MYCOLOGICAL AND AFLATOXIN PROFILE OF HERBAL MEDICINAL PRODUCTS IN NORTH WESTERN-NIGERIA

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NIGERIA

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NOVEMBER, 2014

DECLARATION

I declare that the work in this DISSERTATION entitled **Mycological and aflatoxin profile of herbal medicinal products in North Western Nigeria**has been carried out by me in the Department of Microbiology. The information derived from the literature has been duly acknowledged in the text and a list of references provided.. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

DANLADI ABBA	SIGNATURE	DATE

CERTIFICATION

This dissertation entitled "MYCOLOGICAL AND AFLATOXIN PROFILE OF HERBAL MEDICINAL PRODUCTS IN NORTH WESTERN NIGERIA.". By DANLADI, ABBA meets the regulation governing the award of the degree of Doctor of Philosophy (Ph.D) in Microbiology of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to:
My lovely wife and my children
Also to my mentor (Dr) Alhaji Shehu Abubakar Misau.

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ABSTRACT

The increase in the consumption of natural drugs has made their use a source public health concern. This concern arises from the possibility of the presence of fungal contaminants and the risk of mycotoxins such as aflatoxins. A total of four hundred and thirty two(432) powdered herbal medicinal preparations consisting of twelve (12) each from six(6) localities in each of the six(6) states' of the North Western Nigeria were obtained. These samples were subjected to various mycological analyses. The parameters measured, were grouped as follows: Mycoflora type and their level of contaminations, physicochemical properties (moisture content and pH), molecular identification of aflatoxigenic and level of total aflatoxins. Result indicated that all the samples tested did not comply with the maximum acceptable limit of $2x10^2$cfu/g for fungal load as specified by United State Pharmacopoeia, but the distribution of fungi in the herbal preparations varies from state to state and from species to species. Kaduna and Birnin Kebbi states had significantly (at $p \leq 0.05$) higher mean fungal count of 1.1x105cfu/g and $1.1x10^5$cfu/g respectively. Gusau had the lowest mean value of fungal load of 7.8x104cfu/g. The fungal load in Katsina, Sokoto, Kano and Gusau were not the same but not significantly different ($p \leq 0.05$) as indicated by Analysis Of Variance (ANOVA). The mean of frequency of occurrence of the fungi isolates showed that incidence of *Aspergillus flavus, Aspergillus parasiticus, Aspergillus niger* and *Penicillium* spp were not significantly different at $p \leq 0.05$, but significantly higher than *Aspergillus ochraceus* and *Aspergillus versicolor*. The mean frequency of occurrence of *Rhizopus* spp and *Fusarium* spp were significantly lowest. Fungi of the genus *Aspergillus* spp and *Penicillium* spp were observed to be the most frequently isolated, and among the *Aspergillus* spp, *A. flavus, A. parasiticus, A. niger* were the most frequently occurred fungi and the major contaminants of the herbal medicinal products

in all the six states of the North Western Nigeria. Both toxigenic and atoxigenic strains of *A. flavus* and *A. parasiticus* were identified through molecular characterizations. Approximately, 85.88% of the samples had their moisture content above the stated limit set by Nigerian appropriate regulatory body (National Agency for Food and Drugs Administration and Control). Most of the fungal species were able to grow over a broad pH range, but generally the fungal isolates grew more in number in samples with acidic properties. Chi- square correlation test showed positive correlation between moisture content and fungal load in all the six states while correlation between pH and fungal load in Gusau, Kaduna and Kano were negative. The total aflatoxin value ranged from the highest (>20ppb)in all the six towns to the lowest (1.6ppb). Kastina showed the highest number of samples n=65(90.28%) contaminated with aflatoxin above the acceptable limit of 10ppb.The mean value of total aflatoxin of herbal medicinal preparations is significantly high in Katsina with a value of 18.73ppb, and significantly lowest in Gusau with a value of 9.40ppb. This suggests that Katsina and Gusau had the highest lowest aflatoxin contamination respectively. The investigation also showed that both the mean of fungal count and mean of aflatoxin level were significantly higher($p \leq 0.05$) in the wet season than in the dry season , with a few exceptions as Lalan Area (Gusau) and Old market and Gawon Nama (Sokoto) where the mean fungal count was found to be significantly higher ($p \leq 0.05$) in the dry season than in the wet seasons. In Argungun Motor Park, the mean aflatoxin level was significantly higher ($p \leq 0.05$) in the dry season than in the wet season. There was a significant and positive correlation between total aflatoxin and fungal load ($p \leq 0.05$; $r = +0.123$) in the preparations obtained in the sample area.

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CHAPTER ONE

2.0 GENERAL INTRODUCTION

1.1 Background

Herbal remedies are perhaps the most common form of alternative medicinal intervention. Almost every nation or people have at one time used herbs and preparations of various sorts to treat illnesses and diseases (Barrett, 2003). However, despite the uses of herbs in medicine throughout the centuries, the extracts of only a relatively fewplant species have been carefully studied and there is a minimal amount of information available on their safety and efficacy. For instance, some plant extracts have been shown to have a variety of pharmacological and anti-inflammatory effects (Chainani, 2003).

Sofowora (1993) emphasized that herbal drugs, apart from being unstandadized are cheap, accessible and also enjoy a wider acceptability among the people of developing countries. Furthermore, World Health Organization survey indicated that about 70-80% of the world population particularly in the developing countries rely on non-conventional medicines mainly of herbal sources in their primary healthcare (WHO, 1998).

In Africa, up to 80% of the population use traditional medicine for primary health care with the global market standing at over US $ 60 billion annually and is growing steadily (WHO, 2003).

The most effective method of identifying medicinal plants today is through ethno pharmacological studies. The clinical success of quinine and quinidine isolated from the *Cinchona* tree bark and recently artemisinin from *Artemisia annua* in the treatment of malaria have rekindled interest in medicinal plants as potential sources of novel drugs

(Igoli *et al.,* 2005). Today, artemisinin based combination therapy is recognized as drug of choice for treatment of malaria (WHO, 2006).

Due to its general acceptance, the government of Nigeria, on September 30, 1992, promulgated the Medical and Dental Practitioners (Amendment) Decree No. 78, which placed natural medicine (traditional and alternative medicine) side by side with the orthodox system (Okunade, 2001).

Inspite of its popularity, the quality of the herbal preparations is of great concern and still remains a focus for scientific scrutiny. Studies have confirmed the presence of potential contaminants such as pathogenic fungi that present serious health hazards (Martins *et al.,* 200; Anyanwu, 2010). Practices used in harvesting, handling, storage, production and distribution subject medicinal plants to contamination by various fungi, which may be responsible for spoilage and production of mycotoxins (Tassaneeyakul *et al*., 2004 and Mandeel, 2005).

In spite of the extensive research on the occurrence of mycotoxins in foods, there are few reports available on the incidence of toxigenic mycoflora and mycotoxins in medicinal plants and phytotherapeutic compounds worldwide (Halt, 1998; Costa and Scussel, 2002). Fungi of the genera *Aspergillus* and *Penicillium* were shown to be largely distributed in the Nigerian ecosystem, and are known to contain strains that produce toxins especially mycotoxins (Efuntoye, 1999).

Due to the limited mycological and mycotoxins studies of herbal medicinal preparations in North Western Nigeria, there is the need to screen herbal medicinal products marketed in this part of the country for these hazardous contaminants.

1.2 STATEMENT OFRESEARCHPROBLEM

Herbal medicinal preparations have increasingly been used worldwide during the last few decades as evidenced by rapidly growing global and national markets for herbal drugs. World Health Organization survey indicated that there is massive acceptance of herbal medicinal preparations by people, both in developing and developed countries. Unfortunately, the number of reports of people experiencing negative effects, caused by the use of herbal medicinal preparations, has also been increasing.

Several cases of adverse effects of herbal medicinal preparation have been reported worldwide during the last few years, which are allegedly caused by taking herbal medicinal products prescribed by the practitioners of indigenous systems of medicine (Vartika and Shanta, 2005). These products may be contaminated with excessive microbial contaminants such as pathogenic fungi like *Aspergillus* spp and their by-products (Roy *et. al.,* 1988) which cause various deformities like congenital paralysis, sensori-neural defects, liver and kidney damages e. These contaminants may be related to the source of herbal medicinal preparations, if these are grown under contaminated environment or improperly stored/kept in environmental conditions that could favor the growth of toxigenic fungi which produces aflatoxins.

One of the problems associated with herbal medicines is their poor quality. There are no reports of their compliance with global safety standards. It is noted that insufficient attention is being paid to the quality assurance and control of herbal medicines.

Although WHO has developed guidelines, including the techniques and measures required for the appropriate cultivation, collection and processing of medicinal plants,the farmers and other relevant persons like producers, handlers and processors of herbal drugs are not much aware of WHO's guidelines and they continue their work as before without any quality control measures which results in inferior quality of herbal

drugs with lots of potential contaminants like the pathogenic fungi (Martins *et al*., 2001) that present serious health hazards.

Aflatoxins being the secondary metabolites of fungal origin have attracted worldwide attention due to the significant losses associated with their impact on human and animal health, and consequent national economic implications (Bhat and Vashanti, 1999). Mycotoxicosis is the consequence or effect (disease or pathological abnormalities) of ingesting toxin-contaminated herbal medicinal preparations by man.

The World Development Report of 1993, indicated that diseases caused by mycotoxins lead to reduced life expectancy in developing countries (Miller, 1996). In many parts of Africa, the need to get access to cheap drugs such as herbal medicines outweighs other considerations such as sources, standard and health hazards associated with consumable herbal drugs, and as such, this has made herbal drugs-borne intoxications to be a serious problem in many parts of the continent. According to Miller (1996), 40% of the productivity lost to diseases in developing countries is due to diseases exacerbated by aflatoxins. Regrettably, many of the people in the region are not even aware of the effect of consuming moldy products. Due to the poor education levels and other socio-economic factors, even if steps are taken to make herbal products safe, the consumers will be unwilling to pay the extra costs, and will still prefer to buy the cheaper ones.

The West African countries have tropical climate with an all year round high ambient temperature and relative humidity, which are suitable conditions for the growth of toxigenic moulds resulting in the increased number of mycoflora, qualitatively and quantitatively (Fatih, 2009). The sub-region also has poorly developed infrastructures such as processing facilities, storage, transportation and skilled human resources.

1.3 JUSTIFICATION FOR THE RESEARCH

The increases in the consumption of natural drugs have made their use a source of public health concern and justify reason for the research.

The adverse effects of these crude drugs are directly linked to the presence of contaminats like fungi and mycotoxins. Some of these toxins are even in minute quantities, mutagenic, and carcinogenic in humans (IARC, 2002). The mycotoxins can accumulate both in transit and store.

The fact that 40% productivity lost to diseases in developing countries is due to diseases exacerbated by aflatoxins also justify closer look at herbal medicinal preparations and give reason for the research.The acknowledgement of the fact that plants are the most likely source from which new drugs will be discovered for the treatment not only of recalcitrant old ailments like malaria but also of the latter-day diseases such as HIV/AIDS and the unpredictable and wild influenza that episodically plague us gives additional reason for the research.

Third world countries like Nigeria have come to the realization that total dependence on the use of orthodox drugs is an unrealistic burden on their often fragile economies in the face of the strangle-hold of the multinational companies on orthodox drug supply whose patent rights they own.

The lacuna between the knowledge of WHO developed guidelines for the quality control of herbal drugs which provide a detailed description of the techniques and measures required for the appropriate cultivation and collection of medicinal plants and possible implementation of this knowledge for the purpose of quality assurance of herbal medicinal preparations justify closer look at herbal products.

Aflatoxins have received greater attention than any other mycotoxins because of their demonstrated potent carcinogenic effect in susceptible laboratory animals and their

acute toxicological effects in humans. As it is realized that absolute safety is never achieved, many countries have attempted to limit exposure to aflatoxins by imposing regulatory limits on commodities intended for use as herbal medicinal preparations.

The enormous biodiversity medicinal plants, provides opportunities for research and contribution to knowledge and the expected benefit to humanity provided the required justification for this investigation.

1.4 AIM AND OBJECTIVES.

1.4.1 Aim of the study:

The aim of the study is to determine the mycological quality and possible presence of aflatoxin in herbal medicinal preparations packaged for human consumption in North Western Nigeria.

1.4.3 Specific objectives are to:

1. Determine the level of fungal contamination of herbal medicinal preparations inNorth Western Nigeria.
2. Isolate and identify the fungi present in these preparations
3. Determine the moisture content and pH of herbal medicinal preparations
4. Screen the fungal isolates obtained from the samples fortotal aflatoxin production.
5. Screen the herbal medicinal preparations for the presence of total aflatoxin.
6. Carry out molecular characterization and confirmation of aflatoxigenic fungi isolated.

CHAPTER TWO

2.0 LITERATURE REVIEW.

2.1 Definition: traditional medicines.

Traditional medicine can be described as the total combination of knowledge and practices, whether explicable or not, used in diagnosing, preventing, or eliminating a physical, mental, or social disease and which may rely exclusively on past experience and observation handed down from generation to generation, verbally or in writing (Sofowora, 1993)

Bhushan (2005) reported that World Health Organization defined traditional medicines as diverse health practices, approaches, knowledge and beliefs incorporating plant, animal, and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness.

Traditional Herbal Medicinal products (THMP) registration dossier of (2005) described herbal preparations as one form of traditional medicines that are obtained by subjecting herbal substances (parts of plant) to treatment such as extraction, distillation, expression, fractionation, purification, concentration, or fermentation.

2.2 Classification of herbal medicinal products.

According to Council Directive 65/65/EEC of European Commission (1998) which has been implemented into National law in all member states, herbal products are classified as medicinal products, if they claim therapeutic or prophylactic indications, and are not considered as medicinal products insofar as they do not make these claims. Products not classified as medicinal products belong in most cases to the food or cosmetic area, even though they sometimes contain plants which have pharmacological properties. For example, Senna pods can be marketed as food in Belgium. Furthermore, there are

specific categories of non-medicinal products, e.g. the so-called "therapeutic supplement products" in Austria. Such products are specifically regulated by National law and sometimes require notification to the competent National authority.

2.3 History of herbal medicines.

Herbal medicine is the oldest form of healthcare known to mankind. Herbs have been used by all cultures throughout human history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter, beauty enhancers, and medicines. Kossowan (1991) reported that even before the advent of western orthodox medicine, traditional medicines have been in practice.

Historically, the most important uses of herbs were medicinal and more than fourty percent (40%) of prescribed medicines in the world are of plant origin (Hedberg, 1998). For most of his existence, man had various but limited resources for treating injuries and diseases. Separately and in combination he used any and all of the following: magic and sorcery, prayer, music, crude operations (amputation, bleeding, trepanning), psychotherapy, physical therapy (diet, rest, exercise, fresh aid), and internal and external remedies prepared from plants, animals, and minerals. Of all these, plant remedies represent the most continuous and universal form of treatment, and were the basic sources of therapeutic products for professional and folk medicine from the earliest days until the twentieth century. Much of the medicinal use of plants seems to have been developed through testing on wild animals by trial and error. The same way he learned that some were edible and others poisonous.

Prior to discovery and subsequent synthesis of antibiotics, the herb *Echinacea* (which comes from the plant commonly known as purple coneflower) was one of the most widely prescribed medicines in the United States. For centuries, herbalists prescribed

Echinacea to fight infection. Today, research confirms that the herb boosts the immune system by stimulating the production of disease-fighting white blood cells (Barrett, 2003).

The use of plants as medicine is older than recorded history, as mute witness to this fact the safety and efficacy of herbal preparations such as Ginkgo, St. John's wort, Echinacea, Saw palmetto and Kava were reviewed. Ginkgo has been found to be useful for memory loss and tinnitus; St. John's Wort can reduce the symptoms of mild-to-moderate depression; Echinacea indicates that it may help to prevent or attenuate the symptoms of upper respiratory tract infections. In short- term studies, Saw palmetto was able to reduce the symptoms of benign prostatic hyperplasia while Kava was effective treatment for anxiety (Ernst, 2002a).

It was documented that the early Christian church discouraged the formal practice of medicine, preferring faith healing: but many Greek and Roman writings on medicine, as on other subjects, were preserved by diligent hand copying of manuscripts in monasteries. The monasteries thus tended to become local centers of medical knowledge, and their herb gardens provided the raw materials for simple treatment of common disorders. At the same time, folk medicine in the home and village continues uninterrupted (Janetzand, 1994)

The continuing importance of herbs for the centuries following the Middle Ages was indicated by the hundreds of herbals published after the invention of printing in the fifteenth century. Two of such publications *(APhysical Directory and The English Physician)* are that of Nicholas Culpeper in 1649. These respected herbal pharmacopeias were one of the first manuals that the layperson could use for health care, and it is still widely referred (Janetzand, 1994).

The first U.S Pharmacopoeia was published in 1820. This volume included an authoritative listing of herbal drugs, with descriptions of their properties uses, dosages, and tests of purity. It was periodically revised and became the legal standard for medical compounds in 1906. But as Western medicine evolved in 17th century this marked the beginning of a slow erosion of the pre-eminent position held by plants as sources of therapeutic effects.

In the early 19th century, scientists began extracting and modifying the active ingredients from plants when methods of chemical analysis became available. Later, chemists began making their own version of plant compounds, beginning the transition from raw herbs to synthetic pharmaceuticals. Over time, the use of herbal medicines declined in favors of pharmaceuticals.

2.4 Herbal medicines today.

Today, medicinal plants are greatly embraced by most populations in the world especially in the developing countries because they have been discovered to veil the best medicine that nature can offer to mankind (George and Pamploma-Roger, 1999). Recently, the World Health Organization (WHO) estimated that 4 billion people, the world rely on herbal medicine for some aspect of their primary health care. WHO global stuation report on traditional medicine of 2002 indicates greater data available on the worldwide utilization of herbal/traditional medicines. Moreover, according to statistics and tables within the atlas, herbal medicine is the only traditional, complementary and alternative medicine that was popular in low-income nations as in high-income nations (Bodeker *et al.,* 2005).

Countries in Europe, Asia, and Latin America and Africa use traditional medicine to help meet their primary health needs. WHO (2003) reported that in China, herbal preparations account for over 30%-50% of the total medicinal consumption, and in

Latin America and other industrialized regions, over 50% of the population have used herbal preparations as alternative medicines, while in Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% children with high fever resulting from malaria is the use of herbal medicine at home. Furthermore, studies in Africa and North America have shown that up to 75% of people living with HIV/AIDS used traditional medicines alone or in combination with other medicines for various symptoms or conditions (WHO, 2002).

Cragg (2004) explained that analysis of data on prescriptions dispensed from community pharmacies in United States of America from 1959-1980 indicated that 25% contained plant extracts or active components derived from higher plants. At least 119 chemical substances derived from 90 plant species can be considered as important drugs currently in use in one or more countries. And in countries such as China and India, plants have served as the basis of sophisticated traditional medium systems for thousands of years.Elujoba *et al.* (2005) reported that many medicinal plants even in the crude form are well known in the international markets today and African countries are among the top world producer of such plants. For instance, ginger (*Zingiber officinale*) which is used as spice, carminative and important medicinal product is well known and also produced in Nigeria.

Today, the rampant and prolong usage of herbal preparations world-wide as cited by the World Health Organization and the scientific evidence from randomized clinical trials coupled with the fact that herbal preparations are used unregulated and inappropriately (Sofowora, 1993), and also contain a lot of pathogenic fungi (Adeleye *et al.,* 2005) suggested that some herbal preparations could have negative and dangerous effects on the health status of an individual.

2.5 Medicinal plants and herbal preparations.

Federal Environmental Protection Agency (1992) reported that Nigeria has over 700 species of algae, 80 lower plants and 5,000 higher plants, and out of these, about 300 were listed as being of medicinal value in Western Nigeria alone, and the listed plants are spread over several families including ferns, bryophytes, and some fungi (Adjanahoun*et al*., 1993). The crude extracts of these medicinal plants can be further purified for effective use (Awosika, 1993).

Janetzand (1994) reported that herbal preparations are available in varieties, including fresh, dried, tablets, capsules, or bottled in liquid form. They are available either singly or in mixtures formulated for specific conditions and in whatever form it is, herbal preparation is as good as the quality of the raw herbs from which it was made.

2.6 Herbal medicines and public policy.

WHO (1999) reported that the first and foremost challenge in the world today is the need to reduce greatly the burden of excess mortality and morbidity suffered by the poor as a result of the widening of inequalities between the rich and poor in the area of human health.In order to achieve that, the World Health Organization (WHO), the United Nations Children Education Fund (UNICEF) and other stakeholders identified herbal medicines as one of the most important resources which should be mobilized. It was then proposed that traditional medicine(TM) should be integrated into Primary HealthCare (PHC). These offered the best means available for achieving the goal of health for all in the year 2000 (WHO, 1978).

Balasubramaniam (2000) reported that the year 2000 had reached without achieving the goal (health for all), but seemed to have gone in reverse gear. He also explained that the letdown were attributed to failure in the primary health care sectors due to the fact that since the Alma-Ata Declaration of 1978, no country implemented the primary health

care strategy by given a place for herbal medicines and traditional systems of medicine in their public policies. WHO (1998) reported that it was only China in 1946 and Vietnam 1961 that had done these before the Alma-Ata Declaration.

According to WHO and UNICEF reports of 1978, eighty percent (80%) of the world's population depended on traditional systems of medicine and herbal remedies for their healthcare needs. Unfortunetetly, this healthcare system is not given a place in public policy. And without an officially stated public policy there can be no development of traditional systems of medicine.

WHO (1978) also identified that formulation and implementation of public policies on traditional medicines and integration of these with Primary Health Care are controlled by health professionals trained in modern medicine who have no knowledge of traditional system and view it as "unscientific and very much inferior to modern medicine". These are the reasons for the non-development of traditional systems of medicine.

Four points were highlighted by Balasubramaniam (2000) in his report. These are:

i. In 1985, the Indian Government published a "Manual for Practitioners of Indian Systems of Medicine & Homeopathy". The aim was to explain and describe the involvement of traditional practitioners in family welfare and primary health care (PHC). The entire manual does not contain a single sentence where the traditional practitioner is required to use or draw upon her/his training and experience. The manual treats the traditional practitioner as an unskilled worker whose only task is to refer the patient to a modern health worker or health centre for "proper investigation, diagnosis and treatment"

ii. The South-East Asian Region of WHO (SEARO) consists of 10 countries including India, with about 1.5 billion people, a quarter of the world population and accounts for half of the world poor. In 1993, SEARO published the second evaluation of the global strategy for Health for All by the year 2000. There was no reference to traditional medicine in the case studies of eight countries. A very brief mention was given in the reports from Mongolia and Myanmar.

iii. In the 118 page World Health Report 1995, "Bridging the Gap", all that was written about traditional medicine was mentioned in only three sentences as follows:

"Traditional medicine continues to be an important part of healthcare in many developing countries, and various "alternative" or complementary therapies enjoy a widespread following in developed countries. Traditional medicine has not, however, been incorporated into most national health systems and the potential of services provided by traditional practitioners is far from being fully utilized. There is a continuing need for better assessment of the benefits of alternative forms of medicine including traditional ones."

iv. The World Health Report, 1999 "Making a Difference" has no reference to traditional medicine at all!

2.7 Phytochemicals in medicinal plants.

The phytochemicals which are the active constituents of plants were reported to be available from the extracts obtained from the medicinal plants growing in Nigeria (Ajibola and Motoyoshi, 1992).

The term “phytochemical” refers to a wide variety of compounds produced by plants. They are found in fruits, vegetables, beans, grains, and other plants parts. Scientists have identified thousands of phytochemicals also known as biologically active metabolites in plant materials selected because of local use in traditional medicines (Farnsworth, 1990).

The active constituents of plants have been recognized as non nutritive plant chemicals that are held within them and act as disease-preventing compounds. Although these phytochemicals are not yet classified as nutrients, they are substances known for the prevention and treatment of health conditions, including cancer, heart disease, diabetes, and high blood pressure (George and Pamploma-Roger,1999). Some of the more commonly known phytochemicals include flavonoids, carbohydrates, tannins, cardiac glycosides, saponins, anthraquinones, alkaloids, ascorbic acid, folic acid and vitamin E, and all these aid animal and people by creating a preventive barrier against certain diseases and sicknesses (Ajibola and Motoyoshi, 1992).

2.8 Quality of herbal preparations.

Plants have been used for medicinal purposes in Africa for many centuries. Today, herbal products are being used worldwide in a variety of healthcare settings, as a home remedies, and are often promoted to the public as being “natural” and completely “safe” alternatives to conventional medicines (Adewunmi and Ojewole, 2004). African plants used medicinally are widely assumed to be safe, but many are potentially toxic (Fennell *et al.,* 2004).

Since herbal medicinal products are complex mixtures which originate from biological sources, great care is necessary to guarantee constant and adequate quality. (Adewunmi and Ojewole, 2004) reported that the efficacy, safety and quality of raw medicinal plant materials and plant products depend on intrinsic or external factors, and inadvertent

contamination by microbial or chemical agents during any of the production stages can also affect the quality, safety and efficacy. Medicinal plants collected in the wild may be contaminated by other species or plant parts through misidentification, accidental contamination or intentional adulteration, all of which may have unsafe consequences.

The World Health Organization (WHO, 1998) survey indicated that about 70-80% of the world population particularly in developing countries rely on non- conventional medicines mainly of herbal origins for their primary health care. This is because herbal medicines are accessible and cheap (Sofowora,1993). Therefore, the required standard of quality, safety and efficacy of herbal preparations are also of great concern and should be met (Calixto, 2000)

WHO (1993) explained that quality is the basis of reproducible efficacy and safety of herbal drugs, and to ensure the reliability and repeatability of any research on herbal medicines, the quality of the plant materials or preparations must be determined.

Bauer (1998) reported that the quality criteria for herbal drugs are based on a clear scientific definition of the raw materials and it is difficult to establish comprehensive quality criteria for the herbal drugs due to several aspects that exist with it. However, in order to improve the identity and purity which explain the quality of the herbal drugs, the type of preparation, sensory features, moisture, ash, physical constants, solvent residues, adulteration, biological activity, microbiological contamination as well as foreign materials such as heavy metals, pesticide residues, aflatoxins and radioactivity are to be tested.

Wongwiwat *et al.* (2004) reported the contamination of herbal medicinal products by aflatoxins. Also, adulteration of herbal drugs with synthetic drugs is a potential serious problem which needs to be addressed by adequate regulatory measures. One report from

Taiwan suggested that 24% of the samples analysed were contaminated with at least one conventional pharmacological compound (Ernst, 2002b).

Medicinal plants are associated with a broad variety of microbial contaminants, which are represented by bacteria, fungi and viruses. Inevitably, the microbiological background depends on several environmental factors and exerts an important impact on the overall quality of herbal products and preparations (Wolfgang *et al.,* 2002).

2.9 Mycological quality of herbal medicinal preparations

The quality of the herbal preparations is of great concern. Studies have been carried out and confirmed the presence of potential contaminants such as pathogenic fungi (Martins *et al.,* 2001) that present serious health hazards. Practices used in harvesting, production, handling, distribution and storage make medicinal plants subject to contamination by various fungi, which may be responsible for spoilage and production of mycotoxins (Mandeel, 2005). Potential contaminants such as fungi of the genera *Aspergillus, Penicillium* and *Fusarium* were reported to be present in herbal medicinal products (Anyanwu, 2010).

2.9.1 *Aspergillus* spp.

The genus *Aspergillus* is classified into eight subgenera, and each subgenus is subdivided into several sections that include many related species (Klich, 2006). This is the most frequently traced fungi and the major producer of aflatoxin in herbal medicinal preparations (Bugno *et al.,* 2006).

2.9.2 *Penicillium* spp.

The genus *Penicillium* is commonly found in soil, food, cellulose, grains, paint, carpet, wallpaper, interior fiberglass duct insulation, and decaying vegetation. *Penicillium* may cause hypersensitivity pneumonitis, asthma, and allergic alveolitis in susceptible

individuals. The genus *Penicillium* has several species and is found to be contaminant of herbal medicinal drugs (Anyanwu, 2010). The most common ones include *Penicillium chrysogenum, Penicillium citrinum, Penicillium janthinellum, Penicillium marneffei*, and *Penicillium purpurogenum*.

This fungihas been isolated from patients with keratitis, ear infections, pneumonia, endocarditis, peritonitis, and urinary tract infections. *Penicillium* infections are most commonly exhibited in immunosuppressed individuals. For example, *P. marneffei* is a fungus abundant in Southeast Asia that typically infects immunocompromised patients (Larone, 2002). Infection with *P.marneffei* is acquired via inhalation and initially results in a pulmonary infection and then spreads to other areas of the body (lymphatic system, liver, spleen, and bones), and is often fatal. An indication of infection is the appearance of papules that resemble acne on the face, trunk, and extremities.

Penicillim spp do have the ability to produce mycotoxins. The mycotoxin known as Ochratoxin A, which is nephrotoxic and carcinogenic, may be produced by *Penicillium verrucosum*. Verrucosidin is another mycotoxin produced by this fungus that exhibits neurotoxity. Penicillic acid is another mycotoxin that is nephrotoxic (causes kidney and liver damage).

2.9.3 *Fusarium* spp.

Fusarium is a large genus of filamentousfungi widely distributed in soil and in association with plants. Most species are harmless saprobesand are relatively abundant members of the soil microbial community. Some species produce mycotoxins in cereal crops that can affect human and animal health if they enter the food chain. The main toxins produced by these *Fusarium* species are fumonisins andtrichothecenes.Tthis fungus is often found in humidifiers and has been isolated from water-damaged carpets

and a variety of other building materials. Human exposure may occur through ingestion of contaminated grains and possibly through the inhalation of spores. *Fusarium* spp. is frequently involved with skin, and nail infectionsas well as eye infection (Imamura *et al.,* 2008). More severely it can produce hemorrhagic syndrome (alimentary toxic aleukia) in humans which is characterized by nausea, vomiting, diarrhea, dermatitis, and extensive internal bleeding.

2.9.4 *Rhizopus* spp.

Rhizopus is a cosmopolitan filamentous fungus frequently isolated from soil, decaying fruit and vegetables, animal feces, and old bread. Aside from being known as common contaminants, *Rhizopus* species are also occasional causes of serious, and often fatal, infections in humans (Larone, 2002). Certain species are plant pathogens as well. The genus *Rhizopus* contains several species. The most common ones are, namely, *Rhizopus arrhizus*, *Rhizopus azygosporus*, *Rhizopus microsporus*, *Rhizopus schipperae*, and *Rhizopus stolonifer*.

Morphological features, such as the length of rhizoids and sporangiophores, the shape of columellae, the diameter of sporangia, and the size, shape and surface texture of sporangiospores, help in species differentiation of *Rhizopus*. Maximum growth temperature also varies from species to another.

Rhizopus species are among the fungi causing the group of infections referred to as zygomycosis. Zygomycosis is now the preferred term over mucormycosis for this angio – invasive disease. *Rhizopus arrhizus* is the most common cause of zygomycosis and is followed by *Rhizopus microsporus var. rhizopodiformis*. Zygomycosis infection includes mucocutaneous, rhinocerebral, genitourinary, gastrointestinal, pulmonary, and disseminated infections (Larone, 2002).

2.10 Aflatoxins and aflatoxin contamination of herbal medicines.

Aflatoxins are one of the most potent natural toxins and are recognized as hepatotoxic, carcinogenic, immunosuppressive, and antinutritional contaminants of many staple food commodities (Bommakanti and Waliyar, 2009). The International Agency for Research on Cancer (IARC) has classified aflatoxins as carcinogenic to humans (Group 1) based on sufficient scientific evidence (IARC, 2002b). They are predominantly implicated as a risk factor for the primary liver cancer known as hepatocellular carcinoma (HCC), a common cause of cancer morbidity and mortality in Asia, Africa, and in groups of Asian- and Hispanic-Americans.(Wang *et al.*,2001; Wild and Turner, 2002).

Aflatoxins belong to a group of mycotoxins that are produced as secondary fungal metabolites and excreted into substrates including plants and processed foods intended for human consumption.(Cigic and Prosen, 2009) , and its represent a worldwide threat to public health due to their frequent occurrence in herbal medicinal drugs(Bugno *et al.*,2006). Aflatoxin contamination of staple diets is common in many developing countries and outbreaks have been reported in several parts of Africa and Asia including Kenya, India, Malaysiaand Nigeria (Efuntoye, 1999; Bhat and Vasanthi, 2003; Williams *et al.*,2004), The greatest recorded fatal aflatoxin outbreak occurred in Kenya in 2004 which resulted in 317 cases and 125 deaths. The implicated source was the aflatoxin contaminated homegrown maize which was stored under damp conditions (CDCP, 2004; Lewis *et al.*, 2005; Wagacha and Muthomi, 2008). The largest reported aflatoxin outbreak occurred in western India in 1974 from the consumption of contaminated maize which resulted in 397 cases and 106 deaths (Reddy and Raghavender, 2007).

Aflatoxin contamination in herbal medicinal commodities also has significant economic implications (Bhat and Vashanti, 1999). The Food and Agriculture Organization (FAO)

estimates that 25% of the world's food crops are affected by mycotoxins, particularly aflatoxins (Cornell University Department of Animal Science, 2009). Losses incurred from rejected shipments due to aflatoxin contamination can substantially affect export markets. For instance, the Almond Board of California in 2008 estimated that each rejected almond consignment can cost as much as 10 thousand dollars for demurrage, warehousing, replacement shipments, and other expenses (Bhat and Vashanti. 2003; Almond board, 2008) This economic impact, as well as its frequent occurrence in food and feed and its deleterious effect on human health, have driven producers, manufacturers, regulatory agencies, and researchers to find measures for control and rapid detection in foods (Cheli *et al*., 2009).

2.11 Classification of aflatoxins and their health effect.

Aflatoxins are a group of structurally related toxic compounds produced by certain species of the fungi *Aspergillus*, particularly *Aspergillus flavus* and *Aspergillus parasiticus* (FDA, 2007). Approximately 20 aflatoxins have been isolated from various fungal species; however, only four are commonly found and have been extensively studied with respect to their biological properties and health concerns (Santacroce *et al.*, 2008) .These four aflatoxins are: B_1, B_2, G_1 and G_2.

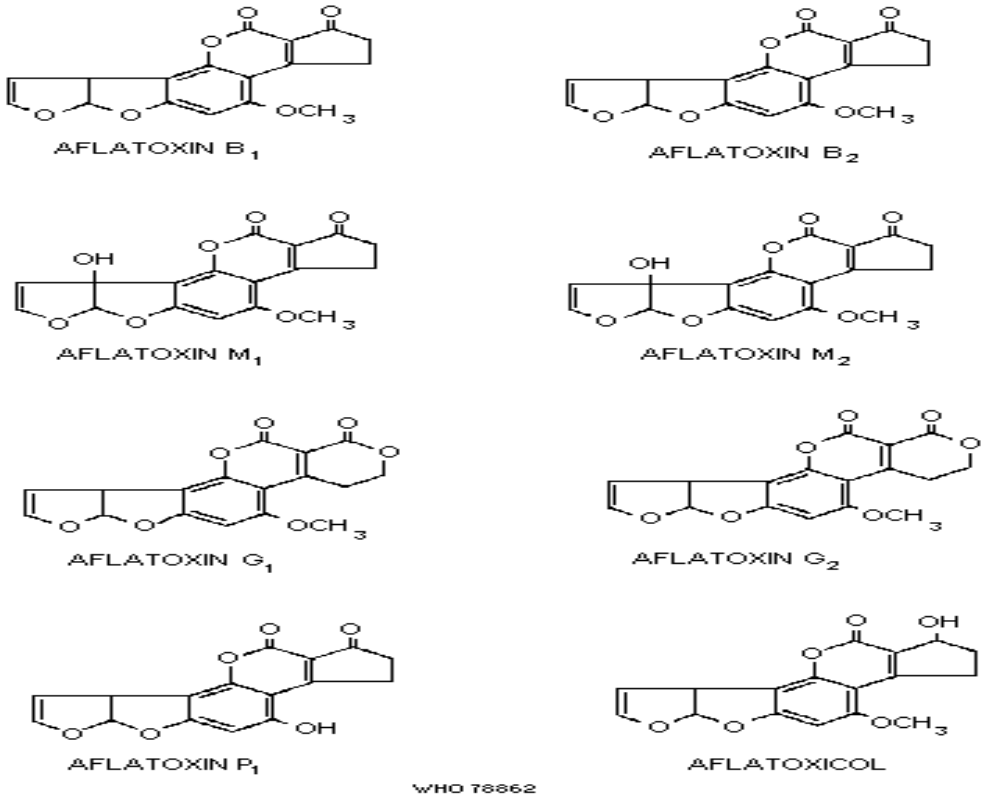


Fig. 1. The structure of some aflatoxins and their metaboiltes.

Aspergillus parasiticus produces all four aflatoxins, while *Aspergillus flavus* produces only B_1 and B_2. The B and G designations of aflatoxins refer to the exhibition of blue and yellow-green fluorescent colors produced by these compounds on thin layer chromatography plates, respectively. The subscript numbers 1 and 2 indicate relative chromatographic mobility of aflatoxins during thin layer chromatography (Watson, 1998; Hussein and Brasel, 2001).

Aflatoxins display variations in the magnitude of toxicity and carcinogenicity. Aflatoxin B_1 is the most toxic followed by G_1, B_2, and G_2 as illustrated by their LD_{50} values for day-old ducklings (Hussein and Brasel, 2001; Reddy and Raghvender, 2007). When aflatoxins B_1 and B_2 are ingested by lactating cows or other ruminants mainly through the consumption of contaminated feed, a proportion of these aflatoxins undergo hydroxylation and are excreted in the milk as aflatoxin metabolites known as aflatoxins

M_1 and M_2. Although these compounds exhibit lower toxicity than the parent molecules, they are significant because of the extensive consumption of cow's milk by infants as well as subsequent contamination of dairy products such as cheese and yogurt through the aflatoxin contaminated milk (Watson, 1998; Pitt, 2000)

2.12 Natural Occurrence of aflatoxin

Aflatoxins occur as natural contaminants in human and animal foods as well as herbal medicinal preparations as a result of fungal contamination of the their sources or raw material during pre and postharvest (WHO, 1998).In another issues, WHO, 1998; Lamanaka *et al.*, 2007; Reddy and Waliyar, 2009 reported that aflatoxin are found in a wide range of foods and raw materials for herbal medicines which include:

- Cereals (maize, sorghum, pearl millet, rice, wheat)
- Oilseeds (peanut, soybean, sunflower, cotton)
- Spices (chillies, black pepper, coriander, turmeric, ginger)
- Tree nuts (almonds, pistachio, walnuts, coconut)
- Dried fruits (sultanas, figs)
- Cocoa beans.
- Raw material for herbal medicines.

Cornell University Department of animal Science (2009) reported that the agricultural commodities which have the highest risk of aflatoxin contamination are: corn, peanuts (groundnuts), cottonseed.

Agricultural commodities grown in tropical and subtropical countries are more prone to aflatoxin contamination. The exportation of such commodities to other countries spreads the contamination and makes aflatoxin a worldwide problem. (Watson,1998).

The United Kingdom Food Standard Administrations (2006) reported that commodities such as peanuts, edible nuts and nut products, dried figs, spices and maize spread the contamination by aflatoxin through international trading.

2.16 Factors favouring aflatoxin production

Aflatoxin development in grains depends on the growth and infestation of the fungi *Aspergillus* (Grybauskas *et al.,* 2000). Fungal invasion and contamination usually begin before harvest and can be enhanced by poor production and harvest conditions. The relative proportions and amounts of the various aflatoxins on food crops depend on several factors (Williams *et al.,* 2004 and U.S. Department of Health and Human Services, 2005)

These factors include:

- **Genotype.**

  Use of crop genotypes that are susceptible to *Aspergillus* infection and insect or microbial infestation increases the risk of aflatoxin contamination. Seed variety should be considered and farmers should consult with the appropriate plant breeding authorities or agricultural extension services to find the best suited cultivar (Codex Alimentarius Commission. 2004)

- **Drought stress.**

  Preharvest aflatoxin contamination is a problem in semi-arid tropics where drought stress amplifies the *Aspergillus* infection and results in groundnut contamination. This is possibly due to loss of the phytoalexin producing capacity of the seed and altered seed composition under drought stress (Katam *et al.,* 2007). Drought stress has also been found to increase the number of *Aspergillus*

spores in the air which significantly increases the possibility of infection, especially during pollination (Grybauskas *et al.,* 2000)

- **Soil types.**

  Crops grown in different soil types may have significantly different levels of aflatoxin contamination. For example, peanuts grown in light sandy soils support rapid growth of the fungi, particularly under dry conditions. Heavier soils result in less contamination of peanuts due to their high water holding capacity which helps the plant to prevent drought stress (Codex Alimentarius Commission, 2004)

- **Insect activity.**

  Insect infestations can increase the occurrence of *Aspergillus* and levels of aflatoxins, especially during pollination in drought-stressed corn. Insect damage to the corn ear allows easy access of *Aspergillus* which subsequently infects the kernels. Insects also transport *Aspergillus* spores to the silks and kernels thereby facilitating aflatoxin contamination (Grybauskas *et al.,* 2000)

- **Growing and storage conditions.**

  Tropical conditions such as High temperature, High Humidity, Monsoons, Unseasonal rains during harvest and Flash floods favor the development of aflatoxins, and contamination is usually higher in plant materials grown in tropical climates than in temperate climates. (Bhat and Vasanthi, 2003). If high moisture and temperature conditions are sustained after harvest, aflatoxins can be produced during storage, especially at moisture content above 12% and temperatures greater than 70^{o}F (Osweiler, 2005). For example, in peanuts and corn, preharvest aflatoxin contamination is favored by high temperatures, prolonged drought conditions, and high insect activity, while postharvest

aflatoxin contamination is facilitated by warm temperatures and high humidity (Cornell University Department of Animal Science, 2009) Poor harvesting practices, improper storage, and improper conditions during transport and marketing can also lead to aflatoxin development and contamination (Bhat and Vasanthi, 2003)

- **Other factors**

  Other crop and field factors which favor aflatoxin production include Specific crop growth stages, Poorfertility, High crop density and Weed competition (Cornell University Department of Animal Science. 2009).

2.17 Aflatoxicoses

Aflatoxicosis is a disease that results from ingestion of aflatoxins in contaminated food or feed. The primary target organ is liver and liver damage occurs in many species, including, birds, fish, rodents, nonhuman primates, and humans (William *et al.,* 2004 and Food and Drugs Administration, 2007) The susceptibility to aflatoxins varies significantly between different species and members of the same species, and is dependent on the following factors: age, sex, weight, Nutritional status of diet, Environmental factors, aflatoxin exposure (dose and duration) and presence of other mycotoxins and pharmacologically active substances(Benett and Klich, 2003; Williams, *et al*., 2004; Food and Drugs Administration,2007)

The risk of cancers due to aflatoxin exposure is based on cumulative lifetime dose. Since all animal species are susceptible to severe toxic effects of aflatoxins, it is often assumed that human health effects are similar to that of animals. According to Food and Drugs Administration (2007), the aflatoxicosis in animals (and possibly in humans) has been categorized in two general forms

1. **Acute**:-This is caused from the consumption of moderate to high levels of aflatoxins.Symptoms may include: Hemorrhage, Acute liver damage, EdemaAlteration in digestion, absorption, and nutrient metabolism and Death
2. **Chronic**:-This is caused from the consumption of low to moderate levels of aflatoxins.It usually results in subclinical effects which are difficult to recognize. Symptoms include: impaired food conversion and slower rates of growth with or without production of clear symptoms.

2.18 Effects of Aflatoxicosis on human health

Humans are exposed to aflatoxins mainly by eating contaminated food. The dose and duration of aflatoxins primarily determines the toxic effects of aflatoxin, usually large doses or long-term exposure to low levels in the diet have significant adverse effects. Symptoms of acute aflatoxicosis in humans include: vomiting, abdominal pain, pulmonary edema, Convulsions, coma *and* Death with cerebral edema and fatty involvement of the liver, kidneys, and heart (William *et al.*, 2004).

2.16 Aflatoxins and Children

Many children in developing countries, including Benin, Gambia, and Togo are malnourished as well as chronically exposed to high levels of aflatoxins from the consumption of contaminated staple foods such as maize and groundnuts. Children exposed to high levels of aflatoxins throughout childhood may become stunted (an indicator of chronic malnutrition) and underweight (an indicator of acute malnutrition). They also become more susceptible to infectious diseases such as malaria, diarrhea, and respiratory infections in childhood and later in life. (Gong *et al.*, 2002; Bhat and Vasanthi, 2003) In addition, it has been assumed that kwashiorkor, a severe

malnutrition disease, may be a form of pediatric aflatoxicosis (Bennett and Klich, 2003). Nursing infants may be exposed to aflatoxins through breast milk. M_1 is the most common aflatoxin detected in breast milk, and in one study its concentration was found to be between 0.02 to 1.12 microgram/liter in breast milk (U.S. Department of Health and Human Services. 2005).

2.18 Effects of Aflatoxicosis on Animal Health

The health impacts of aflatoxin exposure in animals depend on dosage and response. Low doses produce nutritional interferences and immunological suppression, while high doses lead to acute illness and death. These effects are summarized asnutritional effects, immunological suppression and acute effects (Loss of appetite, depression, hemorrhage, diarrhea and Death) as reported (Williams *et al.*, 2004 and Osweiler, 2005).

2.18 Mechanisms of infection

Aflatoxins are associated with both toxicity and carcinogenicity in human populations (Bennett and Klich, 2003). A number of adverse human health effects have been associated with aflatoxins, including hepatotoxicity, liver cancer, kwashiorkor, and Reye's syndrome. However, only a link between aflatoxin exposure and both hepatotoxicity and liver cancer are well established (Eaton and Gallaghar, 1994).

Aflatoxin B1 (AFB1) is the most potent natural carcinogen known and is generally the main aflatoxin produced by toxigenic strains of *Aspergillus* (Bennett and Klich, 2003). AFB1 infection most commonly begins with the ingestion of contaminated food and proceeds through the following four steps:

1. **Metabolic Activation** -- The cytochrome P450 (CYP450) isoenzyme system in the liver metabolizes AFB1 primarily though the process of hydroxylation. The human CYP450 isoforms, CYP3A4 and CYP1A2, catalyze the epoxidation of AFB1 to yield active forms known as AFB1-8, 9-exo-epoxide and AFB1-8,9-endo-epoxide. It is the AFB1-8, 9-exo-epoxide which exerts hepatocarcinogenic effects of AFB1 (Eaton and Gallaghar, 1994; Wild and Turner, 2002; Barceloux, 2008). AFB1 metabolism also results in the formation of aflatoxins Q_1, M_1, and P_1. These metabolites as well as other naturally occurring aflatoxins (G_1, B_2 and G_2) are poor substrates for epoxidation and, consequently, are less mutagenic, toxigenic, and carcinogenic than AFB1 (Wild and Turner,2002).
2. **DNA Modification** -- AFB1-8,9-exo-epoxide is highly unstable molecule which has high affinity for guanine (G) base in the deoxyribonucleic acid (DNA) (Barceloux,2008). It binds to the N7 position of guanine through a covalent bond and forms AFB1-N7-guanine adducts in the target cells (Hussein and Brasel, 2001).These adducts induce DNA mutations primarily through a guanine to thymine transversion in the DNA targeted to the site of the original adduct (Wild and Turner, 2001).
3. **Cell Deregulation** -- The G to T transversion (AGG to AGT) at the third base of codon 249 of the protein 53(p53) tumor suppressor genes in the human liver induces changes in the coding properties of the DNA. The p53 gene is a transcriptional activator which functions as a controller of the cell cycle, and has a role in the apoptosis pathway and DNA repair (Smela *at el*., 2001). The mutations in p53 may cause permanent transcriptional changes as well as interference with RNA synthesis (Hussein and Brasel, 2001; Barceloux, 2008; Wagan et *al.*, 2004). This results in DNA damage, development of DNA lesions,

and consequently cell deregulation (Hussein and Brasel, 2001; Smela *et al.*, 2001).

4. **Development of Hepatocellular Carcinoma** -- Loss of function of p53 give cells a selective growth advantage, since p53 is a tumor suppressor gene. Therefore, the deregulation of p53 leads to tumor formation in the liver known as hepatocelluar carcinoma (HCC). Aflatoxin- induced p53 mutation in liver has been found in up to 50 percent of HCC samples, while AFB1-DNA adducts at other sites within the p53 gene, which induce G to T transversions, were found to be very rare in HCC samples from areas of high aflatoxin exposure (Wild and Turner, 2002).

2.19 Treatment of Aflatoxicosis

Aflatoxicosis is rarely reported in humans and treatment with antibiotics or other drugs has little effect (Food and Drugs Administration, 2007). Cassel *et al.*, 2001; Osweiler, 2005; Cornell University Department of Animal Science, 2009) reported that the treatment options are mainly focused on animals and include the use of following substances in the feed:

1. Chemisorbents such as hydrated calcium aluminosilicate (HSCAS) and mineral clays such as zeolite and bentonite which bind to aflatoxins in feed and prevent their absorption and bioavailability. The levels of usage and effects of these products are:
    - Usually added to feed at the rate of 5 - 10 pounds per ton
    - Reduce effects of aflatoxins on the liver
    - Reduce aflatoxin residues in milk

2. Organic acids such as propionic, benzoic, and sorbic acids as well as their salts calcium propionate, potassium sorbate, and copper sulfate inhibit mold growth in feed.
3. Modified glucan based adsorbents, such as Mycosorb^{TM} and Alltech which may bind aflatoxins in feeds.
4. Increased levels of high quality protein supplements and vitamins A, D, E, and K in the ration as aflatoxin affects protein synthesis and binds to vitamins.

In addition, good management practices should be implemented to reduce stress and to decrease the risk of secondary infections.

2.20 Prevention and Control of Aflatoxin Contamination

The conventional approach to prevent aflatoxin exposure at the individual level is to avoid consumption of contaminated herbal medicinal preparations. However, this approach has not been reported successful in developing countries (Bhat and Vasanthi, 2003; Williams *et al.,* 2004). Therefore, aflatoxin formation in crops can be reduced by several pre- and postharvest good agricultural practices (Grybauskas *et al.,* 2000; Williams *et al.,* 2004).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area and sampling

A total of Four hundred and thirty two (432) samples of powdered herbal medicinal preparations were purchased randomly from identified herbal shops and retail outlets in different parts of Sokoto, Birnin Kebbi, Gusau, Katsina, Kano and Kaduna town. These towns are in the North Central geographical zone of Nigeria. Six (6) samples were collected from each named towns at regular interval of two weeks of every month for one year (January, 2010 to December, 2010). Packaged herbal samples were collected and taken to the laboratory, while those that were not packaged (such as herbal preparations sold by local herbalist) were collected in sterile polythene bags (Pearce *et al.*, 2004). All samples collected from the sites were classified based on their usage, and analysed in the laboratories of Department of Microbiology, Ahmadu Bello University, Zaria and NAFDAC Area Laboratory, Kaduna.

3.2 Media used in the Mycological and aflatoxin analyses.

Peptone water

Potato Dextrose Agar

Agar Agar Medium

Desiccated coconut Nutrient Agar

Neutral Red Desiccated Coconut Agar (NRDCA).

3.3 Preparation of media

3.3.1 Preparation of dehydrated media

All dehydrated media were prepared according to manufacturer instructions. They were mixed with distilled water and dissolved by gentle heat to boil. The media were sterilized in an autoclave (LTE J7090 model, LTE Scientific Ltd, England) at 121^0C for

15 minutes. About 20 ml of the sterile media were dispensed into each of many sterile Petri plates and allowed to cool. The sterility of the prepared media was checked by incubation of the plates at 37^0C for 24 hrs. The sterile agar plates were stored in the refrigerator at 4^0C before use.

3.3.2 Preparation of Neutral Red Desiccated Coconut Agar (NRDCA).

Fresh coconuts were purchased from the market in Sabongari, Zaria, Kaduna state, Nigeria. The hard back was removed and the coconut was chopped into pieces using a grinder and the moisture content was reduced to 15% by putting it into desiccator for few days.

The NRDCA was then prepared by modification of the method of Davis *et al*. (1987) as reported by Atanda *et al.(*2011) as follows: two hundred grams of desiccated coconut was soaked in 1L of hot distilled water for 30min (pH 4.8), blended aseptically in (Marlex Emerald mixer grinder) for 5min and filtered through four layers of cheese cloth, two percent agar(Merck) was added to the filtrate heated to boiling, cooled to about 50^0C and 0.2% of neutral red stain (pH 4.4) was added. The medium was then sterilized at 121^0C for 15min, cooled and poured uniformly(15ml) into sterile Petri dishes(8.5cm) while being vigorously stirred with a sterile stick, care was taken to avoid trapping air bubbles in the medium.

3.4 Fungal analyses

3.4.1 Evaluation of fungal contamination

Ten (10g) of each sample was mechanically homogenized in 90.0 mL of buffered peptone water and shaken vigorously for 2 minutes. Tenfold serial dilution was performed up to 10^{-4} in buffered peptone water and 0.1 mL of the dilution was transferred aseptically to sterilized petri plates containing freshly prepared Potato Dextrose Agar (PDA) with 0.01% chloramphenicol (DIFCO) to inhibit bacterial growth.

Plates were incubated upside down at 26 ± 1^0C for five days. After incubation, enumeration of fungi was performed by surface plating method (USP, 2005). The fungal colonies were counted, recorded and the numbers of colony-forming units (CFU) per gram were calculated. Samples were analyzed using standard aseptic techniques to ensure that no outside contaminants were introduced into the samples.

3.4.2 Isolation and identification of fungi.

Mould colonies representative of all morphologically different types present were inoculated onto Potato Dextrose Agar (PDA) and incubated for 6-8 days at room temperature as described by Harley and Prescott (1993). The pure isolates were identified microscopically by putting 2-3 drops of cotton blue in lacto phenol on the clean microscopic slide and placed a piece of fungus growth on the slide and covered with cover slip (Davise,2002; Pepper, and Gerba, 2004). It was mounted on the microscope and observed usingx10andx40 objectives. Macroscopic (cultural) features like colony color and margins as well as microscopic such as conidia and conidiophores arrangements were examined for species differentiation John, (1986); Philip, (1989); Larone, (1995); Gilman.(2001); Klinch, (2002); Gabriel, (2005); You *et al.*, (2007).

3.4.2.1. *Aspergillus flavus.*

Culturally, colonies identified with yellow or green surface often with black sclerotia and the reverse side of the plate golden at first then later turned red brown, and microscopically the conidiophores was observed to be colourless, rough with biserated phialides, round and radiated vesicles were suspected to be *Aspergillus flavus* (Larone, 1995) .

3.4.2.2. Aspergillus *niger*

Culturally, colonies identified with black surface and white to yellow side and microscopically, the conidiophores were observed to be long, smooth, colourless or brown with biserated philialides and round but columnar head were confirmed to be *Aspergillus niger* (Collier *et al.,* 1998).

3.4.2.3. Aspergillus *parasiticus.*

Culturally, the colonies observed as bright green but without sclerotia. Condiophore had only one set of sterigmata and stalk was spiny only at the top. Globose spiny conidia are produced.this mould is confirmed to be *Aspergillus parasiticus* (Philip, 1989)

3.4.2.4. Aspergillus *ochraceus.*

Culturally on PDA at room temperature the colonies consistently observed to remain bright yellow and sometimes produced purple sclerotia, Conidiophores had two sets of sterigmata; the stalk was spiny with definite yellow pigment in the walls. Conidia were usually smoothed walled and globose to slightly ellipitical. This mould was confirmed to be *Aspergillus ochraceus* (Philip, 1989).

3.4.2.5. Aspergillus *versicolor.*

Culturally on PDA at room temperature the colonies observed grew rapidly reaching 19-22mm in diameter with dark green or blue green and reddish, pale yellowish to uncoloredwithsometimes centrally rising, velvety to rather floccose and with purple reverse. The heads were fragmentary and like penicillum Conidiophores monoverticillate orbiverticillate, Hyaline, smooth-walled, Phialides, flask-shaped and

some phialides directly bored on mycelia. These type of mould were confirmed to be *Aspergillus versicolor* (Gilman, 2001; You *et al.,* 2007).

3.4.2.6. *Penicillium* spp.

Culturally, colonies identified with Greenish blue color, septate hyphae under microscope with conidiophores bearing brush-like conidia were confirmed to be*Penicillium* spp (Burnet, 1976; Gillman, 1957)

3.4.2.7. Fusarium spp.

Culturally, colonies identified with Pink Centered and periphery colony, septate multinucleated hyphae with sickle or canoe shaped conidia in cluster under microscope were confirmed to be *Fusarium* spp (Burnet, 1976; Booth, 1997).

3.4.2.8. Rhizopus spp.

Culturally colonies observed to be Dark-green possessing septate multinucleated hyphae with root-like structure rhizoids under microscope were confirmed to be *Rhizopus* spp. (Raper and Fennel, 1965; Gillman, 1957 and Klinch, 2002).

3.5 Physicochemical analyses.

Moisture content and pH of the samples were determined.

3.5.1 Determination of moisture content.

A Motler Toledo HR73 Halogen moisture analyzer was used to determine the moisture content of the powdered herbal preparations. One (1g) of the sample was weighed into a clean pan (which was a part of the machine accessories). The analysis was done automatically and the reading was shown in percentage on the screen (NAFDAC SOP, 2000).

3.5.2 Determination of pH.

pH was determined using the Hanna pH 211 Microprocessor pH meter(Norris and Ribbons, 1970). Ten (10%) (w/v) solution of the sample was prepared by weighing 10 g of the sample into 200 ml beaker, and then 100 ml of distilled water was added and mixed thoroughly to obtain a homogenous solution. The probe of the pH meter was standardized by using 7.01 buffer solutions. The calibrated probe was then dipped into the ten percent herbal preparation and allowed to stabilize before reading was taken.

3.6. Preliminary screening of aflatoxigenic fungi.

The prepared Neutral Red Desiccated Coconut Agar (NRDCA) plates were inoculated with single spores of fungi isolates (known to produce aflatoxin) and incubated at 30^{o}C for 24hrs, the un-inoculated plates served as control. Thereafter, the plates were observed for some medium characteristics: pigmentation, opacity, transparency and translucency. The reverse side of each plate, was observed under the long wavelength (365nm) UV light for blue/blue green fluorescence (Atanda *et al.*, 2011).The fungi screened to have the potential to produce aflatoxin were sub-cultured for molecular confirmation by partial sequencing (PCR method).

3.7 Aflatoxin Analyses.

3.7.1 Determination of total aflatoxin in herbal medicinal preparations.

The Romer Labs (2004) method using Agra Quant total aflatoxin assay procedure was adopted for the screening of aflatoxin in herbal preparations. The method was a direct competitive enzyme-linked immunosorbent assay (ELISA).

3.7.2 Sample preparation/ extractions for aflatoxin screening.

The method of Romer Labs (2004) as used by NAFDAC was adopted as follows: Fifty grams (50g) of the sample was ground using a Romer Series II Mill and passed through

a 20-mesh screen to obtain about 75% of the Sample. Twenty (20g) of the ground was weighed into a clean jar and 100mL of 70/30 (v/v) methanol/water extraction solution was added into the jar of sample and sealed tightly before shaking on orbital shaker(Model DS2-500E-2; MAx-VWR, USA) for 3mins. In this case, the ratio was always 1:5 samples to extraction solution respectively. The homogenized sample was then allowed to settle, then top layer of the extract was filtered through a Whatman No.1 filter paper and the filtrate was collected for clean-up before the aflatoxin screening.

3.7.3 Clean-up of sample for aflatoxin using push-through format.

Romer laboratory (2003) method was adopted for the clean-up as follows: Five (5ml) of the filtrate was then transferred into glass tube and all was pushed through column (MycoSep224 AflaZon). Two (2ml) of the cleared-filtrate in the MycoSep column was transferred into 5ml beaker then evaporated to dryness in Multi-block heater (Model 2052-1CE; Barristead Lab line, USA) . The sample was then re-dissolved in 400µl mobile phase for ELISA that determines the quantitative level for the presence of total aflatoxin.

3.7.4 Screening of aflatoxin in herbal medicinal preparations.

The ELISA Romer laboratory (2004) method was adopted as follows: An appropriate number of blue/ green- bordered dilution strips were place in micro well strips holder, then equal antibody coated microwell strips were placed in a microwell strips holder. 2ml/strips of aflatoxin conjugate was measured and placed in a separate container from where 200µl of conjugate was pipetted and dispensed into each blue/ green- bordered Dilution well. Then using a single channel pipettor 100µl of each standard or sample was added into the dilution well already containing 200µl of conjugate. Each well was mixed well by carefully pipetting it up and down three times (3x) using 8-channel pipettor for each 8-well strips, then immediately 100µl of the contents from each

Dilution well was be transferred into a corresponding antibody coated microwell and incubated for 5minutes.

The contents of microwell strips were emptied into a waste container and washing was done repeatedly five times and carefully not to dislodge the strips from the holder.After the 5^{th} washing an absorbent was placed on a flat surface, and the microwell strips were tapped to expel excess water, so also the bottom of microwells were dried with a dry towel.

Approximately, 1mL/strip each of substrate from the blue capped bottle and stop solution from the red capped bottle was measured and dispensed into separate regent boat from where 100µL of the substrate was transferred into each micro well strip using 8-channel pipettor, then incubated at room temperature for 5minutes. Then, another 100µL of the stop solution was transferred into each microwell strips containing the substrate using a fresh 8-channel pipettor. The color changed from blue to yellow indicated the presence of aflatoxins.

The microwells were then measured optically using a micro well reader (START FAS Product; Model 303PLUS; Awareness tech, Palm city.) with an absorbance filter of 450nm (OD_{450}) and a differential filter of 630nm. The optical densities of the samples were compared to the optical densities of the standards and an interpretive result was determined.

3.8 Molecular (PCR) confirmation of aflatoxigenic fungi.

3.8.1 Culturing of fungi for DNA extraction.

Fungal strains were cultured for DNA extraction in Erlenmeyer flasks containing 20ml of Sabouraud Broth (Pronadisa, Madrid, Spain) and incubated at 28^0C in an orbital shaker (140 rpm) for 2 days. Mycelia were filtered through Whatman paper no.1 and

kept at −80 ^{0}C for DNA isolation. All genomic DNAs used in this work were tested for suitability for PCR amplification using primers ITS1/ITS4 (White *et al.,* 1990). The amplification programme used was described by Munirul*et al*. (2006).

3.8.2 DNA extraction from aflatoxigenic fungi.

Deoxyribonucleic acid (DNA) of the aflatoxigenic fungal species was extracted according to the method of Munirul*et al.,* (2006). The method involved centrifugation of about 70mg of fungal cells in refrigerated centrifuging machines for 15 seconds and re-suspension in lysis buffer (50mM, Tris-HCl, [PH8.0], 20nm EDTA), and 0.35ml 2% of Sarcosy was added in the buffer added and mixed gently, the mixture was then incubated, after which 50µl of RNase was added at 37^{0}C for 15minutes. A total of 35µl of proteinase K was added and incubated for 1hr at 50^{0}C until lysis was achieved. With the aid of maximum speed setting, the lysate was vortex for two minutes to shear the DNA. This step lowered the molecular weight of DNA molecules to around 20kb, and because of the decrease viscosity, it was also made easier to extract the DNA with phenol, chloroform. In extracting the DNA, double Phenol-chloroform extraction was followed where an equal volume (0.7ml) of phenol, chloroform were mixed with lysate, vortexed for 10 seconds and then centrifuged at 10,000rpm for 10minutes to separate the phases. The top layer was carefully removed with sterile Pasteur pipettes and added to a new micro centrifuged tube (it was ensured that flocculent interphase was taken along). The aqueous DNA was re-extracted with phenol, chloroform one time, before disposing off the phenol (Kay *et al.,* 1994).

Further extractions were done with one volume of chloroform, isomyl alcohol, after which the DNA was precipitated by adding acetate 0.3M (70µl), mixed well before adding equal volume of isopropanol (0.7ml). the mixture was then inverted in the tube 5 times before freezing in an ethanol ice bath for 10 minutes, the supernatant was then

removed with a drawn out Pasteur pipettes. The DNA pellets were obtained by adding 1.0ml of 70% ethanol, vortexed briefly and centrifuged for 5 minutes where the supernatant was then removed with a drawn Pasteur pipette. These were repeated twice to remove residual salts as well as the remaining phenol and chloroform. The two tubes were wrapped with paraffin; poked hole with needle and dried pellets under vacuum to remove ethanol, over drying was avoided. The precipitated DNA was then re-suspended in 50µl "TE" buffer, labeled and stored at -20^0C for further use (Kamalendu, 1990).

3.8.3 Restriction endonuclease digestion of chromosomal DNA.

The DNA isolated from fungi (2µg of chromosomal DNA) was used for the agarose gelelectrophoresis as the undigested control for the next protocol, and also leaving 0.5mg for the PCR experiment. The following order below was followed in adding components of the restriction digest reaction mixture to centrifuge tube, (final volume of 50ml) and 41µl of sterile distilled water.

a. 5µl of 10x Bam HI Buffer (500mM, Tris-Cl, PH8.0, 100M $MgCl_2$, 1000mM Nacl).
b. 3µl of chromosomal DNA.
c. 1µl of Bam HI restriction endonuclease (5 units)

3.8.4 Agarose gel electrophoresis of chromosomal DNA and Restriction endonuclease digestions.

The outside of the gel former was taped. The comb was positioned about 1mm above the surface of the frame by placing an index card between the comb and the gel plate. After removing the index card, about 0.8% agarose gel was poured into the gel frame, after hardening completely of the gel (20-30minutes) the comb were removed gently, making sure gel tray is leveled to prevent the gel from sliding. The gel were transferred to the electrophoresis apparatus and covered with IXTAE buffer (perlitre, 48.4g of

trisma base, 11.42ml of glacial acetic acid, 20ml of 0.5M EDTA, pH 8.0). The DNA samples were mixed with a droplet of loading buffer on paraffin that normally have hydrophobic surface. For the undigested sample (control), 2µl of DNA with 2µl of 6X loading buffer on paraffin. The 0.5µg of the 1kb (1000pb) ladder were loaded in one well, after loading wells with samples the electrophoresis apparatus was connected to power supply and turn the power supply (Hermann and Frischauf, 1987).

3.8.5 Staining and photography of agarose gel of chromosomal DNA restriction endonuclease digestions.

The gel was stained for 20 minutes in the ethidium bromide solution and detained in water for 20 minutes. The U/Vtrans illuminator box was made wet with water and covered with plastic wrap to prevent ethidium bromide from staining the U/V box the gel were moved carefully into U/V box using the protective glasses, the U/V light will be turned on gels were examined, photographed with the Polaroid camera. The photograph were used to determine the distance migrate by each of the bands in comparison with the 1kb ladder (Kohara*et al.,* 1987).

3.8.6. PCR detection of aflatoxigenic fungi genes.

The fungus-specific universal primers ITS1 and ITS4 were used to amplify genes encoding the ITS region (Tarai *et al.,* 2006).The PCR assay was carried out using the following universal Primers ITS1 5’ TCCGTAGGTGAACCTGCGG 3’ and ITS4 3’ TCCTCCGCTTATTGATATGC 5’ intergenic spacer region specific for eukaryotes using method of White *et al.*(1990). The ITS amplifies all fungi for 500pb fragments. The sequence of each of these fragments will differ between species even though the size of the fragments are the same, for the PCR amplification, samples were enriched by incubation in APW (Difco) at 37^0C for 7 hrs and pre-incubated with 0.025% yeast extract (Difco) and 0.002 Nalidixic acid. The broth cultures were centrifuged at

10,000rpm to collect cells pellets. DNA will be extracted from the pelleted cells and subjected to PCR as described by Munirul*et al.* (2006).

Amplification with two primers pairs were performed simultaneously in 0.2ml micro centrifuge tubes.Samples(3µl) were added to the PCR mixture (Final volume 30µl) containing each deoxynucleaside triphosphate at a concentration of 0.21mM, 50mMKCl. 1.0mM $MgCl_2$ 10mM Tris-HCl [pH 8.3], each member of the primers pair at a concentration of 0.27µ and 0.75U of Taq polymerase). The amplification condition used were 5 minutes at 94^0C for initial denaturation of the DNA, 35 cycles consisting of 1 minute at 94^0C, 1 minute at 55^0C (Annealing), and 1 minute at 72^0C (extension) in a DNA Robo cycler gradient temperature cycler(Stratagene, Lajolla,CA). After amplification, 6µl of each reaction mixture were separated by electrophoresis on a 3% agarose gel, and the amplified gene products were visualized under u/v light after staining with ethidiumbromide gel in TAE1X buffer (Tris-acetate 40mM and EDTA1.0mM). The 200bp DNA was used as a molecular size marker

3.9 Statistical Analyses.

Pearson correlation test, ANOVA and Student t-test were used to analyses the data obtained

Pearson correlation test: This statistical analysis was used to ascertain the relationship between the fungal, load, aflatoxin level and the physical parameters analyzed in this study. The Pearson correlation test statistic used as cited by Oyejola (2003) is shown below:

$$r = \frac{n\sum xy - (\sum x)(\sum y)}{\sqrt{n\sum x^2 - (\sum x)^2}\sqrt{n\sum y^2 - (\sum y)^2}}$$

Where: r = correlation coefficient which is used to study the relationship between two variables, and it is a value ranges between $-1 \leq r \leq 1$

X and Y = are the random variables; n = number of observations.

Conditions for the Pearson correlation test statistic: if r is greater than r-critical null hypothesis could be rejected, but if r is less than r-critical the alternative hypothesis is accepted.

Analysis of variance (ANOVA): One way analysis of variance was used, and the statistical formulae and ANOVA table as cited by Oyejola (2003) Condition for the ANOVA test statistic: if F-calculated is greater than F-critical, null hypothesis could be rejected and alternative hypothesis (which says: there is significant difference) could be accepted.

CHAPTER FOUR

4.0 RESULTS

4.1 Classification of herbal samples based on their uses in each of the six towns of the North Western-Nigeria.

Table 4.1 shows the classification of herbal preparations sampled based on their uses and their distribution in each state in the study area. There was a total of four hundred and thirty two(432) herbal samples with seventy two(72) samples in each states. The herbal medicinal preparations used to cure piles were 70(16.20%) being the highest, while those claimed to cure small pox were the least with 21(4.86%). The other preparations used to cure malaria and Stomach ailments were 38(8.80%) each. Those herbal preparations claimed to cure menstrual pain, dysentery/diarrhoea, yellow fever, measles were 22(5.09%), 49(11.34), 42(9.72) and 16(3.70%) respectively. The others include 31(7.18%), 41(9.49%) and 25(5.79%) for hypertension, diabetes and cancer respectively.

4.2 Distributionof herbal drugs samples according to average plate fungal count.

Table 4.2 shows the distribution of fungi in herbal preparations. At a fungal count range of $1.0x10^4$cfu/g to $5.5x10^4$cfu/g, the fungal count observed in all the towns were remarkable; Gusau being the highest with 28(38.89%), Katsina 21(29.17%),Birnin kebbi and Kano had 18(25.00%) each, while Kaduna the lowest with 5(6.94%). At a fungal count range of $6.0x10^4$cfu/gto$10.5x10^4$cfu/g, Sokoto with 49(68.05%) had the highest number of samples followed by Kano with 38(52.78%), Kaduna 31(43.06%), Kastina 28(38.89%), Gusau and Birnin Kebbi with 20(27.78%) each.

Table 4.1: Classification of herbal samples based on their uses in each of the six towns of the North Western-Nigeria.

Uses	TOWNS						
	Gusau town	Sokoto town	Katsina town	Birnin Kebbi town	Kaduna town	Kano town	Total (%)
Typhoid fever	8	5	9	8	5	4	39(9.03)
Menstrual pain	5	3	2	4	2	6	22(5.09)
Antimalarial	5	7	6	5	7	8	38(8.80)
Yellow fever	9	7	8	6	5	7	42(9.72)
Dysentery/diarrhoea	6	7	10	9	11	6	49(11.34)
Measles	3	3	1	1	4	4	16(3.70)
Small pox	2	3	3	4	2	7	21(4.86)
Stomach ailments	7	8	4	7	6	6	38(8.80)
Hypertension	5	6	6	5	5	4	31(7.18)
Diabetes	6	8	7	8	6	6	41(9.49)
Cancer	4	2	5	4	5	5	25(5.79)
Pile	12	13	11	11	14	9	70(16.20)
Total	72	72	72	72	72	72	432(100)

Table 4.2: Distribution of herbal medicinal preparations according to average plate fungal count.

Average aerobic fungal plate count of sample (10^4cfu/gm)	Number and percenztage (%) of samples in each town					
	Gusau town	Kaduna town	Kano town	Katsina town	Sokoto town	Birnin Kebbi town
1.0 – 5.5	28(38.89)	5(6.94)	18(25.00)	21(29.17)	10(13.89)	18(25.00)
6.0 – 10.5	20(27.78)	31(43.06)	38(52.78)	28(38.89)	49(68.05)	20(27.78)
11.0 – 15.5	21(29.17)	32(44.44)	15(20.83)	20(27.78)	13(18.06)	26(36.11)
16.0 – 20.5	3(4.17)	4(5.56)	0(0)	2(2.78)	0(0)	8(11.11)
21.0– 20.5	0(0)	0(0)	1(1.39)	1(1.39)	0(0)	0(0)

4.3 Frequencyof occurrence of fungalisolates in the samplesin six towns of the North Western- Nigeria.

The result presented in Table 4.3 indicates the frequencyof occurrence of fungi in the herbal preparations in six towns of the North Western-Nigeria.

Aspergillus flavus occurred in all the samples obtained from the six towns. The highest frequency was observed in samples from Kano (n=33)45.83%, followed by Gusau (n=32)44.44%, Kaduna (n=31; 43.06%), Birnin Kebbi (n=30)41.67%, while Sokoto and Kastina towns had the frequency occurrence of (n= 29)40.28% and (n=26)36.11% respectively.

Aspergillus parasiticus was present in all the samples of herbal preparations from the six towns under study. The highest frequency was observed in samples (n=40) 55.56%, obtained from Kadunafollowed by Sokoto and Birnin Kebbi (n=33)45.83%, then Kano and Katsina (n= 31)43.06% each.The least occurrence was observed in Gusau (n=25)34.72%.

The frequency of occurrence of *Aspergillus niger* was observed to be highest in samples obtained from Kaduna (n=47)65.27% followed by those fromKano and Sokoto (n=32)44.44% each. Birnin Kebbi (n=28)38.89%, Katsina (n=24)33.33% and Gusau had least (n= 18)25.72%.

Aspergillus ochraceus occurred more frequently in samples obtained from Sokoto (n=23)31.94% followed by those from Birnin Kebbi (n=19)26.39% and Kano (n=18)25.00%. Gusauand Katsina indicated (n=16)22.22% each. The lowest occurrence was observed in Kaduna (n=15)20.83%.

The frequency of occurrence of *Aspergillus versicolor* showed samples from Kano having the highest (n=25)34.72% followed by Gusau (n=20)27.78%.While Kaduna and

Table 4. 3: Frequency of occurrence of fungal isolates in the samples in six towns of the NorthWestern-Nigeria.

Fungi	Number and percentage(%) frequency					
	Kano town	Birnin Kebbi town	Katsina town	Sokoto town	Gusau town	Kaduna town
Asp. Flavus	33(45.83)	30(41.67)	26(36.11)	29(40.28)	32(44.44)	31(43.06)
Asp. parasiticus	31(43.06)	33(45.83)	31(43.06)	33(45.83)	25(34.72)	40(55.56)
Asp. Niger	32(44.44)	28(38.89)	24(33.33)	32(44.44)	18(25.00)	47(65.27)
Asp. ochraceus	18(25.00)	19(26.39)	16(22.22)	23(31.94)	16(22.22)	15(20.83)
Asp. versicolor	25(34.72)	17(23.61)	15(20.83)	18(25.72)	20(27.78)	18(25.00)
*Pen.*Spp	29(40.28)	27(37.50)	35(48.61)	34(47.22)	28(38.89)	37(51.39)
*Fusarium*spp	10(13.89)	27(37.50)	8(11.11)	9(12.50)	13(18.06)	19(26.39)
*Rhizopus*spp	5(6.94)	10(13.89)	3(4.17)	6(8.33)	10(13.89)	10(13.89)
Total(1095)	**183(16.72)**	**191(17.44)**	**158(14.433)**	**184(16.80)**	**162(14.80)**	**217(19.82)**

Asp= Aspergillus; Pen= Penicillium; spp= Species

Sokoto (n=18)25.00% each. The least occurrence was observed in Katsina (n=15)20.83%.

*Penicillium*spphad the highest frequency in samples from Kaduna (n=37)51.39% followed by Katsina (n=35)48.61%. Samples obtained from Sokoto indicated (n=34)47.22%, Kano (n=29)40.28%, Gusau (n=28)38.89% and Birnin Kebbi showed the least of 37.50%(n=37).*Fusarium* sppoccurred less frequently with the highest occurrence in herbal preparations obtained from Birnin Kebbi (n=27)37.50% followed by those from Kaduna (n=19)26.39%. Samples from Gusau showed (n=13)18.06%, Kano (n=10)13.89%, Sokoto and Katsina showed the leastoccurrencewith (n=9)12.50% and (n=8)11.11% respectively. *Rhizopus* spp was least in occurrence in all the states. The highest frequency was observed in Birnin Kebbi, Gusau and Kaduna (n=10)13.89% followed by Sokoto with (n=6)8.33%. Samples obtained from Kano and Katsina had the least frequency with (n=5)6.94% and (n=3)4.17% respectively.

4.4: Mean of frequency of occurrence of fungal isolates in the samples in six towns ofthe North Western Nigeria

Table 4.4 is the result indicating the mean frequency of occurrence of fungi in the herbal medicinal preparations in the North Western Nigeria. The result showed that *Aspergillus parasiticus* had the highest mean frequency of occurrence in the entire North Western Nigeria followed by *Aspergillus flavus*. The least frequency was observed with *Fusarium* spp and *Rhizopus* spp.statistically, the frequency of occurrence of *Aspergillus flavus, Aspergillus parasiticus, Aspergillus niger and Penicillium* spp were observed not to be significantly different ($P \leq 0.05$). *Fusarium* spp occurred frequently but less than *Aspergillus versicolor* and *Aspergillus ochraceus*, even though statistically their mean values were not different. *Rhizopus* spp had the least mean frequency of occurrence in this study.

Table 4. 4: Meanof frequency of occurrence of fungal isolates in samples in six towns ofthe North Western Nigeria

Fungi	Mean ± SEM
Aspergillus flavus	30.17^{a} ± 1.01
Aspergillus parasiticus	32.17^{a} ± 1.97
Aspergillus niger	30.17^{a} ± 4.00
Aspergillus ochraceus	17.83^{b} ± 1.20
Aspergillus versicolor	18.83^{b} ± 1.40
Penicillium spp	31.67^{a} ± 1.71
Fusarium spp	14.33^{b} ± 3.01
Rhizopus spp	7.33^{c} ± 1.26
Total	22.81 ±1.47

Mean values with the same superscripts are not significantly different at $p \leq 0.05$.
SEM= Standard Error Mean
The level of P-value = <0.01

4.5 Percentage moisture content of samples in the six towns of the North Western Nigeria

Fig 4.1 indicates the percentage (%) moisture content analyses of the six towns under studied. The occurrences of number of samples within each moisture content range are described below:

Herbal preparations obtained from Kastina and Gusau that had moisture content range between 1.0-5.09% were 1 and 2 samples respective. Remarkable number of samples was observed at a moisture content range between 5.1-10.09%: Kano had the highest with 44 samples, Sokoto 33, Birnin Kebbi 25, Kastina had 23, while Kaduna and Gusau had 19 and 12 respectively.Herbal preparations obtained that shows moisture content range between 10.1-15.09%: Gusau, Birnin Kebbi, Kaduna and Sokoto showed 26 numbers of herbal samples each, while Kano and Kastina had 23 and 18 respectively.

Herbal preparations obtained between the moisture content range of 15.1 -20.09%: Kano 22, Gusau 20, Kastina 14, Sokoto11 while Birni Kebbi andKano 8 and 7 respectively.Herbal preparations obtained between the moisture content range of 20.1-25.09%: Gusau, Katsina and Birnin Kebbi had 8, 10 and 12 respectively, while Kaduna and sokoto had 5 and 2 respectively. There was no such in Kano.

Herbal preparations obtained between the moisture content ranges of 25.1-30.09%: Gusau and Katsina showed 4 samples each while Birnin Kebbi had only 1. There was no reading from the other towns. Herbal preparations obtained between the moisture content ranges of 30.1-35.09%: Gusau and Kastina showed 1 sample each. There was no reading from the other towns.

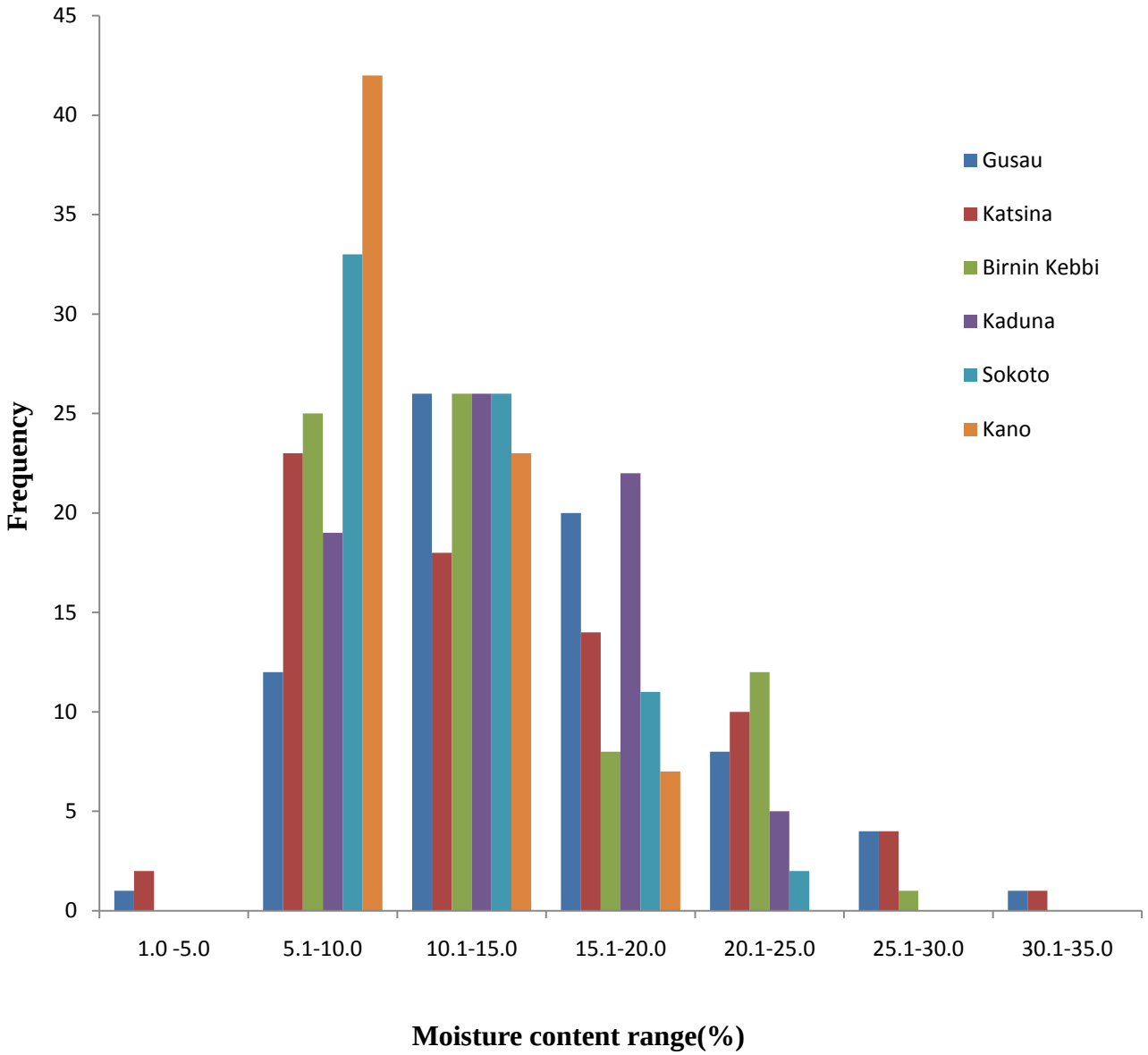


Fig 4.1: Moisture content analysis of samples in North Western-Nigeria.

4.6 Number and percentages (%) of samples within NAFDAC stipulated moisture content limit (8%).

The result in Table 4.5 is the summary of all the moisture content of the herbal preparations analyzed. It shows that those obtained from Gusau, Katsina, Birnin Kebbi, Kaduna, Sokoto and Kano had 66(91.67%), 60(83.33%), 59(81.06), 68(94.44%), 60(83.33) and 58(80.56%) respectively above the 8% maximum moisture content as stipulated by NAFDAC. It is also observed that 6(8.33%), 12(16.67%), 13(18.06), 4(5.56%), 12(16.67%), 14(19.44%) of the herbal preparations in Gusau, Katsina, Kebbi, Kaduna, Sokoto and Kano respectively had moisture contents less than or equal to 8%. In totality, 371(85.88%) of the samples in the six states of the North Western Nigeria had their moisture content above the 8% stated limit by NAFDAC, while only 61(14.12%) samples had their moisture content within the stated limit.

4.7 pH values of the samples in the North Western Nigeria.

Fig 4.2 indicates thepH values of the samples determined. The result shows that at pH range of 1.0-2.1, there were no samples observed but it showed that 4(0.93%), 17(3.94%) and 34(7.87%) of the herbal preparations were within the pH values of 2.1-3.0, 3.1-4.0 and 4.1-5.0 respectively, while 107(24.77%), 85(19.68%) and 79(18.29%) of herbal preparations were within the pH value ranges of 5.1-6.0, 6.1-7.0 and 7.1-8.0 respectively. At the pH ranges of 8.1-9.0, 9.1-10.0 and 10.1-11.0, the number of samples that showed reading were 62(14.35%),50(11.57%) and 3(0.69%) respectively. Only one sample was within the pH range of 11.1-12.0. No samples had pH values between 12.1-13.0 and 13.1-14.0.

Table 4.5: Number and percentages (%) of the sampleswithin NAFDAC stipulated moisture content limit (8%).

Towns	Percentage (%) moisture content limit	
	> 8	≤ 8
Gusau town	66 (91.67)	6 (8.33)
Katsina town	60 (83.33)	12 (16.67)
Birnin Kebbi town	59 (81.94)	13 (18.06)
Kaduna town	68 (94.44)	4 (5.56)
Sokoto town	60 (83.33)	12 (16.67)
Kano town	58 (80.56)	14 (19.44)
Total	371(85.88)	61(14.12)

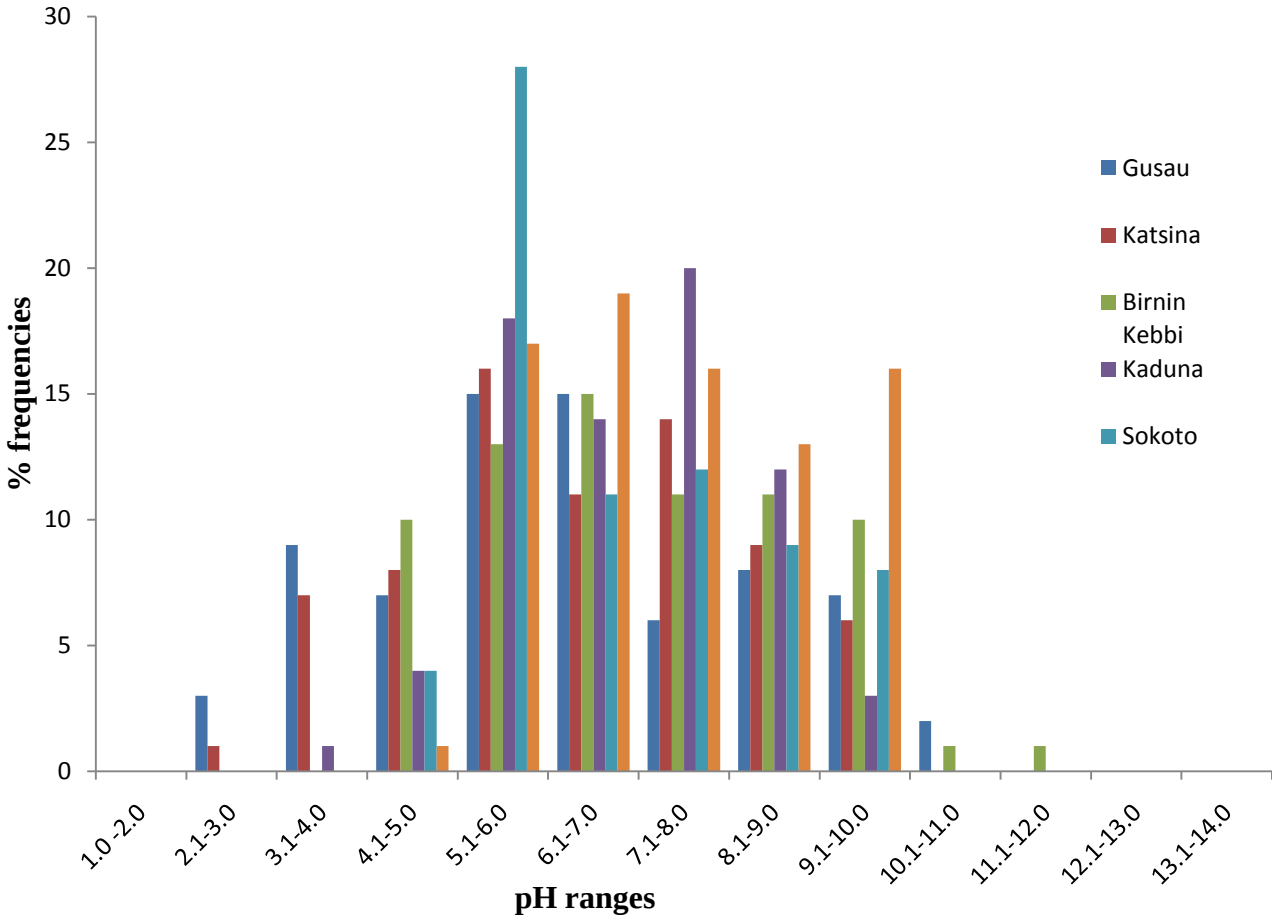


Figure 4.2: pH values of the samples in the North Western -Nigeria.

4.8 Ranges of average fungal count, total aflatoxin, moisture contents and pH values of samples in six towns of the North Western Nigeria.

Table 4.6 shows the ranges of average fungal count, total aflatoxin, moisture content and pH analyzed in six towns. The average fungal count and total aflatoxin in this study ranged from $1.0x10^4$cfu/g - $2.15x10^5$cfu/g and 1.6ppb - >20ppb respectively. The highest average fungal count was observed in sample from Kano with $2.15x10^5$cfu/g, and the lowest fungal count was in Gusau with $1.0x10^4$cfu/g. The lowest range of 1.6ppb of total aflatoxin was observed in Sokoto and Kano, whiles the highest range of 20ppb and above was observed in all the six towns of the North Western Nigeria.

The pH value ranged from 2.42-11.02. The sample with the highest pH value observed in Birnin Kebbi (11.02), while the lowest was observed in Gusau.

4.9 Mean of moisture content, pH and Fungal count in six towns of theNorth West of Nigeria

Table 4.7 shows the means of moisture content, pH and fungal counts/g of herbal medicinal preparations analyzed in six towns. The result showed that the means with the same superscripts were not significantly different ($P \leq 0.05$), while those mean values with different superscripts were significantly different ($P \geq 0.05$). Samples from Kaduna and Birnin Kebbi were observed to havea very high fungal counts that were not significantly different ($P \leq 0.05$). The least fungal load was observed in Gusau. The statistic showed that fungal load in Katsina, Sokoto, Kano and Gusauwere not the same but not significantly different ($P \leq 0.05$). This suggested low level of contamination with fungi when compared with samples from Kaduna and Birnin Kebbi.

The mean values of pH of the herbal preparations from the six towns are illustrated in Table 4.5. Samples from Kano had the highest mean pH value followed by samples from Birnin Kebbi, Kaduna, Sokoto, and Katsina while those from Gusau had the

Table 4.6: Ranges of Average fungal count, total aflatoxin, moisture contents and pHvalues of six towns of the North Western-Nigeria.

Locations	No. of samples	Parameters			
		Average fungal count(cfu/g)	Aflatoxins conc.(ppb)	Moisture content (%)	pH
Gusau town	72	$1.0x10^4$-$1.85x10^5$	3.3 - >20	5.3-26.21	2.42 -10.11
Kastina town	72	$2.0x10^4$-$2.10x10^5$	4.4 - >20	3.75-31.47	2.95-9.78
Birnin Kebbi town	72	$2.0x10^4$-$1.95x10^5$	1.9 - >20	5.78-28.02	4.07-11.02
Kaduna town	72	$2.0x10^4$-$1.70x10^5$	2.1 - >20	5.34-23.80	3.94-9.21
Sokoto town	72	$4.5x10^4$ -$1.25x10^5$	1.6 - >20	7.04-22.09	5.05-9.83
Kano town	72	$2.5x10^4$-$2.15x10^5$	1.6 - >20	5.89-19.38	4.90-9.83

Table 4.7: Mean of moisture content, pH and fungal count in six towns of theNorth West of Nigeria

Locations	Mean ± SEM (p-value)		
	Moisture content	**pH**	**Fungal counts**
Gusau	15.04^{a} ±0.64(0.97)	6.19^{c} ± 0.24(0.90)	$7.8 \times 10^{4\,b}$ ± 5.7(0.97)
Katsina	14.33^{ab} ± 0.79(0.37)	6.39^{bc} ±0.21(0.87)	$8.6 \times 10^{4\,b}$ ± 5.2 (0.19)
Birnin Kebbi	13.21^{b} ± 0.67(0.39)	6.99^{a} ± 0.20(0.83)	$10.9 \times 10^{4\,a}$ ± 8.7(0.36)
Kaduna	13.63^{ab} ± 0.51(0.02)	6.81^{ab} ± 0.15(0.29)	$10.6 \times 10^{4\,a}$ ± 3.9(0.48)
Sokoto	11.48^{c} ± 0.44(0.75)	6.79^{ab} ± 0.17(0.82)	$8.3 \times 10^{4\,b}$ ± 2.6(0.62)
Kano	10.65^{c} ± 0.38(0.83)	7.14^{a} ± 0.15(0.38)	$8.3 \times 10^{4\,b}$ ± 4.1(0.57)
Total	13.06 ± 0.25	6.72 ± 0.08	7.8×10^{4} ± 2.3

Mean values in the same column with same superscripts are significantly the same at $p \leq 0.05$.

lowest pH values. Statistically, the mean values of pH from Kano and Birnin Kebbi were not significantly different (P<0.05), likewise Kaduna and Sokoto. Samples from Katsina and Gusau showed the lowest mean pH value, even though they are significantly different (P<0.05).The mean moisture content observed indicated that samples from Gusau had the highest followed by those from Birnin Kebbi, Katsina and Kaduna. The least mean moisture content was observed in Sokoto and Kano towns.

4.10. Correlationbetween the physical parameters and the fungal load of samples in six towns of the North western Nigeria.

Table 4.8 shows the correlation between the average fungal count and physical parameters analyzed in each town of the North Western-Nigeria. The Pearson correlation test done indicated a positive correlation between moisture content and fungal load in the entire town as indicated by the value of the correlation coefficient(r). The 2-tailed Pearson correlation test between fungal load andpH carried out indicated correlations in all the towns. In Kaduna, Gusau and Kano the correlations were negative, while in the other towns there was positive correlation between the pH and the fungal load.

4.11. Characteristics of aflatoxigenic isolates in herbal medicinal preparations

The result in Table 4.9 shows certain characteristics displayed in Neutral Red Desiccated Coconut Agar (NRDCA) by *Aspergillus* spp that are suspected to be aflatoxigenic. *Aspergillus flavus* and *Aspergillus parasiticus* showed the aflatoxigenic properties by showing a unique characteristic different from non-toxigenic ones. In the medium, *Aspergillus flavus* and *Aspergillus parasiticus* were observed to displayed blue wish color even though the intensity varies, but the background under UV light, the pick time, duration and diameters of the florescent were observed to be the same. As for the *Aspergillus niger, Aspergillus ochraceus* and *Aspergillus versicolor*, there were no characteristic colour change observed on the medium.

Table 4.8: Correlation between the physical parameters and the fungal load of samples in six towns of the North Western-Nigeria.

Towns	Moisture and fungal load		pH and fungal load	
	r value	Level of sig	r value	Level of sig
Gusau	0.669	0.000	-0.056	0.643
Katsina	0.670	0.000	0.183	0.124
Birnin Kebbi	0.752	0.000	0.239	0.043
Kaduna	0.21	0.858	-0.130	0.276
Sokoto	0.804	0.000	0.094	0.434
Kano	0.609	0.000	-0.003	0.982

Correlation coefficient (r) value range between $-1 \leq r \leq 1$
There is significant correlation in all the states at p=0.05 level (2-tailed)

Table 4.9: Characteristics of aflatoxigenic isolates in the samples

Characteristic	*Aspergillus flavus*	*Aspergillus parasiticus*	*Aspergillus niger*	*Aspergillusversicolor*	*Aspergillusochraceus*
Fluorescent colour	Bluewish	Bluewish	No change	No change	No change
Intensity	++	++	-	-	-
Background under UV	pinkish	pinkish	No change	No change	No change
Pick time of fluorescent(h)	72	72	-	-	-
Fluorescent diameter^2(cm)	0.8	0.8	-	-	-
Fluorescent duration(days)	1-5	1-5	-	-	-
Media type	Opaque	Opaque	Opaque	Opaque	Opaque

Intensity of fluorescence under UV light: +++(bright); ++ (Moderate).
- (No intensity or positive result).

4.12. Profile of toxigenic and non-toxigenic *Aspergillus species* in the samples.

Table 4.10 indicates the number and percentages of aflatoxigenic fungi screened. Out of 181 *Aspergillusflavus*screened, seven (3.87%) were aflatoxigenic, while out of 193 *Aspergillus parasiticus* tested for their aflatoxigenic properties, only three (1.55%) were aflatoxigenic.

4.13. Ranges of total aflatoxin in herbal medicines obtained from six towns of the North Western-Nigeria

Table 4.11 indicates the ranges of total aflatoxin in the herbal samples in all the six towns of North Western-Nigeria. Considering the lower range, the highest range of 4.4ppb was observed in Gusau while the lowest 1.6ppb was in Sokoto and Kano metropolis respectively. Greater than the detectable limit of 20ppb as specified by the ELISA kits (AqraQuant total aflatoxin kit) used were observed in all the six towns.

Table 4.10: Profile of toxigenic and non-toxigenic *Aspergillus species* in the samples.

Aspergillus species	No of species Tested	Number and percentage of aflatoxigenic
Aspergillus flavus	181	7(3.87)
Aspergillus parasiticus	193	3(1.55)
Aspergillus versicolor	113	-
Aspergillus niger	181	-
Aspergillus ochraceus	107	-

(aflatoxigenic fungi not detected)-

Table 4.11: Ranges of total aflatoxin in herbal medicines from six town of the North Western-Nigeria.

Towns	No of samples (n)	Ranges Aflatoxins concentrations (ppb)
Gusau	72	3.3 - > 20
Katsina	72	4.4- > 20
BirninKebbi	72	1.9- > 20
Kaduna	72	2.1- > 20
Sokoto	72	1.6- > 20
Kano	72	1.6- > 20

PPb= part per billion

4.14. Number and percentage (%) of the samples within the acceptable limitof 10ppb of total aflatoxin in the six towns of the North Western-Nigeria

Table 4.12 illustrates the number of samples that were positive for aflatoxin and were within the acceptable limit of 10 ppbof total aflatoxins. In each town, a total of seventy two (72) samples were analyzed, and all the samples were positive for aflatoxin. Gusau had the highest positive samples (n=46) 63.89% and were within the acceptable limit of 10ppb of total aflatoxin, while samples from Kastina indicated the lowest (n=7) 9.79%. The number and percentage of herbal preparations from Sokoto, Kano, Birnin Kebbi and Kaduna which were positive for aflatoxin were (n=35)48.61%, (n=27)37.50%, (n=25)34.75% and (n=24)33.33% respectively and were within the acceptable limit of 10ppb of total aflatoxin. The number of samples obtained above the acceptable limit in Kastina, Kaduna, Birnin Kebbi, Kano, Sokoto and Gusau were (n=65)90.28%, (n=48)66.67%, (n=47)65.28%, (n=45)62.50%, (n=37)51.39% and (n=26)36.11% respectively.

4.15. Mean of total aflatoxin concentration (ppb) of samples in six towns of the NorthWestern-Nigeria.

Table 4.13 shows the differences in mean of total aflatoxin of herbal preparations in the sampling areas. The result indicated significant differences ($p<0.001$) of total aflatoxin in the sampling areas. Samples obtained from Katsina had mean of 18.73ppb, while samples from Kaduna and Birnin Kebbi showed mean of 14.47 and 13.21ppb respectively. There were no significant different ($p<0.05$) between Kano and Sokoto with 12.43 and 11.53ppb respectively. Samples from Gusau showed the lowest mean of 9.40ppb of aflatoxin.

Table 4.12: Number and percentage (%) of the samples within the acceptable limitof 10ppb of total aflatoxin in the six towns of the North Western-Nigeria

Towns	Samples tested	Positive samples	Number (%) of herbal preparations within a specified total aflatoxins limit	
			≤10ppb (acceptable limit)	>10ppb acceptable limit
Sokoto	72	72	35 (48.61)	37 (51.39)
Kaduna	72	72	24 (33.33)	48 (66.67)
Kano	72	72	27 (37.50)	45 (62.50)
Gusau	72	72	46 (63.89)	26 (36.11)
Birnin Kebbi	72	72	25 (34.72)	47 (65.28)
Katsina	72	72	7 (9.72)	65 (90.28)

Acceptable limit of total aflatoxin=10ppb (Codex Alimentarius Commission of FAO/ WHO

Table 4.13: Mean of total aflatoxin concentration (ppb) of samples in six towns of the North Western-Nigeria.

Towns	Mean aflatoxin conc (ppb) ± SEM
Kano	$12.426^{c} \pm 0.69$
Kaduna	$14.467^{b} \pm 0.80$
Katsina	$18.726^{a} \pm 0.51$
Birnin Kebbi	$13.207^{bc} \pm 0.75$
Sokoto	$11.526^{c} \pm 0.59$
Gusau	$9.403^{d} \pm 0.66$

Mean values with different superscripts are significantly different $p < 0.001$.

4.16. Mean of Average fungal count of samples obtained from different locations in Kaduna town in dry and wet seasons.

Fig. 4.3 illustrates the meanaverage fungal count of samples from different locations in Kaduna town during both wet and dry seasons. Themean observed in samples obtained during wet season were as follows: Mando garage ($1.35x10^5$cfu/g); U/Rimi market ($1.34x10^5$cfu/g); Malali market ($1.32x10^5$cfu/g); Kasuwar Barci ($1.28x10^5$cfu/g); Kawo motor park ($1.25x10^5$cfu/g), and Central market which had the lowest fungal count of $9.67x10^4$cfu/g. In the dry season, samples from Kawo motor park had the highest mean fungal count of $1.02x10^5$cfu/gand the lowest count was observed in Kasuwar Barci ($7.00x10^4$cfu/g). The overall result of the fungal count of sample in Kaduna indicated mean of 1.2x105cfu/g during wet season which was the highest when compared with $8.8x10^4$cfu/gduring the dry season.

4.17. Mean of total aflatoxin of samples obtained from different locations in Kaduna town in dry and wet season.

Fig. 4.4 shows the mean of total aflatoxin of samples from different locations in Kaduna town during the wet and dry seasons. The highest mean aflatoxin was observed in samples obtained during the wet season with samples from Mando garage having the highest mean of 21ppb followed by those obtained from Kawo motor park (18.92ppb), others were: Central market(18.52ppb); Malali market (16.40ppb); U/Rimi(17.80ppb), and Kasuwar Barci (13.08ppb) indicating the lowest mean aflatoxin level. In the dry season the highest mean aflatoxin of 12.89ppb was observed in U/Rimi followed by those obtained from Kasuwar Barci(12.75ppb); Kawo motor park(11.97ppb); Mando garage(11.63ppb); Central market(10.05ppb), the lowest aflatoxin concentration of 8.60ppb was observed in Malali market. The overall result in Kaduna showed highest mean aflatoxin of 17.62ppb during the wet season compared with 11.32ppb during the dry season.

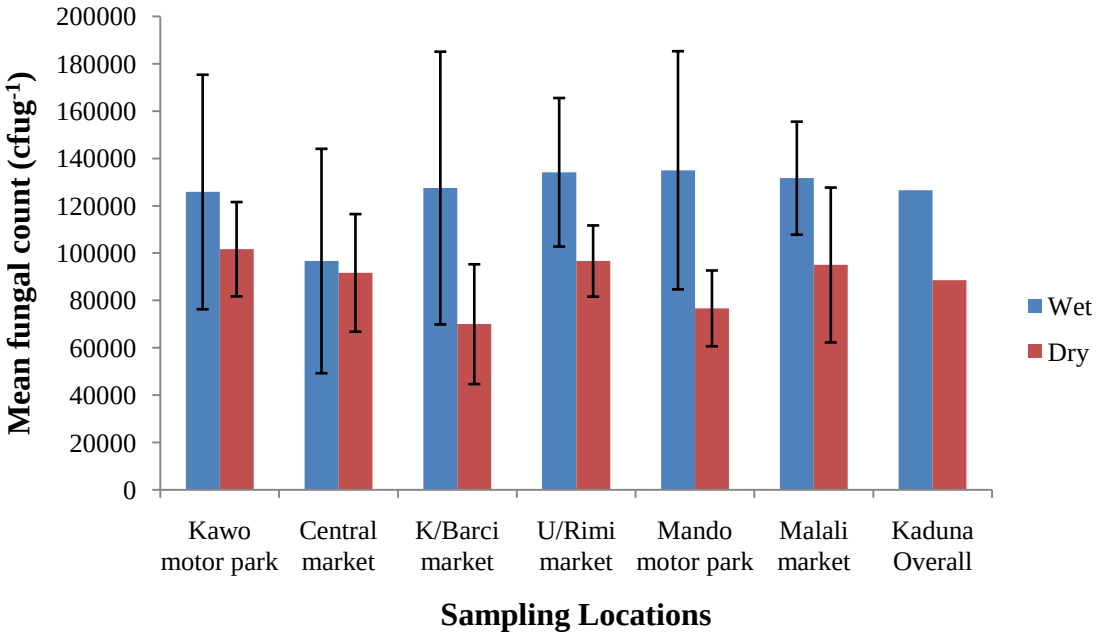


Figure 4.3: Mean of Average fungal count of samples obtained from different locations in Kaduna town in dry and wet seasons at $p \leq 0.05$

I = Error bar explain the strength of differences between the locations.
Level of significant different, P = <0.01

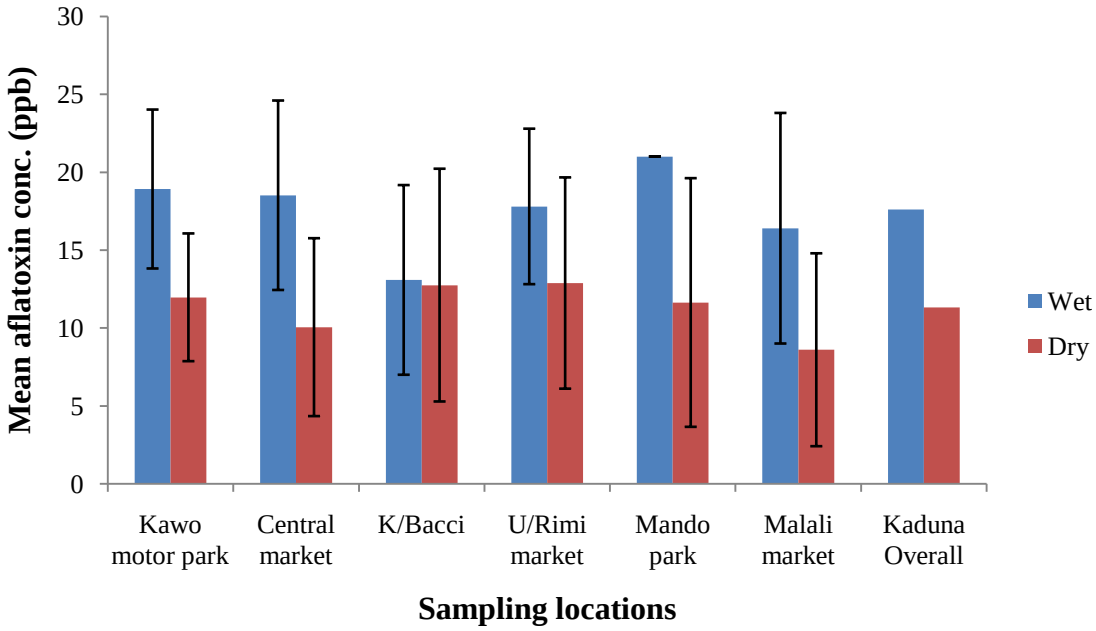


Figure 4.4: Mean of total aflatoxin of samples obtained from different locations in Kaduna town in dry and wet seasons at P≤0.05

I = Error bar explain the strength of differences between the locations.
Level of significant different, P = <0.01

4.18. Mean of average fungal count of samples obtained from different locations in Katsina town in wet and dry seasons.

Fig. 4.5 illustrates the mean of average fungal count of samples from different locations in Katsina town during the wet and the dry seasons. The highest mean fungal count was observed in samples obtained during wet season were as follows: Central motor ($1.38x10^5$cfu/g); Mangal garage ($1.21x10^5$cfu/g); Central market ($1.18x10^5$cfu/g); Katsina State Transport Service (KTSTS)($1.13x10^5$cfu/g); 'Yar Kutungu market($9.17x10^4$cfu/g), and Chake market($8.17x10^4$cfu/g) indicating the lowest fungal count. In the dry season, samples from Mangal garage had the highest mean fungal count of 7.42x104cfu/g followed by KTSTS ($7.08x10^4$cfu/g); and the lowest count was observed in Chakemarket and Central market each with $5.17x10^4$cfu/g. In the overall, Katsina indicated highest mean fungal count of 1.1x105cfu/g during wet season compared with $6.2x10^4$cfu/gduring dry season.

4.19. Mean of total aflatoxin of samples obtained from different locations in Katsina town in wet and dry seasons.

Fig. 4.6 shows total mean aflatoxin levelof samples from different locations in Kastina town during the wet and the dry seasons. The highest mean aflatoxin was observed in samples obtained during wet season with samples from Central Motor Park and Central market having the highest mean aflatoxin level of 21.00ppb each. Other areas: 'Yar Kutungu market (20.58ppb); KTSTS (20.30ppb); Chake market (19.05ppb), and Mangal garage (19.07ppb) indicating the lowest aflatoxin mean. In the dry season, the highest mean aflatoxin value of 21.00ppb was observed in Central Motor Park and the lowest mean aflatoxin of 14.67ppb was observed in Chake market. The overall result in Katsina showed highest mean aflatoxin value of 20.17ppb during the wet season compared with 17.63ppb during the dry season.

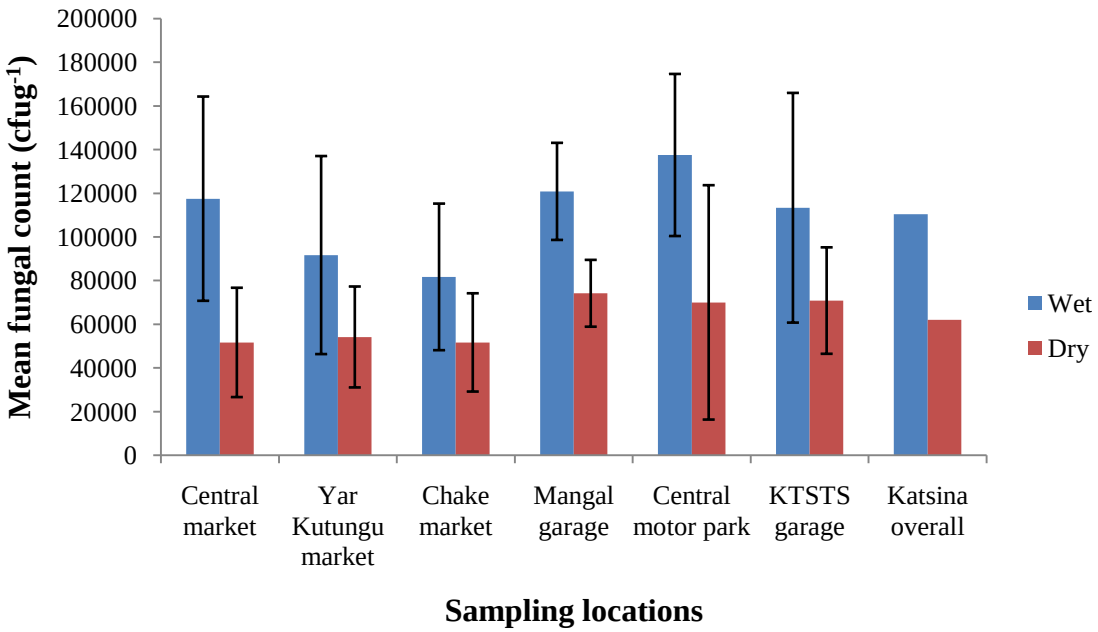


Figure 4.5: Mean of average fungal count of samples obtained from different locations in Katsina town in wet and dry seasons at p≤0.05.

I = Error bar explain the strength of differences between the locations.
Level of significant different, P = <0.01

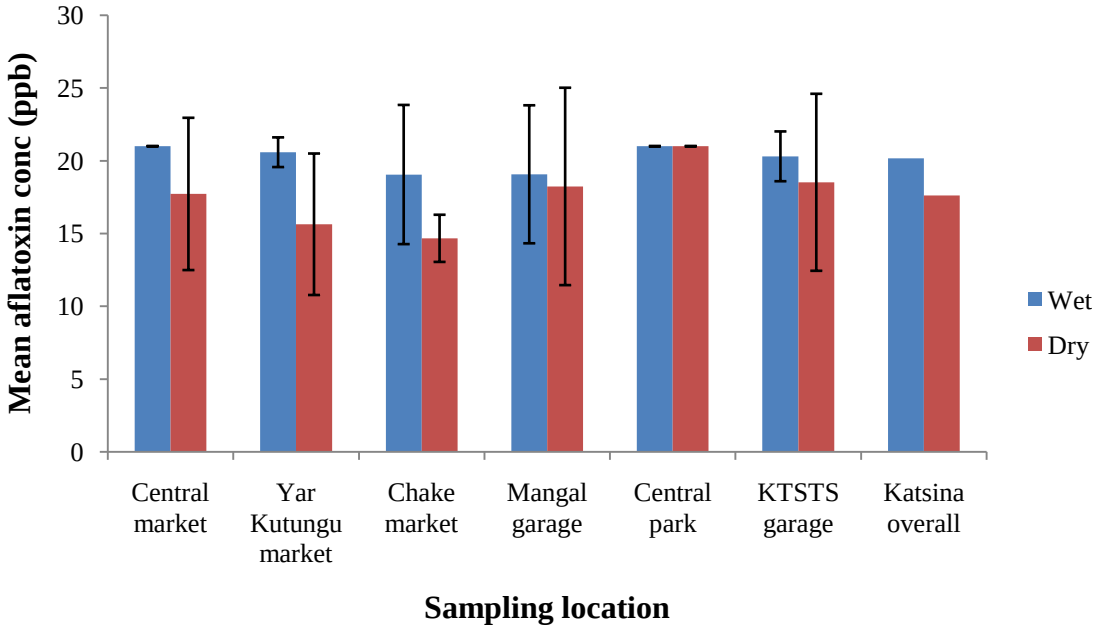


Figure 4.6: Mean of total aflatoxin of samples obtained from different locations in Katsina town in wet and dry seasons at p≤0.05.

I = Error bar explain the strength of differences between the locations.
Level of significant different, P = <0.01

4.20. Mean of average fungal count of samples obtained from different locations in Kano town in wet and dry seasons.

Fig. 4.7 shows the mean of average fungal countof samples from different locations in Kano town during the wet and the dry seasons. The highest mean fungal count was observed in samples obtained during wet season. Samples from Rimi market had the highest mean fungal count of $2.27x10^5$cfu/gfollowed by others: Na'ibawa($1.33x10^5$cfu/g); 'Yankaba market ($1.10x10^5$cfu/g); Anguwa Uku garage($8.42x10^4$cfu/g); Airport road ($8.08x10^4$cfu/g), and Sabongari market($6.83x10^4$cfu/g) having the lowest mean fungal count. In the dry season, samples from Airport road had the highest mean fungal count of $1.70x10^5$cfu/gfollowed by Rimi market($7.75x10^4$cfu/g); Na'ibawa ($7.58x10^4$cfu/g); Anguwa Uku garage($7.08x10^4$cfu/g); 'Yankaba market($6.50x10^4$cfu/g), and the lowest count of $6.26x10^4$cfu/g was observed in Sabongari market. The overall result in Kano indicated highest fungal count of 1.2x105cfu/g during wet season compared with $8.7x10^4$cfu/gduring dry season.

4.21. Mean of total aflatoxin of samples obtained from different locations in Kano town in wet and dry seasons.

Fig 4.8 shows total aflatoxin mean difference of samples of herbal medicinal preparations from different locations in Kano town during both wet and dry seasons. The highest aflatoxin mean difference was observed in samples obtained during wet season with samples from S/gari market had the highest mean of 18.13ppb followed by Airport road (16.50ppb);Na'ibawa market(15.25ppb); A/Uku garage(14.38ppb);'Yankaba market(14.37ppb), and Rimi market (13.53ppb) indicating the lowest aflatoxin mean. In dry season the highest aflatoxin mean of 12.68ppb was observed in Airport road and the lowest aflatoxin of 6.02ppb was observed in Rimi market. The overall result in Kano showed highest mean aflatoxin of 15.36ppb in the wet season compared with 9.42ppbin the dry season.

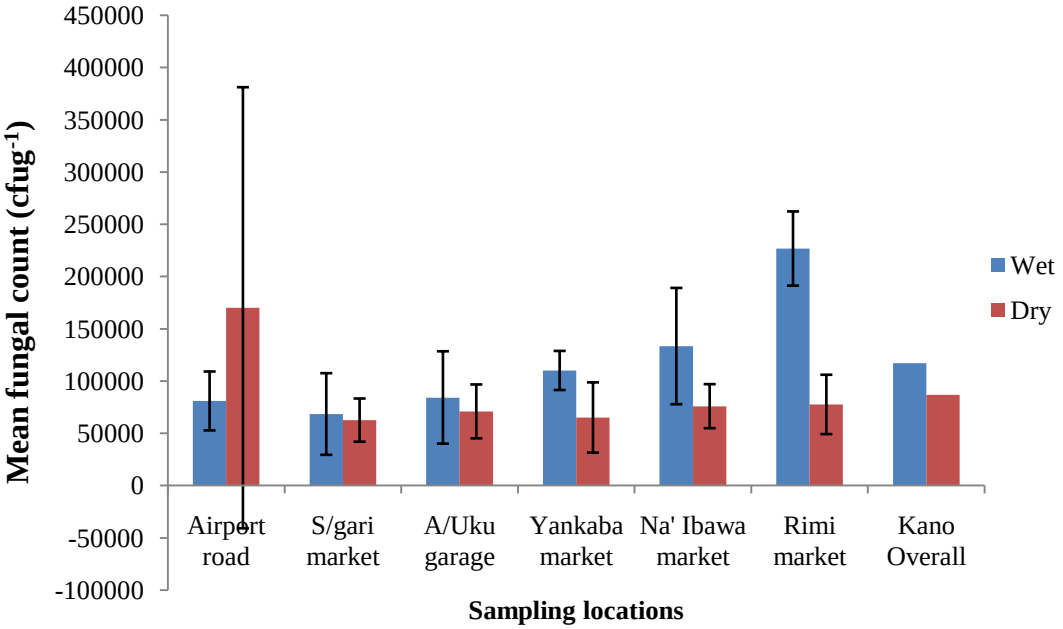


Figure 4.7: Mean of average fungal count of samples obtained from different locations in Kano town in wet and dry seasons at p≤0.05

I = Error bar explain the strength of differences between the locations.
Level of significant different, P = 0.30

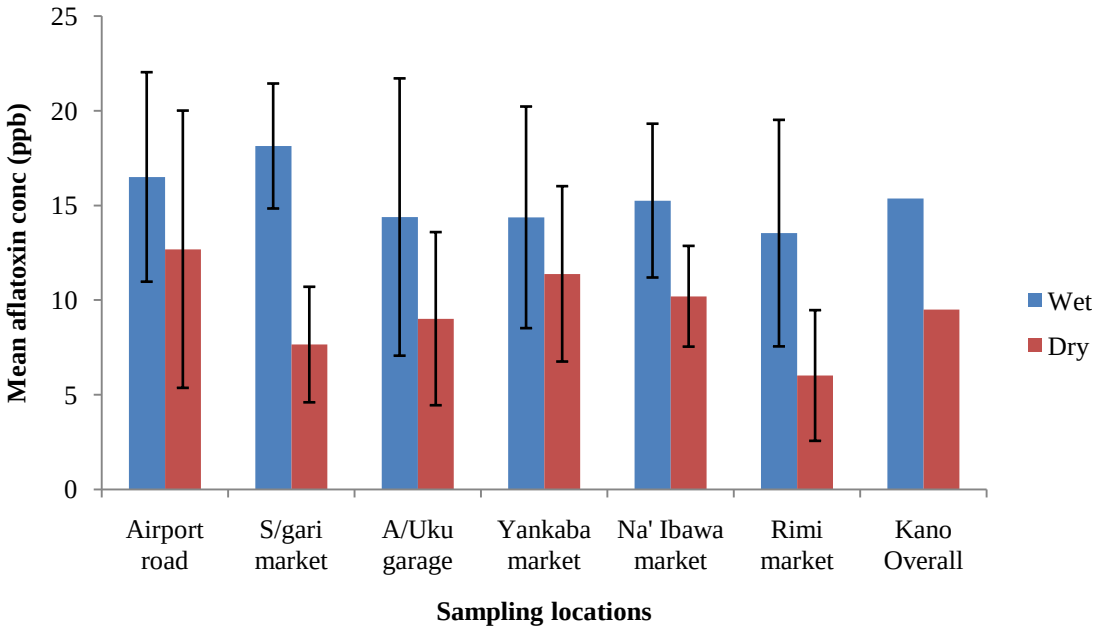


Figure 4.8: Mean of total aflatoxin of samples obtained from different locations in Kano towns in wet and dry seasons at p≤0.05.

I = Error bar explain the strength of differences between the locations.
Level of significant different, P = <0.01

4.22. Mean of average fungal count of samples obtained from different locations in Birnin Kebbi towns in wet and dry seasons.

Fig. 4.9 illustrates the mean average fungal count of samples from different locations in Birnin Kebbi town during the wet and the dry seasons. The highest mean was observed in samples obtained during the wet season. Samples from Argungun Motor Park had the highest fungal mean of $1.85x10^5$cfu/gfollowed by Central market ($1.40x10^5$cfu/g); Jega Motor Park ($1.36x10^5$cfu/g); Old market ($1.29x10^5$cfu/g); Central Motor Park ($9.17x10^4$cfu/g). Samples from Ambursa market had the lowest mean ($8.58x10^4$cfu/g). In the dry season, samples from Old market had the highest mean fungal count of $1.13x10^5$cfu/g followed by those obtained from Argungun Motor Park ($9.0x10^4$cfu/g); Jega motor park ($8.5x10^4$cfu/g); Central market ($8.42x10^4$cfu/g); Ambursa market ($7.83x10^4$cfu/g), and the lowest count of $7.42x10^4$cfu/g was observed in Central motor market. The overall result in B/Kebbi indicated highest mean fungal count of 4.1x105cfu/g during wet season compared with $8.7x10^4$cfu/gduring dry season.

4.23. Mean of total aflatoxin of samples obtained from different locations in Birnin Kebbi town in wet and dry seasons.

Fig. 4.10 shows the total means aflatoxin of samples from different locations in Birnin Kebbi town during the wet and the dry seasons. The highest mean aflatoxin level was observed in samples obtained during wet season with samples from Central motor park haaving the highest mean aflatoxin value of 50.47ppb followed by those obtained from Jega Motor Park (19.30ppb); Ambursa market (16.90ppb); Old market (15.27ppb); Central market (14.83ppb), and Argungun Motor Park (9.70ppb) indicating the lowest mean aflatoxin concentration. In the dry season, the highest mean aflatoxin of 15.78ppb was observed in Central Motor Park and the lowest mean aflatoxin level of 6.58ppb was observed in old market. The overall result in Birnin Kebbi showed highest mean aflatoxin concentration of 15.77ppb during wet season compared with 10.64ppb during dry season.

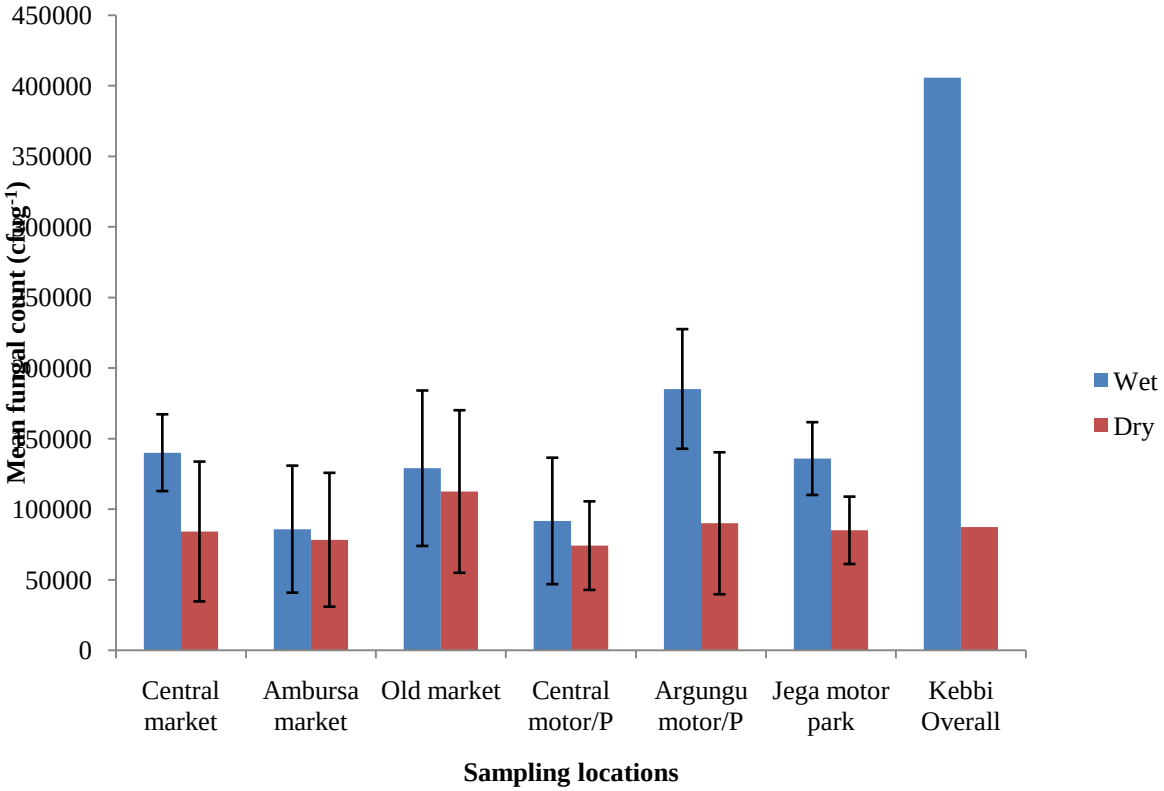


Figure 4.9: Mean of average fungal count of samples obtained fromdifferent locations in Birnin Kebbi town in wet and dry seasons at p≤0.05

I = Error bar explain the strength of differences between the locations.
Level of significant different, P = 0.27

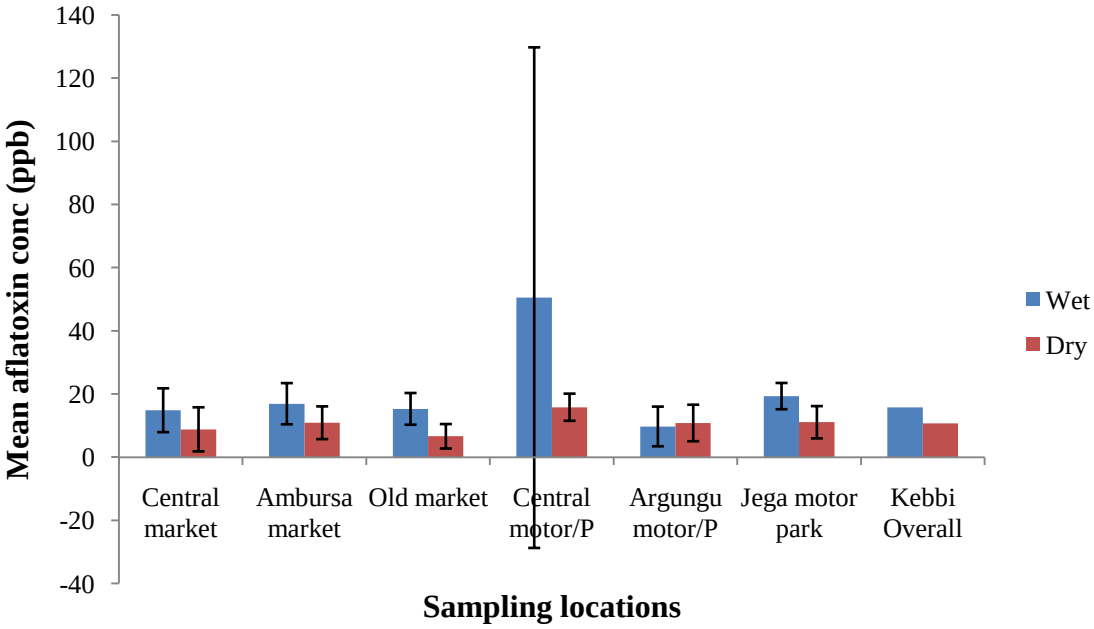


Figure 4.10: Mean of total aflatoxin of samples obtained from different locations in Birnin Kebbi town in wet and dry seasons at p≤0.05.

I = Error bar explain the strength of differences between the locations.
Level of significant different, P = <0.01

4.24. Mean of average fungal count of the samples obtained from different locations in Sokoto town wet and dry seasons.

Fig. 4.11 illustrates the mean average fungal countof samples from different locations in Sokoto town during the wet and the dry seasons. The highest mean fungal count was observed in samples obtained during wet season. Samples from Central market had the highest mean fungal value of $1.05x10^5$cfu/gfollowed by those obtained from Tashar Kura($1.01x10^5$cfu/g); Gawon Nama($9.92x10^4$cfu/g); Kara market($8.92x10^4$cfu/g); Old market ($8.67x10^4$cfu/g). Samples from Arkila area had the lowest mean fungal count ($7.51x10^4$cfu/g). In the dry season, samples from Kara market had the highest mean fungal count of $8.16x10^4$cfu/g and the lowest mean fungal count of 6.00x104cfu/g was observed in Gawon Nama. The overall result in Sokoto indicated highest mean fungal count of 9.3x105cfu/g during the wet season compared with $7.0x10^4$cfu/gduring the dry season.

4.25. Mean oftotal aflatoxin of samples obtained from different location in Sokoto in wet and dry seasons.

Fig. 4.12 shows the total mean aflatoxin of samples from different locations in Sokoto metropolis during the wet and the dry seasons. The highest mean aflatoxin was observed in samples obtained during the wet season with samples from Kara market having the highest mean aflatoxin level of 16.93ppb followed by those obtained from Central market (14.90ppb); Gawon nama (14.25ppb); Arkila area (12.85ppb); Old market (12.50ppb), and Tashar Kura (9.72ppb) indicating the lowest mean aflatoxin level. In dry season, the highest mean aflatoxin level of 12.91ppb was observed in Central market the lowest mean aflatoxin level of 6.48ppb was observed in old market. The overall result in Sokoto showed highest mean aflatoxin level of 13.53ppb in the wet season compared with 9.53ppb in the dry season

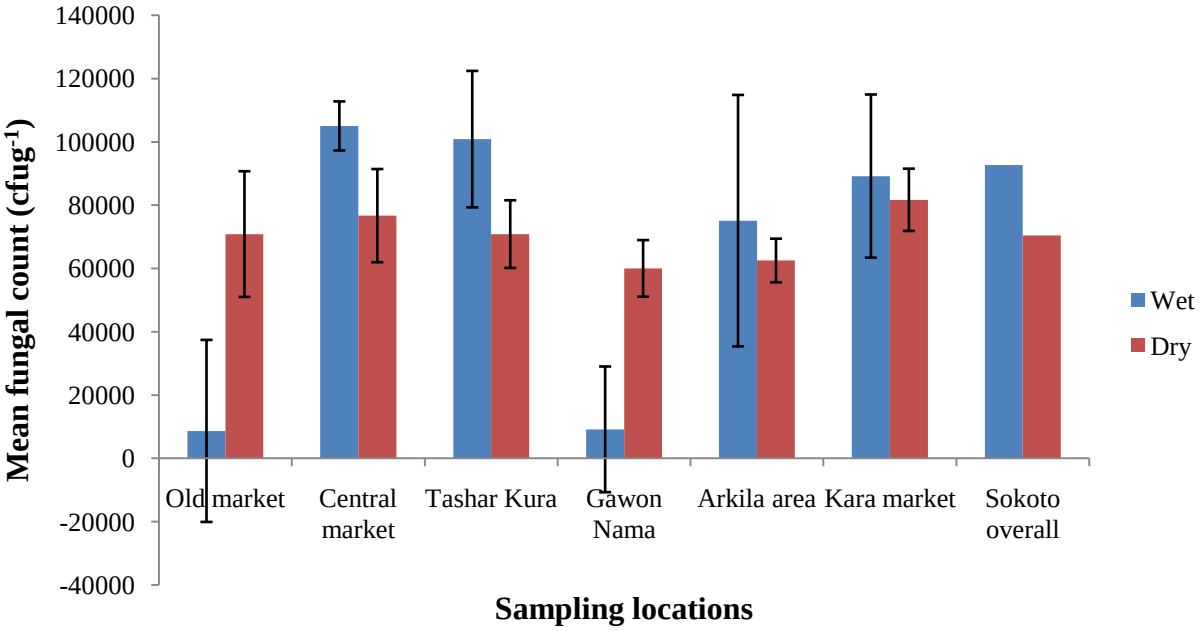


Figure 4.11: Mean of average fungal count of samples obtained from different locations in Sokoto town wet and dry seasons at p≤0.05

I = Error bar explain the strength of differences between the locations.
Level of significant different, P = <0.01

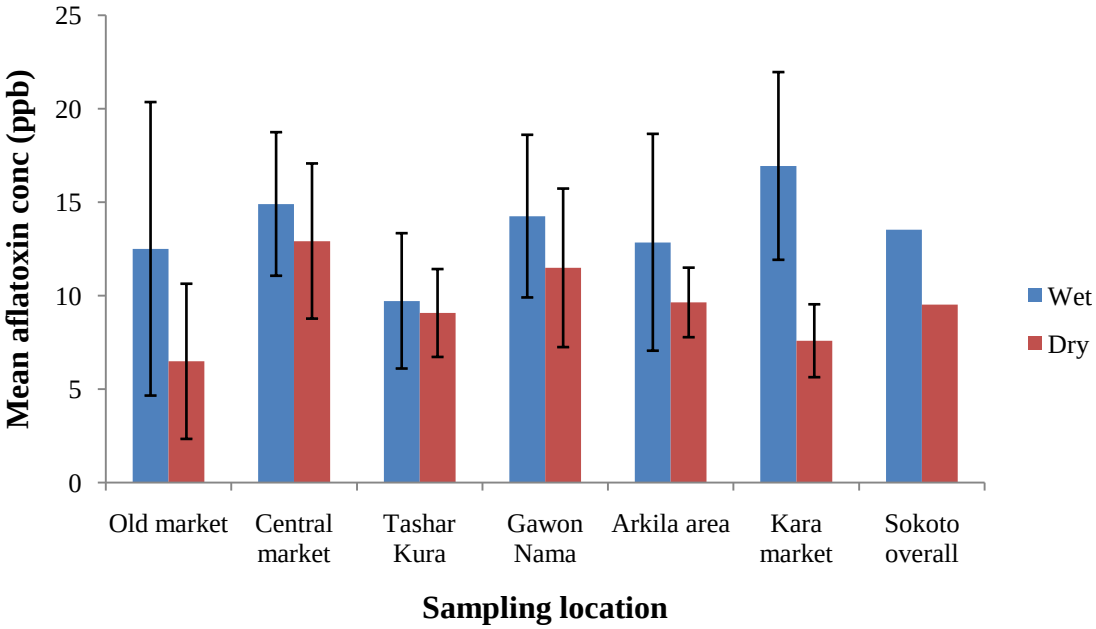


Figure 4.12: Mean oftotal aflatoxin of samples obtained from different location in Sokoto town in wet and dry seasons at p≤0.05.

I = Error bar explain the strength of differences between the locations.
Level of significant different, P = <0.01

4.26. Mean of average fungal count of samples obtained from different locations in Gusau town in wet and dry seasons.

Fig. 4.13 illustrates the mean average fungal count of samples from different locations in Gusau in the wet and the dry seasons. The highest mean fungal count was observed in samples obtained during wet season. Samples obtained from Central market had the highest mean fungal count of 1, 08x105cfu/g followed by those obtained from Old market ($1.00x10^5$cfu/g); Tudun wada ($9.25x10^4$cfu/g); Lalan area ($9.17x10^4$cfu/g); Labilabi ($9.08x10^4$cfu/g). Those obtained from Hospital road had the lowest mean fungal count of $7.50x10^4$cfu/g. In the dry season, samples from Labilabi had the highest mean fungal count of $8.33x10^4$cfu/gfollowed by those obtained from Old market ($7.5x10^4$cfu/g); Lalan area ($7.4x10^4$cfu/g); Hospital road ($6.33x10^4$cfu/g); Central market ($6.08x10^4$cfu/g), and the lowestcount of 3.92x104cfu/g was observed in Tudun wada. The overall result in Gusau indicated highest mean fungal count of $9.29x10^4$cfu/g during the wet season compared with $6.32x10^4$cfu/g during the dry season.

4.27. Mean of total aflatoxin of samples obtained from different locations in Gusau in wet and dry seasons.

Fig. 4.14 shows the total mean aflatoxin of samples from different locations in Gusau town during the wet and the dry seasons. The highest mean aflatoxinwas observed in samples obtained during wet season with samples from Hospital road having the highest mean aflatoxin of 17.03ppb followed by those obtained from Tudun wada(13.27ppb); Labilabi(12.97ppb); Lalan area(11.33ppb); Central market(10.85ppb), and old market (10.65ppb) indicating the lowest mean aflatoxin. In dry season, the highest mean aflatoxin value of 8.17ppb was observed in Hospital road and the lowest mean aflatoxin of 4.57ppb was observed in old market. The overall result in Zamfara showed highest mean aflatoxin value of 12.68ppb during the wet season compared with 6.12ppb during the dry season.

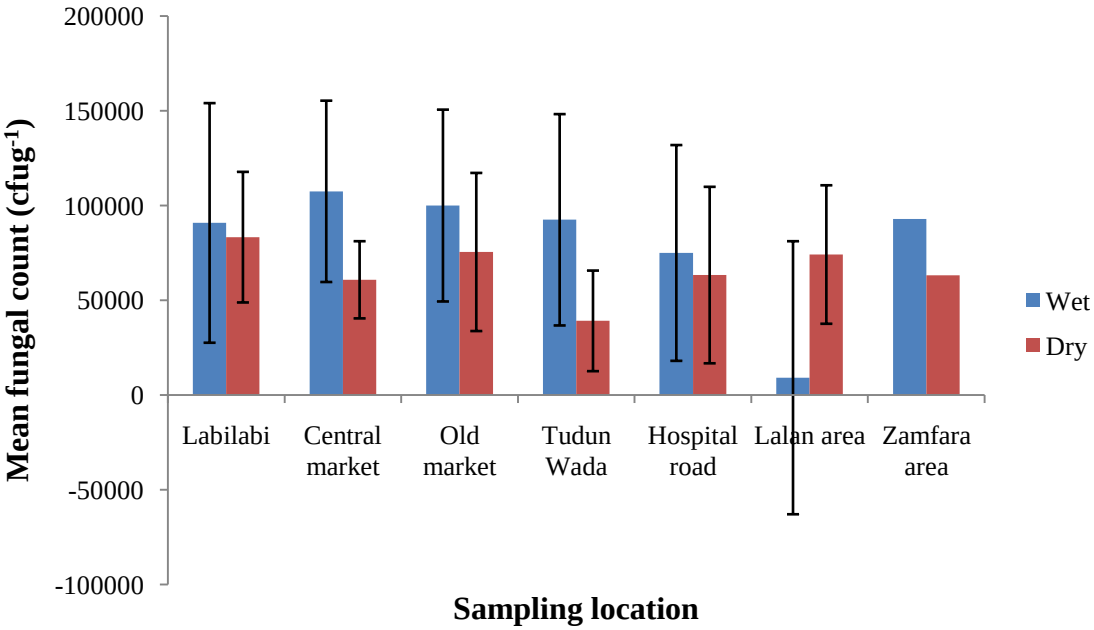


Figure 4.13: Mean of average fungal count of samples obtained from different locations in Gusau town in wet and dry seasons at p≤0.05

I = Error bar explain the strength of differences between the locations.
Level of significant different, P = <0.01

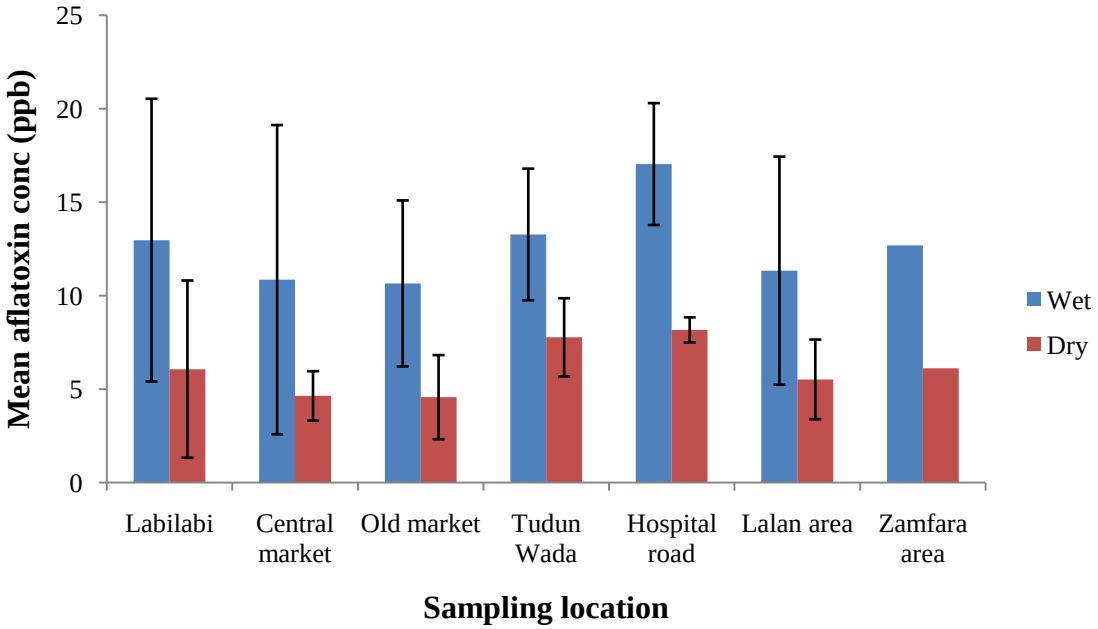


Figure 4.14: Mean of totalaflatoxin of samples obtained from different locations in Gusau town in wet and dry seasons at p≤0.05.

I = Error bar explain the strength of differences between the locations.
Level of significant different, P = <0.01

4.28. Correlation between the total aflatoxin concentration and average fungal load

Table 4.14 shows significant relationship between the total aflatoxin and average fungal loads analyzed in the metropolis of six towns of the North West of Nigeria. The correlation was positive and significant ($p \leq 0.05$; $r = 0.123$).

4.29. Mean of fungal countof samplesobtained from six states of the North Western-Nigeria in wet and dry seasons.

Table 4.15shows the seasonal fungal count of samples in six towns of the North West of Nigeria. The mean fungal count observed during wet season for Sokoto ($9.3x10^5$cfu/g) was the highest followed by those obtained fromBirnin Kebbi ($4.1x10^5$cfu/g); Kano ($1.2x10^5$cfu/g); Kaduna ($1.2x10^5$cfu/g); Katsina ($1.1x10^5$cfu/g). The lowest mean fungal count was observed in samples from Gusau ($9.3x10^4$cfu/g).

In the dry season, the observed result differed, Kaduna had the highest mean fungal count of $8.8x10^4$cfu/g, followed by those obtained from Birnin Kebbi and Kano both with $8.7x10^4$cfu/g; Sokoto ($7.0x10^4$cfu/g), Gusau ($6.3x10^4$cfu/g). The least mean fungal count was observed in Katsina ($6.2x10^4$cfu/g).

4.30. Mean of total aflatoxin of samples obtained from six stown in North Western Nigeria in wet and dry seasons.

Table 4.16 indicates the seasonal mean aflatoxin value of samples in the six town of the North Western-Nigeria. The total mean aflatoxin observed in the wet season for Katsina (20.197ppb) was the highest followed by those obtained from Kaduna(17.619ppb); Kebbi(15.772ppb); Kano(15.361ppb); Sokoto (13.525ppb).The lowest mean aflatoxin value of 12.683ppb was observed in samples from Gusau.

In the dry season, the observed mean of total aflatoxin showed that Kebbi with 10.642ppb had the highest, followed by those obtained from Kaduna (11.314ppb); Kebbi(10.642ppb); Sokoto(9.528ppb); Kano(9.492ppb), and those obtained from Gusau showed the lowest mean of 6.119ppb.

Table 4.14: Correlation between the total aflatoxin concentration and average fungal load

		Aflatoxin Concentration (ppb)	Fungal Load
Aflatoxin Concentration (ppb)	Pearson Correlation	1	0.123*
	Sig. (2-tailed)		.011
	N	432	432
Fungal load	Pearson Correlation	0.123*	1
	Sig. (2-tailed)	.011	
	N	432	432

*. Correlation is significant at the 0.05 level (2-tailed).

N= Total number of samples
Sig= Significancy

Table 4.15: Mean of average fungal countof samples in North Western-Nigeria in wet and dry seasons

States	Mean		p – value
	Wet season(n=72)	**Dry season(n=72)**	
	Average fungal count mean(cfu/g)	Average fungal count mean(cfu/g)	
Kaduna	$1.2x10^{5a}$	$8.8x10^{4\ b}$	<0.001
Katsina	$1.1x10^{5a}$	$6.2x10^{4\ b}$	0.001
Kebbi	$4.1x10^{5a}$	$8.7x10^{4\ a}$	0.274
Kano	$1.2x10^{5a}$	$8.7x10^{4\ a}$	0.301
Sokoto	$9.3x10^{5a}$	$7.0x10^{4\ b}$	<0.001
Zamfara	$9.3x10^{4a}$	$6.3x10^{4\ b}$	0.009
Overall	$3.0\ x10^{5}$	$7.6\ x10^{4a}$	0.095

Means values from the same row with varying superscripts are significantly different at $p \leq 0.05$.

Table 4.16: Mean aflatoxin concentration of samples in North Western-Nigeriain wet and dry season.

Locations	Mean ± SEM		p – value
	Wet season	**Dry season**	
	Aflatoxin (ppb) means	Aflatoxin(ppb) means	
Kaduna	$17.619^{c} \pm 0.96$	$11.314^{d} \pm 1.05$	<0.001
Katsina	$20.167^{c} \pm 0.46$	$17.628^{d} \pm 0.81$	0.001
Kebbi	$15.772^{c} \pm 1.02$	$10.642^{d} \pm 0.94$	0.001
Kano	$15.361^{c} \pm 0.89$	$9.492^{d} \pm 0.80$	0.001
Sokoto	$13.525^{c} \pm 0.90$	$9.528^{d} \pm 0.63$	0.001
Zamfara	$12.683^{c} \pm 0.98$	$6.119^{d} \pm 0.46$	0.001
Overall	15.855±0.40	10.787±0.40	< 0.001

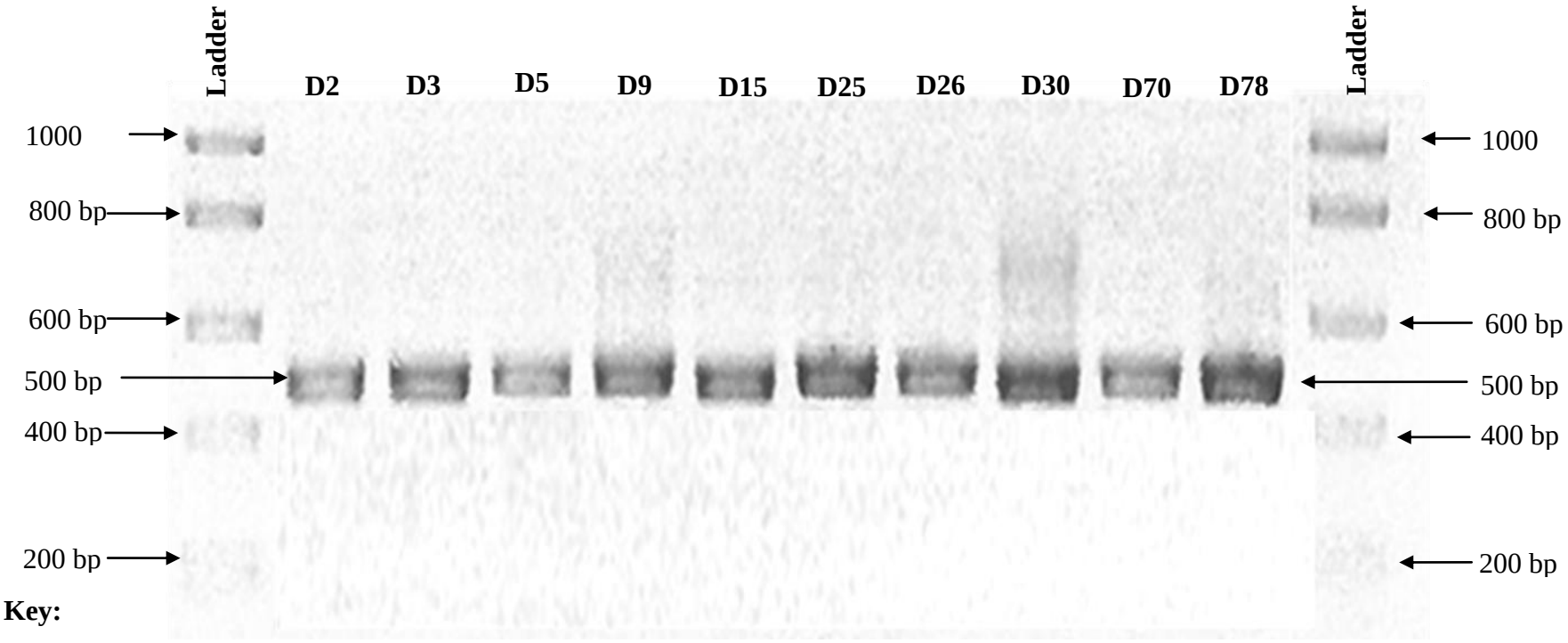
Means values from the same row with varying superscripts are significantly different at $p \leq 0.05$.

4.31 Agarose gel stained with ethidium bromade showing 500pb amplified DNA fragments obtained from aflatoxigenic fungi.

Plate 4.1 shows agarose gel stained with ethidium bromade showing 500pb amplified DNA fragments obtained from aflatoxigenic fungi. Regardless the primers used the PCR amplicon of each tested aflatoxigenic fungishowed similar banding pattern on agarose gel which clearly shows that the fungal species has genes for aflatoxin and subsequently explained their similarity in term of sizes.

4.32. Molecular sequence profile of the target aflatoxigenic fungi screened from the samples.

Table 4.17 indicates the sequence profile of the target fungi screened for their aflatoxigenic properties in herbal medicinal preparations. The sequence of these DNA fragment was observed to be different between species even though the size of the fragments were the same. The result shows the percentage similarities of the target species' genomic DNA with the one in the DNA data bank used during blasting.



Key:

D2, D3, D5, D9, D15, D25, D26, D30, D70, D78= samples showing the same molecular sizes of 500pb.

D2, D3, D5, D9, D15, D25= *Aspergillus flavus*

D26, D30, D70= *Aspergillus parasiticus*

D78 = Standard

Plate 4.1: Amplification products of genes isolated showing rDNA bands of aflatoxigenic fungi (base marker 200b).

Table 4.17: Molecular identification of target fungi isolated from herbal medicinal samples and their rDNA sequences profiles

Sample Codes	rDNA sequences ITS	Species description	Scores (%)	Maximum identity(%)	Accession Number	length
D2	GGTCAACCTGGAAAGATTGTTTTGCGTTCGGCAAGCGCCGGCCGGGCCTACAGAGCGGGTGACAAAGCCCCATACGCTCG AGGATCGGACGCGGTGCCGCCGCTGCCTTTGGGGCCCGTCCCCCCCCGGAGAGGGGACGACGACCCAACACACAAGCCGTG CTTGATGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATG ATTCACGGAATTCTGCAATTCACACTAGTTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTT GAAAGTTTTAACTGATTGCGATACAATCAACTCAGACTTCACTAGATCAGACAGAGTTCGTGGTGTCTCCGGCGGGCGCGG GCCCGGGGCTGAACGCCCCCGGCGGCCATGACGGCGGGCCCGCCGAAGCAACTAAGGTACAGTAAACACGGGTGGGAGG TTGGGCTCGCTAGGAACCCTACACTCGGTAATGATCCTTCCGCAGGTTCACCCTACGGAAG	*Aspergillus flavus*	**795.651**	**99.01**	**IHEM/25132**	**593**
D3	TGATCCGAGGTCAACCTGGAAAGATTGTTTTGCGTTCGGCAAGCGCCGGCCGGGCCTACAGAGCGGGTGACAAAGCCCCA TACGCTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTGGGGCCCGTCCCCCCCCGGAGAGGGGACGACGACCCAACACAC AAGCCGTGCTTGATGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAG ACTCGATGATTCACGGAATTCTGCAATTCACACTAGTTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGAT CCATTGTTGAAAGTTTTAACTGATTGCGATACAATCAACTCAGACTTCACTAGATCAGACAGAGTTCGTGGTGTCTCCGGC GGGCGCGGGCCCGGGGCTGAACGCCCCCGGCGGCCATGACGGCGGGCCCGCCGAAGCAACTAAGGTACAGTAAACACGG GTGGGAGGTTGGGCTCGCTAGGAACCCTACACTCGGTAATGATCCTTCCGCAGGTTCAC	*Aspergillus flavus*	**757.612**	**99**	**UOA/HCPF8 664**	**507**
D5	CTGATCCGAGGTCAACCTGGAAAGATTGTTTTGCGTTCGGCAAGCGCCGGCCGGGCCTACAGAGCGGGTGACAAAGCCCC ATACGCTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTGGGGCCCGTCCCCCCCGGAGAGGGGACGACGACCCAACACA CAAGCCGTGCTTGATGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAA GACTCGATGATTCACGGAATTCTGCAATTCACACTAGTTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAG ATCCATTGTTGAAAGTTTTAACTGATTGCGATACAATCAACTCAGACTTCACTAGATCAGACAGAGTTCGTGGTGTCTCCG GCGGGCGCGGGCCCGGGGCTGAACGCCCCCGGCGGCCATGACGGCGGGCCCGCCGAAGCAACTAAGGTACAGTAAACAC GGGTGGGAGGTTGGGCTCGCTAGGAACCCTACACTCGGTAATGATCCTTCCGCAGGTTCACCCTACGGAAG	*Aspergillus flavus*	**795.537**	**99.01**	**UOA/HCPF5 774**	**505**
D9	CAGAGCGGGTGACAAAGCCCCATACGCTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTGGGGCCCGTCCCCCCCCGGAG AGGGGACGACGACCCAACACACAAGCCGTGCTTGATGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCGGAATACCA GGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACGGAATTCTGCAATTCACACTAGTTATCGCATTTCGCTGCGTTCTT CATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGATTGCGATACAATCAACTCAGACTTCACTAGATCAG ACAGAGTTCGTGGTGTCTCCGGCGGGCGCGGGCCCGGGGCTGAACGCCCCCGGCGGCCATGACGGCGGGCCCGCCGAAGC AACTAAGGTACAGTAAACACGGGTGGGAGGTTGGGCTCGCTAGGAACCCTACACTCGGTAATGATCCTTCCGCAGGTTCA CCCTACGGAAG	*Aspergillus flavus*	**737.008**	**99.175**	**WM12.3**	**612**
D15	GAGGTCAACCTGGAAAGATTGTTTTGCGTTCGGCAAGCGCCGGCCGGGCCTACAGAGCGGGTGACAAAGCCCCATACGCT CGAGGATCGGACGCGGTGCCGCCGCTGCCTTTGGGGCCCGTCCCCCCCCGGAGAGGGGACGACGACCCAACACACAAGCCG TGCTTGATGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGA TGATTCACGGAATTCTGCAATTCACACTAGTTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCATTG TTGAAAGTTTTAACTGATTGCGATACAATCAACTCAGACTTCACTAGATCAGACAGAGTTCGTGGTGTCTCCGGCGGGCGC GGGCCCGGGGCTGAACGCCCCCGGCGGCCATGACGGCGGGCCCGCCGAAGCAACTAAGGTACAGTAAACACGGGTGGGA GGTTGGGCTCGCTAGGAACCCTACA	*Aspergillus flavus*	**757.612**	**99**	**UOA/HCPF5 774**	**505**
D25	TCCGAGGTCAACCTGGAAAGATTGTTTTGCGTTCGGCAAGCGCCGGCCGGGCCTACAGAGCGGGTGACAAAGCCCCATAC GCTCGAGGATCGGACGCGGTGCCGCCGCTGCCTTTGGGGCCCGTCCCCCCCCGGAGAGGGGACGACGACCCAACACACAAG CCGTGCTTGATGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACT CGATGATTCACGGAATTCTGCAATTCACACTAGTTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCC ATTGTTGAAAGTTTTAACTGATTGCGATACAATCAACTCAGACTTCACTAGATCAGACAGAGTTCGTGGTGTCTCCGGCGG GCGCGGGCCCGGGGCTGAACGCCCCCGGCGGCCATGACGGCGGGCCCG	*Aspergillus flavus*	**670.439**	**99.299**	**UOA/HCPF5 774**	**505**

CHAPTER FIVE

5.0 DISCUSSIONS

A total of four hundred and thirty two(432) powdered herbal medicinal preparations consisting of twelve(12) each from six(6) localities in each of the six(6) states' capitals of the North Western Nigeria were obtained. In each localities, six(6) samples were obtained during wet and dry seasons respectively. These samples were subjected to various mycologicalanalyses. The parameters measured, were grouped as follows: Physicochemical properties: (moisture content and $_{P}$H), Mycoflora type and their level of contamination, aflatoxigenic fungi presentand their molecular confirmation, level of aflatoxin in the herbal preparations.

It is generally believed that herbal and natural products are safer than the synthetic or modern medicines, and also have the potential to cure diseases. This research however showed that some indigenous herbal products could be considerably contaminated with various types of fungi.

5.1 Sale and use of herbal medicinal preparations in the North Western-Nigeria.

The preliminary survey indicates that herbalist from the North western Nigeria sell herbal medicinal preparations that are claimed to cure different type of ailments such as typhoid fever, menstrual pain, malaria, yellow fever, dysentery, diarrhea, measles small pox, hypertension diabetes, cancer, pile and stomach ailment, and it is in agreement withFamuyiwa(1993) and World Health Organization(1998) who claimed that about 70-80% of the world population rely on herbal medicinal preparations to cure some diseases. The finding also supported by the study of Samuel (2007); Muazu and Kaita(2008) and Ewere (2009).

5.2Fungal contamination of herbal medicinal preparations obtained from North Western-Nigeria.

Most of the herbal medicinal preparations are used in different forms (such as powders) and they normally carry a large number of various kinds of microbes originating from soil, and are normally adhered to parts of plants (Adeleye *et al.,* 2005).

In this result it is showed that all the four hundred and thirty two herbal medicinal preparation assessed did not comply with the maximum acceptable limit of $2x10^2$ cfu/g as specified by United State Pharmacopoeia (2005). However, the distributions of the herbal medicines according to fungal count vary from town to another but none of the samples in each state conform to the stated limit by US Pharmacopoeia. Considering the stated limit, the results are in agreement with those of previous studies (Roy and Kumari, 1991; Pitt, 2000; Tassaneeyakul *et al.,* 2004; Bugno *et al.,* 2006). The result also shows that samples from Kaduna and Kebbi were observed to have a significantly higher mean fungal count of$1.09x10^5$cfu/g and $1.06x10^5$cfu/g respectively that were not significantly different ($p\leq0.05$), hence suggesting higher contamination with fungi. This finding is in agreement with that of Bugno *et al.,* 2006 and Okunlola *et al.,* 2007. The least mean value of fungal count was observed in Gusau town. The statistical analysis showed that fungal load in Katsina, Sokoto, Kano and Gusauwere not the same but not significantly different ($p\leq0.05$). This suggested low level of contamination with fungi when compared with samples from Kaduna and Birnin Kebbi. The samples were contaminated to varying degrees with fungi may be due to poor practice used in harvesting, handling, storage, production and distribution process (Mandeel, 2005).

5.3 Frequency of occurrence of fungi in herbal medicinal preparations obtained from North Western-Nigeria.

Fungi species were observed to be well distributed in all the herbal medicinal preparations studied as indicated in this finding. Fungi of the genus *Aspergillus* and *Penicillium* were

the most frequently isolated, and this result conform with the work of Bugno*et al.*(2006) who showed that the most commonly observed fungi genera in herbal drugs were *Aspergillus* and *Penicillium.* Although a number of these fungi were identified as common storage fungi that contaminate herbal drugs (Agrios, 1978). In all the samples screened from the six towns, higher level of contamination with *Aspergillus* spp were found, and this suggest that *Aspergillus* spp are considered as the most common contaminant of herbal medicinal products as reported by Martins*et al.* (2001). The highest frequency of occurrence of fungi observed in samples from Kaduna(n=151)69.59%, Kano (n=139)72.02%, Sokoto(n=135)73.37%, Birnin Kebbi(n=127)66.49%, Kastina (n=112)70.89% and Gusau(n=111) 68.52% may be as a result of poor harvesting, processing and storage practice of the handler(s) of the herbal products as reported by Zhang *et al.*(2005). Among the *Aspergillus* spp observed, *A. flavus, A. parasiticus* and*A. niger* were the most frequent suggesting that they are the major contaminants of the herbal medicinal products in all the six towns of the North West of Nigeria. The finding of Abou-Arab *et al.* (1999) and Elshafie *et al.* (1999) supported this discovery in the North Western-Nigeria. The Constant presence of these fungi in the herbal medicines around these areas perhaps due to the fact that their spores are the common air contaminants probably present in the processing and packing area as reported by Lee and Jo(2006) and Zhang *et al.* (2005). *Aspergillus* spp and *Penicillium* spp were found to be higher in frequency of occurrence. Out of one thousand and ninety five (1095) total frequency of occurrence of fungi in the samples, the total frequency of occurrence of *Aspergillus* spp in this study is seven hundred and seventy five(70.77%), *Penicillium* spp(n=190)17.35%; *Fusarium* spp(n=86)7.85% and *Rhizopus* spp(n=44)4.02%. In this finding it could be suggested that *Aspergillus* spp and *Penicillium* spp are the major contaminants of herbal drugs, and the result is in agreement with that of Halt (1998) who indicated the

contamination of herbal drugs with *Penicillium* spp. The variation in the level of contamination of the products with *Penicillium* spp could be due to some growth factors like moisture as reported by Matsushima *et al.* (1958). *Fusarium* spp and *Rhizopus* spp were found in a few samples at low level as showed in this finding(Table 4.3).The presence of these fungi in herbal preparations is reported by Halt (1998) in Croatia; Efuntoye (1996) in Nigeria; Martins *et al*. (2001) in Portuguese; Czech *et al*. (2001) in Australia and Rizzo *et al.* (2004) in Argentina. The low level of contamination by these two fungi be linked with the fact that their spores could survive drying conditions and remain dormant for several months possibly years on the dried herbs after which, with improved moisture levels, germination occurs.(Zhang *et al*.,2005).

The mean of frequency of occurrence of fungal isolates in herbal preparations in six states of the North Western Nigeria shown in table 8 is a clearindication that in this study the incident of *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus niger* and *Penicillium* spp were not significantly different at $p<0.05$ but significantly higher than *Aspergillus ochraceus* and *Aspergillus versicolor.* The result obtained also indicates that *Fusarium* spp and *Rhizopus* spp were significantly the lowest.This suggests that the samples obtained in the North Western Nigeria is heavily contaminated with the fungal species of *Aspergillus* spp and *Penicillium* spp. These results fairly accurate with previous reports that showed *Aspergillus* spp and *Penicillium* spp were the main contaminant and frequently isolated fungi in herbal medicines (Roy and Chourasia, 1990; Kumar and Roy, 1993; Rizzo *et al.,* 2004; Bugno*et al.,* 2006). The fungal contaminants are reported to be the producers of mycotoxins (Halt, 1998; Martin *et al.,* 2001). The incidence of *Fusarium* sppand *Rhizopus* spp showed the least level of occurance than the other fungi. The two isolates generallyrequire higher moisture content for their spores to germinate (Morton,

1978; Zhang *et al.*, 2005; Lee and Jo, 2006).It is on record that *Fusarium* spp could be hazardous through the production of *Fusarium* toxins. (Martins *et al.*, 2001).

5.4 Presence of aflatoxigenic fungi in herbal medicinal preparations.

In these findings, Aflatoxigenic fungi were differentiated it from non-aflatoxigenic ones on Neutral Red Desiccate Coconut Agar (NRDCA) and this is in consonance with the finding of Atanda*et al.*(2011) who indicated that Nutrient Red Desiccated Coconut Agar(NRDCA) was very good for detection of aflatoxigenicfungi.Both aflatoxigenic and non-aflatoxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus* were screened using NRDCA medium.This coincided with the result of Joe and Richard (2002) had earlier reported that some strains of the two species could be non-aflatoxigenic. The in ability of some strains of fungi to produce aflatoxin(even thought they are widely known to be aflatoxigenic) could be due to some genetic modification in their makeup (Tortof and Henikoff, 1991), most especially deletion of the entire aflatoxin biosynthetic gene cluster which carries Afl-1 mutation (Prieto*et al*., 1996), or as a result of production of repressor of aflatoxin gene expression that likely affect *aflR*, which is the transcriptional regulator of the aflatoxin biosynthetic genes(Woloshuk *et al*., 1995). In another studies, silencing of aflatoxin gene cluster by quelling or otherwise affect aflatoxin production in *Aspergillus flavus* (Pickford*et al*. 2002; McDonald *et al*., 2005; Carrie *et al.*, 2007), and culture condition also control expression of the genes for aflatoxin production in *Aspergillus parasiticus* (Guo and Leonard , 1998).

5.5 Moisture content of samples and its effect on fungal growth.

The results of the moisture content in fig. 2 showed that there was remarkable variation among the different herbal preparations sampled. European Agency for the Evaluation of Medicinal Products (1998) suggested that water content should be included in the list of comprehensive specifications for herbal products especially the powdered forms.

Therefore, the maximum moisture content limit of 8% per gram of herbal preparations is satisfactory according to National Agency for Food and Drug Administration and Control (NAFDAC SOP, 2000). In this study, the result of moisture content of the entire North West Nigeria in Fig 4.2 and Table 4.5 showed that 61(14.12%) of the 432 herbal products were within the limit, while 371(85.88%) were higher than the NAFDAC stated limit of 8%. The result in table 4.4 showed that the number of samples within the NAFDAC stated limit in all the six towns were less than the number of samples above the stated limit of 8%. The results obtained showed that even at less than 8% moisture content limit various fungi were found, but at very low moisture content there were very few fungal counts in some of the herbal preparations. The moisture content is acknowledged to play a role in the growth of fungi (Sonia *et al.,* 1998).

5.6 pH value of samples and its effect on fungal growth.

With regards to pH values shown in Fig 3, it could be inferred that most of the samples were neither strongly acidic nor strongly basic, but 37.50%, 37.96% and 26.85% of there acidic, neutral and alkaline respectively. Considering the pH valueas physical parameter that gives an idea as to whether the herbal preparations are acidic or basic, this result is in agreement with those of previous studies (Abba *et al.,* 2009) that herbal medicinal preparations could have acidic, basic or neutral properties

The study also indicates that growth of fungi was observed in all the four hundred and thirty two(432) samples analyzed, In general, it shows that nearly all species observed were able to grow over the entire pH range examined, but *Penicillium* species were observed to be more in number in samples with alkaline properties while *Aspergillus* species grow better in samples that have acidic properties. These results suggest that fungi species can be able to growth favorably under wide ranges of pH at room temperature (Northolt and Bullerman, 1982; Patterson and Damoglou, 1986 and Wheeler *et al.,* 1991).

The mean values of pH from the six towns as illustrated in table 4.7 shows that samples from Kano had the highest pH mean value followed by Samples from Birnin Kebbi, Kaduna, Sokoto, Katsina and Gusau indicated the lowest. Statistically, the mean values of Kano and Kebbi were not significantly different($p \geq 0.05$), likewise Kaduna and Sokoto. Samples from Katsina and Gusau showed the lowest mean value, even though they are significantly different ($p \leq 0.05$).

The mean value of moisture contents observed indicated that samples from Gusau had the highest followed by Birnin Kebbi, then Kastina and Kaduna. The least mean value were observed in Sokoto and Kano. Each of samples exhibited moisture content and pH levelsthat influence the growth of fungi (Wheeler *et al.,* 1991; Sonia *et al.,* 1998), and the practices involved in drying the herbal preparations may be very poor in some states.

5.7 Correlation between fungal count and physicochemical parameters (moisture content and pH).

In this finding, the Pearson chi-square correlation test did at 95% level of confidence shows positive correlations between moisture content and fungal count in all the states as indicated by correlation coefficient(r) in table 4.8. It was observed that at a very low moisture content the average fungal count was low, likewise where moisture content was high the fungal counts was observed to be high. This indicated that moisture is a decisive factor for the growth of fungi (Northolt and Bullerman, 1982). The presence of higher fungal counts and high moisture contents in powder herbal medicinal samples justify favorable impact of high moisture on the fungal growth (Halt, 1998). Diverse abiotic factors such as moisture content operating in the processing and storage conditions as well as chemicals constituents of the herbal drugs might have resulted in variation in mycopopulation in different substrates (Chourasia *et al.,* 2008). Although, the moisture content in some samples is sometimes high but the fungal load is low as compared to otherthat the moisture content low but the fungal load is high, perhaps this could be due to

its greater antifungal activity (Dutta *et al.*, 1998; Chattopadhyay and Bhattacharyya, 2007).

5.8 Seasonal fungal profile.

In this finding, a significantly high($p \leq 0.05$) mean fungal count was observed in herbal medicinal preparations in the wet season compared to the dry season,and this might be due to the fact that during wet season, factors such as moisture content that favors the growth of fungi were optimum and favored the germination of spores and subsequent growth of fungi(Sonia *et al.,* 1998).In all the six states' metropolis, the fungal ranges of$9.3x10^5$cfu/gto $9.3x10^4$cfu/g observed in wet season and 8.8x104cfu/gto $6.2x10^4$cfu/g observed in dry seasons(table 16) were quite above the acceptable limit of $2x10^2$cfu/g stated by United State Pharmacopeia(2005). The high level of contamination of herbal medicines with fungi in the wet season observed could be attributed to higher moisture content and favorable humidity(Sonia *et al.,* 1998). Nevertheless, the high total fungal count in the wet season has been reported previously (Aziz *et al.,* 1998). The seasonal variation in the mean fungal count were significantly higher($P \leq 0.05$) in samples from Kaduna with had the mean fungal count of $1.2x10^5$cfu/g; Katsina ($1.1x10^5$cfu/g); Sokoto ($9.3x10^5$cfu/g) and Gusau ($9.3x10^4$cfu/g) (Table 16) suggesting significantly high level of contamination of herbal medicinal preparations with fungi compared to sampling in dry seasons. Considering the seasonal mean fungal count variation, this result is in agreement with the finding of Okonkwo and Obionu (1981) that showed higher mean fungi count in wet than in dry season. The variations in mean fungal value observed in the sample area were possibly due to unhygienic method of storage (Roy and Chourasia, 1989). Birnin Kebbi and Kano with mean fungal countof 4.1x105cfu/g and $1.2x10^5$cfu/g respectively obtained in wet season showed insignificant difference ($p \leq 0.05$) with the corresponding mean fungal value obtained in dry season suggesting less or no difference in the level of

contamination with fungi and this partially agreed with the previous findings (Mitakakis *et al.,* 1997; Zoppas *et al.,* 2006) who reported that fungi can grow at anytime of the seasons. However, the fungal mean difference due to seasonal or locality/region difference have been reported previously (Abu-Deiyeh *et al.,* 2010).

5.9 Seasonal mean fungal count of samples in all the sampling locations.

In this finding, it was observed that during wet season all the fungal count was significantly high ($p \leq 0.05$). In Kaduna metropolis, samples from Mando garage had the highest mean fungal count of $1.35x10^5$cfu/g(Fiq3) suggesting higher level of contamination with fungi compared with samples from Central market($9.67x10^4$cfu/g).In the dry season, samples from Kawo motor park had the highest mean fungal count of $1.02x10^5$cfu/g, and the lowest fungal count was observed in Kasuwar Barci ($7.00x10^4$cfu/g).The seasonal variations might be due to the fact that during wet season factors such as moisture content that favors the growth of fungi were optimum and favored the germination of spores and subsequent growth of fungi(Sonia *et al.,* 1998).

The result in Katsina metropolis (Fig 6) also shows that samples from Central Motor Park had the highest mean fungal count of $1.38x10^5$cfu/g suggesting significantly higher level of contamination with fungi compared with samples from Chake market($8.17x10^4$cfu/g). A different result in dry season indicated that samples obtained from Mangal garage had the highest level of contamination with mean fungal count of $7.42x10^4$cfu/g, while Chake market and Central market had the lowest level of contamination with fungal count of $5.17x10^4$cfu/geach. However, their means in both wet and dry seasons exceeded the maximum limit 0f $2x10^2$cfug^{-1} stated by United state Pharmacopeia(2005). This result is in agreement with the finding of Okonkwu and Obionu (1981) that showed higher mean count of fungi in wet season than in dry season.

The finding of different outlets in Kano metropolis(fig 8) shows that samples from Rimi market had the highest mean of $2.27x10^5$cfug^{-1} suggesting significantly higher level of

contamination with fungi compared with samples from S/gari market($6.83x10^4$cfu/g). In kano metropolis and in wet season, samples from Rimi market had the highest mean fungal count of $2.27x10^5$cfu/g suggesting significantly higher level of contamination with fungi compared with samples from S/gari market($6.83x10^4$cfu/g).In dry season, samples obtained from Airport road had the highest level of contamination with mean fungal count of $1.70x10^5$cfu/g, while S/gari market had the lowest level of contamination with fungal count of $6.26x10^4$cfu/g. This kind of difference in the fungal means due to seasonal /locality difference has been reported previously (Abu-Deiyeh *et al.,* 2010).

Similarly, the result of different outlets in Birnin Kebbi metropolis (fig 10) shows that in the wet season,samples from Argungun motor park had significantly highest($p \leq 0.05$). mean fungal count of $1.85x10^6$cfug^{-1}suggesting significantly highest ($p \leq 0.05$) level of contamination with fungi ever encountered in this research compared with samples from Ambursa market ($8.85x10^4$cfu/g).In dry season, samples obtained from Old market had the highest level of contamination with mean fungal count of $1.13x10^5$cfu/g, while Central motor park had the lowest level of contamination with fungal count of $7.42x10^4$cfu/g. The high level of contamination of herbal medicines with fungi in the wet season observed could be attributed to higher moisture content and favorable humidity (Sonia *et al.,* 1998). Nevertheless, the high total fungal count in the wet season has been reported previously (Aziz *et al.,* 1998).

The study in Sokoto town (Fig.4.12) shows a different results, it was shown that in wet season, samples from Central market had the highest mean fungal count of $1.05x10^5$cfu/g suggesting higher level of contamination with fungi compared with samples from Old market ($8.7x10^3$cfu/g).In the dry season, samples from Kara market had the highest mean fungal count of $8.16x10^4$cfu/g, while the lowest mean fungal mean of $6.00x10^4$cfu/g was observed in Gawon Nama. In sokoto town, samples obtained in the wet season from Old

market and Gawon Nama showed the lowest means of $8.7x10^3$cfu/g and $9.2x10^3$cfu/g respectively, and this opposed to the previous finding of Okonkwu and Obionu (1981) who showed that fungi count could be higher in the wet season than in the dry season. However, the variations in means value observed of all the six locations in Sokoto town might be as a result of variations in activities such as handling and possibly processing practices or possibly due to unscientific method of storage of herbal drugs by the handlers (Roy and Chourasia, 1989).

The finding in Gusau town (Fig.4.14), showed that during wet season all the mean fungal load was significantly high($p \leq 0.05$),In wet season, it was observed that samples from Central market had the highest mean fungal value of $1.08x10^5$cfu/g suggesting higher level of contamination with fungi than that from hospital road with mean fungal value of 7.50x10cfu/g. In the dry season, samples from Labilabi with fungal mean of $8.33x10^4$cfu/g had higher level of contamination than that of Tudun wada with a mean fungal count of $3.92x10^4$cfu/g. Even though, the mean fungal value obtained in wet and dry seasons exceeded the maximum limit 0f $2x10^2$cfug^{-1} stated by United State Pharmacopeia(2005), this result is in agreement with the finding of Okonkwo and Obionu (1981) that showed higher mean count of fungi in wet season than in dry season.

5.10 Aflatoxin detected in samples obtained from North Western-Nigeria.

Out of 432 herbal medicinal preparations sampled, 62.04% had total aflatoxin above the set stated by Codex Alimentarus Commission of FAO of the UN and adopted by NAFDAC, Nigeria. The total aflatoxin value ranged from the highest (>20ppb) in all the six states to the lowest (1.6ppb) in Sokoto town and Kano town respectively. Kastina town showed the highest number of samples (n=65; 90.28%) contaminated with aflatoxin. This result is in agreement with the work of Roy and Chaurasia, 1990) who reported the contamination of herbal medicinal products by aflatoxin up to level of 20ppb.

The mean value of total aflatoxin of herbal medicinal preparations is significantly high in Katsina metropolis with a value of 18.73ppb, and significantly lowest in Gusau town with a value of 9.40ppb. This suggest highest contamination with aflatoxin in samples obtained from Katsina town , while the lowest was those from Gusau town and the entire study suggest that most samples from North Western Nigeria are highly contaminated with aflatoxin beyond the stated limit of 10ppb approved by NAFDAC, but still some are within the stated limit.

This finding is supported with the report of Roy *et al.*(1988) who reported that herbal medicinal products contained aflatoxin as a natural contaminant and their concentration depend on their natural origins. The variations observed in the level of contamination with aflatoxin in the six states of the North Western Nigeria could be connected to storage period and the sources of these herbal materials, these argument is supported with the previous works (Efuntoye, 1999; Chan, 2003).

5.11. Correlation between fungal load and total aflatoxin.

The Chi-square correlation test showed a significant and positive correlation between total aflatoxin and fungal load(r=+0.123) and this suggest that: Increase of fungal load in herbal medicinal preparations suggest an increase in total aflatoxin concentrations, and is in agreement with the report of Tothill *et al.* (1992); Moretzsohn de Castro *et al.* (2002). Even though, Bugno *et al*. (2006) reported that the presence of mycotoxigenic fungi did not always imply mycotoxins detection but their presence represents a potential risk of contamination with mycotoxins.

5.12. Seasonal aflatoxin profile.

In this finding, it was observed that in metropolises of Kaduna; Katsina; Kano; Birnin Kebbi; Sokoto and Gusau, the total mean aflatoxin value of herbal medicinal preparations

in both wet and dry seasons differed and significantly high (P≤0.05) above the limit of 10ppb stated by Codex Alimentarius Commission (Codex). The level of total aflatoxin was indicated to be higher during wet season than the dry season at 5% level of confidence, and this suggest higher contamination of herbal medicine with aflatoxin during wet season than dry season. This finding could be supported with the fact that level of aflatoxin were significantly higher in wet season than in dry season(Okonkwo and Obionu, 1981), and the high contamination of the preparations might be due unsafe practices.

There was an obviously higher aflatoxin contamination ofsamples obtained from almost all the different locations in the entire six towns of the North Western Nigeria. The mean total aflatoxin were significantly highest (P≤0.05) in wet season especially in samples obtained from Central motor park in Birnin Kebbi (50.47ppb), followed by those from Mando motor park in Kaduna and Central motor park in Kastina (21.0ppb) each. Sabon gari market in Kano(18.13ppb) and Hospital road in Gusau (17.03ppb). The lowest mean of total aflatoxin value was obtained in those samples obtained from Kara market in Sokoto (16.93ppb). This finding could be justified with the significantly higher fungal count value observed in wet season for herbal drugs from those localities, or might be due improper handling of herbal medicinal preparations that led to introduction of those factors that induced the production of aflatoxin by the aflatoxigenic fungi (Yasar*et al.*, 2009). This result is in accordance with the report of Kumar and Roy (1993) who indicated that the level of aflatoxin in herbal drugs was much higher than the tolerable level fixed by the WHO and it could be a risk for patients.

It is also observed that lowest total aflatoxin mean value of 8.0ppb in dry season was obtained in Malali market suggesting least contamination with aflatoxin below the suggested limit of 10ppb set for herbal drugs by Codex. In the overall result of seasonal

variation obtained in Kaduna state, the total aflatoxin mean value of 17.62ppbwas significantly higher($p \leq 0.05$) in wet season than 11.32ppb observed in the dry season. Even though, their mean difference in both wet and dry seasons exceeded the maximum limit 0f 10ppb stated by Codex. This result suggest that herbal drugs in Kaduna is significantly contaminated with aflatoxin away from the acceptable limit of 10ppb, and the contamination might be due lack of awewreness of the method of storage(Roy and Chourasia,1989) , but the report ofPatterson and Damogloau, (1986). Indicated that some toxins may be produced at lower water activities than have been reported on synthetic media.

5.13 Molecular identification of isolated aflatoxigenic fungi.

The bands obtained from the agarose gel electrophoresis were conspicuous and readable, the molecular sizes marker of 1kb when compared with the unknown DNA fragments of the target fungi shows that at 500pb all the bands were at the same horizontal line, indicating similar molecular weight sizes. This explained that all the sample of DNA analyzed were from the same type of fungi, and when partial sequences was done on them, base sequences of varying length and arrangement of the bases were produced which identity were confirmed in BLAST to be *Aspergillus flavus.*

The PCR allows isolation and identification of fungal DNA fragment from genomic DNA when amplified (Weerasekera *et al.,* 2012). In this result PCR generated the anticipated DNA fragment (amplicon) from 500pb DNA fragment. The sequence of each of these fragments differs between species even though the sizes of the fragments were the same. This is because PCR itself used to amplify a segment of DNA that lies between two regions of known sequence (Saiki *et al.,* 1985: Mullis and Faloona, 1987). It requires two oligonucleotide primers that flank the DNA fragment to be amplified and employs repeated cycles of heat denaturation of the DNA, annealing of the primers to their

complementary sequences, and extension of the annealed primers with a thermostable DNA polymerase (Saiki *et al.,* 1988). These primers typically have different sequences, are complementary to sequences that lie on opposite strands of the template DNA, and flank the segment of DNA that is to be amplified. Since the extension products themselves are also complementary to and capable of binding primers, successive cycles of amplification essentially double the amount of target DNA synthesized in the previous cycle (Frey, 1998).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conlusion

The study observed that herbal medicinal practitioners from the North western Nigeria offer for sale herbal medicinal preparations that were claimed to cure different type of ailments without undergoing proper or modern training in the business.Over the year, studies have been carried out on these non-orthodox preparations in different parts of the world with a view to ascertaining wholesome nature of the products. The presence of a wide range of fungi thatare toxigenic in the preparations indicates that considerable work could be made during post- harvest period to eliminate the fungal population.

Although the presence of fungi in the herbal preparations below the stated limit did not imply the safe nature of the product, their presence represents a potential risk of contaminations with aflatoxins. Considering the worldwide increased in the use of herbal products as alternative medicines and the riskof purchase and use of natural products contaminated with moulds suspected to be pathogenic, it is necessary to set appropriate standards and ensure compliance for toxigenic fungi in herbal medicinal preparations and this is to enhance public health in Nigeria.

The setting and percentage moisture content approved by the regulatory agency in Nigeriais itself not enough to reduce the fungal count toacceptable level (2x102cfu/g) set by USP (2005).

The variations of total fungal count in the wet and dry season's shows that different effective strategic management that can reduce the fungal load to the acceptable limit need to be adopted and impose on the practitioners.

These finding will serve the purpose of alerting the consumer and stakeholders in herbal medicinal preparations business on the dangers associated with the herbal products.

6.3 Recommendations

1. Regulatory bodies such as NAFDAC, SON and Consumer Protection Council should be empowered with the necessary facilities to improve the quality of the herbal medicinal products marketed in Nigeria.
2. Mandatory continuing training on the preparations, handling and storage of herbal medicines should be organized regularly for the alternative medicine practitioners to enhance their proficiency in the business.
3. Nigeria as a county should improve research and development with a view to develop our own standard limits on all the parameters associated with safety of these products preparations.
4. An officially stated public policy and adequate legislative support could assist in the proper development of traditional systems of medicine in Nigeria

8.0 REFERENCES.

Abba, D., Inabo, H.I., Yakubu, S.E. and Olonitola, O.S.(2009). Contamination of herbal Medicinal products marketed in Kaduna metropolis with selected Pathogenic bacteria.*African Journal of Traditional, complementary and Alternative Medicines.* **6**(1):70-77.

Abou-Arab, A.A.K., Kawther, M.S., Tantaway, M.E., Badeaa, R.I. and Khayria, N. (1999). Quantity estimation of some contaminants in commonly used medicinal plants in the Egyptian market. *Food Chemistry*. **69**:357-363.

Abu- Deiyeh, M.H., Barham, R., Abu-Elteem, K., Al-Rashid, R. and Shaheen, I. (2010). Seasonal variation of fungal spore populations in the atmosphere of Zarqa area, Jordan. *Aerobiogia.*

Adeleye, I.A., Okogi, G. and Ojo, E.O. (2005). Microbial contamination of herbal Preparationsin Lagos. *Journal of Health, Population and Nutrition.* **23**(3):296-297.

Adewunmi, C.O. and Ojewole, J.A.O. (2004). Safety of traditional medicines, complementary and alternative medicines in Africa. *African Journal Traditional Conplementary and Alternative Medicines.* **1**:1-3.

Adjanahoun, E., Ahiyi, M.R.A., AkeAssi, L., Dramane, K., Eluwade.J.A., Fadoju, S.O., Gbile, Z.O., Goudote, E., Johnson, C.L.A., Keita, A., Morakinyo, O., Ojewole, J.A., Olatunji, O.A. and Sofowora, E.A. (1993). Contribution to Ethno botanical and floristic studies in West Nigeria. O.A.U. Scientific, Technical and Research Commission, Lagos. *Conference paper*.

Agrios N.G.(1978). Plant pathology. Academic Press, New York: pp703.

Ajibola, A. and Motoyoshi, S. (1992). Contribution to the phytochemistry of medicinal plants growing in Nigeria as reported in the 1979-1990 literature-A Review .*African Journal of Pharmacy and Pharmaceutical sciences*. **22**(3): 172-201.

Almond Board of California (2008). The High Cost of Aflatoxins. Retrieved May 15, 2009, http://www.almondboard.com/files/aflatoxin%20fact%20sheet%20final.pdf

Anyanwu, C.U.(2010). Fungal contaminants of powdered herbal drugs soldin parts of Enugu State, South East Nigeria. *Plant Product Research Journal*. **14**:46-50.

Atanda O.O., Ogunrinu M.C. and Olorunfemi F.M. (2011). A neutral red desiccated coconut agar for rapid detection of aflatoxigenic fungi and visual determination of aflatoxins. *World Mycotoxin Journal*. **4**(2):147-155.

Awosika, D.F. (1993). Local medicinal plants and health care of consumers. Clinical plants and health care of consumers. *Clinical Pharmacy and Herbal Medi*cine. **9**: 28-31.

Aziz, N.H., Youssef, Y.A., Moheye, Z.E. and Lofty, A.M. (1998). Contamination of some medicinal plant samples and spices by fungi and their mycotoxins. *Botanical Bulletin of Acadmic Sinences*. **39**(4):279-285.

Balasubramaniam, K. (2000). Herbal Medicine and Public Policy: the 3rd International symposium on herbal medicines in the Caribbean: Organized by the Caribbean Association of Researchers and Herbal Practitioners (Jamaica Division) in collaboration with The National Products Institute of the University of West Indies**. *http://www.ciroap.org/health/health 3.doc*.**

Barrett, B. (2003) Medicinal properties of Echinacea: a critical review. *Phytomedicine.* **10**(1):66-86.

Bauer, R. (1998). Quality criteria and standardization of phytopharmaceuticals: Can acceptable drugs standard be achieved? *Drugs Information Journal.* **32**: 101-110.

Barceloux, D.G., ed. 2008. *Medical Toxicology of Natural Substances*. John Wiley and Sons (Publisher). 327-330. Preview .Retrieved June 3, 2009, from http://tinyurl.com/rb7jue.

Bennett, J.W. and Klich, M. (2003). Mycotoxins. *Clinical Microbiology Reviews*. **16**:497-516.

Bhat R.V. and Vashanti S. (1999).Occurrence of aflatoxins and its economic impact on human nutrition and animal feed.*The New Regulation. Agric. Develop*. **23**: 50-56.

Bhat, R.V. and S. Vasanthi. (2003). Mycotoxins and Food Safety Risk in Developing Countries. *Food Safety in Food Security and Food Trade*. Focus 10, Brief 3. Retrieved May 26, 2009, from http://www.ifpri.org/2020/focus/focus10/focus10_03.pdf.

Bugno, A, Adriana A.B.A., Tatiana, C.P, Terzinha de Jesus, A.P. and Myrna, S. (2006). Occurrence of toxigenic fungi in herbal drugs. *Brazilian Journal of Microbiology*. **37**:47-51.

Bhushan, P. (2005). Traditional Medicine: A novel approach for available, accessible and affordable health care. *Paper for regional consultative on traditional medicine*.WHO Regional office for South-East Asia.

Burnet, J.H. (1976). Fundamentals of mycology.Second edition William Clowes and Sons, Limited. pp221-281.

Bodeker, G., Ong, C. K., Grundy, C., Burford, G., Shein, K. (2005). WHO global atlas of traditional, complementary and alternative medicine. WHO centre for health development, Kobe, Japan:pp46.

Booth, C. (1997). *Fusarium* laboratory guide to the identification of the major species. Common wealth mycological institute (C M I). Kew, Surry, England.

Bommakanti, A.S. and Waliyar, F.(2009). Importance of Aflatoxins in Human and Livestock Health. *International Crops Research Institute for the Semi-Arid Tropics*.Retrieved June 5, 2009, from http://www.aflatoxin.info/health.asp.

Calixto, J.B.(2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines(Phytotherapeutic agents). *Brazilian Journal of Med. Biol.* **33**:179-189.

Carrie, A. S.,Charles, P. W.,Dominique, R.andGary A. P. (2007). Silencing of the Aflatoxin Gene Cluster in a Diploid Strain of*Aspergillus flavus* is Suppressed by Ectopic *aflR* Expression. *Genetic*. **176**(4):2077-2086.

Cassel, E.K., B. Campbell, M. Draper, and B. Epperson.(2001). Aflatoxins Hazards in Grain/ Aflatoxicosis and Livestock.South Dakota State University Cooperative Extension Service. Retrieved June 5, 2009, from http://agbiopubs.sdstate.edu/articles/FS907.pdf.

Centers for Disease Control and Prevention (CDCP) (2004). Outbreak of Aflatoxin Poisoning- Eastern and Central Provinces, Kenya, January-July 2004. Retrieved May 26, 2009, from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5334a4.html

Chan, C. (2003). Some aspect of toxins contaminants in herbal medicines. *Chemosphere.* **52**:1361-1371.

Chainani, W.N. (2003) .Safety and anti-inflammatory activity of Curcumin: a component of tumeric *(Curcuma longa). The Journal of Alternative and complementary Medicine*. **9**(1):161-168.

Chattopadhyay, R.R. and Bhattacharyya, S.K. (2007). Terminalia chebula: An update. *Pharmacognosy Reviews*. **1**:151-156.

Cheli, F.A., Campagnoli, L. Pinotti, G. S. and Dell'Orto.V.(2009).Electronic Nose for Determination of Aflatoxins in Maize.*Biotecnol.Agron. Soc. Environ*. **13**:39-43.

Cigic, I.K. and H. Prosen.(2009). An Overview of Conventional and Emerging Analytical Methods for the Determination of Mycotoxins. *Int. J. Mol. Sci*. **10**:62-115.

Codex Alimentarius Commission.(2004). Code of Practice for the Prevention and Reduction of Aflatoxin Contamination in Peanuts.Retrieved June 3, 2009, from http://www.codexalimentarius.net/download/standards/10084/CXC_055_2004e.pdf.

Cornell University Department of Animal Science. (2009). Aflatoxins: Occurrence and Health Risks. Retrieved May 15, 2009, from http://www.ansci.cornell.edu/plants/toxicagents/aflatoxin/aflatoxin.html.

Chourasia, H.K., Suman, S.K. and Praasad, P. (2008). Mycotoxin fungi and mycotoxins in fastfood of Bihar.*Journal of Mycology and Plant Pathology*. **38**:492-499.

Collier, L., Balows, A. and Sussman, M. (1998). Topley & Wilson's Microbiology and Microbial Infections, 9th ed, vol. 4. Arnold, London, Sydney, Auckland, New York.

Costa, L.L.F and Scussel, V.M.(2002). Toxigenic fungi in beans (*Phaseolus vulgaris* L.) classesblack and color cultivated in the state of Santa Catarina, Brazil.*Brazilian Journal of Microbiology*. **33**:138-144.

Cragg, G.M. (2004). Testimony: *Encyclopedia of medicinal plants*: Education and health library. Editorial safeliz, S.L. pradillo, 6-poligono industrial La Mina E-28770 Colmenar Viejo, Madrid, Spain. **2**: 405.

Czech, E., Kneifel, W. and Kopp, B. (2001). Microbiological status of commercially availablemedicinal herbal drugs- a screening study. *Planta Medicinal*. **67**:263-269.

Davis, N.D., Iyer, S.K. and Diener, U.L.(1987).Improved method of screening for aflatoxin with a coconut agar medium.*Applied Enviromental Microbiology*. **53**:1593-1595.

Davise, H.L. (2002). A guide to identification of fungi. 4thedition. ASM Press, Washington D. C. U.S.A.

Duncan, D.B. (1995). Multiple range and F test. *Biometrics.* **11**:1-42.

Dutta, B.K., Rahman, I., Dass,T.K.(1998). Antifungal activity of Indian plant extracts. *Mycoses*. **41**:535-536.

Eaton, D.L. and E.P. Gallagher.(1994). Mechanisms of Aflatoxin Carcinogenesis. *Annual.Review of Pharmacology and Toxicology*. **34**:135-172.

Efuntoye, M.O. (1996). Fungi associated with herbal drugs during storage. *Mycopathologia*. **136**:115-118.

Efuntoye, M.O.(1999).Mycotoxins of fungal strains from stored herbal plants and mycotoxin contents of Nigerian crude herbal drugs. *Mycopathologia.* **147**:43-48.

Elujoba, A.A., Odeleye, O.M. and Ogunyemi, C.M. (2005). Traditional medicine development for medical and dental primary health care delivery system in Africa. *African Journal of Traditional, Complimentary and Alternative Medicines.* **2**(1):46-61.

El-shafie, A.E., Al-Lawatia,T. and Al-Bahry, S.(1999). Fungi associated with black tea andteaquality in Sultanate of Oman. *Mycopathologia.* **145**:89-93.

Ernst, E. (2002a). The risk- benefit profile of commonly used herbal therapies: Ginkgo, St, John's Wort, Ginseng, Echinacea, Saw palmetto, and Kava.*Annals of Internal Medicine.* **136**(1):42-53.

Ernst, E. (2002b). Adulteration of Chinese herbal medicines with synthetic drugs: a synthetic review. *Journal of Internal Medicine*. **252**:107-113.

European Agency for the Evaluation of Medicinal Products(1998). Note for guidance of quality of herbal medicinal products, EMEA/adhocHMPWG/114/98. http://www.edura.org/emea.html.

European Commission (1998).Council Directives 65/65/EEC.Herbal medicinal products in the European Union. Study carried out on behalf of the European Commission. **http://www.egsp.be.**

Ewere, O.O.(2009). Herbs in orthodox practice: A review by medical students. African *Journal of Traditional, Complimentary and Alternative Medicines*. **6**(2):203-206.

Famuyiwa, O.O. (1993). The efficacy of traditional medicine in the management of diabetesmellitus in South Western Nigeria.Africa Journal of Medical Sciences. **22**(1): 31-37.

Farnsworth, N.R. (1990). The role of ethno pharmacology in drug development, in bioactive compounds from plants (Chadwick, D.J. and Marsh, J., eds.), Wiley, New York. pp2-21.

Fennell, C.W., Lindsey, K. L., McGaw, L. J., Sparg, L. G., Stafford, G. I., Elgorashi, E. E., Grace, O. M. and Van Staden, J. (2004). Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. *Journal of Ethnopharmacology.* **94**:205-217.

Food and Drug Administration.(2007). Foodborne Pathogenic Microorganisms and Natural Toxins Handbook.*Aflatoxins*.Retrieved May 14, 2009, from http://tinyurl.com/y5nwst9.

Fatih, K. (2009). Relationship between airborne fungal allergens and metrological factors in Manisa, Turkey. *Journal of Enviromental Monitoring and Assessment:* pp 1573-2959.

Federal Environmental Protection Agency. (1992). Biological Diversity in Nigeria: A country study. *The Federal Environmental Protection Agency*, Presidency, Abuja, Nigeria.

Frey, B.(1998). Amplification of genomic DNA by PCR. *Method in molecular Medicines.* **13**:143-56.

Gabriel, B.G.(2005). Taxonomical atlas for identification of fungi. 3rd edition, John Wiley publication, Florida, U.S.A. pp 3-7

George, D. and Pamploma-Roger, M.D. (1999).Encyclopedia of medicinal plants.*Education and Health library Spain.* **1**:6-57.

Gillman, J. K. (1957). A manual of soil fungi.Iowa State University Press, Ames, Iowa. U.S.A. pp5-8

Gilman, J.C., (2001). A Manual of Soil Fungi.Oxford and IBH Publishing Corporation, New Delhi, India.

Gong, Y.Y., Cardwell, K., Hounsa, A., Egal, S., Turner, P.C., Hall, A.J. and Wild, C.P.(2002). Dietary Aflatoxin Exposure and Impaired Growth in Young Children from Benin and Togo: Cross Sectional Study. *British Medical Journal*. pp 325:20-21.

Grybauskas, A.P., Thomison, P.R. and Cassel, E.K.(2000). Aflatoxins.Maryland Cooperative Extension, University of Maryland.Retrieved May 15, 2009, from http://dairy.vitaplus.com/pdf/Maryland%20Cooperative%20Extension%3B%20Aflotoxins.pdf.

Guo H. F.and Leonard.T.J.(1998). Culture Conditions Control Expression of the Genes for Aflatoxin and Sterigmatocystin Biosynthesis in *Aspergillusparasiticus*and*A. nidulans.Applied and Enviromental microbiology.* **64**(6):2275-2277**.**

Halt, M. (1998). Moulds and mycotoxins in herb tea and medicinal plants. *European Journal of Epidemiology.* **14**:269-274.

Hedberg, C.I. (1998). Medicinal plants in the Tropics as important Resources. Chancellor College, University of Malawi, Zomba, Malawi. pp88-125.

Hermann, B.G. and Frischaufl, A.M. (1987).Isolation of genomic DNA. Methods *Enzymology*. **152**:180-183.

Hussein, H.S. and Brasel.J.M.(2001). Toxicity, Metabolism, and Impact of Mycotoxins on Humans and Animals. *Toxicology*. **167**:101-134.

Igoli, J.O., Ogaji, O.G.,Tor-Anyiin, T.A. and Igoli, N.P. (2005).Traditional Medicine Practiceamongst the Igede People of Nigeria. Part II. *AfricanJournal of. Traditional,Complimentary and Alternative Medicine.* **2**(2):134-152.

Imamura, Y., Chandra, J., Mukherjee, P.K., Lattif, A.A., Szczotka-Flynn, L.B., Pearlman, E., Lass, J.H., O'Donnell, K. and Ghannoum, M.A. (2008). "*Fusarium* and *Candida albicans* Biofilms on Soft Contact Lenses: Model Development, Influence of Lens Type, and Susceptibility to Lens Care Solutions". *Antimicrob.Agents Chemother.* **52**(1):171–182.

International Agency for Research and Cancer (IARC). (2002a). Aflatoxins.Some traditional herbal medicines, some mycotoxins, naphthalene and styrene.*IARC* Monograph No. 82. Lyon, France.

International Agency for Research on Cancer (IARC) (2002b) – Summaries and Evaluations.Aflatoxins (Group 1).Retrieved May 13, 2009, from http://www.inchem.org/documents/iarc/vol82/82-04.html.

Janetzand, L.A. (1994). Health online; Common medicinal herbs and preparing herbal treatment: In herbal medicine for children. ***http://www.healthy.net/scr/article.asp***

Joe W. D. andRichard J. C. (2002).Effect of application of nontoxigenic strains of *Aspergillusflavus* and *A. parasiticus* on subsequent aflatoxin contamination of peanuts in storage.*Journal of Stored Products Research.* **38**(4):329–339.

John, W. (1986).Introduction to fungi. Second edition, Cambridge University Press, London. Pp.269-282.

Kamalendu, N.(1990). A rapid DNA isolation procedure from petridish grown bacteria isolates. *Nucleic Acid Research.* **18**:6462.

Kay, B.A., Bopp, C.A. and Wells, J.G.(1994).Isolation and identification of fungi/bacteria,p3-25 In Munirul *et al.*, (2006). *Applied and environmental microbiology*. **72**:2185-2190.

Klich, M. A. (2006). Identification of clinically relevant *Aspergilli*.*Medical Mycology*. **44:**127-131.

Kohara, Y.; Akiyama, K. and Isono, K. (1987). The physical map of the whole *E.coli* chromosome. *Cell*. pp50:496.

Kosowan, B. (1991). The wonders of plants. African concord, 14th January, 1991.

Kumar, S. and Roy, A.K. (1993). Occurrence of aflatoxin in some liver curative herbal medicines.*Letters in Applied Microbiology*. **17**:112-114.

Lamanaka, B.T., de Menezes, H.C., Vicente, E, Leite, R.S.F. and Taniwaki, M.H.(2007). Aflatoxigenic Fungi and Aflatoxins Occurrence in Sultanas and Dried Figs Commercialized in Brazil. *Food Control*. **18**:454-457.

Larone, D. H. (1995). Medically Important Fungi - A Guide to Identification, 3rd ed. ASM Press, Washington, D.C. pp1-10.

Larone, D.H. (2002). Medically Important Fungi - A Guide to Identification, 4rd ed. ASM Press, Washington, D.C. pp269-281.

Lee, J.H. and Jo, W.K. (2006). Characteristics of indoor and outdoor bioaerosols at Korean high-rise apartment buildings. *Environmental Research*. **101**(1):11-17.

Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H. and Luber, G et al. (2005).Aflatoxin Contamination of Commercial Maize Products During an Outbreak of Acute Aflatoxicosis in Eastern and Central Kenya. *Environmental Health Perspect*. **113**:1763-1767.

Maiwada J. (2004). Presidential address at the opening ceremony of a 2- day zonal herbal fair/workshop organized by association of lady pharmacists of Nigeria in Abuja. Nasarawa state weekly news paper of August 11, 2004: *Newsday@ nasarawastate.org.*

Mandeel, Q.A. (2005) Fungal contamination of some imported spices. *Mycopathologia*. **159**:291-298.

Martins, H.M., Martins, H.L., Dias, M.L. and Bernardo, F. (2001).Evaluation of microbiological quality of medicinal plants used in natural infusions. *International Journal of Food Microbiology.* **68**:149-153.

Mastushima, T., Itoh, H. and Ikeda, M. (1958). Investigation on the fungal spoilage of crude drugs. *Journal of Japan Botany*. **33**:9-15.

McDonald, T., Brown, D., Keller, N.P.and Hammond, T.M. *(*2005). RNA silencing of mycotoxin production in *Aspergillus* and *Fusarium* species. *Mol. Plant Microbe Interact.* **18:**539–*545*.

Mitakakis, T., Ong, E. K., Stevens, A., Guest, D. and Knox, R.B. (1997). Incidence of Cladosporium, Alternaria and total fungal spores in the atmosphere of Melbourn (Australia) over 3 years. *Aerobiologia*. **13**:83–90.

Morton, J.F. (1987). Orange (Online). Accessed 5 May, 2008. http://www.hort.purdue.edu/newcrop/morton/orange.html

Moretzsohn de Castro, M.F.P, Bragagnolo, N and Valentini T. S.R. (2002). The relationship between fungi Growth and aflatoxin production with ergosterol content of corn grains. *Brazilian journal of Microbiology*. **33**(1):1517-1521

Muazu, J. and Kaita, A.H.(2008). A review of traditional plants used in the treatment of epilepsyamongst the Hausa/ Fulani tribes of Northern Nigeria. *African journalTradional, complementary and alternative medicines*. **5**(4):387-390.

Mullis. K. B. and Faloona, F. (1987). Specific synthesis of DNA in vitro: the polymerase-catalyzed chain reaction. *itMethodsEnzymol*.pp155,335–350.

Munirul, A., Abdus, S., Nur-a-Hasan, A., Bhuiyan, G., Balakrish, N., Siddique, A.K., Sack, D.A., Sunjukta, A., Anwar, H., Bradley, S.R. and Colwell, R.R. (2006). Effect of transport at ambient temperature on detection and isolation of bacteria from environmental samples.*Applied and Enviromental Microbiology.* **72**:2185-2190

NAFDAC Standard Operative Procedure (2006). Immunoaffinity column clean up for total aflatoxins using HPLC. SOP code: MYC/SOP/ 10: pp 1-5

NAFDAC Standard Operating Procedure (2000). Determination of moisture content of herbal Preparation.Central Drug and Vaccine Control (CDVCL). SOP code:HERB/SOP/3: pp 1-2.

Northholt, M.D. and Bullerman, L.B. (1982). Prevention of mould growth and toxin Production through control of environmental conditions. *Journal of Food Protection.* **45**:519-526.

Norris, J.R. and Ribbons, D.W. (1970). Measurement and control of $_{P}$H value: methods in microbiology: *Academic press London*. **2**:39-88.

Okunade, A.O. (2001) The underdevelopment of health care system in Nigeria. Faculty of Clinical Sciences and Dentistry, University of Ibadan. *Vantage publishers Ltd*. Ibadan, Nigeria: pp.43.

Okunlola, A.; Adewoyin, A.B. and Odeku, A.O. (2007). Evaluation of pharmaceutical and microbial qualities of some herbal medicinal products in South Western Nigeria. *Tropical Journal of Pharmaceutical Research.* **6**(1):661-670.

Okonkwo, P.O. and Obioru, C.N. (1981). Implication of seasonal variations in aflatoxin B1 levels in Nigerian market foods. Nutritional Cancer. **3**(1):35-1-359.

Osweiler, G. (2005). Aflatoxins and Animal Health.Veterinary Diagnostic Laboratory, Iowa State University. Retrieved May 15, 2009, from

http://nabc.ksu.edu/assets/factsheet_docs/aflatoxin/Aflatoxins%20and%20animal%20health.pdf.

Oyejola B. A. (2003). Design and analysis of experiment for Biology and agricultural student.Olad publisher.pp.35

Patterson, M. and Damogloau, P. (1986). The effect of water activity and pH on the production of mycotoxins by fungi growing on a bread analogue. *Letters in Applied Microbiology*. **3**:123-125.

Pearce, M.C., Fenlon, D., Low, J.C., Smith, A.W., Knight, H.I., Evan, J., Foster, G., Synge, B.A. and Gunn, G.J. (2004).Distribution of *Escherichia coli* o157 in Bovine faecal parts and its impact on estimates of the prevalence of faecal shedding.*Applied and Environmental microbiology.* **70**:5737-5743.

Pepper, I. L. and Gerba, C. P. (2004).Environmental microbiology.A laboratory manual, 2nd Plant nutrients. May 24-26th, 1992. Islamabad, Pakistan.607-610;pp: 666-681.

Philip, B. M.(1989). Training in mould isolation, identification, handling and evaluation of conditions leading to mycotoxin production: UNDP/FAO/THA/82/004. Reductionof post harvest losses in stored grains. Retrieved June 15, 2002 from http://www.fao.org/docrep/x5036E/x5036E00.htm.

Prescott, L.M. and Harley, J.P. (1993). Basic laboratory and culture techniques, 2nd edition.W.C.Brown Publisher.pp.14-46

Prieto, R..,Yousibova, G.L. and Woloshuk,C.P. (1996) Identification of aflatoxin biosynthesis genes by genetic complementation in an Aspergillusflavus mutant lacking the aflatoxin gene cluster. *Applied.Environmental.Microbiology*. **62:** 3567–3571.

Pickford, A.S., Catalanotto,C., Cogoni, C. and Macino, G.(2002). Quelling in *Neurosporacrassa. Advance Genetics.* **46:**277*–303.*

Pitt, J.I. (2000). Toxigenic fungi and Mycotoxins.*British Medical Bulletin*. **56**:184-192.

Reddy, B.N. and Raghavender, C.(2007).Outbreaks of Aflatoxicosis in India.*African Journal of Food, Agriculture, Nutrition and Development*.ISSN 1684-5374.

Raper, K.B.and Fennel, D.I. (1965).*The genus Aspergillus*.The Williams and Wilkins Company,Baltimore: pp. 357-404

Reddy, S.V. and Waliyar, F. (2009).Properties of Aflatoxin and It Producing Fungi. International Crops Research Institute for the Semi-Arid Tropics.Retrieved May 13, 2009, from http://www.aflatoxin.info/aflatoxin.asp.

Rizzo,I. Vedoya,G. and Maurutto, S. (2004). Assessment of toxigenic fungi on Argentinean medicinal plants.*Microbio Research*. **159**:113-120.

Roy, A.K. and Chourasia, H.K.(1989). Aflatoxin problems in some medicinal plants under storage.*International Journal of Crude Drug Research*. **27**:156-160.

Roy, A.K. and Chourasia, H.K. (1990). Mycoflora, mycotoxin producibility and mycotoxin in traditional herbal drugs in India. *Journal of General and Applied Microbiology*. **36**:295-302.

Roy, A.K. and Kumari, V. (1991). Aflatoxin and citrinin in seeds of some medicinal plan tunder storage. *International Journal of Pharmacognosy*: **29**:62-65.

Roy, A.K., Sinha, K.K. and Chourasia, H. K. (1988). Aflatoxin contamination of some commondrug plants.*Applied and Environmental Microbiology*. **54**(3):842-843.

Romer Lab. (2003). Clean-up foraflatoxin and Zearalenone (Mycosep224 aflZon:Push-through format). www.Romerlabs.com

Romer Lab method (2004). The AgraQuant total aflatoxin assay 1/20. Romer Laboratory Singapore Pte Ltd.1-4: http://www.romerlabs.com.

Saiki, R.K., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T.,Ehrhch, H.A. and Arnheim, N. (1985). Enzymatic amplification of P-globin genoinic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science.* **230**:1350–1354.

Saiki. R. K.,Gelfand, D.H., Stoffel, S.,Scharf, S.J.,Htguchl, R., Horn, G.T., Mullis, K.B. and Erlich, H.A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science.* **239**:487–491.

Samuel, O.O.(2007). Aspects of indigenous Medicine in South Western Nigeria. *Ethno-medicne*. **1**(2):127-133.

Santacroce, M.P., M.C. Conversano, E. Casalino, O. Lai, C. Zizzadoro *et al*. (2008). Aflatoxins in Aquatic Species: Metabolism, Toxicity and Perspectives. *Rev. Fish. Biol. Fisheries*. **18**:99-130.

Smela, M.E., Currier, S.S., Bailey, E.A. and Essigmann, J.M. (2001). The Chemistry and Biology of Aflatoxin B1: from Mutational Spectrometry to Carcinogenesis. *Carcinogenesis*. **22**:535-545.

Sofowora, A. (1993). Medicinal plants and Traditional medicine in Africa. Spectrum books Ltd(pub) Ibadan: pp.50-195.

Sonia, M., Companys, S., Sanchis, V., Ramos, A.J. and Magan, N. (1998). Effect of water

activity and temperature on competing abilities of common maize fungi. *Mycological Research*: **102**(8):959-964.

Tarai, B.,Gupta, A., Ray, P., Shivaprakash, M.R. and Chakrabarti, A. (2006). Polymerase chain reaction for early diagnosis of post-operative fungal endophthalmitis. *Indian.Journal of Medical.Research.* **123**:671-678.

Tassaneeyakul, W., Razzazi-Fazeli, E., Porasuphatana, S., Bohm, J. (2004). Contamination of aflatoxins in herbal medicinal products in Thailand. *Mycopathologia.* **158**:239-244.

Tartof, K.D.,and Henikoff, S. (1991). Trans-sensing effects from Drosophila to humans. *Cell*. **65:**201–*203.*

Tothill, I.E., Harris, D, Magan, N. (1992). The relationship between fungal growth and ergosterol content of wheat grain. *Mycology Research*. **96**:(11)965-970.

Traditional Herbal Medicinal Products (THMP) registration dossier requirement (2005).Definitions and terminology.***http://www.mhra.gov.uk/home/idcplq***

United States Pharmacopeia(USP)(2005) 28. ed. Rockville: United States Pharmacopeial Convention, 2005.

United State Department of Health and Human Services (2005).11th Report on Carcinogens.Substance Profiles.Aflatoxins CAS No. 1402-68-2.Retrieved May 14, 2009, from http://ntp.niehs.nih.gov/ntp/roc/eleventh/profiles/s006afla.pdf.

United Kingdom Food Standards Agency (2006). Foods Contaminated with Aflatoxins Recalled. Retrieved June 3, 2009, from http://www.food.gov.uk/news/newsarchive/2006/may/aflatoxins.

Vartika, R. and Shanta M. (2005). Toxic contamination in herbal drugs.International Society of Environmental Botanists. Environews: *News letter of ISEB India*. 11(4).

Wagacha, J.M. and J.W. Muthomi. 2008. Mycotoxin Problem in Africa: Current Status, Implications to Food Safety and Health and Possible Management Strategies. *Inernational Journal of Food Microbiology*. **124**:1-12.

Wang, J-S, T.Huang, J. Su, F. Liang, Z. Wei *et al*. 2001.Hepatocellular Carcinoma and Aflatoxin Exposure in Zhuqing Village, Fusui County, People's Republic of China.*Cancer Epidemiology, Biomarkers and Prevention*. **10**:143-146. Retrieved May 13, 2009, from http://cebp.aacrjournals.org/cgi/reprint/10/2/143.pdf.

Watson, D.H. (ed). (1998). Natural Toxicants in Food. CRC Press, New York. Preview Retrieved May 15, 2009, from http://tinyurl.com/n26dqe.

Weerasekera M.M., Sissons, C.H.,Wong, L.,Anderson, S., Holmes, A.R. andCannon R.D.(2012). Use of denaturing gradient gel electrophoresis for the identification of mixed oral yeasts in human saliva. *Journal of Medical Microbiology*. 319-330.

White, T.J., Bruns, T., Lee, S. and Taylor, J.W. (1990).Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenics. In *PCR protocols: A Guide to Methods and Application,* eds..Academic press, Inc, New York. pp315-322

Wheeler, K.A., Hurdman, B.F. and Pitt, J.J. (1991). Influence of PH on the growth of some toxigenic*Aspergillus*, *Penicillium* and *Fusarium*. *International journal of Food Microbiology*. **12**(2-3):141-149.

WHO (1978). Primary Health Care; Report of the international conference on primary health care Alma-Ata, USSR: 6-12 September, 1978.

WHO (1993).Research guidelines for evaluating the safety and efficacy of herbal medicines.World Health Organization regional office for the western pacific, Manila.

WHO (1998) Regulatory situation of herbal medicines: A world wide Review, World Health Organization, Geneva.

WHO (1999).Making a Difference. The World Health Report, World Health Organization, Geneva.

WHO (2002).Global situation report on traditional medicine.WHO executive board (111th session) Provisional agenda item 5.7.World Health Organization secretariat.

WHO (2003).Traditional medicine. Media centre world Health Organization. http://www.who.int/mediacentre/factsheets/fs134/en/

Wild, C.P. and Turner, P.C.(2002).The Toxicology of Aflatoxins as a Basis for Public Health Decisions.*Mutagenesis*. **17**:471-481.

Williams, J.H., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M. and Aggarwal, D. (2004). Human Aflatoxicosis in Developing Countries: A Review of Toxicology, Exposure, Potential Health Consequences, and Interventions. The *American Journal of.Clinical Nutrition*. **80**:1106-1122.

Wogan, G.N., Hecht, S.S., Felton, J.S., Conney, A.H. and Loeb, L.A.(2004).Environmental and Chemical Carcinogenesis. *Seminars in.Cancer Biology*. **14**:473-486.

Wolfgang, K., Erich, C. and Brigitte, K. (2002). Microbial contamination of medicinal plants – A review: *Planta Med*. **68**:5-15.

Wongwiwat, T., Ebrahim, R., Supatra, P. and Josef, B. (2004). Contamination of aflatoxins in herbal medicinal products in Thailand. *Mycopathologia*. **158** (2):239-244.

World Health Organisation (2006). Guidelines for treatment of malari Geneva WHO.

Woloshuk, C.P., Yousibova G.L, Rollins J.A, Bhatnagar D. and Payne G.A (1995). Molecular characterization of the Afl-1 locus in *Aspergillus flavus. Applied Environmental Microbiology*. **61**:3019–3023.

Yasar, A., Ahmet, D.D. and Murat, R.A. (2009). Identification of fungal genus and detection of aflatoxin level in second crop corn grain. Journal of Animal and Veterinary Advances. **8**(9):1777-1779.

You, S., Huimin, W., Xiaonan, M., Jun, G., Li, Y., and Hong, G. (2007). Morphological and molecular identification of *Aspergillus Versiculor* D-1 with selective reduction ability. *Journal of Traditional Medicines*. **2**(1): 39-44.

Zhang, Y., Chen, J. and Chen, Y.(2005). Enviromental mycological study and allergicrespiratory disease among tobacco processing workers. *Journal of Occupational Health*. **47**(2):181-187.

Zoppas, B. C., Valencia-Barrera, R.M., Vergamini Duso, S.M., and Ferna0ndez-Gonza0lez, D. (2006). Fungal spores prevalent in the aerosol of the city of Caxias do Sul, Rio Grande do Sul, Brazil, over a 2-year period (2001–2002). *Aerobiologia*. **22**:119–126.

APPENDICES

Appendix A. Physical and fungal analysis of herbal preparations forzamfara State

Sample code	Moisture content/gm of sample (%)	pH	Average fungal count/gm of sample
S-1	10.20	6.00	5.00×10^4
S-2	25.90	7.04	1.20×10^5
S-3	12.61	4.30	9.00×10^4
S-4	8.90	3.70	6.00×10^4
S-5	12.50	4.67	1.50×10^4
S-6	14.50	6.50	7.00×10^4
S-7	16.71	5.70	1.40×10^5
S-8	18.20	5.05	1.30×10^5
S-9	7.89	5.60	3.50×10^4
S-10	9.67	2.67	2.50×10^4
S-11	15.89	8.02	9.50×10^4
S-12	16.30	8.10	7.50×10^4
S-13	20.40	10.07	1.65×10^5
S-14	25.72	6.30	1.50×10^5
S-15	15.90	5.74	6.00×10^4
S-16	9.80	9.07	1.50×10^4
S-17	23.90	9.56	1.30×10^5
S-18	30.27	5.14	1.40×10^5
S-19	24.20	6.87	9.50×10^4
S-20	12.60	4.30	5.00×10^4
S-21	7.98	6.80	4.00×10^4
S-22	18.67	5.74	1.35×10^5
S-23	12.04	7.50	5.00×10^4
S-24	18.70	3.56	1.85×10^5
S-25	16.10	8.30	5.50×10^4
S-26	13.46	7.05	4.00×10^4
S-27	14.86	9.02	5.00×10^4
S-28	19.67	5.85	1.10×10^5
S-29	14.02	9.97	5.50×10^4
S-30	26.21	6.98	1.70×10^5
S-31	17.98	6.08	1.25×10^5
S-32	15.05	2.42	7.50×10^4
S-33	24.10	5.32	1.50×10^5
S-34	10.01	9.61	1.00×10^4
S-35	16.04	5.87	8.00×10^4
S-36	21.01	3.76	1.10×10^5
S-37	12.04	6.46	3.00×10^4

S-38	12.47	4.21	7.00×10^4
S-39	19.07	5.11	1.40×10^5
S-40	12.80	7.90	1.50×10^4
S-41	19.54	4.67	1.20×10^5
S-42	14.92	8.09	2.00×10^4
S-43	19.50	6.08	1.50×10^5
S-44	14.00	9.23	5.50×10^4
S-45	18.32	5.23	1.45×10^5
S-46	14.15	6.86	4.50×10^4
S-47	11.30	8.36	1.80×10^4
S-48	15.30	8.76	1.15×10^5
S-49	10.04	4.02	1.00×10^4
S-50	21.12	7.02	1.25×10^5
S-51	9.97	3.34	3.00×10^4
S-52	10.07	6.02	2.50×10^4
S-53	17.05	3.35	1.00×10^5
S-54	11.37	5.96	7.00×10^4
S-55	12.42	6.58	6.50×10^4
S-56	8.08	9.05	1.50×10^4
S-57	13.08	7.49	6.00×10^4
S-58	3.41	4.07	9.00×10^4
S-59	12.74	5.97	1.50×10^5
S-60	14.70	2.62	6.00×10^4
S-61	8.67	3.65	2.50×10^4
S-62	21.10	5.07	1.25×10^5
S-63	14.02	6.90	8.50×10^4
S-64	9.95	5.03	4.50×10^4
S-65	21.30	3.94	6.00×10^4
S-66	5.63	3.68	1.00×10^4
S-67	17.81	8.74	1.35×10^5
S-68	16.87	6.05	1.45×10^5
S-69	9.10	10.11	5.50×10^4
S-70	15.62	8.96	8.00×10^4
S-71	5.30	3.03	7.00×10^4
S-72	12.04	6.01	1.50×10^4

Appendix B. Physical and fungal analysis of herbal preparations for Katsina state

Sample code	Moisture content/gm of sample (%)	pH	Average fungal count/gm of sample
S-1	9.11	8.80	5.50×10^4
S-2	17.81	6.09	1.45×10^5
S-3	11.09	7.12	9.00×10^4
S-4	5.81	6.71	4.00×10^4
S-5	23.80	9.21	1.85×10^5
S-6	16.60	7.70	9.00×10^4
S-7	8.25	9.70	5.00×10^4
S-8	21.40	8.35	1.40×10^5
S-9	6.57	4.30	3.50×10^4
S-10	8.90	6.07	2.50×10^4
S-11	19.09	3.82	7.50×10^4
S-12	11.67	7.11	6.50×10^4
S-13	15.90	9.12	6.00×10^4
S-14	9.30	7.23	3.50×10^4
S-15	13.07	8.92	8.00×10^4
S-16	7.92	5.92	3.50×10^4
S-17	10.23	5.09	6.00×10^4
S-18	26.90	5.23	1.40×10^5
S-19	21.40	6.65	1.70×10^5
S-20	9.81	7.11	5.00×10^4
S-21	24.70	5.60	1.20×10^5
S-22	13.61	5.76	6.00×10^4
S-23	7.94	7.11	4.00×10^4
S-24	23.12	4.35	1.15×10^5
S-25	6.89	7.40	3.50×10^4
S-26	28.50	8.09	1.05×10^5
S-27	15.08	8.65	6.00×10^4
S-28	16.09	6.50	1.35×10^5
S-29	13.06	8.90	8.50×10^4
S-30	23.90	7.65	9.50×10^4
S-31	9.06	7.05	6.50×10^4
S-32	11.93	3.09	8.50×10^4
S-33	9.05	5.45	3.50×10^4
S-34	24.72	9.25	1.40×10^5
S-35	8.69	5.85	5.00×10^4
S-36	6.70	4.45	2.00×10^4
S-37	13.78	5.78	7.00×10^4
S-38	15.05	4.50	9.00×10^4

S-39	9.06	5.56	6.00×10^4
S-40	10.34	7.12	1.15×10^5
S-41	18.09	6.87	1.50×10^5
S-42	17.82	8.56	1.20×10^5
S-43	10.23	7.05	9.00×10^4
S-44	17.91	4.05	1.15×10^5
S-45	9.54	5.11	5.50×10^4
S-46	7.96	7.85	3.50×10^4
S-47	13.02	3.89	1.05×10^5
S-48	19.05	7.85	9.00×10^4
S-49	10.89	5.59	1.30×10^5
S-50	21.89	8.50	1.25×10^5
S-51	17.18	3.86	1.50×10^5
S-52	23.75	6.05	2.10×10^5
S-53	18.90	4.56	1.40×10^5
S-54	23.67	5.78	1.30×10^5
S-55	11.94	5.89	6.00×10^4
S-56	16.23	9.78	1.10×10^5
S-57	13.90	8.56	8.00×10^4
S-58	9.96	6.07	4.00×10^4
S-59	27.43	5.09	1.10×10^5
S-60	10.89	3.52	8.00×10^4
S-61	6.95	2.95	3.00×10^4
S-62	8.79	6.54	5.50×10^4
S-63	13.89	5.87	1.05×10^5
S-64	11.97	5.12	7.50×10^4
S-65	28.73	3.89	1.20×10^5
S-66	6.89	4.56	2.00×10^4
S-67	3.75	7.56	1.50×10^4
S-68	12.98	6.86	9.50×10^4
S-69	31.47	9.51	1.55×10^5
S-70	9.49	6.51	8.00×10^4
S-71	4.98	4.64	7.00×10^4
S-72	5.58	3.55	2.00×10^4

Appendix C: Physical and fungal analysis of herbal preparations for Kebbi State

Sample code	Moisture content/gm of sample (%)	pH	Average fungal count/gm of sample
S-1	11.5	5.05	1.05×10^5
S-2	24.80	9.50	1.65×10^5
S-3	13.90	6.09	8.00×10^4
S-4	7.56	4.98	6.00×10^4
S-5	21.40	8.45	1.50×10^5
S-6	23.03	7.95	1.40×10^5
S-7	12.05	8.05	9.00×10^4
S-8	9.54	8.34	1.00×10^5
S-9	18.71	6.90	9.50×10^4
S-10	6.04	6.45	3.50×10^4
S-11	11.09	4.56	8.50×10^4
S-12	10.34	7.02	1.25×10^5
S-13	21.80	8.11	1.20×10^5
S-14	7.23	7.21	4.50×10^4
S-15	8.95	6.93	5.00×10^4
S-16	16.01	7.03	1.35×10^5
S-17	14.90	9.24	1.20×10^5
S-18	11.03	5.08	1.20×10^5
S-19	6.74	5.03	4.00×10^4
S-20	6.09	9.90	5.50×10^4
S-21	13.89	11.02	1.30×10^5
S-22	9.46	7.45	4.00×10^4
S-23	5.82	5.92	3.50×10^4
S-24	10.45	6.02	1.20×10^5
S-25	8.99	6.30	4.50×10^4
S-26	20.67	9.12	1.65×10^5
S-27	20.65	8.74	1.40×10^5
S-28	15.70	5.76	1.10×10^5
S-29	11.47	8.90	1.05×10^5
S-30	8.03	7.94	5.00×10^4
S-31	6.98	5.06	3.50×10^4
S-32	17.09	6.90	1.75×10^5
S-33	11.04	4.07	1.20×10^5
S-34	13.94	6.07	1.50×10^5
S-35	7.37	7.45	2.00×10^4
S-36	14.83	4.97	2.00×10^4
S-37	24.02	6.78	1.75×10^5
S-38	9.06	5.98	5.00×10^4

S-39	9.01	5.91	7.00×10^4
S-40	17.93	9.45	1.50×10^5
S-41	9.74	4.09	5.50×10^4
S-42	11.02	7.05	1.05×10^5
S-43	21.90	5.93	1.45×10^5
S-44	28.02	9.03	1.95×10^5
S-45	6.92	4.09	3.50×10^4
S-46	5.97	4.92	5.50×10^4
S-47	12.30	4.96	1.10×10^5
S-48	13.78	9.03	1.40×10^5
S-49	5.78	9.04	5.00×10^4
S-50	7.09	8.90	9.50×10^4
S-51	13.50	6.93	1.60×10^5
S-52	9.34	8.84	7.50×10^4
S-53	5.98	10.04	6.00×10^5
S-54	21.94	8.09	1.60×10^5
S-55	10.54	8.56	7.50×10^4
S-56	24.98	5.82	1.95×10^5
S-57	12.90	7.21	1.05×10^5
S-58	8.39	6.83	3.50×10^4
S-59	21.09	6.03	1.40×10^5
S-60	16.92	5.70	1.20×10^5
S-61	18.05	6.04	1.45×10^5
S-62	18.60	9.81	1.25×10^5
S-63	11.06	4.93	9.50×10^4
S-64	10.34	4.07	8.00×10^4
S-65	8.91	6.93	1.15×10^5
S-66	13.54	5.04	1.00×10^5
S-67	12.93	7.46	9.50×10^4
S-68	9.34	5.93	8.50×10^4
S-69	10.83	7.03	1.10×10^5
S-70	14.96	8.05	1.50×10^5
S-71	23.06	9.11	1.50×10^5
S-72	11.94	6.03	1.25×10^5

Appendix D: physical and fungal analysis of herbal preparations Kaduna State

Sample code	Moisture content/gm of sample (%)	pH	Average fungal count/gm of sample
S-1	15.64	7.06	9.50×10^4
S-2	10.87	5.09	1.10×10^5
S-3	17.49	8.03	1.35×10^5
S-4	11.73	8.31	9.50×10^4
S-5	5.34	7.39	7.00×10^4
S-6	11.70	9.21	1.10×10^5
S-7	18.06	5.09	1.50×10^5
S-8	14.74	9.03	1.20×10^5
S-9	11.02	7.72	7.50×10^4
S-10	9.02	5.08	7.00×10^4
S-11	16.84	6.56	1.35×10^5
S-12	16.01	7.56	1.55×10^5
S-13	23.03	7.94	1.40×10^5
S-14	12.29	5.89	9.50×10^4
S-15	9.04	6.03	2.00×10^4
S-16	23.80	9.00	1.40×10^5
S-17	10.65	7.30	1.05×10^5
S-18	9.56	6.28	6.50×10^4
S-19	12.94	4.65	8.0×10^4
S-20	17.53	6.01	1.25×10^5
S-21	15.00	7.02	1.40×10^5
S-22	5.90	7.40	6.00×10^4
S-23	14.90	7.69	1.15×10^5
S-24	18.24	6.91	1.30×10^5
S-25	14.84	7.94	7.50×10^4
S-26	16.28	6.94	1.05×10^5
S-27	20.65	8.74	1.40×10^5
S-28	16.83	6.53	1.70×10^5
S-29	17.05	8.04	1.20×10^5
S-30	9.57	4.92	7.50×10^4
S-31	8.94	6.43	6.50×10^4
S-32	18.43	7.04	1.45×10^5
S-33	19.05	6.93	1.15×10^5
S-34	7.93	5.93	8.50×10^4
S-35	16.00	8.01	1.15×10^5
S-36	6.47	5.97	3.00×10^4
S-37	11.58	7.09	1.30×10^5
S-38	12.16	6.09	8.50×10^4

S-39	15.83	8.10	1.20×10^5
S-40	19.42	4.82	1.20×10^5
S-41	14.51	6.42	1.05×10^5
S-42	16.03	8.43	1.60×10^5
S-43	19.28	7.83	1.25×10^5
S-44	15.93	5.97	1.05×10^5
S-45	9.73	6.19	1.20×10^5
S-46	9.38	8.83	9.50×10^4
S-47	23.08	7.83	1.45×10^5
S-48	11.94	5.98	1.20×10^5
S-49	11.93	6.26	9.00×10^4
S-50	9.37	5.08	5.00×10^4
S-51	14.51	7.83	1.25×10^5
S-52	13.50	5.81	9.50×10^4
S-53	10.93	5.95	1.05×10^5
S-54	14.95	8.35	1.60×10^5
S-55	9.43	4.03	8.00×10^4
S-56	10.11	7.30	1.25×10^5
S-57	16.90	7.67	1.45×10^5
S-58	12.06	5.29	8.50×10^4
S-59	9.60	5.92	8.0×10^4
S-60	12.79	3.94	1.05×10^5
S-61	8.93	5.89	7.50×10^4
S-62	21.09	8.03	1.75×10^5
S-63	17.56	7.30	1.25×10^5
S-64	19.32	7.04	1.40×10^5
S-65	10.96	6.93	8.50×10^4
S-66	8.01	5.01	4.00×10^4
S-67	8.42	5.21	6.50×10^4
S-68	11.93	5.54	5.50×10^4
S-69	9.03	8.23	9.00×10^4
S-70	10.89	8.91	1.15×10^5
S-71	18.03	5.90	1.20×10^5
S-72	9.06	7.48	7.50×10^4

Appendix E: Physical and fungal analysis of herbal preparations Sokoto State

Sample code	Moisture content/gm of sample (%)	pH	Average fungal count/gm of sample
S-1	22.09	7.01	1.20×10^5
S-2	10.34	7.06	5.00×10^4
S-3	19.02	6.90	9.00×10^4
S-4	14.82	5.09	9.50×10^4
S-5	11.90	4.98	7.50×10^4
S-6	10.93	5.90	7.00×10^4
S-7	19.03	9.01	1.10×10^5
S-8	9.06	5.93	6.00×10^4
S-9	9.84	6.89	7.50×10^4
S-10	7.04	6.59	5.50×10^4
S-11	12.03	7.03	9.00×10^4
S-12	9.04	8.04	7.50×10^4
S-13	10.17	5.93	8.00×10^4
S-14	10.78	5.96	1.05×10^5
S-15	8.32	5.94	6.00×10^4
S-16	9.34	8.54	6.50×10^4
S-17	11.86	7.94	1.20×10^5
S-18	16.90	5.91	1.25×10^5
S-19	18.93	5.94	1.05×10^5
S-20	11.60	5.05	9.00×10^4
S-21	7.83	9.67	5.00×10^4
S-22	19.84	5.92	1.15×10^5
S-23	8.04	6.94	5.00×10^4
S-24	9.04	7.93	8.00×10^4
S-25	7.05	5.92	5.5×10^4
S-26	10.94	8.91	1.10×10^5
S-27	12.73	8.05	9.00×10^4
S-28	9.07	5.91	8.50×10^4
S-29	9.03	6.94	5.50×10^4
S-30	8.08	5.90	6.00×10^4
S-31	14.09	9.80	9.50×10^4
S-32	11.07	5.09	8.50×10^4
S-33	9.04	9.08	6.50×10^4
S-34	9.68	7.05	6.00×10^4
S-35	10.61	7.53	8.00×10^4
S-36	16.93	4.89	1.10×10^5
S-37	12.05	7.93	9.00×10^4
S-38	10.97	5.90	7.50×10^4

S-39	10.84	5.23	7.00×10^4
S-40	9.97	5.09	8.50×10^4
S-41	11.40	9.37	9.00×10^4
S-42	11.95	5.09	1.00×10^5
S-43	18.05	7.93	1.20×10^5
S-44	9.07	5.94	6.50×10^4
S-45	15.08	5.03	1.15×10^5
S-46	9.39	8.04	5.50×10^4
S-47	21.03	5.09	1.25×10^5
S-48	8.93	9.83	7.50×10^4
S-49	11.09	6.95	1.10×10^5
S-50	9.04	7.04	7.50×10^4
S-51	9.04	4.09	9.00×10^4
S-52	8.94	6.84	6.50×10^4
S-53	9.42	8.01	6.00×10^4
S-54	9.07	5.90	7.00×10^4
S-55	13.98	6.87	1.25×10^5
S-56	15.09	5.37	1.05×10^5
S-57	14.92	8.09	9.50×10^4
S-58	7.94	5.90	5.00×10^4
S-59	11.08	5.05	8.50×10^4
S-60	7.04	9.63	6.00×10^4
S-61	7.96	6.21	5.50×10^4
S-62	7.08	7.09	6.50×10^4
S-63	9.06	7.04	8.50×10^4
S-64	10.06	5.95	7.50×10^4
S-65	16.94	8.07	1.20×10^5
S-66	9.87	4.06	7.00×10^4
S-67	11.90	9.04	1.05×10^5
S-68	18.07	5.98	9.50×10^4
S-69	9.86	6.98	7.50×10^4
S-70	11.90	5.90	8.50×10^4
S-71	7.09	8.52	7.00×10^4
S-72	5.18	6.93	4.50×10^4

Appendix F: Physical and fungal analysis of herbal preparations Kano State

Sample code	Moisture content/gm of sample (%)	pH	Average fungal count/gm of sample
S-1	19.34	6.93	6.50×10^4
S-2	14.93	9.03	7.00×10^4
S-3	9.03	6.92	3.00×10^4
S-4	11.85	5.05	7.50×10^4
S-5	9.86	8.34	6.00×10^4
S-6	13.90	6.92	8.00×10^4
S-7	7.83	8.02	4.00×10^4
S-8	5.93	5.93	2.50×10^4
S-9	6.93	5.93	6.50×10^4
S-10	15.97	8.93	1.15×10^5
S-11	8.75	7.93	7.00×10^4
S-12	13.83	8.03	1.00×10^5
S-13	9.04	6.04	6.00×10^4
S-14	9.23	5.94	8.50×10^4
S-15	13.08	7.09	1.20×10^5
S-16	14.09	9.01	1.15×10^5
S-17	7.06	4.90	1.00×10^5
S-18	8.94	7.56	4.50×10^4
S-19	9.75	6.97	7.50×10^4
S-20	10.74	5.82	9.50×10^4
S-21	11.74	9.10	1.10×10^5
S-22	12.83	5.09	8.50×10^4
S-23	9.74	8.32	4.50×10^4
S-24	9.05	7.06	9.00×10^4
S-25	17.07	8.93	1.45×10^5
S-26	14.96	6.91	9.00×10^4
S-27	11.07	6.93	1.10×10^5
S-28	5.89	5.87	4.50×10^4
S-29	11.94	6.81	1.25×10^5
S-30	11.06	6.03	9.00×10^4
S-31	9.43	9.74	7.50×10^4
S-32	9.84	5.76	8.50×10^4
S-33	7.53	5.90	4.50×10^4
S-34	10.95	7.15	6.50×10^4
S-35	9.03	8.19	6.50×10^4
S-36	9.64	7.06	1.00×10^5
S-37	9.07	9.83	5.00×10^4
S-38	19.38	6.98	1.45×10^5

S-39	17.90	6.37	1.10×10^5
S-40	6.89	8.93	5.50×10^4
S-41	8.93	6.94	4.00×10^4
S-42	13.80	5.11	9.50×10^4
S-43	12.90	7.15	1.15×10^5
S-44	7.04	8.73	5.00×10^4
S-45	8.96	6.14	1.05×10^5
S-46	9.32	7.93	5.5×10^4
S-47	9.65	6.09	1.15×10^5
S-48	12.04	8.34	1.55×10^5
S-49	9.84	6.21	8.00×10^4
S-50	9.79	7.74	7.50×10^4
S-51	7.94	6.89	1.00×10^5
S-52	9.05	5.89	6.00×10^4
S-53	15.01	8.03	9.50×10^4
S-54	5.98	5.75	3.00×10^4
S-55	13.68	8.65	9.50×10^4
S-56	11.87	5.91	1.15×10^5
S-57	8.07	7.39	1.05×10^5
S-58	7.98	6.89	5.00×10^4
S-59	12.67	5.25	9.50×10^4
S-60	8.93	6.92	6.00×10^4
S-61	15.98	7.56	1.45×10^5
S-62	8.98	7.90	9.50×10^4
S-63	9.11	7.93	4.50×10^4
S-64	12.90	5.82	2.15×10^5
S-65	6.78	6.93	3.00×10^4
S-66	8.17	7.91	6.00×10^4
S-67	9.06	8.09	9.50×10^4
S-68	13.89	7.95	1.30×10^5
S-69	9.04	5.93	7.00×10^4
S-70	6.09	7.09	4.50×10^4
S-71	11.07	5.09	9.00×10^4
S-72	7.24	9.86	2.50×10^4

Appendix G: Total aflatoxin (ppb) in powdered herbal preparations in Gusau metropolis, Zamfara State

Samples	Absorbance	Total aflatoxin (ppb)
1	1.218	3.3
2	1.182	3.6
3	1.022	5.3
4	0.922	6.8
5	0.634	14.2
6	0.551	18.2
7	1.196	3.5
8	1.186	3.6
9	1.181	3.6
10	0.413	Off curve (>20)
11	0.874	7.6
12	0.596	15.9
13	1.161	3.8
14	1.184	3.6
15	1.199	3.5
16	0.561	17.5
17	0.643	13.9
18	0.927	6.7
19	1.026	5.3
20	1.207	3.4
21	0.601	15.6
22	0.876	7.6
23	0.406	Off curve (>20)
24	1.186	3.6
25	0.6	15.6
26	0.874	7.6
27	0.409	Off curve (>20)
28	1.177	3.6
29	1.17	3.7
30	1.206	3.4
31	1.223	3.3
32	0.555	17.9
33	0.65	13.6
34	0.945	6.4
35	1.045	5.0
36	1.209	3.4
37	0.534	Off curve (>20)
38	1.05	5
39	0.597	15.8
40	0.874	7.6
41	0.409	Off curve (>20)

42	1.168	3.7
43	0.644	13.8
44	0.952	6.3
45	1.036	5.2
46	1.198	3.5
47	1.19	3.5
48	1.207	3.4
49	0.806	9.0
50	1.099	4.4
51	0.989	5.8
52	0.849	8.1
53	0.606	15.4
54	0.652	13.5
55	0.58	16.6
56	0.634	14.2
57	0.838	8.3
58	0.801	9.1
59	0.716	11.4
60	0.812	8.9
61	0.629	14.4
62	0.815	8.8
63	0.938	6.5
64	1.07	4.7
65	0.812	8.9
66	0.63	14.4
67	0.577	16.7
68	0.676	12.7
69	0.805	9.1
70	0.794	9.3
71	0.714	11.4
72	0.808	9.0

Appendix H: Total aflatoxin (ppb) in powdered herbal preparations in Katsina metropolis, Katsina State

Samples	Absorbance	Total aflatoxin (ppb)
1	0.471	Off curve (>20)
2	0.524	Off curve (>20)
3	0.675	12.7
4	0.663	13.1
5	0.525	Off curve (>20)
6	0.489	Off curve (>20)
7	0.402	Off curve (>20)
8	0.467	Off curve (>20)
9	0.434	Off curve (>20)
10	0.578	16.7
11	0.194	Off curve (>20)
12	0.357	Off curve (>20)
13	0.473	Off curve (>20)
14	0.444	Off curve (>20)
15	0.501	Off curve (>20)
16	0.516	Off curve (>20)
17	0.650	13.6
18	0.544	18.5
19	0.522	Off curve (>20)
20	0.400	Off curve (>20)
21	0.436	Off curve (>20)
22	0.337	Off curve (>20)
23	0.193	Off curve (>20)
24	0.396	Off curve (>20)
25	0.647	13.7
26	0.532	Off curve (>20)
27	0.626	14.5
28	0.793	9.3
29	1.101	4.4
30	0.965	6.1
31	0.817	8.8
32	0.602	15.6
33	0.801	9.2
34	0.790	9.4
35	0.695	12.0
36	0.807	9.0
37	0.439	Off curve (>20)
38	0.531	Off curve (>20)
39	0.586	16.3
40	0.529	Off curve (>20)
41	0.514	Off curve (>20)

42	0.496	Off curve (>20)
43	0.441	Off curve (>20)
44	0.503	Off curve (>20)
45	0.347	Off curve (>20)
46	0.348	Off curve (>20)
47	0.188	Off curve (>20)
48	0.409	Off curve (>20)
49	0.364	Off curve (>20)
50	0.471	Off curve (>20)
51	0.577	16.8
52	0.468	Off curve (>20)
53	0.460	Off curve (>20)
54	0.377	Off curve (>20)
55	0.345	Off curve (>20)
56	0.386	Off curve (>20)
57	0.367	Off curve (>20)
58	0.228	Off curve (>20)
59	0.300	Off curve (>20)
60	0.250	Off curve (>20)
61	0.415	Off curve (>20)
62	0.389	Off curve (>20)
63	0.370	Off curve (>20)
64	0.455	Off curve (>20)
65	0.408	Off curve (>20)
66	0.594	15.6
67	0.481	Off curve (>20)
68	0.377	Off curve (>20)
69	0.364	Off curve (>20)
70	0.091	Off curve (>20)
71	0.307	Off curve (>20)
72	0.295	Off curve (>20)

Appendix I: Total aflatoxin (ppb) in powdered herbal preparations in BirninKebbi metropolis, Kebbi State

Samples	Absorbance	Total aflatoxin(ppb)
1	0.726	11.1
2	0.409	Off curve (>20)
3	0.481	Off curve (>20)
4	0.754	10.3
5	1.388	2.1
6	0.984	5.8
7	0.731	11
8	0.339	Off curve (>20)
9	0.505	Off curve (>20)
10	0.861	7.9
11	0.789	9.4
12	0.470	Off curve (>20)
13	0.729	11
14	0.546	18.4
15	0.433	Off curve (>20)
16	0.782	9.6
17	1.413	2.0
18	0.861	7.9
19	0.761	10.1
20	0.506	Off curve (>20)
21	0.523	Off curve (>20)
22	0.881	7.5
23	0.778	9.7
24	0.472	Off curve (>20)
25	0.505	Off curve (>20)
26	0.845	8.2
27	0.943	6.4
28	0.545	18.4
29	0.728	11.0
30	0.567	17.2
31	0.447	Off curve (>20)
32	0.742	10.6
33	1.405	2.0
34	0.854	8.0
35	0.756	10.3
36	0.502	Off curve (>20)
37	0.511	Off curve (>20)
38	0.755	10.3
39	0.849	8.1
40	1.406	2.0
41	0.719	11.3

42	0.473	Off curve (>20)
43	0.576	16.8
44	0.746	10.5
45	0.489	Off curve (>20)
46	0.796	9.2
47	0.858	7.9
48	0.535	Off curve (>20)
49	0.502	Off curve (>20)
50	0.833	8.4
51	0.916	6.9
52	0.524	Off curve (>20)
53	0.499	Off curve (>20)
54	0.718	11.3
55	0.863	7.8
56	1.413	2.0
57	0.733	10.9
58	0.461	Off curve (>20)
59	0.573	16.9
60	0.720	11.3
61	0.736	10.8
62	0.846	8.2
63	0.877	7.6
64	0.571	17.0
65	0.526	Off curve (>20)
66	0.726	11.1
67	0.885	7.4
68	1.424	1.9
69	0.736	10.8
70	0.473	Off curve (>20)
71	0.601	15.6
72	0.466	Off curve (>20)

Appendix J: Total aflatoxin (ppb) in powdered herbal preparations in Kaduna metropolis, Kaduna State

Samples	Absorbance	Total aflatoxin (ppb)
1	0.381	Off curve (>20)
2	0.454	Off curve (>20)
3	0.42	Off curve (>20)
4	0.736	10.8
5	1.078	4.6
6	0.891	7.3
7	0.747	10.5
8	0.399	Off curve (>20)
9	0.105	Off curve (>20)
10	0.003	Off curve (>20)
11	0.003	Off curve (>20)
12	0.003	Off curve (>20)
13	0.385	Off curve (>20)
14	0.752	10.4
15	0.944	6.4
16	1.091	4.5
17	0.743	10.6
18	0.432	Off curve (>20)
19	0.427	Off curve (>20)
20	0.386	Off curve (>20)
21	0.348	Off curve (>20)
22	0.965	6.1
23	0.963	6.1
24	0.476	Off curve (>20)
25	0.418	Off curve (>20)
26	0.913	6.9
27	0.983	5.8
28	0.353	Off curve (>20)
29	0.378	Off curve (>20)
30	0.386	Off curve (>20)
31	0.456	Off curve (>20)
32	0.686	12.3
33	1.137	4.0
34	1.010	5.5
35	0.755	10.3
36	0.363	Off curve (>20)
37	0.411	Off curve (>20)
38	0.912	6.9
39	0.945	6.4
40	0.404	Off curve (>20)
41	0.393	Off curve (>20)

42	0.403	Off curve (>20)
43	0.508	Off curve (>20)
44	0.704	11.7
45	1.095	4.5
46	0.906	7.1
47	0.752	10.4
48	0.378	Off curve (>20)
49	0.729	11
50	0.437	Off curve (>20)
51	0.536	Off curve (>20)
52	0.851	8.1
53	1.044	5.1
54	0.809	9.0
55	0.741	10.7
56	0.399	Off curve (>20)
57	0.376	21
58	0.940	6.5
59	0.621	14.8
60	0.502	Off curve (>20)
61	0.639	14
62	0.829	8.5
63	0.828	8.6
64	0.529	Off curve (>20)
65	0.591	16.1
66	0.399	Off curve (>20)
67	0.409	Off curve (>20)
68	0.869	7.7
69	1.385	2.1
70	0.987	5.8
71	0.787	9.5
72	0.362	Off curve (>20)

Appendix K: Total aflatoxin (ppb) in powdered herbal preparations in Sokoto metropolis, Sokoto State

Samples	Absorbance	Total aflatoxin(ppb)
1	0.583	16.5
2	0.694	12.1
3	0.919	6.8
4	1.466	1.7
5	0.772	9.8
6	0.541	Off curve (>20)
7	0.59	16.1
8	0.704	11.7
9	0.530	Off curve (>20)
10	0.863	7.8
11	0.857	7.9
12	0.733	10.9
13	0.662	13.1
14	0.611	15.2
15	0.530	Off curve (>20)
16	0.775	9.8
17	1.469	1.7
18	0.966	6.1
19	0.713	11.5
20	0.579	16.6
21	0.530	Off curve (>20)
22	0.831	8.5
23	0.835	8.4
24	0.810	8.9
25	0.632	14.3
26	0.602	15.5
27	0.535	Off curve (>20)
28	0.783	9.6
29	1.472	1.6
30	0.968	6.1
31	0.701	11.9
32	0.594	15.9
33	0.807	9.0
34	0.856	8.0
35	0.830	8.5
36	0.541	Off curve (>20)
37	0.683	12.4
38	0.851	8.1
39	0.734	10.9
40	0.761	10.1
41	0.605	15.5

42	0.813	8.9
43	0.959	6.2
44	1.081	4.6
45	0.804	9.1
46	0.630	14.4
47	0.526	Off curve (>20)
48	0.660	13.2
49	0.762	10.1
50	0.673	12.8
51	0.766	10.0
52	0.769	9.9
53	0.599	15.7
54	0.783	9.6
55	0.968	6.1
56	1.094	4.5
57	0.793	9.3
58	0.622	14.7
59	0.534	Off curve (>20)
60	0.665	13.1
61	0.778	9.7
62	0.852	8.1
63	0.735	10.8
64	0.775	9.8
65	0.675	12.7
66	0.541	Off curve (>20)
67	0.619	14.8
68	0.794	9.3
69	1.093	4.5
70	0.963	6.1
71	0.830	8.5
72	0.596	15.9

Appendix L: Total aflatoxin(ppb) in powdered herbal preparations in Kano metropolis, Kano State

Samples	Absorbance	Total aflatoxin (ppb)
1	0.529	Off curve (>20)
2	0.738	10.8
3	0.861	7.9
4	1.434	1.8
5	0.726	11.1
6	0.470	Off curve (>20)
7	0.581	16.5
8	0.592	16.0
9	0.731	10.9
10	0.803	9.1
11	0.845	8.2
12	0.529	Off curve (>20)
13	0.579	16.6
14	0.584	16.4
15	0.456	Off curve (>20)
16	0.732	10.9
17	1.439	1.8
18	0.918	6.8
19	0.752	10.4
20	0.518	Off curve (>20)
21	0.500	Off curve (>20)
22	0.844	8.2
23	0.807	9.0
24	0.746	10.5
25	0.744	10.6
26	0.881	7.5
27	0.889	7.3
28	0.513	Off curve (>20)
29	0.563	17.4
30	0.591	16.0
31	0.454	Off curve (>20)
32	0.751	10.4
33	1.446	1.8
34	0.903	7.1
35	0.735	10.8
36	0.518	Off curve (>20)
37	0.585	16.4
38	0.613	15.1
39	0.467	Off curve (>20)
40	0.750	10.4
41	1.456	1.7

42	0.917	6.9
43	0.673	12.8
44	0.515	Off curve (>20)
45	0.732	10.9
46	0.807	9.0
47	0.801	9.1
48	0.589	16.1
49	0.835	8.4
50	0.878	7.6
51	0.843	8.2
52	0.593	16
53	0.514	Off curve (>20)
54	0.656	13.4
55	0.950	6.3
56	1.474	1.6
57	0.755	10.3
58	0.465	Off curve (>20)
59	0.652	13.5
60	0.650	13.6
61	0.597	15.8
62	0.799	9.2
63	0.813	8.9
64	0.801	9.1
65	0.526	Off curve (>20)
66	0.672	12.8
67	0.951	6.3
68	1.481	1.6
69	0.780	9.6
70	0.461	Off curve (>20)
71	0.634	14.2
72	0.662	13.1

AppendiceM: Determination of correlation between moisture content and fungal loads for samples from six states of the North West of Nigeria

Correlation between moisture content and fungal load for Zamfara samples

```
CORRELATIONS /VARIABLES=MOISTURE_A PH_A FUNGAL_A
/PRINT=TWOTAIL NOSIG /MISSING=PAIRWISE.
```

Correlations

Notes

	Output Created	06-Nov-2011 14:06:02
	Comments	
Input	Data	D:\SPSS DATA 2011\DANLADI NAFDAC 1.sav
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	N of Rows in Working Data File	432
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each pair of variables are based on all the cases with valid data for that pair.
	Syntax	CORRELATIONS /VARIABLES=MOISTURE_A PH_A FUNGAL_A /PRINT=TWOTAIL NOSIG /MISSING=PAIRWISE.
Resources	Processor Time	0:00:00.078
	Elapsed Time	0:00:00.070

[DataSet1] A:\SPSS DATA 2011\DANLADI 1.sav

Correlations

		MOISTURE_A	PH_A	FUNGAL_A
MOISTURE_A	Pearson Correlation	1	.061	.669**
	Sig. (2-tailed)		.610	.000
	N	72	72	72
PH_A	Pearson Correlation	.061	1	-.056
	Sig. (2-tailed)	.610		.643
	N	72	72	72
FUNGAL_A	Pearson Correlation	.669**	-.056	1
	Sig. (2-tailed)	.000	.643	
	N	72	72	72

**. Correlation is significant at the 0.01 level (2-tailed).

Correlation for Samples from Katsina State

CORRELATIONS /VARIABLES=MOISTURE_B PH_B FUNGAL_B
/PRINT=TWOTAIL NOSIG /MISSING=PAIRWISE.

Correlations

Notes

	Output Created	06-Nov-2011 14:06:26
	Comments	
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	N of Rows in Working Data File	432
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each pair of variables are based on all the cases with valid data for that pair.
	Syntax	CORRELATIONS /VARIABLES=MOISTURE_B PH_B FUNGAL_B /PRINT=TWOTAIL NOSIG /MISSING=PAIRWISE.
Resources	Processor Time	0:00:00.156
	Elapsed Time	0:00:00.320

[DataSet1] B:\SPSS DATA 2011\DANLADI NAFDAC 1.sav

Correlations

		MOISTURE_B	PH_B	FUNGAL_B
MOISTURE_B	Pearson Correlation	1	.172	.670**
	Sig. (2-tailed)		.149	.000
	N	72	72	72
PH_B	Pearson Correlation	.172	1	.183
	Sig. (2-tailed)	.149		.124
	N	72	72	72
FUNGAL_B	Pearson Correlation	.670**	.183	1
	Sig. (2-tailed)	.000	.124	
	N	72	72	72

**. Correlation is significant at the 0.01 level (2-tailed).

Correlation for samples from BirninKebbi

CORRELATIONS /VARIABLES=MOISTURE_C PH_C FUNGAL_C
/PRINT=TWOTAIL NOSIG /MISSING=PAIRWISE.

Correlations

Notes

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Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each pair of variables are based on all the cases with valid data for that pair.
	Syntax	CORRELATIONS /VARIABLES=MOISTURE_C PH_C FUNGAL_C /PRINT=TWOTAIL NOSIG /MISSING=PAIRWISE.
Resources	Processor Time	0:00:00.281
	Elapsed Time	0:00:00.320

[DataSet1] C:\SPSS DATA 2011\DANLADI NAFDAC 1.sav

Correlations

		MOISTURE_C	PH_C	FUNGAL_C
MOISTURE_C	Pearson Correlation	1	.262*	.752**
	Sig. (2-tailed)		.026	.000
	N	72	72	72
PH_C	Pearson Correlation	.262*	1	.239*
	Sig. (2-tailed)	.026		.043
	N	72	72	72
FUNGAL_C	Pearson Correlation	.752**	.239*	1
	Sig. (2-tailed)	.000	.043	
	N	72	72	72

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Correlation for samples from Kaduna State

CORRELATIONS /VARIABLES=MOISTURE_D PH_D FUNGAL_D
/PRINT=TWOTAIL NOSIG /MISSING=PAIRWISE.

Correlations

Notes

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	N of Rows in Working Data File	432
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each pair of variables are based on all the cases with valid data for that pair.
	Syntax	CORRELATIONS /VARIABLES=MOISTURE_D PH_D FUNGAL_D /PRINT=TWOTAIL NOSIG /MISSING=PAIRWISE.
Resources	Processor Time	0:00:00.078
	Elapsed Time	0:00:00.181

[DataSet1] D:\SPSS DATA 2011\DANLADI NAFDAC 1.sav

Correlations

		MOISTURE_D	PH_D	FUNGAL_D
MOISTURE_D	Pearson Correlation	1	.205	.021
	Sig. (2-tailed)		.084	.858
	N	72	72	72
PH_D	Pearson Correlation	.205	1	.130
	Sig. (2-tailed)	.084		.276
	N	72	72	72
FUNGAL_D	Pearson Correlation	.021	.130	1
	Sig. (2-tailed)	.858	.276	
	N	72	72	72

Correlation for samples from Sokoto State

CORRELATIONS /VARIABLES=MOISTURE_E PH_E FUNGAL_E
/PRINT=TWOTAIL NOSIG /MISSING=PAIRWISE.

Correlations

Notes

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	N of Rows in Working Data File	432
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each pair of variables are based on all the cases with valid data for that pair.
	Syntax	CORRELATIONS /VARIABLES=MOISTURE_E PH_E FUNGAL_E /PRINT=TWOTAIL NOSIG /MISSING=PAIRWISE.
Resources	Processor Time	0:00:00.171
	Elapsed Time	0:00:00.320

[DataSet1] E:\SPSS DATA 2011\DANLADI NAFDAC 1.sav

Correlations

		MOISTURE_E	PH_E	FUNGAL_E
MOISTURE_E	Pearson Correlation	1	-.125	.804**
	Sig. (2-tailed)		.296	.000
	N	72	72	72
PH_E	Pearson Correlation	-.125	1	-.094
	Sig. (2-tailed)	.296		.434
	N	72	72	72
FUNGAL_E	Pearson Correlation	.804**	-.094	1
	Sig. (2-tailed)	.000	.434	
	N	72	72	72

**. Correlation is significant at the 0.01 level (2-tailed).

Correlation for samples from Kano State

CORRELATIONS /VARIABLES=MOISTURE_F PH_F FUNGAL_F
/PRINT=TWOTAIL NOSIG /MISSING=PAIRWISE.

Correlations

Notes

	Output Created	06-Nov-2011 14:08:26
	Comments	
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	Active Dataset	DataSet1
	Filter	<none>
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	Split File	<none>
	N of Rows in Working Data File	432
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each pair of variables are based on all the cases with valid data for that pair.
	Syntax	CORRELATIONS /VARIABLES=MOISTURE_F PH_F FUNGAL_F /PRINT=TWOTAIL NOSIG /MISSING=PAIRWISE.
Resources	Processor Time	0:00:00.078
	Elapsed Time	0:00:00.120

[DataSet1] F:\SPSS DATA 2011\DANLADI NAFDAC 1.sav

Correlations

		MOISTURE_F	PH_F	FUNGAL_F
MOISTURE_F	Pearson Correlation	1	.092	.609**
	Sig. (2-tailed)		.441	.000
	N	72	72	72
PH_F	Pearson Correlation	.092	1	-.003
	Sig. (2-tailed)	.441		.982
	N	72	72	72
FUNGAL_F	Pearson Correlation	.609**	-.003	1
	Sig. (2-tailed)	.000	.982	
	N	72	72	72

**. Correlation is significant at the 0.01 level (2-tailed).

Appendice N: Correlation between the aflatoxin concentration and fungal load in samples from the six states of North Western Nigeria

```
correlations statistic
  /VARIABLES=Aflatoxin_conc Fungal_ld
  /PRINT=TWOTAIL NOSIG
  /STATISTICS DESCRIPTIVES
  /MISSING=PAIRWISE.
```

Notes

Output Created		20-Mar-2012 12:53:42
Comments		
Input	Active Dataset	DataSet0
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	432
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each pair of variables are based on all the cases with valid data for that pair.
Syntax		CORRELATIONS /VARIABLES=Aflatoxin_conc Fungal_ld /PRINT=TWOTAIL NOSIG /STATISTICS DESCRIPTIVES /MISSING=PAIRWISE.
Resources	Processor Time	00 00:00:00.031
	Elapsed Time	00 00:00:00.156

[DataSet0]

Descriptive Statistics

	Mean	Std. Deviation	N
Aflatoxin Concentration (ppb)	13.2926	6.37935	432
Fungal Load	90608.8241	47074.42673	432

Correlations

		Aflatoxin Concentration (ppb)	Fungal Load
Aflatoxin Concentration (ppm)	Pearson Correlation	1	.123*
	Sig. (2-tailed)		.011
	N	432	432
Fungal Load	Pearson Correlation	.123*	1
	Sig. (2-tailed)	.011	
	N	432	432

*. Correlation is significant at the 0.05 level (2-tailed).

Appendix O: T-test analysis of samples collected at different seasons (Wet and Dry)from different locations in Six states' metropolis of North Western Nigeria

1. Zamfara metropolis;

Location 1: Labilabi

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	90833.33	63199.420	25801.055
	Dry season	6	83333.33	34448.028	14063.349
Total aflatoxin/gm(PPb)	Rainy season	6	12.967	7.5625	3.0874
	Dry season	6	6.067	4.7420	1.9359

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	5.625	.039
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	2.596	.138
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	.255	10	0.804
	Equal variances not assumed	.255	7.730	0.805
Total aflatoxin/gm(PPb)	Equal variances assumed	1.893	10	0.088
	Equal variances not assumed	1.893	8.405	0.093

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	7500.000	29384.898
	Equal variances not assumed	7500.000	29384.898
Total aflatoxin/gm(PPb)	Equal variances assumed	6.9000	3.6441
	Equal variances not assumed	6.9000	3.6441

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-57973.633	72973.633
	Equal variances not assumed	-60675.856	75675.856
Total aflatoxin/gm(PPb)	Equal variances assumed	-1.2196	15.0196
	Equal variances not assumed	-1.4333	15.2333

Location 2: Central market

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	107500.00	47827.816	19525.624
	Dry season	6	60833.33	20351.085	8308.296
Total aflatoxin/gm(PPb)	Rainy season	6	10.850	8.2861	3.3828
	Dry season	6	4.633	1.3216	.5395

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	9.275	.012
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	79.794	.000
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	2.199	10	.053
	Equal variances not assumed	2.199	6.753	.065
Total aflatoxin/gm(PPb)	Equal variances assumed	1.815	10	.100
	Equal variances not assumed	1.815	5.254	.126

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	46666.667	21219.750
	Equal variances not assumed	46666.667	21219.750
Total aflatoxin/gm(PPb)	Equal variances assumed	6.2167	3.4255
	Equal variances not assumed	6.2167	3.4255

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-613.882	93947.215
	Equal variances not assumed	-3884.370	97217.703
Total aflatoxin/gm(PPb)	Equal variances assumed	-1.4159	13.8492
	Equal variances not assumed	-2.4623	14.8957

Location 3: Old market

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	100000.00	50596.443	20655.911
	Dry season	6	75500.00	41707.314	17026.939
Total aflatoxin/gm(PPb)	Rainy season	6	10.650	4.4406	1.8129
	Dry season	6	4.567	2.2518	.9193

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	.191	.672
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	8.541	.015
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	.915	10	.382
	Equal variances not assumed	.915	9.649	.382
Total aflatoxin/gm(PPb)	Equal variances assumed	2.993	10	0.014*
	Equal variances not assumed	2.993	7.412	0.019*

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	24500.000	26769.074
	Equal variances not assumed	24500.000	26769.074
Total aflatoxin/gm(PPb)	Equal variances assumed	6.0833	2.0326
	Equal variances not assumed	6.0833	2.0326

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-35145.214	84145.214
	Equal variances not assumed	-35440.966	84440.966
Total aflatoxin/gm(PPb)	Equal variances assumed	1.5543	10.6123
	Equal variances not assumed	1.3305	10.8361

Location 4: Tudun Wada

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	92500.00	55744.955	22757.783
	Dry season	6	39166.67	26536.139	10833.333
Total aflatoxin/gm(PPb)	Rainy season	6	13.267	3.5217	1.4377
	Dry season	6	7.767	2.0897	.8531

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	7.314	.022
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	.738	.410
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	2.116	10	.060
	Equal variances not assumed	2.116	7.155	.071
Total aflatoxin/gm(PPb)	Equal variances assumed	3.290	10	.008
	Equal variances not assumed	3.290	8.132	.011

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	53333.333	25204.717
	Equal variances not assumed	53333.333	25204.717
Total aflatoxin/gm(PPb)	Equal variances assumed	5.5000	1.6718
	Equal variances not assumed	5.5000	1.6718

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-2826.277	109492.943
	Equal variances not assumed	-6005.044	112671.711
Total aflatoxin/gm(PPb)	Equal variances assumed	1.7750	9.2250
	Equal variances not assumed	1.6557	9.3443

Location 5: Hospital road

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	75000.00	56920.998	23237.900
	Dry season	6	63333.33	46547.467	19002.924
Total aflatoxin/gm(PPb)	Rainy season	6	17.033	3.2556	1.3291
	Dry season	6	8.167	.6653	.2716

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	.654	.437
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	11.440	.007
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	.389	10	.706
	Equal variances not assumed	.389	9.621	.706
Total aflatoxin/gm(PPb)	Equal variances assumed	6.536	10	.000

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	.389	10	.706
	Equal variances not assumed	.389	9.621	.706
Total aflatoxin/gm(PPb)	Equal variances assumed	6.536	10	.000
	Equal variances not assumed	6.536	5.417	.001

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	11666.667	30018.513
	Equal variances not assumed	11666.667	30018.513
Total aflatoxin/gm(PPb)	Equal variances assumed	8.8667	1.3565
	Equal variances not assumed	8.8667	1.3565

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-55218.748	78552.081
	Equal variances not assumed	-55577.778	78911.112
Total aflatoxin/gm(PPb)	Equal variances assumed	5.8441	11.8892
	Equal variances not assumed	5.4588	12.2746

Location 6: Lalan area

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	91666.67	72018.516	29401.436
	Dry season	6	74166.67	36526.246	14911.778
Total aflatoxin/gm(PPb)	Rainy season	6	11.333	6.0961	2.4887

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	91666.67	72018.516	29401.436
	Dry season	6	74166.67	36526.246	14911.778
Total aflatoxin/gm(PPb)	Rainy season	6	11.333	6.0961	2.4887
	Dry season	6	5.517	2.1311	.8700

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	4.815	.053
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	2.372	.155
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	.531	10	.607
	Equal variances not assumed	.531	7.413	.611
Total aflatoxin/gm(PPb)	Equal variances assumed	2.206	10	.052
	Equal variances not assumed	2.206	6.204	.068

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	17500.000	32966.734
	Equal variances not assumed	17500.000	32966.734
Total aflatoxin/gm(PPb)	Equal variances assumed	5.8167	2.6364
	Equal variances not assumed	5.8167	2.6364

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-55954.461	90954.461
	Equal variances not assumed	-59582.775	94582.775
Total aflatoxin/gm(PPb)	Equal variances assumed	-.0576	11.6910
	Equal variances not assumed	-.5833	12.2167

2. Kaduna metropolis

Location 1: Kawo garage

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	125833.33	31530.409	12872.235
	Dry season	6	101666.67	19916.492	8130.874
Total aflatoxin/gm(PPb)	Rainy season	6	18.917	5.1031	2.0833
	Dry season	6	11.967	4.9698	2.0289

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	.275	.611
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	.014	.908
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	1.587	10	.144
	Equal variances not assumed	1.587	8.442	.149
Total aflatoxin/gm(PPb)	Equal variances assumed	2.390	10	.038
	Equal variances not assumed	2.390	9.993	.038

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	24166.667	15225.162
	Equal variances not assumed	24166.667	15225.162
Total aflatoxin/gm(PPb)	Equal variances assumed	6.9500	2.9080
	Equal variances not assumed	6.9500	2.9080

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-9757.108	58090.441
	Equal variances not assumed	-10625.218	58958.551
Total aflatoxin/gm(PPb)	Equal variances assumed	.4705	13.4295
	Equal variances not assumed	.4699	13.4301

Location 2: Central market

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	96668.67	49861.836	20356.009
	Dry season	6	91666.67	24832.774	10137.938
Total aflatoxin/gm(PPb)	Rainy season	6	18.517	6.0829	2.4833
	Dry season	6	10.050	5.7120	2.3319

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	.784	.397
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	.011	.918
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	.220	10	.830
	Equal variances not assumed	.220	7.337	.832
Total aflatoxin/gm(PPb)	Equal variances assumed	2.485	10	.032
	Equal variances not assumed	2.485	9.961	.032

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	5002.000	22740.820
	Equal variances not assumed	5002.000	22740.820
Total aflatoxin/gm(PPb)	Equal variances assumed	8.4667	3.4066
	Equal variances not assumed	8.4667	3.4066

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-45667.705	55671.705
	Equal variances not assumed	-48275.381	58279.381
Total aflatoxin/gm(PPb)	Equal variances assumed	.8763	16.0570

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-45667.705	55671.705
	Equal variances not assumed	-48275.381	58279.381
Total aflatoxin/gm(PPb)	Equal variances assumed	.8763	16.0570
	Equal variances not assumed	.8723	16.0611

Location 3: Kasuwar bacci

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	127500.00	30781.488	12566.490
	Dry season	6	70000.00	25298.221	10327.956
Total aflatoxin/gm(PPb)	Rainy season	6	13.083	6.8738	2.8062
	Dry season	6	12.750	7.4739	3.0512

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	.610	.453
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	.371	.556
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	3.535	10	.005
	Equal variances not assumed	3.535	9.638	.006
Total aflatoxin/gm(PPb)	Equal variances assumed	.080	10	.937
	Equal variances not assumed	.080	9.931	.938

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	57500.000	16266.018
	Equal variances not assumed	57500.000	16266.018
Total aflatoxin/gm(PPb)	Equal variances assumed	.3333	4.1455
	Equal variances not assumed	.3333	4.1455

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	21257.054	93742.946
	Equal variances not assumed	21071.882	93928.118
Total aflatoxin/gm(PPb)	Equal variances assumed	-8.9033	9.5700
	Equal variances not assumed	-8.9120	9.5787

Location 4: U/Rimi

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	134166.67	12416.387	5068.969
	Dry season	6	96666.67	15055.453	6146.363
Total aflatoxin/gm(PPb)	Rainy season	6	17.800	4.9900	2.0372
	Dry season	6	12.883	6.7804	2.7681

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	.004	.949
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	.566	.469

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	134166.67	12416.387	5068.969
	Dry season	6	96666.67	15055.453	6146.363
Total aflatoxin/gm(PPb)	Rainy season	6	17.800	4.9900	2.0372
	Equal variances not assumed				

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	4.707	10	.001
	Equal variances not assumed	4.707	9.650	.001
Total aflatoxin/gm(PPb)	Equal variances assumed	1.431	10	.183
	Equal variances not assumed	1.431	9.188	.186

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	37500.000	7966.946
	Equal variances not assumed	37500.000	7966.946
Total aflatoxin/gm(PPb)	Equal variances assumed	4.9167	3.4369
	Equal variances not assumed	4.9167	3.4369

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	19748.539	55251.461
	Equal variances not assumed	19660.948	55339.052
Total aflatoxin/gm(PPb)	Equal variances assumed	-2.7412	12.5746
	Equal variances not assumed	-2.8340	12.6673

Location 5: Mando motor park

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	135000.00	90774.446	37058.512
	Dry season	6	76666.67	16020.820	6540.472
Total aflatoxin/gm(PPb)	Rainy season	6	21.000	.0000	.0000
	Dry season	6	11.633	7.9776	3.2569

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	2.712	.131
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	13.884	.004
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	1.550	10	.152
	Equal variances not assumed	1.550	5.311	.178
Total aflatoxin/gm(PPb)	Equal variances assumed	2.876	10	.016
	Equal variances not assumed	2.876	5.000	.035

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	58333.333	37631.252
	Equal variances not assumed	58333.333	37631.252
Total aflatoxin/gm(PPb)	Equal variances assumed	9.3667	3.2569
	Equal variances not assumed	9.3667	3.2569

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-25514.321	142180.987
	Equal variances not assumed	-36720.757	153387.424
Total aflatoxin/gm(PPb)	Equal variances assumed	2.1099	16.6234
	Equal variances not assumed	.9947	17.7387

Location 6: Malali market

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	131666.67	19916.492	8130.874
	Dry season	6	95000.00	32710.854	13354.150
Total aflatoxin/gm(PPb)	Rainy season	6	16.400	7.4081	3.0243
	Dry season	6	8.600	6.1864	2.5256

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	2.379	.154
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	.872	.372
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	2.345	10	.041
	Equal variances not assumed	2.345	8.259	.046
Total aflatoxin/gm(PPb)	Equal variances assumed	1.980	10	.076
	Equal variances not assumed	1.980	9.692	.077

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	36666.667	15634.719
	Equal variances not assumed	36666.667	15634.719
Total aflatoxin/gm(PPb)	Equal variances assumed	7.8000	3.9402
	Equal variances not assumed	7.8000	3.9402

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	1830.341	71502.992
	Equal variances not assumed	808.975	72524.358
Total aflatoxin/gm(PPb)	Equal variances assumed	-.9794	16.5794
	Equal variances not assumed	-1.0173	16.6173

3. Kano metropolis.

Location 1: Airport rd/Queen Cinema

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	80833.33	28180.963	11504.830
	Dry season	6	170000.00	211045.019	86158.768
Total aflatoxin/gm(PPb)	Rainy season	6	16.500	5.5267	2.2563
	Dry season	6	12.683	7.3219	2.9891

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	4.449	.061
	Equal variances not assumed		

Total aflatoxin/gm(PPb)	Equal variances assumed	.285	.605
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	-1.026	10	.329
	Equal variances not assumed	-1.026	5.178	.350
Total aflatoxin/gm(PPb)	Equal variances assumed	1.019	10	.332
	Equal variances not assumed	1.019	9.301	.334

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	-89166.667	86923.498
	Equal variances not assumed	-89166.667	86923.498
Total aflatoxin/gm(PPb)	Equal variances assumed	3.8167	3.7451
	Equal variances not assumed	3.8167	3.7451

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-282844.289	104510.956
	Equal variances not assumed	-310316.912	131983.578
Total aflatoxin/gm(PPb)	Equal variances assumed	-4.5279	12.1612
	Equal variances not assumed	-4.6136	12.2470

Location 2: Sabon gari market

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	68333.33	39072.582	15951.315
	Dry season	6	62500.00	20676.073	8440.972
Total aflatoxin/gm(PPb)	Rainy season	6	18.133	3.3049	1.3492
	Dry season	6	7.650	3.0540	1.2468

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	5.797	.037
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	.865	.374
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	.323	10	.753
	Equal variances not assumed	.323	7.597	.755
Total aflatoxin/gm(PPb)	Equal variances assumed	5.706	10	.000
	Equal variances not assumed	5.706	9.938	.000

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	5833.333	18047.007
	Equal variances not assumed	5833.333	18047.007
Total aflatoxin/gm(PPb)	Equal variances assumed	10.4833	1.8371
	Equal variances not assumed	10.4833	1.8371

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-34377.903	46044.570
	Equal variances not assumed	-36170.953	47837.620
Total aflatoxin/gm(PPb)	Equal variances assumed	6.3900	14.5767
	Equal variances not assumed	6.3866	14.5801

Location 3: Anguwa Uku garage

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	84166.67	44205.957	18047.007
	Dry season	6	70833.33	25771.431	10521.143
Total aflatoxin/gm(PPb)	Rainy season	6	14.383	7.3175	2.9874
	Dry season	6	9.017	4.5692	1.8654

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	4.175	.068
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	1.066	.326
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	.638	10	.538
	Equal variances not assumed	.638	8.047	.541
Total aflatoxin/gm(PPb)	Equal variances assumed	1.524	10	.159
	Equal variances not assumed	1.524	8.385	.164

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	13333.333	20889.923
	Equal variances not assumed	13333.333	20889.923
Total aflatoxin/gm(PPb)	Equal variances assumed	5.3667	3.5219
	Equal variances not assumed	5.3667	3.5219

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-33212.316	59878.983
	Equal variances not assumed	-34790.215	61456.882
Total aflatoxin/gm(PPb)	Equal variances assumed	-2.4807	13.2140
	Equal variances not assumed	-2.6905	13.4238

Location 4: 'Yankaba market

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	110000.00	18708.287	7637.626
	Dry season	6	65000.00	33615.473	13723.459
Total aflatoxin/gm(PPb)	Rainy season	6	14.367	5.8538	2.3898
	Dry season	6	11.383	4.6344	1.8920

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	2.914	.119
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	.817	.387
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	2.865	10	.017
	Equal variances not assumed	2.865	7.826	.021
Total aflatoxin/gm(PPb)	Equal variances assumed	.979	10	.351
	Equal variances not assumed	.979	9.500	.352

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	45000.000	15705.625
	Equal variances not assumed	45000.000	15705.625
Total aflatoxin/gm(PPb)	Equal variances assumed	2.9833	3.0481
	Equal variances not assumed	2.9833	3.0481

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	10005.686	79994.314
	Equal variances not assumed	8642.296	81357.704
Total aflatoxin/gm(PPb)	Equal variances assumed	-3.8082	9.7749
	Equal variances not assumed	-3.8570	9.8236

Location 5: Na'ibawa garage

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	133333.33	55647.701	22718.079
	Dry season	6	75833.33	21075.262	8603.940
Total aflatoxin/gm(PPb)	Rainy season	6	15.250	4.0560	1.6558
	Dry season	6	10.200	2.6586	1.0854

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	1.649	.228
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	.561	.471
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	2.367	10	.039
	Equal variances not assumed	2.367	6.405	.053
Total aflatoxin/gm(PPb)	Equal variances assumed	2.551	10	.029
	Equal variances not assumed	2.551	8.627	.032

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	57500.000	24292.774
	Equal variances not assumed	57500.000	24292.774
Total aflatoxin/gm(PPb)	Equal variances assumed	5.0500	1.9799
	Equal variances not assumed	5.0500	1.9799

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	3372.325	111627.675
	Equal variances not assumed	-1042.081	116042.081

Total aflatoxin/gm(PPb)	Equal variances assumed	.6386	9.4614
	Equal variances not assumed	.5416	9.5584

Location 6: Rimi market

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	226666.67	355199.474	145009.578
	Dry season	6	77500.00	28416.544	11601.006
Total aflatoxin/gm(PPb)	Rainy season	6	13.533	5.9762	2.4398
	Dry season	6	6.017	3.4493	1.4082

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	5.242	.045
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	3.332	.098
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	1.025	10	.329
	Equal variances not assumed	1.025	5.064	.352
Total aflatoxin/gm(PPb)	Equal variances assumed	2.668	10	.024
	Equal variances not assumed	2.668	7.999	.028

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	149166.667	145472.888
	Equal variances not assumed	149166.667	145472.888
Total aflatoxin/gm(PPb)	Equal variances assumed	7.5167	2.8170

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	1.025	10	.329
	Equal variances not assumed	1.025	5.064	.352
Total aflatoxin/gm(PPb)	Equal variances assumed	2.668	10	.024
	Equal variances not assumed	7.5167		2.8170

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-174967.127	473300.460
	Equal variances not assumed	-223366.264	521699.597
Total aflatoxin/gm(PPb)	Equal variances assumed	1.2400	13.7933
	Equal variances not assumed	1.0205	14.0128

4. Kastina metropolis.

Location 1: Central market

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	117500.00	46770.717	19094.065
	Dry season	6	51666.67	25033.311	10219.806
Total aflatoxin/gm(PPb)	Rainy season	6	21.000	.0000	.0000
	Dry season	6	17.717	5.2339	2.1367

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	1.562	.240
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	26.159	.000

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	117500.00	46770.717	19094.065
	Dry season	6	51666.67	25033.311	10219.806
Total aflatoxin/gm(PPb)	Rainy season	6	21.000	.0000	.0000
	Equal variances not assumed				

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	3.040	10	.012
	Equal variances not assumed	3.040	7.647	.017
Total aflatoxin/gm(PPb)	Equal variances assumed	1.537	10	.155
	Equal variances not assumed	1.537	5.000	.185

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	65833.333	21657.049
	Equal variances not assumed	65833.333	21657.049
Total aflatoxin/gm(PPb)	Equal variances assumed	3.2833	2.1367
	Equal variances not assumed	3.2833	2.1367

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	17578.421	114088.246
	Equal variances not assumed	15488.532	116178.135
Total aflatoxin/gm(PPb)	Equal variances assumed	-1.4776	8.0443
	Equal variances not assumed	-2.2093	8.7760

Location 2: 'Yar Kutungu market

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	91666.67	45350.487	18514.259
	Dry season	6	54166.67	23112.046	9435.453
Total aflatoxin/gm(PPb)	Rainy season	6	20.583	1.0206	.4167
	Dry season	6	15.633	4.8615	1.9847

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	4.830	.053
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	11.377	.007
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	1.805	10	.101
	Equal variances not assumed	1.805	7.433	.112
Total aflatoxin/gm(PPb)	Equal variances assumed	2.441	10	.035
	Equal variances not assumed	2.441	5.440	.055

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	37500.000	20779.932
	Equal variances not assumed	37500.000	20779.932
Total aflatoxin/gm(PPb)	Equal variances assumed	4.9500	2.0280
	Equal variances not assumed	4.9500	2.0280

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-8800.573	83800.573
	Equal variances not assumed	-11062.240	86062.240
Total aflatoxin/gm(PPb)	Equal variances assumed	.4314	9.4686
	Equal variances not assumed	-.1388	10.0388

Location 3: Chake market

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	81666.67	33565.856	13703.203
	Dry season	6	51666.67	22509.257	9189.366
Total aflatoxin/gm(PPb)	Rainy season	6	19.050	4.7765	1.9500
	Dry season	6	14.667	1.6207	.6616

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	.412	.535
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	2.177	.171
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	1.818	10	.099
	Equal variances not assumed	1.818	8.741	.103
Total aflatoxin/gm(PPb)	Equal variances assumed	2.129	10	.059
	Equal variances not assumed	2.129	6.136	.076

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	30000.000	16499.158
	Equal variances not assumed	30000.000	16499.158
Total aflatoxin/gm(PPb)	Equal variances assumed	4.3833	2.0592
	Equal variances not assumed	4.3833	2.0592

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-6762.415	66762.415
	Equal variances not assumed	-7493.242	67493.242
Total aflatoxin/gm(PPb)	Equal variances assumed	-.2048	8.9715
	Equal variances not assumed	-.6283	9.3950

Location 4: Mangal garage

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	120833.33	22229.860	9075.302
	Dry season	6	74166.67	15302.505	6247.222
Total aflatoxin/gm(PPb)	Rainy season	6	19.067	4.7357	1.9333
	Dry season	6	18.233	6.7769	2.7667

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of	Equal variances assumed	.865	.374

sample	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	.381	.551
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	4.236	10	.002
	Equal variances not assumed	4.236	8.870	.002
Total aflatoxin/gm(PPb)	Equal variances assumed	.247	10	.810
	Equal variances not assumed	.247	8.943	.811

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	46666.667	11017.663
	Equal variances not assumed	46666.667	11017.663
Total aflatoxin/gm(PPb)	Equal variances assumed	.8333	3.3752
	Equal variances not assumed	.8333	3.3752

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	22117.785	71215.549
	Equal variances not assumed	21687.056	71646.277
Total aflatoxin/gm(PPb)	Equal variances assumed	-6.6872	8.3538
	Equal variances not assumed	-6.8094	8.4761

Location 5: Central motor park

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	137500.00	37114.687	15152.008
	Dry season	6	70000.00	53665.631	21908.902
Total aflatoxin/gm(PPb)	Rainy season	6	21.000	.0000[a]	.0000
	Dry season	6	21.000	.0000[a]	.0000

a. t cannot be computed because the standard deviations of both groups are 0.

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	.431	.526
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	2.534	10	.030
	Equal variances not assumed	2.534	8.893	.032

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	67500.000	26638.005
	Equal variances not assumed	67500.000	26638.005

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	8146.825	126853.175
	Equal variances not assumed	7129.419	127870.581

Location 6: KTSTA garage

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	113333.33	52599.113	21473.498
	Dry season	6	70833.33	24375.534	9951.270
Total aflatoxin/gm(PPb)	Rainy season	6	20.300	1.7146	.7000
	Dry season	6	18.517	6.0829	2.4833

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	1.437	.258
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	2.986	.115
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	1.796	10	.103
	Equal variances not assumed	1.796	7.053	.115
Total aflatoxin/gm(PPb)	Equal variances assumed	.691	10	.505
	Equal variances not assumed	.691	5.790	.516

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	42500.000	23667.254
	Equal variances not assumed	42500.000	23667.254
Total aflatoxin/gm(PPb)	Equal variances assumed	1.7833	2.5801
	Equal variances not assumed	1.7833	2.5801

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-10233.927	95233.927
	Equal variances not assumed	-13379.170	98379.170
Total aflatoxin/gm(PPb)	Equal variances assumed	-3.9655	7.5322
	Equal variances not assumed	-4.5860	8.1527

5. Birnin kebbi metropolis

Locaton 1: Central market

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	140000.00	27202.941	11105.554
	Dry season	6	84166.67	49539.546	20224.435
Total aflatoxin/gm(PPb)	Rainy season	6	14.833	6.9425	2.8343
	Dry season	6	8.783	6.9769	2.8483

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	1.893	.199
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	.483	.503
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	2.420	10	.036
	Equal variances not assumed	2.420	7.764	.043
Total aflatoxin/gm(PPb)	Equal variances assumed	1.506	10	.163

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	2.420	10	.036
	Equal variances not assumed	2.420	7.764	.043
Total aflatoxin/gm(PPb)	Equal variances assumed	1.506	10	.163
	Equal variances not assumed	1.506	10.000	.163

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	55833.333	23072.952
	Equal variances not assumed	55833.333	23072.952
Total aflatoxin/gm(PPb)	Equal variances assumed	6.0500	4.0182
	Equal variances not assumed	6.0500	4.0182

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	4423.593	107243.074
	Equal variances not assumed	2344.156	109322.510
Total aflatoxin/gm(PPb)	Equal variances assumed	-2.9031	15.0031
	Equal variances not assumed	-2.9032	15.0032

Location 2: Ambursa market

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	85833.33	44990.740	18367.393
	Dry season	6	78333.33	47399.015	19350.567
Total aflatoxin/gm(PPb)	Rainy season	6	16.900	6.5161	2.6602
	Dry season	6	10.867	5.1794	2.1145

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	.129	.727
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	1.271	.286
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	.281	10	.784
	Equal variances not assumed	.281	9.973	.784
Total aflatoxin/gm(PPb)	Equal variances assumed	1.775	10	.106
	Equal variances not assumed	1.775	9.516	.108

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	7500.000	26679.684
	Equal variances not assumed	7500.000	26679.684
Total aflatoxin/gm(PPb)	Equal variances assumed	6.0333	3.3982
	Equal variances not assumed	6.0333	3.3982

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-51946.041	66946.041
	Equal variances not assumed	-51967.919	66967.919

Total aflatoxin/gm(PPb)	Equal variances assumed	-1.5383	13.6050
	Equal variances not assumed	-1.5909	13.6576

Location 3: Old market

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	129000.00	55063.600	22479.620
	Dry season	6	112500.00	57597.743	23514.180
Total aflatoxin/gm(PPb)	Rainy season	6	15.267	5.0186	2.0488
	Dry season	6	6.583	3.8665	1.5785

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	.210	.657
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	1.687	.223
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	.507	10	.623
	Equal variances not assumed	.507	9.980	.623
Total aflatoxin/gm(PPb)	Equal variances assumed	3.357	10	.007
	Equal variances not assumed	3.357	9.389	.008

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	16500.000	32530.755
	Equal variances not assumed	16500.000	32530.755
Total aflatoxin/gm(PPb)	Equal variances assumed	8.6833	2.5864
	Equal variances not assumed	8.6833	2.5864

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of	Equal variances assumed	-55983.038	88983.038

sample	Equal variances not assumed	-56002.907	89002.907
Total aflatoxin/gm(PPb)	Equal variances assumed	2.9205	14.4462
	Equal variances not assumed	2.8693	14.4974

Location 4: Central motor park

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	91666.67	44795.833	18287.822
	Dry season	6	74166.67	31371.431	12807.333
Total aflatoxin/gm(PPb)	Rainy season	6	50.467	79.2325	32.3465
	Dry season	6	15.783	4.3000	1.7555

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	4.460	.061
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	5.429	.042
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	.784	10	.451
	Equal variances not assumed	.784	8.954	.453
Total aflatoxin/gm(PPb)	Equal variances assumed	1.071	10	.309
	Equal variances not assumed	1.071	5.029	.333

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of	Equal variances assumed	17500.000	22326.491

sample	Equal variances not assumed	17500.000	22326.491
Total aflatoxin/gm(PPb)	Equal variances assumed	34.6833	32.3941
	Equal variances not assumed	34.6833	32.3941

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-32246.523	67246.523
	Equal variances not assumed	-33046.037	68046.037
Total aflatoxin/gm(PPb)	Equal variances assumed	-37.4953	106.8619
	Equal variances not assumed	-48.4420	117.8086

Location 5: Argungu motor park

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	1851666.67	4236969.042	1729735.368
	Dry season	6	90000.00	50299.105	20534.524
Total aflatoxin/gm(PPb)	Rainy season	6	9.700	6.2820	2.5646
	Dry season	6	10.800	5.7755	2.3578

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	6.076	.033
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	.000	.988
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	1.018	10	.332
	Equal variances not assumed	1.018	5.001	.355
Total aflatoxin/gm(PPb)	Equal variances assumed	-.316	10	.759
	Equal variances not assumed	-.316	9.930	.759

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	1761666.667	1729857.252
	Equal variances not assumed	1761666.667	1729857.252
Total aflatoxin/gm(PPb)	Equal variances assumed	-1.1000	3.4838
	Equal variances not assumed	-1.1000	3.4838

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-2092695.484	5616028.817
	Equal variances not assumed	-2684696.108	6208029.441
Total aflatoxin/gm(PPb)	Equal variances assumed	-8.8623	6.6623
	Equal variances not assumed	-8.8697	6.6697

Location 6: Jega motor park

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	135833.33	25771.431	10521.143
	Dry season	6	85000.00	23874.673	9746.794
Total aflatoxin/gm(PPb)	Rainy season	6	19.300	4.1641	1.7000
	Dry season	6	11.033	5.1095	2.0859

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	.121	.735
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	.101	.757
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	3.544	10	.005
	Equal variances not assumed	3.544	9.942	.005
Total aflatoxin/gm(PPb)	Equal variances assumed	3.072	10	.012
	Equal variances not assumed	3.072	9.609	.012

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	50833.333	14342.052
	Equal variances not assumed	50833.333	14342.052
Total aflatoxin/gm(PPb)	Equal variances assumed	8.2667	2.6909
	Equal variances not assumed	8.2667	2.6909

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	18877.251	82789.416
	Equal variances not assumed	18852.016	82814.651
Total aflatoxin/gm(PPb)	Equal variances assumed	2.2709	14.2624
	Equal variances not assumed	2.2376	14.2957

6. Sokoto metropolis

Location 1: Old market

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	86666.67	28751.812	11737.878
	Dry season	6	70833.33	19853.631	8105.211
Total aflatoxin/gm(PPb)	Rainy season	6	12.500	7.8473	3.2036
	Dry season	6	6.483	4.1460	1.6926

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	1.453	.256
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	2.899	.119
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	1.110	10	.293
	Equal variances not assumed	1.110	8.885	.296
Total aflatoxin/gm(PPb)	Equal variances assumed	1.661	10	.128

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	1.110	10	.293
	Equal variances not assumed	1.110	8.885	.296
Total aflatoxin/gm(PPb)	Equal variances assumed	1.661	10	.128
	Equal variances not assumed	1.661	7.590	.137

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	15833.333	14264.369
	Equal variances not assumed	15833.333	14264.369
Total aflatoxin/gm(PPb)	Equal variances assumed	6.0167	3.6233
	Equal variances not assumed	6.0167	3.6233

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-15949.661	47616.328
	Equal variances not assumed	-16498.743	48165.409
Total aflatoxin/gm(PPb)	Equal variances assumed	-2.0565	14.0899
	Equal variances not assumed	-2.4180	14.4513

Location 2: Central market

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	105000.00	7745.967	3162.278
	Dry season	6	76666.67	14719.601	6009.252
Total aflatoxin/gm(PPb)	Rainy season	6	14.900	3.8393	1.5674
	Dry season	6	12.917	4.1499	1.6942

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	3.200	.104
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	.000	.989
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	4.172	10	.002
	Equal variances not assumed	4.172	7.572	.004
Total aflatoxin/gm(PPb)	Equal variances assumed	.859	10	.410
	Equal variances not assumed	.859	9.940	.410

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	28333.333	6790.516
	Equal variances not assumed	28333.333	6790.516
Total aflatoxin/gm(PPb)	Equal variances assumed	1.9833	2.3080
	Equal variances not assumed	1.9833	2.3080

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	13203.120	43463.546
	Equal variances not assumed	12518.961	44147.705
Total aflatoxin/gm(PPb)	Equal variances assumed	-3.1592	7.1259
	Equal variances not assumed	-3.1635	7.1301

Location 3: Tashar Kura

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	100833.33	21544.528	8795.517
	Dry season	6	70833.33	10684.880	4362.084
Total aflatoxin/gm(PPb)	Rainy season	6	9.717	3.6224	1.4788
	Dry season	6	9.067	2.3509	.9597

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	16.364	.002
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	.819	.387
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	3.056	10	.012
	Equal variances not assumed	3.056	7.319	.017
Total aflatoxin/gm(PPb)	Equal variances assumed	.369	10	.720

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	3.056	10	.012
	Equal variances not assumed	3.056	7.319	.017
Total aflatoxin/gm(PPb)	Equal variances assumed	.369	10	.720
	Equal variances not assumed	.369	8.577	.721

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	30000.000	9817.784
	Equal variances not assumed	30000.000	9817.784
Total aflatoxin/gm(PPb)	Equal variances assumed	.6500	1.7630
	Equal variances not assumed	.6500	1.7630

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	8124.613	51875.387
	Equal variances not assumed	6988.361	53011.639
Total aflatoxin/gm(PPb)	Equal variances assumed	-3.2781	4.5781
	Equal variances not assumed	-3.3683	4.6683

Location 4 :Gawon Nama area

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	99166.67	19853.631	8105.211
	Dry season	6	60000.00	8944.272	3651.484
Total aflatoxin/gm(PPb)	Rainy season	6	14.250	4.3547	1.7778
	Dry season	6	11.483	4.2433	1.7323

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	4.201	.068
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	.001	.978
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	4.406	10	.001
	Equal variances not assumed	4.406	6.949	.003
Total aflatoxin/gm(PPb)	Equal variances assumed	1.115	10	.291
	Equal variances not assumed	1.115	9.993	.291

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	39166.667	8889.757
	Equal variances not assumed	39166.667	8889.757
Total aflatoxin/gm(PPb)	Equal variances assumed	2.7667	2.4822
	Equal variances not assumed	2.7667	2.4822

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	19359.054	58974.279
	Equal variances not assumed	18114.603	60218.730

Total aflatoxin/gm(PPb)	Equal variances assumed	-2.7641	8.2974
	Equal variances not assumed	-2.7646	8.2979

Location 5: Arkila area

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	75083.33	39711.984	16212.350
	Dry season	6	62500.00	6892.024	2813.657
Total aflatoxin/gm(PPb)	Rainy season	6	12.850	5.8030	2.3691
	Dry season	6	9.633	1.8587	.7588

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	10.588	.009
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	4.736	.055
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	.765	10	.462
	Equal variances not assumed	.765	5.301	.477
Total aflatoxin/gm(PPb)	Equal variances assumed	1.293	10	.225
	Equal variances not assumed	1.293	6.015	.243

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	12583.333	16454.694
	Equal variances not assumed	12583.333	16454.694

Total aflatoxin/gm(PPb)	Equal variances assumed	3.2167	2.4876
	Equal variances not assumed	3.2167	2.4876

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-24080.009	49246.676
	Equal variances not assumed	-29002.683	54169.350
Total aflatoxin/gm(PPb)	Equal variances assumed	-2.3261	8.7594
	Equal variances not assumed	-2.8666	9.2999

Location 6: Kara market

Group Statistics

	Season	N	Mean	Std. Deviation	Std. Error Mean
Average fungal count/gm of sample	Rainy season	6	89166.67	25771.431	10521.143
	Dry season	6	81666.67	9831.921	4013.865
Total aflatoxin/gm(PPb)	Rainy season	6	16.933	5.0182	2.0487
	Dry season	6	7.583	1.9539	.7977

Independent Samples Test

		Levene's Test for Equality of Variances	
		F	Sig.
Average fungal count/gm of sample	Equal variances assumed	3.310	.099
	Equal variances not assumed		
Total aflatoxin/gm(PPb)	Equal variances assumed	6.094	.033
	Equal variances not assumed		

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Average fungal count/gm of sample	Equal variances assumed	.666	10	.520
	Equal variances not assumed	.666	6.425	.529

Total aflatoxin/gm(PPb)	Equal variances assumed	4.253	10	.002
	Equal variances not assumed	4.253	6.482	.005

Independent Samples Test

		t-test for Equality of Means	
		Mean Difference	Std. Error Difference
Average fungal count/gm of sample	Equal variances assumed	7500.000	11260.797
	Equal variances not assumed	7500.000	11260.797
Total aflatoxin/gm(PPb)	Equal variances assumed	9.3500	2.1985
	Equal variances not assumed	9.3500	2.1985

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
Average fungal count/gm of sample	Equal variances assumed	-17590.620	32590.620
	Equal variances not assumed	-19618.085	34618.085
Total aflatoxin/gm(PPb)	Equal variances assumed	4.4514	14.2486
	Equal variances not assumed	4.0660	14.6340

Appensix P: Analysis of variance (ANOVA): One way analysis of variance was used, and the statistical formulae and ANOVA table as cited by Oyejola (2003) are shown below:

$SS_{TOTAL} = SS_{TRMT} + SS_{error}$

$SS_{TOTAL} = \sum y^2_{i.j} - C.F$

$SS_{TRMT} = \sum y_{i.}/r - C.F.$

Where $y_{i.}$ = Treatment. And $C.F = (GT)^2/N$

TABLE 18: Anova Table used in statistical camparison of two variables.

SOURCE OF VARIABLES	DEGREE OF FREEDOM	SUM OF SQUARES (SS)	MEAN SQUARE (MS)	F-CALCULATED
TRMT between group	t-1	$\sum y_{i.}/r - C.F.$	$SS_{TRMT} / t-1$	
ERROR within group	N-t	$SS_{TOTAL} - SS_{TRMT}$	$SS_{TOTAL}-SS_{TRMT}/N-t$	MS_{TRMT}/MS_{ERR0R}
TOTAL	N-1	$\sum y^2_{i.j} - C.F$		

Key: TRMT= treatment between groups.
EERROR = error within groups.
SS_{TOTAL} = Sum of square total.
SS_{TRMT} = Sum of square between groups.
SS_{ERROR} = Sum of square within groups.
MS_{TRMT} = Mean square between groups.
MS_{ERROR} = Mean square within groups.



Plates 2: Herbal medicinal products displayed for sale in Gusau town.



Plate 3: Containers for the preparation of herbal medicine in Kano town.



Plate 4: *Aspergillus niger* grown on PDA at room temperature on the 5thof incubation.



Plate 5: *Aspergillus flavus* grown on PDA at room temperature on the 5th day of incubation.



Plate 6: *Aspergillus niger* grown on PDA at room temperature on 5th day of incubation.

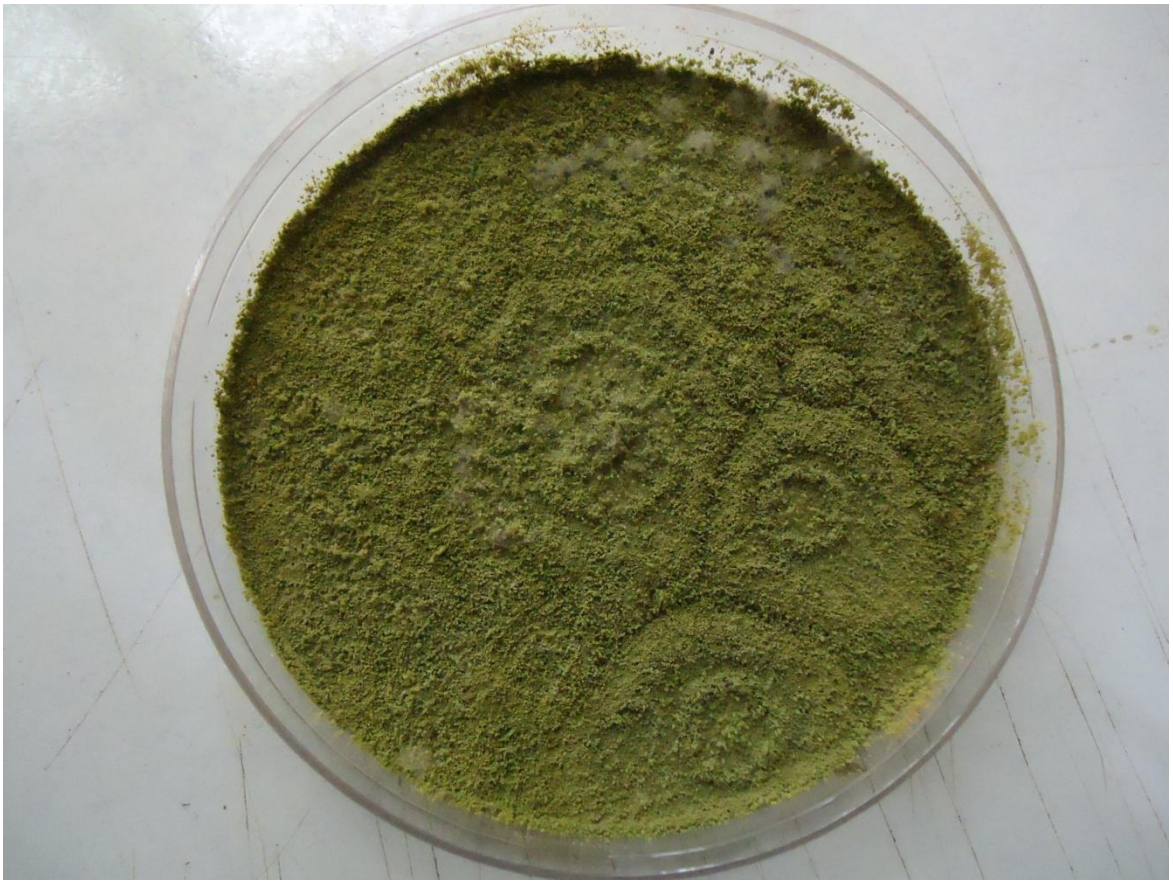


Plate 7: *Aspergillus Parasiticus* growm on PDA at room temperature on 5th day of incubation.

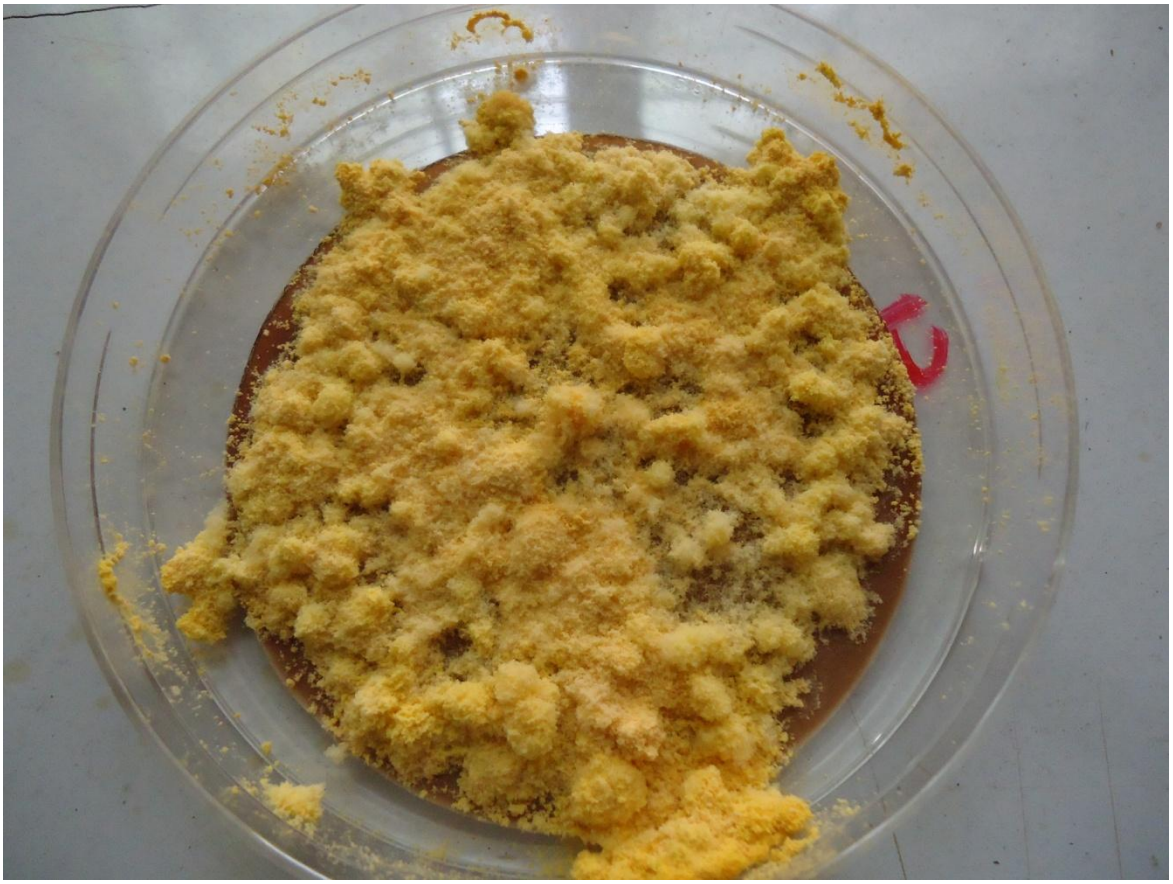


Plate : 8 *Aspergillus versicolor* grown on PDA at room temperature on 5th day of incubation.

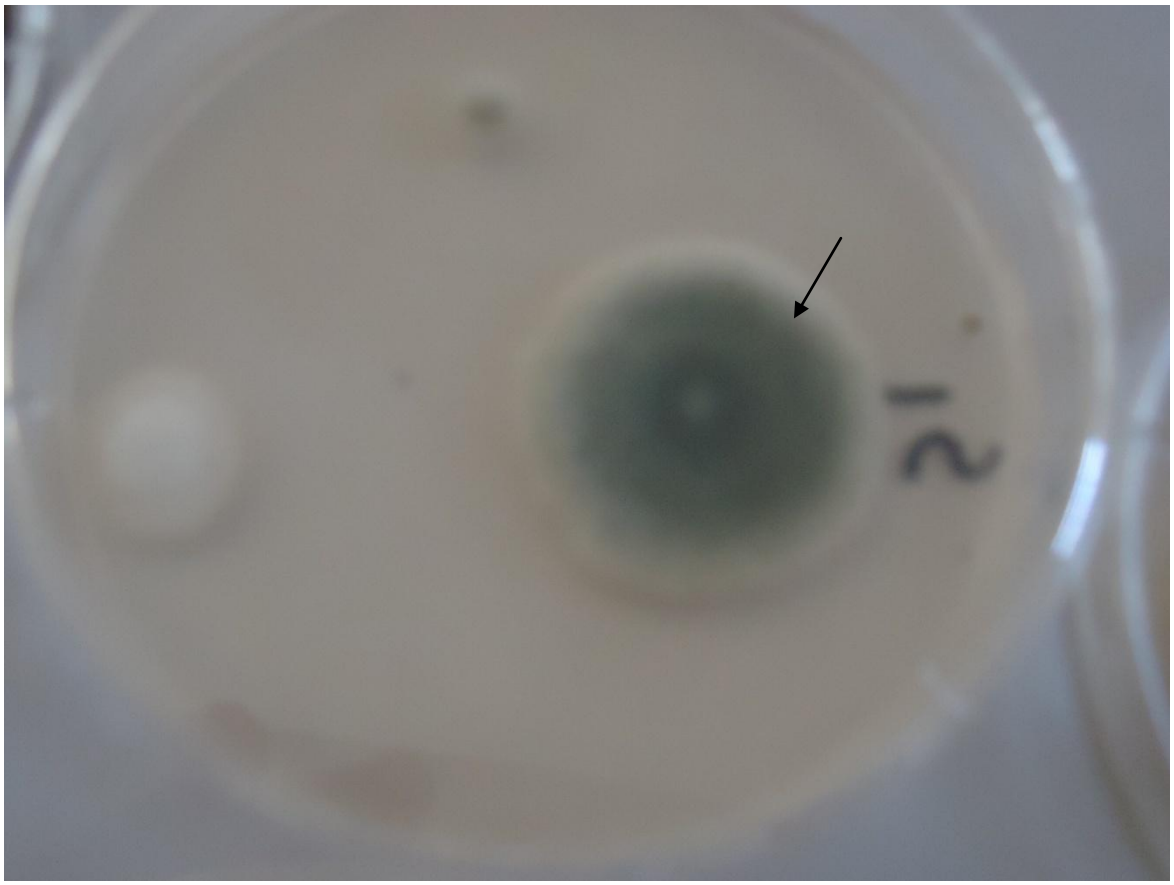


Plate 9: Arrow showing *Penicillium* spp grown on PDA at room temperature on 5th day of incubation.



Plate: 10 *Fusarium* spp grown on PDA at room temperature on 5th day of incubation.