Sudan University of Science and Technology

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Detection and Identification of seed-borne- fungi of Some sorghum varieties under different storage Condition in Sudan

كشف وتعريف الفطريات المحمولة علي بذور بعض أصناف الذرة تحت ظروف تخزين مختلفة في السودان

A thesis submitted in partial fulfillment of the requirements for the M.Sc. Degree in Plant Protection

By:

Ashraf Mohi Eldien Mohammed Dawod

Sudan University of Science and Technology

Department of Plant Protection

B.Sc. Agric. (Honors), 2003.

Supervisor:

Dr. Ibrahim Saeed Mohamed

Department of Plant Protection

College of Agricultural Studies -Shambat

Sudan University of Science and Technology

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الآية

قال تعالي:

بسم الله الرحمن الرحيم

إِنِّي أَرَىٰ سَبْعَ بَقَرَاتٍ سِمَانٍ يَأْكُلُهُنَّ سَبْعٌ عِجَافٌ وَسَبْعَ سُنْبُلَاتٍ خُضْرٍ وَأُخَرَ يَابِسَاتٍ يَا أَيُّهَا الْمَلَأُ أَفْتُونِي فِي رُؤْيَايَ إِن كُنتُمْ لِلرُّؤْيَا تَعْبُرُونَ **[43]** قَالُوا أَضْغَاثُ أَحْلَامٍ وَمَا نَحْنُ بِتَأْوِيلِ الْأَحْلَامِ بِعَالِمِينَ **[44]** وَقَالَ الَّذِي نَجَا مِنْهُمَا وَادَّكَرَ بَعْدَ أُمَّةٍ أَنَا أُنَبِّئُكُمْ بِتَأْوِيلِهِ فَأَرْسِلُونِ **[45]** يُوسُفُ أَيُّهَا الصِّدِّيقُ أَفْتِنَا فِي سَبْعِ بَقَرَاتٍ سِمَانٍ يَأْكُلُهُنَّ سَبْعٌ عِجَافٌ وَسَبْعِ سُنْبُلَاتٍ خُضْرٍ وَأُخَرَ يَابِسَاتٍ لَعَلِّي أَرْجِعُ إِلَى النَّاسِ لَعَلَّهُمْ يَعْلَمُونَ **[46]** قَالَ تَزْرَعُونَ سَبْعَ سِنِينَ دَأَبًا فَمَا حَصَدْتُمْ فَذَرُوهُ فِي سُنْبُلِهِ إِلَّا قَلِيلًا مِمَّا تَأْكُلُونَ **[47]** ثُمَّ يَأْتِي مِن بَعْدِ ذَٰلِكَ سَبْعٌ شِدَادٌ يَأْكُلْنَ مَا قَدَّمْتُمْ لَهُنَّ إِلَّا قَلِيلًا مِمَّا تُحْصِنُونَ **[48]** ثُمَّ يَأْتِي مِن بَعْدِ ذَٰلِكَ عَامٌ فِيهِ يُغَاثُ النَّاسُ وَفِيهِ يَعْصِرُونَ **[49]**

صدق الله العظيم

سورة يوسف(43-49)

Dedication

To My:

Family,

Teachers

And every Friends.

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All thanks are due to Almighty Allah (SWT) who gave me health and strength, and helped me tremendously to produce this work.

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List of Contents

List of Tables

Title **Page No.**

List of Figures

Title **Page No.**

ABSTRACT

Sorghum is considered one of the major cereal crop and staple food as well for millions of the poorest and most food insecure people in many part of the world and in Sudan as well. The production and consumption of sorghum is constrained by several factors, the most important among which are seed-borne fungal species. In addition to causing quantitative losses, these spoilage fungi produce highly toxic and carcinogenic mycotoxins. In view of the negative public health and economic impacts of fungi producing mycotoxins associated with sorghum grains, this study, aimed at exploring and investigating on presence of pathogenic fungi associated with seeds in samples of sorghum collected from Gadaref, and Rabak in Sudan under different storage conditions. The results obtained revealed that irrespective of source of seed samples, the association of seed borne fungi with sorghum grains in different varieties appears to be a prevalent situation. The four most prevailing seed borne fungi recorded across sorghum varieties and storage facilities were the storage ones; *Aspergillus flavus, Aspergillus Niger, Alternaria solani and Phoma longum* with varying level of incidences ranging from 3.33 to 63.3%. The significantly high percentage incidence was given by the fungus *P. longum* (63.3%) in variety Fetarita. The predominance of some spoilage fungi under modern silo storage was attributed to infection prior to storage. The significance of these results was discussed.

الملخص

يعتبر الذرة احد أهم محاصيل الحبوب والغذاء الرئيسي أيضا للملايين من الفقراء ولمعظم الناس الغير امنين غذائيا في كثير من أجزاء العالم وأيضا في السودان. هنالك عدة عوامل تعوق إنتاج وإستهلاك الذرة الأهم من بينها أنواع الفطريات المحمولة على البذور. بالإضافة إلى الخسائر في الكمية. هذه الفطريات المفسدة تنتج سموم فطرية ذات سمية عالية ومسرطنة. على ضوء التأثير السلبي على الصحة العامة والاقتصاد لهذه الفطريات المنتجة للسميات الفطرية المرتبطة بحبوب الذرة,هدفت هذه الدراسة إلى استكشاف والتحقق من وجود فطريات مرتبطة بعينات بذور للذرة جمعت منطقتي القضارف وربك تحت وسائل تخزين مختلفة. النتائج التي تم الحصول عليها أظهرت انه بغض النظر عن مصدر عينات البذور, وجد أن ارتباط الفطريات المحمولة على حبوب الذرة أنها هي الصفه السائدة. أجناس الفطريات الأربعة المحمولة على البذور الأكثر تواجدا التي تم كشفها والتعرف عليها عبر أصناف الذرة ووسائل التخزين هي *Aspergillus flavus, Aspergillus Niger, Alternaria solani and Phoma longum* وبدرجات مختلفة تتراوح بين 63.3 و 3.33%. أعلى نسبة إصابة هامة كانت بواسطة الفطر *P. longum* (63.3%) في الصنف فتريتة يليه *Aspergills flavus* بنسبه (43%) في الصنف عكر.التواجد الغالب لبعض الفطريات المفسدة تحت ظروف الصوامع الحديثة ربما يعزى إلى الإصابة قبل التخزين. لأهمية هذه النتائج تمت مناقشتها.

CHAPTER ONE

INTRODUCTION

Sorghum grain [*Sorghum bicolor* L. (Monech)] is the fifth most important cereal in the world after wheat, rice, maize and barley (Reddy, *et al.*, 2004). In fact, it is one of the major cereal crop and staple food as well for millions of the poorest and most food insecure people in the Semi-Arid Tropics of Africa and Asia and an important feed grain and fodder crop in the Americas and Australia. The greatest diversity in both cultivated and wild types of Sorghum is found in north-eastern tropical Africa. The crop may have been domesticated in that region, possibly Ethiopia (ICRISAT, 1993)

According to FAO/stat (2006), 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 in irrigated and rain-fed agriculture and used as staple food for human beings and an important feed grain and fodder crop for animals. Industrial uses include extraction of many products such as starch, oil, alcohol, sugar, and sugary juices (Khatab *et al.*, 2000). According to Government of sudan and FAO/WFP (2011) 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.

Common seed and seedling rot diseases in sorghum are caused by soil- and seed-borne Aspergillus, Fusarium, Pythium, Rhizoctonia and Rhizopus spp. (Taylor, 2003.) (Reddy, *et al.,* 2004).

The major constraints facing the productivity and availability of healthy food crops worldwide are the losses and spoilage caused by plant pathogens, insects, nematodes and parasitic weeds. Common seed diseases in sorghum are caused by soil- and seed-borne Aspergillus, Fusarium, Pythium, Rhizoctonia and Rhizopus spp. The threat to food crops from fungal pathogens has now reached a level that outstrips that posed by bacterial and viral diseases (Berger, 1977).

The joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) initiated a project to determine current levels of fungal contamination in Sudanese sorghum in order to identify ways to reduce this problem.

This is holding true particularly in the humid tropics being major spoilage agents of food crops (Olusegun, *et al.,* 2013). Moreover, seed borne fungi 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. Other seed-borne fungi were also most frequently isolated from pear millet seeds such as *Alternaria alternata*, *Fusarium semitectum*, and *Curvularia lunata* (Azhar, *et al.*, 2011.)

In Sudan, Haq Elamin NH *et al*., 1988; Ali, 1989 and Yousif, M.A.*et al*., 2010 reported that several seed borne fungi associated with seeds of food grains are known to limit utilization of these crops, of which *Aspergillus ssp*. are the most important. In fact, these fungi and their secondary metabolites are one of the most important food crops spoilage agents in the Sudan.

Because of its extensive use as human foods and livestock feeds, the microbiology and safety of sorghum grains is a very important area. The sources of microbial contamination of sorghum are many, but all are traceable to the environment in which grains are grown, handled, and processed. Microorganisms that contaminate cereal grains may come from air, dust, soil, water, insects, rodents, birds, animals, humans, storage and shipping containers, and handling and processing equipment.

In view of the negative public health and economic impacts of fungi producing mycotoxins associated with sorghum grains, this study, aimed at exploring and investigating on presence of pathogenic fungi associated with seeds in samples of sorghum collected from Gadaref, and Rabak in Sudan under different storage conditions and with following objectives:-

- To investigate the occurrence of fungal contaminants associated with sorghum grains
- To detect and identify the seed borne mycoflora associated with seeds of sorghum grains
- To study the effect of storage facilities on grain health

CHAPTER TWO

LITERATURE REVIEW

2.1 Sorghum

2.1.1 Scientific classification

Kingdom: Plantae

Division : Magnoliophyta

Class : Liliopsida

Order : Poales

Family : Poaceae

Genus : *Sorghum*

Species : *bicolor*

Sorghum (*Sorghum bicolor* L.) is the fifth most important world cereal and an important native cereal in Africa (FAO, ICRISAT, (1996);(Murty and Renard, 2001). Sorghum is also the dietary staple of more than 500 million people in more than 30 countries (National Academic Science, 1996). Total world production of sorghum was estimated at about 54 million tons (FAO, 2004). An annual production of about 70 million metric tons of grains from 50 million hectares of land has been reported (NAS, 1996). In Sudan, cereals include sorghum, millet, wheat, maize, telebon and rice. Among these grains only the surplus of sorghum and millet are exported while wheat and rice are imported, but there is a good potential for export of surplus maize in future (Mahmoud, 2001). Sorghum is the most important world cereal crop (More, *et al*., 1992), and

is one of the major crops grown in the tropics and subtropics (Hall, 1970). In Sudan, it ranks first in production with an area of 3.6 million hectares and a total annual production of 5.5 million tones (FAO, 1982). Sorghum is the staple food in many countries including the Sudan (Shazali and Ahmed, 1998). It is the staple food in most regions of this country. It contains a reasonable amount of proteins, ash, carbohydrates, oil and fibre (Drich and Pran, 1987).

Grain sorghum contains more protein (108 g/kg) than corn (92 g/kg), but slightly less protein than wheat (125 g/kg). Its carbohydrate content is about the same as thatof wheat or corn (720 g/kg) for sorghum and wheat and (716 g/kg) for corn. Its fat content is about 31 g/kg compared with 17 g/kg and 45 g/kg for wheat and corn respectively (Reed, 2005).

2.1.2 Description

Sorghum is an upright, short-day, summer annual that is a member of the Poaceae family. The grass blades are flat, stems are rigid, and there are no creeping rhizomes. Sorghum has a loose, open panicle of short, few-flowered racemes. As seed matures, the panicle may droop. Glumes vary in color from red or reddish brown to yellowish and are at least three quarters as long as the elliptical grain.

Brown (Kearney and Peebles, 1969; Barkworth, 2003). Sorghums exhibit different heights and maturity dates depending on whether they are grain sorghums (Sorghum bicolor ssp. bicolor), forage sorghums (Sorghum bicolor), Sudangrass (*Sorghum bicolor ssp*. drummondii), or sorghum-Sudangrass hybrids (Sorghum bicolor x Sorghum bicolor var. sudanense). Growth characteristics also vary depending on the location grown, inputs, and agronomic practices. In general, forage sorghums are taller plants with later maturity dates and more vegetative growth than grain

sorghums. Sudangrass and sorghum-Sudangrass hybrids fall in between grain sorghums and forage sorghums in height (Undersander, 2003).

2 .1.3. Taxonomy

Sorghum (Sorghum bicolor L. Moench) is belonging to the Tribe Andropogonae of the family Poaceae. The genus Sorghum has been classified into five subgenera: Eusorghum, Chaetosorghum, Heterosorghum, Para-sorghum and Stiposorghum (Garber, 1950). Although this classification is convenient, however it does not stand for evolutionary relationships (Dillon *et al.* 2004).

The Eu-sorghum section is originated from Africa or Asia (Doggett 1976, DuVall and Doebley 1990). Sections Chaetosorghum and Heterosorghum consist of S. macrospermum and S. laxiflorum and both of these species are annuals and polyploids (Lazarides *et al.* 1991, Wu 1990). Section Stiposorghum includes ten species indigenous to northern Aus and tralia (Lazarides *et al.* 1991). Para-sorghum Section is comprised seven African, Asian, Australian and Central American species. The basic number of chromosome of species in each section is five. The species belong to Parasorghum and Stiposorghum are mostly diploid (2n = 10), however a few species are tetraploid or hexaploid.

Sorghum includes three species, S. halepense, S. propinquum and S.bicolor Sorghum halepense is also known as Johnsongrass, derived from anatural cross between *S. arundinaceum* and *S. propinquum* (Doggett 1976). Sorghum propinquum is a perennial species related to S. bicolor (Chittenden *et al.* 1994).

By using Harlan and deWet‘s system which is based on spikelet morphology, Sorghum bicolor has been classified into five races. These races consist of Bicolor, Guinea, Caudatum, Kafir, and Durra. Owing to

the variability found in each race an additional classification scheme was developed. This new classification amalgamates the Harlan and deWet‘s classification with working groups (sub-races) based on- head opening which resulted in the classification of seventy working groups (Dahlberg *et al*. 2004). Early bicolor sorghum is believed to have arisen from the subspecies verticilliforum in central Africa (Dahlberg, 1995). The races; Caudatum, Kafir, Guinea, and Durra were created by the crossing of early bicolor with the wild forms of sorghum. It is believed that the Guinea race has been evolved when the Bicolors came into contact with the wild *S. arudinaceum*. The Caudatum race is also believed to develop from a cross between an early domesticated Bicolor and wild sorghum (Dahlberg2000). The Kafir race is thought to be developed from crosses between Bicolor in northern Africa with wild verticilliflorum that was carried from east toward south by the Bantu speakers of Africa (Dahlberg1995). The Durra race is thought to be originated in Ethiopia as a result of crossing between early bicolor and with wild *S. aethiopicum* which allowed it to cope with drier conditions (Dahlberg, 1995).

2 .1.4. Uses

Sorghum is used as a drought tolerant, summer annual rotational cover crop either alone or seeded in a warm season cover crop mixture. There are multiple cultivars of sorghum available for use as a cover crop including sorghum-Sudan grass hybrids (Sorghum bicolor x Sorghum bicolor var. sudanense). However, all sorghum and Sudan grass-related species have the potential to smother weeds, suppress nematode species, and penetrate compacted subsoil (Clark, 2007). Sorghum cover crops can also be used as livestock forage in a cropping system (Magd off and Van Es, 2009). Sorghum-Sudan grass hybrids can produce up to 4,000-5,000 pounds of dry matter per acre (Clark, 2007). Sorghum cover crops can

also be used as livestock forage in a cropping system (Magdoff and Van Es, 2009). Sorghum-Sudan grass hybrids can produce up to 4,000-5,000 pounds of dry matter per acre (Clark, 2007).

Sorghums are quick growing grasses that have the potential to shade out and/or smother weed populations when planted at a high density. In addition, root exudates of sorghum have been shown to reduce the growth of weeds such as velvet leaf, thorn apple, redroot pigweed, crabgrass, yellow foxtail and barnyard grass (Stapleton *et al.*, 2010).

sorghum as a food in developing as well as in developed countries is discussed. A particular emphasis is made on the impact of starch and starch degrading enzymes in the use of sorghum for some African foods, e.g. “tô”, thin porridges for infants, granulated foods “couscous”, local beer “dolo”, as well agro-industrial foods such as lager beer and bread. Key words: sorghum, amylase, b-amylase, starch, infant porridge, beer, couscous, dolo, .(Doggett1988,**).**

The Sorghum is a acquisition of good quality grain is fundamental to produce acceptable food products from sorghum and while playing crucial role in food security in Africa, it is also source of income of house-hold (Anglani, 1998).

2.1.5. Sorghum environmental requirement:

2.1.5.1. Water: Sorghum can successfully grow under rainfall ranging

between 400-800 mm annually. This range is easily found in Gadarif area.

2.1.5.2. Soils: sorghum can be grown in sandy (qoz) to heavy clays. Sorghum can tolerate saline and alkaline soils. However, high salinity reduces the level of crop yields.

2.1.5.3. Temperature: The appropriate temperature for sorghum production falls within the range of 26-30 degrees centigrade. It requires dry weather and can tolerate high temperature. However, high temperature affects sorghum crop yield, particularly during sowing date.

2.1.5.4. Sowing date: The optimum sowing date for sorghum lies within July and early August.

2.2. Seed borne diseases:

Seeds are regarded as highly effective means for transporting plant pathogens over long distances. Numerous examples exist in agriculture literature for the international spread of plant diseases as a result of the importation of seeds that were infected or contaminated with pathogens (Agarwal & Sinclair, 1996).

Seed-borne diseases have been found to affect the growth and productivity of crop plants (Kubiak & Korbas, 1999; Weber *et al*., 2001; Dawson & Bateman, 2001). A seed-borne pathogen present externally or internally or associated 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 (Khanzada *et al*., 2002; Bateman & Kwasna, 1999).

. Seed is the basic unit of production for the world's food crop. Inrecent years seed has become an international commodity used to exchang egermplasm around the world. Seed is ,however, also an efficient means of introducing plant pathogens into a new area as well as providing ameans of their survival from one cropping season to another (Walcott *et al*., 2003). Seed health testing is thus routinely carried out in most

countries for domestic seed certification, quality assessment and plant quarantine(FAO, 2010).

Seed health is a well recognized factor in the modern agricultural science for desired plant population and good harvest (Rahman *et al*., 2008). Seedborne pathogens are a continuing problem and may even be responsible for the re-emergence of diseases of the past as well as the introduction of diseases into new areas (Gitaitis and Walcott, 2007). Seed-borne pathogens present a serious threat to seedling establishment (Walcott, 2003). In today's global economy, seed accounts more than ever for the movement of plant pathogens across vast distances, natural barriers, and political borders (Gitaitis and Walcott, 2007). The quality of planted seeds has a critical influence on the ability of crops to become established and to realize their full potential of yield and value (McGee, 1995).

Seed-borne fungi are one of the most important biotic constrains in seed production worldwide. They are responsible for both pre and post-emergence death of grains, affect seedling vigor, and thus cause some reduction in germination and also variation in plant morphology (Van Du *et al*., 2001; Niaz and Dawar, 2009).

Fungi outnumber all other types of pathogens that attack plants and causea very serious economic impact on agricultural production due to their ability to induce diseases of cultivated crops that result in important yield losses (Paplomatas, 2006).

There are three primary organizations that publish standardized seed health test methods for use in international trade. These are International Seed Testing Association (ISTA), International Seed Health Initiative (ISHI), and in the United States, the National Seed Health System

(NSHS) (Munkvold, 2009). Two of the most important concepts in seed health testing are sensitivity and selectivity, which are inextricably linked. For example, increasing the selectivity of semi selective media may decrease the recovery efficiency of all or some strains of the target organism.

In contrast, increasing selectivity may reduce the number of nontargetorganisms that act as competitors and/or inhibitors that interfere with the assay, and thus increase the detection sensitivity (Roumagnace *et al*., 2000; Toussaint *et al*.,(2001). A semi selective medium may have a higher mean plating efficiency than a standard growth medium because standard media are complex and often

become toxic, perhaps due to the accumulation of peroxides or other secondary metabolites (Block *et al*., 1998)

2.3. Seed borne diseases of sorghum

Sorghum have been a number of seed borne diseases of which the major ones are explained in detail below.

2.3.1 Phoma sorghina

Sorghina causes leaf spot and blight of sorghum. In the field, the pathogen causes leaf lesions that measure approximately 5 x 2.5 mm. Lesions are generally parallel-sided with dark brown margins and light brown necrotic centres. Coalesced lesions usually result in tattered leaf tissue. Leaf margins are frequently necrotic. Pycnidia may form in the necrotic tissue (Zillinsky, 1982). Prolonged periods of continuous wetting are requisite for infection and symptom development (Wiese, 1977).

The seed borne inoculum of the fungus causes considerable damage. The fungus is carried as pycnidia and as dormant mycelium. Infected seeds can be detected by visual examination and incubation tests.

Numerous pycnidia can be seen on dry seeds under a magnifying lens. The pycnidia are dark brown to black with the size of a pinhead and can be scattered throughout the surface of incubated seed (Mathur and Kongsdal, 2000; Mathur, Ram and Mathur, 1973). Whenthe seed is heavily infected, the fungus can rupture the seed coat giving the seed a cankerousor warty appearance (Zillinsky, 1982). Sometimes fungal growth on incubated seed consists only of mycelium and chlamydospores. The mycelium is profuse, fluffy to dense, and is often very variable in colour. Sometimes pycnidia are produced on the aerial mycelium. Conidia are hyaline, single-celled, variable in shape and they measure 1.4 - 4.4 x 3.5 - 8.8 µm in diameter and are straight. Chlamydospores are frequently produced on the aerial mycelium and directly on the seed surface. They resemble Alternaria spp.spores, and are sometimes irregular in shape (Ahmed and Reddy, 1993).

Control of fungus includes selection of seed from noninfected plants combined with seed treatment. Seed treatment with thiram, captan or mancozeb (Dithane M-45) fungicides at about 3 g (a.i) / kg is advised to reduce the seed borne inoculum (Mathur and Manandhar, 1993).

2.3.2 *Bipolaris sorghicola* (Lefebvre & Sherwin) Alcorn)

The pathogen causes seed decay, seedling blight, leaf spot, and head mold of sorghum and pearl millet.

Young plants and maturing plants are most susceptible to foliar blight. Seedling blight is more pronounced at temperatures of 25 ^{o}C and less. The disease is widely distributed and found in countries such as the

United States, Hawaii, India, Japan, Zimbabwe and Zambia (Frederiksen, 1986).

The foliar symptoms vary from brown flecks, fine linear streaks, small oval spots, large irregular oval to almost rectangular spots measuring 1 - 10 x 0.5 - 3 mm depending on variety (Frederiksen, 1986).

Lesions may expand and coalesce to form very long interveinal lesions. They may be solid dark brown but usually become tan or greyish brown with a more or less distinct dark brown border.

Infected seeds can be detected by incubation tests. The fungus grows on the incubated seeds producing mycelium, conidiophores, and conidia. Conidiophores are usually single or in small groups on the infected seed. The most distinguishing character of this species is that the primary conidia while still attached to the conidiophores frequently bear long secondary conidiophores on which small secondary conidia are produced (Ahmed and Reddy, 1993).

The pathogen can be controlled through seed treatment with ferbam at about 2.5 g (a.i) / kg seed (Almekinders and Louwaars, 1999).

2.3.3. *Fusarium moniliforme*:

Fusarium moniliformecauses head blight, stalk rot of sorghum, and twisted top or top rot of pearl millet (Leslie, Pearson, Nelson and Toussoun, 1990; Wu and Mathur, 1987).

Infected seed can be detected by visual examination and incubation tests. A white powdery fungal growth can be seen on dry infected sorghum seed. Sometimes, infected seeds of white-seeded sorghum cultivars have a pinkish or violet tinge (Ram, Neergaard and Mathur, 1970). Profusely infected seeds are reduced in size and weight, and do not germinate

(Rheeder, Marasas and Van Wyk, 1990). The fungus usually produces awhite to light orange powdery growth consisting of aggregated or loosely scattered chains of microconidia on incubated seed. Sometimes the microconidia may beproduced on monophialides in false heads. The microconidia are hyaline, one to two-celled, 2 - 4 x 5 - 12 µm, and appear as beaded chains. They are oval to club-shaped with a flattened base. When the microconidia are not produced in chains, they might be confused with those of *F. oxysporum*. However, the phialides are longer and narrower in *F. moniliformethan* in *F. oxysporum* (Ahmed and Reddy, 1993).

Macroconidia are produced in pale orange sporodochia, which can be obscured by the mycelium and the abundant chains of microconidia. Macroconidia are produced on macroconidiophores. They are hyaline, 3 - 7 septate, 1.5 - 4 x 20 - 82 µm, slender, almost straight, and taper towards either end. They are slightly hooked at the tip, thin-walled, with the apicalcell slightly curved and tapering to a point, and may be either distinctly or slightly foot-shaped at the basal cell (Frederiksen, 1986).

Control can be effected by discarding infected seeds and also seed treatment with carbendazim or a mixture of benomyl + thiram (Benlate-TIR), or carbendazim at about 2 g (a.i) / kg is advised (Ahmed and Reddy, 1993)

2.3.4. *Aspergillus ssp*

2.3.4. 1. Synonym or Cross Reference:

Aspergillosis, farmer’s lung, A. fumigatus, A. flavus, A. niger, A. nidulans, A. terreus.

2.3.4.2 Characteristics:

The genus Aspergillus belongs to the class Euascomycetes of the Phylum Ascomycota ,They consist of anamorphic (asexual) species with known or presumed telomorphic forms in the family Trichocomaceae , The genus Aspergillus includes seven subgenera, each containing several species. Aspergillus spp. contain approximately 184 species, 40 of which have been reported to cause human or animal infections. Aspergillus spp. reproduce by producing conidia on uniseriate or biseriate phialides. Aspergillus colonies grow rapidly, producing white, green, yellow, or black colonies (Verweij, P.E. and Brandt.2007)

2.3.4.3 Pathogenicity/ Toxicity:

Aspergillus spp. includes many species, about 40 of which have been implicated in human or animal infections , Aspergillosis is a common term used to describe infections caused by different species of Aspergillus, Most cases of aspergillosis are caused by A. fumigatus, with A. flavus and A. niger being the second most common pathogenic Aspergillus spp. Worldwide, Diseases caused by Aspergillus spp. include clinical allergies (allergic bronchopulmonary aspergillosis, rhinitis, Farmers's lung), superficial and local infections (cutaneous infections, otomycosis, tracheobronchitis), infections associated with damaged tissue (aspergilloma, osteomyelitis), and invasive pulmonary and extrapulmonary infections , Invasive infections due to *Aspergillus spp*. occur mainly in immunocompromised individuals and are the most severe forms of infections caused by *Aspergillus spp*. , Invasive aspergillosis is most commonly caused by *A. fumigatus,* but other species such as A. flavus, *A. nidulans,* and *A. terreus* have also been reported to cause invasive infections. (Verweij, P. E. and Brandt, M. E .2007). Invasive

infections primarily involve the sino-pulmonary tract, with lung being the most common site of invasion, Clinical signs suggestive of invasive sinusitis include fever, facial pain, headache, asymmetric facial swelling, epistaxis, proptosis, cranial nerve abnormalities, ischemia of the palate, and bone erosion ، Fever, cough, and dyspnea are the most common but non-specific symptoms of invasive pulmonary aspergillosis, Vascular invasion may also occur and may manifest as pleural chest pain ، If left untreated, hematogenous dissemination involving any organ may occur. The most serious condition is the involvement of the CNS, leading to seizures or stroke .(Segal, 2009)

2.3.5. *Alternaria. sp.*

Alternaria contains most of the small-spored Alternaria species with concatenated conidia. Almost 60 morphological or host-specific species can be assigned to this section, including the type species of the genus Alternaria, A. alternata (Woudenberg *et al.* 2013). *Alternaria alternata* is known as the cause of leaf spot and other diseases in over 100 host species of plants (Rotem 1994), but also as postharvest disease in various crops (Coates & Johnson 1997) and of upper respiratory tract infections and asthma in humans (Kurup *et al.* 2000). Other important plant pathogens in sect. Alternaria include A. longipes, the causal agent of brown spot of tobacco, A. mali, the causal agent of Alternaria blotch of apple, *A. gaisen*, the causal agent of black spot of Japanese pear and *A. arborescens*, the causal agent of stem canker of tomato. The first descriptions of the *A. alternata*, *A. tenuissima*, *A. cheiranthi* and *A. brassicicola* species-groups, based on sporulation patterns, were made by Simmons (1995). More recent molecular-based studies revealed that Alternaria species cluster in several distinct species clades, now referred to as sections (Lawrence *et al.* 2013,Woudenberg *et al.* 2013), which do

not always correlate with the species-groups that were delineated based on morphological characteristics. Currently, 26 Alternaria sections are recognised based on molecular phylogenies (Woudenberg, 2013 and Woudenberg *et al*., 2014Grum-Grzhimaylo *et al.* 2015). So far, species within sect. Alternaria have been mostly described based on morphology and / or host-specificity; yet the molecular variation between them is minimal. The standard gene regions used for the delimitation of Alternaria species are not able to delineate species within sect. Alternaria (Peever *et al.*, 2004 and Andrew *et al.*, 2009). Multiple molecular methods have been tested or proposed for distinguishing the small-spored Alternaria species, including random amplified polymorphic DNA (Roberts *et al.* 2000), amplified fragment length polymorphism (Somma *et al.* 2011), selective subtractive hybridisation (Roberts *et al.* 2012) and sequence characterised amplified genomic regions (Stewart *et al.* 2013a). However, none of these methods successfully distinguished all morphospecies described within sect. Alternaria.

2.3.6. Colletotrichum graminicola

Colletotrichum graminicolacauses sorghum anthracnose that is one of the most important sorghum diseases limiting grain production worldwide (Vaillancoaurt and Hanau, 1991). The extent of damage or yield loss due to anthracnose is usually related to the degree of host susceptibility, the environment, the aggressiveness of the pathogen, and the physiological status of the host (Maude, 1996).

The disease is serious on sorghum, maize and rye. The leaf blightphase of the disease can limit production, with reductions in grain yield of 50 % or more in severe epidemics. Losses are greater when alternating wet and dry cycles occur with dry temperatures. The foliar phase of anthracnose

results in small, elliptic to circular spots, usually 5mm or less in diameter. These spots develop small, circular, straw coloured centres with wide margins that are red, orange or tan depending on the cultivar (Frederiksen, 1986). The disease may defoliate sorghum plants and reduce growth and further development leading to plant death in severe cases. The fungus can overwinter on seed as dry acervuli and within the seed as a dormant mycelium. Seeds harvested from diseased plants are likely to carry the fungus (Wiese, 1977).

Infected seeds can be detected by visual examination and incubation tests. Dry seeds show visible symptoms of infection, in the form of dark brown to black acervuli scattered on their surface (Chaudhary and Mathur, 1986). These acervuli are irregular in shape and consist of dark setae.

Sometimes acervuli are also formed on the glumes. On incubated seed, the fungus produces numerous acervuli, which are rounded or elongate, separate or confluent, superficial, erumpent, with conspicuous multicellular, darkly pigmented setae, and 70 - 300 µmin diameter. The acervuli consist of a gelatinous or mucoid, salmon orange coloured conidial mass. Conidiophores are hyaline, 8 - 20 µm long, and 4 - 8 µm broad. Individual conidia are hyaline, single-celled, spindle-shaped, with acute apices, and measure 19 - 28.9 x 3.3 - 4.8 µm. Setae are brown with a dark swollen base and a pale rounded tip (Ahmed and Reddy, 1993).

Control of the pathogen includes conducting pre-export crop health inspections during crop growth and discarding the mouldy seeds. Seed treatment with benomyl at about 2 g (a.i) / kg seed reduces the seed borne inoculum (Ahmed and Reddy, 1993).

2.4. Storage methods of sorghum in the Sudan

The storage methods used for sorghum and millet in the Sudan, can be divided into four categories (Mustafa, 1983; Shazali, 1998).

2.4.1. Farmers' stores

Traditional grain storage structures are frequently of poor construction and are susceptible to storage insects pest's attack. Their suitability depends on the climate of the region as well as the local customs of the farmers and they include:

2.4. 2. Sweiba

A sweiba is a cylindrical container made of mud or a mixture mud and twigs. Generally sweibas are raised off the ground to a height of up to one meter upon strong wooden platforms supported by poles (with or without rat guards). It is covered with a conical thatched roof with wide eaves. The average diameter is 1-2 meters with a similar height. The storage capacity is usually less than one ton. Sweibas are common in northern and western Sudan (FAO, 1987).

2.4. 3. Gugus

Similar to sweibas, but are madefrom plant material such as split, interwoven bamboo twigs or grass. Sorghum and millet are often stored lossely. Long-term storage is areal problem because of insect infestations. They are widely used in southern Sudan, probably due to the high humidity. The disadvantages of this system are: the roof is not tight, outlet and inlet cracks allow insect pests to complete their life cycle and escape, no ventilation, sanitation is poor not well protected against thieves (FAO, 1987).

2.4. 4. In-hut storage

These are thatched roof grass huts known as "Guttia" made of wood, chaff and grass. Bagged grain is stored directly on the ground.

In-hut storage is common in rural Sudan. Initial cost is low, suitable for small farmers and rural population, easy to construct and maintain and humidity and temperature are more constant. The most important problem of this system is that, the roof is not tight enough, thus penetration of pest is very easy (FAO, 1987).

2.4. 5. Matmoras (underground pits)

Storage of bulk grain in matmoras is common in the drier areas of central Sudan where rainfall is erratic. Matmoras are invariably cylindrical in shape 2-5 meters indiameter and 1-1.5 m deep. They may be unlined or lined with empty spikelets (Butab) and/or straw matting. The capacity of farmer's matmoras varies between 2 to 25 tons. Insect infestations are reduced by depleted oxygen levels caused by insects, fungi or the grain itself. In many cases, the grains are kept well for 3-5 years. Shazali *et al*. (1996) reported that the loss due to insect infestations was less in matmoras (1.4%) than in above ground stores (3.7%) and the most dominant species in matmuras were *T. castaneum*, *R. dominica* and *Cryptoleste ssp*. However, the losses due to moulds were high (FAO, 1987).

2.4. 6. Traders/Merchants stores

Local trader's stores are small room-like structures with an average size of 4 x 5 meters. They are made of mud or fired bricks and cement with corrugated iron roofing. Such stores are often of poor structure and bad storage hygiene. Bagged grain is usually stacked up to the ceiling and

against all walls, making inspection and disinfestations procedures difficult (FAO, 1987).

2.4. 7. Central stores (large-scale warehouses)

These are hanger type warehouses with concrete flooring and either concrete or galvanized iron walls and iron roof. Bags of grain (90 kg) are stacked on wooden dungage (pallets). They vary in shape and capacity, however, the size may be up to 100 x 40 x 9 meters, with capacity of up to one hundred thousand tons. The disadvantage of this system is that loading and discharge are costly, protection against pests is poor, temperature monitoring and fumigation are difficult and prevention of rain water is not well (FAO, 1987).

2.4. 8. Silo storage:

Above-ground concrete or metal silo structures have not been used for hermetic storage, but have been rendered airtight for use with carbon dioxide or nitrogen mixtures to reduce the amount of oxygen present in the structures and thus limit insect and microbial growth. Silo storage was adopted in the Sudan for the first time in 1967, when two modern concrete silos were constructed in Gedaref (100.000 tons) and Port Sudan (500,000 tons) (FAO, 1987).

2.5. Management of seed-borne fungal diseases/pathogens

The control of seed-borne fungal pathogens can be considered broadly in terms of exclusion and elimination of inoculum (Maude, 1996). Exclusion strategies include; use of legislation, the isolation of seed production areas, the setting of minimum inoculum tolerance levels for seeds and breeding for resistance, while direct eliminatory measures include, control of organisms by seed treatments and crop treatments. Disease

control may be achieved by single or by combined strategies contained within the concepts of exclusion and elimination (Maude, 1996).

2.5.1 Seed treatment

Seed treatment is a generic term (Scott, 1989) which does not specify the application method but indicates that seeds are subjected to a compound of chemical, nutrient, hormone, etc treatments; a process (such as wetting or drying) or to various energy forms (e.g. radiation, heat, magnetism, electricity). Research has shown that seedborne pathogens can be controlled substantially using various physical, mechanical, chemical, botanical, and other methods (Messiaen, 1992).

2.5.2 Effects of seed treatment on control of seed-borne pathogens

2.5.2.1 Physical control measures

Cowpea seeds naturally infected with Macrophomina phaseolina and Fusarium equiseti given hot water treatment at eight different temperatures showed reduced infection frequency (Sinha and Khare, 1977). Duration of each treatment was 5, 10, 15 and 20 minutes. The most effective treatment to check both pathogens was dipping of seeds in water at 46 degree Celsius for 20 minutes.). Baker (1972) reported that dry heat has been used to eliminate or reduce artificial or natural bacterial contamination of seeds often with little impairment of seed germination.

According to Megahed and Moore (1969) exposure of infected Prunus seeds to radiation at doses of (20, 000 rad) reduced the transmission of Prumus Necroti Ringspot Virus (PNRSV) and Prunus Dwarf Virus (PDV).

2.5.2.2 Mechanical control measures

Sheppard (1983) during his investigation, revealed that the use of furrow rather than overhead irrigation system in low rainfall areas further restricts the spread of splash-dispersal pathogens including *Xanthomonas* campestris pv. Phaseoli, Phaseoli suyringae pv. *Phaseolicola* and *Colletotrichum lindemuthianum* on phaseolus bean, Septoria apiicola on celery *Xanthomonas campestris* pv. Campestris and *Phoma lingam* on cabbage and Ascohyta spp. on peas.

2.5.2.3 Biological control measures

Biological control had attained importance in modern agriculture, due to attempts to reduce hazards of intensive use of chemicals for pests and disease control (Tuber and Baker, 1988). Trichoderma spp have shown to inhibit Macrophomena phaseolina growth on PDA. (Mahaber et al., 1995). Okigbo and Ikediugwu (2000) showed that Trichoderma viride displaced the naturally occurring mycoflora on the surface of yam tubers. Single application of Trichoderma viride effectively controlled the normal tuber surface mycoflora throughout six months storage, greatly reducing rotting. Okigbo (2002) also used Bacillus subtilis to control pathogens that affect white yam (Dioscorea rotundata) and it was reported that Bacillus subtilis displaced the naturally occurring mycoflora on the surface of yam tubers as was observed in yams with Trichoderma viride.

2.5.2.4 Chemical control measures

Attempts have been made to reduce seedborne infection by chemical treatment of the seeds and some successes have been reported. Seed dressings are used to eliminate most surface infestation of seeds but have relatively little effect on internally borne organisms (Jackson, 1963).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study location:

The sorghum seeds samples collected from different locations and storage conditions in Sudan were investigated in the laboratory of plant pathology, Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology during February-April, 2016. The aim of this study was to detect and identify seed borne fungi associated with seeds samples of four varieties of sorghum, namely, Dabar, Mogod, Wad Akar and Fetarita, collected from Gadaref and Rabak grain storage facilities and to explore the impact of storage conditions on seeds health in each location.

All materials except seeds, 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. Collection of samples:

A total of 12 seed samples, one from each of the four sorghum varieties in each of the two locations plus one combined sample including the four varities of sorghum from each of the two types of storage facilities in each location. The samples were collected from grains market' seed stocks, silos and Warehouse at Gadaref and Rabak. One random and homogeneous sample of one kilo gram of seeds was secured from each of the 12 samples. Seed 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.

3.3. Methods for the detection of seed borne fungal pathogens:

3.3.1. Dry Seed Inspection:

As described by the international seed testing association (ISTA Rules,1966), a sample of four hundred (400) seeds of each seed sample were randomly selected and examined under stereoscopic binocular microscope (25-4x) and by magnified lens and naked eye. The samples were examined for impurities, plant debris, weed seeds, discoloration and malformation.

3.3.2. Blotter method:

For the detection of seed borne fungi, standard blotter method as described by the International Seed Testing Association (ISTA 1996), was used for the detection of the seed-borne fungi associated with each seed sample. The seed samples in their various forms according to their variety and location were then platted on moistened filter papers (dia. 9.0 cm) in 9.0 cm sterilized plastic Petri-dishes. Twenty five seeds were plated from each sample, 15 arranged at the periphery of the plate and 10 at the centre. A total of two seed samples per variety and location, with three replications, were used and then kept in dark place for seed germination.

After seven days of incubation, seeds were then examined for fungal growth under a stereo microscope. Fungi identification by habit character was supplemented by microscopic examination of spores and fruiting

bodies using a compound microscope. Other identification aids were Burgess *et al.,* (1994); Mathur SK, SB Mathur, P Neergaard (1975); Agarwal *et al.,* (1989) and Mathur and Kongsdal (2003). Infection levels were recorded as the percentage of infected seeds in a sample

3.3.3. Agar Method:

All seed samples (from varieties and locations) were pre-treated with sodium hypochlorite 1% solution for 5 minutes then washed three times with sterilized distilled water (SDW) and dried between tow filter papers. The seed samples were then plated in the sterilized glass Petri-dishes on potato dextrose agar medium (PDA).

The plates were incubated for seven days at 25^0C. On the 8^{th} day the seeds were examined under light microscopes using slides preparation.

3.3.4 Isolation and identification:

Specimen of the detected of each fungus was taken randomly from each sample. These specimens were identified on the basis of colony characteristics and microscopic examinations. Standard books and research papers were consulted during the examination of these fungi (Aneja, 2004; Rifai, 1969; Barnet and Hunter, 1999).The binocular compound microscope was also used to determine the type of fungus in each plate. Fungi identified and their percentage frequency (PF) of occurrence was calculated by applying the following formula:

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

3.3.5 Effect of storage condition on fregceney of occurrence percentageson funge seed sample of sorghum –(Agar – Blotter mothod)

3.3.6 Statistical analysis

Data collected were subjected to analysis of (ANOVA) .The experiment Model ,Tow Factor completely Randmized Design (CRD) and significantly means were separated using least significant Different (LSD) at probabililty(0.05).

CHAPTER FOUR

RESULTS

4.1. Detection and occurrence percentage of fungal species on seeds of different sorghum genotypes using Agar method

The mean occurrence percentages of seed borne fungi of sorghum samples revealed by the Agar Method are given in Tables 1, 2, and Figure 1 and 2) respectively. Out of the 12 seed samples, 6 from each location, tested for occurrence of seed borne fungi, a total of 7 genera of 6 species of fungi were recorded (Table 1 and 2).

The seed borne fungi identified were *Aspergillus flavus, Aspergillus niger, Penicillium digitatum, Rhizopus nigricans, Alternaria solani, Fusarium solani* and *Phoma longum.* Moreover, the most predominant seed borne fungus recorded across varieties was the storage fungi, *Aspergillus flavus* with varying level of occurrence ranging from 3.33 to 43.3% (Fig. 1).

Most samples tested for seed borne fungi gave a number of fungi with varying level of occurrence that varied with sorghum genotype (Table, 1). However, the highest occurrence of seed borne fungi was recorded in variety Akar and Fetarita where six species were detected (Table, 1) and the significantly high percentage incidence was given by the fungus *P. longum* (63.3%) in variety Fetarita.

Table 1: Mean occurrence percentage of seed borne fungi on various seed samples of sorghum using Agar method

Fungi spp.	Sorghum varieties				Overall mean
	Dabar	Mogod	Akar	Fetarita	
Aspergillus flavus	20.00^{cde}	3.33^{de}	43.33^{b}	3.33^{de}	17.50^{AB}
Aspergillus niger	0.00^{e}	3.33^{de}	6.67^{de}	0.00^{e}	2.50^{D}
Rhizopus nigricans	0.00^{e}	33.33^{bc}	13.33^{de}	6.67^{de}	13.33^{BC}
Penicillium digitatum	6.67^{de}	0.00^{e}	6.67^{de}	13.33^{de}	6.67^{CD}
Alternaria solani	0.00^{e}	0.00^{e}	6.67^{de}	10.00^{de}	4.17^{CD}
Phoma longum	0.00^{e}	10.00^{de}	23.33^{cd}	63.33^{a}	24.17^{A}
Fusarium solani	3.33^{de}	0.00^{e}	0.00^{e}	0.00^{e}	0.833^{D}
Overall	4.286^{B}	7.143^{AB}	14.29^{A}	13.81^{A}	
C.V%	11.52%				
$Lsd_{0.05}$	6.812^{*}				
SE±	2.405				

Means bearing different superscripts are significantly different (P≤0.05) according to DMRT.

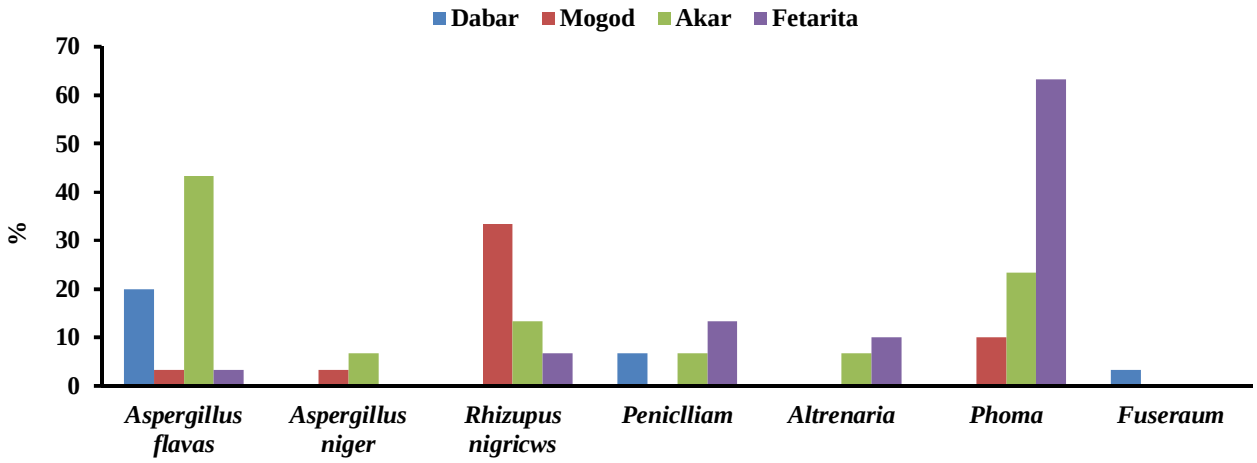

Fig. (1): Agar method (sorghums)

Fig 1: Mean occurrence percentage of seed borne fungi on various seed sample of sorghum using Agar method

4.2. Detection and occurrence percentage of fungal species on seeds samples of different sorghum genotypes using Filter method

The results obtained by filter method revealed similarities in number of fungi detected by filter method (Table, 2 and Fig. 2). Thus, all species of fungi recorded by Agar method was recorded by Filter method. Five genera of seed borne fungi were identified in all samples of seeds whereas 6 species were occurred. Fungi identified were *Aspergillus flavus, Aspergillus niger, Penicillium digitatum, Rhizopus nigricans, Alternaria solani, Fusarium solani and Phoma longum.* Among fungi, the most predominant seed borne ones recorded across sorghum varieties seeds samples were the storage fungi, *Aspergillus flavus, Aspergillus niger, Alternaria solani and Phoma longum* with varying level of occurrence (Table 1-4) ranging from 3.33 by *A. solani* to 36.67% by *P. longum*.

The highest percentage frequency of occurrence of the seed borne fungi in seed samples recorded was given by *A. flavus and P. longum* (36.67%).

Table 2: Mean occurrence percentage of seed borne fungi on various seed samples of sorghum using Filter method

Fungi	Sorghum cultivars				Overall mean
	Dabar	Mogod	Akar	Fetarita	
Aspergillus flavus	26.67^{ab}	36.67^{a}	23.33^{abc}	6.67^{cd}	23.33^{A}
Aspergillus niger	30.00^{a}	23.33^{abc}	23.33^{abc}	10.00^{bcd}	21.67^{A}
Rhizopus nigricans	0.00^{d}	6.67^{cd}	0.00^{d}	0.00^{d}	1.667^{C}
Penicillium digitatum	0.00^{d}	3.33^{cd}	0.00^{d}	0.00^{d}	0.833^{C}
Alternaria solani	10.00^{bcd}	3.33^{cd}	6.67^{cd}	23.33^{abc}	10.83^{B}
Phoma longum	3.33^{cd}	6.67^{cd}	36.67^{a}	36.67^{a}	20.83^{A}
Overall	11.67^{A}	13.33^{A}	15.00^{A}	12.78^{A}	
C.V%	7.88%				
$Lsd_{0.05}$	6.976^{*}				
SE±	2.453				

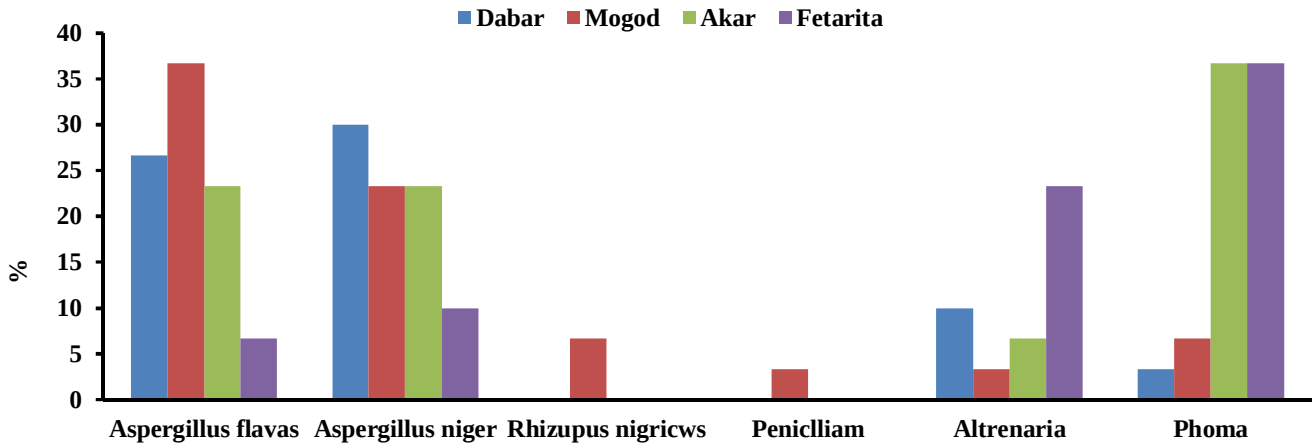

Fig. (2): Filter method (sorghums)

Fig 2: Mean occurrence percentage of seed borne fungi on various seed sample of sorghum using Filter method

4.3. Effect of storage conditions on frequency of occurrence percentages of fungi on seeds samples of sorghum using Agar method

Results of detection of fungi in sorghum seed samples collected from two types of storage facilities (Modern Silos and Warehouse) in Gadaref and Rabak using Agar method were shown in table,3 and figure, 3. Most samples tested for seed borne fungi gave a number of fungi with varying level of occurrence. The level of occurrence varied with location and storage facility (Table, 3). Four genera of seed borne fungi were identifies in samples from locations and storage faculties including five species namely, *Aspergillus flavus, Aspergillus niger, Alternaria solani, Fusarium solani and Phoma longum*

Generally, the occurrence percentages of fungi were low and there is no significant difference in occurrence between location and storage facilities. However, the most predominant seed borne fungi recorded in samples across locations and storage facilities seeds samples were *A. niger and Alternaria solani* with varying level of occurrence (Table, 3) ranging from 6.67 and 23.3%. Moreover, the highest level of occurrence was recorded by *A. solani* (23.33 %) in samples of seeds from Rabak stores.

Table 3: Frequency of occurrence percentages of fungi on seeds samples of sorghum from different locations and storage facilities using Agar method

Fungi spp.	Locations and storage condition				Overall mean
	Gadaref		Rabak		
	GM	GS	RM	RS	
Aspergillus flavus	10.00^{ab}	0.00^{b}	13.33^{ab}	13.33^{ab}	9.167^{A}
Aspergillus niger	3.33^{ab}	6.67^{ab}	13.33^{ab}	3.33^{ab}	6.667^{A}
Alternaria solani	6.67^{ab}	6.67^{ab}	6.67^{ab}	23.33^{a}	10.83^{A}
Phoma longum	16.67^{ab}	13.33^{ab}	0.00^{b}	16.67^{ab}	11.67^{A}
Fusarium solani	6.67^{ab}	3.33^{ab}	3.33^{ab}	0.00^{b}	3.33^{A}
Overall	8.667^{A}	6.000^{A}	7.333^{A}	11.33^{A}	
C.V%	7.799*				
Lsd$_{0.05}$	2.728				
SE±	10.00^{ab}				

Means bearing different superscripts are significantly different (P≤0.05) according to DMRT.

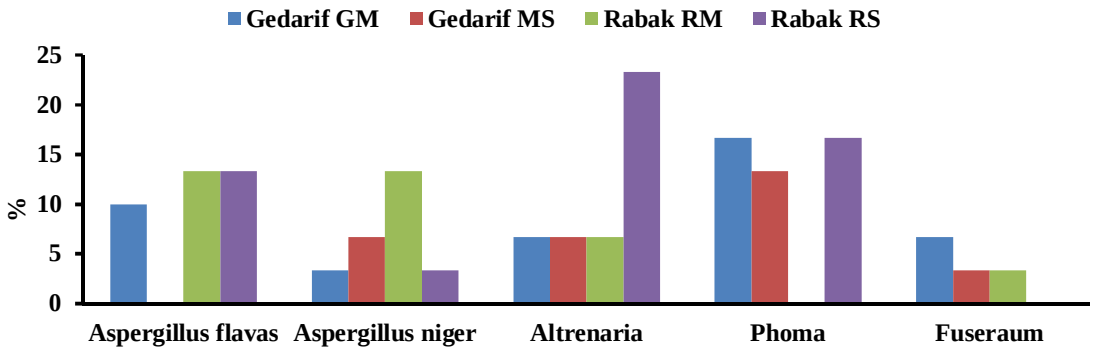

Fig. (3): Agar method (locations)

Fig 3: Frequency of occurrence percentages of fungi on seeds samples of sorghum from different locations and storage facilities using Agar method

4.4. Effect of storage conditions on frequency of occurrence percentages of fungi on seeds samples of sorghum using Fitter method

Results in table,4 and figure, 4 showing the fungi detected in sorghum seed samples collected from Silos and stores in Gadaref and Rabak using Filter method. Generally, the level of occurrence of fungi varies with location and storage facility. Four species of fungi were detected in all locations namely, *Aspergillus flavus, Aspergillus niger, Alternaria solani* and *Phoma longum.*

The most predominant seed borne fungus among species of fungi recorded in seed samples across locations and storage facilities was *Phoma longum* with varying level of occurrence (Table, 4) ranging from 20.00 to 36.00%. Its occurrence percentages were significantly high compared to other species.

Table 4: Frequency of occurrence percentages of fungi on seeds samples of sorghum from different locations and storage facilities using Filter method

Fungi spp.	Storage condition in each location				Overall mean
	Gadaref		Rabak		
	GM	MS	RM	RS	
Aspergillus flavus	3.33^{c}	10.00^{bc}	13.33^{bc}	13.33^{bc}	10.00^{B}
Aspergillus niger	10.00^{bc}	6.67^{c}	3.33^{c}	13.33^{bc}	8.33^{B}
Alternaria solani	6.67^{c}	10.00^{bc}	6.67^{c}	16.67^{bc}	1.00^{B}
Phoma longum	36.67^{a}	23.33^{abc}	30.00^{ab}	20.00^{abc}	27.50^{A}
Overall	14.17^{A}	12.50^{A}	13.33^{A}	15.83^{A}	
C.V%	5.28%				
$Lsd_{0.05}$	8.738^{*}				
SE±	3.033				

Means bearing different superscripts are significantly different (P≤0.05) according to DMRT.

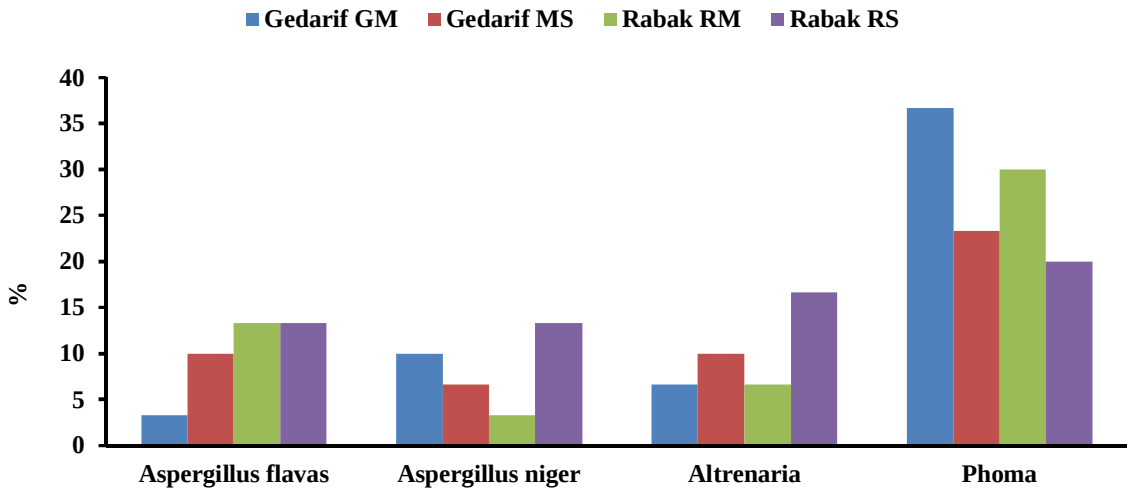

Fig. (4): Filter method (locations)

Fig 4: Frequency of occurrence percentages of fungi on seeds samples of sorghum from different locations and storage facilities using Agar method

CHAPTER FIVE

DISCUSSION

Sorghum [*Sorghum bicolor* (L.) Monech] is the principal staple food in the Sudan. Pathogen free healthy seed is critically needed for desired crop production and consumption. Many plant pathogens are seed-borne, which can cause enormous crop losses. Fakir (1983) reported that out of 16% annual crop losses due to plant diseases, at least 10% loss is incurred due to seed-borne diseases. Coincidentally important or devastating crop diseases are seed-borne and caused by fungi. It has also been

Accordingly, contamination of grain by seed borne fungi cannot be ignored. In Sudan, the risk encountered due to these fungi 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 and Eltayeb and Sana, 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. This study was conducted to investigate the occurrence of seed borne fungi associated with four genotypes of sorghum seed samples collected from main growing centers in Sudan (Gadaref and Rabak) and storage facilities over there. The results revealed that irrespective of source of seed samples, the association of seed borne fungi with sorghum grains in different varieties appears to be a prevalent situation. Generally, all seeds samples tested either with Agar or Filter method as described by the International Seed Testing Association (ISTA, 1976) were associated with at least four

known spoilage species of fungi (*Aspergillus flavus, Aspergillus niger, Alternaria solani and Phoma longum.* These results are in agreement with those of Syed Danis, *et al*., (2013); Kamal and Mughal (1968) and Khan *et 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 also showed that *Aspergillus spp*. were the most prevailing seed borne fungi recorded across tested varieties. The common occurrence of seed borne fungi like *Aspergillus* had been widely reported by Haq Elamin NH *et al.*, 1988 and Martin *et al.*, (1984).

The data also revealed high load of seed borne fungi in some varieties seeds or in some storage facilities compared to others could be attributed to favorable storage conditions for the different fungi in 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 the predominance of some seed-borne fungal species e.g. *Aspergillus* spp. and *Phoma sp*. even under modern silo condition and the occurrence percentages of the later were significantly high compared to other species. This could be attributed to pre-infection of seeds before storage. Similar results were reported by Mashilla (2004) who demonstrated that seed borne fungal species of *Aspergillus spp*. and *Phoma spp*. were associated with sorghum seeds before storage.

Conclusion:

Based on the foregoing, few perspectives seem to emerge:-

- Spoilage-pathogen free healthy seed is critically needed for desired crop production and consumption. In this study, seven fungal genera were encountered in wide range of incidence percentage in 12 seed samples of sorghum verities collected from Gadaref and Rabak under different storage facilities.

- Of the fungi occurred in seed samples, the four most prevailing seed borne fungi recorded across sorghum varieties and storage facilities were the storage ones; *Aspergillus flavus, Aspergillus Niger, Alternaria solani and Phoma longum* with varying level of incidences.

- Predominance of some spoilage fungi under modern silo storage attributed to infection prior to storage.

Recommendations:

The prospective, on the other hand, are envisaged to address, in the first place:-

- 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.

- 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.

- Introduction of seed health testing of major food grains should incorporated in the national seed quality system.

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Appendices

Statistical analysis

1- Sorghums agar

A N A L Y S I S O F V A R I A N C E T A B L E

S. of Var.	d.f	SS	MS	F-cal	P-value
Factor A	3	1546.429	515.476	4.2451	0.0090
Factor B	6	5440.476	906.746	7.4673	0.0000
AB	18	11111.905	617.328	5.0839	0.0000
Error	56	6800.000	121.429		
Total	83	24898.810			

Coefficient of Variation: 11.52%

Duncan's Multiple Range Test

Overall sorghum/location

LSD value = 6.812 SE = 2.405 at alpha = 0.050

Original Order				Ranked Order			
Mean	1 =	4.286	B	Mean	3 =	14.29	A
Mean	2 =	7.143	AB	Mean	4 =	13.81	A
Mean	3 =	14.29	A	Mean	2 =	7.143	AB
Mean	4 =	13.81	A	Mean	1 =	4.286	B

Overall fungi

Duncan's Multiple Range Test

LSD value = 9.012 SE = 3.181 at alpha = 0.050

Original Order				Ranked Order			
Mean	1 =	17.50	AB	Mean	6 =	24.17	A
Mean	2 =	2.500	D	Mean	1 =	17.50	AB
Mean	3 =	13.33	BC	Mean	3 =	13.33	BC
Mean	4 =	6.667	CD	Mean	4 =	6.667	CD
Mean	5 =	4.167	CD	Mean	5 =	4.167	CD
Mean	6 =	24.17	A	Mean	2 =	2.500	D
Mean	7 =	0.8333	D	Mean	7 =	0.8333	D

Interaction

Duncan's Multiple Range Test

LSD value = 18.02 SE = 6.362 at alpha = 0.050

Original Order Ranked Order

```
 Mean    1 =    20.00    CDE    Mean   27 =    63.33  A
 Mean    2 =   0.0000      E    Mean   15 =    43.33   B
 Mean    3 =   0.0000      E    Mean   10 =    33.33   BC
 Mean    4 =    6.667     DE    Mean   20 =    23.33
CD
 Mean    5 =   0.0000      E    Mean    1 =    20.00
CDE
 Mean    6 =   0.0000      E    Mean   25 =    13.33
DE
 Mean    7 =    3.333     DE    Mean   17 =    13.33
DE
 Mean    8 =    3.333     DE    Mean   26 =    10.00
DE
 Mean    9 =    3.333     DE    Mean   13 =    10.00
DE
 Mean   10 =    33.33   BC      Mean   19 =    6.667
DE
 Mean   11 =   0.0000      E    Mean   16 =    6.667
DE
 Mean   12 =   0.0000      E    Mean   18 =    6.667
DE
 Mean   13 =    10.00     DE    Mean    4 =    6.667
DE
 Mean   14 =   0.0000      E    Mean   24 =    6.667
DE
 Mean   15 =    43.33   B       Mean    7 =    3.333
DE
 Mean   16 =    6.667     DE    Mean    9 =    3.333
DE
 Mean   17 =    13.33     DE    Mean   22 =    3.333
DE
 Mean   18 =    6.667     DE    Mean    8 =    3.333
DE
 Mean   19 =    6.667     DE    Mean    2 =   0.0000
E
 Mean   20 =    23.33    CD     Mean   14 =   0.0000
E
 Mean   21 =   0.0000      E    Mean   21 =   0.0000
E
 Mean   22 =    3.333     DE    Mean    5 =   0.0000
E
 Mean   23 =   0.0000      E    Mean   23 =   0.0000
E
 Mean   24 =    6.667     DE    Mean    3 =   0.0000
E
 Mean   25 =    13.33     DE    Mean   11 =   0.0000
E
 Mean   26 =    10.00     DE    Mean   12 =   0.0000
E
 Mean   27 =    63.33  A        Mean    6 =   0.0000
E
```

```
 Mean   28 =   0.0000      E    Mean   28 =   0.0000
E
```

2- Sorghums filter

```
          A N A L Y S I S   O F   V A R I A N C E   T A B
L E
S. of Var.        d.f         SS         MS       F-cal
P-value
------------------------------------------------------------
--------------------
Factor A          3       104.167        34.722
0.3205
Factor B          5      6290.278      1258.056
11.6128   0.0000
AB               15      5770.833       384.722
3.5513   0.0004
Error            48      5200.000       108.333
------------------------------------------------------------
--------------------
Total            71     17365.278
------------------------------------------------------------
--------------------
Coefficient of Variation: 7.88%
Duncan's Multiple Range Test
LSD value = 6.976     SE = 2.453      at alpha = 0.050
     Original Order                Ranked Order
 Mean    1 =    11.67  A    Mean    3 =    15.00  A
 Mean    2 =    13.33  A    Mean    2 =    13.33  A
 Mean    3 =    15.00  A    Mean    4 =    12.78  A
 Mean    4 =    12.78  A    Mean    1 =    11.67  A
Duncan's Multiple Range Test
LSD value = 8.543     SE = 3.005      at alpha = 0.050
      Original Order                Ranked Order
 Mean    1 =    23.33  A      Mean    1 =    23.33  A
 Mean    2 =    21.67  A      Mean    2 =    21.67  A
 Mean    3 =    1.667    C    Mean    6 =    20.83  A
 Mean    4 =   0.8333    C    Mean    5 =    10.83   B
 Mean    5 =    10.83   B     Mean    3 =    1.667    C
 Mean    6 =    20.83  A      Mean    4 =   0.8333    C
Duncan's Multiple Range Test
LSD value = 17.09     SE = 6.009      at alpha = 0.050
      Original Order                Ranked Order
 Mean    1 =    26.67  AB      Mean    7 =    36.67  A
 Mean    2 =    30.00  A       Mean   18 =    36.67  A
 Mean    3 =   0.0000     D    Mean   24 =    36.67  A
 Mean    4 =   0.0000     D    Mean    2 =    30.00  A
 Mean    5 =    10.00   BCD    Mean    1 =    26.67  AB
 Mean    6 =    3.333    CD    Mean   23 =    23.33  ABC
 Mean    7 =    36.67  A       Mean   13 =    23.33  ABC
```

Mean	8 =	23.33	ABC	Mean	8 =	23.33	ABC
Mean	9 =	6.667	CD	Mean	14 =	23.33	ABC
Mean	10 =	3.333	CD	Mean	5 =	10.00	BCD
Mean	11 =	3.333	CD	Mean	20 =	10.00	BCD
Mean	12 =	6.667	CD	Mean	9 =	6.667	CD
Mean	13 =	23.33	ABC	Mean	19 =	6.667	CD
Mean	14 =	23.33	ABC	Mean	12 =	6.667	CD
Mean	15 =	0.0000	D	Mean	17 =	6.667	CD
Mean	16 =	0.0000	D	Mean	10 =	3.333	CD
Mean	17 =	6.667	CD	Mean	6 =	3.333	CD
Mean	18 =	36.67	A	Mean	11 =	3.333	CD
Mean	19 =	6.667	CD	Mean	4 =	0.0000	D
Mean	20 =	10.00	BCD	Mean	3 =	0.0000	D
Mean	21 =	0.0000	D	Mean	16 =	0.0000	D
Mean	22 =	0.0000	D	Mean	15 =	0.0000	D
Mean	23 =	23.33	ABC	Mean	22 =	0.0000	D
Mean	24 =	36.67	A	Mean	21 =	0.0000	D

3- Locations agar

A N A L Y S I S O F V A R I A N C E T A B L E

S. of Var.	d.f	SS	MS	F-cal	P-value
Factor A	3	233.333	77.778	0.6965	
Factor B	4	550.000	137.500	1.2313	0.3130
AB	12	1583.333	131.944	1.1816	0.3286
Error	40	4466.667	111.667		
Total	59	6833.333			

Coefficient of Variation: 12.58%

Duncan's Multiple Range Test

LSD value = 7.799 SE = 2.728 at alpha = 0.050

Original Order				Ranked Order			
Mean	1 =	8.667	A	Mean	4 =	11.33	A
Mean	2 =	6.000	A	Mean	1 =	8.667	A
Mean	3 =	7.333	A	Mean	3 =	7.333	A
Mean	4 =	11.33	A	Mean	2 =	6.000	A

Duncan's Multiple Range Test

LSD value = 8.719 SE = 3.051 at alpha = 0.050

Original Order				Ranked Order			
Mean	1 =	9.167	A	Mean	4 =	11.67	A
Mean	2 =	6.667	A	Mean	3 =	10.83	A

Mean	3 =	10.83	A	Mean	1 =	9.167	A
Mean	4 =	11.67	A	Mean	2 =	6.667	A
Mean	5 =	3.333	A	Mean	5 =	3.333	A

Duncan's Multiple Range Test

LSD value = 17.44 SE = 6.101 at alpha = 0.050

Original Order				Ranked Order			
Mean	1 =	10.00	AB	Mean	18 =	23.33	A
Mean	2 =	3.333	AB	Mean	4 =	16.67	AB
Mean	3 =	6.667	AB	Mean	19 =	16.67	AB
Mean	4 =	16.67	AB	Mean	12 =	13.33	AB
Mean	5 =	6.667	AB	Mean	11 =	13.33	AB
Mean	6 =	0.0000	B	Mean	16 =	13.33	AB
Mean	7 =	6.667	AB	Mean	9 =	13.33	AB
Mean	8 =	6.667	AB	Mean	1 =	10.00	AB
Mean	9 =	13.33	AB	Mean	5 =	6.667	AB
Mean	10 =	3.333	AB	Mean	3 =	6.667	AB
Mean	11 =	13.33	AB	Mean	7 =	6.667	AB
Mean	12 =	13.33	AB	Mean	8 =	6.667	AB
Mean	13 =	6.667	AB	Mean	13 =	6.667	AB
Mean	14 =	0.0000	B	Mean	10 =	3.333	AB
Mean	15 =	3.333	AB	Mean	15 =	3.333	AB
Mean	16 =	13.33	AB	Mean	2 =	3.333	AB
Mean	17 =	3.333	AB	Mean	17 =	3.333	AB
Mean	18 =	23.33	A	Mean	6 =	0.0000	B
Mean	19 =	16.67	AB	Mean	14 =	0.0000	B
Mean	20 =	0.0000	B	Mean	20 =	0.0000	B

4- Locations filter

A N A L Y S I S O F V A R I A N C E T A B L E

S. of Var.	d.f	SS	MS	F-cal	P-value
Factor A	3	72.917	24.306	0.2201	
Factor B	3	2956.250	985.417	8.9245	0.0002
AB	9	985.417	109.491	0.9916	
Error	32	3533.333	110.417		
Total	47	7547.917			

Coefficient of Variation: 5.28%

Duncan's Multiple Range Test

LSD value = 8.738 SE = 3.033 at alpha = 0.050

Original Order				Ranked Order			
Mean	1 =	14.17	A	Mean	4 =	15.83	A
Mean	2 =	12.50	A	Mean	1 =	14.17	A
Mean	3 =	13.33	A	Mean	3 =	13.33	A
Mean	4 =	15.83	A	Mean	2 =	12.50	A

Duncan's Multiple Range Test

LSD value = 8.738 SE = 3.033 at alpha = 0.050

Original Order				Ranked Order			
Mean	1 =	10.00	B	Mean	4 =	27.50	A
Mean	2 =	8.333	B	Mean	1 =	10.00	B
Mean	3 =	10.00	B	Mean	3 =	10.00	B
Mean	4 =	27.50	A	Mean	2 =	8.333	B

Duncan's Multiple Range Test

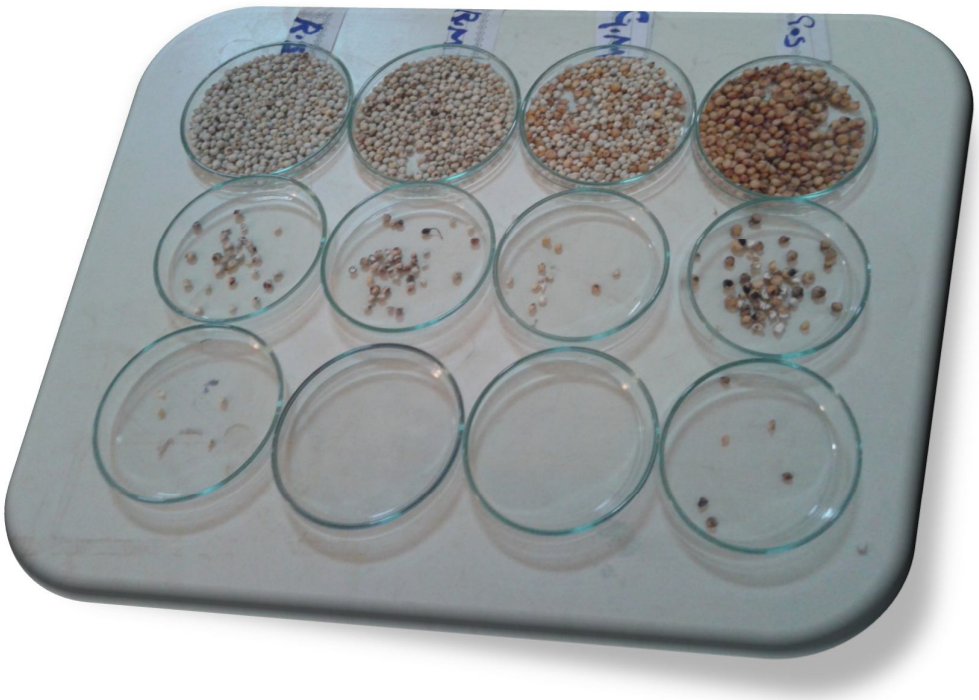
LSD value = 17.48 SE = 6.067 at alpha = 0.050

Original Order				Ranked Order			
Mean	1 =	3.333	C	Mean	4 =	36.67	A
Mean	2 =	10.00	BC	Mean	12 =	30.00	AB
Mean	3 =	6.667	C	Mean	8 =	23.33	ABC
Mean	4 =	36.67	A	Mean	16 =	20.00	ABC
Mean	5 =	10.00	BC	Mean	15 =	16.67	BC
Mean	6 =	6.667	C	Mean	9 =	13.33	BC
Mean	7 =	10.00	BC	Mean	13 =	13.33	BC
Mean	8 =	23.33	ABC	Mean	14 =	13.33	BC
Mean	9 =	13.33	BC	Mean	7 =	10.00	BC
Mean	10 =	3.333	C	Mean	5 =	10.00	BC
Mean	11 =	6.667	C	Mean	2 =	10.00	BC
Mean	12 =	30.00	AB	Mean	3 =	6.667	C
Mean	13 =	13.33	BC	Mean	11 =	6.667	C
Mean	14 =	13.33	BC	Mean	6 =	6.667	C
Mean	15 =	16.67	BC	Mean	1 =	3.333	C
Mean	16 =	20.00	ABC	Mean	10 =	3.333	C

Dry Seed Inspection:

Method Dry seeds inspection of different Varieties			
Varieties	Healthy	UNH	Seed Depress
D	330	60	10
F	346	50	4
A	376	24	0
M	347	53	0

Method Dry seeds inspection of different location			
location	Healthy	UNH	Seed Depress
GS	343	48	9
GM	388	12	0
RM	361	39	0
RS	364	29	7



Sterilization seed sorghum