بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Agricultural Studies

Department on Plant Protection

Exploration of Contamination Level of *Aspergillus spp* on sorghum (*Sorghum bicolor* L. (Moench)) Seeds in Khartoum North and Omdurman Grains Central markets

إستكشاف مستوي التلوث بفطر الإسبيرجلس على بذور الذرة الرفيعة في الأسواق المركزية للحبوب بالخرطوم شمال وامدرمان

A thesis Submitted in Partial Fulfillment of the Requirements for the B. Sc. Degree in Plant Protection.

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, 2020

الآية

بسم الله الرحمن الرحيم

قال تعالي:

وَإِذْ قَالَ رَبُّكَ لِلْمَلائِكَةِ إِنِّي جَاعِلٌ فِي الأَرْضِ خَلِيفَةً قَالُوا أَتَجْعَلُ فِيهَا مَنْ يُفْسِدُ فِيهَا وَيَسْفِكُ الدِّمَاءَ وَنَحْنُ نُسَبِّحُ بِحَمْدِكَ وَنُقَدِّسُ لَكَ قَالَ إِنِّي أَعْلَمُ مَا لا تَعْلَمُونَ (30)وَعَلَّمَ آدَمَ الأَسْمَاءَ كُلَّهَا ثُمَّ عَرَضَهُمْ عَلَى الْمَلائِكَةِ فَقَالَ أَنْبِئُونِي بِأَسْمَاءِ هَؤُلاء إِنْكُنتُمْ صَادِقِينَ(31) قَالُوا سُبْحَانَكَ لا عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ(32)

صدق الله العظيم

الآيات (30-32 سورة البقرة)

Dedication

To all my family

To all my teachers

To all my colleagues and friends

With love and respect.

Acknowledgement

First of all, I render my gratitude and praise to the Almighty Allah. I wish also to express my sincere gratitude to our supervisor **Dr. Ibrahim Saeed Mohamed** for his helpful guidance, encouragement and supervision of this research.

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Abstract

This study was conducted under plant pathology laboratory conditions of Plant Protection Department, College of Agricultural Studies, Sudan University of Science and Technology to investigate the level of contamination of sorghum seeds collected from two different locations, in Khartoum state, Khartoum North (KN) and Omdurman (OM) central grains markets) with *Aspergillus spp*. fungi. The study revealed that the *Aspergillus spp*. fungi were recorded in all samples collected from the two locations with varying level of contamination. The mean percentage frequency of occurrence of *Aspergillus spp*. in seeds samples ranged from 10% in Khartoum North to 9% in Omdurman. The highest level of fungus contamination was recorded in samples collected from Omdurman. The most prevailing seed borne fungi recorded across seeds samples was *Aspergillus niger.* The study showed that the mean percentage frequency of occurrence of this fungus in seeds samples increased with days of incubation. It was recorded as 9 and 10 four days after incubation in KN and OM. It is worth mentioning that, the findings of this study are therefore, important as they highlighted the need for adhering to effective measures that aimed at reducing seed-borne fungi incidence in stable food crops seeds in Sudan.

ملخص الدراسة

أجريت هذه الدراسة بمعمل امراض النبات الخاص بقسم وقاية النباتات بكلية الدراسات الزراعية -جامعة السودان للعلوم والتكنولوجيا بغرض إستكشاف فطر الاسبرجلس ودرجة التلوث على بذور الذرة الرفيعه التي جمعت من مناطقتين مختلفتين للاسواق المركزية للحبوب بولاية الخرطوم هي الخرطوم بحري وأمدرمان. اوضحت الدراسة أن فطر الاسبرجلس قد سجل تواجده فى كل العينات التى جمعت من المنطتين وبنسب تلوث متفاوته. إذ تفاوت متوسط نسبة تواجد الاسبرجلس فى عينات الحبوب من 9 في الخرطوم شمال إلى 10 في امدرمان. اعلى نسبة تلوث بالفطر سجلت فى العينات التي جمعت من امدرمان. كما أن اكثر فطر تواجدأ على البذور وفى كل العينات هو اسبيرجلس نيقر. أظهرت الدراسة ايضأ ان نسبة تواجد هذا الفطر تزداد مع ايام الحضن. الجدير ذكره ان اهمية نتائج هذه الدراسة تكمن فى انها سلطة الضوء على الحوجة الى تطبيق اجراءات فعالة تهدف الى تقليل الاصابة بالفطريات المحمولة على البذور فى محاصيل الغذاء بالسودان.

CHAPTER ONE

INTRODUCTION

Cereal grains and edible oil seeds are important human food resources and livestock feeds worldwide. In fact, cereal grains are food staples in many, if not most, countries and cultures and are the raw materials of many of our foods. However, sorghum [*Sorghum bicolor* L. (Moench)] is one of the major cereal crops and staple food as well for millions of people in the Semi-Arid Tropics of Africa and Asia (ICRISAT (1993).

In the Sudan, sorghum is produced mainly in rain-fed agriculture in addition to irrigated schemes. The cereal harvest for the 15 northern states of the Republic of the Sudan is estimated at 5.707 million MT, comprising 4.606 million MT of sorghum (FAO/WHO, 2011). In fact, sorghum is used in Sudan as food for human beings and animals. Industrial uses include extraction of many products such as starch, oil, alcohol, sugar, and sugary juices (Khatab *et al*., 2000).

The major threats facing the productivity and availability of healthy food crops worldwide are the losses and spoilage caused by plant pathogens, insects, nematodes and parasitic weeds. Among these, are fungi that contaminate grains of food crops.

In fact, seed borne fungi associated with seeds of food crops and edible oil continues to represent a major human health risk throughout the world and particularly in the humid tropics being major spoilage agents of food crops (Olusegun, *et al.*, 2013). These seed borne fungi associated with seeds of food grains, of which *Aspergillus ssp*. are the most important, and their secondary metabolites are ones of the most important food crops spoilage agents in the Sudan (Haq Elamin N. H. *et al*., 1988; Ali, 1989; Yousif M.A. *et al*., 2010).

The ingestion of such contaminated grains by animals and human beings has enormous public health significance, because these toxins are capable of causing diseases in man and animals (Bhat and Vasanth, 2003).

The above mentioned facts, reflect the potential of risk of food crops contamination with spoilage pathogens a situation that necessitate more scientific studies to be carried out in order to help overcoming the risk involved.

It is in view of this that the current study aimed at exploring and investigating on i) presence of pathogenic fungi, namely, *Aspergillus spp*. associated with seeds in samples of Sorghum crop collected from central markets of cereal grains in Khartoum States in Sudan and (ii) the level of contamination of sorghum seeds with *Aspergillus spp*. fungi with following objectives:-

- To investigate the occurrence of *Aspergillus spp*. fungi associated with seeds of Sorghum crop;
- To determine the level of contamination of sorghum seeds with these fungi.

CHAPTER TWO

LITERATURE REVIEW

2.1. Food grains

Food crops are ones of the most important foods in many part of the world. Among these cereal ones *play an important role in food security*. According to FAO statistic, Mahmud *et al*., (1995), sorghum considered one of the main food grains used for food. The common name of sorghum in Sudan is “Aish” which means life and the crop is used as food for human beings, food for the animals. Industrial uses include extraction of many products such as starch, oil, alcohol, sugar, and sugary juices (Khatab and Hassan 2000).

2.2 *Sorghum bicolor* L. (Moench)

2.2.1 Scientific classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Liliopsida

Order: Poales

Family: Poaceae

Genus: Sorghum

Species: bicolor

Sorghum [*Sorghum bicolor* L. (Moench)] is one of the major cereal crop and staple food as well for millions of the people and most of those who are insecure in the Semi-Arid Tropics of Africa and Asia. The crop may have been domesticated in that region, possibly Ethiopia (ICRISAT, 1993) .

According to ICRISAT (1993) the total area cultivated by sorghum in the entire world is 106 million feddans and the fives top countries area wise are India, Sudan, USA, Nigeria and China. The areas under cultivation in these countries represent 66% of the total world areas cultivated by sorghum. In the Sudan sorghum is produced mainly in rain-fed agriculture. The cereal harvest for the 15 northern states of the Republic of the Sudan is estimated at 5.707 million MT, comprising 4.606 million MT of sorghum Vast acreage are cultivated in mechanized crop production Schemes in Gadarif, Damazin, Blue Nile state and both Kordofan and Darfur states .the crop is also grown in irriga ted Schemes of Gazera and Rahad as important crop in rotation. In the traditional rain fed, sorghum is cultivated in Kordofan , Darfur, and White Nile. But in Butana and Blue Nile it is produced mechanically by rains.

Sorghum is affected by a range of fungal seed borne diseases including ergot (*Claviceps* africana), seed rot (*Fusarium moniliforme*), zonate leaf spot (*Gloeocercospora* sorghi), downy mildew (*Sclerospora* sorghi), loose smut (*Sphacelotheca* cruenta), covered smut (Sphacelotheca sorghi), leaf spots (*Phoma* sorghina), Bipolaris bicolor, anthracnose (*Colletotrichum* graminicola), grey leaf spot (*Cercospora* sp.) and contaminant (e.g. *Aspergillus spp.* (Almekinders and Louwaars, 1999; Kaula and Chisi, 2002 and Neergaard, 1979). Of all these diseases and contaminants, common seed and seedling rot diseases in sorghum are caused by soil- and seed-borne Aspergillus, Fusarium, Pythium, Rhizoctonia and Rhizopus spp. (Taylor, 2003).

Aspergillus spp represent high risk to both human and animals. Therefore, seed health testing is a prerequisite to minimize this risk by assessing the quality of seed before it is eaten as food or sown (International Seed Testing Association (ISTA), 1985).

2.2.2 Seeds aflatoxin contaminant fungi

The importance of seed borne pathogens to crop quality and quantity cannot be ignored. Results by Bipen *et al.,* (1999) showed that there was a significant decrease in oil content of sunflower seeds infected with Rhizopus oryzae. Wanyera (1998) analyzed wheat seed and concluded that fungal infection led to abnormal seedlings and dead seeds. Aflatoxin contamination and its associated risks to humans, wild animals and livestock and reduced grain quality have been reported by several authors (Haq Elamin NH *et al*., 1988; Diaz *et al*., 1998; Thompson 2000 and Yousif *et al*., 2010).

2.2.3 Aflatoxin

Aflatoxin is a naturally occurring mycotoxin that can poison humans and almost all animals and remains the biggest barrier for export due to food safety concerns. Produced by fungi in the *Aspergillus spp.* The toxin can contaminate a variety of agricultural commodities but most commonly food grains and edible oils (IARC, 2002). Poisoning primarily occurs through ingestion of contaminated food and milk, but it can also occur as a result of occupational exposure in agricultural workers and for those in oil mills and granaries (ICRISAT, 2010).

The level of toxicity may either be acute—when large amounts of the toxin are consumed in short periods—or chronic due to ingestion over long periods of time. *Acute toxicity* may result in death and/or inhibition of carbohydrate and lipid metabolism but this type of poisoning is most common in livestock due to the large amounts of poison that needs to be ingested for the symptoms to occur. When animals consume aflatoxin contaminated feed they produce milk contaminated by an aflatoxin metabolite that is known to be carcinogenic, producing tumors and liver cancer in test animals (ICRISAT, 2010).

Acute toxicity has been re uring subsequent drying and storage. About 30 species of Aspergillus or their teleomorphs are associated with food spoilage, these include: *Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius, Aspergillus ochraceus, Aspergillus candidus, Aspergillus restrictus, Aspergillus penicillioides, Aspergillus niger, Aspergillus carbonarius, Aspergillus fumigatus, Aspergillus clavatus,* and *Aspergillus carbonarius, and Aspergillus versicolor*. (Haq Elamin, 1988).However, Haq Elamin NH et al., (1988); Ali, (1989); Yousif M. A.et al., (2010), and Olusegun,(2013) reported that Aspergillus species tend to be associated more with tropical and warm temperate crops, for example oilseeds and nuts, since they prefer to grow at relatively high temperatures. They concluded that, *Aspergillus flavus, Aspergillus parasiticus* and aflatoxins typically affect oilseeds, including groundnuts, soya, tree nuts, maize and various oilseed-based animal feed stocks - cotton seed cake, copra, sunflower, but can also affect rice, wheat, sorghum, figs, coffee and sweet potatoes, for example. Aflatoxins are also noted in milk, via contaminated animal feed.

The role of *Aspergillus* species in food spoilage is well-established. Mycotoxins produced by *Aspergillus flavus* include aflatoxins and cyclopiazonic acd. Other important mycotoxins from aspergilli include ochratoxin A and patulin. Some Aspergilli have an ascomycete teleomorphic (sexual) stage; for example, *Eurotium*, *Neosartorya*, and *Emericella*; Many Aspergilli are xerophilic and present particular problems during commodity harvest, and during subsequent drying and storage.(Cutler.1991).

2.3.1 Growth and Distribution

Aspergillus species are highly aerobic and are found in almost all oxygen-rich environments, where they commonly grow as molds on the surface of a substrate, as a result of the high oxygen tension. Commonly, fungi grow on carbon-rich substrates such as monosaccharides (such as glucose) and polysaccharides (such as amylose). *Aspergillus* species are common contaminants of starchy foods (such as bread and potatoes), and grow in or on many plants and trees. In addition to growth on carbon sources, many species of *Aspergillus* demonstrate oligotrophy where they are capable of growing in nutrient-depleted environments, or environments in which there is a complete lack of key nutrients. *A. niger* is a prime example of this; it can be found growing on damp walls, as a major component of mildew (Machida, *et al.*, 2010)

2.3.2 Commercial importance

Species of *Aspergillus* are important medically and commercially. Some species can cause infection in humans and other animals. Some infections found in animals have been studied for years. Some species found in animals have been described as new and specific to the investigated disease and others have been known as names already in use for organisms such as saprophytes. More than 60 *Aspergillus* species are medically relevant pathogens. (Williams & Wilkins Company, 1926.)

For humans there is a range of diseases such as infection to the external ear, skin lesions, and ulcers classed as mycetomas. Other species are important in commercial microbial fermentations. For example, alcoholic beverages such as Japanese sake are often made from rice or other starchy ingredients (like manioc), rather than from grapes or malted barley. Typical microorganisms used to make alcohol, such as yeasts of the genus *Saccharomyces*, cannot ferment these starches, and so *koji* mold such as *Aspergillus oryzae* is used to break down the starches into simpler sugars. Members of the genus are also sources of natural products that can be used in the development of medications to treat human disease (Bennet, 2010).

Perhaps the largest application of *A. niger* (Plate,1) is as the major source of citric acid; this organism accounts for over 99% of global citric acid production, or more than 1.4 million tons per annum. *A. niger* is also commonly used for the production of native and foreign enzymes, including glucose oxidase and hen egg white lysozyme. In these instances, the culture is rarely grown on a solid substrate,

Although this is still common practice in Japan, but is more often grown as a submerged culture in a bioreactor. In this way, the most important parameters can be strictly controlled, and maximal productivity can be achieved. It also makes it far easier to separate the chemical or enzyme of importance from the medium, and is therefore far more cost-effective.

2.4 *Aspergillus flavus*

Aspergillus flavus is a fungus. It grows by producing thread like branching filaments known as hyphae. Filamentous fungi such as *A. flavus* are sometimes called molds. A network of hyphae known as the mycelium secretes enzymes that break down complex food sources. The resulting small molecules are absorbed by the mycelium to fuel additional fungal growth. The unaided eye cannot see individual hyphae, but dense mats of mycelium with conidia (asexual spores) often can be seen. The ear of maize below shows the growth of the fungus covering four maize kernels. When young, the conidia of *A. flavus* appear yellow green in color. As the fungus ages the spores turn a darker green (Scheidegger, and Payne. 2003). Growth of the fungus on a food source often leads to contamination with aflatoxin, a toxic and carcinogenic compound. *Aspergillus flavus* is also the second leading cause of aspergillosis in humans. Patients infected with *A. flavus* often have reduced or compromised immune systems (Richard, and Payne. 2003).

2.4.1 Constraints in Sorghum grains food (Aflatoxin)

The primary constraint affecting grains food is aflatoxin, a naturally occurring toxin that can infect a number of crops including sorghum and can result in acute and chronic poisoning in humans and animals on ingestion. The health impacts of ingestion in humans include stunted growth and development as well as an increased risk in liver cancer (IARC, 2002; ICRISAT). Many countries have recognized the severity of impact on human health and the need to set and/or meet aflatoxin requirements. However, the investment required to do this is considerable. For instance, U.S. producers spend in excess of $27 million USD annually and even more during years of drought to meet aflatoxin standards (USDA, 2008). Consequently, ingesting infected sorghum daily either through sorghum grain food or other staples that can be contaminated by aflatoxin e.g. Millet or sesame produced in subsistence level farming or purchased from other commercial producers.

2.4.2 Seed Health Testing

Seed health testing for the presence of seed borne pathogens is an important step in management of crop diseases. This is simply because seed-borne diseases have been fund to affect the quality and quantity of food crops. According, the important of seed health testing cannon be under estimated. Seed-borne diseases have been found to affect the growth and productivity of crop plants. Seed-borne pathogens present or associated externally with the seed as contaminant may cause seed abortion , seed rot, seed necrosis, reduction or elimination of germination capacity as well as seedling damage resulting in development of disease at later stages of plant growth by systemic or local infection. Seeds are regarded as highly effective means for transporting plant pathogens over long distances. Besides, the mold fungi which grow on the seed substratum produce mycotoxins which are hazardous to humans and animals (Halt, 1994).

Several reports about seed-borne mycoflora on sorghum, [Soetan et al.(2006), Garish *et. al.*, (2004)] have been published. Post-harvest fungal infection, according to farmers, has been one of the constraints for mass production of these grains and less seed germination and viability. Mathur et. al., (1975) observed reduction in germination rate of sorghum and pearl millet due to Alternaria alternata, Aspergillus spp., Rhizopus spp., Curvularia lunata and Fusarium equiseti present in or on seed surface .

Seed health testing constitutes part of the seed certification and plant quarantine practices aimed at reducing the distribution of seed borne pathogens by both national and international trade of seeds. Mathur and Kongsdal (2003) also reported that percent frequency of seed-borne fungal pathogens were more in pearl millet as compared to sorghum .Groundnut seed mycoflora: Results of fungal identification in showed that all the seed samples were contaminated with various fungal pathogens , Fungal pathogens identified included Alternaria, Aspergillus, Fusarium, Helminthosporium and Rhizopus ,All the seed samples were found to be infected by Aspergillus whereas five samples with Fusarium. The test also shows loss in seed germination and symptom development in seedlings and can be used to evaluate seed treatments.

2.5 Management of fungal contaminants associated with seeds

Several effective ways for prevention and control of fungal contaminants associated with seed crops and their dangerous mycotoxin have been discussed by many researchers (FAO, 1979; Sanders et al., 1981 and WHO, 1988). In their recommendations they concentrate on optimization of cultural practices, development of resistant varieties, biological control and physical treatments. Farmers should be aware of pre-harvesting preparation of the field and environments, drying of commodities after post harvest is the most economical and effective means for farmers. Chemical treatments such as alkalinization and ammonization are well-recognized and industrially used. They call for International cooperation through authorized organizations to promoted and support efforts aiming the benefits for the economics and health of people of all the nations. Fungal pathogens associated with food grains are major problem of many economically important food crops. Some are soil-borne pathogen, which can live in the soil for long periods of time, so rotational cropping is not a useful control method. It can also spread through infected dead plant material, so cleaning up at the end of the season is important (Jones et.al., 1982). One of the control methods is to improve soil conditions because soil borne pathogens spreads faster through soils that have high moisture and bad drainage. Other control methods include removing infected plant tissue to prevent over win (Smith, et. al., 1988). Control of the disease using soil and systemic fungicides to eradicate the pathogen from the soil, flood, fallowing, and using clean seeds each year are very common methods (Booth, 1971). Thomas (1998) reported that it is difficult to find a biological control method because research in a green house can have different effects than testing in the field. However, the best control method found for soil borne fungi. Are planting resistant varieties, although not all have been bred for every forma specialis. A group of studies were carried out to investigate the antifungal activity of plant extract. In fact the antifungal activities of some plants extracts in controlling different pathogens have been reported by several workers who pointed out that the active compounds present in plants were influenced by many factors which include the age of plant, extracting solvent, method of extraction and time of harvesting plant materials (Tewarri and Nayak, 1991; Amadioha, 2000; Okigbo, 2005). Babu Joseph (2008) reported that Antifungal activity of the in vitro efficacy of different plant extracts viz., *Azadirachta indica*, *Artemisia annua*, *Eucalyptus globulus; Ocimum sanctum* and *Rheumemodi* were found to control wilt pathogens. Varma et al., 2002 also reported that extracts of tulsi (20%) was found to be least effective in inhibition of growth of *Fusarium.* The crude extracts of six plants viz, *Alliumsativum*, *Capsicumannuum*, *Artimesiavulgaris*, *Eupatorium adenophorum, Gaultheria fragrantissima* and *Phyllanthus emblica* were found to have activity against the fungus*Fusariumsolani* (Asha, *et. al.*,2009). Igbinosa (2009) investigated the ability of the crude stem extracts of *J. curcas* to inhibit the growth of fungi and bacteria is an indication of its broad spectrum antimicrobial potential which may be employed in the management of microbial infections. Aiyelaagbe and Ekunday (2000) investigated in-vitro and in-vivo the antifungal properties of *Jatroph acurcas* and *Ricinus cumunis* seed extracts in the control of mycelia growth and rot development of yam caused by *Fusarium verticilliodes* and *Aspergillus flavus r*eported that these plants possess antimicrobial activity. In Sudan, ten Sudanese plants were screened for their antibacterial activity, seven of them showed promising results , The fenugreek oilwas also found to inhibit Salmonella typhimurium (Sulieman, 2009). Despite of the growth of global market for herbal products, following complementary and/or alternative medicines, homeopathy, health foods and natural-pharmaceuticals therapy, yet the majority of these herbs were not assessed for quality, safety and efficacy.

2.6 Fungicide (Tilt ® 250EC)

Tilt fungicide 250ec is systemic fungicide is recommended for the control of many important plant diseases , multiple purpose in fungi control for controlled the Powdery mildew in different vegetables (tomato 15mililiter\100liter) and used with wilt fungal disease. SYNGENTA encourages responsible resistance management to ensure effective short term control of the fungal diseases on this label Tilt ® 250ec. The number reported in Sudan is 524.

2.7 Storage:- in temperature little from (10-25).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study location

This study was conducted in the laboratory of plant pathology, Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology during March, 2020. The aim of the study was to explore the distribution and level of contamination of sorghum grains with *Aspergillus spp*. in seeds samples collected from central markets of cereal grains in Khartoum State (Omdurman and Khartoum North). The details of the materials and methods used or technique followed during the course of this study are explained below.

3.2 Materials, tools and equipments used in the study

- Camera
- Marker pen
- Petri-dishes
- Microscope

- Slide
- Potato dextrose agar (pda)
- Filter papers

All materials except seeds, which used in the experiments, were sterilized using 70% ethyl alcohol. Formalin (10%) was used for Petri dishes sterilization. Cotton blue and lacto phenol were used for staining of the fungal cytoplasm and for providing a light blue background, against which the walls of hyphae can readily be seen (Aneja, 2004).

3.2.1 Collection of samples

A total of five labeled seed samples of sorghum seeds, one Kg each, were collected from each of the two central food grins markets in Khartoum State (Omdurman and Khartoum North). The samples were kept in paper sacks and brought to pathology laboratory. In the laboratory, one random and homogeneous sample of one kilo gram of seeds was secured separately from each of the two locations. Seed samples were drawn according to international standards for seed testing association (ISTA, 1966). Labeled samples were kept separately in sealed paper bags and stored at 5^0C refrigerator for further analysis.

3.2.2 Dry Seed Inspection

Hundred (100) seeds of each sample were randomly selected from each location and examined for impurities, plant debris, weed seeds, discoloration and malformation under stereoscopic binocular microscope (25-4x) and by magnified lens and naked eye according to the international seed testing association. Only normal seeds were used for seed borne Aspergillus fungi testing (ISTA Rules, 1966).

3.2.3 Incubation procedures:

The seed samples were tested by the standard blotter method using nutrient agar method for detection of *Aspergillus* spp. as described by IST.

3.3 Methods for the detection of *Aspergillus spp*. fungi

3.3.1 Blotter method

For the detection of seed borne Aspergillus fungi associated with each seed sample, standard blotter method as described by the International Seed Testing Association (ISTA 1966), was used. The seed samples were then platted on nutrient agar medium in sterilized plastic Petri-dishes (dia. 9.0 cm) in 9.0 cm. Fifty seeds were plated from each sample, ten seeds per plate. A total of five plates per location, five replications, and then kept in dark place for growth of the fungi.

After three days of incubation, seeds were then examined for Aspergillus fungi growth under a stereo microscope for three successive days. Fungi identification by habit character was supplemented by microscopic examination of spores and fruiting bodies using a compound microscope. Other identification aids were Standard books and research papers were consulted during the examination of these fungi (Rifai, (1969); P. Neergaard (1975); Agarwal *et al.,* (1989): Burgess *et al.,* (1994); Barnet and Hunter, (1999); Mathur and Kongsdal (2003) and Aneja, (2004). The binocular compound microscope was also used to determine the type of fungus in each plate. Aspergillus fungi identified and their percentage frequency (PF) of occurrence at each count in each sample was calculated by applying the following formula:

PF = (No. of seeds on which fungus appear / Total number of seeds) X 100

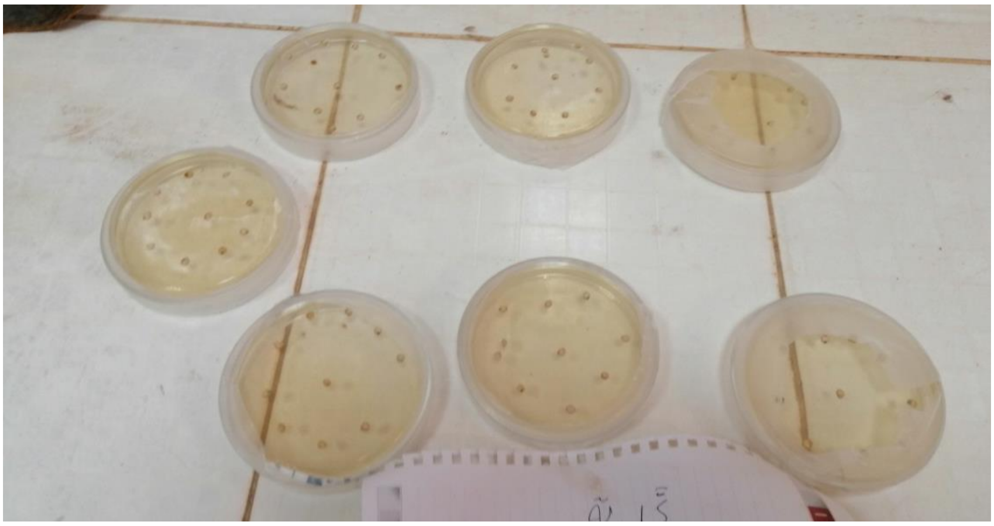
3.3.2 Agar Method:

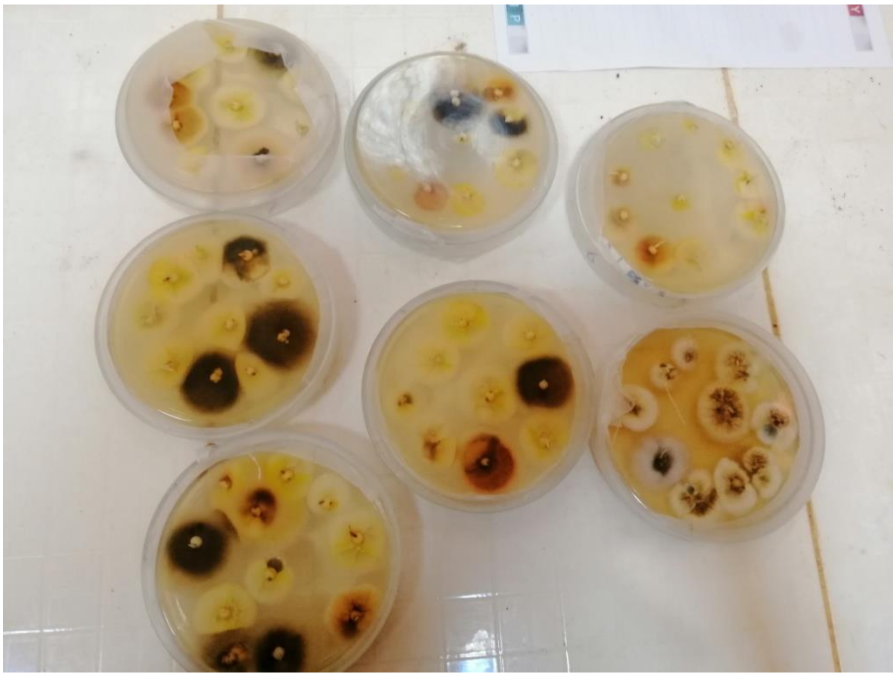
3.3.4 Slide preparation and identification

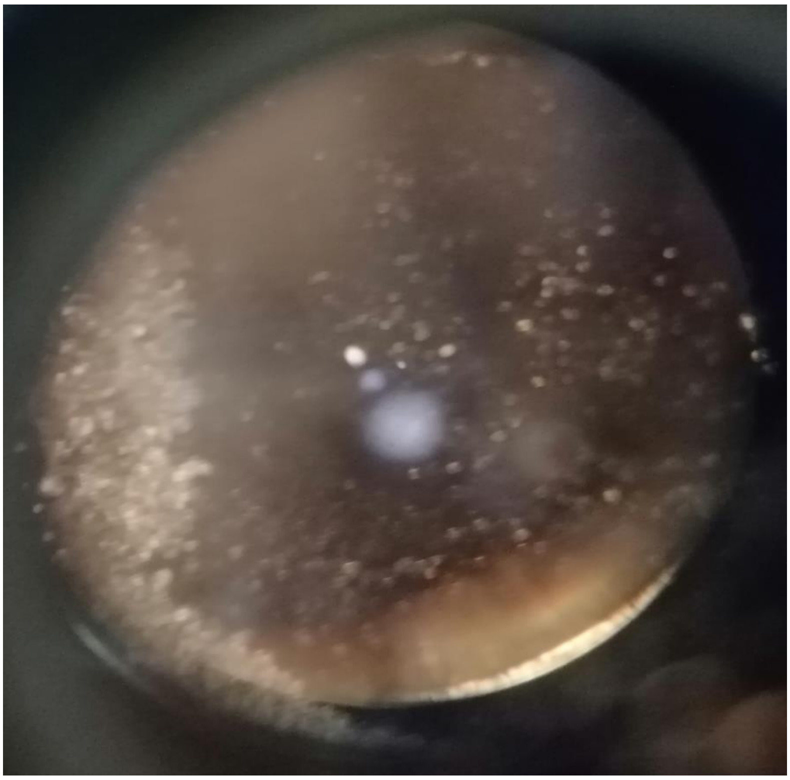
The samples of fungus were taken randomly from each plate samples. These samples were identified on the basis of colony characteristics and microscopic examinations on PDA.

3.3.5 Pure culture

An amount of the mycelium of *Aspergillus spp.* were picked and cultured into sterilized glass Petri-dishes (9.0 cm in diameter) containing N media for further identification with the help of various keys (Raper and Fennel, 1965; Booth, 1971; Barnett & Hunter 1972 and Ellis, 1980). Fungal growth continued for 7-10 days and then kept in the refrigerator as a stock for future investigation.







CHAPTER FOUR

RESULTS

This study was conducted under laboratory conditions of plant protection Department, College of Agricultural Studies, Sudan University of science and Technology during February, 2020, to investigate the distribution and level of contamination of sorghum grains with Aspergillus fungi on seeds samples collected from two locations (Omdurman and Khartoum North) of grain central markets in Khartoum State.

Table 1, shows the mean percentage frequency (PF) of occurrence of *Aspergillus spp*. in samples of Sorghum seeds from the two different locations and distribution revealed by the standard blotter method and (AM) for detection of *Aspergillus* spp. as described by IST after four days from incubation.

Generally, test of all sorghum seeds samples revealed that the fungus was found distributed in the two locations. The level of contamination was not varying too much from one location to another and ranging from 9 to 10%. However, among the two locations, seeds samples obtained from Omdurman 10 % and Khartoum North 9%.

The microscopic examination revealed that the most predominant seed borne fungus recorded across the seeds samples from the three locations was *Aspergillus niger*.

Table 4. 1 Mean percentage frequency (PF) of occurrence of *Aspergillus spp*. and distribution in samples of Sorghum seeds from different locations four days after incubation

Locations	R1	R2	R3	R4	R5	Mean
Khartoum North	6	5	3	6	7	9
Omdurman	7	4	5	7	7	10

CHAPTER FIVE

DISCUSSION

The study was carried out to investigate the distribution and level of contamination of sorghum grains with Aspergillus spp. collected from three central grain markets (Omdurman and Khartoum North) in Khartoum State.

The threat from contaminants fungi to crop quality and quantity cannot be ignored. The risk encountered have been reported by several authors (Haq Elamin NH *et al.,* 1988; Ali, 1989; Saber *et al.,* 1998; El-Naghy *et al*., 1998; Osman *et al*., 1999; Holbrook *et al*., 2000; Thompson and Henke 2000; Yousif M.A.*et al*., 2010).

Azhar *et al., (*2011) reported that the seed mycoflora of most concern are produced by species within the genera of *Aspergillus*, *Fusarium*, and *Penicillium* that frequently occur in major food crops in the field and continue to contaminate them during storage, including cereals, oil seeds, and various fruits.

Peter, W. (2014) reported that fungi are destructive agents causing losses of agricultural commodities in many zones of the world, ranking alongside insects and weeds for crop loss or yield reduction. They can occur on growing in-field crops as well as harvested commodities, leading to damage ranging from rancidity, odour, flavour changes, loss of nutrients, and germ layer destruction. This can result in a reduction in the quality of grains, as well as gross spoilage and possible mycotoxin production. Fungi are often referred to as moulds in this context, to differentiate them from single celled yeasts.

This study demonstrated categorically that irrespective of load of seed borne fungi, their association with food crops seeds in different locations of Khartoum State appears to be a prevalent situation. Apart from the control which was not recorded, level of contamination with *Aspergillus spp*. were recorded on seeds samples tested with standard blotter method as described by the International Seed Testing Association (ISTA 1966) revealed that all other seeds from different locations were contaminated with the test fungi. These results are in agreement with those of Syed Danis, *et al*., (2013); Kamal and Mughal (1968) and Khan e*t al*., (1974), who reported the presence of Aspergillus, Penicillium, Alternaria, Fusarium, and Rhizopus, species in seeds of food crops. The results also corroborate those of Khan and Bhutta (1994); Bhutta and Hussain (1999) and Singh (1983) who reported the occurrence of Aspergillus, Penicillium and Fusarium spp. were common associates of seeds crops.

The results showed that the most prevailing seed borne fungi recorded across samples tested seeds was *Aspergillus Niger* with varying level of contamination. However, the common occurrence of seed borne fungi like Aspergillus and Penicillium had been widely reported by Haq Elamin NH *et al*., 1988 and Martin *et al*., (1984).

The slight difference in the level of contamination with seeds borne fungi in some seeds or in some location (10%) compared to other location demonstrated by this study could be attributed to favorable weather conditions (high humidity) for Aspergillus spp. different environments. The implications of this variation was highlighted in the report of Bandyopadhyay (1986) who determined that prevailing conditions at harvest and storage were responsible for incidence of spoilage fungi. Moreover, the present result showed that all the samples tested were associated with *Aspergillus niger* fungus which was known to be a predominant fungi of groundnut in relevant studies. Mathur *et al.,* (1975) and Mukherjee *et al.,* (1992) also found that *Aspergillus* were the predominant storage fungi of groundnut seeds.

Conclusion

- The food crop seeds besides being of high quality and purity should also must be free from contaminant fungi. In this study *Aspergillus spp*. were encountered in wide range of incidence percentage in all samples of sorghum, collected from the three locations, in Khartoum State of Sudan.
- The most prevailing seed borne fungi recorded across samples seeds was the storage ones; *Aspergillus Niger*, with close level of contamination.

Recommendations

- There is a real need for proper cultural practices during the growing season accompanied with timely harvest and proper storage of the grain after harvests are critical management tools for minimizing mycotoxin contamination.

- Establishment of seed borne fungi mapping through continuous seed health analysis for sorghum, across Sudan and to be updated regularly so that research will target potentially important ones.
- The efforts should be guided by the results obtained by other African countries which succeeded in developing a non aflatoxin producing species of Aspergillus to commercial stage to compete with Aflatoxin producing fungus.
- More investigation needs to be done to determine consistency of the seed borne fungi isolated across locations to determine percentage incidences and severity under favorable conditions.
- Reduction of grains moisture content could help greatly reducing the level of contaminants fungi

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ported in many African countries. Most recently, 2.3m bags of maize from Kenya have been declared unfit for human consumption by the government due to presence of high levels of lethal aflatoxins, which have killed at least one child (BBC News, 2010). The crop was harvested in the drought- and famine-prone Eastern Province and went bad because farmers lacked the appropriate storage facilities.

Chronic toxicity is more common in humans but symptoms such as lowered milk or egg production in livestock and stunting of development in humans may not be attributed to aflatoxin. Although the full implications of exposure are unknown due to lack of medical testing and study, there is evidence of strong correlations between aflatoxin exposure and liver cancer, particularly in areas with endemic infection of hepatitis B and C viruses (Williams and Mc Donald, 1983; Dawson and Bateman, 2001; IARC, 2002 and Islam *et al*., 2009).)

2.3 *Aspergillus* spp.

The role of *Aspergillus* species in food spoilage is well-established (Haq Elamin NH *et al*., 1988; Ali, 1989; Yousif M.A.et al., 2010and KRN Reddy, 2010). Many Aspergilli are xerophilic and present particular problems during commodity harvest, and d