Sudan University of Science and Technology

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Effect of Application of Some Agronomic Practices on infection by *Aspergillus flavus* and yield of Groundnut*(Arachis hypogaea L.)*

أثر تطبيق بعض العمليات الزراعية في الاصابة بفطر الأسبيرجلس فلافس وانتاج محصول الفول السوداني

By

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استهلال

قال تعالى:

أَنَّا صَبَبْنَا الْمَاءَ صَبًّا * ثُمَّ شَقَقْنَا الْأَرْضَ شَقًّا * فَأَنْبَتْنَا فِيهَا حَبًّا * وَعِنَبًا وَقَضْبًا * وَزَيْتُونًا وَنَخْلًا * وَحَدَائِقَ غُلْبًا * وَفَاكِهَةً وَأَبًّا * مَتَاعًا لَكُمْ وَلِأَنْعَامِكُمْ .

صدق الله العظيم

سورة عبس الآيات (25-32)

DEDICATION

This work is dedicated to all my family specially to my parents , my wife and to my young sister.

Acknowledgment

I am greatly thank ful to Allah who enabled me to accomplish this work.

I wish to express my sincere thanks and appreciation to my supervisor Dr. Ibrahim Saeed Mohammed and co supervisor Dr.Samia osman Yaguob for their invaluable advice ,guidance, patience and encouragement throughout the research process and for their effective help inthe preparation of this thesis.

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ABSTRACT

Groundnut plays an important role in the livelihood of poor people and in the rural economy of many developing countries. Infection of the crop with, Aflatoxin caused by*Aspergillus flavus*, hampers international trade and adversely affects health of consumers of nuts and their products. No single approach for control of *Aspergillusflavus* in groundnut was proved to be effective and without drawback. The objective of this study was to investigate the effect of an Integrated Agronomic Practices Management on infection by *Aspergillus flavus*inaddition to Growth and yield in Groundnut. Treatments which includes single dose of Jatropha Seed Cake (2.5 t/ha), sulfur (119kg/ha), super phosphate (119 kg/ha), ammonium sulphate (119 kg/ha) and control were applied to two groundnut cultivars under three harvesting periods(90-100-110days)after sowing laid out in a randomized complete block design arranged in a split-plots replicated three times for two successive seasons.

Experiments were conducted for two seasons 2011/12-2012/13 in the demonstration farm of the College of Agricultural Studies, Sudan University of Science and Technology, Khartoum North, Shambat. The results showed that fertilizers treatments among them and interaction between varieties and fertilizers significantly reduced infection of groundnut by *Aspergillus flavus* and revealed significant increase on leaf area index, plant height, number of primary branches, number of pods/plant, germination % and yield /ha as well.The lowest infection (5.8% and 6.9%) in pods and 1.2% in seeds were obtained by the interaction of Fertilizers and varieties whereas the highest infection (27.3%) was countered in the control. The two tested cultivars showed similar behavior towards the fungus infection during vegetative and reproductive growth stage except for plant height, primary branches, yield and yield components. The yield increased with delayed harvest as well. The current findings were considered promising and encouraging for more research studies that aims at minimizing the risk of contamination with fungi producing mycotoxins in Sudan.

ملخص الاطروحة

يلعب الفول السودانى دوراً هاماً فى معيشة الطبقات الفقيرة وايضاً الاقتصاد القومي لعدد من الدول النامية .

ان اصابة المحصول بالأفلاتوكسن المتسبب من فطر الاسبيرجلس فلافس ، أدى الى اثارسلبية على صحة مستهلكى الفول ومنتجاته مما اعاق التجارة العالمية للمحصول . ليس هنالك منهج واحدلمكافحة هذا الفطر اثبت فعاليته بدون معوقات واثارجانبية.

الهدف من هذه الدراسة هو بحث اثر الادارة المتكاملة للعمليات الفلاحية على الاصابة بفطر الاسبيرجلس وايضاً على نمو وانتاجية الفول السودانى .

المعاملات تضمنت جرعة واحدة من كيكة بذور الجاتروفا (2.5 طن للهكتار) و119كيلو للهكتار لكل من(الكبريت والسيوبرفوسفات وكبريتات الامونيا) اضافة الى الشاهد تم اخضاع صنفين من الفول السودانى لهُذه المعاملات المختلفة و من ثم حصدتبعد 90-100-110يوم من الزراعة

اختبرت المعاملات المختلفة بواسطة التصميم العشوائْ القطاعى الكامل الذى تم تكراره ثلاث مرات لموسمين متتاليين . وكما وضعت الأصناف فى الاحواض الريْيسية والاسمدة فى الاحواض الفرعية .تم إجرْا التجارب بمزرعة كلية الدراسات الزراعية لجامعة السودان بالخرطوم شمال بشمبات.

أظهرت النتائج أن معاملات الاسمدة فيما بينها والتفاعل بين العينات والاسمدة أدى الى تقليل اصابة الفول السودانى بفطر الاسبيرجلس فلافس بدرجة معنوية كما نتج عن ذلك زيادة معنوية فى كل من مساحة الاوراق ،طول النبات ،عدد الافرع ،عدد حبوب الفول بالنبات الواحد ،نسبة الانبات والانتاجية .

أقل اصابة (5.8% و6.9%) فى حبوب الفول و 1.2% فى البذور وجدت فى التفاعل (التداخل) بين الاسمدة والأصناف فى حين أن أعلى اصابة (27.3%) كانت فى الشاهد .أظهرت الاصناف سلوكاً متشابهاً نحو الاصابة بالفطر وأثناء النمو الخضرى وفترة الازهار ما عدا فى حالة طول النبات والتفرع الأولى والانتاجية كما زادت الانتاجية مع تاخر الحصاد .

النتايج الحالية تعتبر واعدة ومشجعة لمزيد من البحث الذى يهدف الى تقليل مخاطر الاصابة بالفطريات المنتجة للسموم فى السودان.

CHAPTER ONE

1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.,) is a major oilseed crop widely grown in tropical and subtropical regions of the world, and is a native of South American legume not known to the Old World in pre-Columbian times. Portuguese navigators are credited with introducing the crop to the western coast of Africa from Brazil, but it is not known (Mahmoud *et al.*, 1995 and Wiess, 2000).

The crop is the 13th most important food crop of the world and the 4th most important source of edible oil of the world 3 rd most important source of vegetable protein (Taru, *et al.*, 2010). Major groundnut growing countries are confined to the tropical countries ranging from 40º N to 40º S. Major groundnut producing countries are: China (40.1%), Sudan(30%).India(16%), Nigeria (8.2%), U.S.A (5.9%)and Indonesia (4.1) (Nwokoro, 1996).

Worldwide, approximately 25.7 million tons of groundnuts are produced annually from about 21 million hectares of cropped land. Asia alone produces 17.9 million tons, 70% of global production. Africa produces another 20%. About 60% of Africa's production comes from Western Africa (FAO, 2006).

It is an annual legume which is also known as peanut, earthnut, monkey-nut and goobers. Groundnut seeds (kernels) contain 40-50% fat, 20-50 % protein and 10-20 % carbohydrate. Groundnut seeds are nutritional source of vitamin E, niacin, falacin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium. Groundnut kernels are consumed directly as raw, roasted or boiled kernels or oil extracted from the kernel is used as culinary oil). It is also used as animal feed (oil pressings, seeds, green material and straw) and industrial raw material (oil cakes and fertilizer). These multiple uses of groundnut plant make it an excellent cash crop for domestic markets as well as for foreign trade in several developing and developed countries (Nwokoro, 1996).

In Sudan Groundnut plays an important role in the diets of rural populations, particularly children, because of its high nutritive value, protein content was found(21-30%), fat (41-52%), and carbohydrate (11-27%). It is also rich in calcium, potassium, phosphorus, magnesium and vitamin E and the by-product of oil extraction is an important ingredient in livestock feed. Groundnut haulms are nutritious and widely used for feeding livestock. The total area under groundnut production is approximately one million ha with an average yield of 855 kg/ha(Mahmoud *et al.,* 1995 and ARC, 2003-2010).

The yield and quality of groundnut is affected by a wide array of biotic facters. One of the major constraints facing the productivity and availability of healthy groundnut produce worldwide are the losses and spoilage caused by fungi, bacteria, viruses, insects, nematodes and parasitic weeds. In fact, the threat to this crop from *Aspergillus spp*. which produce secondary metabolites called Aflatoxin has now reached a level that outstrips that posed by other biotic and abiotic factors (Berger, 1977).These fungi continue 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). The Food and Agriculture Organization (FAO) estimates that 25 % of the world's food crops are affected by food contaminants, of which the most notorious are those resulted from *Aspergillusspp*. (Anon, 1989)

In Sudan, the impact of these fungi and their secondary metabolites as food contaminants is well-established (Haq Elamin *et al.*, 1988; Ali, 1989; Yousif *et al.,* 2010 and Reddy KRN. 2010). Ahmed(1981) indicated that *Aspergillusflavus* was isolated from twenty six samples (43.33%) out of the total number of samples investigated. Younis and Malik (2003) who studied contamination in Sudanese groundnut and groundnut products found that the percentage of contamination was 2%, 64%, 14% and 11% for kernels, butter, cake and roasted groundnuts, respectively.

Obviously, the infection of groundnuts by various *Aspergillusspp*. not only results in reduction in crop yield and quality but also contamination of produce with poisonous fungal secondary metabolites called mycotoxins. These substances arise from the secondary metabolism of fungi in response to a wide range of genetic and environmental factors and are capable of causing diseases in man and animals (Agrios,2005)

The foregoing reflect the potential of risk of contamination of groundnuts and its by-products with *Aspergillispp*.a situation that necessitate more scientific studies to be carried out in order to help overcoming the risk involved.

In most cases in order to control plant pathogens and to protect the crop produce against them, chemical control methods are in practice. Although the use of chemicals has helped increase of yields obtained (Ali, 1996), but one of the major problems with the constant use of chemicals is that resistance can be induced in target organisms in addition to contamination of the environment with very toxic substances (Okigbo, 2004; Carvalho, 2004). This has initiated the exploration of safe alternate methods of control.

Obviously, no single approach for control of *Aspergillusspp*.contaminants of groundnuts was proved to be effective and without drawback. Therefore, integrated management approaches are the only solution to minimize the risk of contaminants. These approaches should include minimum use of chemicals for checking the pathogen population, optimization of pre and post cultural practices e.g. fertilization, scheduling of harvest and use of resistant varieties.

In view of this, the current study aimed at exploring and investigating on theUse of different groundnut varieties (ii) Proper plant nutritionand (iii) Scheduling of harvesting in order to formulate an integrated approach for minimizing the negative impactof *Aspergillus spp*. on groundnuts with following objectives:-

- To investigate the resistivity of some groundnuts varietiesto the infection by *Aspergillus spp.*

- To study the effect of organic and inorganic fertilizers on plant health, yield and consequently minimization of *Aspergillus spp*. incidence

- To evaluate the influence of harvesting time on contamination of groundnuts with *Aspergillus spp*.
- To develop Integrated Management Approach for minimizing mycotoxin contamination.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Groundnut

Groundnuts (*Arachis hypogaea*) family leguminaceae, also known as peanuts or monkey nuts, are the edible seeds of a legume plant that grow to maturity in the ground. The crop is cultivated in nearly 100 countries, over 90% of which are developing countries; the groundnut is a food staple and valuable cash crop for millions of households (CGIAR, 2004-2005). It is the 13th most important food crop and 4th most important oilseed crop of the world (Taru, 2010). Groundnut seeds (kernels) contain 40-50% fat, 20-50 % protein and 10-20 % carbohydrate. Groundnut seeds are a nutritional source of vitamin E, niacin, falacin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium. Groundnut kernels are consumed directly as raw, roasted or boiled kernels or oil extracted from the kernel is used as culinary oil (Feuell, 1966). It is also used as animal feed (oil pressings, seeds, green material and straw) and industrial raw material (oil cakes and fertilizer). These multiple uses of groundnut plant make it an excellent cash crop for domestic markets as well as for foreign trade in several developing and developed countries

The crop which believed to be originated from South America (Wiess 2000) is one of the most popular and universal crops cultivated in more than 100 countries in the six continents (Nwokoro 1996); the groundnut is staple food and valuable cash crop for millions of households (CGIAR, 2004-2005). It is the 13th most important food crop and 4th most important oilseed crop of the world (Taru, 2010). According to FAO (2006) it is grown in 25.2 million hectares with a total production of 35.9 million metric tons Major groundnut growing countries are India (26%), China (19%) and Nigeria (11%). Its cultivation is mostly confined to the tropical countries extending from 40º N to 40º S. Major groundnut producing countries are: China (40.1%), India (16.4%), Nigeria (8.2%), U.S.A (5.9%) and Indonesia (4.1%), (Wiess, 2000).

2.1.1. Groundnut cultivation in Sudan

In Sudan, groundnut is one of the major sources of adible oil production for local consumption and exports (salih, 1986). The crop is primarily used for oil extraction in Sudan. It is consumed directly because of its high food value It plays an important role in the diets of rural populations, particularly children, because of its high contents of protein , fat , and carbohydrate . It is also rich in calcium, potassium, phosphorus, magnesium and vitamin E and the by-product of oil extraction is an important ingredient in livestock feed. Groundnut haulms are nutritious and widely used for feeding livestock (IARC and ICRISA, 2002). The earliest forms of groundnut introduced belonged to the subspecies hypogaea. The small-seeded runner types were established on the sandy soils of western Sudan and the bunch types were grown along the Blue Nile on heavy clays. These two groups of groundnut form the land varieties in Sudan. Varieties of the subspecies fastigiata (varieties *vulgaris* and *fastigiata*) were introduced about sixty years ago as part of an improvement programme. Of these *fastigiata* introductions, the cultivar Barberton, primarily because of its early maturity, quickly replaced the late maturing land varieties of the runner type in the northern parts of Kordofan and Darfur.

However, Sudan is one of the major groundnut producing countries. The total area under groundnut production is approximately one million ha with an average yield of 855 kg/ha. The crop is grown under irrigation mainly in central Gezira, New Halfa scheme, some Northern region and Kassala. Under rain fed the crop grown in western Sudan in Kordofan and Darfor regions (ARC, 2010).

2.2 Constraints in Groundnut Production

The primary constraint facing local consumption and export of groundnuts worldwide and in Africa in particular is Aflatoxin associated with spoiled crop with *Aspergillus spp*. In fact, Aspergillus infection and subsequent contamination of groundnut with Aflatoxin is a major limitation in groundnut production. Aflatoxin is secondary metabolites produced by fungi in response to environmental conditions. Infection by Aflatoxin-producing fungi can occur at any stage, from pre-harvest to storage (ICRISAT, 1987). In fact it is a naturally occurring toxin that can spoil a number of crops including groundnuts 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). Countries of import, as well as producers like the US and Argentina, have recognized the need to 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).

2.2.1 *Aspergillus* spp.

*Aspergillus*is classified of order Eurotiales and family Trichocomaceae, is a genus of molds, of which about 200 species have been identified and which found in various climates worldwide. The role of *Aspergillus* species in food spoilage is well-established (Haq Elamin *et al.*, 1988; Ali, 1981; Yousif *et al.*, 2010 and (Reddy et al.,2011).

Many Aspergillus are xerophilic and present particular problems during commodity harvest, and during subsequent drying and storage. About 30 species of Aspergillus or their teleomorphs are associated with food spoilage, these include: *A. flavus, A. parasiticus, A. nomius, A. ochraceus, A. candidus, A.restrictus, A. penicillioides, A.niger, A. carbonarius, A. fumigatus,* *A.clavatus*, and *A. carbonarius, and A. versicolor (Peter, 2010.)* However, Haq Elamin *et al.*, (1988); Ali, (1989); Yousif *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, *A. flavus*, *A. parasiticus* and aflatoxins typically affect oilseeds, including groundnuts, soya, tree nuts, maize and various oilseed-based animal feedstocks (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.

2.2.2Aflatoxin

Aflatoxin is the name for a group of toxins known as B1, B2, G1, G2, M1 and M2 (carcinogenic compounds) that are produced mainly by two fungi called *Aspergillus flavus* and *Aspergillus parasiticus* (Kurtzman *et al.,* 1987; CAST, 2003). These toxins occur naturally and have been found in a wide range of commodities used for animal and human consumption. Depending on their levels, toxins can severely affect the liver and induce a human carcinogen, i.e., causes cancer. In many developing countries, Aflatoxin is a major health risk to both humans and animals due to the high levels of the contaminated products consumed (Wright *et al*., 2002).The toxin can contaminate a variety of agricultural commodities but most commonly maize, peanuts, cottonseed, sesame and tree nuts (Yousif *et al*., (2010);Olusegun (2013) and) 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 reported in many African countries as well as India, China, Thailand and others. 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).

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 (IARC, 2002).

Aflatoxin contamination of groundnut is a major problem in most of the groundnut production regions across the world. It is mostly influenced by the occurrence of drought during the late seed filling duration. Toxicity of groundnut from Aflatoxin endangers the health of humans and animals and lowers market value (Abdalla *et al.,* 2005).

2.3 Effect of fertilization

David (2009) who investigated the impact of fertilizer on mycotoxin contamination of field crops demonstrated that plant nutrition is critical for overall plant health and for reducing the risk of mycotoxin contamination. On the other hand he added that, in general, poor fertilization increases the risk of mycotoxin contamination.

2.3.1 Effect of improved agricultural practices on contamination and yield of groundnuts

It is well established that appropriate cultural practices during the growing season accompanied with timely harvest and proper storage of the grain after harvest are critical management tools for good plant health, high yield and minimizing contamination of groundnut with mycotoxin producing fungi (David, 2009).

Safa (2008) investigate the effect of some cultural practices as a component of Integrated Pest Management (IPM) to control tomato insect pests and simultaneously increase the yield clearly demonstrated the positive effect of these practices.Futhermore, Agrios.(2005)Who assess the effect of some cultural practices on infection by scab disease of potato reported a significantly positive correlation between economic losses due to infection and cultivated variety, seed source and fertilizer application.

Moreover, substantial evidence showed that groundnut responds well to additional inorganic and organic fertilizers application.

2.3.2Phosphorous (P)

Phosphorus is very important nutrient element for crop health and yield. It plays an important role in physiological processes of plants. As P source, single superphosphate is the most suitable fertilizer for groundnut in Nigeria Lombin*et al.,* (1985). Sulfur deficiency in legume crops affects not only yield, but also the nutritional quality and health of the seeds (Jamal *et al.,* 2010).

The positive effect of phosphorus fertilizers application on both quantity and quality of groundnut was well established by many researchers. Tomar *et al.,* (1990) observed significant increase in pod yield in groundnut with application of 40kg P_2O_5/ha, when rainfall was well distributed. Application of phosphorous significantly increased plant height, kernel/ plant, dry weight/plant and shelling percentage.

Sinha (1970) observed that placement of superphosphate either in contact or 3-5 cm below the seed was equally effective and significantly superior to that of broadcast application in the uptake of fertilizer phosphorous and vigour, but not in the dry matter weight or total phosphorous content of the plant.

Tandom and Rego (1988) reported that the (P) requirement per unit yield is highest for oilseeds as compared with other field crops. The end result of these reactions is conversion of complex phosphates to monobasic forms in plant rhizosphere thus rendering them available to plants. The known phosphate-solubilizing microorganisms include bacteria, fungi and Actinomycetes (Mahadi, 1993).

Crop inoculation with “phosphor- bacteria” an inoculants impregnated with cells of the bacterium bacillus moratorium var. Phosphaticum has been shown to enhance crop productivity and health although the accruing gains have in many cases been accredited to the production of growth- promoting compounds and antifungal antibiotics however, no research reports are available on work carried out on this aspect of bio-fertilization in the Sudan.

Restricted soil moisture has been reported to be the primary factor for low/ erratic response in such cases , Correlation of available P with pod yield and quality indicated that subsoil fertility made an important contribution to nutrient uptake by groundnut (Agrios. (2005).

2.3.3 Sulphur (S)

Sulphur plays an important role in plant metabolism. Freney (1967) reported that sulphur is known to be required for the amino acid cysteine, cystine and methionine in building proteins, for biotin in carbon dioxide fixation, for thiamine in the decarboxylation of x-keto acid, for glutathione in the conversion of methyl glyoxal to lactic acid and in triose phosphate oxidation, for coenzyme A in the metabolism of fatty acids, for lipoic acid in the decarboxylation of x-keto acids, and for methionine as a source of methyl groups.

Singh *et al.*, (1990) reported that application of 20kg S/ha as elemental to the soil before sowing produced 25% more pods and 16% more fodder in groundnut. Also Singh *et al.*, (1970) observed that both quantity and quality of groundnut improved significantly irrespective of the form of sulphur applied.

Tandom and Rego (1988) reported that significant responses of groundnut to the application of sulphur at all yield parameters Hago and Salama (1987) reported that when sulphur was applied at rates of 50, 100 or 150 kg/ha, either at sowing, at flowering or in two equal splits at sowing and flowering, shoot dry weight, total sulphur content of leaves, nodule number per plant and pod yield and quality were all significantly increased. However, nodule dry weight was unaffected. The greatest response was at or below 50 kg S/ha applied at sowing.

Omer et al., (1970) reported that the application of gypsum with and without nitrogen, phosphorous and potassium significantly increased the yield of pods and total mineral constituents in groundnut plants besides improving yield and quality of nuts. The results were more pronounced with combined treatments of gypsum and phosphorous supplied at 38.4 kg P_2O_5/ha. It was suggested that gypsum should be added at 720 kg/ha or even more in the fruiting zone at the early blooming stage to increase pod development and health as well as yield. It was also recommended that 24 kg N/ha should be given at the early stage of growth, and 38.4 kg P_2O_5/ha and 57.6 kg K_2O/ha need to applied in the rooting zone before planting.

2.3.4Organic manure

Organic manure is very important, as it contains both major and minor elements necessary for plant growth, and improvement of the physical, chemical and biological properties of the soil Ganapathy S.G.(2014).

The byproduct of oil extraction from *Jatropha curcas* seeds and kernel is called seedcake. It is containing highly toxic protein that is not suitable for animal's feeds, although good for organic manure; it is being used as an organic fertilizer Sirinophakun P.(2014). The cake is rich in nitrogen (3.2%) phosphorus (1.4%) and potassium (1.3%) and can be used as manures (Keremane BG.(2003)and Openshaw K.A(2000) .In Sudan Limited studies have been reported on potential of Jatropha Seed Cake to be used as organic fertilizers. Accordingly, the use of Jatropha seed cake in fields as manure fertilizer should be carefully considered.

However, it does have potential as good organic manure (Staubmann et al., 1999; Gubitz et al., 1999), replacing chemical fertilizer since it has nitrogen content similar to that of neem oil cake, castor bean, cow/chicken manure. The nitrogen content ranges from 3.2 to 3.8% (Juillet et al., 1955; Moreira, 1979; Vohringer, 1987). Application showed phytotoxicity, expressed as reduced germination, when high rates of up to 5 tonnes ha-1 was used. The GTZ project in Mali carried out a fertilizer trial with pearl millet where the effects of manure (5 tonnes/ha), physic nut oil cake (5 tonnes/ha) and mineral fertilizer (100 kg ammonium phosphate and 50 kg urea/ha) on pearl millet were compared (Henning et al., 1995). Pearl millet yields per hectare were maximum (1366 kg) in physic nut oil cake treatment. As the costs for mineral fertilizer were higher than those of the oil cake (Henning *et al.*, 1995), it is appreciated by the farmers and can be sold for 10 FCFA per kg (US $ 0.02/kg).

2.4Use of resistant varieties

In Sudan Ahmed (1981) reported that the variety NA2/40 show marked resistance to *A. flavus* in comparison with varieties 5B2 and Ashford. He added that The Aflatoxin content of three varieties was 10, 40 and 60 mg/kg attributed to the impenetrability of protective shell. However, Studies on varieties resistance to *Aspergillusflavus* for three different varieties of groundnuts in Nigeria showed no evidence of varietal resistance to *A. flavus* infection (Agrios,(2005).

2.5 Management of *Aspergillusspp.* in groundnut

It is well established that appropriate cultural practices during the growing season accompanied with timely harvest and proper storage of the grain after harvesting are critical management tools for good plant health, high yield and minimizing contamination of groundnut with mycotoxin producing fungi (David, 2009).

2.6Effect of soil moisture

Craufurd *et al.,* (2006), under Nigerian conditions demonstrated the significant relationship between Aflatoxin concentration and plant extractable soil water (using CROPGROW model). This Model formed the basis for developing a decision support system to predict Aflatoxin concentration in groundnut. Nageswara Rio *et al.,* (2004) have used a similar approach to model the risk of contamination of Aflatoxin in Queensland, Australia, using the crop simulation model APSIM and have shown how farmers in Queensland can manage Aflatoxin given a Decision Support System (DSS).

2.6.1Effect of timely harvesting

In Queensland, Rachaputi *et al.,* (2002) identified early harvest and threshing as best management practices for minimizing Aflatoxin contamination under high Aflatoxin risk conditions. They added that, in general, early sowing or early harvest and even supplementary irrigation (if available) are possible ameliorating practices for reducing Aflatoxin risk.

2.6.2Varietal selection

The choice of a groundnut variety for any particular area depends on matching the variety with the length of the growing season (Ali and Malik, 1992). Groundnut varieties whose growth cycle is longer than the duration of growing season at a particular location either fail to mature or mature at a time when soil is too hard to dig the pods. Moreover, in a majority of the groundnut growing regions, drought stress affects groundnut production (Bailey, 1999). In Indian conditions ICGV 86699, K-134 and TMV-2 were considered as drought tolerant (Reddy and Setty, 1995). Ali and Malik (1992) reported that ICGS (E) 52 and ICGS (E) 56 as promising short duration varieties that could escape end of season drought in rain fed areas of Pakistan. Schilling and Misari (1992) reported that short duration and erect varieties like 55-437 released in Niger, Nigeria, Chad, Gambia and Cameroon; and varieties 73-30 and 73-73 released in West Africa and ICGS (E) 30 and ICGS (E) 60 released in Botswana are drought tolerant to combat the risk of contamination with Aflatoxin. Attempts to develop Aflatoxin resistant varieties have been carried out by many researchers (Petit, 1986; Waliyar *et al.,* 1994; Upadhyaya *at al.,* 2004). Ahmed (1981) who screened groundnut for resistance to *Aspergillus flavus*,reported marked resistance to *A. flavus* among tested varieties.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Study location

Field experiments were conducted in Demonstration farm, College of Agricultural Studies, Sudan University of Science and Technology, Shambat, Khartoum North, Sudan for two consecutive seasons 2011/12- 2012/13in autumn. Laboratory investigations were carried in plant pathology lab, Department of Plant Protection of the college. Shambat is located at longitude 32 35"E and latitude 15 31"N, within the semi-desert region. Climate of the locality is semi-desert and tropical with low relative humidity.

All materials, which used in the experiments, were sterilized using 70% ethyl alcohol. Formalin (10%) was used for Petri plate 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. Layout of the Experiment and Land Preparation

The experiment was designed in split plotsdesign replicated three times. Two cultivars of groundnut (Sodri =V1) and (Gebish=V2) and four types of fertilizers were applied; (F1) control without fertilizer, (F2) organic fertilizer (Jatropha Seed Cake, (JSC) of 2.5 t/ha, and three inorganic fertilizers: (F3) pure sulfur of 119 kg/ha, (F4) super phosphate of 119 kg/ha and (F5) Ammonia sulphate of 119 kg/ha. After maturing (110 days from sowing), three harvesting periods were taken (every 10 days), early, medium and late. The field was prepared according to the methodadopted by Engineering Department of Agriculture.

3.3 Source of Seeds and fertilizers

Two cultivars of groundnut certified seed (Sodri = V1) and (Gebish= V2) were obtained from Al –Obied Research Center (North Kordofan), and Arabian Company for Seeds, Khartoum. Ammonia sulfate ,pure sulfate and super phosphate were obtained from crops production department, College of Agricultural Studies, Sudan University. Jatropha seeds were obtained fromEnergy Research Central,Suba-Khartoum .

3.3.1 Land preparation

The field was prepared according to adopted method by engineering department of agriculture, Sudan university- Shambat. Started by ploughing using disc plough then left for one month to dry out and then harrowed using disc harrow after that it was leveled divided into main plots and subplots each of four lines which measured four meter in length and 50cm apart.

3.3.2 Seed sowing

The seeds were sown in July 12th, and July 21th respectively for two seasons, 3 seeds per hole were used then thinned to two seedlings per hole. During July until August (about two months) it was irrigated by rain-fed then irrigation was applied every 15 days. In rest season the three harvesting time were done in October, 11- 21th and first November, and for second season were done in October, 24th, and November in 3-13th.

3.4 Data Collection

3.4.1 Pre-harvest data

Observations were taken from 10 plants selected randomly in each subplot. Data was recorded on pre and post harvesting stage. The data recorded during pre-harvest; were percentage of germination after 3 weeks and 5 weeks, leaf area index after 90 days. The harvest stage data were: height of plant, number of primary and secondary branches, and number of pods per plant. The yield of threshed seeds from one meter of each plots were taken and transformed into kg/ha.

3.4.2 Post-harvest data

One random and homogeneous sample of 100 pods was secured from each sub plot (treatment). Pod samples were drawn according to international standards for seed testing association (ISTA, 1966). Collected samples were labeled and kept separately in sealed paper bags and transported to the laboratory where they were stored at 5^0C refrigerator for further analysis.

3.5 Dry seed inspection

Each of the samples selected were examined under stereoscopic binocular microscope (25-4x) and by magnified lens and naked eye according to the international seed testing association (ISTA Rules, 1966). The samples were examined for impurities, plant debris, weed seeds, discoloration and malformation.

3.6 Incubation procedures:-

The samples were tested by the standard blotter method and (PDA) potato dextrose agar method for detection of *Aspergillusflavus* fungus as described by ISTA.

3.7 Detection and identification of *Aspergillusflavus*

3.7.1 Blotter method

For the detection of *Aspergillusflavus* in each sample standard blotter method as described by the International Seed Testing Association (ISTA 1996) was used. Five pods and five seeds of dehulled pods from each sample were then platted on moistened filter papers (dia. 9.0 cm) in 9.0 cm sterilized plastic

Petri-dishes and arranged 3 pods at the periphery of the plate and 2 at the centre. Similar arrangement was made for seeds testing. Each test was replicated three times and then kept in dark place for seed germination.

After seven days of incubation, pods and seeds were then examined for fungal growth under a stereo microscope. *Aspergillusflavus* identified by its habit of growth and supplemented by microscopic examination of spores and fruiting bodies using a compound microscope. Other identification aids were Burgess *et al.*, (1994); Mathur (1975);Agarwal *et al.*, (1996) and Mathur and Kongsdal (2003). Infection levels were recorded as the percentage of infected pods and seeds as well in a sample

3.7.2. Agar Method

Pods and seeds samples were plated in sterilized glass Petri-dishes on potato dextrose agar medium (PDA). In case of pods and before being plated, the samples was pre-treated with sodium hypochlorite (Naocl) 1% solution for 5 minutes then washed three times with sterilized distilled water (SDW) and dried between tow filter papers. All Petri dishes were then sealed by a thin layer of Para film to prevent post contamination and incubated for seven days at 25^{0}C. On the 8^{th} day the pods and seeds were examined under light microscopes using slides preparation for further identification of the fungus.

3.7.3 Slide preparation and identification

The fungus was taken randomly from each sample of seeds. These samples were identified on the basis of growth characteristics and microscopic examinations. Standard books and research papers were consulted during the examination of the fungus (Aneja, 2004; Rifai, 1969; Barnet and Hunter, 1999).The binocular compound microscope was also used to confirm the occurrences of the *A. flavus* in each plate. The percentage incidence of *A. flavus* was calculated by applying the following formula:

PI = (No. of pods and seeds on which fungus appear / Total number of pods or seeds) X 100.

3.8Statistical Analysis

The collected data were subjected to standard statistical analysis. The procedure of analysis of variance tests and means were statically separated using Duncans multiple Range test according to the description of Gomez and Gomez (1984). The data was analyzed by MSTAT-C Statistical Package.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Effect of fertilization, varieties and harvest time on infection of groundnut pods by *Aspergillus flavus*

The results of the effect of Jatropha Seed Cake (JSC) and different inorganic fertilizers on infection of pods of two groundnut cultivars by *A. flavus* under three harvesting periods for two successive seasons was presented in table 1a & 1b. The results showed that interaction between cultivars and fertilizers had significant difference. In the first season, the lowest infection values (6.9%, 9.7% and 14.4) were obtained by F3V1 fallowed by F2V1 and F4V1respectively in first harvest which are significantly different from F1V1 (the control) that showed the highest infection rate 24.3. In this season also, the interactions F5V2 and F4V2 showed significantly different lower infection rate than the control (F1V2) and the F1 (control) showed the highest infection rate among all treatments in first harvest of first season. These results were in line with(**Gebreselassie et al., 2014) who demonstrated that** the integrated agronomic management practices showed significant reduction of *A. flavus* infection on groundnut where **application of DAP fertilizer as source for Phosphorus and gypsum as source for Ca is ones of the components.**

Table, 1a: Effect of fertilization, varieties and harvest time on percentage infection of pods by *Aspergillus flavus season* 2011/12

	infection of pods %								
	First harvest			Second harvest			Third harvest		
Treatments	V1(Sodri)	V2(Gebish)	Mean	V1(Sodri)	V2(Gebish)	Mean	V1(Sodri)	V2(Gebish)	Mean
F1(Control)	24.3 a	25.3 a	24.8 a	16.9 a	12.7 abc	14.8 a	20.7 a	14.2 ab	17.5 a
F2(JSC)	09.7 bc	25.3 a	17.5 ab	15.4 ab	09.7 bc	12.5 a	12.3 ab	05.8 a	09.1 a
F3(Sulfur)	06.9 c	25.3 a	16.1 ab	15.4 ab	15.4 ab	15.4 a	14.2 ab	18.9 a	16.5 a
F4(Super ph.)	14.4 b	15.4 b	15.4 b	17.6 a	15.4 ab	16.5 a	18.9 a	12.3 ab	15.6 a
F5(Amonium sulfate)	23.3 a	12.7 bc	18.0 ab	18.1 a	12.7 abc	15.4 a	18.8 a	15.6 ab	17.2 a
Mean	15.9 a	20.8 a		16.7 a	13.2 a		17.0 a	13.4 a	
CV%			68.8			87.7			77.6
LSD V			6.91			6.68			11.17
LSD F			9.05			8.75			15.80
LSD V*F			20.6			33.2			22.10

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

* Data in parentheses transformed using square root transformation$\sqrt{X + 0.5}$ before analysis

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

In the second season the lowest infection rates were shown by F2V1 (05.8%) for the three harvest periods and F5V1 (12.3%) and F4V2 in the second and third harvest period respectively. However, for the two seasons there were no significant differences between cultivars. **Similar results were also obtained by Nigam et al., (2009) who reported that** pre and post harvest management practices can significantly reduce Aflatoxin contamination in farmers' fields. They further added that, despite global efforts, progress in Aflatoxin resistance breeding has been limited due to the low level of resistance to different components of resistance (preharvest seed infection and Aflatoxin production, and *in vitro* seed colonization by *A. flavus*), lack of reliable screening protocols, and limited understanding of genetics of resistance. Obviously, as reported by Dietzgen (1999) that Aflatoxin contamination can be minimized 'on-farm' using a combination of agronomic and genetic strategies including: optimal harvesting management, appropriate plant density and use of peanut varieties that escape end-of-season drought stress.

Table, 1b: Effect of fertilization, varieties and harvest time on percentage infection of pods by *Aspergillus flavus* *season*2012/13

	infection of pods %								
	First harvest			Second harvest			Third harvest		
Treatments	V1(Sodri)	V2(Gebish)	Mean	V1(Sodri)	V2(Gebish)	Mean	V1(Sodri)	V2(Gebish)	Mean
F1(Control)	20.7 abc	27.3 ab	24.0 a	12.7 cd	18.1 bc	15.4 a	12.7 abc	11.9 abc	12.3 a
F2(JSC)	05.8 d	22.1 abc	14.0a	01.2 e	26.1 a	13.7 a	06.9 c	15.4 ab	11.2 a
F3(Sulfur)	17.9 abcd	29.1 a	23.5 a	20.3 ab	13.9 bcd	17.1 a	12.7 abc	17.6 a	15.1 a
F4(Super ph.)	14.2 bcd	15.6 abcd	14.9 a	12.7 cd	12.7 cd	12.7 a	09.7 bc	06.9 c	08.3 a
F5(Amonium sulfate)	12.3 cd	18.9 abcd	15.6 a	06.9 de	12.7 cd	09.8 a	15.4 ab	17.6 a	16.5 a
Mean	14.2 a	22.6 a		10.7 a	16.7 a		11.5 a	13.9 a	
CV%			70.3			89.8			99.3
LSD V			9.9			6.7			6.9
LSD F			12.3			8.8			9.0
LSD V*F			17.3			14.2			27.0

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

* Data in parentheses transformed using square root transformation$\sqrt{X+0.5}$ before analysis

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

The effect of Jatropha Seed Cake (JSC) and different inorganic fertilizers on infection of seeds of two groundnut cultivars by *Aspergillus flavus* under three harvesting periods for two successive seasons was presented in tables 2a & 2b. Although the result revealed no significant difference between cultivars but highly significant differences were obtained among fertilizers treatments and the interaction of cultivars and fertilizers. In first season the lowest significantly different fungal infection was shown by F2V1 (9.7%) and F2V2 (12.7) in first and second harvest period respectively whereas F1 (The control) in first harvest showed the highest infection (31.1%). In second harvest F4 showed the highest values and F2 is the lowest. Finally in third harvest F4 gave the highest infection and F5 is the lowest. The highest Infection of groundnut seeds by the pathogen in second season were showed by F3 and the lowest infection was observed in (F1 and F4), F5, and (F2 and F1) for first, second and third harvest respectively.

Table, 2a: Effect of fertilization, varieties and harvest time on percentage infection of seeds by *Aspergillus flavus* *season*2011/12

	SeedsInfection %								
	First harvest			Second harvest			Third harvest		
Treatments	V1(Sodri)	V2(Gebish)	Mean	V1(Sodri)	V2(Gebish)	Mean	V1(Sodri)	V2(Gebish)	Mean
F1(Control)	31.9 ab	30.0 ab	31.1a	15.4 ef	30.0 a	22.7 ab	20.3 b	18.1 b	19.2 ab
F2(Jatropha)	09.7 d	25.3 bc	17.5c	23.3 bc	12.7 f	18.0 b	22.5 b	21.1 bc	21.8 ab
F3(Sulfur)	21.1 c	33.0 a	27.1ab	17.6 de	26.6 ab	22.1 ab	18.1 b	15.4 bc	16.8 ab
F4(Super ph.)	26.1 bc	12.7 d	19.4 bc	23.9 bc	23.9 bc	23.9 a	21.1 b	31.0 a	26.1 a
F5(Amonium sulfate)	28.3 ab	21.1 c	24.7 abc	25.3 ab	21.1 cd	23.2 ab	21.1 b	09.7 c	15.4 b
Mean	23.4 a	24.5 a		21.1 a	22.9 a		20.6 a	19.1 ab	
LSD V			6.2			4.3			6.9
LSD F			8.1			5.6			9.1
LSD V*F			10.0			8.9			16.7

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

* Data in parentheses transformed using square root transformation$\sqrt{X + 0.5}$ before analysis

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

Table, 2b: Effect of fertilization, varieties and harvest time on percentage infection of seeds by *Aspergillus flavus* *season*2012/13

	SeedsInfection %								
	First harvest			Second harvest			Third harvest		
Treatments	V1(Sodri)	V2(Gebish)	Mean	V1(Sodri)	V2(Gebish)	Mean	V1(Sodri)	V2(Gebish)	Mean
F1(Control)	10.8 d	18.0 cd	14.4 b	21.4 bcd	19.7 bcd	20.6 ab	09.7 d	14.7 cd	12.2 b
F2(JSC)	23.9 abc	23.3 abc	23.6 ab	19.3 bcd	23.7 ab	21.5 ab	09.7 d	10.8 d	10.2 b
F3(Sulfur)	31.6 a	28.8 ab	30.2 a	23.5 abc	29.3 a	26.4 a	21.5 ab	25.8 a	23.7 a
F4(Super ph.)	12.3 d	17.8 cd	15.0 b	26.1 ab	16.6 cd	21.3 ab	22.6 ab	12.3 d	17.4 ab
F5(Amonium sulfate)	16.5 cd	22.5 bc	19.5 b	14.7 d	21.1 bcd	17.9 b	21.1 ab	18.6 bc	19.9 a
Mean	19.0 a	22.1 a		21.0 a	22.1 a		16.9 a	16.5 a	
CV%			69.4			53.5			62.7
LSD V			7.8			6.3			5.7
LSD F			10.2			8.3			7.5
LSD V*F			14.1			34.8			34.9

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

* Data in parentheses transformed using square root transformation$\sqrt{X + 0.5}$ before analysis

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

4.2 Effect of fertilization, varieties and harvest time on growth and yield parameters of groundnut

Similarly, the effect of Jatropha Seed Cake (JSC), sulfur, super phosphate and ammonium sulphate fertilizer on germination % of two groundnut cultivars (Sodri and Gebish) were presented in Table (3a) for season 2011/12 and Table (3b) season 2012/13. There were no significant difference between two cultivars for 3 and 5 weeks of sowing, and among the fertilizers for 5 weeks of sowing, but there were significant differences among fertilizers x cultivars (F×V) for two seasons for 3 and 5 weeks of sowing. Sulfur fertilizer with V1 (Sodri) and super phosphate with V2 (Gebeish) gave the highest values for first and second seasons respectively. Among fertilizers for two seasons in 3 weeks of sowing application of sulfur and (JSC) obtained the highest values. These results demonstrate that groundnut responded well to application of organic and inorganic fertilizes. The findings of this study were in line with Rumbidzai and Mabwe (2014).

Table, 3a: Effect of Jatropha Seed Cake and different fertilizers on germination % of two groundnut cultivars season 2011/12

germination %						
	3 WS			5WS		
Treatments	V1(Sodri)	V2(Gebish)	Means	V1(Sodri)	V2(Gebish)	Means
F1(Control)	70.0 b	69.0 b	69.5 ab	77.5 bc	81.3 b	79.4 a
F2(Jatropha)	65.0 bc	64.6 bc	64.8 ab	71.3 c	76.8 bc	74.1 a
F3(Sulfur)	82.9 a	58.7 c	70.8 a	89.2 a	69.5 c	79.4 a
F4(Super ph.)	66.7 bc	63.8 bc	65.2 ab	75.2 bc	71.7 c	73.4 a
F5(Amonium sulphate)	69.6 b	49.6 d	59.6 b	70.6 c	72.8 bc	71.7 a
Means	70.8 a	61.1 a		76.8 a	74.4 a	75.6
CV%			21.5			18.9
LSD V			7.8			7.8
LSD F			10.2			10.2
LSD V*F			62.7			54.1

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

* Data in parentheses transformed using square root transformation$\sqrt{X + 0.5}$ before analysis

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

Table 3b. Effect of Jatropha Seed Cake and different fertilizers on germination % of two groundnut cultivars season 2012/13.

germination %						
	3 WS			5WS		
Treatments	V1(Sodri)	V2(Gebish)	Means	V1(Sodri)	V2(Gebish)	Means
F1(Control)	45.8 c	60.3 b	53.1 b	36.3 c	50.4 ab	43.4 a
F2(Jatropha)	54.6 b	60.4 b	59.5 a	46.9 b	50.7 ab	48.8 a
F3(Sulfur)	41.9 c	65.4 b	53.7 b	31.7 c	50.5 ab	41.1 a
F4(Super ph.)	46.1 c	68.9 a	57.5 ab	37.2 c	56.8 a	47.0 a
F5(Amonium sulphate)	57.5 b	57.5 b	57.5 ab	46.4 b	46.4 b	46.4 a
Means	49.9 a	62.5 a		39.7 a	51.0 a	
CV%			31%			34.6%
LSD V			4.86			11.30
LSD F			9.63			8.57
LSD V*F			26.7			15.00

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

* Data in parentheses transformed using square root transformation$\sqrt{X + 0.5}$ before analysis

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

The results of leaf area index were presented in Table 4. There were significant difference in first season between cultivars, fertilizers and interaction among cultivars and fertilizers. Variety V2 (Gebish), F3 (Sulphur) and the interaction of V2×F4 gave the highest values. While in the second season the significant differences were noticed only among the interaction of cultivars and fertilizers and V1×F2 showed the highest values. These results confirmed that of Hago and Salama (1987) who reported that when sulphur was applied at rates of 50, 100 or 150 kg/ha, either at sowing, at flowering or in two equal splits at sowing and flowering, shoots, total sulphur content of leaves, nodule number per plant and pod yield and quality were all significantly increased.

Table, 4: Effect of Jatropha Seed Cake (JSC) and different fertilizers on leaf area index of two groundnut cultivars seasons 2011/12-2012/13.

leaf area index						
	2011/12			2012/13		
Treatments	V1(Sodri)	V2(Gebish)	Means	V1(Sodri)	V2(Gebish)	Means
F1(Control)	29.2 efg	30.6 def	29.9 b	34.7 b	29.3 de	31.9 a
F2(Jatropha)	32.3 cd	32.6 cd	32.5 ab	38.1 a	28.7 e	33.4 a
F3(Sulfur)	35.2 ab	30.6 def	33.4 a	36.2 ab	31.7 cd	33.9 a
F4(Super ph.)	27.8 ef	36.4 a	32.1 ab	34.0 bc	28.8 e	31.4 a
F5(Amonium sulphate)	28.4 fg	33.8 bc	31.1 ab	34.4 b	30.3 de	32.3 a
Means	30.6 b	32.9 a		35.5 a	29.7 a	
CV%	13.2			14.1		
LSD V			1.5			2.5
LSD F			2.3			3.3
LSD V*F			3.0			13.7

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

* Data in parentheses transformed using square root transformation $\sqrt{X + 0.5}$ before analysis

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

Tables 5a and 5b showed the results of effect of JSC and different fertilizers on plant height of two groundnut cultivars for three harvesting periods. The results indicated no significant differences between cultivars except in third harvest of second season where V2 (Gebeish) gave the highest value. Among fertilizers treatments all of them revealed highly significant difference except the first harvest of second season. In general, F4 (super phosphate fertilizers)showed the highest records in first harvest of first season and second harvest of second season also with control in second and third harvest of first season, but in second season third harvest F2(JSC) showed the highest value. The interaction of cultivars with fertilizers showed significant difference for all parameters and F4 and F1 obtained the highest values except in the third harvest of second season F2 gave the higher value. The results reported by Kaba *et al.,(2014)*who studied the *i*nter-relationships of yield and components of yield at different stages of maturity in three groundnuts (*Arachis hypogaea* L.) varieties were in agreement with this study findings.

Table 5a. Effect of Jatropha Seed Cake and different fertilizers on plant height of two groundnut cultivars season 2011/12.

leaf area index									
	First harvest			Second harvest			Third harvest		
Treatments	V1(Sodri)	V2(Gebish)	Means	V1(Sodri)	V2(Gebish)	Means	V1(Sodri)	V2(Gebish)	Means
F1(Control)	18.1 cd	19.8 bc	18.9 b	21.3 c	23.3 b	22.3 a	21.3 cde	26.1 a	23.7 a
F2(Jatropha)	20.4 b	17.3 d	18.8 b	19.6 def	20.4 cde	19.9 b	21.9 cd	20.3 ef	21.1 b
F3(Sulfur)	18.7 bcd	20.2 b	19.4 b	19.2 ef	22.8 b	21.0 ab	20.1 ef	19.6 f	19.9 b
F4(Super ph.)	22.3 a	23.3 a	22.8 a	19.5 ef	24.7 a	22.1 a	22.7 bc	23.6 b	23.2 a
F5(Amonium sulphate)	19.5 bc	19.8 bc	19.7 b	18.9 f	21.0 cd	19.9 b	20.7 def	19.8 ef	20.2 b
Means	19.8 a	20.1 a		19.7 a	22.4 a		21.3 a	21.9 a	
CV%			16.4			11.7			12.2
LSD V			1.79			1.35			1.45
LSD F			2.3			1.77			1.89
LSD V*F			3.6			9.4			2.7

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

* Data in parentheses transformed using square root transformation$\sqrt{X + 0.5}$before analysis

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

Table 5b. Effect of Jatropha Seed Cake and different fertilizers on plant height of two groundnut cultivars season 2012/13.

	plant height								
	First harvest			Second harvest			Third harvest		
Treatments	V1(Sodri)	V2(Gebish)	Means	V1(Sodri)	V2(Gebish)	Means	V1(Sodri)	V2(Gebish)	Means
F1(Control)	19.0 d	22.5 bc	20.8 a	18.1 d	21.6 c	19.9 cd	18.8 e	24.7 bc	21.8 ab
F2(Jatropha)	20.5 cd	25.9 a	23.2 a	21.5 c	26.1 b	23.8 ab	21.6 d	27.4 a	24.5 a
F3(Sulfur)	23.1 b	22.5 bc	22.8 a	18.6 d	25.9 b	22.3 bc	18.6 e	25.6 ab	22.1 ab
F4 (Super ph.)	20.4 cd	24.1 ab	22.2 a	23.0 c	29.6 a	26.3 a	23.2 cd	24.1 bc	23.7 ab
F5(Amonium sulphate)	21.8 bc	21.7 bc	21.7 a	17.9 d	18.8 d	18.4 d	21.7 d	21.7 d	21.7 b
Means	20.9 a	23.3 a		19.9 a	24.4 a		20.8 b	24.7 a	
CV%			19.2			21.3			16.3
LSD V			0.78			2.52			2.66
LSD F			2.33			3.31			2.03
LSD V*F			3.05			7.60			13.7

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

* Data in parentheses transformed using square root transformation $\sqrt{X + 0.5}$ before analysis

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

The results showed the effect of JSC and different fertilizers on number of primary branches of two groundnut cultivars seasons 2011/12-2012/13 were presented in Table 6a-6b for two seasons respectively. There were no significant differences between cultivars for three different harvesting time except in season two second harvest and V1(Sodri) gave higher record than V2(Gebish). Among fertilizers in the first season the results showed that F2 (JSC) in first and third harvest and F4 (Super phosphate) gave the highest values with significant differences. Meanwhile, second season showed slight difference in first and second harvest and no significant difference in third harvest for fertilizers treatment. The results of interaction of cultivars with fertilizers showed that F2 for two cultivar in first harvest and V1F4 in second harvest and V2 F2 for thirds harvest had the highest values with significant differences. The interaction in season two showed results similar to results of fertilizers with difference in V1which gave the highest values in first and second harvest, but in thirds harvest the interaction V1F4 gave significant difference. Singh *et al.,* (1990) reported that application of 20kg S/ha as elemental to the soil before sowing produced 25% more pods and 16% more branches in groundnut.

Table, 6a: Effect of Jatropha Seed Cake and different fertilizers on number of primary branches of two groundnut cultivars season under three harvest periods 2011/12.

	No.primary branches								
	First harvest			Second harvest			Third harvest		
Treatments	V1(Sodri)	V2(Gebish)	Means	V1(Sodri)	V2(Gebish)	Means	V1(Sodri)	V2(Gebish)	Means
F1(Control)	4.5 bc	4.1 c	4.5 bc	4.1 d	4.5 cd	4.3 c	5.0 bc	4.8 bcd	4.9 ab
F2(Jatropha)	5.6 a	5.3 a	5.6 a	4.7 c	5.2 b	4.9 ab	5.1 b	5.7 a	5.4 a
F3(Sulfur)	5.1 ab	3.9 c	5.1 ab	4.7 d	5.2 b	4.9 ab	4.3 de	4.9 bc	4.6 b
F4(Super ph.)	4.5 bc	4.2 c	4.5 bc	5.8 a	4.6 cd	5.1 a	4.9 bc	4.5 cde	4.7 b
F5(Amonium sulphate)	4.5 bc	4.5	4.5 bc	4.4 cd	4.6 cd	4.5 bc	4.8 bcd	4.2 c	4.5 b
Means	4.8 a	4.4 a		4.7 a	4.8 a		4.8 a	4.8 a	
CV%			24			17			16.5
LSD V			0.61			0.44			0.43
LSD F			0.79			0.58			0.57
LSD V*F			2.5			2.6			1.5

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

* Data in parentheses transformed using square root transformation$\sqrt{X + 0.5}$ before analysis

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

Table 6b. Effect of Jatropha Seed Cake and different fertilizers on number of primary branches of two groundnut cultivars season 2012/13.

	No.primary branches								
	First harvest			Second harvest			Third harvest		
Treatments	V1(Sodri)	V2(Gebish)	Means	V1(Sodri)	V2(Gebish)	Means	V1(Sodri)	V2(Gebish)	Means
F1(Control)	6.1 a	4.7 cd	5.4 a	6.2 a	4.3 de	5.3 a	5.0 bc	4.6 cd	4.8 a
F2(Jatropha)	5.3 b	3.8 e	4.6 b	4.2 e	3.9 e	4.0 b	5.4 abc	5.2 bc	5.3 a
F3(Sulfur)	4.1 de	5.4 b	4.8 ab	5.3 bc	3.8 e	4.6 ab	5.8 ab	5.0 bc	5.4 a
F4(Super ph.)	4.5 cd	5.0 bc	4.7 ab	4.9 cd	5.2 bc	5.1 a	6.3 a	4.0 d	5.2 a
F5(Amonium sulphate)	6.2 a	4.1 de	5.2 ab	5.7 ab	5.0 bcd	3.3 a	5.8 ab	5.1 bc	5.5 a
Means	5.2 a	4.6a		5.3 a	4.4 b		5.7 a	5.7 a	
CV%			20.2			24.0			32.5
LSD V			0.71			0.83			0.93
LSD F			0.54			0.63			1.21
LSD V*F			2.20			0.66			2.31

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

* Data in parentheses transformed using square root transformation $\sqrt{X + 0.5}$ before analysis

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

Data on numbers of pods/plants were presented in table 7a-7b. The results showed no significant differences between cultivars for two seasons in all harvest time. Fertilizer treatment showed no significant differences in first harvest of first season and second and third harvest of second season. In first season super phosphate gave the significant difference among treatments. In second season F5 gave the highest values.. The interaction treatment revealed highly significant difference for three harvests for two seasons. In general third harvest of two seasons had the highest number of pods/plant and V1F4 of third harvest of first season obtained the heaviest numbers of pods/plant which was 22.8 pods /plant. These results were in agreement with Tomar *et al.,* (1990) who observed significant increase in pod yield in groundnut with application of 40kg P_2O_5/ha. Similar results were obtained by Kaba *et al.,(2014)*who studied the *i*nter-relationships of yield and components of yield at different stages of maturity in three groundnuts (*Arachis hypogaea* L.) varieties and reported that the genotypes obtained an increasing trend in the number of mature pods as harvesting date delayed. Similar results were obtained by Kaba *et al.,(2014)*who studied the *i*nter-relationships of yield and components of yield at different stages of maturity in three groundnuts (*Arachis hypogaea* L.) varieties and reported that the genotypes obtained an increasing trend in the number of mature pods as harvesting date delayed.

Table7a. Effect of Jatropha Seed Cake and different fertilizers on number of pods/plant of two groundnut cultivars season 2011/12.

	No. of pods/plant								
	First harvest			Second harvest			Third harvest		
Treatments	V1(Sodri)	V2(Gebish)	Means	V1(Sodri)	V2(Gebish)	Means	V1(Sodri)	V2(Gebish)	Means
F1(Control)	5.4 cd	8.1 a	6.7 a	8.0 b	05.0 d	06.5 b	13.1 bc	10.9 cd	12.0 ab
F2(Jatropha)	7.0 abcd	7.5 ab	7.3 a	5.5 cd	08.1 b	06.8 b	10.6 cde	12.0 cd	11.3 b
F3(Sulfur)	5.5 cd	5.3 cd	5.4 a	7.6 bc	08.0 b	07.8 ab	09.6 de	15.8 b	12.7 ab
F4(Super ph.)	5.6 bcd	5.0 d	5.3 a	9.0 ab	11.0 a	10.0 a	22.8 a	07.7 e	15.2 a
F5(Amonium sulphate)	7.1 abc	5.3 cd	6.2 a	8.7 ab	07.2 bcd	07.9 ab	13.4 bc	09.3 de	11.5 ab
Means	6.1 a	6.2 a		7.8 a	7.8 a		13.9 a	11.2 a	
CV%			53.3			51.5			40.7
LSD V			1.80			2.10			2.80
LSD F			1.55			2.20			3.66
LSD V*F			2.37			4.10			7.80

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

* Data in parentheses transformed using square root transformation $\sqrt{X + 0.5}$ before analysis

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

Table 7b. Effect of Jatropha Seed Cake and different fertilizers on number of pods/plant of two groundnut cultivars season 2012/13.

	No. of pods/plant								
	First harvest			Second harvest			Third harvest		
Treatments	V1(Sodri)	V2(Gebish)	Means	V1(Sodri)	V2(Gebish)	Means	V1(Sodri)	V2(Gebish)	Means
F1(Control)	05.9 d	09.1 b	07.5 bc	12.5 bc	15.8 a	14.2 a	12.4 cd	17.9 a	15.1 a
F2(Jatropha)	07.4 bcd	06.8 cd	07.1 c	11.5 c	14.6 ab	13.1 a	14.4 bc	14.4 bc	14.4 a
F3(Sulfur)	08.4 bc	10.9 b	09.7 ab	11.2 c	15.0 a	13.1 a	11.9 cd	14.9 b	13.4 a
F4(Super ph.)	10.9 a	07.8 bc	09.3 ab	11.9 c	15.7 a	13.8 a	11.9 d	18.3 a	15.1 a
F5(Amonium sulphate)	10.9 a	10.9 a	10.9 a	11.7 c	14.3 ab	12.9 a	15.4 b	10.3 d	12.9 a
Means	8.7 a	9.1 a		11.8 a	15.1 a		13.2 a	15.2 a	
CV%			33.5			26			29.3
LSD V			6.60			2.00			2.27
LSD F			8.89			2.63			2.97
LSD V*F			14.53			7.10			23.9

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

* Data in parentheses transformed using square root transformation $\sqrt{X + 0.5}$ before analysis

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

The yield kg/ha data of two groundnut cultivars under different fertilizers application and for three harvests in two successive seasons were presented in table 8a-8b for two seasons. The result showed that yield increased with advanced in harvest time for two seasons, and the yield of third harvest is the best of the first and second harvest. V2 (Gebish) gave slight big yield compared with V1 (Sodri) with significant difference in first and third harvest of first season and first harvest of second seasons. Among fertilizers treatment in second seasons (Table, 8b), the results showed that F2 for three harvests gave the highest yield and also the interaction gave the same results with variation in cultivars where V2F2 gave the highest values. In first season Table 8a, F5 (Amonium sulphate) gave the highest values for three harvests. The interaction of cultivars and fertilizers in this season gave significant differences. The results obtained by Migawer and Soliman (2001) who investigated the performance of two peanut cultivars and their response to NPK fertilization in newly reclaimed loamy sand soil were in line with results of current study.

Table 8a. Effect of Jatropha Seed Cake and different fertilizers on yield (kg/ha) of two groundnut cultivars season 2011/12

	yield (kg/ha)								
	First harvest			Second harvest			Third harvest		
Treatments	V1(Sodri)	V2(Gebish)	Means	V1(Sodri)	V2(Gebish)	Means	V1(Sodri)	V2(Gebish)	Means
F1(Control)	250.2 f	445.7 de	347.9 c	309.3 f	695.5 cd	0502.4 d	0760.5 e	1224.0 cde	0992.3 e
F2(Jatropha)	791.2 b	360.3 ef	575.8 ab	942.3 cd	450.8 ef	069.60 e	2675.3 ab	0936.5 de	1805.9 c
F3(Sulfur)	967.3 a	455.7 cde	711.5 a	1331.5 a	705.0 de	1018.3 b	2710.5 ab	1621.8 c	2166.2 b
F4(Super ph)	390.7 ef	595.0 cd	492.8 bc	430.0 f	895.2 cd	0662.6 c	1331.0 cd	1569.3 c	1450.2 d
F5(Amonium sulphate)	831.0 ab	609.5 c	720.3 a	1119.5bc	1424.7 a	1272.1 a	2761.1 a	2254.5 b	2507.8 a
Means	646.1 a	493.2 b		826.5 a	834.2 a		2047.7 a	1521.2 b	
CV%			50.7%			57.3%			49.5%
LSD V			132.0			2216			584.7
LSD F			340.9			572.2			150.0
LSD V*F			157.9			260.3			483.1

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

* Data in parentheses transformed using square root transformation$\sqrt{X + 0.5}$ before analysis

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

Table, 8b: Effect of Jatropha Seed Cake and different fertilizers on yield (kg/ha) of two groundnut cultivars season 2012/13

yield (kg/ha)									
	First harvest			Second harvest			Third harvest		
Treatments	V1(Sodri)	V2(Gebish)	Means	V1(Sodri)	V2(Gebish)	Means	V1(Sodri)	V2(Gebish)	Means
F1(Control)	124.7 e	126.5 e	193.1 d	327.3 d	0242.8 de	285.1 c	792.3 c	0444.3 d	618.3 cd
C	062.7 f	677.5 a	370.1 a	089.5 f	1607.7 a	848.6 a	191.2 e	1721.2 a	956.2 a
F3(Sulfur)	090.0 g	377.2 b	193.1 d	085.7 f	0713.7 b	399.7 b	142.7 e	1340.5 b	741.6 bc
F4(Super ph.)	242.7 a	119.5 e	186.8 c	162.7 ef	0274.0 d	218.3 c	556.5 d	0558.5 d	557.5 d
F5(Amonium sulphate)	325.0 c	196.7 d	260.8 b	512.7 c	0432.5 c	472.6 b	719.7 c	0794.8 c	757.3 b
Means	152,7 a	299.5 b		235.6 a	654.19 a		480.5 a	971.9 a	
CV%			38.9%			35.5%			32.6%
LSD V			71.8			666.4			884.2
LSD F			18.5			172.1			228.3
LSD V*F			48.4			86.4			129.4

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

* Data in parentheses transformed using square root transformation$\sqrt{X + 0.5}$ before analysis

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

Obviously, the effect of different fertilizers in form of Jatropha Seed Cake, super phosphate, sulfur and ammonium sulphate as ones of an integrated agricultural management practices on infection of groundnut by *Aspergillus flavus* and on yield components gave positive results with significant difference. David (2009) reported that appropriate cultural practices during the growing season accompanied with timely harvest and proper storage of the grain after harvest are critical management tools for good plant health, high yield and minimizing contamination of groundnut with Aflatoxin producing fungi. In Queensland, Rachaputi *et al.,* (2002) identified early harvest and threshing as best management practices for minimizing Aflatoxin contamination under high Aflatoxin risk conditions. They added that, in general, early sowing or early harvest and even supplementary irrigation (if available) are possible ameliorating practices for reducing Aflatoxin risk.

Moreover, Shiyam (2012) who studied growth and yield response of groundnut (*Arachis hypogaea* L.) to plant densities and phosphorus concluded that phosphorus rate increased the number of filled pods and seed yield. Also Jat and Alhawat (2010) found that application of FYM and S fertilization increased the yield and yield attributed of groundnut. Dash *et al.,* (2014) confirmed that sulfur application increased pod yield of groundnut. On the other hand, Tajeswara (2013) found that sulfur application significantly influenced the growth and yield attributed characters, yield and oil content over control regardless of the source and levels of sulfur.

4.3Conclusion

Groundnut plays an important role in the livelihoods of poor people and in the rural economy of many developing countries. Aflatoxin contamination in peanut seeds, caused by *Aspergillus flavus*, hampers international trade and adversely affects health of consumers of peanut and its products. In Sudan, groundnut is very important oil seed. The quality and productivity of the crop depends on proper selection of variety, fertilizer management and proper management practices. In view of the foregoing results and discussion no single approach for control of *Aspergillusflavus* in groundnut was proved to be effective and without development of toxity. Therefore, integrated management strategies are the only solution to minimize the risk of contamination with Aflatoxin producing fungi. This study demonstrated that integration of appropriate agronomic practices during the growing season accompanied with timely harvest and improved cultivar are critical management tools for good plant health, high yield and minimizing infection of groundnut with *Aspergillus flavus*.

It is demonstrated that using Jatropha Seed Cake with different inorganic fertilizers; sulfur, superphosphate and ammonium sulphate gave significant differences in reducing *A. flavus* infection and in growth, yield and yield components of groundnut. On the other hand, delayed harvesting periods gave good results in growth and yield of groundnut. The two cultivars of groundnut obtained similar results without clear variation in their growth. Thus additional studies under more conductive condition are needed forbetter understand of the relation between agricultural practices and the fungus (*A. flavus*) to minimize its risk and to increase growth and yield of crops.

4.4Recommendations

- It is vital to create awareness on the ill effects of aflatoxins and benefits of toxin free groundnut.
- Aflatoxin produced by *Aspergillusspp*. cannot be eliminated from food or feed supplies; however, their levels can be substantially reduced using appropriate agronomic practices during the growing season accompanied with timely harvest
- Optimum plant nutrition is critical for overall plant health and for reducing the risk of Aflatoxin producing fungi
- Harvesting groundnut at the appropriate time is essential to minimize pods moisture as well as the risk of Aflatoxin contamination.
- The wide spread of Aflatoxin warrants more investigation to be done for better understand of the interrelation between management practices and the fungi producing Aflatoxin.

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