth. For example, as the moisture level rises, the range of temperature over which the fungi will develop becomes broader. Fungi growing on stored grain can cause major damage that leads to quality reduction and, as a consequence, huge economic losses. The major types of damage caused by fungi are: initiation of heating processes in the bulk; reductions in technological qualities (e.g. suitability for milling or baking); losses in quality (e.g. discoloration, high levels of free fatty acids, mustiness and caking); reductions in germination ability; nutritional losses; production of toxic secondary metabolites – i. e. mycotoxins (Magan and Olsen, 2004).

#### 1.4. *Aspergillus* in Food and Feeds

The occurrence of *Aspergillus* in foods and feeds is dependent on the substrate and environmental factors such as water activity, temperature, pH, redox potential, presence of preservatives and microbial competition. *Aspergillus* species and teleomorphs of *Aspergillus* (*Eurotium* and *Neosartorya*) associated with various foods. Because of their soil borne nature and ability to grow at low water activity, *Aspergillus* species are common contaminants of various cereal grains. The presence of *Aspergillus* and *Eurotium* in processed, dehydrated foods is mostly due to their ability to grow at lower water activities ( $a_w$  0.7-0.8) (Pitt and Hocking, 1997; Chang et al., 1999).

#### 1.5. Mycoses and Mycotoxicoses

The growth of fungi on animal hosts produces the diseases collectively called mycoses. Mycoses range from merely annoying (e.g., athlete's foot) to life-threatening (e.g., invasive aspergillosis). The fungi that cause mycoses can be divided into two categories, primary pathogens (e.g., *Coccidioides immitis* and *Histoplasma capsulatum*)



and opportunistic pathogens (e.g., *Aspergillus fumigatus* and *Candida albicans*). Primary pathogens affect otherwise healthy individuals with normal immune systems. Opportunistic pathogens produce illness by taking advantage of debilitated or immunocompromised hosts. The majority of human mycoses are caused by opportunistic fungi (Van and Magee, 2001). Some infections remain localized, while others progress to systemic infection. For many mycoses, the ordinary portal of entry is through the pulmonary tract, but direct inoculation through skin contact is not uncommon. Mycoses are frequently acquired via inhalation of spores from an environmental reservoir or by unusual growth of a commensal species that is normally resident on human skin or the gastrointestinal tract. These commensal species become pathogenic in the presence of antibacterial, chemotherapeutic, or immunosuppressant drugs, human immunodeficiency virus infection, and other predisposing factors (Calderone and Cihlar, 2002).

Mycotoxicoses is the term used for poisoning associated with exposures to mycotoxins. In contrast to mycoses, mycotoxicoses are examples of "poisoning by natural means" such as dietary, respiratory, dermal, and other exposures to toxic fungal metabolites produce the diseases and thus are analogous to the pathologies caused by exposure to pesticides or heavy metal residues. The symptoms of a mycotoxicosis depend on the type of mycotoxin; the concentration and length of exposure; as well as age, health, and sex of the exposed individual (Bennett and Klich, 2003). The synergistic effects associated with several other factors such as genetics, diet, and interactions with other toxics have been poorly studied. Therefore it is possible that vitamin deficiency, caloric deprivation, alcohol abuse, and infectious disease status can all have compounded effects with mycotoxins (Bennett and Klich, 2003). Mycotoxins have the potential for both acute and chronic health effects via ingestion, skin contact, and inhalation. Acute toxicity generally has a rapid onset and an obvious toxic response, while chronic toxicity



is characterized by low-dose exposure over a long time period, resulting in cancers and other generally irreversible effects. The majority of mycotoxicoses, on the other hand, result from eating contaminated foods. Skin contact with mold- infested substrates and inhalation of spore borne toxins are also important sources of exposure. Thus mycotoxicoses are the diseases caused by mycotoxins; mycotoxicology is the study of mycotoxins. While all mycotoxins are of fungal origin, not all toxic compounds produced by fungi are called mycotoxins. The target and the concentration of the metabolite are both important. Fungal products that are mainly toxic to bacteria (such as penicillin) are usually called antibiotics. Fungal products that are toxic to plants are called phytotoxins by plant pathologists (Bennett and Klich, 2003). These toxins can enter the blood stream and lymphatic system; they inhibit protein synthesis, damage macrophage systems, inhibit particle clearance of the lung, and increase sensitivity to bacterial endotoxin (Godish, 2001).

#### **1.6. Human Health Effects**

The ingestion of mycotoxin in animals and humans causes toxic effects, which depends upon many factors including defence mechanism, metabolism, mechanism of action, intake levels and duration of exposure (Hussein and Brasel, 2001; Galvano et al., 2001). The contamination of mycotoxin in feed and foods received much attention for the safety of humans and animals. The major mycotoxins *i.e.* aflatoxins, trichothecenes, zearalenone, fumonisins and OTA have been comprehensively reviewed for their effects on human and animals (Dhavan and Choudary, 1995; Sabino et al., 1999; Trucksess et al., 1999; Visconti et al., 1999; Samira and Nadine, 2004). Aflatoxin is associated with both toxicity and carcinogenicity in human and animal populations (Eaton and Groopman, 1994). The diseases caused by aflatoxin consumption are loosely called



aflatoxicoses. Acute aflatoxicosis results in death; chronic aflatoxicosis results in cancer, immune suppression, and other "slow" pathological conditions (Bennett and Klich, 2003). It has been hypothesized that kwashiorkor, a severe malnutrition disease, may be a form of pediatric aflatoxicosis (Hendrickse, 1997). Further, aflatoxin might be involved in Reye's syndrome, an encephalopathy, and fatty degeneration of the viscera in children and adolescents (Hayes, 1980). Aflatoxigenic fungi infect animals and humans that often are immune compromised. Systemic aspergillosis by aflatoxigenic fungi was considered to contribute to immunosuppression (Mori et al., 1998). Human hepatic neoplasms, gastrointestinal disorders and liver cell carcinoma are due to aflatoxins having been shown in many epidemiological studies in china, Philippines and South Africa (Goeger, 1999). In Kenya it has reported that, recently aflatoxins caused about 123 deaths and affected large area geographically (CDC, 2004).

#### 1.7. Aflatoxins

Aflatoxins are secondary metabolites produced by toxic species such as *Aspergillus flavus* and *Aspergillus parasiticus*. Both these fungi under favorable conditions of humidity and temperature can contaminate feed and foods by producing aflatoxins (AFs) during shipment and storage condition, at pre and post harvest stage (Bushby and Wogan, 1984, Lopez and Ramos, 2002; Peraica et al., 1999). In grains containing high carbohydrate content such as corn, cotton, peanut and wheat, the *Aspergillus* can produce AFs under optimum conditions of moisture and temperature (Jaimez et al., 2000). The four types of aflatoxins (B1, B2, G1 and G2) occur naturally in different foods. These toxins were commonly found in cottonseed, maize, peanuts and pistachios (Molyneux et al., 2007). Among the mycotoxins, AFs causes maximum toxicity as well as high carcinogenic effect. These toxins not only affect the economy of



food and agriculture but also the health of humans and animals (Hwang et al., 2004). In epidemiological survey, the chances of hepatocellular carcinoma along with hepatitis B virus increased because it is associated with AF contamination (IARC, 2002). Among 18 different types of aflatoxins identified, major members are aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>, M<sub>1</sub> and M<sub>2</sub>. Aflatoxin B<sub>1</sub> is produced most abundantly and also is most toxic followed by G<sub>1</sub>, B<sub>2</sub> and G<sub>2</sub> (Krishnamurthy and Shashikala, 2006).

Aflatoxin B<sub>1</sub> and B<sub>2</sub> fluoresce blue (hence the title B) under UV-light while aflatoxin G<sub>1</sub> and G<sub>2</sub> fluoresce green (hence the title G). *A. flavus* and *A. pseudotamarii*, produce only the B aflatoxins, but at times *A. flavus* isolates have been reported to produce G aflatoxins. *Aspergillus nomius*, *A. bombycis* and *A. parasiticus* produce all four major aflatoxins. Various studies have determined that aflatoxins are synthesized in two stages from malonyl CoA, first with the formation of hexanoyl CoA, followed by formation of a decaketide anthraquinone (Minto and Townsend, 1997; Yabe et al., 1999).

Dried fruit, tree nuts, spices and cereals are commonly associated with aflatoxins. Contamination is widespread in the warm, humid areas of Africa, Asia and Latin America. However, aflatoxins are found in temperate areas of North America and Europe as well. The rate and degree of aflatoxin contamination are dependent on temperature, humidity, soil and storage conditions (Boutrif 1997). Pakistan is situated in subtropical region and its environmental conditions are conducive to aflatoxin production in agricultural products and have shown to be responsible for acute and chronic toxic effects in animals as well as in human beings (Mansoor et al., 1989).

#### 1.8. Aflatoxigenic Species and Their Identification

Filamentous fungal species in *Aspergillus* section Flavi more commonly known as the *Aspergillus flavus* Group and are of great economic importance (Chang et al., 1999). Several species, not only cause food spoilage, but also produce potent toxins. The most



significant mycotoxins produced by these fungi are aflatoxins. Aflatoxins are produced by *Aspergillus flavus*, *A. parasiticus* and *Aspergillus nomius* and more recently *A. pseudotamarii* and *A. bombycis* (Peterson et al., 2001; Bennett and Klich, 2003; Mishra and Das, 2003). *Aspergillus nomius* and *A. bombycis* produce both the type B and G aflatoxins but not cyclopiazonic acid (CPA). *Aspergillus parasiticus* is distinguished from *A. bombycis* by its typically dark green colour on Czapek agar (Peterson et al., 2001). In comparison to other aflatoxigenic *Aspergillus* spp., *Aspergillus pseudotamarii* has orange-brown colony colour. It only produces the B type aflatoxins. *Aspergillus pseudotamarii* grows at 37 °C with no growth at all at 42°C. As mentioned, *Aspergillus flavus* and *A. parasiticus* typically grow moderately at 42°C and rapidly at 37 °C and have much smaller conidia than those of *Aspergillus pseudotamarii* (Ito et al., 2001). Characteristics of widely known aflatoxigenic *Aspergillus* namely, *A. flavus*, *A. parasiticus* and *A. nomius* are summarized [Table 2.2].

**Table 1.1: Key characteristics of aflatoxin producing fungi**

| Characteristics             | <i>Aspergillus flavus</i>                                  | <i>Aspergillus parasiticus</i>                           | <i>Aspergillus nomius</i>   |
|-----------------------------|------------------------------------------------------------|----------------------------------------------------------|-----------------------------|
| Conidiophore arrangement    | Mostly biserial                                            | Mostly uniserial                                         | Mostly biserial             |
| Conidia                     | Smooth to moderately roughened, variable in size (3 - 8µm) | Conspicuously roughened, less variable in size (4 - 7µm) | Similar to <i>A. flavus</i> |
| Sclerotia                   | Large, globose                                             | Large, globose                                           | Small, elongated            |
| Colony colour               | Green                                                      | Dark yellow-green                                        | Green                       |
| Aflatoxins produced         | B1, B2                                                     | B1, B2, G1, G2                                           | B1, B2, G1, G2              |
| Cyclopiazonic acid produced | Yes most isolates                                          | No                                                       | No                          |



### **1.9. Economic Significance of Aflatoxin Contamination**

The worldwide extent of contamination of commodities is not totally understood often because of reluctance to report its occurrence. Aflatoxin contamination in food for human consumption as well as in feed for livestock has been found in many geographically diverse regions of the world. Such contamination has serious food safety and economic implications for the agriculture industry. National economies are affected adversely both by the losses incurred by crop and livestock producers when aflatoxin contaminated crops are destroyed due to regulatory restrictions and by the multiplier effect this would have on other industries as a result of the reduced spending power of producers. Because of the health concern, regulatory guidelines of 20 parts per billion aflatoxin for food or feed substrate is the maximum allowable limit imposed by the U.S. Food and Drug Administration for consumption and for interstate shipment of foods and feeds. In some European countries aflatoxin levels are regulated below five parts per billion (Sejakhosi et al., 2004).

### **1.10. Factors Affecting Aflatoxin Biosynthesis**

Aflatoxins arise naturally when an aflatoxin-producing strain of *Aspergillus* grows on a substrate in a geographical area where environmental conditions are suitable for the development of the fungus. Some strains of aflatoxigenic *Aspergillus* species do not produce aflatoxins, so the mere presence of mouldiness is not, of itself, indicative of toxin production. In view of the fact that the *A. flavus* are capable of development over a wide range of temperature or substrates of high carbohydrate content, agricultural commodities and their products are vulnerable to contamination (Egel et al., 1994).

#### **1.10.1. Effect of Aeration**

Production of aflatoxin by *A. flavus* cultures grown on a medium of groundnuts in atmospheres depleted of oxygen is greatly decreased when compared with controls in



normal atmospheres (Dobson and Sweeney, 1998). These findings are indicative of the fact that aflatoxin production is an aerobic process. The effects of various levels of atmospheric gases, carbon dioxide, oxygen and nitrogen on aflatoxin production under conditions of varying temperature and humidity were investigated (Diener and Davis, 1967). It was stated that aflatoxin production in sound, mature, groundnut kernels decreased with increasing concentrations of carbon dioxide from 0.03 to 100 %. Reducing the oxygen concentration generally decreased aflatoxin production. A significant decrease in mycotoxin production resulted when the oxygen was reduced from 5 to 1 % regardless of the carbon dioxide concentration (Diener and Davis, 1967).

#### 1.10.2. Effect of Temperature

The temperature requirements of the *Aspergillus flavus* group of fungi have also been investigated. The optimum temperature for aflatoxin production by *Aspergillus bombycis* and *A. nomius* was 25 °C on Czapek agar (Peterson et al., 2001). The optimum temperature for aflatoxin production by *A. flavus* was 25 °C on both groundnuts and liquid medium, whereas that for *A. parasiticus* extended from 25 to 30 °C. The incubation period for maximum production of aflatoxin was from seven to 15 days. It was also observed that a change in the proportions of aflatoxins B1 and G1 produced by *A. parasiticus*, with changes in temperature. A reduction in the amount of aflatoxin G1 produced was observed with increased temperature (Diener and Davis, 1967).

#### 1.10.3. Effect of Relative Humidity

Relative humidity (R.H.) is a crucial factor for growth of *Aspergillus flavus* and aflatoxin production. The optimum relative humidity for fungal growth is 85 % or greater (Dobson and Sweeney, 1998). The lowest relative humidity for aflatoxin production on peanuts is 83 %, with yields increasing up to 99 % relative humidity. It was also observed



that significant levels of aflatoxins were produced at 97 to 99 % R.H. at temperatures of 20 to 35 °C. Maximum production of aflatoxin on groundnuts was obtained with kernel moisture contents of 25 %, at a temperature of 30 °C (Diener and Davis, 1967).

#### 1.10.4. Effect of Water Activity

The optimum water activity ( $a_w$ ) for growth of *Aspergillus flavus* is near 0.996, with the minimum being 0.80 - 0.82. Aflatoxins are generally produced in greater quantity at higher water activity values (0.98 to 0.99) with toxin production apparently ceasing at or near  $a_w$  0.85 (Sejakhosi et al., 2004).

#### 1.11. Key Environmental Conditions Affecting Production of Aflatoxin

In warm and humid subtropical and tropical conditions maize ears are ideal conditions for colonization and dominance of *A. flavus/parasiticus* species, resulting in the formation of aflatoxins. The aflatoxin group is carcinogenic and thus contamination results in significant economic impact. In groundnuts the presence of drought stress can lead to cracking of the pods and ingress by *A. flavus* and *A. parasiticus* resulting in significant aflatoxin accumulation (Magan and Olsen 2004).

The conditions conducive to germination, growth and aflatoxin production by *A. flavus* and *A. parasiticus* on in vitro media are shown [Fig 1.2]. This shows again that germination occurs over a wider range than that for growth, with the aflatoxin production range narrower than that for growth. Optimum conditions for aflatoxin production by these two species are at 33 °C and 0.99  $a_w$ ; while that for growth is 35 °C and 0.95  $a_w$ . It was showed that the impact of environmental factors on growth of *A. flavus*, *A. parasiticus* and *A. oryzae* was similar with minima of 0.82  $a_w$  at 25, 0.81 at 30 and 37 °C. However, this study did not include comparison of aflatoxin production by *A. flavus* and *A. parasiticus* (Magan and Olsen 2004).



It is worthwhile noting that in maize often the predominant species isolated was *A. flavus* with only about 10 % of isolates *A. parasiticus*. In groundnuts, the majority of isolates are usually *A. flavus* with *A. parasiticus* isolates about 30 % of the strains. Interestingly, studies in the USA suggest that *A. flavus* isolates almost always produce aflatoxin B1 and sometimes B2 in in vitro culture. However, *A. parasiticus* produces B1, B2, G1 and G2 under the same conditions (Magan and Olsen 2004).

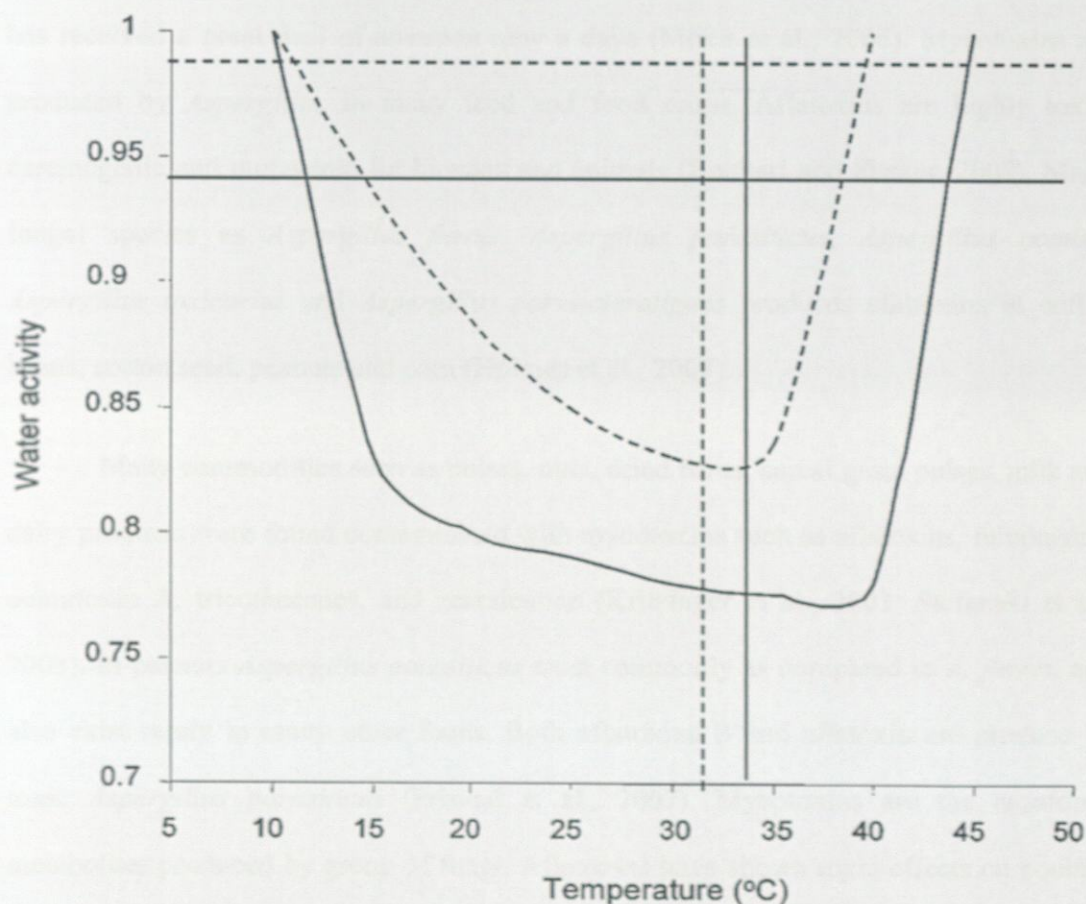


Fig 1.2: Comparison of profiles for growth and aflatoxin production by *Aspergillus flavus* and *A. parasiticus* (adapted from Hill et al., 1985). Optima for growth (for aflatoxin production (----)) are indicated by straight lines.

### 1.12. Contamination in Agricultural Commodities

In most developing countries, export crops are the main source of foreign exchange. Most of these crops are cereals, fruits, vegetables and oil seeds that are highly susceptible to fungal growth and mycotoxin production (Saeed et al., 2004). Due to High incidence of aflatoxin in agricultural commodities, which leads to important economical losses, the problem of food and feed contaminated by aflatoxin is of current concern and has received a great deal of attention now a days (Miren et al., 2006). Mycotoxins are produced by *Aspergillus* in many feed and food crops. Aflatoxins are highly toxic, carcinogenic and mutagenic for humans and animals (Bokhari and Shaker, 2008). Many fungal species as *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus nomius*, *Aspergillus toxicarius* and *Aspergillus parvisclerotigen* produces aflatoxins in coffee beans, cotton seed, peanuts and corn (Holmes et al., 2008).

Many commodities such as pulses, nuts, dried fruits, cereal grain pulses, milk and dairy products were found contaminated with mycotoxins such as aflatoxins, fumonisins, ochratoxin A, tricothecenes, and zearalenone (Kritzinger et al., 2003; Stefanaki et al., 2003). In peanuts *Aspergillus parasiticus* exist commonly as compared to *A. flavus*, and also exist rarely in many other foods. Both aflatoxins B and aflatoxin are produce by toxic *Aspergillus parasiticus* (Frisvad et al., 2007). Mycotoxins are the hazardous metabolites produced by group of fungi. Aflatoxins have shown toxic effects on poultry, live stock and human beings (Gowda et al., 2003, Passone et al., 2005).

Carcinogenic, neurotoxic and immunosuppressive effects occur in animals and humans due to utilization of feed and food contaminated by mycotoxins (Atroshi et al., 2002). The presence and concentration of aflatoxins food products including ground nuts, cereals and grains depends upon humidity and temperature conditions in the storage or in



field (Dhavan and Choudhary, 1995; Juan et al., 2008). Aflatoxins are the derivatives of difuranocoumarin and are produced by many strains of *Aspergillus parasiticus* and *Aspergillus flavus*. These most hazardous toxins have shown contamination in various feed and food products in many parts of the world (Bennett and Klich, 2003). Contamination of aflatoxins in agricultural commodities has received world wide importance due to their toxic effects on animal and human health and as well as their role in international import and export. In tropical countries the food contamination by fungi normally occurs before, during or after the time of harvest as well as in storage conditions (Ellis et al., 1991). Cotton, maize, peanuts and tree nuts are mainly contaminated due to the presence of these fungi (Yu et al., 1995).

Among the contaminated commodities maximum contamination was noticed in pistachio nuts (Molyneux et al., 2007; Pittet, 1998). Among mycotoxins, aflatoxins contamination in agricultural products has reported in preliminary surveys at Morocco (Zinedine et al., 2006). The most important arable crops in the Republic of Croatia are wheat and maize. They are frequently contaminated by moulds which, in favourable conditions, may produce mycotoxins (Jurjević et al., 2000).

Basmati rice (*Oryza sativa* Linn.) is the second most produced cereals in the world, wheat being the first. The annual world production of these two cereals is estimated at 500 million tons and Pakistan is one of the Asian countries wherein rice is cultivated and used as a basic food item. China heads this list with an annual production of 180 million tons (Sindarroz, 2003). It has reported that in many countries the contamination of rice with mycotoxins occurred (El-Gohary, 1996; Patel et al., 1996). Mycotoxin contamination has been found in about 25 % of cereals utilized in the world (Devegowda et al., 1998). Contamination of agricultural commodities with aflatoxins has



been reported in many crops including corn, rice, wheat, peanuts (Aly, 2002). The presence of mycotoxins in animal feed and cereal grains has been reported at worldwide (Shöllenberger et al., 1999; Dutta and Das, 2001). The contamination of maize by *Aspergillus flavus* took place frequently, which in turn produced aflatoxin B<sub>1</sub>, caused cytotoxic as well as carcinogenic affect in animals and human beings (Lewis 1999; Palanee et al., 2000). The fungus such as *Aspergillus ochraceus* and *Penicillium verrucosum* produced ochratoxins. It has also reported that the occurrence of high concentration of ochratoxin was found in the food of Balkans, due to which a disease endemic nephropathy has reported in the blood of people (Fink-Gremmel, 1995; Marth, 1992).

Soybean (*Glycine max* L.) is one of the most important oilseed crops cultivated. In India the seeds of soybean are heavily contaminated by the dominant fungi, *Aspergillus flavus* (Anon, 2005; Krishnamurthy and Raveesha, 1996). It has reported that aflatoxicoses caused many diseases in humans and animals, due to the utilization of feed and food contaminated by aflatoxins (Reddy and Raghavender, 2007). Feed contaminated with aflatoxins when ingested caused potentially hazardous effects on animal production. The toxic metabolites which excreted in eggs, meat and eggs of animals have also shown toxic effects (Ramos and Hernandez, 1997).

The presence of aflatoxins has been detected especially in peanuts and derived products, cereals, almonds, walnuts and spices whereas, the presence of OTA was documented in corn, barley, wheat, rice, coffee and wine (Dhavan and Choudary, 1995; Sabino et al., 1999; Trucksess et al., 1999; Visconti et al., 1999; Samira and Nadine, 2004). During the years spanning 1977–1994, high aflatoxin levels (more than 20 mg/kg) in maize were reported. Colonization of maize crops prior to harvest in the field (preharvest) by aflatoxigenic fungi often resulted in spoilage and aflatoxin accumulation



in post harvest grains during storage. In southern China, in Fujian and Guangxi Province, a comprehensive survey on aflatoxins in grains (maize and rice) was performed. Aflatoxin contamination generally occurs in the tropical and sub-tropical regions of the world but aflatoxin accumulation in food and feedstuff in northern China (Shandong and Henlongjiang Provinces) was reported. Some feedstuffs and maize from Liaoning Province were found to contain aflatoxins (Zuoxin et al., 2006). It has been reported that highest level of contamination as 107 µg/kg of aflatoxin B1 in pistachio. Analysed 29 samples of pistachio with shell and found the contamination by aflatoxin B1 in the range of zero to 165 µg/kg. In the year 1997 Dutch authorities reported that a large number of pistachio from Iran were contaminated with unacceptable levels of aflatoxins, this resulted in temporary import ban of Iranian pistachio in to the European Union from September 1997 (Abdulkadar et al., 2000).

### **1.13. Decontamination or Prevention Strategies**

Mycotoxins have detrimental effects, so a number of strategies have been developed to help prevent the growth of mycotoxigenic fungi as well as to decontaminate and/or detoxify mycotoxin contaminated foods and animal feeds. These strategies include, the prevention of mycotoxin contamination, detoxification of mycotoxins present in food and feed and inhibition of mycotoxin absorption in the gastrointestinal tract. The prevention of mycotoxin contamination prior to harvest or during post harvest and storage is not always possible necessitating decontamination before the use of such materials for food and feed purposes. Therefore various detoxification processes play an important role in helping prevent exposure to the toxic and carcinogenic effect of mycotoxins (Bulent et al., 2006). Detoxification of mycotoxins is typically achieved by



removal or elimination of the contaminated commodities or by inactivation of the toxins present in these commodities by physical, chemical, or biological methods (Sinha, 1998). It is generally accepted that mycotoxin levels in food needs to be reduced as low as technologically possible. Many physical, chemical and biological methods have been applied for the removal and biosynthesis of aflatoxins, and also growth of aflatoxigenic moulds inhibition. However, few of these have practical applications (Moss, 1992; Buser & Abbas, 2002; Lúias et al., 2002; Plorunhosa et al., 2002). Many methods have been applied in order to control or inhibit mould growth and maize contamination by aflatoxin, although strategies to control growth of mould and mycotoxin contamination has to be considered (Munkvold and Desjardin, 1997), for plants products postharvest methods are also recommended (Bankole and Adebajo, 2003).

The prevention of mycotoxin production includes all phases of food and feed production, because the mould contamination may occur in the field, during storage, as well as in transport. Adequate storage with optimal temperature and humidity of grains and relative humidity and the hygiene in silos may decrease the growth of toxicogenic moulds (Maja et al., 2002).

#### **1.13.1. Biological Decontamination**

Several herbal as well as some chemicals materials were tested in order to reduce production of aflatoxin and growth of mould, due to which some herbal compounds and chemicals showed inhibition of fungus *Aspergillus* growth and reduction in synthesis of aflatoxins. A mushroom extract showed inhibition of the formation of AFB1 -8, 9-epoxide (Lee et al., 2003). The application of competitive, non aflatoxigenic strains of *A. flavus* and *A. Parasiticus* has clearly been shown to reduce the aflatoxin contamination of agricultural commodities such as peanuts, rice, maize and cottonseed mainly through



competition for substrate and through the production of inhibitory metabolites (Cleveland et al., 2003; Dorner et al., 2003). It has been reported that the extracts of many medicinal and wild plants were evaluated against fungi producing aflatoxins, the antimicrobial properties in many species of plants have also been tested (Arora and Ohlon, 1997). Antipyretic, analgesic and anti-inflammatory properties have reported in *Nigella sativa* (Al-Ghamdi, 2001). The Kalongi (*Nigella sativa*) crude extracts showed antimicrobial effects on resistant bacteria of different antibiotics (Morsi, 2000). It has been investigated that natural antioxidants treatment for aflatoxin reduction occurred world wide. These finding verified that the metabolic pathways of microorganisms for aflatoxins was elucidated (Huwig et al., 2001). The antifungal and antimicrobial activities have reported for of *N. sativa* Crude extracts (Morsi, 2000; Khan et al., 2003) and antifungal activities. In Uganda many postharvest techniques have been investigated for the improvement in the quality and shelf life studies of the harvested produce (Agona and Nadhy, 1998).

Many plant species like tobacco, tagetes, tephrosia and neem (*Azadirachta indica*) were found effective for dry cereals and legumes against insect pests. The insect pests such *Acanthoscelides obtectus* and *Sitophilus*, which are common for beans and maize, respectively have been controlled by application of Neem leaves powder and kernel (Agona and Muyinza, 2000). The fungitoxic activities have been reported by several extracts of plants, while some of them i.e. cloves, cinnamon, Chinese cassia and thymes along with their mixture showed complete reduction in aflatoxins synthesis (El-Maraghy, 1995; Awuah, 1999; Yin and Tsao, 1999).

The antipyretic, antitumour, antimalarial and antifungal activities have been reported for different parts of neem tree, neem kernels, extracts of leaf and kernels during storage of grains (Awuah and Kpodo, 1996; Ragasa et al., 1997; Bagchi et al., 1999;



Sinha et al., 1999; Srinivasan et al., 2001). To reduce the toxic effect of aflatoxin B<sub>1</sub>, microorganisms as an antagonist have been used as treatment by biological means (Cho and Kang, 2000). Natural plant extracts and spices are also known to prevent mould growth and mycotoxin production (Dobson, 2002; Juglal et al., 2002; Paranagama et al., 2003). Specifically extracts from Egyptian plants namely, *Lupinus albus* (Leguminosae), *Ammi visnaga* (Umbelliferae), and *Xanthium pungens* (Compositae), have been shown to inhibit the growth of an aflatoxin of *A. flavus* in a dose-dependent manner (Mahmood, 1999). The inhibitory effects exerted by spices and herbs may rely at least in part on phenolic compounds such as coumarins and flavonoids (Juglal et al., 2002). There have been many reports on the ability of lactic acid bacteria to inhibit the mutagenic potential of mycotoxins. For example *Lactobacillus* sp has been shown to inhibit mutagenic potential of AFB<sub>1</sub>, AFB<sub>2</sub> and AFG<sub>1</sub> in the range of 56.6 - 77.4 %. *Flavobacterium aurantiacum* can significantly remove AFB<sub>1</sub> from a liquid medium and a variety of food products including milk, peanuts, and corn without leaving toxic by- products (Bulent et al., 2006).

#### 1.13.2. Physical Decontamination

In order to reduce partly or totally the contamination of mycotoxins and food processing, treatment by heat has been reported. More than 40 % contamination level in peanuts and corn being reduced by applying conventional methods of roasting (Samarajeeva, 1990; Taha et al., 2001), while more than 90 % reduction was found by roasting in a microwave oven (Pluyer, 1987). Different cooking methods such as pressure cooker, cooking with excess water and normal cooking have been evaluated for the reduction in contamination level of aflatoxins, although 75 % reduction in initial quantity was found effective by applying pressure cooker heating (Rehana, 1979).



Heating and roasting of peanuts reduced more than 40 % level of AFs (Rustom, 1997), whereas extrusion cooking method reported 33 % reduction in the level of aflatoxins (Buser and Abbas, 2002). There are several physical methods of decontamination of agricultural products known to us such as the removal of damaged grains or of a part of contaminated crop, washing procedures, radiation, ultrasound and extraction with organic solvents. It is very difficult to remove AFs since they are heat resistant and soluble in intermediate polar solvents (Maja et al., 2002). The removal of damaged parts of a crop (usually mould contaminated) is possible when contamination is uneven or partial. Physical removal of discoloured, damaged or inadequately developed peanut kernels significantly decreases the concentration of aflatoxins, fumonisins and ergot alkaloids (Scott, 1998). Although this is the most widely used decontamination technique in the peanut and pistachio industry (Pearson et al., 2001), it is not practical for maize and cottonseed (Scott, 1998).

Blanching and electronic eye colour sorting of raw peanuts contaminated by *Aspergillus flavus*, and damaged peanut kernels decrease the concentration of aflatoxins down to under 5 µg / kg (De Koe, 1999). Fluorescent sorting can be used for maize, cottonseed and dried figs, but it is ineffective for the decontamination of peanuts (Scott, 1998). Flotation may lower high concentrations of aflatoxins in contaminated maize and peanuts by as much as 90 %, because contaminated seeds float on water (Phillips et al., 1994).

Radiation has proven useful in the post harvest control of mycotoxins in storage, in addition however ionizing involving x - rays and γ rays have been extensively investigated as methods for the degradation of mycotoxins (Scott, 1998). Irradiation is a non-thermal treatment and is increasingly being referred to as “cold pasteurization” given



that it can eliminate food borne pathogens without increasing in product temperature, and that overall average doses of 10 kGy presents no toxicological hazard (Aziz and Moussa, 2002). 74.8 % of aflatoxins (B1 + B2) were degraded by heating at 100 °C for 1 h at a moisture content of 30 % in contaminated cottonseed meal, while 32.7 % of the degradation occurred in a similar meal containing 6.6 % moisture, under similar conditions. It has been suggested that the presence of moisture in foods helps in opening the lactone ring in AFB1 to form a terminal carboxylic acid; which then undergoes a heat induced decarboxylation. Reductions in the levels of contamination in peanuts, in the range of 50 – 60 % and 32 – 40 % for AFB1 and AFG1 respectively; after microwave roasting at 0.7 kw for 8.5 min (Maja et al., 2002).

An enzyme treatment of peanuts meal followed by microwave treatment at 1 kw for 15 min increased the degradation level of AFB1 from 53 % to 97 % without affecting the organoleptic qualities of the meal (Das and Mishra, 2000), while microwave roasting at 900 watt for 1.5 min has been shown to reduce AFB1 levels by 50.3 %, 30.5 %, and 38.5 % in unsalted, 2 % and 5 % salted peanuts, respectively (Ozkarsli, 2003). There are certain regulations for the maximum level of aflatoxins in many countries, whereas USA, European union have suggested level below than 20 ppb, while Japan and Korea suggested 10 ppb limit for aflatoxins in their foods (Chiavaro et al., 2001).

#### **1.13.3. Chemical Decontamination**

A wide range of chemicals have been shown to reduce, destroy, or inactivate mycotoxins. These chemicals include (a) acids (e.g. hydrochloric acid), (b) bases (e.g. ammonia, sodium hydroxide), (c) oxidizing agents (e.g. hydrogen peroxide, ozone), (d) reducing agents (e.g. bisulfite), (e) chlorinating agents (e.g. sodium hypochlorite and chlorine dioxide), and (f) miscellaneous reagents such as formaldehyde (Maja et al.,



2002). Organic solvents (ethanol, isopropanol, methoxymethane) effectively remove aflatoxins from different types of food products. In addition to the limiting high cost of organic solvents, these compounds are not practical for industrial use because they themselves are removed from the treated products with difficulty (Phillips et al., 1994). Hydrogen peroxide and ammonia are mostly used to remove aflatoxins from feed. It has been shown that these methods do not leave toxic metabolites of mycotoxins in feed, but the ammonia reduces its nutritional value by decreasing lysine and sulphur containing amino acids (Scott, 1998). Ammoniation is considered safe and practical for the decontamination of aflatoxins in feed, and it is used in some states of the USA, Mexico, France, Senegal, Sudan, and Brazil (Guerre, 2000). In chemical decontamination methods, treatment by application of ammonia in many raw materials showed high efficacy in order to decontaminate aflatoxins (Buser and Abbas, 2002).

Feed additives reduce mycotoxin bioavailability by binding them in the gastrointestinal system. Although a number of adsorbents are shown to be active in vitro, this is not predictive for their activity in vivo. Hydrated sodium calcium aluminosilicates, zeolites, bentonite, active charcoal, clays, synthetic anion exchange resins (cholestyramine), and alfalfa fibre have already established their efficiency. Zeolites are hydrated alkaline aluminosilicates that adsorb AFB1 from feed (Maja et al., 2002; Piva et al., 1995). Chemical detoxification is very effective, but it does not meet the FAO requirements, because some compounds leave behind their toxic metabolites and others reduce the nutritional value of treated food and feed.

#### **1.14. Worldwide Limits for Aflatoxins in Food**

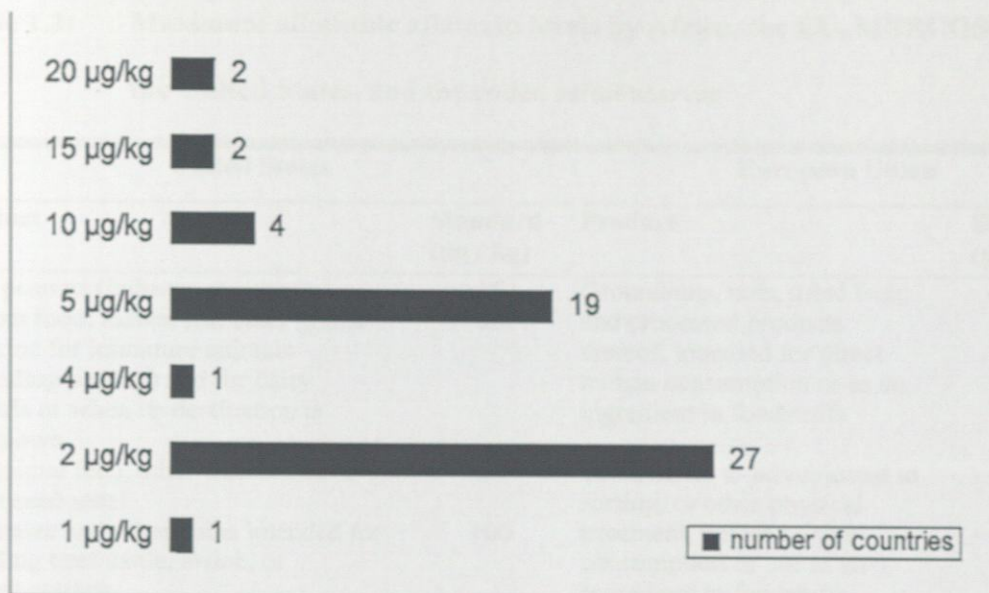
Naturally occurring toxins such as aflatoxins pose profound challenges to food safety in both developed and developing countries. The knowledge that aflatoxins can



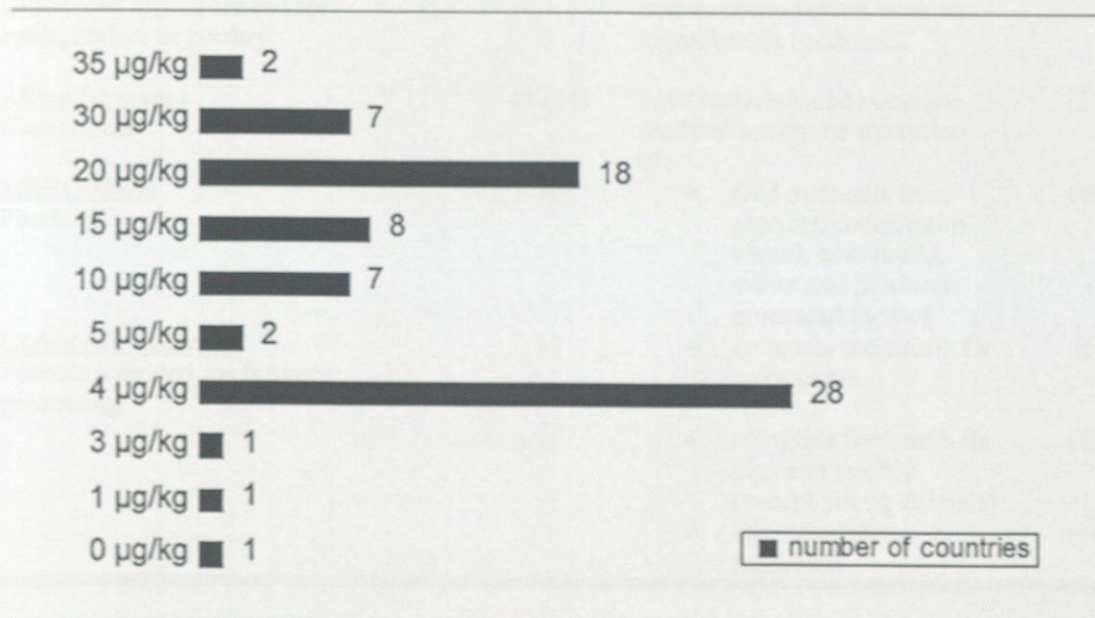
have serious effects on humans and animals has led many countries to establish regulations on aflatoxins in food and feed in the past few decades to safeguard public health, as well as the economic interests of producers and traders. A wide range of aflatoxin standards and corresponding regulatory requirements exist worldwide. In general, more stringent aflatoxins standards are found in wealthy industrialized countries with more developed market economies than in developing countries where subsistence farming still prevails. Countries in the European Union (EU) have historically had the most stringent regulations for aflatoxins in the world. Their newly adopted harmonized aflatoxin standards have set tolerance levels much lower than those in the developing countries and the Codex Alimentarius [Table 1.2 and 1.3].

The range of limits was narrowed a little ( $1\mu\text{g/kg}$ ) and the median is at  $2\mu\text{g/kg}$ , a limit now in force in 27 countries. Most of these countries belong to the EU. Another major limit is visible at  $5\mu\text{g/kg}$ , followed by 19 countries, spread over Africa, Asia/Oceania, Latin America and Europe [Fig. 1.2]. In 2002 many countries regulated aflatoxins in food with limits for the sum of the aflatoxins B1, B2, G1 and G2. The limits range from  $1\text{--}35\mu\text{g/kg}$ , whereas the most frequently occurring limit is at  $4\mu\text{g/kg}$  (applied by 28 countries), again a limit found back in the harmonized regulations in the EU, EFTA and candidate EU countries, where dual limits both for aflatoxin B1 and for total aflatoxins are enforced. Another major peak occurs at  $20\mu\text{g/kg}$ , applied by 18 countries [Fig 1.3] (Magan and Olsen, 2004), with half of them in Latin America (where it is also a MERCOSUR harmonized limit), and several in Africa. Also the USA, one of the first countries to establish an aflatoxin action limit, follows the  $20\mu\text{g/kg}$  limit. More than 50 countries have established or proposed regulations for controlling aflatoxins in foods and feeds, and at least 15 countries have levels of other mycotoxins (Rajasekaran et al., 2005).





**Fig 1.2:** Frequency distribution of specific regulatory limits for aflatoxin B1 in food in 56 countries, in 2002



**Fig 1.3:** Frequency distribution of specific regulatory limits for the sum of aflatoxins B1, B2, G1 and G2 in food in 75 countries, in 2002

**Table 1.2: Maximum allowable aflatoxin levels by Africa, the EU, MERCOSUR, the United States, and the codex alimentarius**

| United States                                                                                                                                    |                    | European Union                                                                                                                               |                    |
|--------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|----------------------------------------------------------------------------------------------------------------------------------------------|--------------------|
| Product                                                                                                                                          | Standard (µg / kg) | Product                                                                                                                                      | Standard (µg / kg) |
| Raw peanuts (industry standard)                                                                                                                  | 15                 | Groundnuts, nuts, dried fruit, and processed products thereof, intended for direct human consumption or as an ingredient in foodstuffs       | 4 (2)              |
| Human food, maize, and other grains intended for immature animals (including poultry) and for dairy animals or when its destination is not known | 20                 |                                                                                                                                              |                    |
| For animal feed, other than maize or cottonseed meal                                                                                             | 20                 | Groundnuts to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs           | 15 (8)             |
| For maize and other gains intended for breeding beef cattle, swine, or mature poultry                                                            | 100                |                                                                                                                                              |                    |
| For maize and other grains intended for finishing swine of 100 pounds or greater                                                                 | 200                | Nuts and dried fruit to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs | 10 (5)             |
| For maize and other grains intended for finishing beef cattle and for cottonseed meal intended for cattle, swine, or poultry                     | 300                | Cereals and processed products thereof, intended for direct human consumption or as an ingredient in foodstuffs                              | 4 (2)              |
| <u>Africa (average)</u><br>Groundnuts                                                                                                            | 44 (14)            | Feed materials and complete feedstuffs with the exception of:                                                                                | (50)               |
| <u>MERCOSUR</u><br>Foodstuffs                                                                                                                    | 20                 | • feed materials from peanuts, copra, palm-kernel, cottonseed, maize and products processed thereof                                          | (20)               |
| <u>Codex Alimentarius</u><br>Peanuts intended for further processing                                                                             | 15                 | • complete feedstuffs for dairy cattle                                                                                                       | (5)                |
|                                                                                                                                                  |                    | • complete feedstuffs for pigs and poultry (except young animals)                                                                            | (20)               |
|                                                                                                                                                  |                    | • other complete feedstuffs                                                                                                                  | (10)               |

Note: Standards reported are for total aflatoxin B1+B2+G1+G2. Figures in parentheses are separate standards for aflatoxin B1.

EU standards are after harmonization in 2002.

Sources: Dohlman 2003, Otsuki et al. 2001, and FAO 2004.



Table 1.3: Maximum aflatoxin levels in different food commodities

| Products                          |                                            |                        | EU Aflatoxin<br>Maximum<br>Levels<br>(2006) |                             | EU Aflatoxin<br>Maximum<br>Levels<br>(amended, 2010) |                             |
|-----------------------------------|--------------------------------------------|------------------------|---------------------------------------------|-----------------------------|------------------------------------------------------|-----------------------------|
|                                   |                                            |                        | B1                                          | Total<br>(B1,B2,<br>G1, G2) | B1                                                   | Total<br>(B1,B2,<br>G1, G2) |
| Tree nuts                         | Almonds,<br>pistachios,<br>Apricot kernels | Ready-to eat           | 2                                           | 4                           | 8                                                    | 10                          |
|                                   |                                            | For further processing | 5                                           | 10                          | 12                                                   | 15                          |
|                                   | Hazelnuts,<br>Brazil nuts                  | Ready-to eat           | 2                                           | 4                           | 5                                                    | 10                          |
|                                   |                                            | For further processing | 5                                           | 10                          | 8                                                    | 15                          |
|                                   | Other tree nut                             | Ready-to eat           | 2                                           | 4                           | 2                                                    | 4                           |
|                                   |                                            | For further processing | 5                                           | 10                          | 5                                                    | 10                          |
| Oilseeds<br>(Not for<br>crushing) | Peanuts                                    | Ready-to eat           | 2                                           | 4                           | 2                                                    | 4                           |
|                                   |                                            | For further processing | 8                                           | 15                          | 8                                                    | 15                          |
|                                   | Other Oilseeds                             | Ready-to eat           | -                                           | -                           | 2                                                    | 4                           |
|                                   |                                            | For further processing | -                                           | -                           | 8                                                    | 15                          |
| Cereals                           | Corn                                       | Ready-to eat           | 2                                           | 4                           | 2                                                    | 4                           |
|                                   |                                            | For further processing | 5                                           | 10                          | 5                                                    | 10                          |
|                                   | Rice                                       | Ready-to eat           | 2                                           | 4                           | 2                                                    | 4                           |
|                                   |                                            | For further processing | -                                           | -                           | 5                                                    | 10                          |
|                                   | Other Cereals                              | Ready-to eat           | 2                                           | 4                           | 2                                                    | 4                           |
|                                   |                                            | For further processing | -                                           | -                           | -                                                    | -                           |
| Spices                            | Capsicum spp.                              | Ready-to eat           | 5                                           | 10                          | 5                                                    | 10                          |
|                                   | Chillies                                   | Ready-to eat           | 5                                           | 10                          | 5                                                    | 10                          |
|                                   | Black pepper                               | Ready-to eat           | 5                                           | 10                          | 5                                                    | 10                          |
|                                   | Red pepper                                 | Ready-to eat           | 5                                           | 10                          | 5                                                    | 10                          |
|                                   | Ginger                                     | Ready-to eat           | 5                                           | 10                          | 5                                                    | 10                          |
|                                   | Turmeric                                   | Ready-to eat           | 5                                           | 10                          | 5                                                    | 10                          |
|                                   | Mixture of<br>spices above                 | Ready-to eat           | 5                                           | 10                          | 5                                                    | 10                          |

Note: Maximum levels are for aflatoxin B1 and total aflatoxin B1+B2+G1+G2.  
Commission Regulation (EU) No 165/2010 published on February 27, 2010  
amends Regulation (EC) No 1881/2006 setting maximum levels for aflatoxins.

Sources: Gerda & Kurt (2010) Report Number: E50018, EU-27;  
Commission Regulation (EU) 2006 & 2010

### 1.15. Aims and Objectives of the Present Study

- To investigate the contamination level of aflatoxins in cereals, beans, dried fruits and nuts.
- To create awareness about hazards of these toxins to human/animal health by quantifying the incidence and severity of food contamination.
- To establish a data collection on the occurrence of these toxins in Pakistani foods, due to the significant health risks associated with the presence of mycotoxins, especially aflatoxins in foods.
- To apply method suitable for the control of aflatoxins contamination and quality control of food.
- To develop inexpensive, practical method to eliminate or reduce the levels of aflatoxin in food to permissible limits.
- To enable small-scale farmers in the semi-arid tropics and stockholders to make available aflatoxin-free food; which in turn will enhance the export.
- Consequently the project will not only help in alleviating the toxic effects of these toxins but will also improve the quality of the dietary products.
- To improve the nutritional status and enhance the utilization of cereals, dried fruits and nuts; in turn will be the backbone of the economy of the country.



# CHAPTER – II

## MATERIALS

## AND

## METHODS

## **2. MATERIALS AND METHODS**

### **2.1. Chemical and Reagents**

All the reagents and solvents of HPLC grade were used and obtained from Merck Darmstadt, Germany. Deionized water of LC grade was obtained by filtering through 0.45  $\mu\text{m}$  filter having Waters Millipore system Milford, MA, USA. An ultrasonic water bath (Model EIA CP104, Italy) was used for 20 minutes to degassed water and solvents. Phosphate buffer saline (PBS) and trifluoroacetic acid (TFA) used were from Sigma, St. Louis, MO, USA. Standards of aflatoxin B1 (2.02  $\mu\text{g/ml}$ ), aflatoxin B2 (0.520  $\mu\text{g/ml}$ ), aflatoxin G1 (2.01  $\mu\text{g/ml}$ ) and aflatoxin G2 (0.500  $\mu\text{g/ml}$ ) were purchased from Biopure (Tecknopark Tullin, Austria). Standard stock solutions of AFB1 and AFB2 of concentrations 1  $\mu\text{g/ml}$  each were prepared by diluting in benzene/ acetonitrile (98:2; v/v). These stock solutions were kept in refrigerator at  $-4\text{ }^{\circ}\text{C}$  in dark as aflatoxins gradually breakdowns under UV light.

### **2.2. Sampling of Dried Fruits and Nuts**

Samples of dried fruits and nuts ( $n = 180$ ) includes were; 20 dried apricot, 20 dates, 10 dried figs, 15 mulberries, 10 dried raisins, 15 apricot kernels, 10 almonds with shells, 10 almonds without shells, 10 peanut with shells, 10 peanuts without shells, 10 pistachios with shells, 10 pistachios without shells, 10 walnuts with shells, 10 walnuts without shells and 10 pine nuts with shell) were collected or purchased from retail shops and local markets of Northern areas and different locations of Pakistan during the period from January to November 2009.



### 2.3. Sampling of Cereals and Beans

Samples of cereals and beans ( $n = 160$ ) includes were 20 rice, 20 rice broken, 20 wheat, 15 maize, 10 barley and 10 sorghum, 10 red kidney beans, 15 split peas, 10 chick peas, 10 cow peas and 20 soybean) were purchased from retail shops and local markets of different locations of Pakistan during the period from January to November 2009. The collected samples ranging from 2 to 3 Kg were brought to Mycotoxin Laboratory at Pakistan Council of Scientific and Industrial Research Laboratories Complex Peshawar, Pakistan.

As the amount of mycotoxins has not uniform distribution in all the food, so in order to obtain effective distribution of samples and maximum reduction of particle size for analyses an acceptable procedure for sampling is adapted. The 500 g representative samples were taken from each collected sample after through mixing. Finally a 200 g subsample was subjected to fine grinding in a Grinding mill, Model RAS Mill EQMMR 1017 Romer, Labs, Inc., MO, USA, and passed it through No. 20 mesh size sieve or prepared paste and immediately dispensed into plastic bags. These samples were kept under  $-4^{\circ}\text{C}$  till analyses for aflatoxins.

### 2.4. Apparatus

The HPLC system consisted of Hitachi model L-2000 equipped with two pumps L-2130, autoinjector L-2200 and fluorescence detector L-2458 was used for this study obtained from Macao, Japan. The guard column, Intersil ODS - 3,  $5\ \mu\text{m}$  (GL sciences Inc. Tokyo, Japan) was placed between the auto injector and the separative column, Intersil ODS - 3 (25 cm x 4.6 mm I.D.,  $5\ \mu\text{m}$ , GL science, Tokyo, Japan) column. Photochemical reactor, UVE (LC Tech, Germany) for post column derivatization of Aflatoxin was equipped between separative column and fluorescence detector.

## **2.5. Extraction Procedure**

The analyses of aflatoxins in dried fruits, edible nuts, cereals and beans were carried out by reported method of Stroka et al. (2000). Briefly, in 10 gram of pistachio, peanut, and walnut added one gram NaCl along with 40 ml of methanol / water (80:20). In mixture added 20 ml of cyclohexane and blended for 3 minutes. Poured into separating funnel to separate the two phases and than the cyclohexane was removed. Samples of dried fruits were extracted only with 40 ml of methanol/water (80:20). Extracts were filtrated through Whatman filter paper No. 4.

## **2.6. Immunoaffinity Column Clean-up**

An aliquot of 10 ml was diluted with 60 ml of PBS buffer (pH 7.4). An immune affinity column IAC AflaTest, Vicam, USA was conditioned with 10 ml of PBS buffer by gentle syringe pressure at a flow rate of 5 ml / min. Then, the mixture of the filtrate diluted extract (70 ml) was applied to the IAC column (1 - 2 drops per second), followed by a washing with 20 ml of bi distilled water and then dried with air. Aflatoxins were then slowly eluted from the IAC with 2 ml methanol into a glass vial.

## **2.7. LC Analysis**

The mobile phase consisted of acetonitrile / methanol / water (8:27:65, v/v/v). Degassing of the mobile phase was done by sonication. The column Intersil ODS - 3 (25 cm x 4.6 mm I.D., 5  $\mu$ m, GL science, Tokyo, Japan) was connected as LC column. The column was maintained at 40 °C with a flow rate of 0.8 ml / min. The aflatoxins were detected at the excitation and emission wavelengths of 365 and 450 nm, respectively. The injection volume was 20  $\mu$ l. For quantitation of aflatoxins in samples, a separate



calibration curve was established for each aflatoxin. Triplicate samples were used for setting calibration curve, determining LODs and extraction recovery.

## **2.8. Performance of Method**

The procedure for extraction of the aflatoxins was optimized. Spiking of all uncontaminated samples with standard solution of aflatoxins was carried out for recovery studies. In this regard samples of dried fruits and edible nuts were spiked in triplicate with AFB1, AFG1, AFB2 and AFG2 in different concentration i.e. 0.5, 2.5, 5.0 and 25  $\mu\text{g} / \text{kg}$ . The extraction and analyses of these spiked samples were carried out by above mentioned procedure. The limits of detection (LOD) in samples of dried fruits contains 5  $\text{mg} / \text{kg}$  AFB1, AFG1, AFB2 and AFG2 was calculated by signal-to-noise ratio as  $S / N = 3$  and  $S / N = 10$ . The LOD were 0.5  $\mu\text{g} / \text{kg}$  for AFB1 and AFG1 and 1.0  $\mu\text{g} / \text{kg}$  for AFB2 and AFG2.

## **2.9. Application of Methods for Decontamination**

The decontamination by different methods was conducted. In this regard three techniques were evaluated to decontaminate the mycotoxin (aflatoxin, ochratoxin-A) contaminated agricultural commodities.

### **2.9.1. Biological Method**

In a preliminary study chemicals, herbal compounds/spices and plants at different weights (0.1, 0.2 and 0.5 g) each were evaluated for the growth inhibition of toxic strains of two fungus i.e. *Aspergillus flavus* (Accession No.1000) and *Aspergillus parasiticus* (Accession No.1002). Their effects on the reduction of aflatoxins production were also evaluated in vitro.

## 2.9.2. Collection of Samples

### 2.9.2.1. Chemicals

Nine chemicals are given below;

1. Ammonia solution, 20 % ( $\text{NH}_3$ )  
 $M = 17.03 \text{ g/mol}$
2. Benzoic acid ( $\text{C}_7\text{H}_6\text{O}_2$ ) (Merk, Germany)  
 $M = 122.12 \text{ g/mol}$
3. Citric acid – ( $\text{C}_6\text{H}_8\text{O}_7$ ) (Scharlau, Spain)  
 $M = 192.13 \text{ g/mol}$
4. Copper sulphate ( $\text{CuSO}_4$ ) – Extra pure (Sigma-Aldrich, Germany)  
 $M = 159.60 \text{ g/mol}$
5. Potassium metabisulfite ( $\text{K}_2\text{S}_2\text{O}_5$ -98%) (Sigma-Aldrich, Germany)  
 $M = 222.33 \text{ g/mol}$
6. Propionic acid ( $\text{C}_3\text{H}_6\text{O}_2$ ) (Merck, Germany)  
 $M = 74.08 \text{ g/mol}$
7. Sodium chloride ( $\text{NaCl}$ ) (Merck)  
 $M = \text{g/mol}$
8. Sodium propionate ( $\text{C}_2\text{H}_5\text{CO}_2 \text{ Na}$ -99%) (Aldrich)  
 $M = 96.06 \text{ g/mol}$
9. Urea ( $\text{CH}_4\text{N}_2\text{O}$ ) (Scharlau, Spain)  
 $M = 60.06 \text{ g/mol}$

### 2.9.2.2. Herbal Compound/Spices

Nine (09) samples of different herbal compounds/spices, known for their medicinal value in traditional medicine and generally available in Pakistan were selected



for the study. The list of herbal compound/spices, the family to which they belong and parts used for antifungal activity is presented [Table 2.1].

#### **2.9.2.3. Plants**

Nine (09) samples of different plant species, generally available in Pakistan were selected for the study and identified by Botany Department, University of Peshawar, Pakistan. The list of plants tested for antifungal activity, the family to which they belong and parts used is presented [Table 2.2].

### **2.9.3. Processing of Samples**

#### **2.9.3.1. Chemicals**

Chemicals in different weights (0.1 g, 0.2 g and 0.5 g) were added to the freshly prepared growth media of *Aspergillus flavus* (Accession No.1000) and *Aspergillus parasiticus* (Accession No.1002) separately in 250 ml Erlenmeyer flasks containing 100 ml sterile potato dextrose agar (PDA). The concentration obtained was 0.1, 0.2 and 0.5 % for each chemical respectively.

#### **2.9.3.2. Herbal Compounds/Spices**

The herbal compounds (ajowain, clove, kalonji) and spices (coriander, cinnamon and turmeric) after sterilization were dried in hot air oven for 1-2 days at 50 °C, ground to powder form in warring blender and sieved with 1 mm mesh. These samples along with kalonji oil, clove oil and neem oil were added to the growth media of *A. flavus* and *A. parasiticus* separately in 250 ml Erlenmeyer flasks containing freshly prepared 100 ml sterile potato dextrose agar (PDA). The concentration obtained was 0.1, 0.2 and 0.5 % for each sample respectively.

**Table 2.1:** Common and scientific names of some herbal compounds/spices used to detect their antifungal activities.

| Common name | Scientific name             | Family        | Part used of plant |
|-------------|-----------------------------|---------------|--------------------|
| Ajowain     | <i>Trachyspermum ammi</i>   | Apiaceae      | Seed               |
| Clove       | <i>Eugenia caryophyllus</i> | Myrtaceae     | Flower buds        |
| Clove       | <i>Eugenia caryophyllus</i> | Myrtaceae     | Seed oil           |
| Cinnamon    | <i>Cinnamomum burmani</i>   | Lauraceae     | bark               |
| Coriander   | <i>Coriandrum sativum</i>   | Apiaceae      | fruit              |
| Kalonji     | <i>Nigela sativa</i>        | Ranunculaceae | Seed               |
| Kalonji     | <i>Nigella sativa</i>       | Ranunculaceae | Oil                |
| Neem        | <i>Azadirachta indica</i>   | Meliaceae     | Seed oil           |
| Turmeric    | <i>Curcuma longa</i>        | Zingiberaceae | Rhizome            |

**Table 2.2:** Common and scientific names of some plants used to detect their antifungal activities.

| Common name | Scientific name            | Family         | Part used of plant |
|-------------|----------------------------|----------------|--------------------|
| Carrot      | <i>Daucus carota</i>       | Apiaceae       | Whole              |
| Garlic      | <i>Allium satium</i>       | Amaryllidaceae | Bulb               |
| Ginger      | <i>Zingiber officinale</i> | Zingiberaceae  | Rhizome            |
| Lemon grass | <i>Cymbopogon citratus</i> | Poaceae        | Leaf               |
| Mint        | <i>Mentha longifolia</i>   | Lamiaceae      | Leaf               |
| Neem        | <i>Azadirachta indica</i>  | Meliaceae      | Leaf               |
| Onion       | <i>Allium cepa</i>         | Amaryllidaceae | Bulb               |
| Sour orange | <i>Citrus aurantium</i>    | Rutaceae       | Peel               |
| Tulsi       | <i>Ocimum sanctum</i>      | Lamiaceae      | Leaf               |



### 2.9.3.3. Plants

The plants were obtained from the local market and immediately kept in refrigerator until starting the experiments. The samples were brought to the laboratory and the healthy parts of the plants were washed thoroughly 2-3 times with running tap water, then with 2% sodium hypochlorite solution and subsequently with sterile distilled water. Different weights of these plants (1.0 g, 2.0 g, and 5.0 g) were dried in hot air oven for 1-2 days at 50 °C, ground to powder form in warring blender and sieved with 1 mm mesh. These samples were added to the growth media of *Aspergillus flavus* and *Aspergillus parasiticus* separately in 250 ml Erlenmeyer flasks containing freshly prepared 100 ml sterile potato dextrose agar (PDA). The concentration obtained was 0.1, 0.2 and 0.5 % for each sample respectively.

### 2.9.4. Screening of Materials for Anti Fungal Activities

In biological decontamination, the study on inhibition of fungal (*Aspergillus flavus* and *Aspergillus parasiticus*) growth and inhibition in aflatoxin production were evaluated in vitro. The anti-fungal activity of some chemicals, herbal compounds/spices and plants at different concentrations were evaluated.

#### 2.9.4.1. Fungal Strains

The pathogenic fungal strains as *Aspergillus flavus* (Accession No.1000) and *Aspergillus parasiticus* (Accession No.1002) were purchased from Institute of Mycology and Plant Pathology; University of the Punjab, Pakistan was used throughout this study.

#### 2.9.4.2. Preparation of Spore Suspension

A potato dextrose agar (PDA) slant (Oxide, USA) was used for maintaining the cultures of each fungus at  $25 \pm 2$  °C for 7 days, then spores were removed from the

sporulating colonies and poured in sterile distilled water contains Tween 80, 0.1 % (v/v). The suspension of spore was transferred into a sterile bottle and the number of spores was counted by an improved Neubauer Haemocytometer.

#### 2.9.4.3. Inhibition of Fungal Growth

All the processed samples were thoroughly mixed with the medium (PDA) after autoclaving, then media (100 ml) was transferred into 9 cm petri dishes, each in triplicate and then cooled. The media without tested samples served as control. After complete solidification of the medium to these petri-dishes, 0.1 ml diluted spore suspension ( $25 \times 10^7$  CFU / ml) of *Aspergillus parasiticus* (Accession No.1002) and *Aspergillus flavus* (Accession No.1000) were added, spread, sealed with parafilms and incubated for 7 days at 27–30 °C for the control reach of full growth. Anti-fungal properties of the samples were judged by counting the colonies on the 7<sup>th</sup> day. Fungal growth was measured for each colony and Percent Inhibition (I %) of the fungal growth was calculated according to the following formula:

$$\% I = (\text{growth in control} - \text{growth in treatment} / \text{growth in control}) \times 100$$

The samples which tested the mycelial growth inhibition for *A. flavus* and *A. parasiticus* were further subjected to poisoned food techniques at different concentrations against pathogenic tested fungi. This selection was based on the significant antifungal activity of the screened samples.

#### 2.10. Evaluation of Anti Fungal Activities on Reduction of Total Aflatoxin

##### Production in Maize

The sterile maize grains (50 g) were weighed to a series of 500 ml conical flasks. The initial moisture content of the substrate was adjusted to 23 % by adding sterile



distilled water. Based on the results of preliminary screening, different concentrations (0.1, 0.2 and 0.5%) of the selected test samples were added to these flasks in triplicate, except for the control flasks. All flasks were then autoclaved at 121 °C for 15 min, inoculated with 0.1 ml spore suspension ( $25 \times 10^7$  CFU / ml) of *Aspergillus parasiticus* (Accession No.1002) and *Aspergillus flavus* (Accession No.1000) and incubated separately for each samples at 27–30 °C in the dark for 7 days.

#### **2.10.1. Fungal Spore Count**

A representative sample, one gram from each flask was used for spore counting. Spores of *A. flavus* and *A. parasiticus* were harvested from and maize using sterile distilled water and Tween 80 (0.1 %). The suspension of spore was filtered and transferred to a sterile bottle; the numbers of spores were counted using a haemocytometer and represented as (log CFU / g). The remaining samples in flasks were autoclaved at 121 °C for 15 minutes, dried in oven, grinded to powder form and analyzed for total aflatoxin.

#### **2.10.2. Aflatoxin Analysis**

After autoclaving each flask took 25 gram of sample in triplicate and analyzed for total aflatoxin as previously described method (Stroka et al., 2000).

#### **2.10.3. Statistical Analysis**

One way analysis of variance (ANOVA) was applied to data obtained for spore counts and analyses of aflatoxin for each sample separately. The mean values were tested for al significance difference by Duncan's Multiple Range Test (DMRT) at the 5% level (Snedecor and Cochran, 1989).

## **2.11. Physical Method for Decontamination of Aflatoxin B1 in Different Wheat Varieties**

### **2.11.1. Collection of Samples**

Total 30 samples of different local wheat varieties such as Auqab, Ghaznavi-98, Saleem- 2000, Kyber-87, Pirsabak-2005 were acquired from NIFA Peshawar and Agricultural University Peshawar, Khyber Pukhtoonkhwa.

### **2.11.2. Processing of Samples**

Wheat varieties were artificially contaminated with aflatoxin B1 (AFB1). For washing, the contamination levels employed on wheat varieties, [(Auqab, Ghaznavi-98) and (Saleem-2000, Kyber-87, Pirsabak-2005)] were 43 µg/kg and 46 µg/kg, respectively, for AFB1. For heating treatment, the contamination levels employed on dried wheat samples [(Auqab, Saleem-2000) and (Ghaznavi-98, Kyber-87, Pirsabak-2005)] were 100 µg/kg and 55 µg/kg, respectively, for AFB1, while on the wet wheat samples [(Auqab), (Saleem-2000) and (Pirsabak-2005)] were 28.6 µg/kg, 14.86 µg/kg and 18.56 µg/kg, respectively. Adequate amount of the standard dissolved in benzene were dripped on to 100 g portions of samples held in 600 mL beakers, taking precautions so as to avoid contaminating the wall. After 24 h washing and heating of contaminated wheat was done for the reduction of aflatoxin level.

### **2.11.3. Washing and Heating**

Twenty gram wheat sample contaminated with aflatoxin B1 was added in 200 mL water and stirred for 10, 20 and 30 minutes at 155 rpm. After that the sample was heated in an oven at various temperatures for 30, 60 and 90 minutes, the concentration of AFB1



was analyzed by TLC. Soaking of wheat in water was done to obtain 10 % moisture content of wet wheat, and heated at 100 °C for 30 minutes. Then, homogenization of wheat and analysis of aflatoxin B1 was carried out by TLC by standard method (AOAC, 2000). The wet and dry wheat samples were analyzed for AFB1 in the same way.

## **2.12. Chemical Analyses**

### **2.12.1. Chemicals and Standards**

The analytical grade chemicals were used of BDH (Poole, England), Sigma (St. Louis, USA) and Merck (Darmstadt, Germany). Standard of aflatoxin B1 (2.02 µg/mL) was purchased from Biopure (Tecknopark Tullin, Austria). Standard stock solution of AFB1 of concentration 1 µg / mL was prepared by diluting in benzene/ acetonitrile (98:2; v/v). This stock solution was then stored at 4 °C in refrigerator.

### **2.12.2. Determination of Aflatoxin B1**

The determination of aflatoxin B1 carried out by standard method (AOAC, 2000) by thin layer chromatography. Briefly, 250 mL acetone/ water (85:15 v/v) was added in 50 g sample, blended for 3 minutes and filtered through Whatman filter paper No. 4. to obtain clear filtrate in beaker. After that, 170 mL of sodium hydroxide (0.02 N) was added in 30 mL ferric chloride in one flask, while 3 g copper carbonate were added to the 150 mL filtrate in other flask, mixed both the flask together and filtered. About 150 ml filtrate poured into separating funnel contains 250 mL of sulphuric acid (0.03 %) and extracted twice with 10 mL of chloroform. Lower chloroform extract layer was transferred to another separating funnel containing 100 mL of potassium hydroxide (0.02 M), swirled gently for 30 sec and left for layer separation. The chloroform extract layer was collected in a vial and 8 mL of it was dried by evaporating on heating block at 45 °C

with stream of nitrogen. The dry residue was redissolved in 200 µl of benzene / acetonitrile (98:2 v/v) and spotted along with standards on precoated silica gel plates (Merck, Germany), developed with solution of chloroform / xylene / acetone (60 : 30 : 10 ; v / v / v). The identification was performed under long wave ultraviolet light ( $\lambda=365$  nm) and quantified by visual comparison of spots. Confirmation of the identity of AFB1 was carried out with the spray of 50 % sulphuric acid and using the trifluoroacetic acid (TFA) reaction (Scott, 1984) and its amount was calculated.

### 2.12.3. Statistical Analysis

The data was analyzed statistically by calculating significance differences by using Student's t-test.). The data was expressed as average mean  $\pm$  SD (n=3). Method of recovery varied from 94 to 104 % and the quantification limits was 1 µg/kg for AFB1 (Steel et al., 1997; Simionato et al., 2003).

## 2.13. Application of Cooking Methods for Reduction of Aflatoxin B1 and Ochratoxin-A in Rice

### 2.13.1. Processing of Samples

A total of eighteen polished basmati rice (*Oryza sativa* Linn.) samples were artificially contaminated with AFB<sub>1</sub> and OTA. The contamination levels employed on 6 samples per each treatment were 20 µg/kg for AFB<sub>1</sub> and 30 µg/kg for OTA both when added individually and simultaneously [Table 2.3]. Adequate amounts of the standards dissolved in benzene were dripped on to 50 g portions of samples held in 600 ml beakers, taking care to avoid contaminating the wall. After 24 hours, the samples were submitted to the three types of cooking.



**Table 2.3: Layout of the experiment**

| Toxins                | Methods                   | No. of rice samples | Toxin levels (µg / kg) |    |        |
|-----------------------|---------------------------|---------------------|------------------------|----|--------|
|                       |                           |                     | T1                     | T2 | T3     |
|                       | Normal cooking            |                     |                        |    |        |
| AfB <sub>1</sub>      | Cooking with excess water | 6                   | 20                     |    | -      |
|                       | Microwave oven cooking    |                     |                        |    |        |
|                       | Normal cooking            |                     |                        |    |        |
| OTA                   | Cooking with excess water | 6                   | -                      | 30 | -      |
|                       | Microwave oven cooking    |                     |                        |    |        |
|                       | Normal cooking            |                     |                        |    |        |
| AFB <sub>1</sub> +OTA | Cooking with excess water | 6                   | -                      | -  | 20 +30 |
|                       | Microwave oven cooking    |                     |                        |    |        |

Where; T1= First treatment; T2= Second treatment; T3= Third treatment

### 2.13.2. Cooking Methods

The artificially contaminated polished rice samples were submitted to three types of cooking. Each experiment was repeated or replicates six times. The cooking times used for the heat treatments were established as being those required for the rice to present a proper texture for ingestion.

#### *Normal Cooking*

This method is in practice in Pakistan. Approximately 50 g of artificially contaminated rice were cooked in 100 ml water on a gas stove. Nine minutes were required to cook the rice after it had boiled.

### *Cooking with Excess Water*

The same procedure described above was carried out but with twice the amount of water (200 ml). After cooking, the excess water was drained off. The total cooking time was 9 minutes for boiling.

### *Microwave Oven Cooking*

About 50 g contaminated rice was transferred to a special pan used to cook rice in a microwave oven and 100 ml water added. The total cooking time was 9 minutes at medium potency (0.45 kW).

## **2.13.3. Chemical Analyses**

The analytical grade chemicals were used of BDH (Poole, England), Sigma (St. Louis, USA) and Merck (Darmstadt, Germany).

### **2.13.3.1. Standards of Aflatoxin B<sub>1</sub> and Ochratoxin A**

Standards of aflatoxin B<sub>1</sub> (2.02 µg / ml) and Ochratoxin A (10 µg / ml) were of Biopure (Tecknopark Tullin, Austria). Standard stock solutions of AFB<sub>1</sub> and OTA of concentrations 1 µg / ml each were prepared by diluting in benzene / acetonitrile (98:2; v / v). These stock solutions were kept at -4 °C in refrigerator.

### **2.13.3.2. Determination of Aflatoxin B<sub>1</sub>**

Aflatoxin B<sub>1</sub> was determined according to standard method by thin layer chromatography as previously describes (Scott, 1984; AOAC, 2000).



### 2.13.3.3. Determination of Ochratoxin-A

Ochratoxin-A was determined by TLC according to standard method of (AOAC, 2000). Briefly, 250 ml of methanol and 30 ml aqueous potassium chloride (4 %) were added to 50 gm test sample and extracted using blender for 3 minutes at low speed and filtered through whatman filter paper No 4. The filtrate collected in 600 ml beaker containing 150 ml of ammonium sulphate (30 %) and filtered. 150 ml filtrate was taken into a separating funnel contains distilled water (100 ml) and twice extracted with 10 ml of chloroform. The chloroform extract layer was collected in a vial and 8 mL of it, was dried by evaporating on heating block at 45 °C with stream of nitrogen on a heating block. The dry residue was redissolved in 200 µl of benzene / acetonitrile (98 : 2 ; v / v) and spotted along with standards on precoated silica gel plates (Merck, Germany), developed with toluene / ethyl acetate / formic acid (50 : 40 : 10 ; v / v / v) in a saturated chamber. The identification was performed under long wave ultraviolet light ( $\lambda=365$  nm) and quantified by visual comparison of OTA standards spots. Confirmation of the identity of AFB<sub>1</sub> was carried out with the spray of 50 % sulphuric acid and using the trifluoroacetic acid (TFA) reaction (Scott, 1984) and its amount was calculated.

Confirmation of the identity of OTA was carried out with the spray of 20 % solution of aluminum chloride (Scott, 1984). Six determinations for each sample were carried out and standard deviation was calculated (Steel et al. 1997). Method recovery varied from 94 to 104 % for AFB<sub>1</sub> and 80 to 105 % for OTA. The quantification limits were 1 and 4 µg/kg for AFB<sub>1</sub> and OTA, respectively (Simionato et al., 2003)

## CHAPTER – III

# RESULTS

# AND

# DISCUSSION



### 3. RESULTS AND DISCUSSION

#### 3.1. Method Performance

The applied analytical method showed good performance. The analysis of aflatoxins in all the samples represented valid recoveries as per commission directives (European Commission, 2002). The recoveries results for aflatoxins were found 70 % at 2.5 - 5.0  $\mu\text{g/kg}$  [Table 3.1, 3.2, 3.3 and 3.4]. The monitoring of AFs has been reported by many methods such as, TLC (Otta et al., 2000), ELIZA (Ghali, et al., 2007) and HPLC with mass or fluorescence detectors (Blesaa et al., 2003; Ventura et al., 2004). The confirmation of aflatoxin by HPLC is still an official method with fluorescence detection.

#### 3.2. Occurrence of AFs in Analyzed Dried Fruits Samples

Aflatoxins analyzed in 75 samples of dried fruits, their results are summarized [Table 3.5]. The percentage of all samples contaminated with total AFs in dried fruits are also presented [Fig 3.1]. It is evident from the results that four samples of dried apricot, two samples of dates, five samples of dried fig, four samples of dried mulberries and two samples of raisins were found contaminated with aflatoxins. The concentration of total AFs and individual AFs in all the samples are given [Table 3.6].

Among contaminated samples of dried apricot, one sample (10.1  $\mu\text{g} / \text{kg}$ ) was more than the limit (4  $\mu\text{g} / \text{kg}$ ) for total aflatoxins suggested in regulations of EU (European Commission, 2006a) whereas three samples were ranging from 1.5 to 3.2  $\mu\text{g} / \text{kg}$  had aflatoxins below the suggested limit. The contamination level of individual AFs in dried apricot are also presented [Fig 3.1a]. In dates, only two samples were contaminated with aflatoxins having concentrations ranging from 2.1 to 3.2  $\mu\text{g} / \text{kg}$ , were above the suggested limit. The contamination level of individual AFs are also presented [Fig 3.1b].

**Table 3.1: Recoveries of aflatoxins in dried fruits**

| Commodities    | Mean recovery <sup>a</sup> ± RSD |            |            |            |
|----------------|----------------------------------|------------|------------|------------|
|                | AFB1                             | AFG1       | AFB2       | AFG2       |
| Dried apricot  | 91.7 ± 2.7                       | 84.5 ± 3.2 | 85.7 ± 2.1 | 88.3 ± 4.1 |
| Dried fig      | 84.5 ± 2.1                       | 86.7 ± 4.3 | 89.5 ± 1.9 | 85.7 ± 3.7 |
| Dried mulberry | 86.7 ± 2.5                       | 87.3 ± 4.5 | 90.4 ± 1.6 | 89.5 ± 2.6 |
| Dates          | 90.5 ± 1.6                       | 90.4 ± 3.7 | 83.5 ± 1.8 | 92.5 ± 3.2 |
| Raisins        | 83.5 ± 2.5                       | 86.5 ± 2.7 | 92.4 ± 3.5 | 90.2 ± 2.5 |

<sup>a</sup> Number of replicates: N=3

RSD: relative standard deviation

**Table 3.2: Recoveries of aflatoxins in edible nuts**

| Dried fruits             | Mean recovery <sup>a</sup> ± RSD |            |            |            |
|--------------------------|----------------------------------|------------|------------|------------|
|                          | AFB1                             | AFG1       | AFB2       | AFG2       |
| Apricot kernels          | 83.5 ± 2.1                       | 87.3 ± 3.4 | 88.5 ± 1.9 | 85.7 ± 2.5 |
| Almonds with shell       | 87.5 ± 4.2                       | 83.7 ± 2.6 | 85.7 ± 2.1 | 86.4 ± 1.6 |
| Almonds without shell    | 85.7 ± 2.4                       | 88.2 ± 1.7 | 89.5 ± 2.0 | 91.5 ± 1.9 |
| Walnuts with shell       | 86.4 ± 1.9                       | 89.6 ± 1.9 | 92.5 ± 1.9 | 84.5 ± 3.2 |
| Walnuts without shell    | 91.5 ± 3.2                       | 85.7 ± 2.0 | 90.2 ± 3.5 | 86.7 ± 2.1 |
| Peanuts with shell       | 90.4 ± 4.2                       | 91.7 ± 2.6 | 83.7 ± 3.2 | 87.3 ± 2.7 |
| Peanuts without shell    | 83.5 ± 1.8                       | 84.5 ± 3.3 | 87.5 ± 2.6 | 83.7 ± 3.4 |
| Pistachios with shell    | 85.6 ± 1.5                       | 86.7 ± 1.8 | 84.5 ± 2.6 | 88.2 ± 2.5 |
| Pistachios without shell | 89.5 ± 2.6                       | 90.5 ± 2.3 | 88.6 ± 4.0 | 92.4 ± 1.8 |

<sup>a</sup> Number of replicates: N=3

RSD: relative standard deviation



**Table 3.3: Recoveries of aflatoxins in cereals**

| Commodities | Mean recovery <sup>a</sup> ± RSD |            |            |            |
|-------------|----------------------------------|------------|------------|------------|
|             | AFB1                             | AFG1       | AFB2       | AFG2       |
| Rice        | 90.7 ± 2.6                       | 85.5 ± 3.1 | 83.7 ± 2.0 | 87.4 ± 3.1 |
| Rice broken | 84.5 ± 2.0                       | 86.4 ± 4.1 | 89.3 ± 1.4 | 84.7 ± 3.2 |
| Wheat       | 85.7 ± 2.5                       | 88.3 ± 4.0 | 90.6 ± 1.6 | 89.5 ± 2.6 |
| Maize       | 90.5 ± 1.5                       | 90.1 ± 3.3 | 82.5 ± 1.5 | 91.7 ± 3.1 |
| Barley      | 83.7 ± 2.5                       | 84.5 ± 2.5 | 91.4 ± 2.5 | 90.6 ± 2.4 |
| Sorghum     | 82.5 ± 2.2                       | 87.3 ± 3.4 | 87.5 ± 1.8 | 83.7 ± 2.7 |

<sup>a</sup> number of replicates: n=3  
RSD: relative standard deviation

**Table 3.4: Recoveries of aflatoxins in beans**

| Commodities      | Mean recovery <sup>a</sup> ± RSD |            |            |            |
|------------------|----------------------------------|------------|------------|------------|
|                  | AFB1                             | AFG1       | AFB2       | AFG2       |
| Red kidney beans | 84.5 ± 2.0                       | 86.4 ± 3.1 | 84.3 ± 1.2 | 84.7 ± 3.2 |
| Split pea        | 87.5 ± 1.7                       | 88.2 ± 4.1 | 90.4 ± 1.4 | 89.5 ± 2.6 |
| Chickpea         | 91.5 ± 1.3                       | 90.1 ± 3.3 | 82.5 ± 1.5 | 90.4 ± 3.0 |
| Cowpea           | 82.7 ± 2.7                       | 84.5 ± 2.5 | 90.4 ± 2.2 | 87.6 ± 2.3 |
| Soybean          | 90.2 ± 1.6                       | 86.4 ± 2.4 | 84.7 ± 3.0 | 89.4 ± 3.4 |

<sup>a</sup> number of replicates: n=3  
RSD: relative standard deviation

**Table 3.5: Total aflatoxins in dried fruits**

| Commodities      | Sample<br>size/positive | Contamination (µg/kg)    |              | Mean value<br>(µg/kg) |
|------------------|-------------------------|--------------------------|--------------|-----------------------|
|                  |                         | Below limit              | Above limit  |                       |
| Dried apricot    | 20/4                    | 3 (1.5-3.2) <sup>a</sup> | 1 (10.8)     | 4.55                  |
| Dates            | 20/2                    | 2 (2.1-2.9)              | -            | 2.5                   |
| Dried fig        | 10/5                    | 2 (0.8-3.7)              | 3 (4.5-12.5) | 5.81                  |
| Dried mulberries | 15/4                    | 4 (1.0-3.5)              | -            | 2.22                  |
| Raisins          | 10/2                    | 1 (2.6)                  | 1 (7.5)      | 5.05                  |

<sup>a</sup> total aflatoxin range

**Table 3.6: Total and individual levels in positive samples of dried fruits**

| Commodities      | Mean AFs<br>(µg / kg) | Aflatoxins (µg / kg) |      |      |      |      |
|------------------|-----------------------|----------------------|------|------|------|------|
|                  |                       | Range                | AFB1 | AFB2 | AFG1 | AFG2 |
| Dried apricot    | 4.55                  | 1.5                  | 0.8  | 0.7  | ND   | ND   |
|                  |                       | 2.7                  | 1.5  | ND   | 2.2  | ND   |
|                  |                       | 3.2                  | 0.7  | 1.2  | ND   | 1.3  |
|                  |                       | 10.8                 | 3.5  | 2.0  | 3.0  | 2.3  |
| Dates            | 2.50                  | 2.1                  | 2.1  | ND   | ND   | ND   |
|                  |                       | 2.9                  | 1.4  | ND   | 1.5  | ND   |
| Dried fig        | 5.81                  | 0.8                  | 0.8  | ND   | ND   | ND   |
|                  |                       | 3.7                  | 1.7  | 2.0  | ND   | ND   |
|                  |                       | 4.5                  | 0.8  | 1.2  | 2.5  | ND   |
|                  |                       | 7.55                 | 4.0  | 3.5  | ND   | ND   |
|                  |                       | 12.5                 | 5.2  | 2.6  | 3.2  | 1.5  |
| Dried mulberries | 2.22                  | 1.0                  | ND   | ND   | 1.0  | ND   |
|                  |                       | 2.0                  | ND   | ND   | 0.7  | 1.3  |
|                  |                       | 2.4                  | 1.4  | 1.0  | ND   | ND   |
|                  |                       | 3.5                  | 1.0  | 0.8  | ND   | 1.7  |
| Raisins          | 5.05                  | 2.6                  | 1.6  | 1.0  | ND   | ND   |
|                  |                       | 7.5                  | 3.5  | 2.5  | 1.5  | ND   |



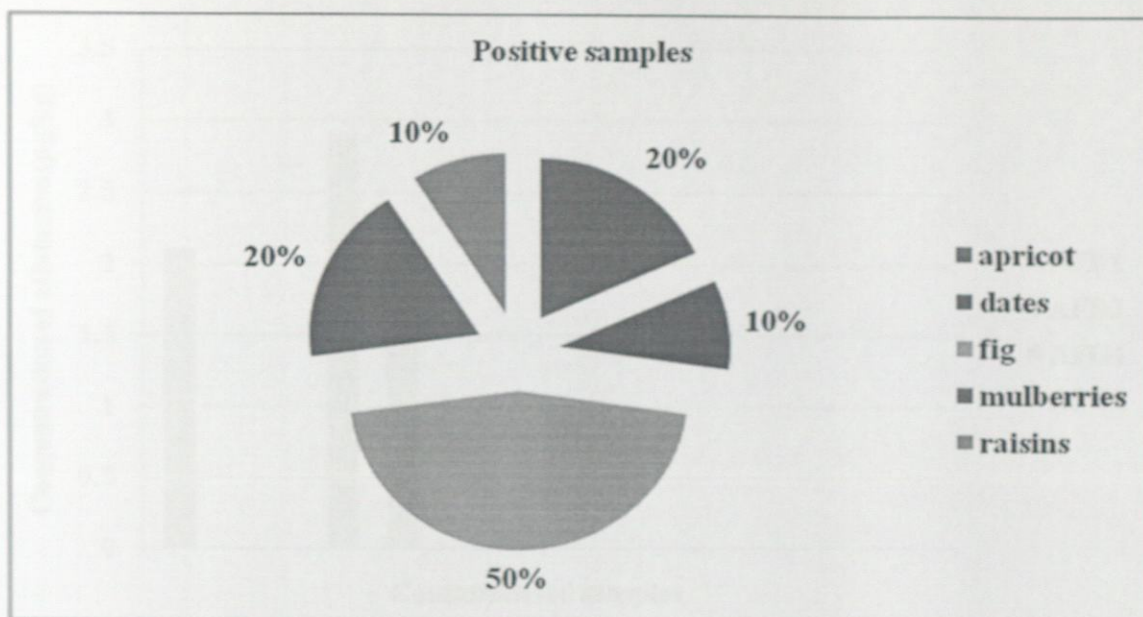


Fig 3.1: Aflatoxins level in contaminated dried fruits

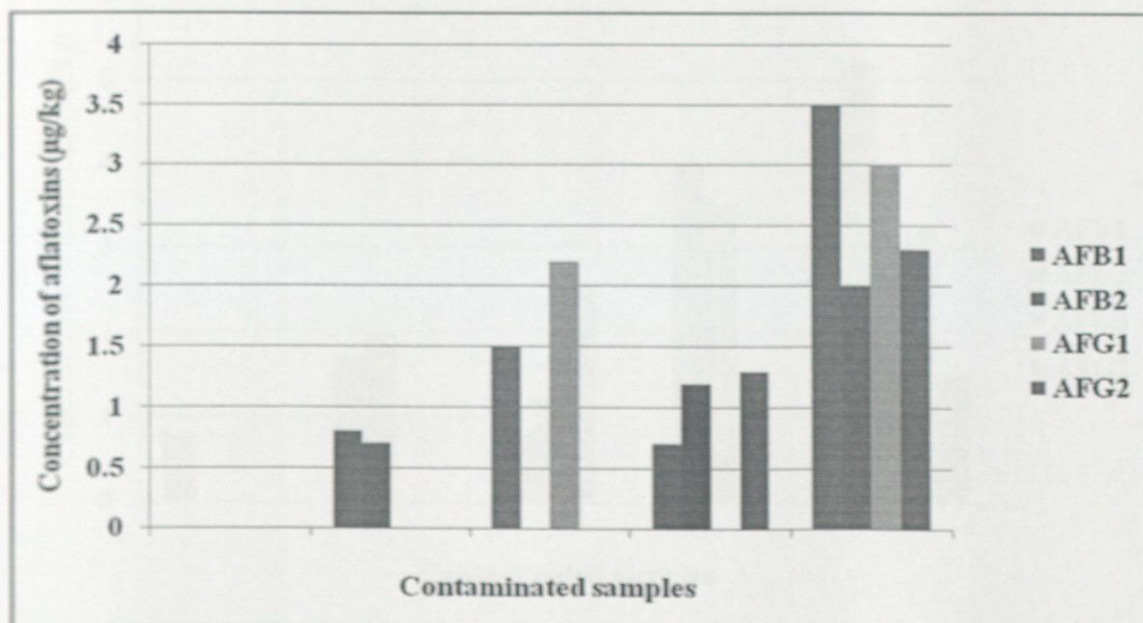


Fig 3.1a: Aflatoxins level in contaminated dried apricot

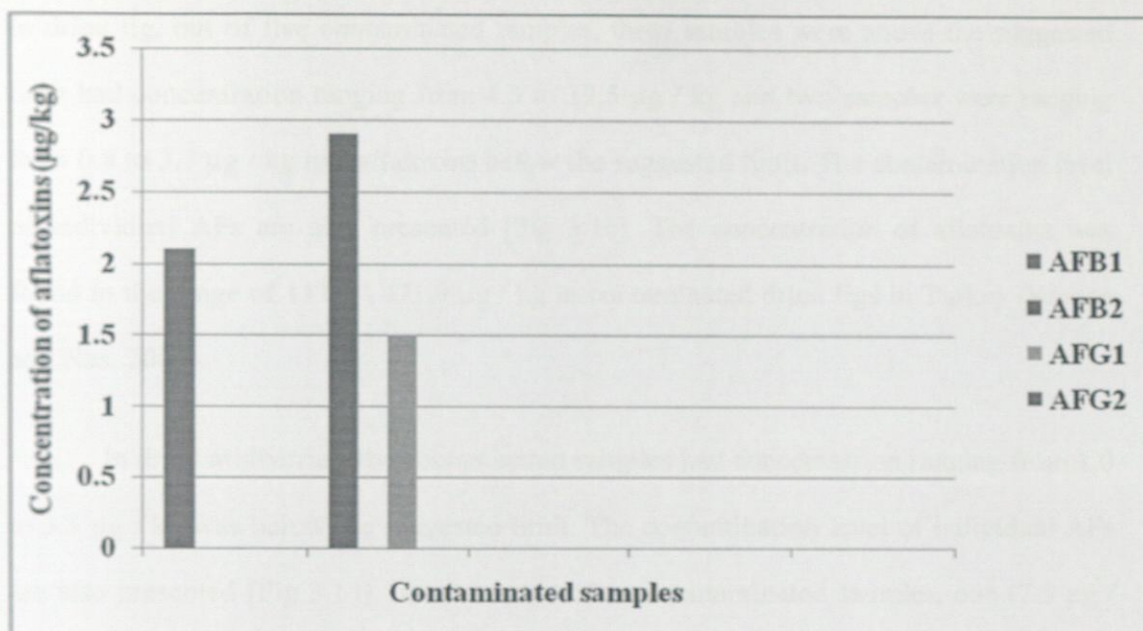


Fig 3.1b: Aflatoxins level in contaminated dates

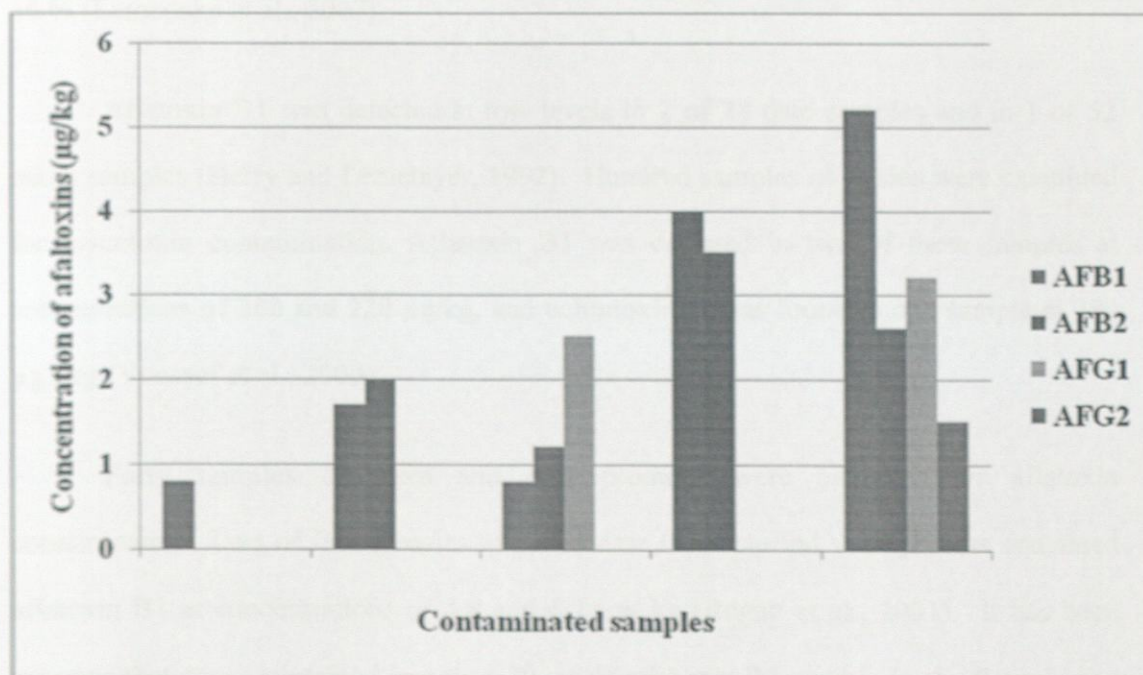


Fig 3.1c: Aflatoxins level in contaminated dried fig



In dried fig, out of five contaminated samples, three samples were above the suggested limit had concentration ranging from 4.5 to 12.5  $\mu\text{g} / \text{kg}$  and two samples were ranging from 0.8 to 3.7  $\mu\text{g} / \text{kg}$  had aflatoxins below the suggested limit. The contamination level of individual AFs are also presented [Fig 3.1c]. The concentration of aflatoxins was found in the range of 117.9 - 471.9  $\mu\text{g} / \text{kg}$  in contaminated dried figs in Turkey (Karaca and Nas, 2006).

In dried mulberries, the contaminated samples had concentration ranging from 1.0 to 3.5  $\mu\text{g} / \text{kg}$  was below the suggested limit. The contamination level of individual AFs are also presented [Fig 3.1d]. In raisins, out of two contaminated samples, one (7.5  $\mu\text{g} / \text{kg}$ ) was above and one (2.6  $\mu\text{g}/\text{kg}$ ) had aflatoxins below the suggested limit. The contamination level of individual AFs are also presented [Fig 3.1e]. It has been reported that in Brazil, the contamination level of aflatoxin B1 and B2 in dried grapes was found 16 % (Lamanaka et al., 2007).

Aflatoxin B1 was detected at low levels in 2 of 28 date samples and in 1 of 52 raisin samples (Herry and Lemetayer, 1992). Hundred samples of raisins were examined for mycotoxin contamination. Aflatoxin B1 was detected in two of these samples at concentrations of 300 and 220  $\mu\text{g}/\text{kg}$ , and ochratoxin A was found in one sample at 250  $\mu\text{g} / \text{kg}$  (Youssef et al., 2000).

Forty samples of dates and date products were analyzed for aflatoxin contamination. Two of five samples of pitted date fruits stuffed with peanuts contained aflatoxin B1 at concentrations of 4.8 and 6.2  $\mu\text{g} / \text{kg}$  (Ragab et al., 2001). It has been reported that copra contained less than 20  $\mu\text{g}$  of aflatoxin B1 per kg. Both aflatoxin and free fatty acid contents increased with increasing degrees of contamination with *A. flavus* (Head et al., 1999).

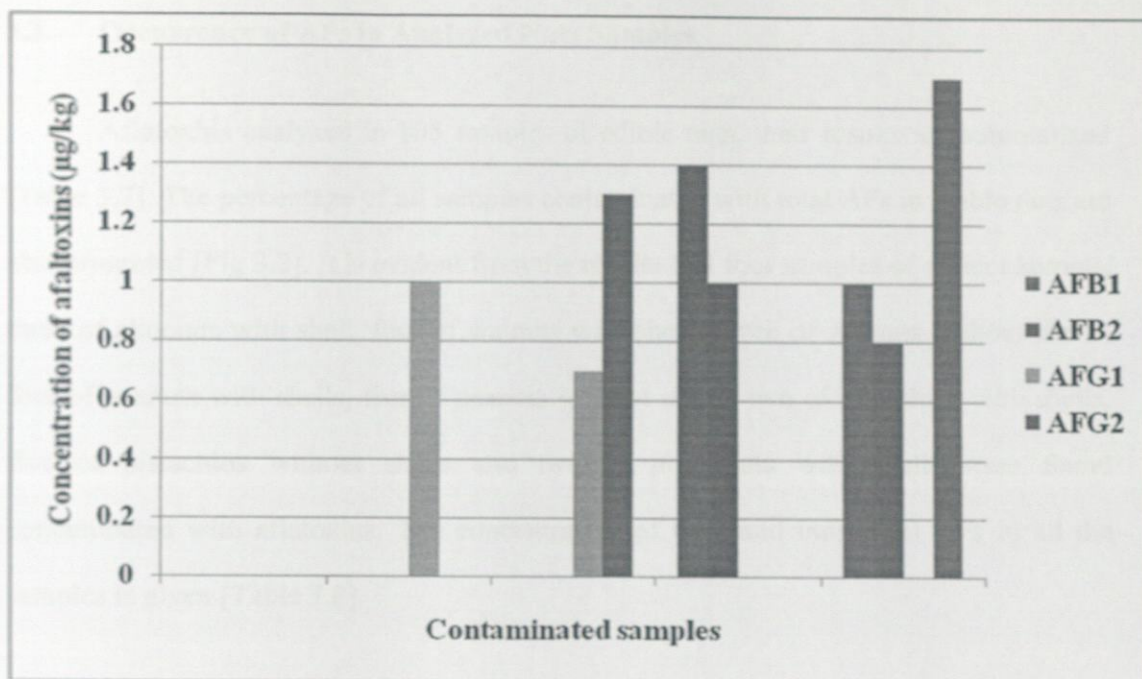


Fig 3.1d: Aflatoxins level in contaminated dried mulberries

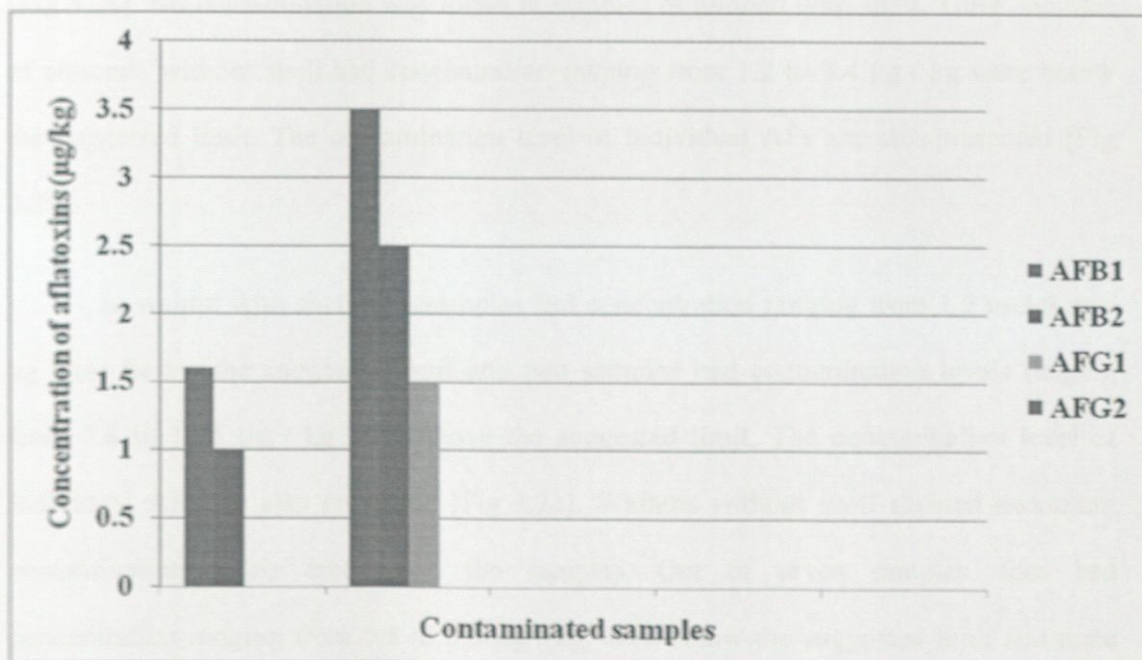


Fig 3.1e: Aflatoxins level in contaminated raisins



### 3.3. Occurrence of AFs in Analyzed Nuts Samples

Aflatoxins analyzed in 105 samples of edible nuts, their results are summarized [Table 3.7]. The percentage of all samples contaminated with total AFs in edible nuts are also presented [Fig 3.2]. It is evident from the results that four samples of apricot kernels, three of almonds with shell, four of walnuts with shell, seven of walnuts without shells, four of peanuts with shells, five of peanuts without shells, two of pistachios with shells, five of pistachios without shells and two of pine nuts with shells were found contaminated with aflatoxins. The concentration of total and individual AFs in all the samples is given [Table 3.8].

In apricot kernels, out of four contaminated samples, one ( $5.6 \mu\text{g} / \text{kg}$ ) was above the suggested limit and three showed aflatoxins ranging from  $0.7$  to  $2.8 \mu\text{g} / \text{kg}$  were below the suggested limit. The contamination level of individual AFs are also presented [Fig 3.2a]. No contamination was found in samples of almond with shell. Three samples of almonds without shell had concentration ranging from  $1.2$  to  $3.4 \mu\text{g} / \text{kg}$  were below the suggested limit. The contamination level of individual AFs are also presented [Fig 3.2b].

In walnut with shell, two samples had concentration ranging from  $1.5$  to  $3.0 \mu\text{g} / \text{kg}$  were below the suggested limit and two samples had contamination levels ranging from  $7.8$  to  $13.5 \mu\text{g} / \text{kg}$  were above the suggested limit. The contamination level of individual AFs are also presented [Fig 3.2c]. Walnuts without shell showed maximum contamination levels among all the samples. Out of seven samples, four had concentration ranging from  $0.8$  to  $3.6 \mu\text{g} / \text{kg}$  were below the suggested limit and three had concentration ranging from  $6.0$  to  $10.8 \mu\text{g} / \text{kg}$ , above suggested limit fixed for aflatoxins by European legislations (European Commission, 2006a). It is evident from

Table 3.7 that levels of total aflatoxin contamination were less in pistachio with shell compares to pistachio without shell. This may be because the pistachio kernels are protected and safe in the microenvironment beneath the shell, therefore less chances for cross contamination, growth of mould and toxin production. The reason for occurrence of toxin in few samples might be preharvest contamination in the field and not due to cross-contamination, poor post harvest storage and handling. Pistachios without shell were found highly contaminated with aflatoxin. The reasons for this might be preharvest contamination in the field followed by cross-contamination after deshelling, poor storage and handling resulting in conditions favorable for mould growth and toxin production (Abdulkadar et al, 2000).

**Table 3.7: Total aflatoxins in edible nuts**

| Commodities              | Sample<br>size/positive | Contamination ( $\mu\text{g} / \text{kg}$ ) |              | Mean value<br>( $\mu\text{g} / \text{kg}$ ) |
|--------------------------|-------------------------|---------------------------------------------|--------------|---------------------------------------------|
|                          |                         | Below limit                                 | Above limit  |                                             |
| Apricot kernels          | 15/4                    | 3 (0.7-2.8) <sup>a</sup>                    | 1 (5.6)      | 2.65                                        |
| Almonds with shell       | 10/0                    | -                                           | -            | -                                           |
| Almonds without shell    | 10/3                    | 3 (1.2-3.4)                                 | -            | 2.13                                        |
| Walnuts with shell       | 10/4                    | 2 (1.5-3.0)                                 | 2 (7.8-13.5) | 6.45                                        |
| Walnuts without shell    | 10/7                    | 4 (0.8-3.6)                                 | 3 (6.0-10.8) | 3.43                                        |
| Peanuts with shell       | 10/4                    | 3 (1.5-2.5)                                 | 1 (14.5)     | 5.10                                        |
| Peanuts without shell    | 10/5                    | 3 (0.7-3.8)                                 | 2 (6.4-12.8) | 5.20                                        |
| Pistachios with shell    | 10/2                    | 2 (1.2-3.0)                                 | -            | 2.10                                        |
| Pistachios without shell | 10/5                    | 3 (2.0-3.6)                                 | 2 (9.5-14.0) | 6.34                                        |
| Pine nuts with shell     | 10/2                    | 2 (2.6-3.9)                                 | -            | 3.25                                        |

<sup>a</sup> total aflatoxin range



**Table 3.8: Total aflatoxin and individual levels in positive samples of edible nuts**

| Commodities              | Mean AFs<br>( $\mu\text{g} / \text{kg}$ ) | Aflatoxins ( $\mu\text{g} / \text{kg}$ ) |      |      |      |      |
|--------------------------|-------------------------------------------|------------------------------------------|------|------|------|------|
|                          |                                           | Range                                    | AFB1 | AFB2 | AFG1 | AFG2 |
| Apricot kernels          | 2.65                                      | 0.7                                      | 0.7  | ND   | ND   | ND   |
|                          |                                           | 1.5                                      | 0.8  | 0.7  | ND   | ND   |
|                          |                                           | 2.8                                      | 2.8  | ND   | ND   | ND   |
|                          |                                           | 5.6                                      | 1.6  | 2.0  | 2.0  | ND   |
| Almonds with shell       | -                                         |                                          |      |      |      |      |
| Almonds without shell    | 2.13                                      | 1.2                                      | ND   | ND   | 1.2  | ND   |
|                          |                                           | 1.8                                      | ND   | ND   | 1.0  | 0.8  |
|                          |                                           | 3.4                                      | 1.4  | 2.0  | ND   | ND   |
| Walnuts with shell       | 6.45                                      | 1.5                                      | 1.5  | ND   | ND   | ND   |
|                          |                                           | 3.0                                      | 1.0  | ND   | 2.0  | ND   |
|                          |                                           | 7.8                                      | 3.4  | 2.0  | 1.4  | 1.0  |
|                          |                                           | 13.5                                     | 6.0  | 3.5  | 2.5  | 1.5  |
| Walnuts without shell    | 4.33                                      | 0.8                                      | 0.8  | ND   | ND   | ND   |
|                          |                                           | 1.0                                      | 1.0  | ND   | ND   | ND   |
|                          |                                           | 1.5                                      | ND   | 1.5  | ND   | ND   |
|                          |                                           | 3.6                                      | 2.0  | 1.6  | ND   | ND   |
|                          |                                           | 6.0                                      | 3.0  | ND   | 3.0  | ND   |
|                          |                                           | 6.6                                      | 1.8  | 2.4  | 1.4  | 1.0  |
| Peanuts with shell       | 5.10                                      | 10.8                                     | 4.0  | 2.0  | 2.8  | 2.0  |
|                          |                                           | 1.5                                      | 1.5  | ND   | ND   | ND   |
|                          |                                           | 1.9                                      | 1.0  | 0.9  | ND   | ND   |
|                          |                                           | 2.5                                      | 1.3  | ND   | 1.2  | ND   |
| Peanuts without shell    | 5.20                                      | 14.5                                     | 5.5  | 4.0  | 3.2  | 1.8  |
|                          |                                           | 0.7                                      | 0.7  | ND   | ND   | ND   |
|                          |                                           | 2.3                                      | 1.3  | 1.0  | ND   | ND   |
|                          |                                           | 3.8                                      | 2.0  | ND   | 1.8  | ND   |
|                          |                                           | 6.4                                      | 3.4  | 1.5  | 1.5  | ND   |
| Pistachios with shell    | 2.10                                      | 12.8                                     | 5.5  | 3.0  | 2.3  | 2.0  |
|                          |                                           | 1.2                                      | ND   | ND   | ND   | 1.2  |
|                          |                                           | 3.0                                      | ND   | ND   | 2.0  | 1.0  |
|                          |                                           |                                          |      |      |      |      |
| Pistachios without shell | 6.34                                      | 2.0                                      | 2.0  | ND   | ND   | ND   |
|                          |                                           | 2.6                                      | 1.6  | 1.0  | ND   | ND   |
|                          |                                           | 3.6                                      | ND   | 2.2  | ND   | 1.4  |
|                          |                                           | 9.5                                      | 3.0  | 2.5  | 3.0  | 1.0  |
|                          |                                           | 14.0                                     | 6.8  | 3.2  | 2.0  | 2.0  |
| Pine nuts with shell     | 3.25                                      | 2.6                                      | 1.4  | 1.2  | ND   | ND   |
|                          |                                           | 3.9                                      | 2.5  | 1.4  | ND   | ND   |

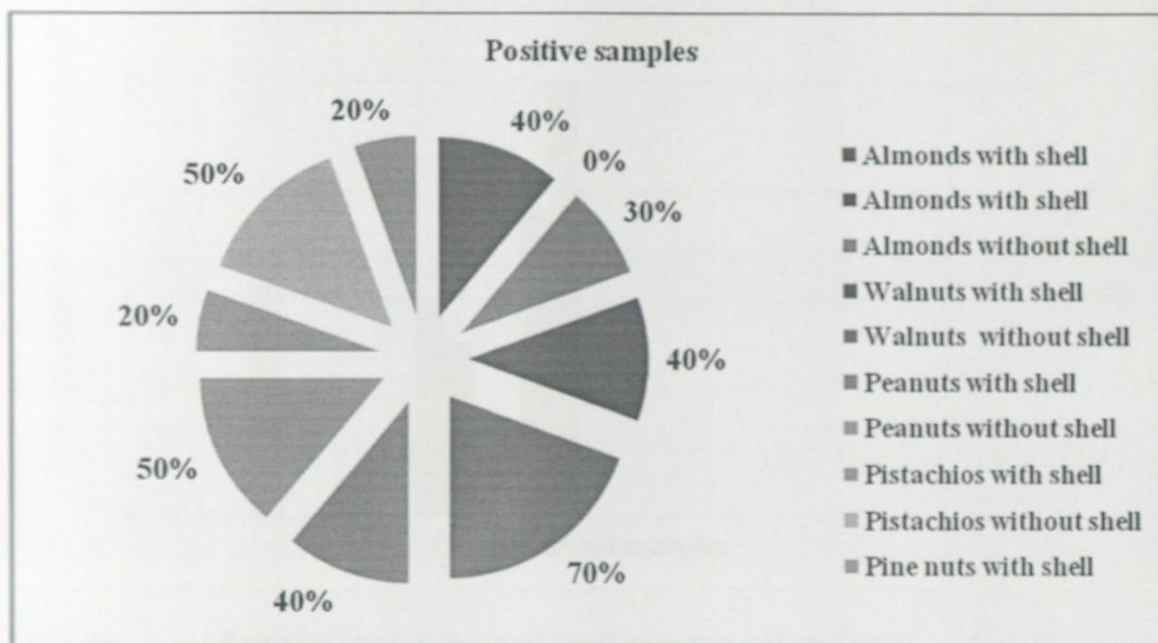


Fig 3.2: Aflatoxins level in contaminated edible nuts

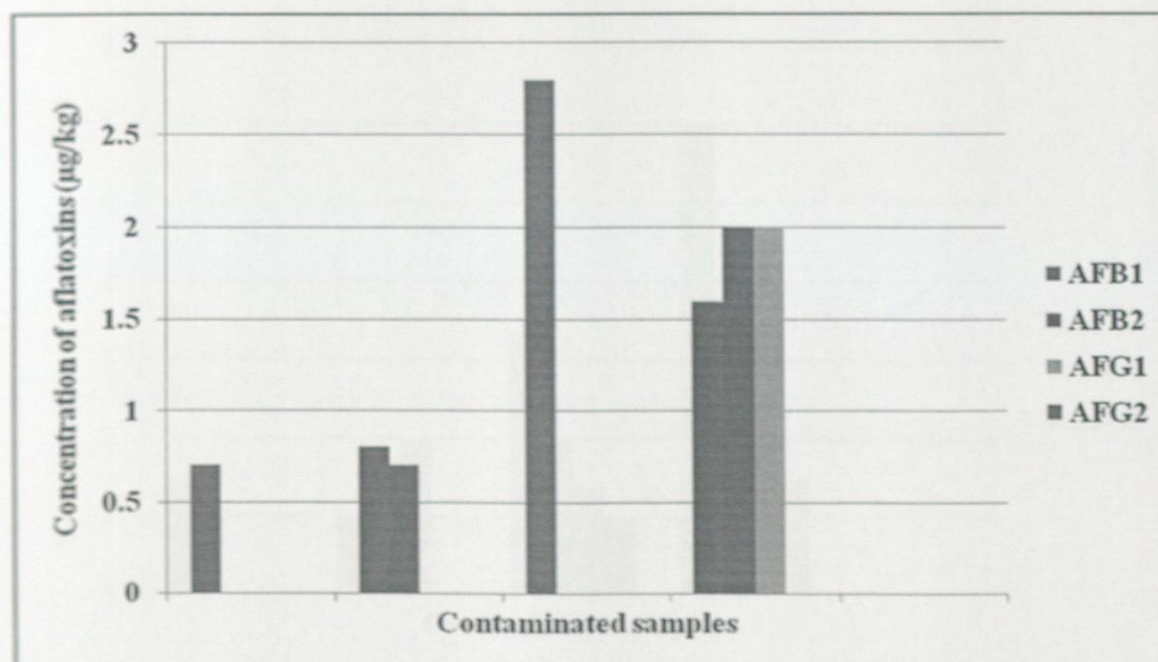


Fig 3.2a: Aflatoxins level in contaminated apricot kernel



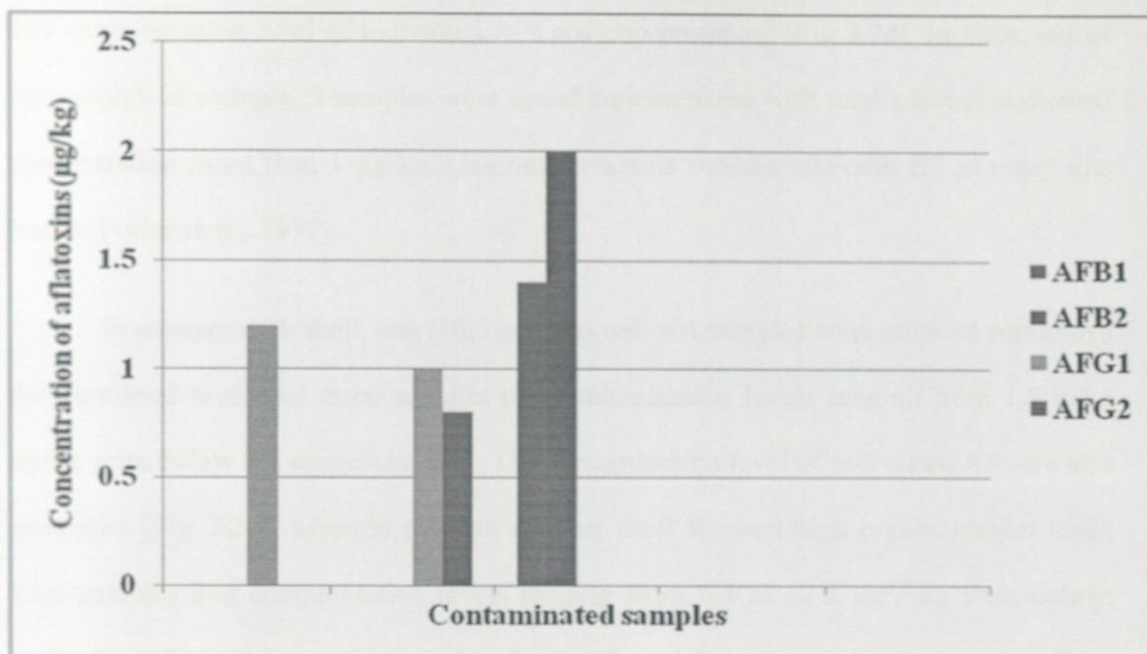


Fig 3.2b: Aflatoxins level in contaminated almonds without shell

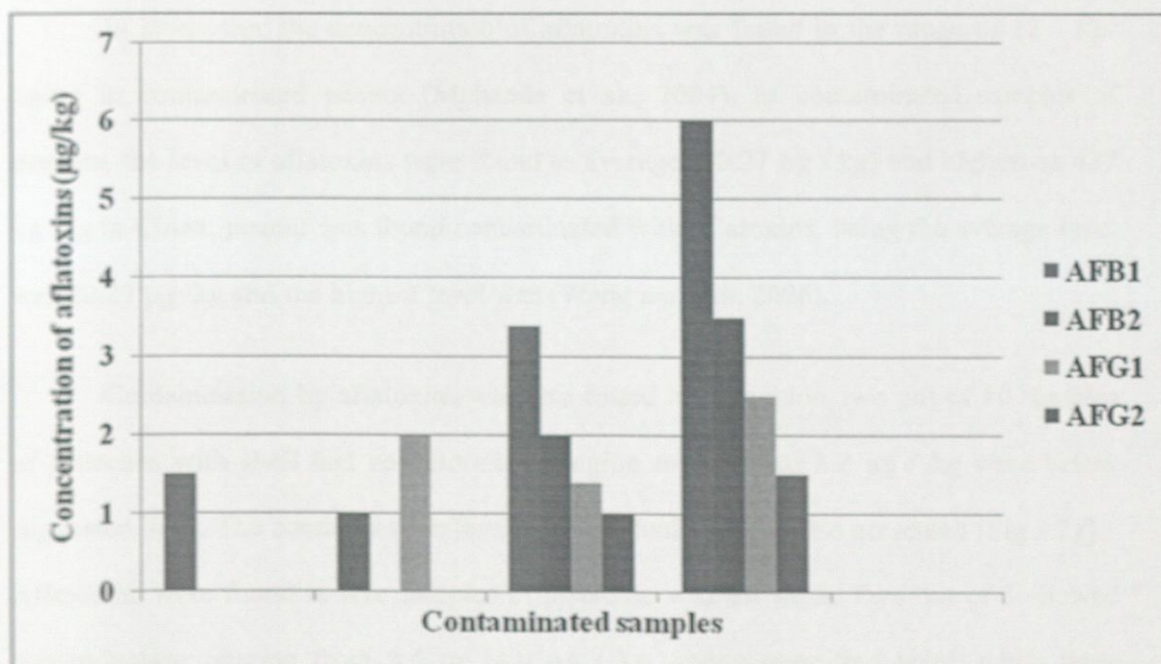


Fig 3.2c: Aflatoxins level in contaminated walnuts with shell

The contamination level of individual AFs are also presented [Fig 3.2d]. In USA, out of 40 samples of walnuts, 3 samples were found contaminated with total aflatoxins showed concentration more than 1  $\mu\text{g/kg}$ . Unusually walnuts contain aflatoxin B2 in many lots tested (Fuller et al., 1977).

In peanuts with shell, one (14.5  $\mu\text{g/kg}$ ) out of 4 samples contaminated was above the permitted level and three samples had contamination levels ranging from 1.5 to 2.5  $\mu\text{g/kg}$  were below the suggested limit. The contamination level of individual AFs are also presented [Fig 3.2e], whereas peanuts without shell showed high contamination level. Two samples had contamination levels ranging from 6.4 to 12.8  $\mu\text{g/kg}$  were below; while three had concentration ranging from 0.7 to 3.8  $\mu\text{g/kg}$ , above suggested limit fixed for aflatoxins. The contamination level of individual AFs are also presented [Fig 3.2f]. The peanut is one of the most susceptible foodstuffs to contaminate by toxicogenic fungi producing aflatoxins (Escobar and Regueiro, 2002; Mphande et al., 2004).

In Botswana, the concentration of aflatoxins was found in the range of 12 - 329  $\mu\text{g/kg}$  in contaminated peanut (Mphande et al., 2004). In contaminated samples of peanuts, the level of aflatoxins were found as average (80.27  $\mu\text{g/kg}$ ) and highest as 437  $\mu\text{g/kg}$  in China, peanut was found contaminated with aflatoxins, being the average level was 80.27  $\mu\text{g/kg}$  and the highest level was (Wang and Liu, 2006).

Contamination by aflatoxins was also found in pistachios, two out of 10 samples of pistachio with shell had concentration ranging from 1.2 to 3.2  $\mu\text{g/kg}$  were below suggested limit. The contamination level of individual AFs are also presented [Fig 3.2g]. Aflatoxins were found in five samples of pistachio without shell. Two out of 5 showed contamination ranging from 9.5 to 14.0  $\mu\text{g/kg}$ , above permitted level, while three showed contamination ranging from 2.0 to 3  $\mu\text{g/kg}$  were below the suggested limit. The



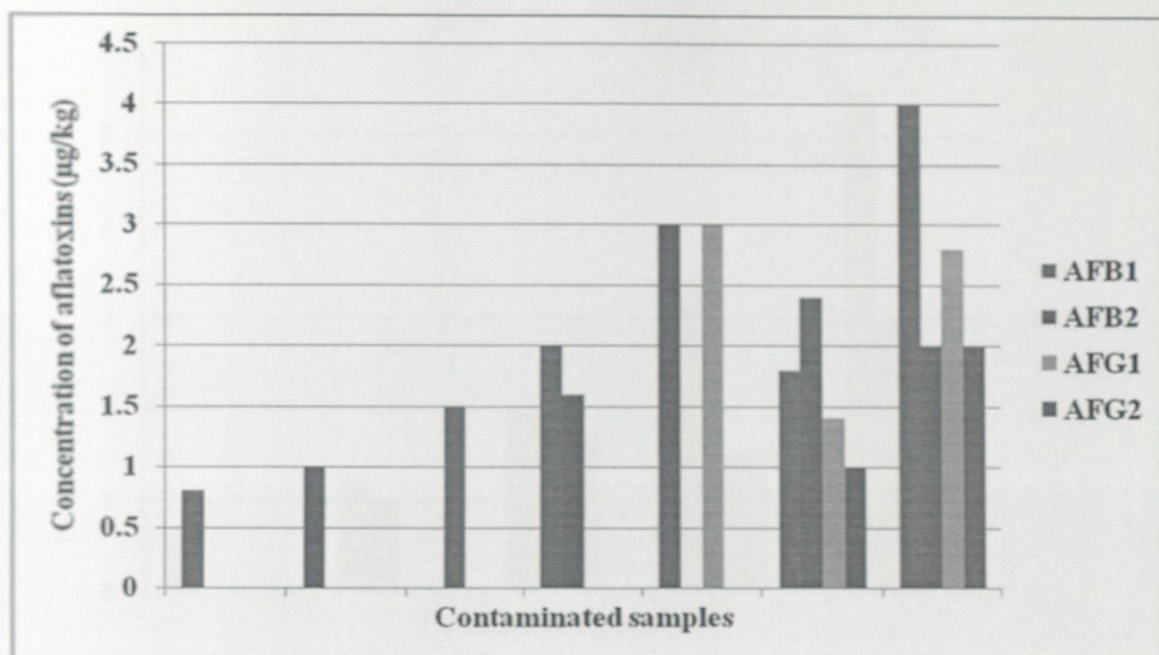


Fig 3.2d: Aflatoxins level in contaminated walnuts without shell

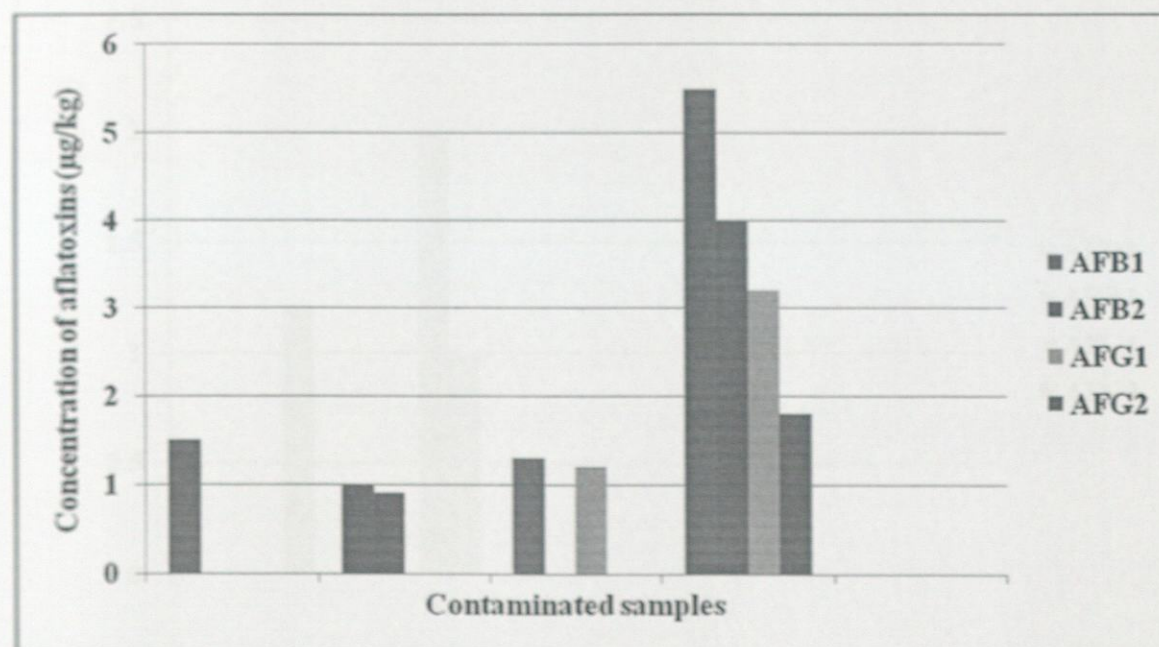


Fig 3.2e: Aflatoxins level in contaminated peanuts with shell

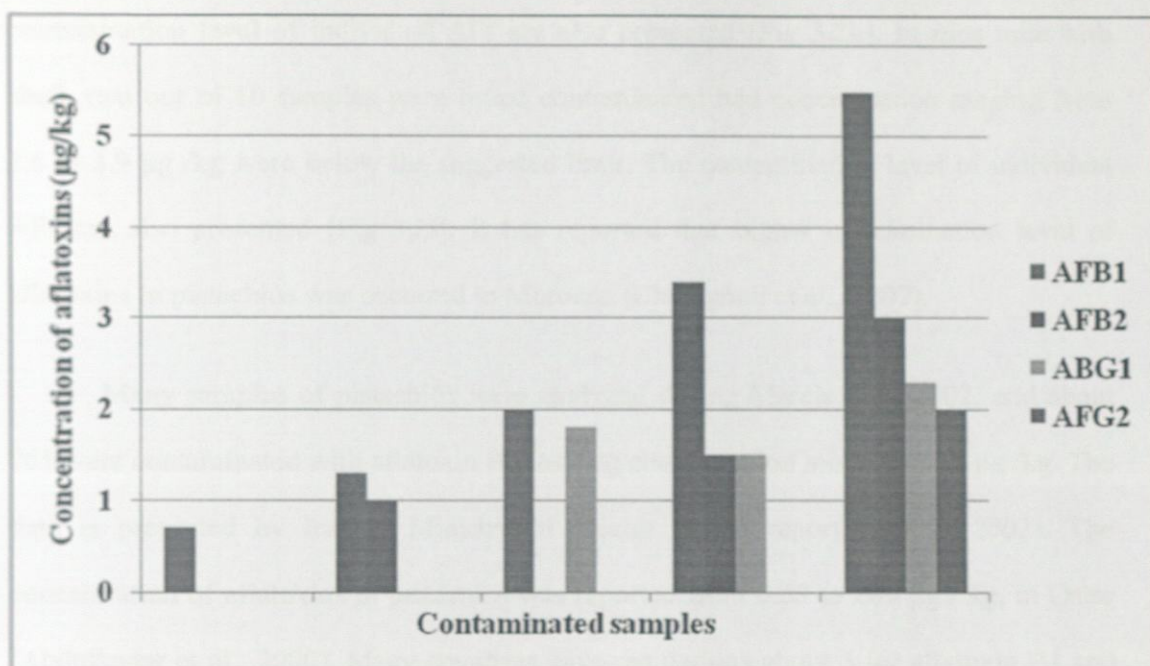


Fig 3.2f: Aflatoxins level in contaminated peanuts without shell

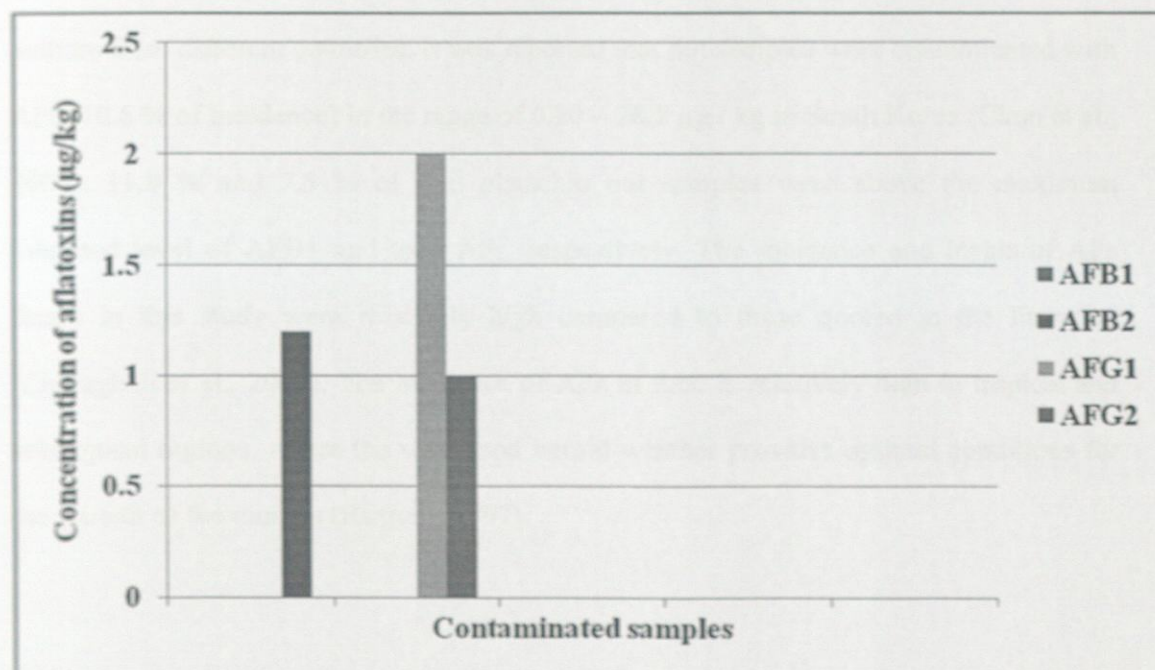


Fig 3.2g: Aflatoxins level in contaminated pistachios with shell



contamination level of individual AFs are also presented [Fig 3.2h]. In pine nuts with shell, two out of 10 samples were found contaminated had concentration ranging from 2.6 to 3.9  $\mu\text{g} / \text{kg}$  were below the suggested limit. The contamination level of individual AFs are also presented [Fig 3.2i]. It has reported that higher contamination level of aflatoxins in pistachios was occurred in Morocco (Cheraghali et al., 2007).

Many samples of pistachios were analyzed during March 2001-2002, and about 761 were contaminated with aflatoxin B1, having concentration more than 10  $\mu\text{g} / \text{kg}$ . The data is presented by Iranian Ministry of Health in his report (FDCL, 2002). The concentration of aflatoxins in pistachios was reported from 0.53 to 289  $\mu\text{g} / \text{kg}$ , in Qatar (Abdulkadar et al., 2000). Many countries have regulations about 5 for aflatoxin B1 and 20 for total aflatoxins (B1, B2, G1 and G2) in food, while in EU this limit is 2 and 4  $\mu\text{g} / \text{kg}$  respectively (Van Egmond and Jonker, 2004; European Commission, 2006b).

The occurrence of AFs in different types of nuts has been reported by several authors from different countries. It was reported that nut samples were contaminated with AFs (10.6 % of incidence) in the range of 0.20 – 28.2  $\mu\text{g} / \text{kg}$  in South Korea (Chun et al., 2007). 11.8 % and 7.5 % of Iran pistachio nut samples were above the maximum tolerated level of AFB1 and total AFs, respectively. The incidence and levels of AFs found in this study were relatively high compared to those quoted in the literature (Cheraghali et al., 2007). The incidence of AFs in food is relatively high in tropical and subtropical regions, where the warm and humid weather provides optimal conditions for the growth of the moulds (Rustom, 1997).

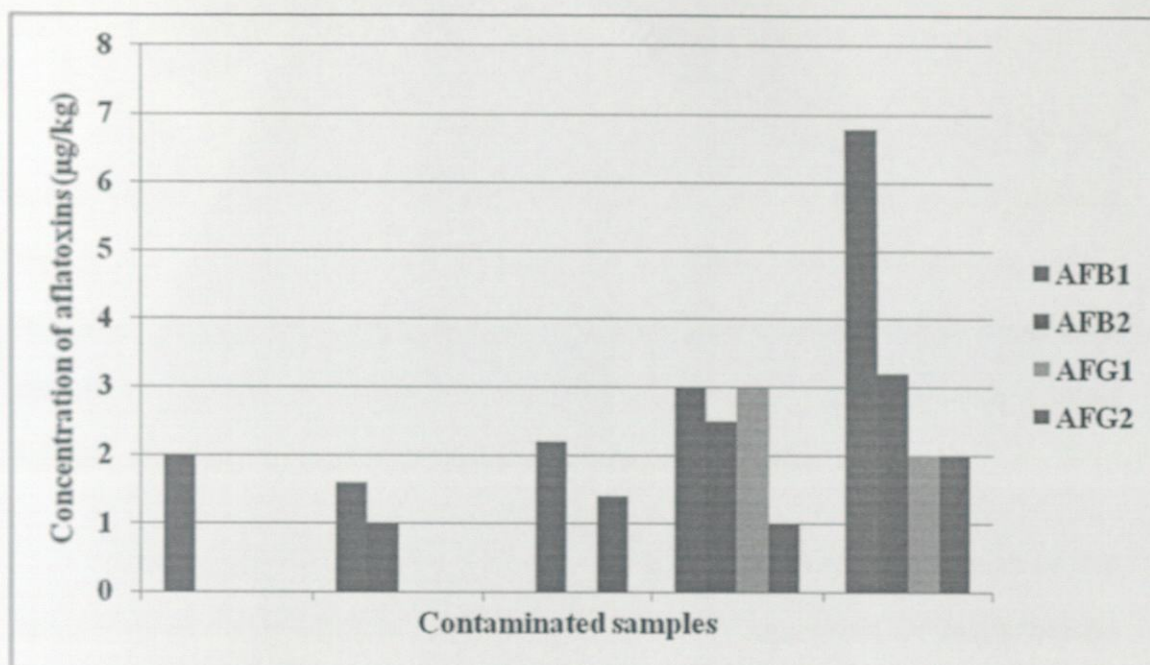


Fig 3.2h: Aflatoxins level in contaminated pistachios without shell

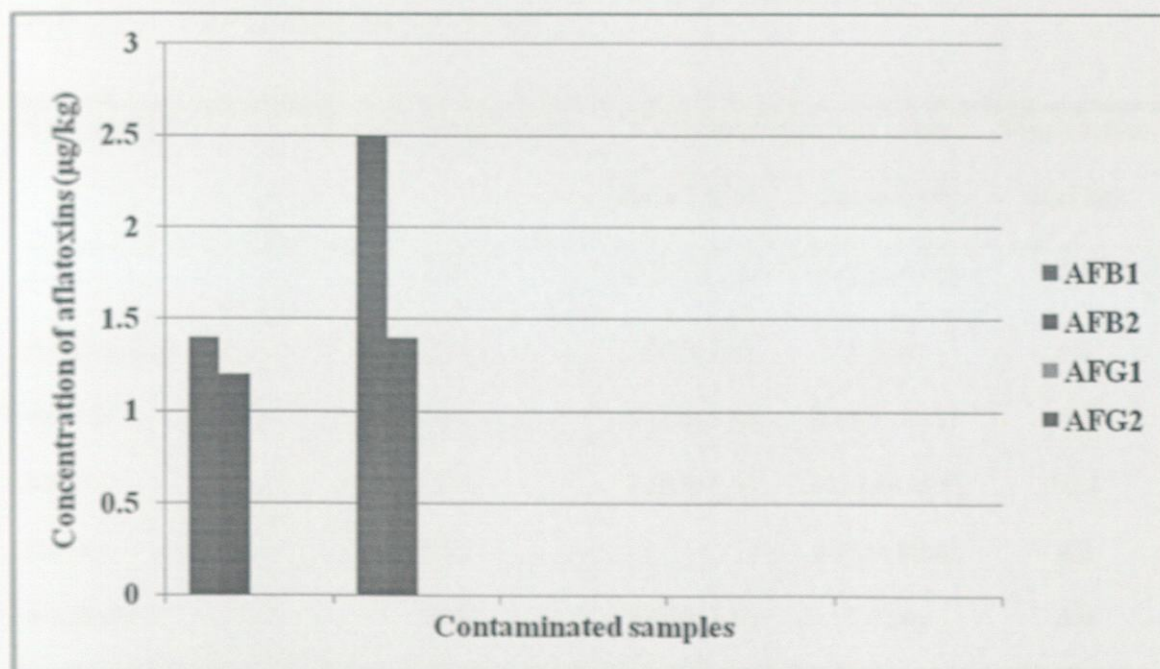


Fig 3.2i: Aflatoxins level in contaminated pine nuts



### 3.4. Occurrence of Aflatoxins in Analyzed Cereals Samples

Aflatoxin analyzed in 95 samples of cereals is summarized [Table 3.9]. The percentage of all samples contaminated with total AFs in edible nuts are also presented [Fig 3.3]. It is evident from the results that five samples of rice, three samples of rice broken, four samples of wheat, six samples of maize, two samples of barley and three samples of sorghum were found contaminated with aflatoxins. The concentration of total AFs and individual AFs in all the samples are given [Table 3.10].

Among contaminated samples of rice, two samples were ranging from 5.6 to 10.8  $\mu\text{g/kg}$  were above the suggested limit (4  $\mu\text{g/kg}$ ) set by EU regulations for total aflatoxins (European Commission, 2010), where as three samples were ranging from 1.5 to 3.2  $\mu\text{g/kg}$  had aflatoxins below the suggested limit. The contamination level of individual AFs are also presented [Fig 3.3a].

Table 3.9: Total aflatoxins in cereals

| Commodities | Sample size/positive | Contamination ( $\mu\text{g} / \text{kg}$ ) |               | Mean value<br>( $\mu\text{g}/ \text{kg}$ ) |
|-------------|----------------------|---------------------------------------------|---------------|--------------------------------------------|
|             |                      | Below limit                                 | Above limit   |                                            |
| Rice        | 20/5                 | 3 (1.5-3.2) <sup>a</sup>                    | 2 (5.6-10.8)  | 4.6                                        |
| Rice broken | 20/3                 | 2 (2.1-2.9)                                 | 1 (8.0)       | 4.3                                        |
| Wheat       | 20/4                 | 2 (1.8-3.6)                                 | 2 (5.5-15.5)  | 6.6                                        |
| Maize       | 15/6                 | 3 (3.0-8.5)                                 | 3 (13.0-18.5) | 10.4                                       |
| Barley      | 10/2                 | -                                           | 2 (6.0-12.6)  | 9.3                                        |
| Sorghum     | 10/3                 | 2 (2.0-3.6)                                 | 1 (9.4)       | 5.0                                        |

<sup>a</sup> total aflatoxin range

Table 3.10: Total aflatoxin and individual levels in positive samples of cereals

| Commodities | Mean AFs<br>( $\mu\text{g} / \text{kg}$ ) | Aflatoxins ( $\mu\text{g} / \text{kg}$ ) |      |      |      |      |
|-------------|-------------------------------------------|------------------------------------------|------|------|------|------|
|             |                                           | Range                                    | AFB1 | AFB2 | AFG1 | AFG2 |
| Rice        | 4.6                                       | 1.5                                      | ND   | 1.5  | ND   | ND   |
|             |                                           | 2.0                                      | ND   | 2.0  | ND   | ND   |
|             |                                           | 3.2                                      | 2.0  | 1.2  | ND   | ND   |
|             |                                           | 5.6                                      | 0.8  | 1.8  | 2.0  | 1.0  |
|             |                                           | 10.8                                     | 4.8  | 2.5  | 1.5  | 2.0  |
| Rice broken | 4.3                                       | 2.1                                      | 1.1  | 1.0  | ND   | ND   |
|             |                                           | 2.9                                      | 1.4  | 1.5  | ND   | ND   |
|             |                                           | 8.0                                      | 2.6  | 3.4  | 2.0  | ND   |
| Wheat       | 6.6                                       | 1.8                                      | 1.8  | ND   | ND   | ND   |
|             |                                           | 3.6                                      | 2.0  | 1.6  | ND   | ND   |
| Maize       | 10.4                                      | 3.0                                      | ND   | ND   | 2.0  | 1.0  |
|             |                                           | 4.5                                      | ND   | 1.5  | 3.0  | ND   |
|             |                                           | 8.5                                      | 2.5  | 3.2  | 2.8  | ND   |
|             |                                           | 13.0                                     | 5.0  | 3.8  | 3.0  | 2.0  |
|             |                                           | 15.0                                     | 7.2  | 4.8  | 1.5  | 1.5  |
|             |                                           | 18.5                                     | 6.4  | 5.1  | 4.0  | 3.0  |
| Barley      | 9.3                                       | 6.0                                      | 4.0  | ND   | 2.0  | ND   |
|             |                                           | 12.6                                     | 5.6  | 2.5  | 4.5  | ND   |
| Sorghum     | 5.0                                       | 2.0                                      | 2.0  | ND   | ND   | ND   |
|             |                                           | 3.6                                      | 2.4  | ND   | 1.2  | ND   |
|             |                                           | 9.4                                      | 3.4  | 2.5  | 3.5  | ND   |



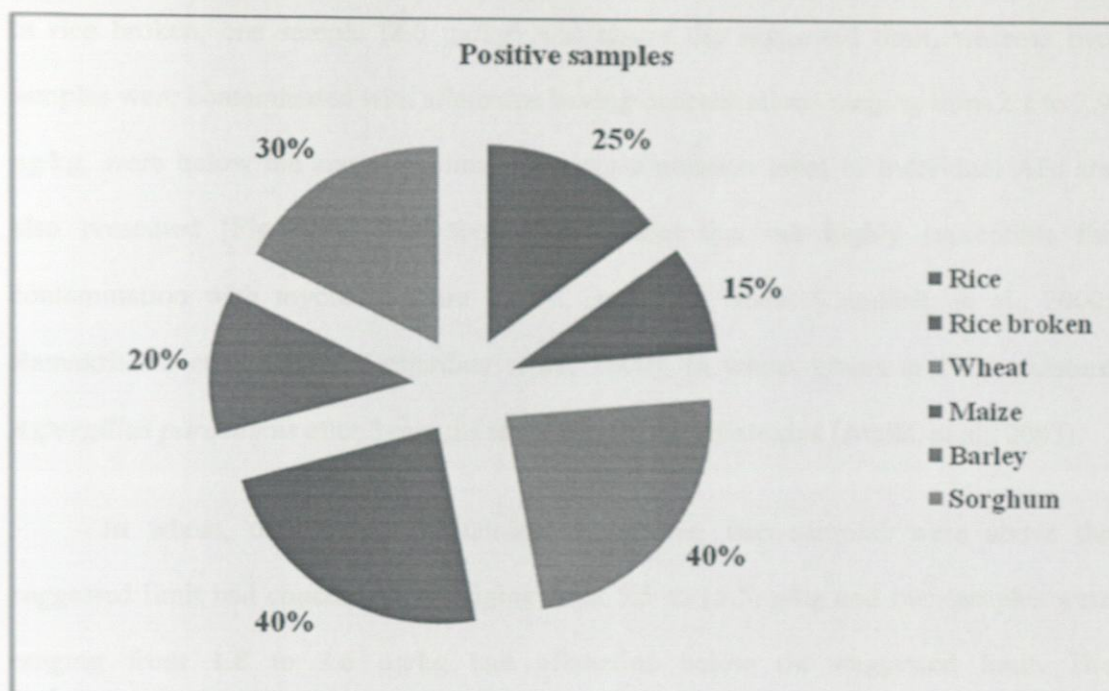


Fig 3.3: Aflatoxins level in contaminated cereals

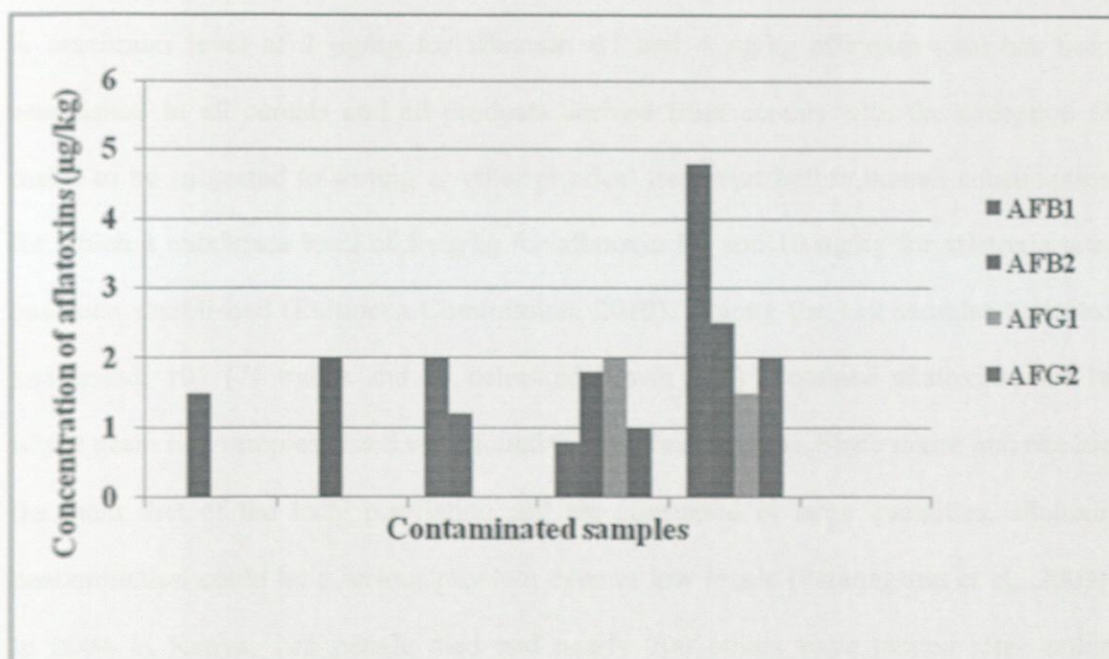


Fig 3.3a: Aflatoxins level in contaminated rice

In rice broken, one sample (8.0 µg/kg) was above the suggested limit, whereas two samples were contaminated with aflatoxins having concentrations ranging from 2.1 to 2.9 µg/kg, were below the suggested limit. The contamination level of individual AFs are also presented [Fig 3.3b]. The food commodities that are highly susceptible for contamination with mycotoxins are barley, corn, and wheat (Candlish, et al., 2000; Ramakrishna et al., 1990; Desjardins et al., 2000). In wheat grains at 14% moisture *Aspergillus parasiticus* after 3 months storage produces aflatoxins (Atalla, et al., 2003).

In wheat, out of four contaminated samples, two samples were above the suggested limit had concentration ranging from 5.5 to 15.5 µg/kg and two samples were ranging from 1.8 to 3.6 µg/kg had aflatoxins below the suggested limit. The contamination level of individual AFs are also presented [Fig 3.3c]. In grains rich with carbohydrates such as cotton, corn, peanuts and wheat *Aspergillus* genus produced AFs under optimum condition of moisture and temperature (Jaimez et al., 2000).

A maximum level of 2 µg/kg for aflatoxin B1 and 4 µg/kg aflatoxin total has been established in all cereals and all products derived from cereals with the exception of maize to be subjected to sorting or other physical treatment before human consumption for which a maximum level of 5 µg/kg for aflatoxin B1 and 10 µg/kg for aflatoxin total has been established (European Commission, 2010). Among the 110 samples collected and tested, 107 (71 maize and 36 dehusked brown rice) contained aflatoxins. All 16 whole grain rice samples tested were found to contain aflatoxins. Since maize and rice are the main diet of the local population and are consumed in large quantities, aflatoxin contamination could be a serious problem even at low levels (Paranagama et al., 2003). In 2004 in Kenya, 125 people died and nearly 200 others were treated after eating aflatoxin contaminated maize (Lewis et al., 2005).



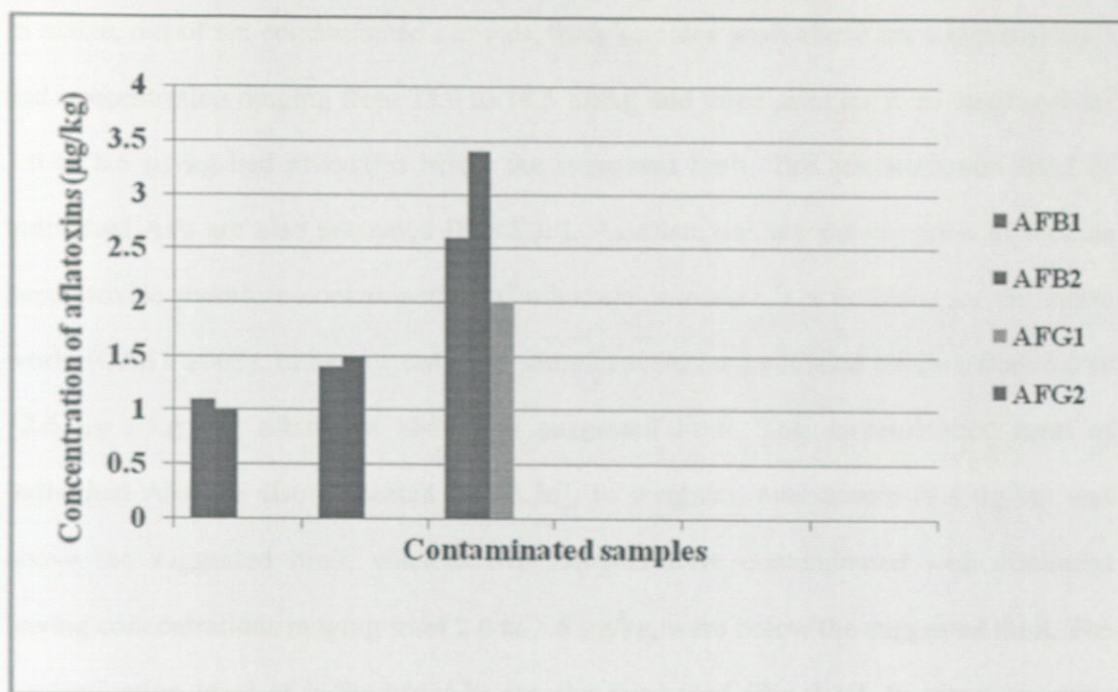


Fig 3.3b: Aflatoxins level in contaminated rice broken

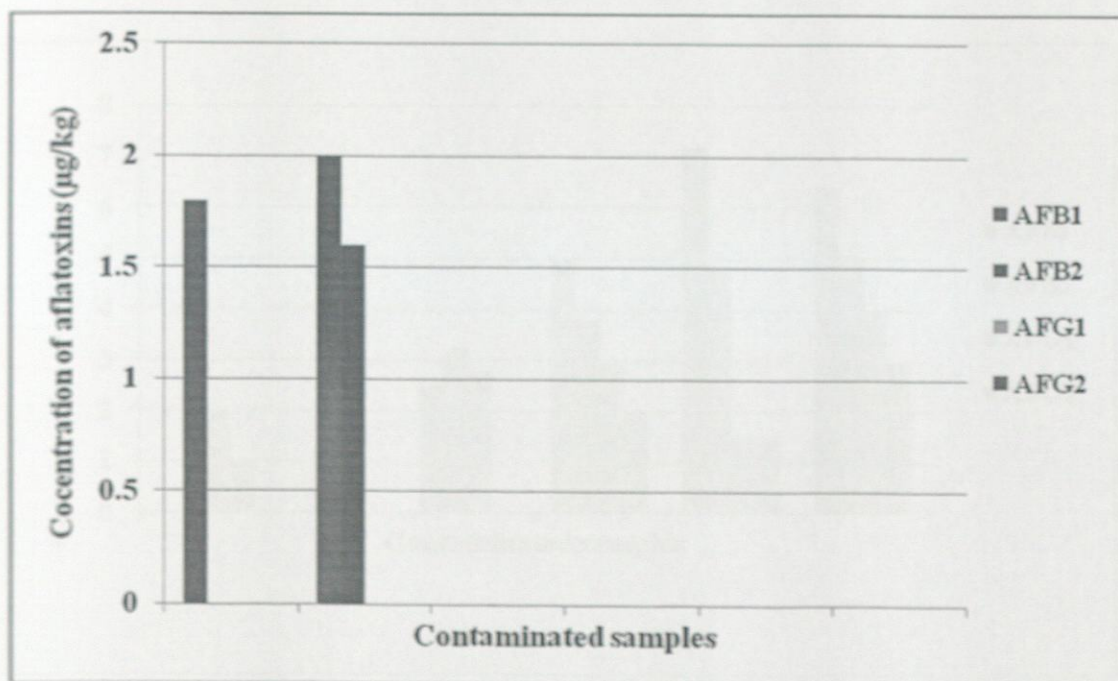


Fig 3.3c: Aflatoxins level in contaminated wheat

In maize, out of six contaminated samples, three samples were above the suggested limit had concentration ranging from 13.0 to 18.5  $\mu\text{g/kg}$  and three samples were ranging from 3.0 to 8.5  $\mu\text{g/kg}$  had aflatoxins below the suggested limit. The contamination level of individual AFs are also presented [Fig 3.3d]. As aflatoxins are carcinogenic as well as hepatotoxins therefore contamination of aflatoxin in maize is a problem for the entire world (CAST 2003). In barley, only two samples were contaminated ranging from 6.0 to 12.6  $\mu\text{g/kg}$  had aflatoxins above the suggested limit. The contamination level of individual AFs are also presented [Fig 3.3e]. In sorghum, one sample (9.4  $\mu\text{g/kg}$ ) was above the suggested limit, whereas two samples were contaminated with aflatoxins having concentrations ranging from 2.0 to 3.6  $\mu\text{g/kg}$ , were below the suggested limit. The contamination level of individual AFs are also presented [Fig 3.3f]. In many countries presence of aflatoxins naturally were found in commodities such as sorghum and pistachio (Abdulkadar, 2000; Cheraghali, 2007; Ghali, 2007).

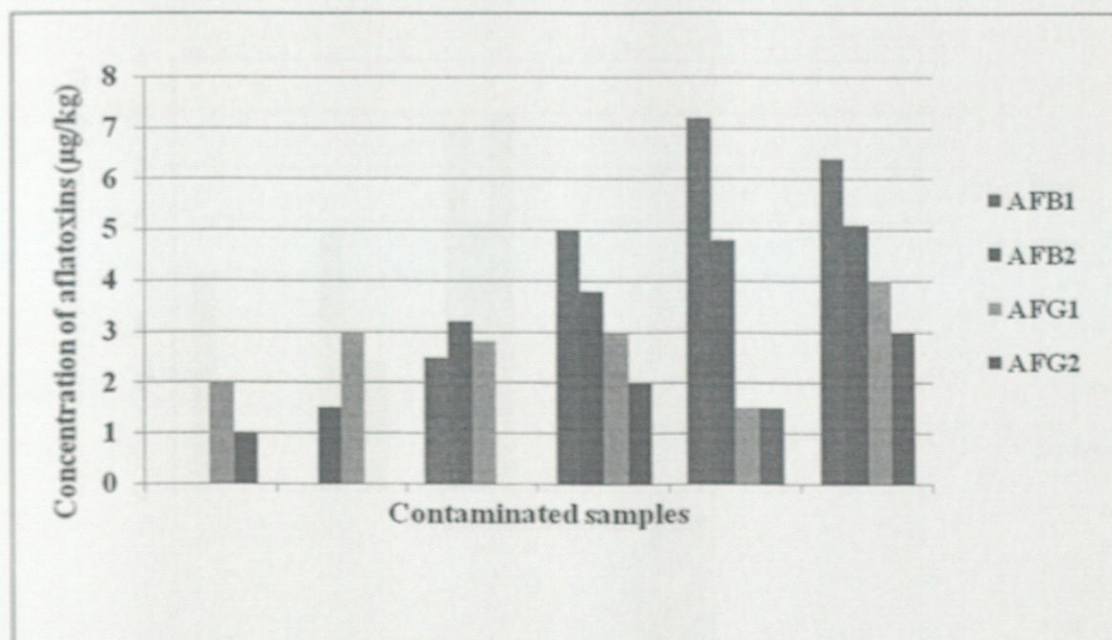


Fig 3.3d: Aflatoxins level in contaminated maize



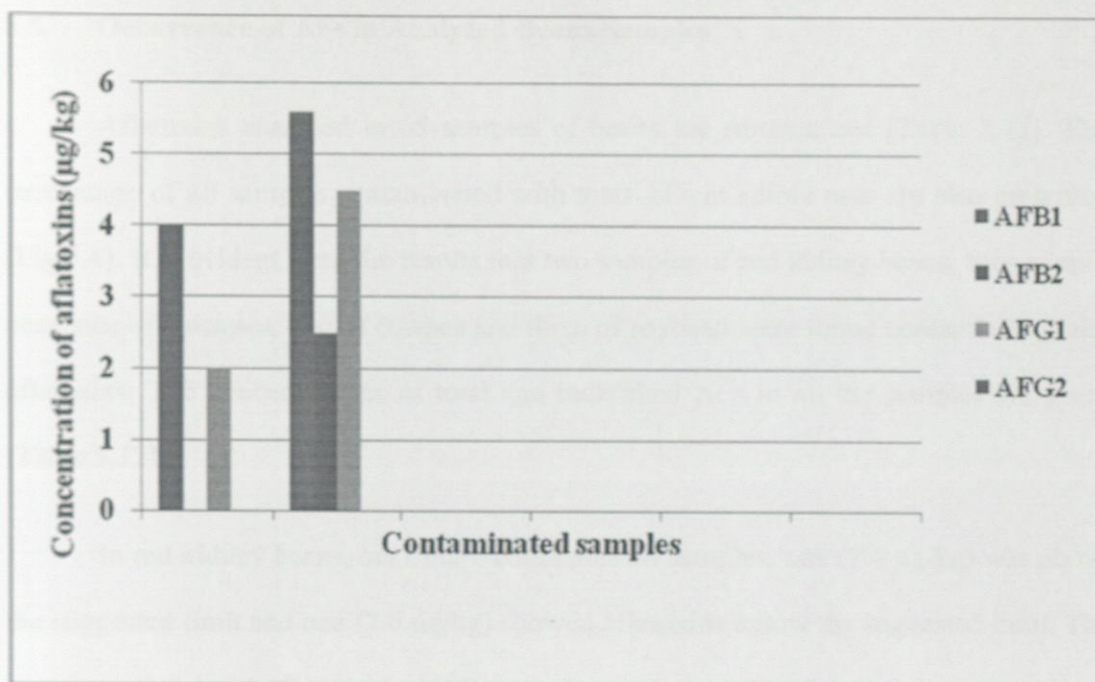


Fig 3.3e: Aflatoxins level in contaminated barley

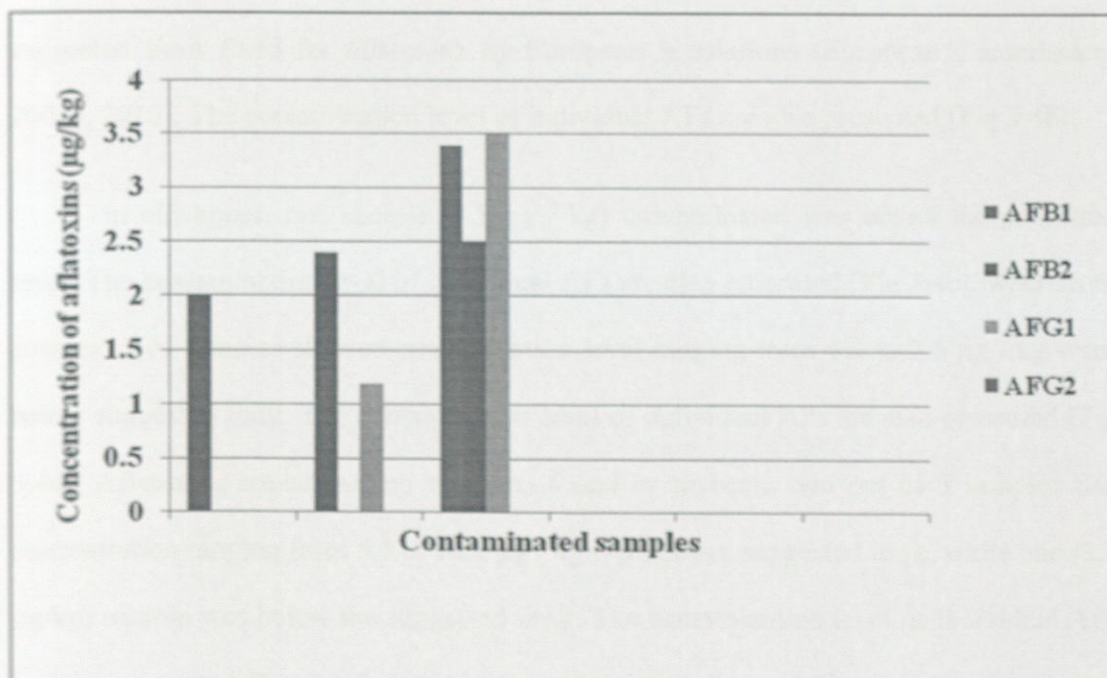


Fig 3.3f: Aflatoxins level in contaminated sorghum

### 3.5. Occurrence of AFs in Analyzed Beans Samples

Aflatoxins analyzed in 65 samples of beans are summarized [Table 3.11]. The percentage of all samples contaminated with total AFs in edible nuts are also presented [Fig 3.4]. It is evident from the results that two samples of red kidney beans, four of split peas, one of chickpea, two of cowpea and three of soybean were found contaminated with aflatoxins. The concentrations of total and individual AFs in all the samples are given [Table 3.12].

In red kidney beans, out of two contaminated samples, one (7.4  $\mu\text{g/kg}$ ) was above the suggested limit and one (2.6  $\mu\text{g/kg}$ ) showed aflatoxins below the suggested limit. The contamination level of individual AFs are also presented [Fig 3.4a]. Split peas showed maximum contamination levels among all the samples, out of four contaminated samples one (9.5  $\mu\text{g/kg}$ ) was above the suggested limit, whereas, three samples were contaminated had concentrations ranging from 1.5 to 3.0  $\mu\text{g/kg}$ , were below the suggested limit fixed for aflatoxins by European legislations (European Commission, 2006a, 2010). The contamination level of individual AFs are also presented [Fig 3.4b].

In chickpeas, one sample (2.5  $\mu\text{g} / \text{kg}$ ) contaminated was below the permitted level. The contamination level of individual AFs are also presented [Fig 3.4c], whereas in cowpeas two samples showed contamination level ranging from 1.8 to 2.6  $\mu\text{g} / \text{kg}$  were below suggested limit. The contamination level of individual AFs are also presented [Fig 3.4d]. Aflatoxins contamination was also found in soybean, two out of 3 samples had concentration ranging from 5.5 to 10.5  $\mu\text{g} / \text{kg}$  were above suggested limit, while one (3.2  $\mu\text{g/kg}$ ) sample was below the suggested limit. The contamination level of individual AFs are also presented [Fig 3.4e]. It has been reported that due to utilization of contaminated food commodities, toxicity indicated in humans (Williams et al., 2004). In wide rage of



feed and foodstuff including dried fruits such as nuts and pistachios; cereals such as wheat barley sorghum and some spices contains total aflatoxins B1, B2, G1 and G2 (Maurice and Moss, 2002).

**Table 3.11: Total aflatoxins in beans**

| Commodities      | Sample size/positive | Contamination ( $\mu\text{g} / \text{kg}$ ) |              | Mean value<br>( $\mu\text{g} / \text{kg}$ ) |
|------------------|----------------------|---------------------------------------------|--------------|---------------------------------------------|
|                  |                      | Below limit                                 | Above limit  |                                             |
| Red kidney beans | 10/2                 | 1 (2.6) <sup>a</sup>                        | 1 (7.4)      | 5.0                                         |
| Split pea        | 15/4                 | 3 (1.5-3.0)                                 | 1 (9.5)      | 4.1                                         |
| Chickpea         | 10/1                 | 1 (2.5)                                     | -            | 2.5                                         |
| Cowpea           | 10/2                 | 2 (1.8-2.6)                                 | -            | 2.2                                         |
| Soybean          | 20/3                 | 1 (3.2)                                     | 2 (5.5-10.5) | 6.4                                         |

<sup>a</sup>total aflatoxin range

**Table 3.12: Total aflatoxin and individual levels in positive samples of beans**

| Commodities      | Mean AFs<br>( $\mu\text{g} / \text{kg}$ ) | Aflatoxins ( $\mu\text{g} / \text{kg}$ ) |      |      |      |      |
|------------------|-------------------------------------------|------------------------------------------|------|------|------|------|
|                  |                                           | Range                                    | AFB1 | AFB2 | AFG1 | AFG2 |
| Red kidney beans | 5.0                                       | 2.6                                      | 1.6  | 1.0  | ND   | ND   |
|                  |                                           | 7.4                                      | 3.4  | 2.0  | 2.0  | ND   |
| Split pea        | 4.1                                       | 1.5                                      | 0.7  | 0.8  | ND   | ND   |
|                  |                                           | 2.5                                      | 1.5  | ND   | 1.0  | ND   |
|                  |                                           | 3.0                                      | ND   | ND   | 3.0  | ND   |
| Chickpea         | 2.5                                       | 2.5                                      | 2.5  | ND   | ND   | ND   |
| Cowpea           | 2.2                                       | 1.8                                      | ND   | ND   | 1.0  | 0.8  |
|                  |                                           | 2.6                                      | 1.6  | ND   | 1.0  | ND   |
| Soybean          | 6.4                                       | 3.2                                      | 1.9  | 1.3  | ND   | ND   |
|                  |                                           | 5.5                                      | 3.0  | 1.5  | 1.0  | ND   |
|                  |                                           | 10.5                                     | 4.5  | 2.6  | 2.4  | 1.0  |

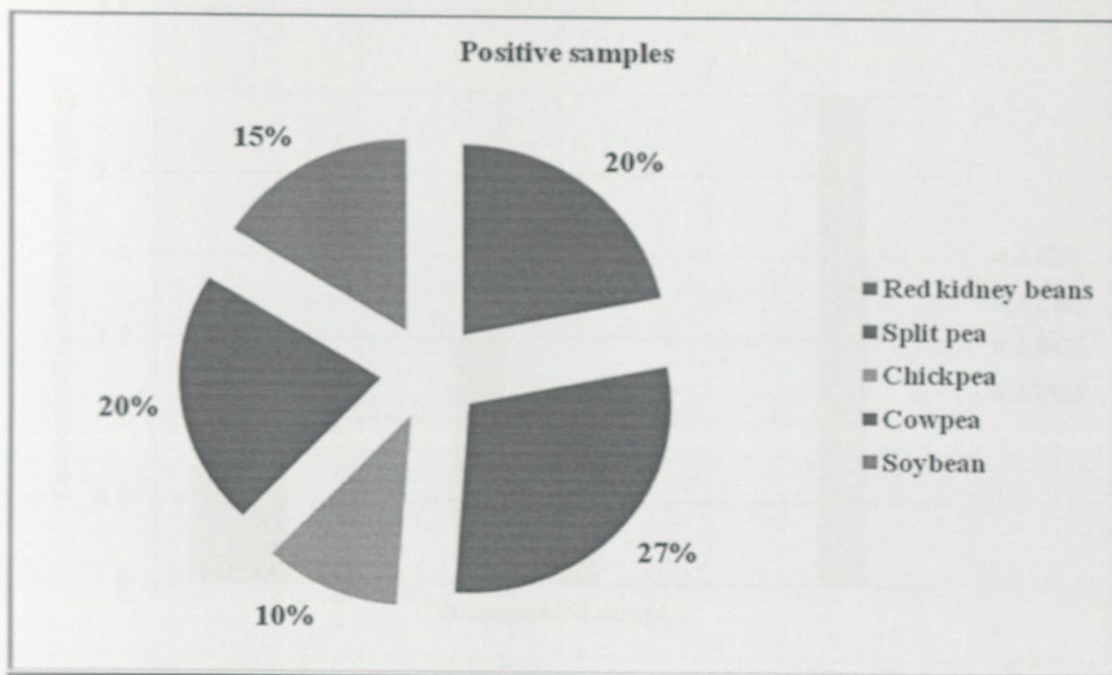


Fig 3.4: Aflatoxins level in contaminated beans

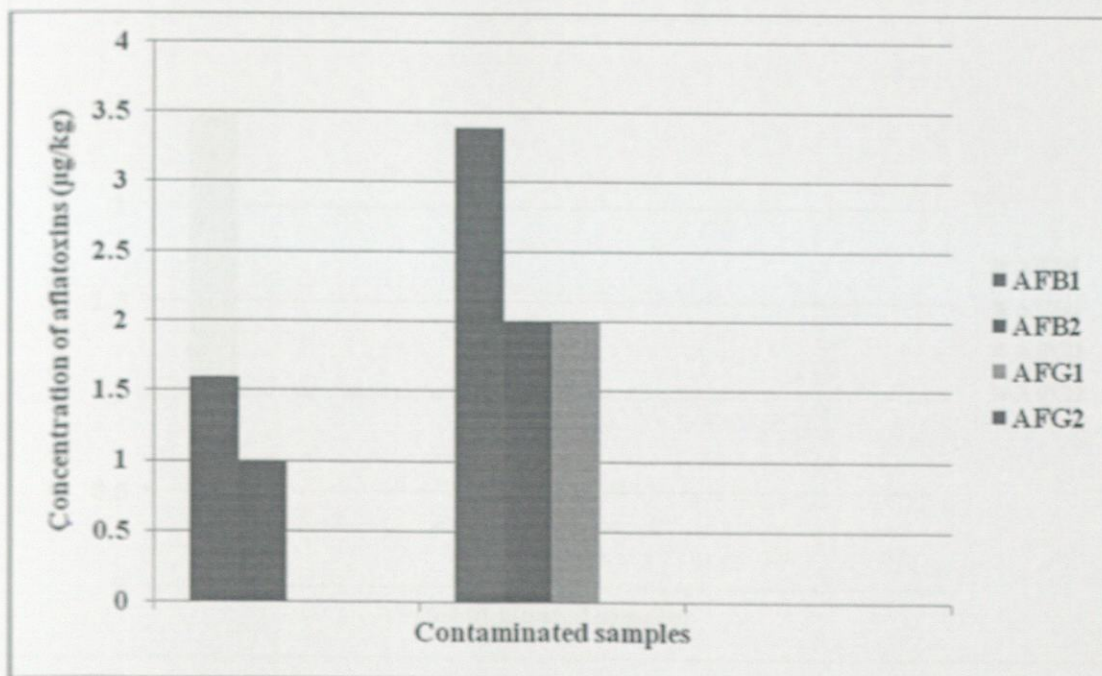


Fig 3.4a: Aflatoxins level in contaminated red kidney beans



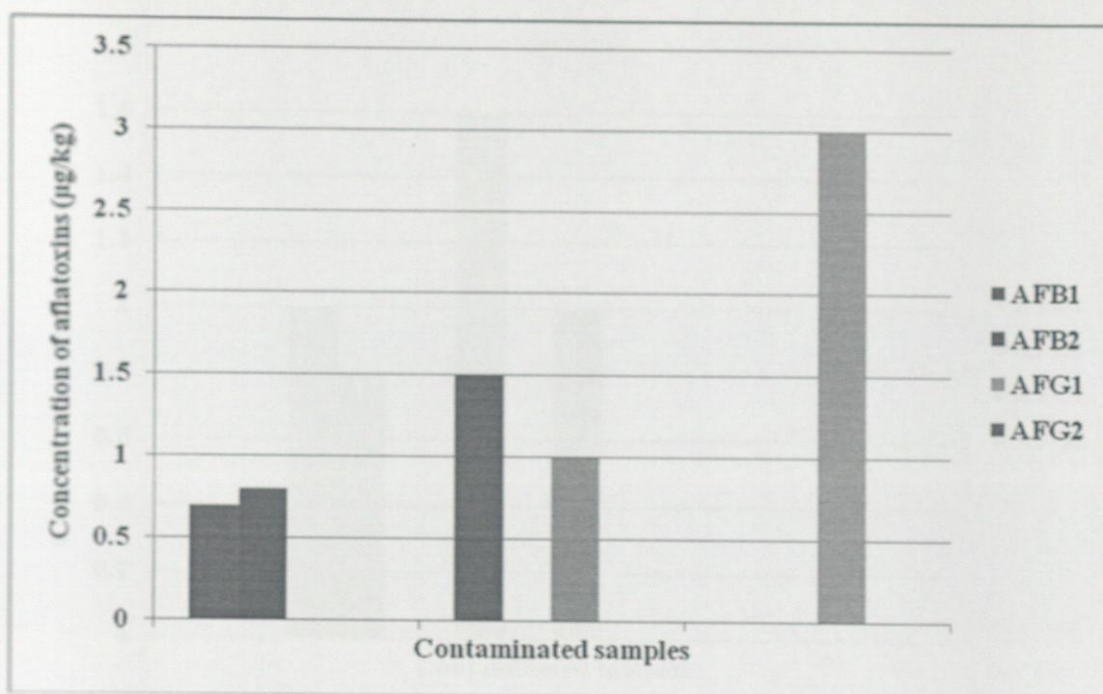


Fig 3.4b: Aflatoxins level in contaminated split peas

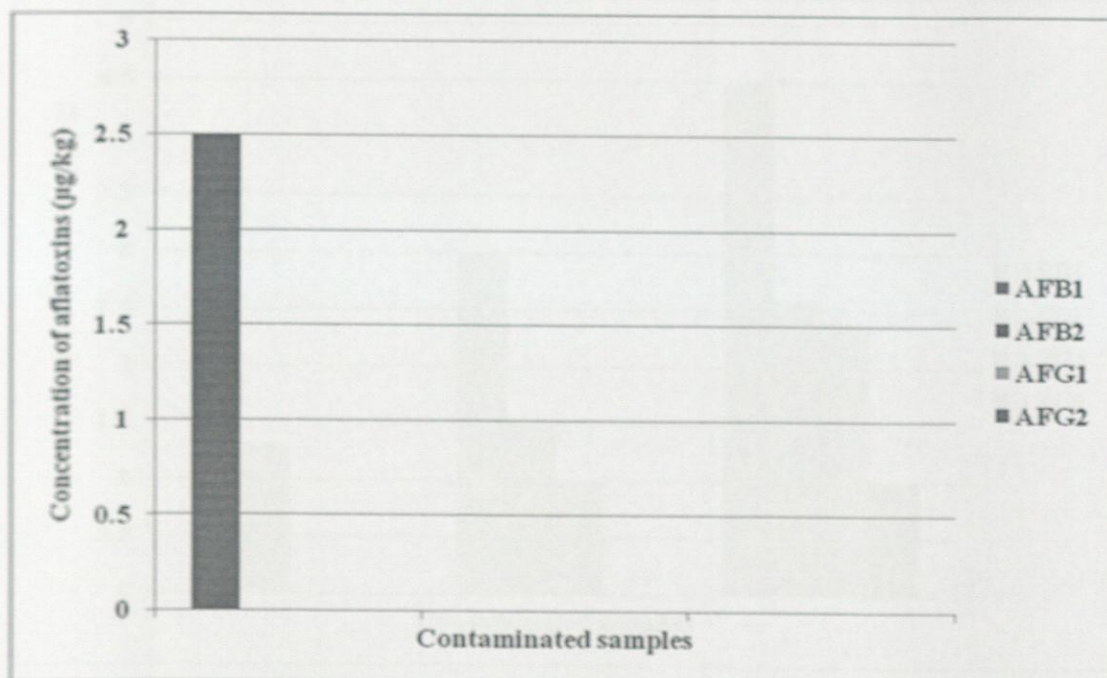


Fig 3.4c: Aflatoxins level in contaminated chick pea

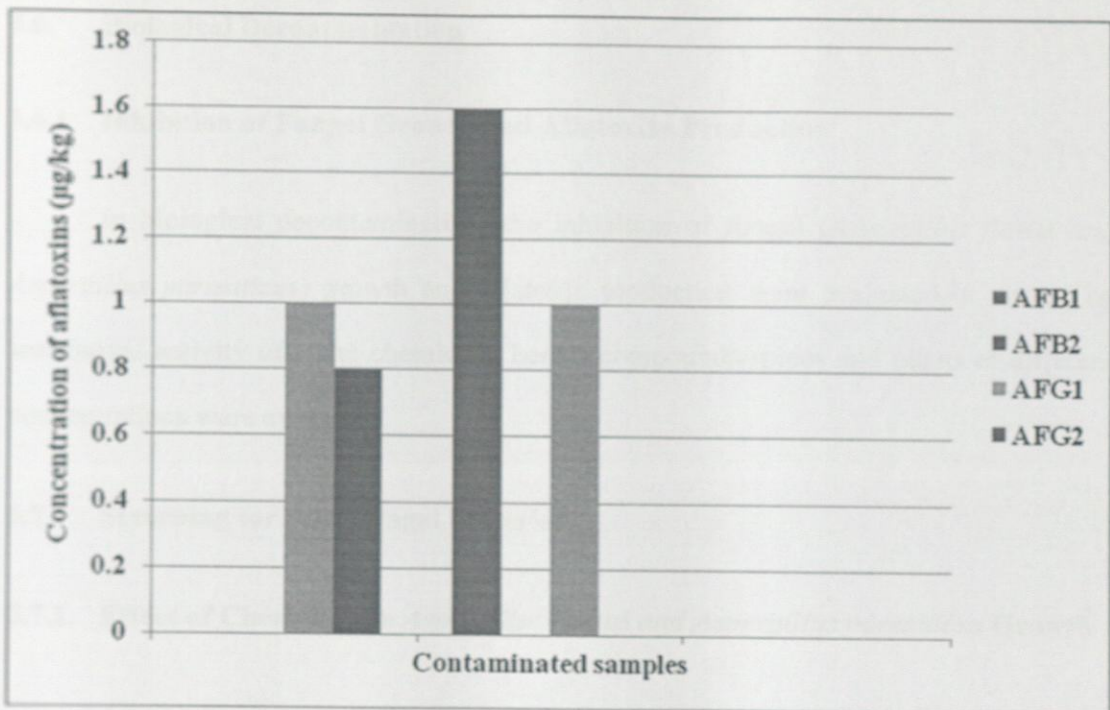


Fig 3.4d: Aflatoxins level in contaminated cowpea

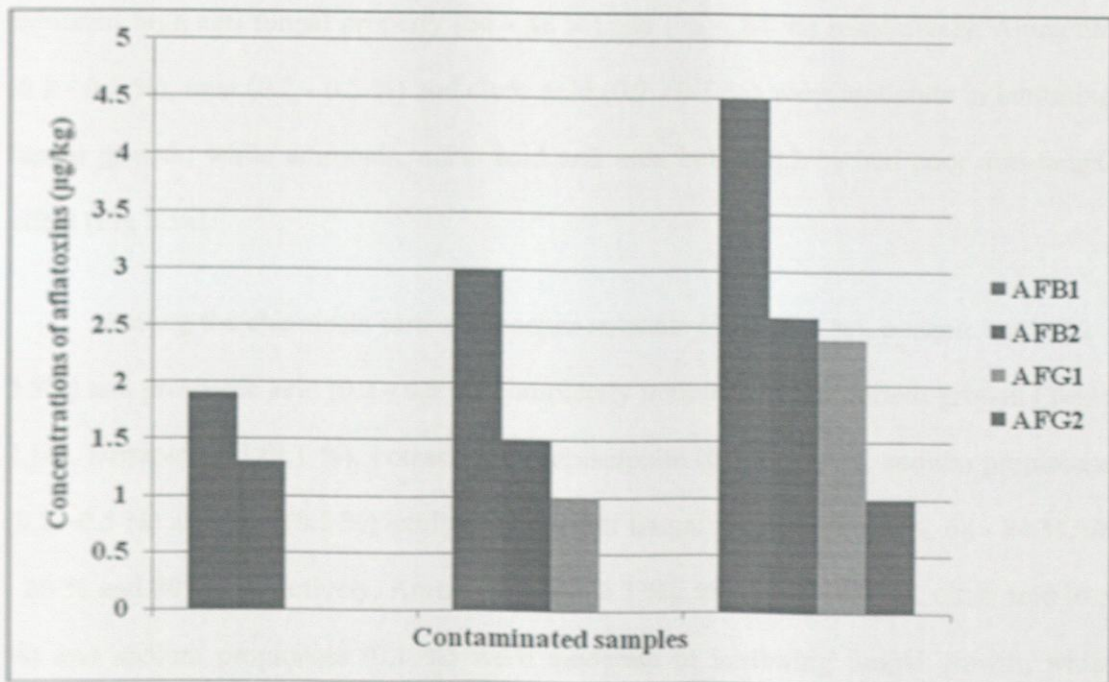


Fig 3.4e: Aflatoxins level in contaminated soybeans



### 3.6. Biological Decontamination

#### 3.6.1 Inhibition of Fungal Growth and Aflatoxins Production

In biological decontamination, the inhibition of fungal (*Aspergillus flavus* and *Aspergillus parasiticus*) growth and aflatoxin production were evaluated in vitro. The anti-fungal activity of some chemicals, herbal compounds/spices and plants at different concentrations were evaluated.

#### 3.7. Screening for Anti Fungal Materials

##### 3.7.1. Effect of Chemicals on *Aspergillus flavus* and *Aspergillus parasiticus* Growth

Among the chemicals screened, benzoic acid (0.1 – 0.5 %), propionic acid (0.1 – 0.5 %) and copper sulphate (0.2 – 0.5 %) completely inhibited *A. flavus* growth [Table 3.13]. Potassium metabisulphite (0.1 – 0.5 %) and sodium propionate (0.2–0.5 %) exhibited high anti fungal property (64 - 88 %) and (76 – 84 %) respectively. Ammonia (0.2 - 0.5 %), urea (0.2 - 0.5 %) and citric acid (0.2 - 0.5 %) were moderate in inhibiting fungal growth, while ammonia, citric acid and urea below 0.2 % had poor anti-fungal effect [Fig 3.5a].

Among the chemicals screened copper sulphate (0.1 – 0.5 %), benzoic acid (0.2 - 0.5%) and propionic acid (0.2 - 0.5 %) completely inhibited *A. parasiticus* growth [Table 3.14]. Benzoic acid (0.1 %), potassium metabisulphite (0.1 – 0.5 %), sodium propionate (0.2 –0.5 %) and urea (0.5 %) exhibited high anti fungal property as 96 %, 68 - 84 %, 68 - 80 % and 80 % respectively. Ammonia (0.2 - 0.5 %), urea (0.1 - 0.2 %), citric acid (0.5 %) and sodium propionate (0.1 %) were moderate in inhibiting fungal growth, while ammonia (0.1 %), citric acid (0.1 - 0.2 %) and urea (0.1 %) showed poor antifungal

property against fungal growth [Fig 3.5b]. Except for sodium chloride and urea the antifungal activity of all the remaining chemicals were evaluated. Propionic acid is used to inhibit mould growth. Its disadvantage is that it is a corrosive, which makes it dangerous for handling (Kiessling et al., 1982). The disadvantages of ammoniation are the relatively long period of aeration and its cost which can increase the price of the product by 5 – 20 % (Coker, 1998).

**Table 3.13:** Efficacy of some chemicals to inhibit *Aspergillus flavus* growth

| Chemicals         | Concentration <sup>a</sup> | Spore count <sup>b</sup> | Percentage inhibition |
|-------------------|----------------------------|--------------------------|-----------------------|
| Control           | –                          | 25 ± 1.65                | –                     |
| Ammonia           | 0.1                        | 19 ± 1.20                | 24                    |
| (v/v)             | 0.2                        | 9 ± 0.50                 | 66                    |
|                   | 0.5                        | 5 ± 0.48                 | 80                    |
| Benzoic acid      | 0.1                        | 0                        | 100                   |
| (w/v)             | 0.2                        | 0                        | 100                   |
|                   | 0.5                        | 0                        | 100                   |
| Citric acid       | 0.1                        | 18 ± 0.80                | 28                    |
| (w/v)             | 0.2                        | 15 ± 0.75                | 40                    |
|                   | 0.5                        | 11 ± 0.55                | 56                    |
| Copper sulphate   | 0.1                        | 5 ± 0.35                 | 80                    |
| (w/v)             | 0.2                        | 0                        | 100                   |
|                   | 0.5                        | 0                        | 100                   |
| Potassium         | 0.1                        | 9 ± 0.40                 | 64                    |
| metabisulphite    | 0.2                        | 7 ± 0.55                 | 72                    |
| (w/v)             | 0.5                        | 3 ± 0.15                 | 88                    |
| Propionic acid    | 0.1                        | 0                        | 100                   |
| (v/v)             | 0.2                        | 0                        | 100                   |
|                   | 0.5                        | 0                        | 100                   |
| Sodium propionate | 0.1                        | 14 ± 0.60                | 44                    |
| (w/v)             | 0.2                        | 6 ± 0.45                 | 76                    |
|                   | 0.5                        | 4 ± 0.30                 | 84                    |
| Sodium Chloride   | 0.1                        | 25 ± 1.55                | 0                     |
| (w/v)             | 0.2                        | 24 ± 1.40                | 4                     |
|                   | 0.5                        | 23 ± 1.55                | 8                     |
| Urea              | 0.1                        | 15 ± 0.70                | 40                    |
| (w/v)             | 0.2                        | 9 ± 0.50                 | 64                    |
|                   | 0.5                        | 6 ± 0.25                 | 76                    |

<sup>a</sup> Percentage of potato dextrose agar.

<sup>b</sup> Colony forming units.



Table 3.14: Efficacy of some chemicals to inhibit *Aspergillus parasiticus* growth

| Chemicals                            | Concentration <sup>a</sup> | Spore count <sup>b</sup> | Percentage inhibition |
|--------------------------------------|----------------------------|--------------------------|-----------------------|
| Control                              | —                          | 25 ± 1.70                | —                     |
| Ammonia<br>(v/v)                     | 0.1                        | 20 ± 1.20                | 20                    |
|                                      | 0.2                        | 10 ± 0.40                | 60                    |
|                                      | 0.5                        | 6 ± 0.25                 | 76                    |
| Benzoic acid<br>(w/v)                | 0.1                        | 1 ± 0.20                 | 96                    |
|                                      | 0.2                        | 0                        | 100                   |
|                                      | 0.5                        | 0                        | 100                   |
| Citric acid<br>(w/v)                 | 0.1                        | 19 ± 0.80                | 24                    |
|                                      | 0.2                        | 16 ± 0.55                | 36                    |
|                                      | 0.5                        | 12 ± 0.45                | 52                    |
| Copper sulphate<br>(w/v)             | 0.1                        | 0                        | 100                   |
|                                      | 0.2                        | 0                        | 100                   |
|                                      | 0.5                        | 0                        | 100                   |
| Potassium<br>metabisulphite<br>(w/v) | 0.1                        | 8 ± 0.35                 | 68                    |
|                                      | 0.2                        | 6 ± 0.32                 | 76                    |
|                                      | 0.5                        | 4 ± 0.15                 | 84                    |
| Propionic acid<br>(v/v)              | 0.1                        | 2 ± 0.10                 | 92                    |
|                                      | 0.2                        | 0                        | 100                   |
|                                      | 0.5                        | 0                        | 100                   |
| Sodium Chloride<br>(w/v)             | 0.1                        | 25 ± 1.40                | 0                     |
|                                      | 0.2                        | 27 ± 1.25                | 8                     |
|                                      | 0.5                        | 27 ± 0.88                | 8                     |
| Sodium propionate<br>(w/v)           | 0.1                        | 16 ± 0.75                | 44                    |
|                                      | 0.2                        | 17 ± 0.60                | 68                    |
|                                      | 0.5                        | 16 ± 0.54                | 80                    |
| Urea<br>(w/v)                        | 0.1                        | 15 ± 0.85                | 40                    |
|                                      | 0.2                        | 10 ± 0.70                | 60                    |
|                                      | 0.5                        | 5 ± 0.50                 | 80                    |

<sup>a</sup> Percentage of potato dextrose agar.

<sup>b</sup> Colony forming units.

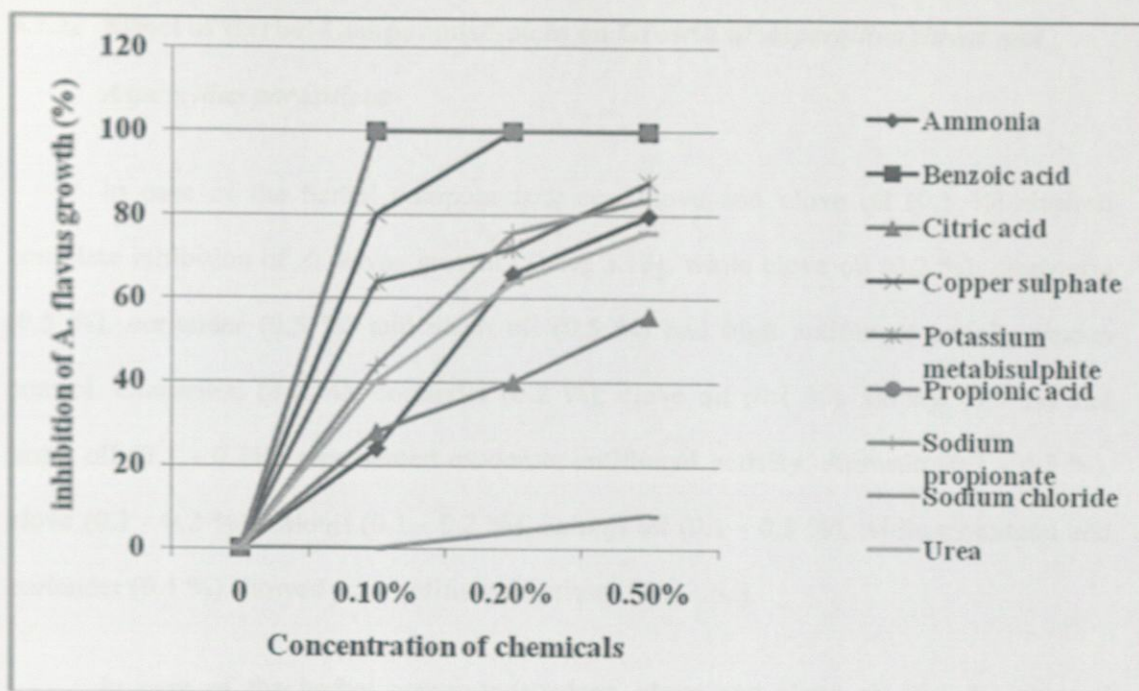


Fig 3.5a: Percent inhibition of *Aspergillus flavus* growth by different chemicals

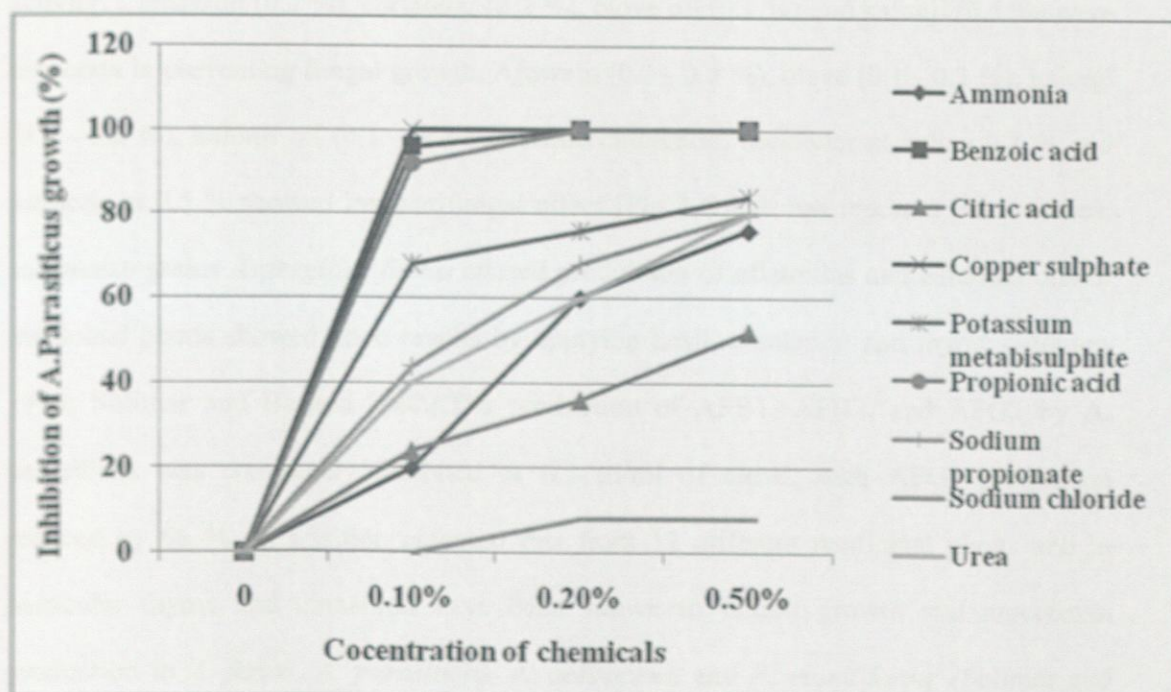


Fig 3.5b: Percent inhibition of *Aspergillus parasiticus* growth by different chemicals



### 3.7.2. Effect of Herbal Compounds/Spices on Growth of *Aspergillus flavus* and *Aspergillus parasiticus*

In case of the herbal compounds/spices, clove and clove oil (0.5 %) showed complete inhibition of *A. flavus* growth [Table 3.15], while clove oil (0.2 %), cinnamon (0.5 %), coriander (0.5 %) and neem oil (0.5 %) had high antifungal activity versus control. Cinnamon (0.2 %), coriander (0.2 %), clove oil (0.1 %), kalonji (0.5 %) and neem oil (0.1 - 0.2%) represented moderate antifungal activity. Ajowain (0.2 - 0.5 %), clove (0.1 - 0.2 %), kalonji (0.1 - 0.2 %), kalonji oil (0.1 - 0.5 %), while cinnamon and coriander (0.1 %) showed poor antifungal activity [Fig 3.6a].

In case of the herbal compounds/spices, clove and clove oil (0.5 %) showed complete inhibition of *A. parasiticus* growth [Table 3.16], while clove oil (0.2 %), cinnamon (0.5 %), coriander (0.5 %) and neem oil (0.2 - 0.5 %) showed high antifungal activity. Cinnamon (0.2 %), coriander (0.2 %), clove oil (0.1 %) and kalonji (0.5 %) were moderate in preventing fungal growth. Ajowain (0.2 - 0.5 %), clove (0.1 - 0.2 %), kalonji (0.1 - 0.2 %), kalonji oil (0.1 - 0.5 %), while cinnamon, coriander at below 0.2 % and turmeric at 0.5 % showed low antifungal effect [Fig 3.6b]. It has reported that in wheat and maize grains *Aspergillus flavus* caused production of aflatoxins and essential oils of medicinal plants showed good results by applying basil, cinnamon and thyme (Montes, 1998; Solimar and Badeaa 2002). The production of AFB1, AFB2, and AFG2 by *A. parasiticus* was completely inhibited at 0.1 µl/ml of clove, with AFG1 production reduced by 66 %. In addition essential oils from 12 different medicinal plants and in particular thyme and cinnamon have been shown to inhibit growth and mycotoxin production in *A. flavus*, *A. parasiticus*, *A. ochraceus*, and *F. moniliforme* (Solimar and Badeaa, 2002).

Table 3.15: Efficacy of some herbal compounds/spices to inhibit *Aspergillus flavus* growth

| Herbal Compounds/<br>Spices | Concentration <sup>a</sup> | Spore count <sup>b</sup> | Percentage inhibition |
|-----------------------------|----------------------------|--------------------------|-----------------------|
| Control                     | —                          | 25 ± 1.60                | —                     |
| Ajowain<br>(w/v)            | 0.1                        | 25 ± 1.45                | 0                     |
|                             | 0.2                        | 21 ± 1.60                | 16                    |
|                             | 0.5                        | 16 ± 0.58                | 36                    |
| Clove<br>(w/v)              | 0.1                        | 18 ± 1.20                | 28                    |
|                             | 0.2                        | 16 ± 0.75                | 36                    |
|                             | 0.5                        | 0                        | 100                   |
| Clove oil<br>(v/v)          | 0.1                        | 15 ± 0.66                | 40                    |
|                             | 0.2                        | 9 ± 0.35                 | 64                    |
|                             | 0.5                        | 0                        | 100                   |
| Cinnamon<br>(w/v)           | 0.1                        | 16 ± 1.10                | 36                    |
|                             | 0.2                        | 12 ± 0.85                | 52                    |
|                             | 0.5                        | 3 ± 0.12                 | 88                    |
| Coriander<br>(w/v)          | 0.1                        | 17 ± 0.45                | 32                    |
|                             | 0.2                        | 15 ± 0.50                | 40                    |
|                             | 0.5                        | 7 ± 0.60                 | 72                    |
| Kalonji<br>(w/v)            | 0.1                        | 19 ± 1.45                | 24                    |
|                             | 0.2                        | 18 ± 1.20                | 28                    |
|                             | 0.5                        | 12 ± 0.75                | 52                    |
| Kalonji oil<br>(v/v)        | 0.1                        | 21 ± 1.30                | 16                    |
|                             | 0.2                        | 20 ± 1.10                | 20                    |
|                             | 0.5                        | 20 ± 1.55                | 20                    |
| Neem oil<br>(v/v)           | 0.1                        | 14 ± 0.76                | 44                    |
|                             | 0.2                        | 11 ± 0.45                | 56                    |
|                             | 0.5                        | 2 ± 0.25                 | 92                    |
| Turmeric<br>(w/v)           | 0.1                        | 25 ± 1.60                | 0                     |
|                             | 0.2                        | 23 ± 1.35                | 8                     |
|                             | 0.5                        | 22 ± 0.95                | 12                    |

<sup>a</sup> Percentage of potato dextrose agar.

<sup>b</sup> Colony forming units.



Table 3.16: Efficacy of some herbal compounds/spices to inhibit *Aspergillus parasiticus* growth

| Herbal Compounds/<br>Spices | Concentration <sup>a</sup> | Spore count <sup>b</sup> | Percentage inhibition |
|-----------------------------|----------------------------|--------------------------|-----------------------|
| Control                     | —                          | 25 ± 2.05                | —                     |
| Ajowain<br>(w/v)            | 0.1                        | 25 ± 1.45                | 0                     |
|                             | 0.2                        | 22 ± 1.60                | 12                    |
|                             | 0.5                        | 16 ± 1.80                | 36                    |
| Clove<br>(w/v)              | 0.1                        | 17 ± 1.20                | 32                    |
|                             | 0.2                        | 15 ± 0.65                | 40                    |
|                             | 0.5                        | 0                        | 100                   |
| Clove oil<br>(v/v)          | 0.1                        | 14 ± 0.30                | 44                    |
|                             | 0.2                        | 10 ± 0.25                | 60                    |
|                             | 0.5                        | 0                        | 100                   |
| Cinnamon<br>(w/v)           | 0.1                        | 15 ± 1.20                | 40                    |
|                             | 0.2                        | 11 ± 0.45                | 56                    |
|                             | 0.5                        | 3 ± 0.15                 | 88                    |
| Coriander<br>(w/v)          | 0.1                        | 16 ± 0.70                | 36                    |
|                             | 0.2                        | 14 ± 0.65                | 44                    |
|                             | 0.5                        | 6 ± 0.50                 | 76                    |
| Kalonji<br>(w/v)            | 0.1                        | 19 ± 1.25                | 24                    |
|                             | 0.2                        | 18 ± 1.16                | 28                    |
|                             | 0.5                        | 12 ± 0.54                | 52                    |
| Kalonji oil<br>(v/v)        | 0.1                        | 21 ± 1.55                | 16                    |
|                             | 0.2                        | 21 ± 1.40                | 16                    |
|                             | 0.5                        | 19 ± 1.35                | 24                    |
| Neem oil<br>(v/v)           | 0.1                        | 13 ± 0.75                | 48                    |
|                             | 0.2                        | 10 ± 0.40                | 60                    |
|                             | 0.5                        | 2 ± 0.30                 | 92                    |
| Turmeric<br>(w/v)           | 0.1                        | 25 ± 1.70                | 0                     |
|                             | 0.2                        | 22 ± 1.45                | 12                    |
|                             | 0.5                        | 16 ± 0.85                | 24                    |

<sup>a</sup> Percentage of potato dextrose agar.

<sup>b</sup> Colony forming units.

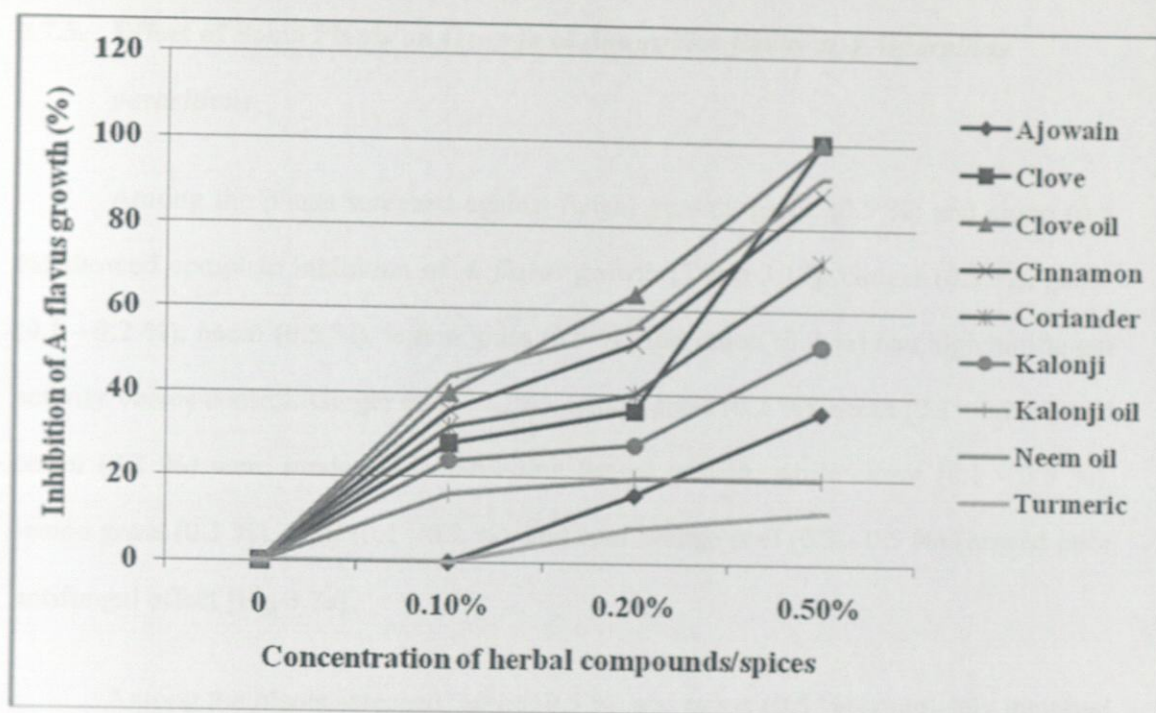


Fig 3.6a: Percent inhibition of *Aspergillus flavus* growth by different herbal compounds/spices

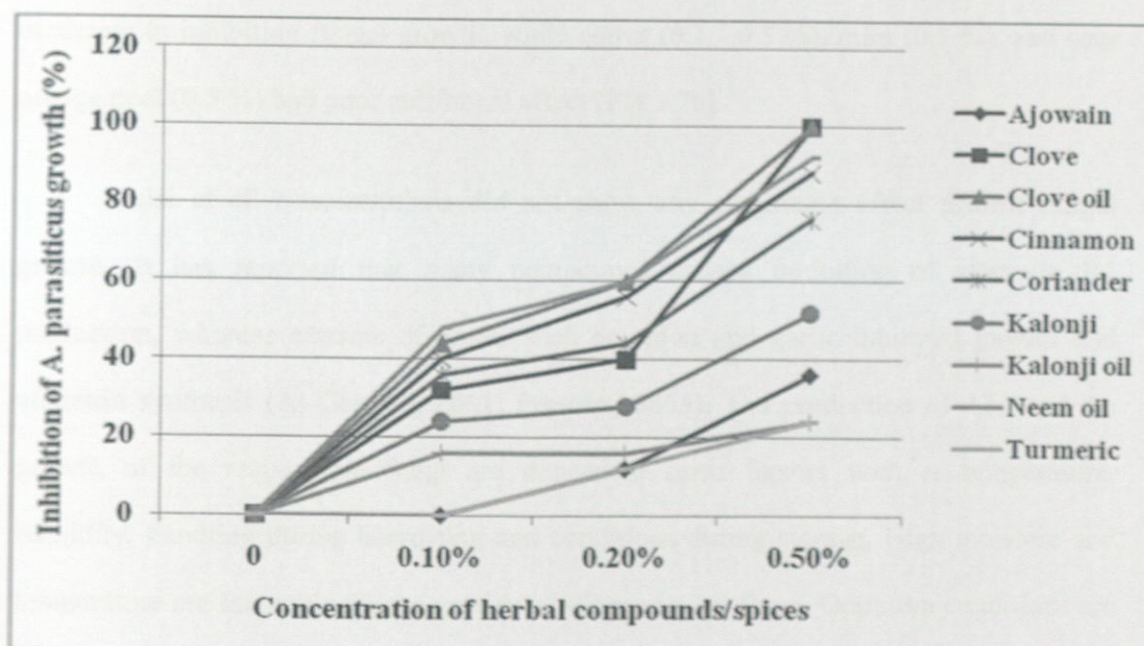


Fig 3.6b: Percent inhibition of *Aspergillus parasiticus* growth by different herbal compounds/spices



### 3.7.3. Effect of Some Plants on Growth of *Aspergillus flavus* and *Aspergillus parasiticus*

Among the plants screened against fungal growth, garlic (0.5 %) and onion (0.5 %) showed complete inhibition of *A. flavus* growth [Table 3.17]. Ginger (0.5 %), garlic (0.1 - 0.2 %), neem (0.5 %), lemon grass (0.5 %) and onion (0.2 %) had high antifungal activity verses control. Ginger (0.1 - 0.2%), lemon grass (0.2 %), neem (0.1 - 0.2 %) and onion (0.1 %) were moderate in inhibiting fungal growth, while carrot (0.1 - 0.5 %), lemon grass (0.1 %), mint (0.1 - 0.2 %), and sour orange peel (0.2 - 0.5 %) showed poor antifungal effect [Fig 3.7a].

Among the plants screened, garlic (0.5 %) and onion (0.5 %) completely inhibited *A. parasiticus* growth [Table 3.18]. Ginger (0.5 %), garlic (0.1 - 0.2 %), neem (0.2 - 0.5 %), lemon grass (0.5 %) and onion (0.2 %) had high antifungal activity. Ginger (0.1 - 0.2 %), lemon grass (0.1 - 0.2 %), mint (0.5 %), neem (0.1 %) and onion (0.1 %) were moderate in inhibiting fungal growth, while carrot (0.1 - 0.5 %), mint (0.1 %), and sour orange peel (0.5 %) had poor antifungal effect [Fig 3.7b].

Tulsi at all concentrations did not show any significant effect against fungal growth. It has reported that many compounds caused inhibition of aflatoxin B1 production, whereas extracts of plants such as onion and garlic inhibited growth and aflatoxin synthesis (Al-Ghamdi, 2001; Prescott, 2005). The production of AFs and the growth of the responsible fungi are dependent upon factors such as temperature, humidity, handling during harvesting and conditions during storage. High moisture and temperature are favorable for the growth of AF producing fungi. Optimum conditions are 16–24% moisture at 20–38 °C. However, it is reported that AF production can also take place at temperatures as low as 7–12 °C (Belma et al., 2007).

Table 3.17: Efficacy of some plants to inhibit *Aspergillus flavus* growth

| Plants                    | Concentration <sup>a</sup> | Spore count <sup>b</sup> | Percentage inhibition |
|---------------------------|----------------------------|--------------------------|-----------------------|
| Control                   | —                          | 25 ± 1.80                | —                     |
| Carrot<br>(w/v)           | 0.1                        | 19 ± 2.30                | 24                    |
|                           | 0.2                        | 16 ± 1.48                | 36                    |
|                           | 0.5                        | 15 ± 1.25                | 40                    |
| Garlic<br>(w/v)           | 0.1                        | 7 ± 0.45                 | 72                    |
|                           | 0.2                        | 5 ± 0.30                 | 80                    |
|                           | 0.5                        | 0                        | 100                   |
| Ginger<br>(w/v)           | 0.1                        | 11 ± 0.50                | 56                    |
|                           | 0.2                        | 9 ± 0.45                 | 64                    |
|                           | 0.5                        | 4 ± 0.15                 | 84                    |
| Lemon grass<br>(w/v)      | 0.1                        | 14 ± 0.56                | 44                    |
|                           | 0.2                        | 11 ± 0.45                | 56                    |
|                           | 0.5                        | 5 ± 0.20                 | 80                    |
| Mint<br>(w/v)             | 0.1                        | 15 ± 0.60                | 40                    |
|                           | 0.2                        | 15 ± 0.53                | 40                    |
|                           | 0.5                        | 10 ± 0.25                | 60                    |
| Neem<br>(w/v)             | 0.1                        | 12 ± 0.80                | 52                    |
|                           | 0.2                        | 9 ± 0.55                 | 64                    |
|                           | 0.5                        | 3 ± 0.05                 | 88                    |
| Onion<br>(w/v)            | 0.1                        | 10 ± 0.35                | 60                    |
|                           | 0.2                        | 4 ± 0.20                 | 84                    |
|                           | 0.5                        | 0                        | 100                   |
| Sour orange peel<br>(w/v) | 0.1                        | 25 ± 2.15                | 0                     |
|                           | 0.2                        | 20 ± 1.75                | 16                    |
|                           | 0.5                        | 18 ± 0.95                | 28                    |
| Tulsi<br>(w/v)            | 0.1                        | 25 ± 1.90                | 0                     |
|                           | 0.2                        | 23 ± 1.65                | 8                     |
|                           | 0.5                        | 22 ± 1.40                | 12                    |

<sup>a</sup> Percentage of potato dextrose agar;

<sup>b</sup> Colony forming units.



Table 3.18: Efficacy of some plants to inhibit *Aspergillus parasiticus* growth

| Plant                     | Concentration <sup>a</sup> | Spore count <sup>b</sup> | Percentage inhibition |
|---------------------------|----------------------------|--------------------------|-----------------------|
| Control                   | —                          | 25 ± 1.65                | —                     |
| Carrot<br>(w/v)           | 0.1                        | 20 ± 1.56                | 20                    |
|                           | 0.2                        | 17 ± 1.28                | 32                    |
|                           | 0.5                        | 15 ± 1.20                | 40                    |
| Garlic<br>(w/v)           | 0.1                        | 6 ± 0.54                 | 76                    |
|                           | 0.2                        | 4 ± 0.35                 | 84                    |
|                           | 0.5                        | 0                        | 100                   |
| Ginger<br>(w/v)           | 0.1                        | 12 ± 0.60                | 52                    |
|                           | 0.2                        | 10 ± 0.55                | 60                    |
|                           | 0.5                        | 4 ± 0.36                 | 84                    |
| Lemon grass<br>(w/v)      | 0.1                        | 12 ± 1.30                | 52                    |
|                           | 0.2                        | 11 ± 0.85                | 56                    |
|                           | 0.5                        | 6 ± 0.32                 | 76                    |
| Mint<br>(w/v)             | 0.1                        | 14 ± 0.70                | 44                    |
|                           | 0.2                        | 12 ± 0.65                | 52                    |
|                           | 0.5                        | 9 ± 0.54                 | 64                    |
| Neem<br>(w/v)             | 0.1                        | 13 ± 1.10                | 48                    |
|                           | 0.2                        | 7 ± 0.68                 | 72                    |
|                           | 0.5                        | 2 ± 0.25                 | 92                    |
| Onion<br>(w/v)            | 0.1                        | 11 ± 0.85                | 56                    |
|                           | 0.2                        | 3 ± 0.30                 | 88                    |
|                           | 0.5                        | 0                        | 100                   |
| Sour orange peel<br>(w/v) | 0.1                        | 25 ± 2.15                | 0                     |
|                           | 0.2                        | 22 ± 1.75                | 12                    |
|                           | 0.5                        | 18 ± 1.36                | 28                    |
| Tulsi<br>(w/v)            | 0.1                        | 25 ± 1.56                | 0                     |
|                           | 0.2                        | 23 ± 1.40                | 8                     |
|                           | 0.5                        | 23 ± 1.20                | 8                     |

<sup>a</sup> Percentage of potato dextrose agar;

<sup>b</sup> Colony forming units.

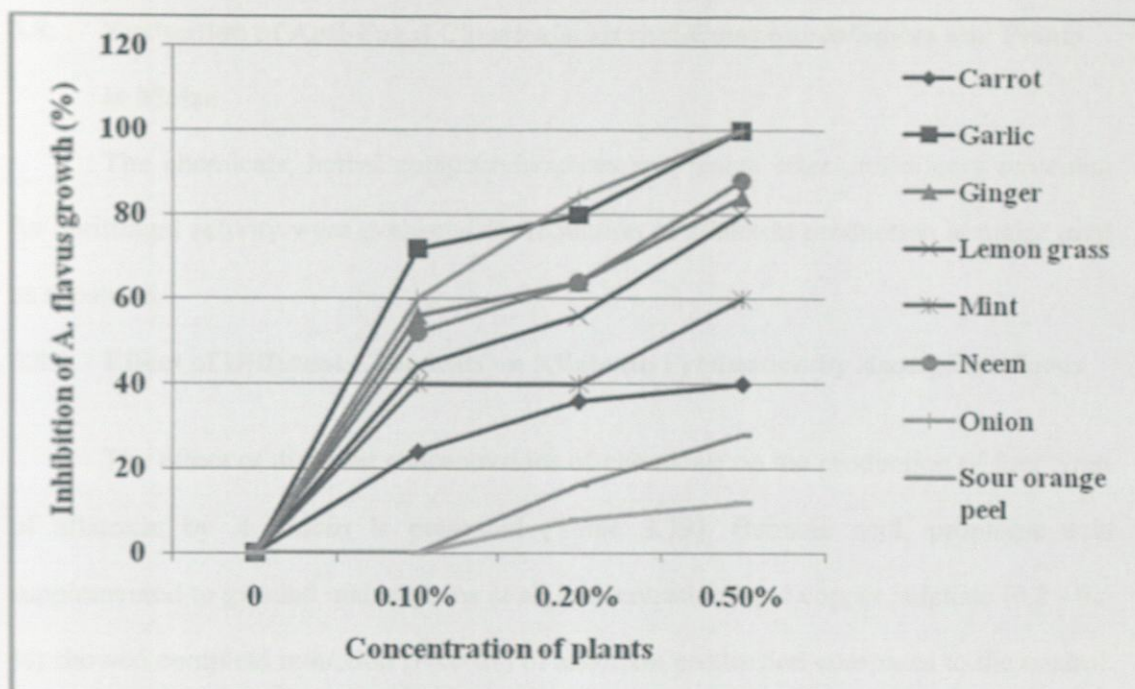


Fig 3.7a: Percent inhibition of *Aspergillus flavus* growth by different plants

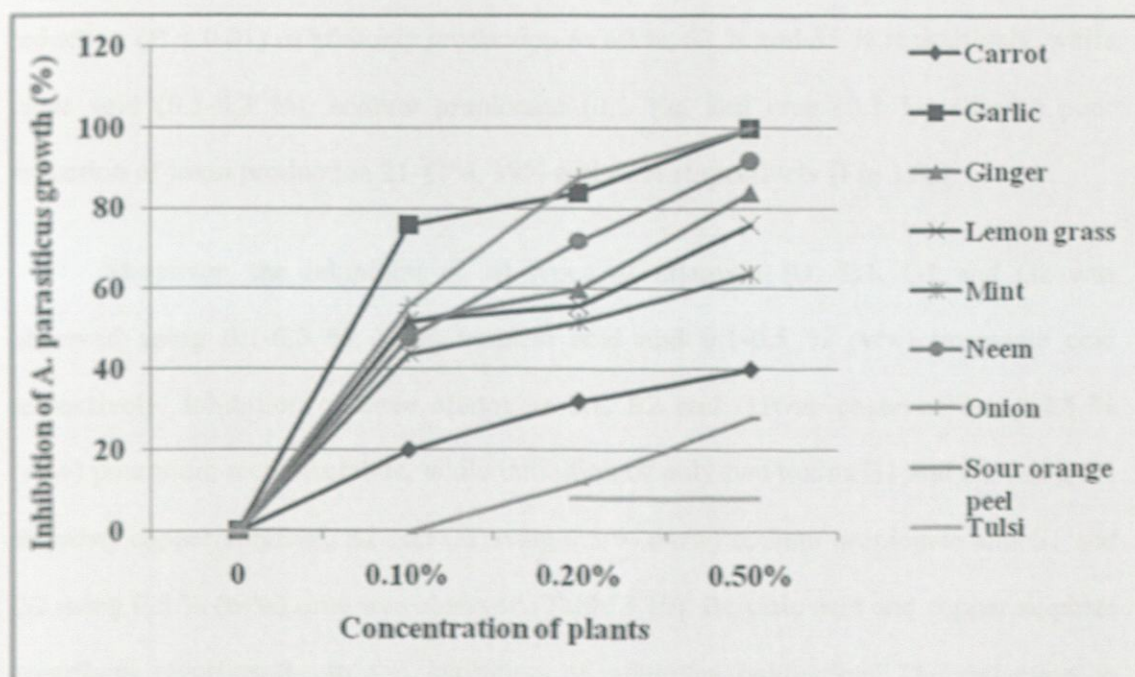


Fig 3.7b: Percent inhibition of *Aspergillus parasiticus* growth by different plants



### 3.8. Evaluation of Anti-Fungal Chemicals, Herbal Compounds/Spices and Plants in Maize

The chemicals, herbal compounds/spices and plants after preliminary screening for antifungal activity were evaluated for reduction of aflatoxin production in maize used as substrate.

#### 3.8.1. Effect of Different Chemicals on Aflatoxin Production by *Aspergillus flavus*

The effect of different concentrations of chemicals on the production of four types of aflatoxin by *A. flavus* is presented [Table 3.19]. Benzoic acid, propionic acid supplemented to grinded maize grains at all concentrations and copper sulphate (0.2 - 0.5 %) showed complete reduction ( $P < 0.01$ ) of aflatoxin production compared to the control. Sodium propionate (0.2-0.5 %), potassium metabisulphite (0.2-0.5%), copper sulphate (0.1%) and urea (0.5 %) showed high reduction ( $P < 0.01$ ) of aflatoxin production. Citric acid (0.5%), potassium metabisulphite (0.1%) and urea (0.2%) showed moderate reduction ( $P < 0.01$ ) of aflatoxin production as 60 %, 62 % and 65 % respectively, while citric acid (0.1-0.2 %), sodium propionate (0.1 %), and urea (0.1 %) showed poor reduction of toxin production 21-41%, 39% and 42% respectively [Fig 3.8a].

Moreover, the inhibition of all types of aflatoxin B1, B2, G1 and G2 was observed using 0.1-0.5 % (w/w) benzoic acid and 0.1-0.5 % (v/w) propionic acid respectively. Inhibition of three aflatoxins B1, B2 and G1 was observed using 0.5 % (w/w) potassium metabisulphite, while inhibition of only two toxins G1 and G2 using 0.1 % (w/w) copper sulphate, B2 and G2 using 0.5 % (w/w) sodium propionate and G1 and G2 using 0.5 % (w/w) urea was observed [Table 3.19]. Benzoic acid and copper sulphate contribute significantly to the inhibition of aflatoxin production. The reduction in aflatoxin G1 synthesis occurred due to addition of caffeine to media (Bokhari 2009).

**Table 3.19: Effect of different concentration of selected chemicals on aflatoxin production by *Aspergillus flavus***

| Chemicals                            | Concentration<br>(% of maize) | AfB1<br>(ppb) | AfB2<br>(ppb) | AfG1<br>(ppb) | AfG2<br>(ppb) | Cumulative<br>reduction<br>(%) |
|--------------------------------------|-------------------------------|---------------|---------------|---------------|---------------|--------------------------------|
| Control                              | 0.0                           | 20±0.60       | 20±0.50       | 20 ±0.45      | 20 ±0.40      |                                |
| Benzoic acid<br>(w/w)                | 0.1                           | ND            | ND            | ND            | ND            | 100                            |
|                                      | 0.2                           | ND            | ND            | ND            | ND            | 100                            |
|                                      | 0.5                           | ND            | ND            | ND            | ND            | 100                            |
| Citric acid<br>(w/w)                 | 0.1                           | 14±0.20b      | 12.4±0.30b    | 18.2±0.20c    | 13.0±0.34b    | 28                             |
|                                      | 0.2                           | 9.5±0.15a     | 10.5±0.18a    | 15.0±0.32b    | 12.2±0.28a    | 41                             |
|                                      | 0.5                           | 9.0±0.25a     | 10.0±0.22a    | 13.0±0.25a    | ND            | 60                             |
| Copper sulfate<br>(w/w)              | 0.1                           | 8.0±0.10a     | 5.6±0.09a     | ND            | ND            | 83                             |
|                                      | 0.2                           | ND            | ND            | ND            | ND            | 100                            |
|                                      | 0.5                           | ND            | ND            | ND            | ND            | 100                            |
| Potassium<br>Metabisulphite<br>(w/w) | 0.1                           | 4.4±0.08a     | 4.0±0.06b     | 9.0±0.15b     | 13.0±0.21c    | 62                             |
|                                      | 0.2                           | ND            | 3.0±0.02a     | 8.5±0.09a     | 12.5±0.19b    | 70                             |
|                                      | 0.5                           | ND            | ND            | ND            | 5.6±0.07a     | 93                             |
| Propionic acid<br>(v/w)              | 0.1                           | ND            | ND            | ND            | ND            | 100                            |
|                                      | 0.2                           | ND            | ND            | ND            | ND            | 100                            |
|                                      | 0.5                           | ND            | ND            | ND            | ND            | 100                            |
| Sodium<br>propionate<br>(w/w)        | 0.1                           | 15.5±0.3b     | 12.5±0.19b    | 11.2±0.15b    | 9.6±0.16a     | 39                             |
|                                      | 0.2                           | 9.0±0.10a     | 6.0±0.10a     | 5.0±0.04a     | ND            | 75                             |
|                                      | 0.5                           | 7.8±0.14a     | ND            | 5.0±0.03a     | ND            | 84                             |
| Urea<br>(w/w)                        | 0.1                           | 15±0.42c      | 12.0±0.35c    | 9.0±0.20b     | 10.4±0.12b    | 42                             |
|                                      | 0.2                           | 12±0.25b      | 8.0±0.26b     | 5.0±0.08a     | 3.0±0.03a     | 65                             |
|                                      | 0.5                           | 10±0.16a      | 7.6±0.18a     | ND            | ND            | 78                             |

Means ±SD with different letter (a–c) in a column under each chemical differ ( $P < 0.01$ )

ND: not detected.



### 3.8.2. Effect of Different Chemicals on Aflatoxin Production by *Aspergillus parasiticus*

The effect of different concentrations of chemicals on the production of four types of aflatoxin by *A. parasiticus* is presented [Table 3.20]. Copper sulphate at all concentrations while benzoic acid and propionic acid supplemented at 0.2-0.5 % to grinded maize grains showed complete reduction ( $P < 0.01$ ) of aflatoxin production compared to the control. Benzoic acid (0.1 %), propionic acid (0.1 %), potassium metabisulphite (0.2-0.5 %), sodium propionate (0.5 %) and urea (0.5 %) showed high reduction ( $P < 0.01$ ) of aflatoxin production. Citric acid (0.5%), potassium metabisulphite (0.1%) sodium propionate (0.2 %) and urea (0.2 %) showed moderate reduction ( $P < 0.01$ ) of aflatoxin production (57 %), (65 %), (70 %) and (66 %) respectively, while citric acid (0.1-0.2 %), sodium propionate (0.1 %), and urea (0.1 %) showed poor reduction of toxin production (25-38 %), (41 %) and (44 %) respectively [Fig 3.8b].

Moreover, the inhibition of all types of aflatoxin B1, B2, G1 and G2 was observed using 0.1-0.5 % (w/w) copper sulphate, 0.2-0.5 % (w/w) benzoic acid and 0.2-0.5 % (v/w) propionic acid respectively. Inhibition of three aflatoxins B1, B2 and G1 was observed using 0.5 % (w/w) potassium metabisulphite and 0.1 % (w/w) benzoic acid, while inhibition of only two aflatoxin G1 and G2 using 0.1 % (v/w) propionic acid, 0.5 % (w/w) sodium propionate and 0.5 % (w/w) urea was observed [Table 3.20]. In addition, copper sulphate, benzoic acid and propionic acid contribute significantly to the inhibition of aflatoxin production. Benzoic acid proved more effective than propionic acid, as the highest effect of benzoic acid was recorded at a concentration of 0.1 % (w/w) inhibiting three types, while that of propionic acid at 0.1 % (v/w) inhibited only two types [Table 3.20].

**Table 3.20: Effect of different concentration of selected chemicals on aflatoxin production by *Aspergillus parasiticus***

| Chemicals                            | Concentration<br>(% of maize) | AfB1<br>(ppb) | AfB2<br>(ppb) | AfG1<br>(ppb) | AfG2<br>(ppb) | Cumulative<br>reduction<br>(%) |
|--------------------------------------|-------------------------------|---------------|---------------|---------------|---------------|--------------------------------|
| Control                              | 0.0                           | 20±0.60       | 20±0.50       | 20±0.45       | 20±0.40       |                                |
| Benzoic acid<br>(w/w)                | 0.1                           | ND            | ND            | ND            | 3.2±0.12a     | 96                             |
|                                      | 0.2                           | ND            | ND            | ND            | ND            | 100                            |
|                                      | 0.5                           | ND            | ND            | ND            | ND            | 100                            |
| Citric acid<br>(w/w)                 | 0.1                           | 15±0.55       | 12.0±0.30     | 18.5±1.2c     | 14.5±0.23b    | 25                             |
|                                      | 0.2                           | 13±0.32       | 10.4±0.22     | 17.0±0.60b    | 9.2±0.12a     | 38                             |
|                                      | 0.5                           | 10.4±0.2a     | 9.0±0.12      | 15.0±0.45a    | ND            | 57                             |
| Copper sulfate<br>(w/w)              | 0.1                           | ND            | ND            | ND            | ND            | 100                            |
|                                      | 0.2                           | ND            | ND            | ND            | ND            | 100                            |
|                                      | 0.5                           | ND            | ND            | ND            | ND            | 100                            |
| Potassium<br>Metabisulphite<br>(w/w) | 0.1                           | 3.5±0.05a     | 3.5±0.05      | 9.0±0.15b     | 12.0±0.20c    | 65                             |
|                                      | 0.2                           | ND            | 3.0±0.02      | 8.6±0.12a     | 10.0±0.18b    | 73                             |
|                                      | 0.5                           | ND            | ND            | ND            | 8.0±0.07a     | 90                             |
| Propionic acid<br>(w/w)              | 0.1                           | 3.2±0.08a     | 2.6±0.03      | ND            | ND            | 94                             |
|                                      | 0.2                           | ND            | ND            | ND            | ND            | 100                            |
|                                      | 0.5                           | ND            | ND            | ND            | ND            | 100                            |
| Sodium<br>propionate<br>(w/w)        | 0.1                           | 15±0.15c      | 12.2±0.19     | 11.0±0.20b    | 9.0±0.06a     | 41                             |
|                                      | 0.2                           | 10±0.10b      | 9.0±0.14      | 5.0±0.05a     | ND            | 70                             |
|                                      | 0.5                           | 9±0.12a       | 6.2±0.08      | ND            | ND            | 81                             |
| Urea<br>(w/w)                        | 0.1                           | 13.4±0.2c     | 12.0±0.15     | 9.0±0.10b     | 10.4±0.15b    | 44                             |
|                                      | 0.2                           | 11.2±0.2b     | 9.0±0.14      | 5.0±0.06a     | 2.0±0.02a     | 66                             |
|                                      | 0.5                           | 9.0±0.08a     | 4.6±0.10      | ND            | ND            | 83                             |

Means ±SD with different letter (a-c) in a column under each chemical differ ( $P < 0.01$ )

ND: not detected.



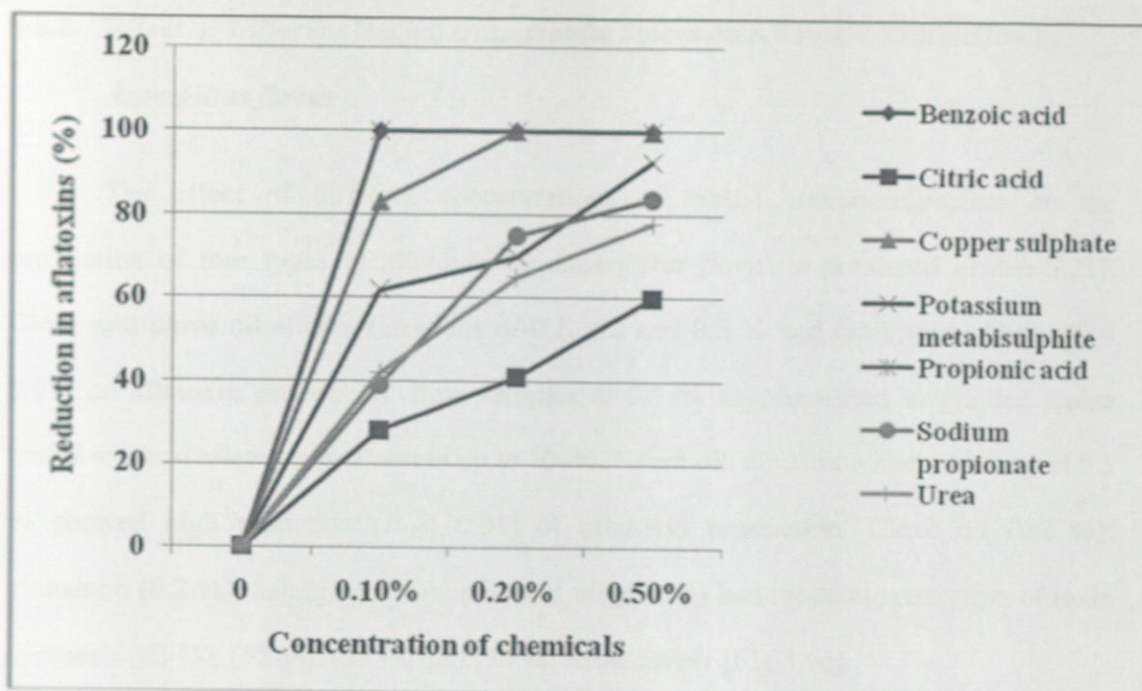


Fig 3.8a: Effect of different concentration of selected chemicals on aflatoxin production by *Aspergillus flavus*

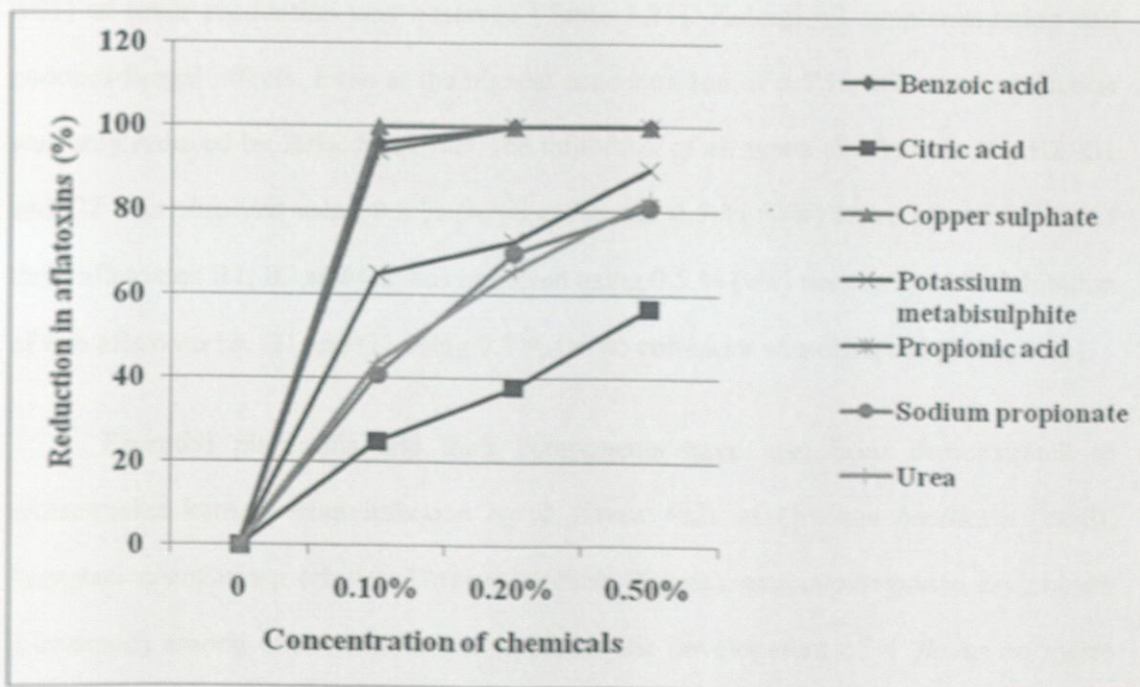


Fig 3.8b: Effect of different concentration of selected chemicals on aflatoxin production by *Aspergillus parasiticus*

### 3.8.3. Effect of Different Herbal Compounds/Spices on Aflatoxin Production by *Aspergillus flavus*

The effect of different concentrations of herbal compounds/spices on the production of four types of aflatoxin by *Aspergillus flavus* is presented [Table 3.21]. Clove and clove oil at concentrations of 0.1, 0.2 and 0.5 % had inhibitory effects ( $P < 0.01$ ) on aflatoxin production. Both samples at 0.5 % supplemented in grinded maize grains reduced aflatoxin production up to 100%. Neem oil, cinnamon and coriander at 0.5 % showed high reduction ( $P < 0.01$ ) of aflatoxin production. Clove oil (0.2 %), cinnamon (0.2 %), kalonji (0.5 %) and neem oil (0.2 %) had moderate reduction of toxin synthesis (63 %), (52 %), (53 %) and (56 %) respectively [Fig 3.9a].

In Clove (0.1-0.2 %), clove oil (0.1 %), cinnamon (0.1 %), coriander (0.1-0.2 %), kalonji (0.1-0.2 %), kalonji oil (0.1-0.5 %) and neem oil (0.1 %) poor reduction ( $P < 0.01$ ) of toxin production was observed [Table 3.21]. Kalonji oil supplementation had poor anti-fungal effects. Even at the highest concentration of 0.5 %, aflatoxin production was only reduced by 20%. Moreover, the inhibition of all types of aflatoxin B1, B2, G1 and G2 was observed using 0.5 % (w/w) clove and 0.5 % (v/w) clove oil. Inhibition of three aflatoxins B1, B2 and G2 was observed using 0.5 % (v/v) neem oil, while inhibition of two aflatoxin i.e. G1 and G2 using 0.5 % (w/w) coriander was observed [Table 3.21].

Essential plant oils and their components have also been demonstrated to protect maize kernels from infection by *A. flavus*. Oils of *Ocimum basilicum* (basil), *Syzygium aromaticum* (clove), *Thymus vulgaris* (thyme), and *cinnamomum zeylanicum* (cinnamon) among others are known to inhibit the development of *A. flavus* on maize kernels (Montes and Carvajal 1998).



**Table 3.21:** Effect of different concentration of selected herbal compounds/spices on aflatoxin production by *Aspergillus flavus*

| Herbal Compounds /Spices | Concentration (% of maize) | AfB1 (ppb) | AfB2 (ppb) | AfG1 (ppb) | AfG2 (ppb) | Cumulative reduction (%) |
|--------------------------|----------------------------|------------|------------|------------|------------|--------------------------|
| Control                  | 0.0                        | 20±0.60    | 20±0.50    | 20±0.45    | 20±0.40    |                          |
| Clove (w/v)              | 0.1                        | 17.5±1.10b | 14.9±0.65b | 13.7±0.30b | 11.5±0.25b | 28                       |
|                          | 0.2                        | 15.0±0.70a | 13.2±0.30a | 13.0±0.25a | 10.0±0.17a | 36                       |
|                          | 0.5                        | ND         | ND         | ND         | ND         | 100                      |
| Clove oil (v/v)          | 0.1                        | 14.0±0.52b | 12.0±0.37b | 13.0±0.21  | 8.2±0.19a  | 41                       |
|                          | 0.2                        | 13.0±0.40a | 8.6±0.21a  | 9.0±0.10   | ND         | 63                       |
|                          | 0.5                        | ND         | ND         | ND         | ND         | 100                      |
| Cinnamon (w/v)           | 0.1                        | 16.0±0.35a | 14.0±0.40c | 12.0±0.13c | 10.0±0.20b | 35                       |
|                          | 0.2                        | 16.0±0.30a | 12.0±0.28b | 7.0±0.10b  | 3.4±0.06a  | 52                       |
|                          | 0.5                        | ND         | 7.3±0.09a  | 2.3±0.14a  | ND         | 88                       |
| Coriander (w/v)          | 0.1                        | 13.0±0.28c | 17.4±0.75c | 15.0±0.25b | 9.0±0.09b  | 32                       |
|                          | 0.2                        | 12.6±0.25b | 14.4±0.40b | 14.0±0.30a | 7.0±0.10a  | 40                       |
|                          | 0.5                        | 11.2±0.20a | 11.2±0.30a | ND         | ND         | 72                       |
| Kalonji (w/v)            | 0.1                        | 15.2±0.32a | 16.0±0.52b | 15.0±0.50b | 14.6±0.19b | 24                       |
|                          | 0.2                        | 17.8±0.40c | 13.2±0.24a | 12.6±0.36a | 12.4±0.23a | 30                       |
|                          | 0.5                        | 16.0±0.44b | 13.0±0.29a | 12.0±0.33a | 12.0±0.30a | 53                       |
| Kalonji oil (v/v)        | 0.1                        | 20.0±1.30b | 20.0±1.25b | 13.4±0.27b | 12.2±0.25a | 18                       |
|                          | 0.2                        | 19.2±0.85a | 19.6±1.18a | 13.2±0.25a | 12.0±0.18a | 20                       |
|                          | 0.5                        | 19.0±0.77a | 19.8±0.90a | 13.2±0.20a | 12.0±0.86a | 20                       |
| Neem oil (v/v)           | 0.1                        | 6.8±0.20b  | 15.0±0.50b | 15.4±0.42b | 8.6±0.10a  | 44                       |
|                          | 0.2                        | 6.2±0.15a  | 14.0±0.45a | 15.0±0.38b | ND         | 56                       |
|                          | 0.5                        | ND         | ND         | 6.4±0.12a  | ND         | 92                       |

Means ±SD with different letter (a–c) in a column under each compound differ ( $P < 0.01$ )

ND: not detected.

#### 3.8.4. Effect of Different Herbal Compounds/Spices on Aflatoxin Production by *Aspergillus parasiticus*

The effect of different concentrations of herbal compounds/spices on the production of four types of aflatoxin by *Aspergillus flavus* is presented [Table 3.22]. Clove and clove oil at concentrations (0.1, 0.2 and 0.5 %) had inhibitory effects ( $P < 0.01$ ) on aflatoxin production. Both samples at 0.5% supplemented in grinded maize grains reduced aflatoxin production up to 100 %. Neem oil, cinnamon and coriander at 0.5 % showed high reduction ( $P < 0.01$ ) of aflatoxin production as 94 %, 90 % and 75 % respectively. Clove oil (0.2 %), cinnamon (0.2 %), kalonji (0.5 %) and neem oil (0.2 %) had moderate reduction of toxin synthesis as 65%, 54%, 54% and 58%, respectively [Fig 3.9b]. Clove (0.1-0.2 %), clove oil (0.1 %), cinnamon (0.1%), coriander (0.1-0.2 %), kalonji (0.1-0.2 %), kalonji oil (0.1-0.5 %) and neem oil (0.1 %) showed poor reduction ( $P < 0.01$ ) of toxin production [Table 3.22].

Kalonji oil supplementation had poor anti-fungal effects. Even at the highest concentration of 0.5 %, aflatoxin production was only reduced by 24 %. Moreover, the inhibition of all types of aflatoxin B1, B2, G1 and G2 was observed using 0.5 % (w/w) clove and 0.5 % (v/w) clove oil. Inhibition of three aflatoxins B1, B2 and G2 was observed using 0.5 % (v/v) neem oil, while inhibition of only two, aflatoxin G1 and G2 using 0.5 % (w/w) coriander was observed [Table 3.22]. It has reported that application of clove, cumin, black pepper, peppermint caused inhibition of mycotoxins production. The attributed to the presence of eugenol (antitoxic compound) in cloves and cinnamons (Juglal et al. 2002; Bokhari, 2007b), while in a similar study spice oils of eugenol, cinnamon, oregano, mace, nutmeg, tumeric, and aniseed displayed antifungal activity against *A. parasiticus* (Juglal et al., 2002).



**Table 3.22:** Effect of different concentration of selected herbal compounds/spices on aflatoxin production by *Aspergillus parasiticus*

| Herbal Compounds /Spices | Concentration (% of maize) | AfB1 (ppb) | AfB2 (ppb) | AfG1 (ppb) | AfG2 (ppb) | Cumulative reduction (%) |
|--------------------------|----------------------------|------------|------------|------------|------------|--------------------------|
| Control                  | 0.0                        | 20±0.60    | 20±0.50    | 20±0.55    | 20±0.65    |                          |
| Clove (w/v)              | 0.1                        | 17.4±0.44b | 15.5±0.52b | 14.0±0.35b | 12.3±0.28b | 26                       |
|                          | 0.2                        | 16.0±0.30a | 13.0±0.41a | 12.2±0.30a | 9.2±0.20a  | 37                       |
|                          | 0.5                        | ND         | ND         | ND         | ND         | 100                      |
| Clove oil (v/v)          | 0.1                        | 15.0±0.32b | 12.0±0.25b | 14.2±0.23b | 10.0±0.18a | 40                       |
|                          | 0.2                        | 10.5±0.20a | 8.5±0.18a  | 9.0±0.10a  | ND         | 65                       |
|                          | 0.5                        | ND         | ND         | ND         | ND         | 100                      |
| Cinnamon (w/v)           | 0.1                        | 15.0±0.36b | 14.2±0.26c | 12.2±0.15c | 9.0±0.20b  | 37                       |
|                          | 0.2                        | 14.6±0.26a | 13.4±0.19b | 10.0±0.20b | 5.2±0.10a  | 54                       |
|                          | 0.5                        | ND         | 6.0±0.06a  | 2.0±0.04a  | ND         | 90                       |
| Coriander (w/v)          | 0.1                        | 15.0±0.20c | 16.5±0.45c | 13.6±0.15b | 8.5±0.30b  | 33                       |
|                          | 0.2                        | 12.6±0.32b | 13.2±0.20b | 12.0±0.19a | 7.0±0.25a  | 44                       |
|                          | 0.5                        | 10.2±0.15a | 9.8±0.14a  | ND         | ND         | 75                       |
| Kalonji (w/v)            | 0.1                        | 18.2±0.62b | 15.0±0.33c | 14.0±0.25c | 14.4±0.41c | 23                       |
|                          | 0.2                        | 18.0±0.50b | 12.4±0.26b | 13.0±0.19b | 11.0±0.20b | 32                       |
|                          | 0.5                        | 12.0±0.25a | 9.5±0.12a  | 8.3±0.07a  | 7.0±0.08a  | 54                       |
| Kalonji oil (v/v)        | 0.1                        | 20.0±1.12b | 20.0±1.40b | 14.4±0.32b | 10.4±0.25a | 19                       |
|                          | 0.2                        | 19.0±1.10a | 19.0±1.23a | 14.2±0.28b | 10.2±0.30a | 22                       |
|                          | 0.5                        | 18.6±0.83a | 19.0±0.96a | 13.2±0.80a | 10.0±0.75a | 24                       |
| Neem oil (v/v)           | 0.1                        | 8.0±0.18b  | 14.0±0.42b | 15.0±0.35c | 7.0±0.08a  | 45                       |
|                          | 0.2                        | 7.2±0.15a  | 12.0±0.30a | 14.4±0.25b | ND         | 58                       |
|                          | 0.5                        | ND         | ND         | 4.8±0.05a  | ND         | 94                       |

Means ±SD with different letter (a–c) in a column under each compound differ ( $P < 0.01$ )

ND: not detected.

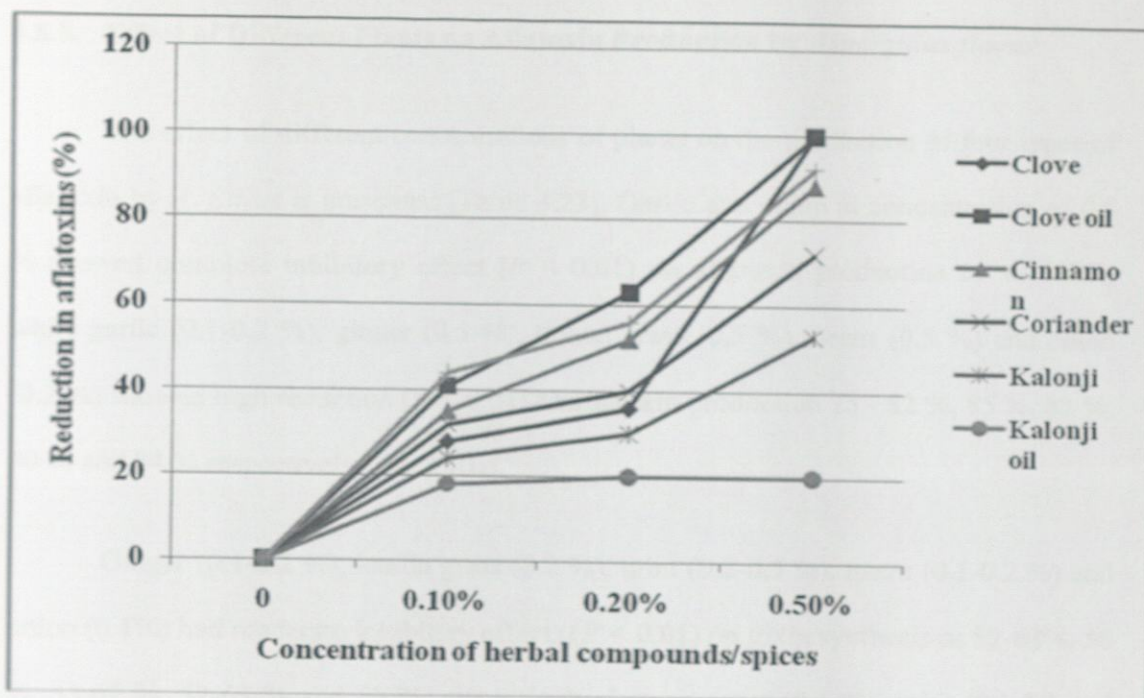


Fig 3.9a: Effect of different concentration of selected herbal compounds/spices on aflatoxin production by *Aspergillus flavus*

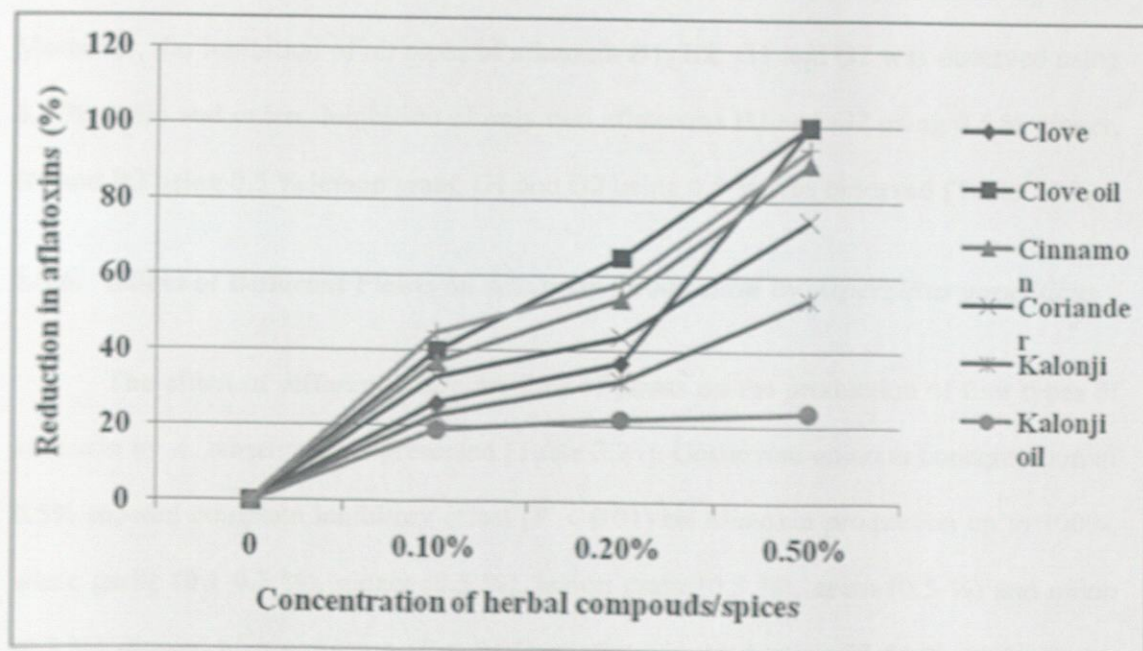


Fig 3.9b: Effect of different concentration of selected herbal compounds/spices on aflatoxin production by *Aspergillus parasiticus*



### 3.8.5. Effect of Different Plants on Aflatoxin Production by *Aspergillus flavus*

The effect of different concentrations of plants on the production of four types of aflatoxin by *A. flavus* is presented [Table 3.23]. Garlic and onion at concentration of 0.5 % showed complete inhibitory effect ( $P < 0.01$ ) on aflatoxin production up to 100%, while garlic (0.1-0.2 %), ginger (0.5 %), lemon grass (0.5 %), neem (0.5 %) and onion (0.2 %) showed high reduction ( $P < 0.01$ ) of aflatoxin production 75 - 82 %, 85 %, 82 %, 90 % and 84 % respectively [Fig 3.10a].

Ginger (0.1-0.2 %), lemon grass (0.2 %), mint (0.2-0.5 %), neem (0.1-0.2 %) and onion (0.1%) had moderate inhibitory effects ( $P < 0.01$ ) on toxin synthesis as 52-63%, 56 %, 53-62 %, 52-63 % and 60 %, respectively. Lemon grass (0.1 %), mint (0.1 %) and sour orange peel (0.1-0.5 %), showed poor reduction ( $P < 0.01$ ) of toxin production (Table 3.23). Sour orange peel supplementation had lowest anti-fungal effects. Even at the highest concentration of 0.5 %, aflatoxin production was only reduced by 35%. Moreover, the inhibition of all types of aflatoxin B1, B2, G1 and G2 was observed using 0.5 % garlic and onion. Inhibition of only two aflatoxins B2 and G2 using 0.5 % ginger, B1 and B2 using 0.5 % lemon grass, G1 and G2 using 0.5 % was observed [Table 3.23].

### 3.8.6. Effect of Different Plants on Aflatoxin Production by *Aspergillus parasiticus*

The effect of different concentrations of plants on the production of four types of aflatoxin by *A. parasiticus* is presented [Table 3.24]. Garlic and onion at concentration of 0.5% showed complete inhibitory effect ( $P < 0.01$ ) on aflatoxin production up to 100%, while garlic (0.1-0.2 %), ginger (0.5 %), lemon grass (0.5 %), neem (0.5 %) and onion (0.2 %) showed high reduction ( $P < 0.01$ ) of aflatoxin production 77-84 %, 87 %, 86 %, 91 % and 85 %, respectively.

Table 3.23: Effect of different concentration of selected plants on aflatoxin production by *Aspergillus flavus*

| Plants                       | Concentration<br>(% of maize) | AfB1<br>(ppb) | AfB2<br>(ppb) | AfG1<br>(ppb) | AfG2<br>(ppb) | Cumulative<br>reduction<br>(%) |
|------------------------------|-------------------------------|---------------|---------------|---------------|---------------|--------------------------------|
| Control                      | 0.0                           | 20±0.60       | 20±0.50       | 20±0.45       | 20±0.40       |                                |
| Garlic<br>(w/v)              | 0.1                           | 9.5±0.15b     | 5.5±0.10b     | 3.0±0.07a     | 2.0±0.04a     | 75                             |
|                              | 0.2                           | 7.0±0.10a     | 4.2±0.06a     | 3.2±0.09a     | ND            | 82                             |
|                              | 0.5                           | ND            | ND            | ND            | ND            | 100                            |
| Ginger<br>(w/v)              | 0.1                           | 18.0±1.10c    | 10.4±0.40b    | 6.0±0.12c     | 4.0±0.10b     | 52                             |
|                              | 0.2                           | 12.3±0.55b    | 9.0±0.30a     | 5.0±0.18b     | 3.3±0.08a     | 63                             |
|                              | 0.5                           | 8.0±0.12a     | ND            | 4.0±0.10a     | ND            | 85                             |
| Lemon grass<br>(w/v)         | 0.1                           | 6.0±0.10b     | 16.6±0.50b    | 15.0±0.35c    | 8.0±0.25b     | 43                             |
|                              | 0.2                           | 2.2±0.04a     | 14.0±0.35a    | 13.0±0.20b    | 6.0±0.19a     | 56                             |
|                              | 0.5                           | ND            | ND            | 8.4±0.12a     | 6.0±0.10a     | 82                             |
| Mint<br>(w/v)                | 0.1                           | 14.2±0.25c    | 16.0±0.46c    | 10.0±0.28c    | 7.0±0.15c     | 41                             |
|                              | 0.2                           | 10.0±0.18b    | 14.3±0.30b    | 7.0±0.22b     | 6.3±0.09b     | 53                             |
|                              | 0.5                           | 6.2±0.10a     | 13.7±0.25a    | 6.2±0.12a     | 4.3±0.08a     | 62                             |
| Neem<br>(w/v)                | 0.1                           | 16.0±0.35c    | 12.4±0.30c    | 6.6±0.20b     | 3.4±0.10a     | 52                             |
|                              | 0.2                           | 13.6±0.20b    | 8.0±0.21b     | 5.0±0.15a     | 3.0±0.12a     | 63                             |
|                              | 0.5                           | 3.0±0.05a     | 5.0±0.07a     | ND            | ND            | 90                             |
| Onion<br>(w/v)               | 0.1                           | 11.5±0.14b    | 9.5±0.19b     | 4.0±0.06a     | 3.1±0.11b     | 60                             |
|                              | 0.2                           | 6.8±0.12a     | 5.0±0.08a     | ND            | 1.0±0.04a     | 84                             |
|                              | 0.5                           | ND            | ND            | ND            | ND            | 100                            |
| Sour orange<br>peel<br>(w/v) | 0.1                           | 20.0±1.32c    | 20.0±1.20c    | 20.0±1.14c    | 20.0±1.20c    | 0                              |
|                              | 0.2                           | 16.0±0.85b    | 17.2±0.98b    | 18.0±1.10b    | 12.0±0.56b    | 21                             |
|                              | 0.5                           | 12.5±0.50a    | 13.0±0.62a    | 15.0±0.50a    | 11.5±0.32a    | 35                             |

Means ±SD with different letter (a–c) in a column under each plant differ ( $P < 0.01$ )  
 ND: not detected.



Table 3.24: Effect of different concentration of selected plants on aflatoxin production by *Aspergillus parasiticus*

| Plants                       | Concentration<br>(% of maize) | AfB1<br>(ppb) | AfB2<br>(ppb) | AfG1<br>(ppb) | AfG2<br>(ppb) | Cumulative<br>reduction<br>(%) |
|------------------------------|-------------------------------|---------------|---------------|---------------|---------------|--------------------------------|
| Control                      | 0                             | 20±0.60       | 20±0.50       | 20±0.45       | 20±0.40       |                                |
| Garlic<br>(w/v)              | 0.1                           | 8.4±0.21b     | 5.0±0.10b     | 3.0±0.12b     | 2.0±0.07a     | 77                             |
|                              | 0.2                           | 6.0±0.15a     | 4.4±0.07a     | 2.4±0.08a     | ND            | 84                             |
|                              | 0.5                           | ND            | ND            | ND            | ND            | 100                            |
| Ginger<br>(w/v)              | 0.1                           | 18.0±0.55b    | 10.4±0.40b    | 5.0±0.20a     | 4.2±0.12b     | 53                             |
|                              | 0.2                           | 10.4±0.30a    | 9.0±0.25a     | 5.0±0.15a     | 2.0±0.09a     | 67                             |
|                              | 0.5                           | 10.4±0.25a    | ND            | ND            | ND            | 87                             |
| Lemon grass<br>(w/v)         | 0.1                           | 6.0±0.18a     | 17.0±1.10b    | 16.2±0.50c    | 8.0±0.15c     | 41                             |
|                              | 0.2                           | ND            | 14.0±0.75a    | 15.0±0.41b    | 7.0±0.13b     | 55                             |
|                              | 0.5                           | ND            | ND            | 6.0±0.20a     | 5.2±0.10a     | 86                             |
| Mint<br>(w/v)                | 0.1                           | 15.0±0.30c    | 16.0±0.50c    | 10.0±0.32c    | 7.0±0.22c     | 40                             |
|                              | 0.2                           | 9.0±0.12b     | 14.4±0.35b    | 7.0±0.30b     | 6.4±0.14b     | 54                             |
|                              | 0.5                           | 6.4±0.10a     | 13.0±0.24a    | 6.0±0.20a     | 4.2±0.08a     | 63                             |
| Neem<br>(w/v)                | 0.1                           | 16.0±0.40c    | 12.0±0.20b    | 7.0±0.18b     | 5.0±0.20b     | 50                             |
|                              | 0.2                           | 14.4±0.32b    | 8.0±0.16a     | 5.0±0.10a     | 3.0±0.17a     | 62                             |
|                              | 0.5                           | 7.2±0.18a     | ND            | ND            | ND            | 91                             |
| Onion<br>(w/v)               | 0.1                           | 13.5±0.20b    | 9.5±0.15b     | 5.2±0.12a     | 4.6±0.07a     | 59                             |
|                              | 0.2                           | 6.0±0.17a     | 6.0±0.09a     | ND            | ND            | 85                             |
|                              | 0.5                           | ND            | ND            | ND            | ND            | 100                            |
| Sour orange<br>peel<br>(w/v) | 0.1                           | 20.0±1.35     | 20.0±1.22c    | 20.0±1.16c    | 20.0±1.20c    | 0                              |
|                              | 0.2                           | 15.0±0.95     | 17.2±0.87b    | 17.0±0.80b    | 12.4±0.56b    | 23                             |
|                              | 0.5                           | 12.0±0.45     | 13.0±0.72a    | 15.4±0.65a    | 10.0±0.32a    | 37                             |

Means ±SD with different letter (a–c) in a column under each plant differ ( $P < 0.01$ )  
 ND: not detected.

Ginger (0.1-0.2 %), lemon grass (0.2 %), mint (0.2-0.5 %), neem (0.1-0.2 %) and onion (0.1 %) had moderate inhibitory effects ( $P < 0.01$ ) on toxin synthesis as 53-67 %, 55 %, 54-63 %, 50-62 % and 59 %, respectively. Lemon grass (0.1 %), mint (0.1 %) and sour orange peel (0.2-0.5 %), showed poor reduction ( $P < 0.01$ ) of toxin production [Table 3.24]. Sour orange peel supplementation had lowest anti-fungal effects. Even at the highest concentration of 0.5 %, aflatoxin production was only reduced by 37 % [Fig 3.10b].

Moreover, the inhibition of all types of aflatoxin B1, B2, G1 and G2 was observed using 0.5 % (w/w) garlic and onion. Inhibition of three aflatoxins B2, G1 and G2 was observed using 0.5 % (w/w) ginger and neem. Inhibition of only two aflatoxins B1 and B2 using 0.5 % (w/w) lemon grass, while G1 and G2 using 0.2 % (w/w) onion was observed (Table 3.24). In addition, garlic, ginger, onion and neem contribute significantly to the inhibition of aflatoxin production. Onion proved more effective than lemon grass, as the highest effect of onion was recorded at a concentration of 0.2 % (w/w) inhibiting two types, while that of lemon grass at 0.5 % (w/w) inhibited two types of aflatoxin [Table 3.24].

Neem leaf extracts caused 10 – 15 % more inhibition of aflatoxin production by *A. parasiticus* as compared to its seed extract (Razzaghi et al., 2005). Neem oil at 0.1 %, 0.2 %, 0.5 % and 1.0 % had moderate inhibitory effect on fungal growth and aflatoxin production compared to the control (Gowda et al., 2004). Neem leaf extract on growth of *A. parasiticus* and production of aflatoxin and reported that the inhibition of aflatoxin synthesis by neem leaf extract was found to be time and dose dependent (Ghorbanian et al., 2008).



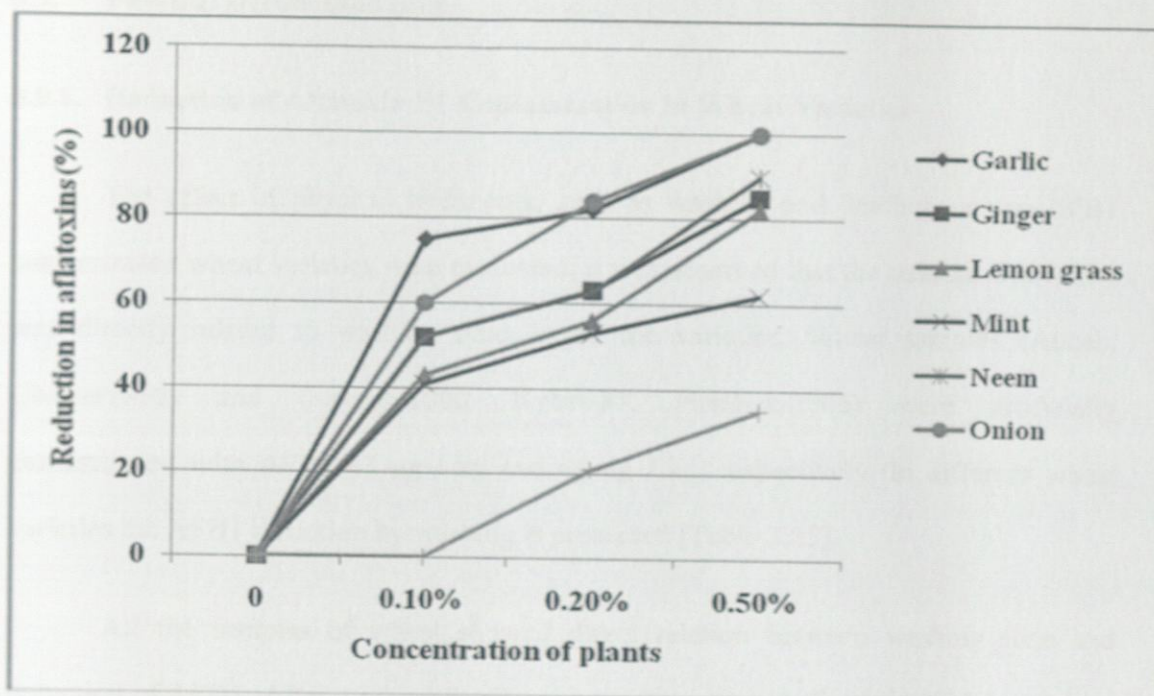


Fig 3.10a: Effect of different concentration of selected plants on aflatoxin production by *Aspergillus flavus*

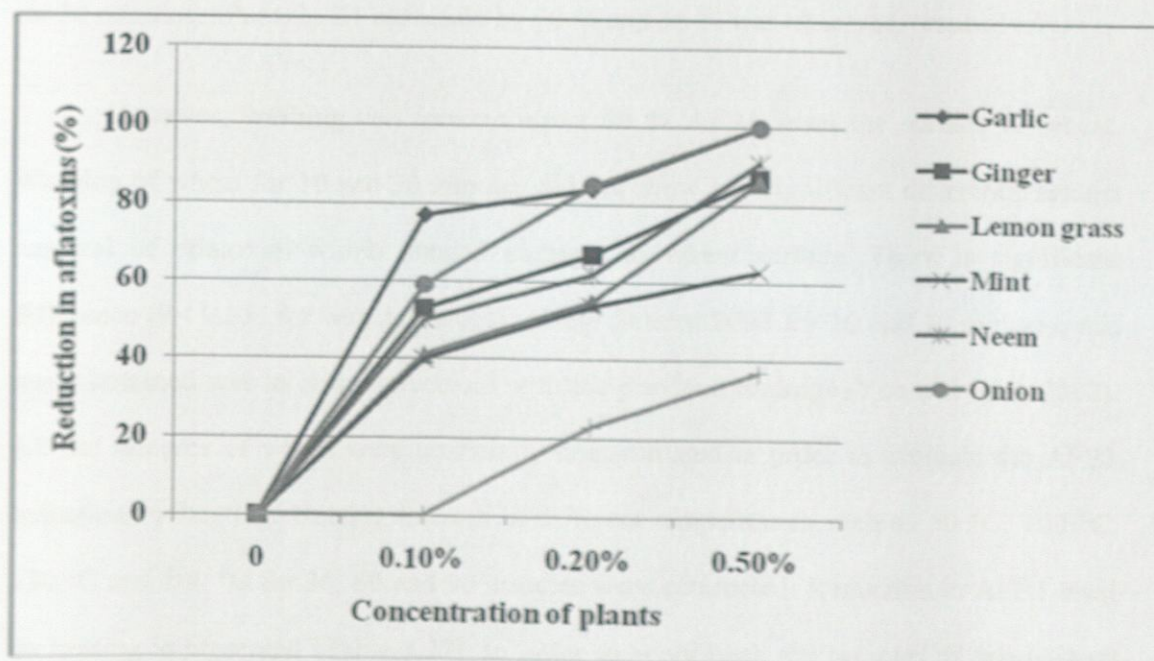


Fig 3.10b: Effect of different concentration of selected plants on aflatoxin production by *Aspergillus parasiticus*

### 3.9. Physical Decontamination

#### 3.9.1. Reduction of Aflatoxin B1 Contamination in Wheat Varieties

The effect of physical treatments, such as washing and heating, on the AFB1 contaminated wheat varieties were evaluated, it was observed that the reduction of AFB1 was directly related to washing time in all the varieties. Wheat samples (Auqab, Ghaznavi-98) and (Saleem-2000, Kyber-87, Pirsabak-2005) were artificially contaminated with AFB1 43  $\mu\text{g} / \text{kg}$  and 46  $\mu\text{g} / \text{kg}$ , respectively. In different wheat varieties the AFB1 reduction by washing is presented [Table 3.25].

All the samples of wheat showed direct relation between washing time and reduction of AFB1. After washing for 30 minutes, the concentration of AFB1 in samples of wheat such as Saleem- 2000, Kyber-87and Pirsabak-2005 showed reduction by 62 %, 56 %, and 58%, respectively. When wheat samples Auqab and Ghaznavi-98 were washed for 30 minutes, aflatoxin B1 reduction by 60 % and 56 % was observed, respectively.

However, washing can remove about 40 % AFB1 from the surface of wheat. Washing of wheat for 10 and 30 minutes did not show any significant difference against removal of aflatoxin, which bonded strongly to wheat surface. There is significant difference ( $P < 0.05$ ) for washing wheat variety Saleem 2000 for 20 and 10 minutes, this result obtained was in close agreement with the previous findings (Yeo and Kim, 2002). All the samples of wheat were artificially contaminated in order to evaluate the AFB1 reduction by heating. Heating in oven at different temperatures such as 50 °C, 100 °C, 150 °C and 200 °C for 30, 60 and 90 minutes were conducted. Reduction in AFB1 level by heating is presented [Table 3.27]. In order to apply heat, the samples of dried wheat were selected. It was found that AFB<sub>1</sub> reduction at temperature 200 °C was higher than



100 °C or below. At 150 °C, heating of Auqab variety for 30, 60 and 90 minutes showed AFB1 reduction 60 %, 75 % and 82 %, respectively. Heating at 200 °C, AFB1 reduction occurred more than 90%. There was a significant difference between AFB1 reductions by heating at 100 °C as compared to 50 °C for 60 minutes ( $P<0.05$ ).

The heating showed almost same effect on Ghaznavi as well as on other wheat varieties. Greater decrease in AFB1 was apparent in Saleem-2000 by heating at 150 °C. However, heating of foods over 100 °C is not good enough to reduce AFs. It has reported that different types of heating, oven heating is effective than microwave heating for aflatoxins reduction (Soliman, 2002; Soliman et al., 2001). The varieties, such as Auqab, Pirsabak-2005 and Saleem-2000 (moisture 10%) were subjected to heating at 100 °C for 30 min to investigate AFB1 reduction by moisture content [Table 3.26]. The initial levels of AFB1 in Auqab, Saleem-2000 and Pirsabak-2005 were 28.6 and 18.5 µg/L, respectively and after heating the reduction of AFB1 in these varieties was 40%, 47% and 43%, respectively. In comparison with the dried wheat samples, reduction of AFB1 in wet samples of Auqab, Saleem-2000 and Pirsabak-2005 varieties was higher by 20%, 24% and 18%, respectively.

A significant difference was found by heating under the same condition the wet Saleem-2000 varieties as compared to the dried variety ( $P<0.05$ ). Destruction of aflatoxins depends upon the moisture content in food (Torres et al., 2001; Mendez-Albores et al., 2004). For detoxification of aflatoxins opening of lactone ring is important step (Buser and Abbas, 2002). The sensitivity of mycotoxins to heat treatment is affected by many factors including the moisture content, pH, and ionic strength of food. Roasting or frying for periods of up to 30 minutes at temperatures 150 – 200 °C can result in residual levels of between 20 – 60 % (Bulent et al., 2006).

Table 3.25: Aflatoxin B<sub>1</sub> reduction in wheat varieties through washing for different time periods

| Varieties     | Initial Level of AFB1 (µg/kg) | After 10 min        |               | After 20 min        |               | After 30 min        |               |
|---------------|-------------------------------|---------------------|---------------|---------------------|---------------|---------------------|---------------|
|               |                               | Final level (µg/kg) | Reduction (%) | Final level (µg/kg) | Reduction (%) | Final level (µg/kg) | Reduction (%) |
| Auqab         | 43                            | 21.3 ± 1.7*         | 50            | 21.2 ± 0.5          | 50            | 17.2 ± 2.6          | 60            |
| Ghaznavi-98   | 43                            | 25.2 ± 2.5          | 41            | 19.4 ± 0.4          | 55            | 18.9 ± 1.1          | 56            |
| Saleem-2000   | 46                            | 22.8 ± 1.2          | 50            | 18.2 ± 1.5**        | 60            | 17.1 ± 3.0          | 62            |
| Kyber-87      | 46                            | 25.0 ± 3.1          | 45            | 23.6 ± 2.4          | 48            | 20.0 ± 8.9          | 56            |
| Pirsabak-2005 | 46                            | 24.4 ± 3.0          | 46            | 21.9 ± 4.4          | 52            | 18.8 ± 3.0          | 58            |

\* Mean ± standard deviation (n = 3); \*\* P < 0.05

Table 3.26: Aflatoxin B<sub>1</sub> reduction for 30 min at 100 °C in wet wheat

| Moisture (%) | Auqab                         |                     |               | Saleem-2000                   |                     |               | Pirsabak-2005                 |                     |               |
|--------------|-------------------------------|---------------------|---------------|-------------------------------|---------------------|---------------|-------------------------------|---------------------|---------------|
|              | Initial level of AFB1 (µg/kg) | Final level (µg/kg) | Reduction (%) | Initial level of AFB1 (µg/kg) | Final level (µg/kg) | Reduction (%) | Initial level of AFB1 (µg/kg) | Final level (µg/kg) | Reduction (%) |
| 10           | 28.6                          | 17.0 ± 3.0          | 40            | 14.8                          | 7.8 ± 2.3           | 47**          | 18.5                          | 10.5 ± 2.0          | 43*           |

The values are mean ± standard deviation (n=3); \* P < 0.05; \*\* P < 0.01



Table 3.27: Effect of heating for different time periods on AFB<sub>1</sub> reduction in dried wheat

| Temperature (°C) | Initial level of AFB <sub>1</sub> (µg/kg) | After 30 min                   |               | After 60 min                   |               | After 90 min                   |               |
|------------------|-------------------------------------------|--------------------------------|---------------|--------------------------------|---------------|--------------------------------|---------------|
|                  |                                           | AFB <sub>1</sub> level (µg/kg) | Reduction (%) | AFB <sub>1</sub> level (µg/kg) | Reduction (%) | AFB <sub>1</sub> level (µg/kg) | Reduction (%) |
| Auqab            | 100                                       |                                |               |                                |               |                                |               |
| 50               |                                           | 98.0 ± 3.0                     | 2             | 95.0 ± 3.3                     | 5             | 82.0 ± 4.0                     | 18            |
| 100              |                                           | 80.0 ± 1.9                     | 20            | 69.2 ± 2.4*                    | 31            | 57.3 ± 1.7*                    | 42            |
| 150              |                                           | 40.0 ± 2.5**                   | 60            | 25.0 ± 2.0**                   | 75            | 17.6 ± 0.7*                    | 82            |
| 200              |                                           | 4.0 ± 0.2**                    | 96            | 11.0 ± 1.0**                   | 90            | 7.0 ± 0.3**                    | 93            |
| Ghaznavi-98      | 55                                        |                                |               |                                |               |                                |               |
| 50               |                                           | 51.6 ± 3.6                     | 6             | 52.7 ± 2.0                     | 4             | 49.0 ± 0.4                     | 11            |
| 100              |                                           | 45.6 ± 3.2                     | 17            | 36.0 ± 1.6*                    | 34            | 38.4 ± 2.8*                    | 30            |
| 150              |                                           | 25.9 ± 2.3**                   | 53            | 18.0 ± 1.8**                   | 67            | 12.0 ± 2.9**                   | 78            |
| 200              |                                           | 5.0 ± 0.7**                    | 91            | 6.4 ± 0.3**                    | 88            | 4.7 ± 0.8**                    | 91            |
| Saleem2000       | 100                                       |                                |               |                                |               |                                |               |
| 50               |                                           | 95.0 ± 5.0                     | 5             | 93.0 ± 3.3                     | 7             | 82 ± 15.5                      | 18            |
| 100              |                                           | 77.0 ± 1.9                     | 23            | 70.0 ± 2.4*                    | 30            | 62 ± 4.7*                      | 38            |
| 150              |                                           | 38.6 ± 3.5**                   | 61            | 24.0 ± 2.0**                   | 76            | 12.0 ± 0.7*                    | 88            |
| 200              |                                           | 5.0 ± 0.2**                    | 95            | 9.0 ± 1.0**                    | 91            | 6.0 ± 4.9**                    | 94            |
| Kyber-87         | 55                                        |                                |               |                                |               |                                |               |
| 50               |                                           | 52.0 ± 3.6                     | 5             | 52.0 ± 2.0                     | 5             | 47.2 ± 0.4                     | 14            |
| 100              |                                           | 44.0 ± 3.2                     | 20            | 45.0 ± 1.6*                    | 18            | 35.0 ± 2.8*                    | 36            |
| 150              |                                           | 24.2 ± 2.3**                   | 56            | 18.9 ± 1.8**                   | 66            | 12.2 ± 2.9**                   | 77            |
| 200              |                                           | 6.4 ± 0.7**                    | 88            | 4.2 ± 0.3**                    | 92            | 3.7 ± 0.8**                    | 93            |
| Pirsabak-2005    | 55                                        |                                |               |                                |               |                                |               |
| 50               |                                           | 51.5 ± 3.6                     | 6             | 50.0 ± 2.0                     | 9             | 49.3 ± 0.4                     | 10            |
| 100              |                                           | 46.5 ± 3.2                     | 15            | 42.3 ± 1.6*                    | 23            | 33.5 ± 2.8*                    | 39            |
| 150              |                                           | 24.5 ± 2.3**                   | 55            | 14.6 ± 1.8**                   | 73            | 11.5 ± 2.9**                   | 79            |
| 200              |                                           | 2.8 ± 0.7**                    | 95            | 3.6 ± 0.3**                    | 93            | 2.0 ± 0.8**                    | 96            |

The values are mean ± standard deviation (n = 3); \* P < 0.05; \*\* P < 0.01

### 3.9.2. Effect of Different Cooking Methods on the Reduction of Aflatoxin B<sub>1</sub> and Ochratoxin-A in Rice.

Polished basmati rice (*Oryza sativa* Linn.) samples, artificially contaminated with AFB<sub>1</sub> (20 µg / kg), ochratoxin A (30 µg / kg), and AFB<sub>1</sub> (20 µg / kg) and OTA (30 µg / kg) simultaneously. These contaminated samples were then submitted to normal cooking, cooking with excess water and microwave oven cooking. For these three processes the cooking time was 9 minutes, the time necessary for the final product to present an adequate texture.

Basmati rice samples artificially contaminated with AFB<sub>1</sub>, the highest reduction was observed for rice cooked in excess water (87.5 %) followed by that cooked by normal cooking (84 %) and finally by the microwave oven cooked sample (72.5 %). Cooking in excess water was also the most effective way of reducing contamination with OTA, showing an average reduction (86.6 %). Normal cooking presented an average reduction (83 %) and the microwave oven (82.4 %) as can be seen [Tables 3.28, 3.29 and 3.30].

In the samples artificially contaminated with both toxins AFB<sub>1</sub> and OTA, the highest reductions were observed for rice cooked in excess water (87.5 and 83.0 %) followed by that cooked by normal cooking (82.5 and 76.6 %), and finally by the microwave oven cooked sample (77.6 and 75.9 %) as can be seen in Tables 3.28, 3.29 and 3.30, respectively. From the results obtained in this study, microwave oven cooking was shown to be less effective in reducing contamination with the mycotoxins studied than cooking on a gas stove. The effect of normal cooking, cooking in excess water and cooking under pressure, on the concentrations of AFB<sub>1</sub> in artificially and naturally contaminated rice samples were reported, as cooking under pressure for 5 minutes



(proportion rice: water was 1:1) resulted in average reductions in contamination of 70.7 and 70.5 % for the artificially and naturally contaminated rice samples, respectively. Normal cooking for 30 minutes (proportion rice: water was 1: 4) resulted in an average reduction of 47.5 % for the naturally contaminated samples and 49.0 % for the artificially contaminated samples. Cooking in excess water (proportion rice: water was 1: 8) resulted in higher toxin losses, with average reductions of 75.7 and 74.5 % for the artificially and naturally contaminated rice, respectively [Rehana et al., 1979].

Degradation by heat treatment depends on the type of mycotoxin and its concentration, the extent of binding between the mycotoxin and the food constituents, the degree of heat penetration, as well as the heating temperature and the processing time. Heat supplied in the form of high energy microwaves can also destroy mycotoxins (Bulent et al., 2006). The increased moisture content enhances the destruction of AFs during cooking or baking. In addition, AFs are stable up to their melting points when heated without moisture (Mann et al., 1967).

**Table 3.28:** Percentage reduction ( $\pm$  SD) of aflatoxin B<sub>1</sub> and ochratoxin A levels in artificiallycontaminated polished basmati rice samples (n = 6) by normal cooking.

| Mycotoxins                    | Initial level<br>( $\mu\text{g} / \text{kg}$ ) | Levels after cooking<br>( $\mu\text{g} / \text{kg}$ ) | Reduction<br>(%) |
|-------------------------------|------------------------------------------------|-------------------------------------------------------|------------------|
| Aflatoxin B <sub>1</sub>      | 20                                             | $3.2 \pm 0.20$ (< 1 – 5.7)                            | 84.0             |
| Ochratoxin - A                | 30                                             | $5.1 \pm 0.15$ (< 4 – 9.1)                            | 83.0             |
| Aflatoxin B <sub>1</sub><br>+ | 20                                             | $3.5 \pm 0.22$ (< 1 – 6.2)                            | 82.5             |
| Ochratoxin A                  | 30                                             | $7.0 \pm 0.30$ (4.3 – 10.4)                           | 76.6             |

Quantification limit of  $1 \mu\text{g} / \text{kg}$  for AFB<sub>1</sub> and  $4 \mu\text{g} / \text{kg}$  for ochratoxin A  
Values in parenthesis are the contamination range (minimum to maximum).

**Table 3.29:** Percentage reduction ( $\pm$  SD) of aflatoxin B<sub>1</sub> and ochratoxin A levels in artificially contaminated polished basmati rice samples (n = 6) by cooking in excess water.

| Mycotoxins               | Initial level                 | Levels after cooking          |  | Reduction |
|--------------------------|-------------------------------|-------------------------------|--|-----------|
|                          | ( $\mu\text{g} / \text{kg}$ ) | ( $\mu\text{g} / \text{kg}$ ) |  | (%)       |
| Aflatoxin B <sub>1</sub> | 20                            | 2.5 $\pm$ 0.10 (ND – 6.0)     |  | 87.5      |
| Ochratoxin - A           | 30                            | 4.0 $\pm$ 0.20 (ND – 4.0)     |  | 86.6      |
| Aflatoxin B <sub>1</sub> | 20                            | 2.5 $\pm$ 0.25 (< 1 – 6.5)    |  | 87.5      |
| +                        |                               |                               |  |           |
| Ochratoxin A             | 30                            | 5.1 $\pm$ 0.30 (< 4 – 6.8)    |  | 83.0      |

Quantification limit of 1 $\mu\text{g} / \text{kg}$  for AFB<sub>1</sub> and 4 $\mu\text{g} / \text{kg}$  for ochratoxin A  
 Values in parenthesis are the contamination range (minimum to maximum)

**Table 3.30:** Percentage reduction ( $\pm$  SD) of aflatoxin B<sub>1</sub> and ochratoxin A levels in artificially contaminated polished basmati rice samples (n = 6) by microwave oven cooking.

| Mycotoxins               | Initial level                 | Levels after cooking          |  | Reduction |
|--------------------------|-------------------------------|-------------------------------|--|-----------|
|                          | ( $\mu\text{g} / \text{kg}$ ) | ( $\mu\text{g} / \text{kg}$ ) |  | (%)       |
| Aflatoxin B <sub>1</sub> | 20                            | 5.5 $\pm$ 0.40 (< 1 – 12.3)   |  | 72.5      |
| Ochratoxin - A           | 30                            | 5.3 $\pm$ 0.30 (< 4 – 9.1)    |  | 82.4      |
| Aflatoxin B <sub>1</sub> | 20                            | 4.5 $\pm$ 0.25 (< 1 – 8.2)    |  | 77.6      |
| +                        |                               |                               |  |           |
| Ochratoxin - A           | 30                            | 7.2 $\pm$ 0.45 (< 4 – 14.6)   |  | 75.9      |

Quantification limit of 1 $\mu\text{g} / \text{kg}$  for AFB<sub>1</sub> and 4 $\mu\text{g} / \text{kg}$  for ochratoxin-A



# CONCLUSIONS

#### 4. CONCLUSIONS

Following conclusion has been made based in this carried out study:

- The results shows that among contaminated samples in dried fruits, one dried apricot, three dried fig, one raisins, one apricot kernels, two walnuts with shell, three walnuts without shell, one peanuts with shell, two peanuts without shell and two pistachios without shell samples showed concentration higher than the limit for total aflatoxins 4 µg/kg, according to European Union standards.
- Among contaminated samples in cereals and beans, two rice, one rice broken, two wheat, three maize, two barley, one sorghum, one red kidney bean, one split pea and two soybeans showed concentration higher than the limit.
- Samples were evaluated, among which most of these commodities produced in Pakistan are safe for consumption and for national and international trading without restriction. Exposure to afatoxins is not a problem solely for rural communities, with very low income of developing countries, but may also be a problem for wealthy countries if they lack the legislative measures to control the levels of afatoxins in food.
- The data obtained can be used as basis for risk analysis of aflatoxins hazard in different kinds of agricultural commodities of Pakistan. It is so important to establish the permanent controlling and monitoring program from the production until consumption of these commodities in order to minimize the contamination problem of AFs.



- Decontamination by different methods was also conducted. In this regard biological and physical methods were evaluated to decontaminate the mycotoxin (aflatoxins) contaminated agricultural commodities, to prevent the threat to human health and the risk of great economic loss.
- In biological decontamination, some materials such as chemicals, herbal compounds/spices and plants were showed the inhibitory effect on the growth of *Aspergillus flavus* / *Aspergillus parasiticus* and total aflatoxins production.
- On the basis of inhibitory effect on growth of fungus, sodium propionate and potassium metabisulphite showed high, while ammonia, urea and citric acid were moderate in inhibiting fungal growth. Clove, clove oil, cinnamon, coriander and neem oil represented high, while kalonji and neem oil showed moderate antifungal activity. Among the plants garlic and onion showed complete inhibition, while ginger, neem and lemon grass had high antifungal activity.
- On the basis of reduction in aflatoxin production in maize used as substrate, the chemicals benzoic acid, copper sulphate and propionic acid showed maximum antifungal activity against aflatoxins production by both the fungus, while potassium metabisulphite, sodium propionate and urea showed moderate antifungal effect.
- Among the herbal compounds/spices, clove, clove oil and neem oil showed the best antifungal activity than cinnamon and coriander. Among the selected plants, garlic, onion and neem showed best antifungal activity as compared to ginger, lemon grass and mint.

- In physical decontamination, application of oven heating and washing shows reduction in contamination level of AFB<sub>1</sub> in different wheat varieties. Heating in oven reduced the concentration of AFB<sub>1</sub> more effectively as compared to washing. The wet wheat showed more reduction in the concentration of aflatoxin B<sub>1</sub> by heating than the dried wheat.
- Moreover, the cooking of rice in an excess of water showed maximum reduction on the levels of AFB<sub>1</sub> and OTA in artificially contaminated basmati rice samples than normal cooking and microwave oven cooking. Thus in order to make unusable contaminated commodity into usable form, feasible methods for AFB<sub>1</sub> and OTA decontamination by physical techniques are developed.
- Some chemicals, herbal compounds/spices and plants evaluated in this study significantly suppressed fungal growth and production of aflatoxins. These may yield one of the most effective strategies for prevention of mycotoxin contamination.
- Thus some herbal compounds/spices and some chemicals as preservatives may successfully replace synthetic pesticides and provide an alternative method to protect our staple food from the toxic fungal contamination. Overall, it is clear that no single method/technique will be suitable for all foods. The effectiveness of a method depends on the nature of the food, as well as the type of mycotoxin and its concentration level.



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## REFERENCES

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### International Paper Published

1. Ghosia Lutfullah, Arshad Hussain. Studies on contamination level of aflatoxins in some dried fruits and nuts of Pakistan. Food control, 22, 426-429 (2011)  
Impact Factor: 2.463 (ELSEVIER)
2. Ghosia Lutfullah, Arshad Hussain. Studies on contamination level of aflatoxins in some cereals and beans of Pakistan. Food Control, 23, 32-36 (2012)  
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### National Paper Published

3. Arshad Hussain, Ghousia Lutfullah. Reduction of Aflatoxin B1 and Ochratoxin- A levels in polished basmati rice (*Oryza sativa* Linn.) by different cooking methods, Journal of the Chemical Society of Pakistan, 31 (6), 911-915 (2009)
4. Arshad Hussain, Ghosia Lutfullah, Shafqatullah. Reduction of aflatoxin B1 contamination in Pakistani wheat varieties by physical methods, Pak. J. Sci. Ind. Res. Ser. B: Biol. Sci. 54 (1), (2011)