بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Examination of Fungal Contaminants of Wheat, Rice, Barely and Maize Grains

فحص الملوثات الفطرية لحبوب القمح، الأرز، الشعيرو الذرة الشامي

A thesis submitted in partial fulfillment of the requirements for the

M.Sc. degree in plant protection

By

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الآية

قال تعالى :

﴿أَوَلَمْ يَرَ الَّذِينَ كَفَرُوا أَنَّ السَّمَوَاتِ وَالْأَرْضَ كَانَتَا رَتْقًا فَفَتَقْنَاهُمَا وَجَعَلْنَا مِنَ الْمَاءِ كُلَّ شَيْءٍ حَيٍّ أَفَلَا يُؤْمِنُونَ ﴾

صدق الله العظيم

سورة الأنبياء الآية ﴿30﴾

Dedication

To My

Family ***for their kind support and encouragements****,*

Teachers

And my Friends.

Acknowledgements

Thanks above all to Almighty Allah the most gracious merciful, who gave me health to complete this study. I most express my sincere gratitude and appreciation to my Supervisor Dr**. Mawahib Ahmed Elsiddig**. I am also grateful Dr. Nayla Haroun for their help to complete research. Thanks also extended to my father, mother, sister, brother and all family. Finally, I wish to express sincere appreciation to colleagues and friend.

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Abstract

Seed borne fungi present on /in the seed as contaminants with the seed debris adversely affect seed viability, germination, emergence, plant growth vigor and eventually production and productivity. In the present study, seed borne fungi of four local cereal seeds i-e Maize, Barely, Wheat and Rice were collected randomly from four location in Khartoum Estate examined and recovered from the seeds. The results obtained revealed that all the seeds of all samples were highly contaminated with four fungal genera namely *Aspergillus, Penicillum, Alternaia* and *Rhizopus,* collected samples were examined by different test methods. In maize, *Aspergillus* recorded the highest fungi percent in filter paper test (28.67%) and Agar method (28.33%) followed by *Penicillium, Alternaria* and *Rhizopus* (26.67, 17.33 and 5.33) respectively. The genus *Aspergillus* and *Penicillum* were the most prevalent genera followed by *Alternaria* and *Rhizopus.* Therefore, there is urgent need for development of proper standard laboratory seed testing methods, fungal eradication measures, and adopting strong legislations and quarantine regulations. The use of certified and high grade seeds is a priority.

الملخص

الفطريات التي تحملها البذور الموجودة على / في البذور كمواد ملوثة ببقايا البذور تؤثر سلبًا علي حيوية البذور وإنباتها وظهورها وعلى نمو النبات ، والإنتاج والإنتاجية. في هذه الدراسة ، تم جمع الفطريات المنقولة بالبذور لأربع بذور حبوب محلية مثل الذرة ، الشعير ، القمح والأرز بشكل عشوائي من أربعة مواقع في ولاية الخرطوم تم فحصها من البذور. أظهرت النتائج التي تم الحصول عليها أن جميع البذور في جميع العينات كانت شديدة التلوث بأربعة أجناس فطرية وهي *Aspergillus Penicillum Alternaria Rhizopus* , العينات المجمعة تم فحصها بطرق اختبار مختلفة. في الذرة الشامية، سجل *Aspergillus* أعلى نسبة فطرية في إختبار ورق الترشيح (28.67٪) وطريقة أجار (28.33٪) تليها *Penicillum Alternaria Rhizopus* (26.67 ، 17.33 و 5.33) على التوالي. كان جنس *Aspergillus* و *Penicillum* أكثر الأجناس انتشارًا يليه *Alternaria* و *Rhizopus*. لذلك ، هناك حاجة ملحة لتطوير طرق إختبار البذور المختبرية القياسية المناسبة ، وإجراءات القضاء على الفطريات ، وإعتماد تشريعات قوية ولوائح الحجر الصحي. يعد إستخدام البذور المعتمدة وعالية الجودة من الأولوية.

CHAPTER ONE

INTRODUCTION

Cereals can be defined as a grain or edible seed of the grass family Graminae (Bender & Bender 1999). Cereals are grown for their highly nutritious edible seeds, which are often referred to as grains. Some cereals have been staple foods both directly for human consumption and indirectly via livestock feed since the beginning of civilization (BNF, 1994). Cereals are the most important sources of food (FAO, 2002), and cereal based foods are a major source of energy, protein, vitamins and minerals for the world population. Generally, cereals are cheap to produce, easily stored and transported, and do not deteriorate readily if kept dry.

Grains develop from flowers or florets and, although the structures of the various cereal grains are different, there are some typical features. The embryo (or germ) is a thin-walled structure, containing the new plant. It is separated by the *scutellum* (which is involved in mobilization of food reserves of the grain during germination) from the main part of the grain *endosperm.* The endosperm consists of thin-walled cells, packed with starch grains. If the cereal grain germinates, the seedling uses the nutrients provided by the endosperm until the developments of green leaves that allow photosynthesis to begin (Kent & Evers 1994). The endosperm is surrounded by the aleuronic, consisting of one or three cell layers (wheat, rye, oats, maize and sorghum have one; rice and barley three). The outer layers of the grain are the pericarp (derived from the ovary of the flower) which surround the seed coat (the testa). The outer thick-walled structures form the bran.

Wheat is a major cereal crop in many parts of the world. It belongs to the *Triticum* family, of which there are many thousands of species (Kent & Evers 1994).It is grown as both a winter and aspring cereal and, owing to the number of species and varieties and their adaptability, It is grown in many

countries around the world. The great wheat-producing countries of the world include the USA, China and Russia; extensive wheat growing occurs in India, Pakistan, the European Union (EU), Canada, Argentina and Australia. It is estimated that 556.4 million tons of wheat have been produced in 2003, accounting for 30% of the world's cereal production (FAO 2003).

Wheat plants are largely susceptible to various pests and diseases *al*. Seeds from the time of their inception at flowering of the parent plants until they germinate and develop into seedlings, are prone to attack by various microbial agents (Harman,1983). Fungi associated with seeds prove to be hazardous for the seed itself, or the new plant created from it. The associated fungi may be pathogenic, weak parasites or saprophytes. The study of seed-borne fungi is very important to determine the health of grains and to protect them from seed-borne pathogens.

Rice (*Oryza sativ*) is an important crop, forming a staple food for many of the world's population, especially those living in Asia. Rice is produced mainly for use as human food, including breakfast cereals, and in Japan it is also used to brew sake (Kent & Evers 1994). There is a huge number of rice varieties (100 000) but only a few are grown widely (*e.g.* Varieties of the improved semi-dwarf plant type with erect leaves (Bender & Bender1999). Maize*(Zea mays* L.), also referred to as corn, originated in the Western Hemisphere (Fast & Caldwell 2000). It is cheap form of starch and is a major energy source for animal feed (Macrae *et al*., 1993).

The maize kernel (the reproductive seed of the plant) has four main parts – the germ, the endosperm, the pericarp and the tip cap. Production in the USA exceeds that in any other country (Fast & Caldwell 2000) and much research has been done in the USA on the maize genome (see section 4.2 for more on genetic modification).

Barley (*Hordeum vulgare*), is a resilient plant, tolerant of a range of conditions, which may have been cultivated since 15000BC(Fast & Caldwell 2000). Cultivated barley is mainly grown for animal feed, especially for pigs,

malting and brewing in the manufacture of beer and for distilling in whisky manufacture. A small amount of barley is used for food. Pearled barley is eaten in soups and stews in the UK and in the Far and Middle East; Barley is also used in bread (as flour) and grounds porridge in some countries (Kent & Evers 1994).Stored barley grains are subjected to infection by many species of microorganisms. Although there are a few species of bacteria and yeasts that can infect the stored barley grains, the main storage microorganisms are species of fungi. Therefore, the present study aim to detect and identify seed borne fungi associated with seed of cereals grain (viz Wheat, Rice, Barely and Maize) in Khartoum Estate Grains Methods.

CHAPTER TWO

LITERATURE REVIEW

Cereals are the edible seeds or grains of the grass family, Gramineae. A number of cereals are grown in different countries, including rye, oats, barley, maize, triticale, millet and sorghum. On a worldwide basis, wheat and rice are the most important crops, accounting for over 50% of the world's cereal production. All of the cereals share some structural similarities and consist of an embryo (or germ), which contains the genetic material for a new plant, and an endosperm, which is packed with starch grains.

2.1 Wheat

2.1.1 Classification

Cultivated wheat (*Triticum* sp L.) belongs to genus *Triticum* in sub class Commelinidae*, order* Poales of family Poaceae. The cultivation of wheat (*Triticum* sp.) reaches far back into history. The most archaeological records show that agriculture began around 10.000 B.C. where Iraqi people settled into villages near the Tigris and in the regions known as the Fertile Crescent (Lev-Yadun *et al*., 2000;). Wheat was one of the first domesticated food crops and for 8000 years has been the basic staple food of the major civilization of the world (Tanno and Wilcox, 2006). Its production leads all crops, including rice, maize and potatoes and is grown in almost all the temperate and subtropical regions of the world (Agrawal and Sinclair, 1996). Wheat plants are largely susceptible to various pests and diseases Seeds from the time of their inception at flowering of the parent plants until they germinate and develop into seedlings, are prone to attack by various microbial agents (Harman,1983).

Fungi associated with seeds prove to be hazardous for the seed itself, or the new plant created from it. The associated fungi may be pathogenic, weak

parasites or saprophytes. The study of seed-borne fungi is very important to determine the health of grains and to protect them from seed-borne pathogens

Wheat is affected by a range of fungal seed-borne pathogens including wheat bunt (*Tilletia* spp.), Septoria seedling blight (*Phaeospher ianodorum*), *Fusarium* seedling blight (*Microdoch iumnivale*), loose smut (*Ustilago tritici*), foliar diseases such as leaf blotch (*Septoriatritici*, Teleomorph: *Mycosphaerell agraminicola*), yellow rust (*Pucciniastriiformis*), root rots (*Fusarium culmorum, F.graminearum*) and Fusarium root crown and foot rots (*Fusarium* spp.), black stem rust (*Pucciniagraminisf. sp. tritici*), Fusarium head blight (*Fusarium* spp.) and root rot and black point of wheat caused by *Bipolarissorokiniana*, teleomorph: *Cochliobolussativus* (Acharya *et al*., 2011).

More concern was focused on wheat smut diseases caused by *Tilletia*s spp, their dissemination and control (Hassan, 2006; Hassan and Shams-Allah (2010); Hassan *etal*.2010b). Rust disease caused by *Pucciniarecondita* was also investigated (Hassan *et al*.2010a). *Fusarium* species (*F.graminearum* and *F.pseudograminearum*) causing crown rot disease was also reported by Root rot disease in wheat caused by *Helminthosporium sativum* (current name:*Bipolarissorokiniana*) and its control was also investigated reported for the first time 8 fungal species on durum and soft wheat grains from Iraq. Wheat is generally not classed by variety. Instead classes are used, based on the time of year the wheat is grown and the milling and baking quality of the flour produced. Within each class there is a group of different varieties of wheat with similar characteristics. Most of the wheat produced is used for human consumption and because of its unique properties, a large range of ingredients and foods are produced, including wheat germ, spelt (a coarse type of wheat), couscous, cracked wheat

As a food crop, wheat ranks second to sorghum. Present annual consumption is around one million tons and estimated per capita

consumption is 33 kg per annum. During the sixties, wheat production was confined to Northern Sudan where an area of 30,000 feddans was cultivated. Wheat has expanded over years to other parts of the country and its area reached 800,000 feddans during the early nineties. Recent changes in agricultural policies have resulted in a sharp decline in the area under wheat, whereby in the year 2000 this area was in the vicinity of only 200,000 feddans.

2.2 Barley

2.2.1 Scientific Classification and Etymology

Kingdom: Plantae, Subkingdom:

Tracheobionta, Superdivision Spermatophyta, Division Magnoliophyta, Class: Liliopsida, Subclass Commelinidae, Order Cyperales, Family Poaceae, Genus: *Hordeum* Species *vulgar*

Barley (*Hordeumvulgare* L.) one of the most important cereal crops in the world. It is widely grown fourth cereal and among top ten crop plants in the world. Barley was mainlycultivated and used for human food supply in the last century but nowadays it is significantly grown as animal feed, malt products and human food respectively. In addition, barley is very well known as a model crop for plant breeding methodology, genetics, cytogenetics, pathology, virology and biotechnology studies (Hockett and Nilan 1985;).Barley is mainly produced in unfavorable climate and soil conditions of the world. Wide adaptation to these conditions mentioned above, versatile utility mainly for animal feed and food and superiority for malt and beer industry as a raw material are the main reasons that enable barley to be commonly cultivated crop plant over centuries. Barley is cultivated in highly diverse regions of the world from 330 m below sea level near the Dead Sea in the Middle East up to 4200 m on Atipano and the Andes in Bolivia. Fertile Crescent of the Middle East consisting of Turkey, Iran, Iraq and Lebanon has

been reported as original area of cultivation and most likely origin of barley, the most ancient crop of cereals (Harlan, 1979). According to the excavations, barley was domesticated in the Nile River Valley of Egypt at least 17.000 year ago (Wendorf *et al*., 1979)

2.2.2 Socio economic impact of the crop

Barley is very important cereal in terms of 132 million tons production, 55 million ha acreage and 2.4 t/ha yield in the world. Barley production is generally and drastically affected by environmental and seasonal conditions. Considering the reasons, production, acreage and yield data are reported below as a-three year average. It is clearly seen from that nearly 74% of world barley production is met by ten leading countries during the last three year period (1998).The barley head or spike is made up of spikelet's, which are attached to the rachis in an alternating pattern.

The outer layers of the barley kernel consist of a husk, completely covering the grain; the pericarp (to which the husk is tightly joined in most species); the testa or seed coat and the aleurone.

2.2.3 Post-Production Operations

2.2.3.1 Pre-harvest Operations

Physiological maturity, 10 or 15 days are required to harvest barley with combine intemperate dry lands. If this duration is exceeded, crop will get too dry and then cause shattering at harvest. Harvesting time should be decided when barley stem becomes dry enough to be broken by hand easily in semi-arid and arid areas. In humid regions seed moisture and hardness should be checked before deciding harvest by using teeth or using moisture meter.

In some areas rainfalls may force to postpone the harvest, but harvest before rainfall should be preferred, as seed after drying following rainfall may be discolored. In addition, delayed harvest can lead to yield losses. Klinner and Bigger (1972) found that yield loss of barley increased from 3.5% to 9.5 % as a result of delay in harvest date in the same location but loss increase was very low with wheat crop.

In humid or irrigated areas generally six-row and logging resistant varieties should be chosen.

2.2.3.2 Storage microorganisms

Stored barley grains are subjected to infection by many species of micro organisms. Although there are a few species of bacteria and yeasts that can infect the stored barley grains, the main storage microorganisms are species of fungi.

The most important fungal species causing spoilage of barley in storage belong to the genus of *Aspergillus* and *Penicillium*. In general *Aspergillus* species can be adapted to conditions without free water and can grow at lower humidity R.H.70% (Dube, 1990) whereas *Penicillium* species are abundant mainly in grains with high moisture content stored at lower temperatures. Similar to *Penicillium* spp., species of *Rhizopus*, *Mucor* and *Nigrospora*c an also invade the high moisture grains before or during the storage (Sauer *et al.*, 1992). There are many other less important species of fungi that can be isolated from barley grains stored under unfavorable conditions. However, only the species of *Aspergillus*, *Penicillium* and *Alternaria*were indicated to be significant.

The means and time of invasion of the grains by storage fungi are significant for the establishment of management strategies. In general, it is considered that the wet weather conditions near the harvest time would favor invasion of grains by storage fungi. However, found no storage fungi growing from the surface sterilized barley seeds collected from barley fields of Minnesota in a

wet and showery season. Sauer (1992) reviewed the studies on time of invasion of grains by storage fungi and indicated that the fungi causing damage to grains in storage do not invade the grains to any significant degree or extent before harvest. Therefore it may be concluded that the storage fungi contaminate the grains during or after harvest, as the conidia of *Aspergillus* and *Penicillium* species are present in the air. Here, the procedures and conditions during harvest, transportation andstorage determine the extent of the invasion of grains by storage fungi.

Aspergillus and *Penicillium* species may be seen worldwide, but *Aspergillus* spp. is more of a problem in tropical countries while *Penicillium* spp. species are more abundant in tropical countries . However, their occurrence on barley grains is not limited to geographical regions and they occur in all parts of the world providing the favorable storage conditions. The limiting factors for their occurrence and severity are mainly crop husbandry practices, quality and moisture of grains and Characteristics of storage facilities.

2.3 Maize

2.3.1 Classification

Kingdom: Planta, Division: Magnoliophyta,Class: Liliopsida

Order: Poales,Family: Poaceae, Genus : Zea ,Species Zea mays

2.3.2 General Description, Cultivation, Use as a Crop Plant and Hybrid Production

Maize grains are vulnerable to attack by several genera of fungi such as *Fusarium, Aspergillus, Penicillium and Helminthosporium* from ripening, through harvest and storage. These fungi that play an important role in deterioration can be divided into field and storage fungi. Based on their occurrence on cereal grains, field fungi are those contaminate or invade grains in the field, often during or after ripening, and during harvesting operations. These include genera such as Fusarium, Alternaria and Helminthosporium on

grains. Storage fungi that develop on grains during storage commonly fall into two genera, i.e. Aspergillus and Penicillium; field fungi can increase on grains under exceptional moist storage conditions.

*Zea*is a genus of the family *Graminae*(*Poaceae*), commonly known as the grass family. Maize (*Z. mays* L.) is a tall, monecious annual grass with overlapping sheaths and broad conspicuouslydistichous blades. Plants have staminate spikelets in long spike-like racemes that form largespreading terminal panicles (tassels) and pistillate inflorescences in the leaf axils, in which the spikelets occur in 8 to 16 rows, approximately 30 long, on a thickened, almost woody axis (cob).The whole structure (ear) is enclosed in numerous large fallacious bracts and a mass of long styles (silks) protrude from the tip as a mass of silky threads (Hitchcock and Chase, 1971).

Pollen is produced entirely in the staminate inflorescence and eggs, entirely in the pistillateinflorescence. Maize is wind pollinated and both self and cross pollination are usually possible.

Shed pollen usually remains viable for 10 to 30 minutes, but can remain viable for longer durations under favorable conditions.

Cultivated maize is presumed to have been derived from teosinte (*Z. mexicana*) and is thought to have been introduced into the old world in the sixteenth century. Maize is cultivated worldwide and represents a staple food for a significant proportion of the world's population. No significant native toxins are reported to be associated with the genus *Zea*(International Food Biotechnology Council, 1990).

Maize has been cultivated by the indigenous peoples of North America, including Canada, for thousands of years. The modern era of maize hybrid production in Canada began in the U.S. where research conducted in the early part of this century proved that hybrid maize could produce a yield superior to open-pollinated varieties (Sprague and Eberhart, 1977). Gradually, hybrid-derived varieties replaced the open-pollinated types in Canada in the 1930's

and 1940's. In Canada, maize is grown primarily in Ontario and Quebec. Farmers must purchase new supplies of hybrid seed each season.

Maize is planted when soil temperatures are warm (greater than or equal to 10/ C) usually early to mid-May in southern Ontario (OMAF, 1994) and Quebec (MAPAQ, 1984). Optimum yields occur when the appropriate hybrid maturity and population density are chosen. In addition, exogenous sources of nitrogen fertilizer are generally applied and weed and insect control measures are generally recommended. Choice of the appropriate hybrid for the intended growing area helps to ensure that the crop will mature before frost halts the growth of the plant at the end of the season; hybrids are categorized according to the amount of "heat units" that will be required for maturity. The maize-growing areas of Canada are illustrated on maps that indicate the number of heat units that they receive (e.g., OMAF, 1994; MAPAQ, 1984). Therefore, a hybrid developed for a specific heat unit zone, will not mature in (cooler) areas that receive fewer" heat units". Traditional cultivation practices in maize often result in bare soil' which is susceptible to erosion by wind or water; increasingly, "no till" maize is being grown in an effort to reduce this soil loss. For more information on specific cultivation practices, please contact the appropriate Canadian provincial agricultural authorities.

In 1993, there were more than 24 million ha planted to the crop in North America. Maize grown in North America is predominantly of the yellow dent type, a commodity crop largely used to feed domestic animals, either as grain or silage. The remainder of the crop is exported or processed by wet or dry milling to yield products such as high fructose maize syrup and starch or oil, grits and flour. These processed products are used extensively in the food industry, for example, maize starch serves as a raw material for an array of processed foods, and in industrial manufacturing processes. Since the early 1980's a significant amount of grain has also been used for fuel ethanol production. The by-products from these processes are often used in animal feeds.

For a full discussion of the uses of maize see .The production of hybrid seed requires the development and maintenance of inbred lines and subsequent controlled crosses to produce commercial seed. Self-pollination is essential for inbred development while controlled cross pollination is mandatory for hybrid seed production.

Mechanisms have been developed to ensure the correct form of pollination for each process and to prevent genetic contamination of seed stocks (Wych, 1988).

In breeding nurseries, receptive ear shoots are protected from unwanted pollination by ear shoot bags that cover the silks. Pollen is contained and collected in bags that cover the tassels.

Controlled hand pollinations are then made by exposing the ear shoot on the selected female parent and covering it with the bag containing pollen from the selected male parent.

Breeder or foundation seed is produced from self pollinated seed after the eighth or ninth generation of inbreeding. A high degree of self-pollination is assured by planting in blocks that are isolated by a distance of at least 200 meters from any other contaminating source of pollen.

Hybrid seed production is accomplished by in terplanting rows of the male and female in bred parents (e.g., one row of male to four female rows). Hybrid seed production requires isolation similar to that for foundation seed. Self pollination of the female parent is prevented through

detasseling prior to pollen shed or by the use of male sterile females.

Genetic conformity of inbreds and hybrids is monitored and assured through grow-outs of representative seed lots and laboratory screening using such criteria as is ozyme profiles. The most important fungal species causing spoilage of barley in storage belong to the genus of *Aspergillus* and *Penicillium*. In general *Aspergillus* species can be adapted to conditions without free water and can grow at lower humidity R.H.70% (Dube, 1990)

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Diseases detected in zea maize seeds were grains are vulnerable to attack by several genera of fungi such as Fusarium, Aspergillus, Penicillium and Helminthosporium from ripening, through harvest and storage. These fungi that play an important role in deterioration can be divided into field and storage fungi. Based on their occurrence on cereal grains, field fungi are those contaminate or invade grains in the field, often during or after ripening, and during harvesting operations. These include genera such as Fusarium, Alternaria and Helminthosporium on grains. Storage fungi that develop on grainsduring storage commonly fall into two genera, i.e. Aspergillus and Penicillium; field fungi can increase on grains under exceptional moist storage conditions.

2.3.3 The Centers of Origin of the Species

It is generally agreed that teosinte (*Z. mexicana*) is an ancestor of maize, although opinions vary as to whether maize is a domesticated version of teosinte, (Galinat, 1988).

Teosinte is an ancient wild grass found in Mexico and Guatemala. Because it has differentiated into various races, species and plant habits, taxonomic classification is still a matter of controversy. classified the annual teosintesinto two subspecies of *Z. mays*: ssp. *mexicana*(including races Chalco, Central Plateau andNobogame) and ssp. *parviglumis*-var. *parviglumis*(race Balsas) and var. *huehuetenan gensis* (race Huehuetenango) and the species *Z. luxurians*(race Guatemala). The perennial teosintesfrom Jalisco, Mexico are separated into two more species according to ploidy, *Z.*

*perennis*and *Z.diploperennis*. Although there are hundreds of different varieties, the four main categories of commercial importance are:

(1) Dent maize (identified by the dent in the crown of the kernel);

(2) Flint maize (hard, round kernels);

(3) Sweet corn (a dent-type maize);

(4) Popcorn (flint-type maize which expands when heated).

2.4 Rice

2.4.1Classification

Kingdom : Plantae Division :Magnoliophyta, Class: Liliopsida

Order : Poales, Family : Gramineae o Poaceae,Tribe : Oryzeae

Genus: *Oryzasativa*

Rice (*Oryza sativa* L.) is a plant belonging to the family of grasses, Gramineae (Poaceae). It is one of the three major food crops of the world and forms the staple diet of about half of the world's population.

The global production of rice has been estimated to be at the level of 650 million tones and the are aunder rice cultivation is estimated at 156 million hectares . Asia is the leader in rice production accounting for about 90% of the world's production. Over 75% of the world supply is consumed by people in Asian countries and thus rice is of immense importance to food security of Asia. The demand for rice is expected to increase further in view of expected increase in the population. India has a long history of rice cultivation. Globally, it stands first in rice area and second in rice production, after China. It contributes 21.5 percent of global rice production. Within the country, rice occupies one quarter of the total cropped area, contributes about 40 to 43 percent of total food grain production and continues to play a vital role in the national food and livelihood security system. India is one of the leading exporter of rice, particularly basmati rice. *O. sativa* has many ecotypes or cultivars adopted to various environmental conditions.

The morphology, physiology, agronomy, genetics and biochemistry of *O. sativa* have been intensely studied over a long time. More than 40,000 varieties of rice had been reported worldwide.

Crop improvement research in case of rice had been started more than a century back. Extensive adoption of higher yielding varieties has enabled many countries in Asia to achieve sustained self-sufficiency in food.

In India rice is grown under four ecosystems: irrigated, rainfed lowland, rain fed upland and flood prone.

More than half of the rice area (55%) is rainfed and distribution wise 80% of the rainfed rice areas are in eastern India, making its cultivation vulnerable to vagaries of monsoon. Rice is a nutritious cereal crop, used mainly for human consumption. It is the main source of energy and

is an important source of protein providing substantial amounts of the recommended nutrient intake of zinc and niacin. Rice protein is biologically the richest by virtue of its high true digestibility (88%) among cereal proteins and also provides minerals and fibre. Calories from rice are particularly important for the poor accounting for 50-80% of the daily caloric intake. Rice can also be used in cereals, snack foods, brewed beverages, flour, oil (rice bran oil), syrup and religious ceremonies to name a few other uses. Rice is also believed to have medicinal properties and used in many countries for the same including in India.

Rice is classified primarily based on its grain size and shape. Uniform standard grain classification is used in India for grouping the varieties into 5 groups based on the length/length-breadth ratio of the kernel.

This classification has been developed by the Ramaiah Committee in 1965 which was appointed by Government of India. Similar international classification has also been developed by International Rice

Research Institute (IRRI), Philippines which also takes into consideration the grain length-width (breadth)ratio. As per Indian classification, rice varieties

are grouped as Long Slender and Long Bold where the length is 6 mm and above and the length-breadth ratio is either 3 and above or less than 3 respectively. Likewise the varieties which are classified as Short Slender and Short Bold where the length is less than 6mm and the length-breadth ratio is more than 3 or less than 2.5 respectively. There is a Medium Slender category which has a grain length of less than 6 mm and the length-breadth ratio between 2.5 to 3.Amylose content varies from 2% to more than 25% and varieties with low (2-19%), intermediate (20-25%) and high (>25%) amylose content are available in all grain types. However, only in case of japonicas, short bold or round grains in general have only low (<20%) and very low (2-8%) amylose content. Husk, bran and broken rice are the by-products of the rice milling industries. These by-products can be used in better and profitable manner both for industrial human and animal consumption. Rice husk constitutes the largest by-product of rice milling and one fifth of the paddy by weight consists of rice husk. Rice husk has a considerable fuel value for a variety of possible industrial uses. Hence, the major use of husk at the moment is as boiler fuel. Rice husk is also a rich source of silica. Rice bran is the most valuable by-product of the rice milling industry. It is obtained from the outer layers of the brown rice during milling. Rice bran consists of pericarp, aleurone layer, germ and a part of endosperm. Rice bran can be utilized in various ways. It is a potential source of vegetable oil, feed, fertilizers etc. Rice bran oil is one of the healthiest oil for human consumption most of these infectious problems are seed-borne in nature which causes enormous losses both in storage as well as in the field. These pathogens are known to cause damage at different stages like storage, seed germination, seedling establishment, vegetative growth and reproductive phase. The infected seeds may fail to germinate, transmit disease from seed to seedling and from seedling to growing plants (Fakir *et al.* 2002). Most seed borne diseases like brown leaf spot, rice blast, stem rot and bacterial leaf blight are caused by the pathogens like Drechsleraoryzae, Fusariummoniliforme,

Pyriculariaoryzae, Rhizoctoniasolani, Sarocladiumoryzae, Sclerotiumoryzae, Trichoconiellapadwickii and Xanthomonascompestrispvoryzae (Khan *et al*. 1990, Gill *et al*.
1999),

2.4.2Geographic Origin

The centre of origin and centres of diversity of two cultivated species *O. sativa* and *O. glaberrima* have been identified using genetic diversity, historical and archaeological evidences and geographical distribution.

It is generally agreed that river valleys of Yangtze, Mekonrivers could be the primary centres of origin of *O. sativa* while Delta of Niger River in Africa as the primary centre of origin of *O. glaberrima*. The foothills of the Himalayas, Chhattisgarh, Jeypore Tract of Orissa, northeastern India, northern parts of Myanmar and Thailand, Yunnan Province of China etc., are some of the centres of diversity for Asian cultigens. The Inner delta of Niger River and some areas around Guinean coast of the Africa are considered to be centre of diversity of the African species of *O. glaberrima* (Chang, 1976; Oka, 1988).

O. sativa and *O. glaberrima*are believed to have evolved independently from two different progenitors, viz.

*O. nivara*and *O. barthii* and they are believed to be domesticated in South or South East Asia and tropical West Africa respectively. The progenitors of *O. sativa* are considered to be the Asian AA genome diploid species and those of *O. glaberrima*to be African AA genome diploid species *O. barthii* and *O. longistaminata* as indicated in various reviews by Chang, 1976 *O. sativa*, is considered to have occurred in 7,000 BC . It has spread and diversified to form two ecological groups, Indica and Japonica (Oka, 1988). There are other studies indicating that the two groups were derived independently from the domestication of two divergent wild rices in China and India, respectively (Second, 1982; 1986).The rice grain consists of an outer protective coating (referred to as the hull or husk) and the edible rice caryopsis.

Brown rice consists of the outer layers of pericarp (which contains pigment), seed coat, the embryo and the endosperm (comprising the aleurone layer which encloses the embryo, subaleurone layer and the starchy or inner endosperm).

2.4.3Fungal diseases of Rice

2.4.3 .1 Rice Blast [*Magnaporthegrisea (Pyriculariaoryzae)*]

Blast is caused by the fungus *Magnaporthegrisea*(*Pyricularia oryzae)*. Blast fungus can infest any organ of the plant. Young seedlings, leaves, panicles and other aerial parts of the adult plant are affected and so often called as leaf blast, neck blast, or panicle blast. The fungus produces spots orlesions on leaves, nodes, panicles, and collar of the flag leaves. Leaf spots are of spindle-shaped with brown or reddishbrownmargins, ashy centers, and pointed ends. Infection of panicle base causes rotten neck or neck rot and causes the panicle to fall off.

2.4.3.2 Sheath Blight (*Rhizoctoniasolani*)

Sheath blight of another major fungal disease in rice caused by *Rhizoctoniasolani***.** Symptoms become apparent attillering or flowering stage and affect all plant parts above water line. Spots or lesions first develop near the water level(in flooded fields) or soil (in upland fields) and spots initially appear on the leaf sheath. Spots may be oval or ellipsoidal and measure 1-3 cm long. Lesions on the leaf blade are usually irregular and banded with green, brown, and orange coloration. When several such lesions are continuous on a greenish tissue, it almost looks like a snake skin from a distance, so it is also known as snake skin disease.

2.4.3.3 False Smut (*Ustilaginoideavirens)*

False smut is caused by *Ustilaginoideavirens* and is characterized by large orange to olive-green fruiting structure son one or more grains of the mature panicle. The symptoms of false smut are visible only after flowering. The pathogen grows in the ovary and transforms it into large, yellowish and

velvety green balls, which become greatly enlarged at later stage. The spore balls are covered by a membrane in the early stages, which bursts with further growth and the loose velvety pseudo morphs become visible.

2.4.3.4 Brown Spot *(Bipolarisoryzae)*

The disease symptoms of brown spot causes by *Bipolarisoryzae*are seen on leaves and glumes of maturing plants. Symptoms also appear on young seedlings and the panicle branches in older plants. Brown leaf spot is a seed-borne disease. The fungus causes brown, circular to oval spots on the leaves. Leaf spots may be evident shortly after seedling emergence and continue to develop until maturity.

2.4.3.5. Black Sheath Rot (*Gaeumannomycesgraminis)*

Gaeumannomycesgraminis attacks the crown, lower leaf sheaths, and roots of the rice plant causing a dark brown to black discoloration of the leaf sheaths from the crown to considerably above the water line. As the discolored, infected sheaths decay, tiny, black the fungal reproductive structures (perithecia) form within the tissue. The disease is usually observed late in the main crop season and may cause reduced tillering, poor grain fill, and lodging.

2.4.3.6 Bakanae Disease (*Gibberellafujikuroi)*

Gibberellafujikuroi causes bakanae disease in which infected plants become several inches taller than normal plants in seedbed and field. The plants become thin with yellowish green leaves and pale green flag leaves. Dying of the seedlings occurs at early tillering reduced tillering and drying leaves at late infection occurs.

The genera *Aspergillussensulato* and *Penicilliumsensulato* containa high number of very diverse species. These species produce a large number of exometabolites, also known as secondary metabolites.

Exometabolites are small molecules produced during morphological and chemical differentiation that are outward directed, i.e., secreted or deposited in or on the cell wall, and accumulated in contrast to endometabolites

(primary metabolites), that are fluctuating in concentration (the fluxome), and either transformed into other end ometabolites or feeding into exometabolites, exoproteins, exopolysaccharides, and morphological structures.

While endometabolites can be found in almost all species of fungi(and most other kinds of organisms), exometabolites, exoproteins, and exopolysaccharides are taxonomically restricted, being produced in species-specific profiles. Some metabolites can occur both as endo- and exometabolites, for example citric acid. When citric acid is part of the mitochondrial fluxome, it should be regarded as an end ometabolite, but when citric acid is secreted and accumulated (Andersen et al., 201) as in *Aspergillusniger*, it must be regarded as an exometabolite. Accumulation of citric acid requires that there is a reductive pathway for it in the cytosol and that it canbe secreted to the surroundings via an exporter. Thus the transport from the mitochondria to the cytosol, the cytosolic reduction, and the secretion requires a dedicated gene cluster. Such a gene cluster has been found in for example *A. terreus* that is codingfor accumulating and secreting itaconic acid (Van der Straat *et al*., 2014), but the gene cluster for citric acid accumulation has not been described yet. Some species related to *Aspergillus* and *Penicillium*, such as *Xeromycesbisporus*, (Leong et al., 2014). In *Aspergillus* most species produce a large number of exometabolites, but some stress selected species, such as *A. penicillioide s*and *A. restrictus*, have only been reported to produce asperglaucide and cristatinA,and the related arestrictin A and B (Itabashi *et al*., 2006). However,the closely related xerotolerant/xerophilic species in the *Aspergillus* subgenus *Aspergillus* (formerly *Eurotium*) produce a high number of exometabolites in the ascomata, making them chemically very diverse (Slack *et al*., 2009).

Species of the genus *Alternaria*are common field fungi, including both saprotrophytic and plant pathogenicspecies that may affect cereal, vegetables and fruit crops in the field or cause postharvest decay (6, 24, 40). Additionally, some *Alternaria*species have a hightoxigenic potential as they

are capable of producing toxic secondary metabolitescalledmycotoxins (24, 45) which do humans or animals unavoidably ingest.

The most known mycotoxinsarealternariols, altenuene, altertoxins, and tenuazonic acid (17, 19) which are considered as a potential cause of many cancers (5, 8, 35).

The genus *Alternaria* was originally described by Nees (28),including nearly 100 species of dematiaceoushyphomycetes (11). The taxonomy of *Alternaria* is primarily based on the morphology and development of conidia and conidiophores, and to a lesser degree on host plant association. Indeed, morphology is still the most reliable method to identify *Alternaria* at the species level, but misidentifications are known to occur because the morphological method requires a skilled specialist and takes diligence and time(2). Thus, various molecular methods have been developed to help and facilitate differentiation between *Alternaria* species. These methods include: analysis of ribosomal DNA (rDNA) sequences to establish molecular phylogenetic relationships within many groups of fungi(27, 42) or by using the mitochondrial small subunit (SSU) rDNA sequence method (18) In the light of the 2009's survey, we targeted to identify the *Alternaria* species recovered from durum wheat during harvest in the main producing cereal regions in Tunisia. Indeed, an understanding of the genetic diversity in pathogen can provide a valuable basis for designing efficient and durable disease management strategy. In this research, we amplified the ITS1-5.8S-ITS2 region of the rDNA of many Tunisian *Alternaria* isolates using the polymerase chain reaction (PCR). These rDNA fragments were then sequenced and analyzed as a way of identifying *Alternaria* species.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study location

This study was conducted in the laboratory of Plant Pathology Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology during February –April, 2016.The aim of this study was to detect and identify seed borne fungi associated with seed of cereals grain (Wheat, Rice, Bearly and Miaze) collected from three different locations in local market of Khartoum (Al hagyousif, Khartoum Bahri and Omdurman) stored four a year.

3.2 Collections of samples

The cereals seed stocks on shells were obtained from grains market seed stocks in each location(Al haguosif, Khartoum bahri and Omdurman).The maize, wheat, barely, and rice samples were obtained from Random and homogenous sample of three kilo grams was secured form each of in of the four crops the three locations according to international standers for seed testing association (ISTA, 1966). Collected samples were labeled and kept separately in sealed paper bag and transformed to the laboratory where they were stored at ±1-2^{o}c refrigerator for further investigations.

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

3.3 Detection and isolation of seed borne fungi

3.3.1 Dry seed inspection

A sample of 400 seeds of each seed sample were randomly selected and examined under microscope and by magnified lens and naked eye according to the international seed testing association (ISTA) .The samples were also examined for impurities, plant debris, weed seed, discoloration and malformation.

3.3.2Methods for the detection of seed borne fungal pathogens

The seed samples were tested by the standard filter paper and agar methods. The fungi identified and their percentage of occurrence was calculated by applying following formula:

Pf= (No. of contminated seeds on which fungus appear/Total number of seeds)x100.

All the detection methods of seed borne fungi were adopted as described by ISTA 1966. The normal and discolored seeds were tested separately for seed borne fungi.

3.3.2.1Blotter method

For the detection of seed borne fungi, standard blotter method as described by the international seed testing association (ISTA, 1996), was used. All the samples were platted on moistened filter papers in 9.0cm sterilized plastic Petri-Dishes. Five seeds were plated for each sample, three were arranged at the periphery of plate and 2 at the center, Each sample was replicated four times and then kept in dark place for seed germination after seven days of incubation, Seeds were examined for fungal growth under steromicroscope. Fungi identification by habit character was supplemented by microscopic examination of spores and fungi bodies using acompound microscope. Other

identifications were used according to methods described Agarwal *et al*., (1989); Buregers *et al*.,(1994)., Mathursk, SD Mathur, PNeergard (1975)., and Mathur . Incidence levels were recorded as the percentage of contaminated seeds.

3.3.2.2 Agar method

All seed samples was pre-treated with sodium hypochlride (NaOCl)1% for 5minutes then washed three times with sterilized distilled water(SDW) and dried between tow filter papers. The seed samples, (five for each crop)were seeded in PDA medium (Potato Dextrose Agar), in sterilized glass Petri-dishes.

The plates were incubated for seven days in incubator at±1-2 C^{o}. Then the seeds were examined under light microscopes using slides.

3.4 Slide preparation and identification

The samples of fungi were taken randomly from each crop samples. These samples were identified on the basis of colony characteristics and microscopic examinations using standard books and research paper (Aneja, 2004.,Barntt and Hunter, 1999., and Rifai.1969). The binocular compound microscope was also used to determine the type of fungus in each plate.

3.5 Data analysis

Completely randomized design was adopted in this study analysed.

All the collected data were determined by Analyses of Variance (ANOVA) using a completeiy randomized desing. The significance ($p<0.05$) of differences between treatments were determined, using the Duncan's Multiple Range test of Statistical Analysis.

CHAPTER FOUR

RESULTS

This study was conducted under laboratory conditions, during February – April, 2016 to investigate the occurrence seed borne mycoflora associated with seeds samples of four food crops collected from three local markets in Khartoum State in Sudan. The methods used in detection that Dry inspection, Blotter method and agar plate method,

4.1 Dry Seed Inspection

Dry inspection of the Cereal grains collected from different areas of Khartoum State. Discolored seeds. Besides that, impurities were found mixed with all seed samples. These were identified as dust particles, stones, pieces of straw and plant debris. However, with the exception of the few discolored seeds no disease like symptoms was observed in the dry inspected seeds. Presented in (Table 1)

Table 1. Dry inspection of grains

Crop	Healthy (%)	Contaminated (%)	Malformed (%)	Plant debris (%)	Total (%)
Maize	73	22	5	0	100
Barely	75	18	6	1	100
Wheat	70	15	9	6	100
Rice	80	10	7	3	100

4.2 Blotter method and agar plate method in maize

Four samples of cereal grain collected from seed market, fungi isolated include Aspergillus, Penicillium, Alternaia, Rhizopus.Table (2),plate (1and 2).Four fungal species (Aspergillus, Penicillum, Alternaia and Rhizopus) were detected in Miaze. The fungi Aspergillus recorded the highest fungi percent in Filter paper (28.67%) and Agar method(28.33%) Followed by Penicillium, Alternaria and Rhizopus(26.67,17.33and5.33) respectively.

Table 2. Fungal genera obtained from Maize grains collected from study area

Method	**Filter Pepper**			**Agar Method**		
Fungi	MIAZE B	MIAZE H	MIAZE O	MIAZE B	MIAZE H	MIAZE O
Aspergillus	42.00[a]	28.67[a]	22.00[a]	28.33[a]	28.33[a]	11.00[a]
Penicillum	11.67[b]	23.00[ab]	26.67[a]	15.33[a]	13.00[ab]	1.33[a]
Alternaria	17.33[b]	14.33[ab]	15.33[ab]	15.33[a]	5.67[b]	1.33[a]
Rhizopus	0.00[b]	0.00[b]	0.00[b]	5.33[a]	3.33[b]	0.67[a]
CV%	9.20	9.88	6.05	19.08	9.95	23.07
SE±	8.58	12.11	7.32	24.96	9.65	6.82

Key:

O= Omdurman

H= Al hagyousif

B= Khartoum bahri

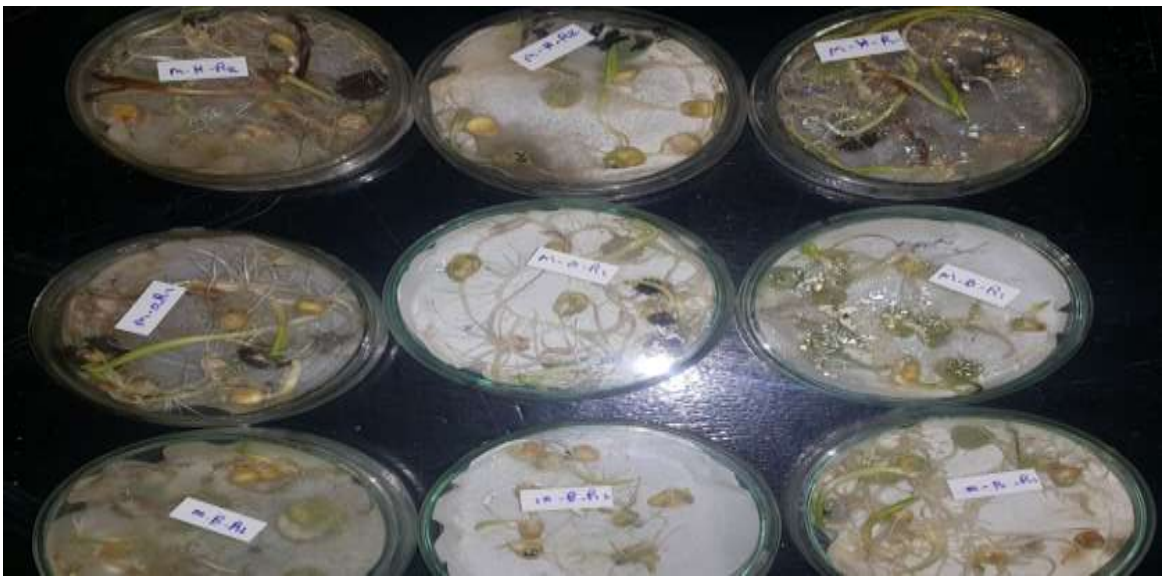

Plate 1. Seeds tested by the filterpapper for maize seeds

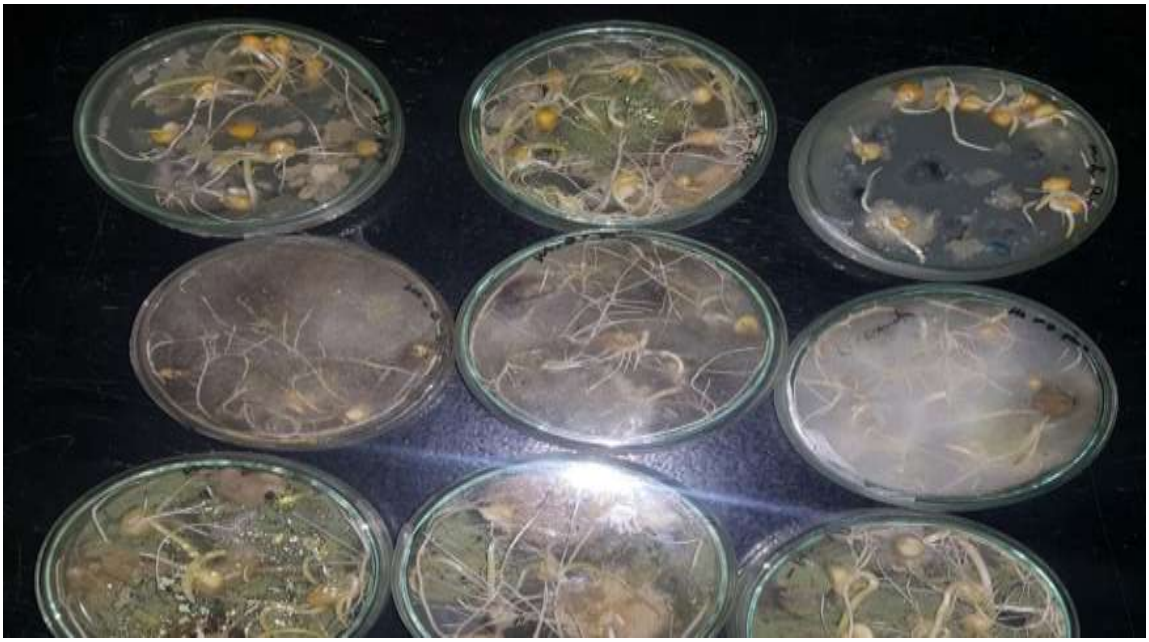

Plate 2. Seeds tested by the Blotter method for maize seeds

4.3 Detection of Fungi from wheat Seeds

Fungi species detected from wheat seeds are shown in Table (3).The most common fungi species detected from the wheat seeds is *Aspergillusniger* whether tested by the Blotter or Agar methods. Other fungi detected include Penicillium, Rhizopus and Alternaria (Plate 3 and 4)

Table 3. Fungal genera obtained from Wheat grains collected from study area

Method	**Filter Pepper**			**Agar Method**		
Fungi	**Wheat B**	**Wheat H**	**Wheath O**	**Wheat B**	**Wheat H**	**Wheath O**
Aspergillus	11.67^{a}	8.00^{a}	18.00^{a}	27.33^{a}	21.33^{a}	19.67^{a}
Penicillum	10.67^{a}	1.33^{b}	15.33^{a}	12.33^{ab}	9.00^{ab}	18.67^{ab}
Alternaria	3.67^{a}	0.33^{b}	11.00^{a}	0.33^{b}	4.33^{ab}	7.67^{ab}
Rhizopus	0.00^{a}	0.00^{b}	0.00^{a}	0.00^{b}	0.33^{b}	3.33^{b}
CV%	10.14	30.31	12.09	12.08	11.10	7.64
SE±	5.69	2.57	10.14	10.38	7.94	6.81

Key:

O=Omdurman

H=Al hagyousif

B=Khartoum bahri

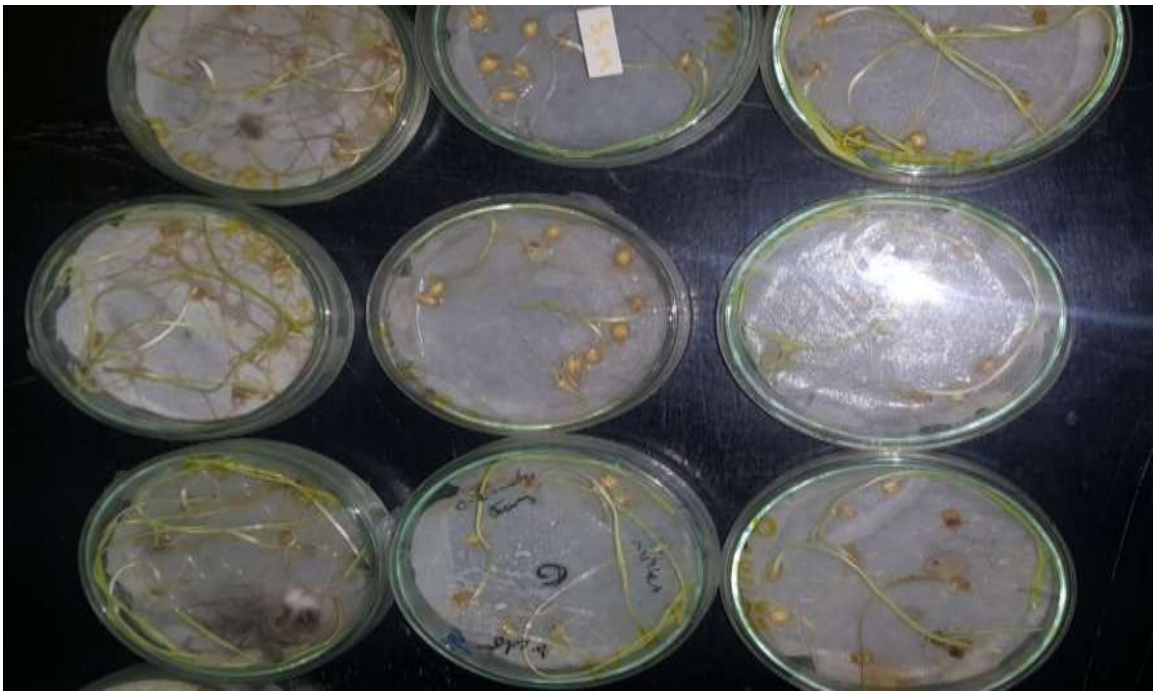

Plate 3. Seeds tested by the Blotter test for wheat seeds

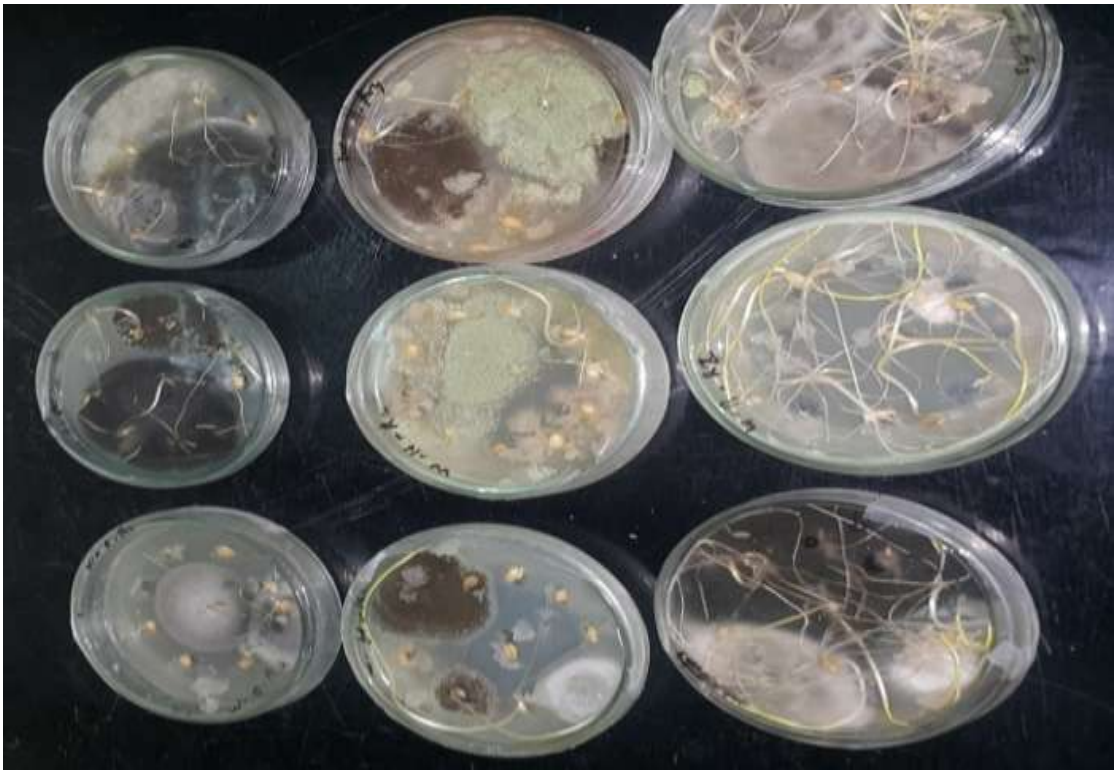

Plate 4. Seeds tested by Agar plate method for wheat seeds

4.4 Detection of Fungi from Barely Seeds:

Fungi species detected from barely seeds are shown in Table (4).The most common fungi species detected from the seeds is *Aspergillusniger* whether tested by the Blotter or Agar methods. Other fungi detected include Penicillium, Rhizopus and Alternariano significant difference between Filterpaper and Agar method. In the case of Penicillium, Rhizopus and Alternaria plate (5&6)

Table 4. Fungal genera obtained from Barely grains collected from study area

Method	Filter Pepper			Agar Method		
Fungi	**Bearly B**	**Bearly H**	**Bearly O**	**Bearly B**	**Bearly H**	**Bearly O**
Aspergillus	11.67[a]	11.33[a]	16.67[a]	33.67[a]	30.00[a]	39.00[a]
Penicillum	11.00[a]	8.00[a]	8.67[a]	2.00[b]	18.67[ab]	0.33[b]
Alternaria	4.67[a]	0.00[a]	4.33[a]	0.67[b]	0.33[b]	0.00[b]
Rhizopus	0.00[a]	0.00[a]	0.00[a]	0.00[b]	0.33[b]	0.00[b]
CV%	12.53	19.37	15.84	7.59	9.75	24.69
SE±	6.00	7.70	9.26	5.75	8.03	10.01

Key:

O=Omdurman

H=Al hagyousif

B=Khartoum bahri

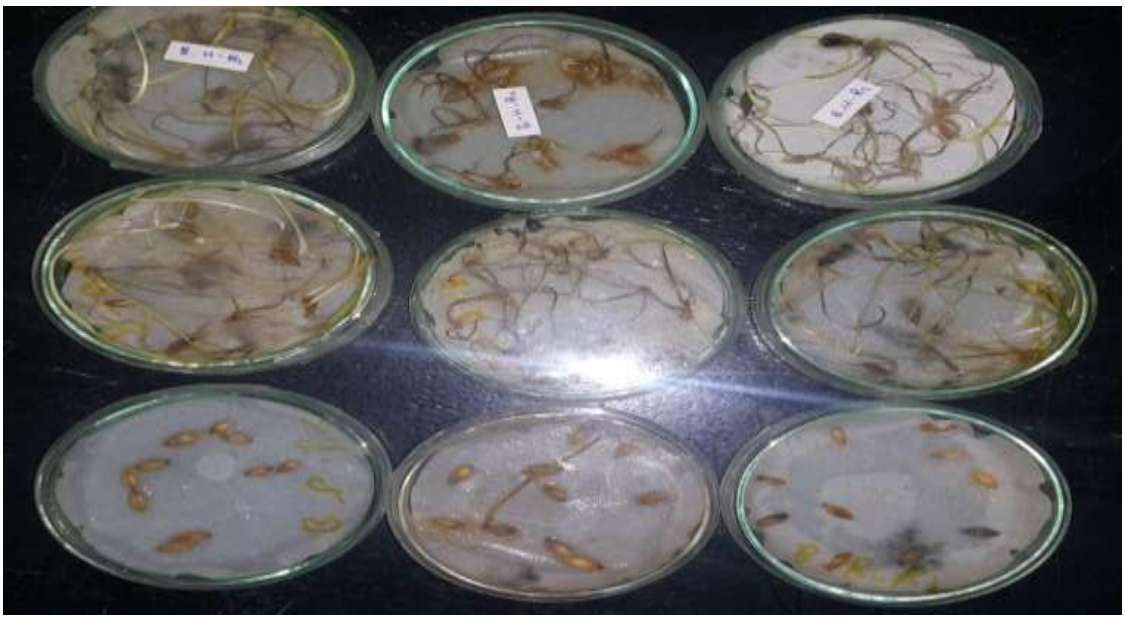

Plate 5. Blotter method for barely seeds

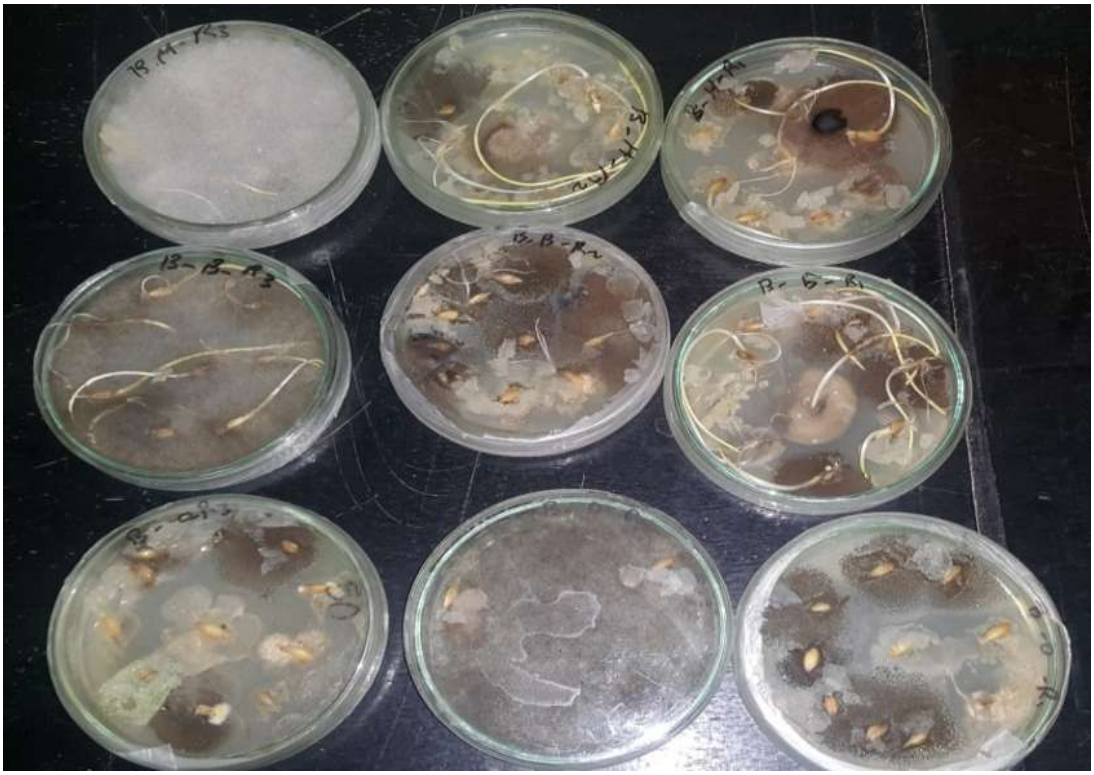

Plate 6. Seeds tested by the Agar method for barely seeds

4.5 Detection of Fungi from rice seeds

Table (5) show four fungal species (Aspergillus, Penicillum, Alternaria and Rhizopus) were detected in Rice . The fungi Aspergillus recorded the highest fungi percent in Filter papper and Agar method Followed by Penicillium, Alternaria and Rhizopus but no significant difference between fungi growth on Filter papper and Agar method(plate 7&8)

Table 5. Fungal genera obtained from Rice grains collected from study area

Method	Filter Pepper			Agar Method		
Fungi	**Rice B**	**Rice H**	**Rice O**	**Rice B**	**Rice H**	**Rice O**
Aspergillus	11.67[a]	6.00[a]	10.33[a]	1.33[a]	0.00[a]	14.00[a]
Penicillum	5.33[a]	4.67[a]	3.67[ab]	1.33[a]	0.00[a]	0.00[a]
Alternaria	0.33[a]	0.00[a]	0.33[b]	1.33[a]	0.00[a]	0.00[a]
Rhizopus	0.00[a]	0.00[a]	0.00[b]	0.00[a]	0.00[a]	0.00[a]
CV%	23.71	21.41	28.64	32.29	0.00	21.98
SE±	8.45	4.62	3.76	1.08	0.00	9.20

Key:

O=Omdurman

H=Al hagyousif

B=Khartoum bahri

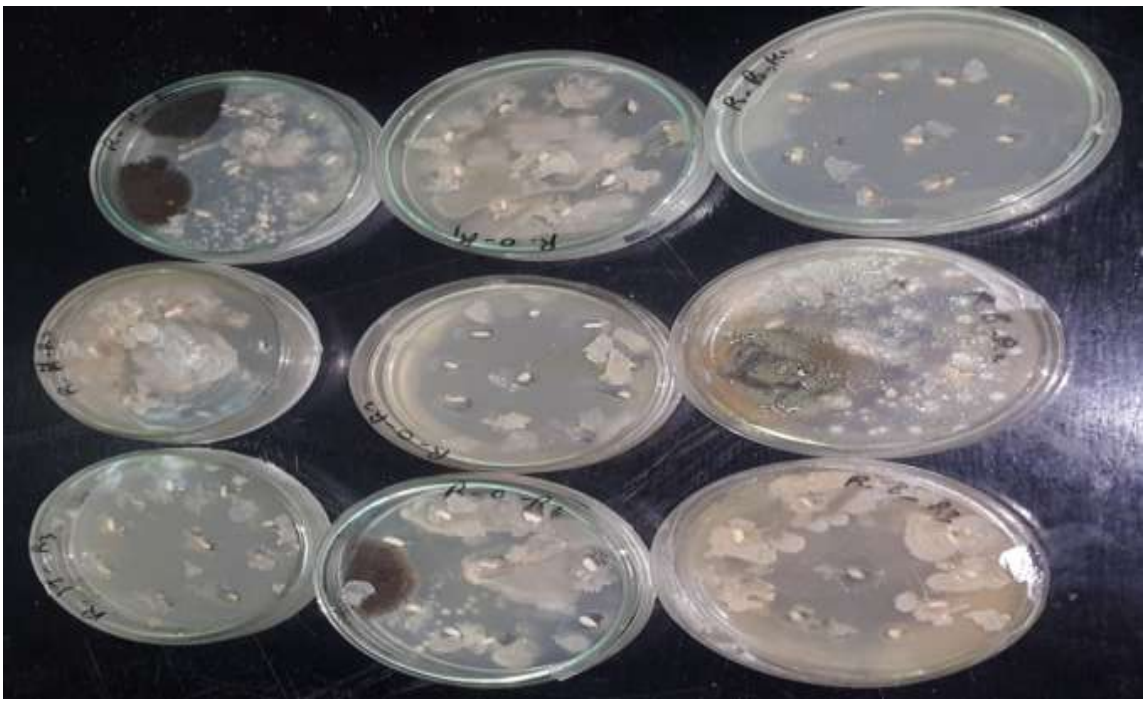

Plate 7. Seeds tested byagar method for rice seeds

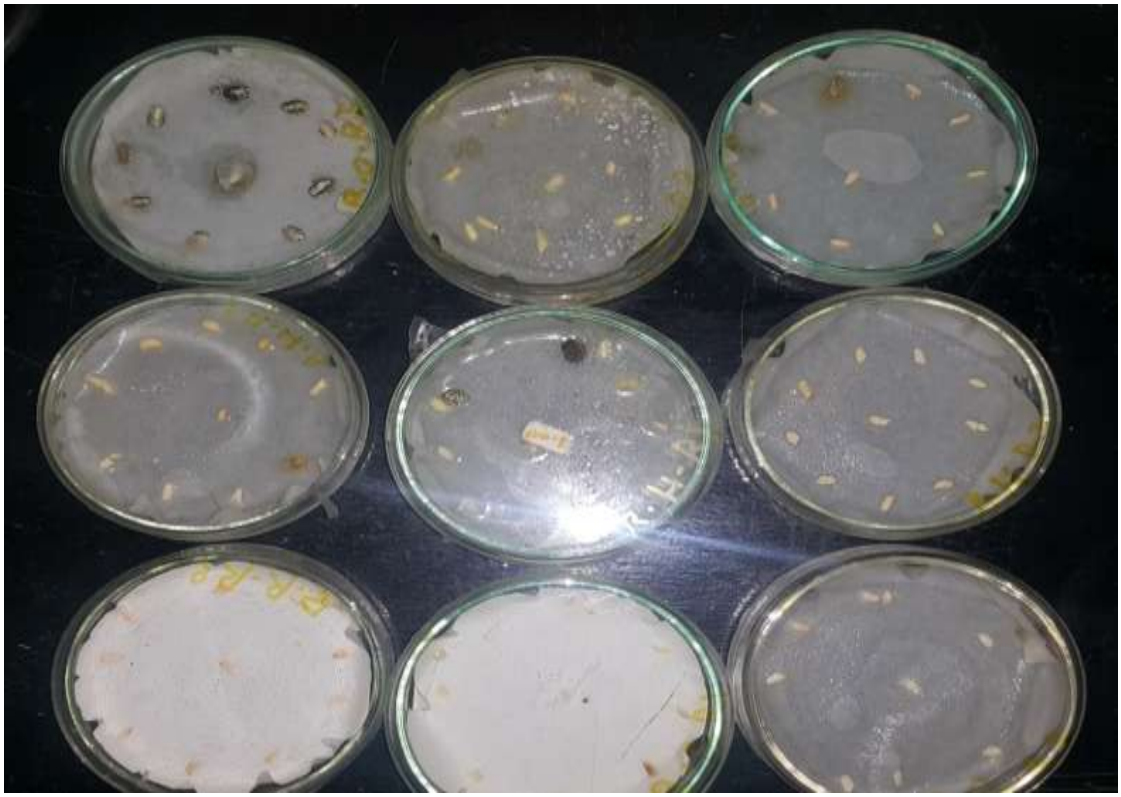

Plate 8. Seeds tested by Blotter method for rice seeds

CHAPTER FIVE

DISCUSSION

This study was conducted in the laboratory of plant pathology department of plant protection, college of agricultural studies, Sudan University of science and technology during February –April, 2016.The aim of this study was to detect and identify seed borne fungi associated with seed of cereals grain(Wheat, Rice, Bearly and Miaze) collected from three different locations in local market of Khartoum(Al hagyousif, Khartoum bahri and Omdurman) stored since season 2015.The dry inspection tests revealed the presence of a few (less than1%) discolorated seeds. In similar tests Neegaard (1977) reported that dry seeds may show symptoms in varying degrees due to necrosis or discoloration from stain produced by various seed-borne micro-organisms. The results of the present study revealed a high incidence of seed-borne Aspergillus. Niger and A. flavus. Working with the seeds of sorghum in the Sudan, Abuagla (2001)also reported high incidence of A. niger and A. flavus. Moreover, a high incidence of A. niger and A. flavus from seeds stored in high temperature. In comparison of the different methods used in the present study the Agar plate tests gave excellent mycelial growth and conidial sporulation of the fungi detected. As explained by Mrs. Awatif (Personal Communication) such results are expected as the agar media provide the necessary nutrients for the growth of the fungi. The results of the present work also revealed that beside A. nigerother less prevalent fungi include Pencilliumsp, Alternaria and Rhizopus. As previously stated by Malone and Muskett (1964) such results are expected from seeds stored under high temperature and high humidity conditions. Hence, the need for proper storing, seed health testing and seed sterilization should be a routine practice before sowing of above seeds.

Recommendations

1. In view of the abundant seed contamination reported in the present study and in other previous studies it is high time that the seed health testing should be applied before sowing both local and imported seeds.
2. It is also recommended that stores for seeds should meet the international storage conditions.
3. Seed dressing with contact and/or systemic fungicides should be a routine procedure in all the agricultural section.
4. The result obtained in this thesis showed the need for further research and investigation to provide a satisfactory explanation to these results.

References

Abuagla, S.I. (2001). Seed-borne fungi of important food crops of the Gezira Scheme, Sudan. M.Sc., Thesis, Dept. of Crop Protection, Fac. of Agric., Univ. of Khartoum, 24-69.

Acharya, K., Dutta, AK and Pradhan (2011). *Bipolarissorokiniana*(Sacc.) Shom. The most destructive wheat fungal pathogen in the warmer areas .AJCS 5(9):1064-1071.

Agarwal, P.C., Carmen, N. and Mather, S.B. (1989).Seedborne diseases and seed health testing of rice. Danish Government Institute of seed pathology for Developing Counteries, Copenhagen, Denmark. PP106.

Agrawal ,VK and Sinclair, JB. (1996). Principles of pathology .CRC press Inc. Boca Raton, pp 539

Andersen, M. R., Salazar, M. P., Schaap, P. J., van de Vondervoort, P. J. I., Culley, D., Thykaer, J. (2011). Comparative genomics of citric-acid producing *Aspergillusniger* ATCC 1015 versus enzyme-producing CBS 513.88.*Genome Res.*

Aneja KR (2004). Exeperiments in Microbiology, plant pathology and Biotechnology, Fourth edition, New International (P) limited publishers, India.121-128.

Barentt, H.M. and Hunter, B.B. (1999). IIIustratedgenraofn imperfect fungi edition. Prentice Hall Inc.

Bender D.A & Bender A.E (1999). Benders' Dictionary of Nutrition and Food *Technology*, 7th edn. Woodhead Publishing, Abington.

Bennett JW. Mycotechnology: the role of fungi in biotechnology. J Biotechnol 1998;66:101–7.

Bhutta, A. R., 1997. Comparison of cotton seed health testing method and their economics ..Pakistan Cotton, 32(3): 146-153.

Blunden, G., Roch, O.G., Rogers, D.J., Coker, R.D., and Bradburn, N. (1991).Mycotoxins in food. Med. Lab. Sci. 48: 271-282.

BNF (British Nutrition Foundation) (1994) *Starchy Foods in the Diet*.

Buregers, L.W., Summerell B.A., Bullock S., Gott K.P and Backhouse D. (1994).Laboratory Manual for Fusarium Research. University of Sydeny, 3rdEd. 133pp Boiron P.2009, Campignonstoxinogeneetmycotoxicoses. http;// WWW.ispb.univ-lyonl.fr. Consulte le 5 Janavier 2009.Punithalingam E., 1985, Description of pathogenic fungi and bacteria. Plant pathology, 55,1234.

Chang, T. T. (1976). The origin, evolution, cultivation, dissemination and diversification of Asian and African rices.*Euphytica,* 25 (1): 425-441.

Dube, D.C., 1990. An introduction to fungi.Vikas publishing house pvt ltd., 608pp.

Fakir, G. A., I. Hossain, M. U. Ahmed, M. A. U Doullah and M. Alam, (2002). Quality of farmers Boro and T. Aman rice seeds collected before sowing from Bogra, Rajshahi and Rangpur districts of Bangladesh. A paper presented in the review and planning meeting of the Rice Seed Health Improvement (SHIP), PETRRA project held on 17-18 April at BRRI, Gazipur , Bangladesh.

FAO (Food and Agriculture Organization) (2002) *World Agriculture: Towards 2015/2030.Summary Report*. FAO, Rome.

FAO (Food and Agriculture Organization) (2003) *Food Outlook. No.*

FAO Global Information and Early Warning System on Food and Agriculture. FAO, Rome.

Fast R.B & Caldwell E.F (2000). *Breakfast Cereals and How They Are Made*, 2nd edn. American Association of Cereal Chemists, St. Paul.

Galinat, W.C. 1988. The origin of corn.In G.F Sprague and J.W. Dudley, Eds. Corn and corn improvement. Agronomy Monographs No.18; pp. 1- American Society of Agronomy: Madison, Wisconsin

Gill, M. A., A. Wahid, M. S. Javed and T. Z. Khan , .(1999) Major diseases of rice crop in the Punjab and their management strategies. In: Proc. 2nd Natl. Conf. Plant Pathol., Sept. 27-29, 1999 , Univ. Agri. Faisalabad.

Harlan, J.R.1979. On the origin of barley.*In* Origin, Botany, culture, winter hardness, Genetics, Utilization, Pests. Agric. Handb. 338.US. Dept. Agr. Washington, D.C., pp. 10-36.

Harman, GE. (1983). Mechanisms of seed infection and pathogenesis. Phytopathology 73:326- 329.

Hassan, MS and Shams – Allah, SA..(2010). Dissemination of wheat covered smut in Iraq. Anbar J.Agric .Sci. 4: 469 – 473.

Hitchcock, A.S. and A. Chase. (1971). Manual of the grasses of the United States Volume 2. p. 790-796. Dover Publications: N.Y.

Hockett, E.A., and Nilan R.A.1985.Genetics.*In* Barley. D.C. Rasmusson, ed. American Society of Agronomy, Madison, WI, pp 187-230.

International Food Biotechnology Council. (1990). Biotechnologies and food: assuring the safetyof foods produced by genetic modification. Regulatory Toxicology and Pharmacology 12: S1-S196.

ISTA (1966). International Ruies of seed Testting; pros. In. seed, Test. Assoc. 32:565-589.

June 1999, Konya, Turkey, GurcanOfset Printhouse ISBN: 975-487-071-3, pp: 77-86 (in Turkish).

Kent N.L & Evers A.D (1994) *Kent's Technology of Cereals*, 4th edn. Elsevier, Oxford

Khan, S. A., S. A. Anwar, A. B. Bhutta, (1990). Studies on seed borne fungi, bacteria, and nematodes of rice in the Punjab. Pak. J. Sci. Ind.

Klinner, W.E. and G. Bigger, 1972. Some effects of harvest date and designfeatures of cutting table on the front losses of combine harvesters. *Journ.Agr. Eng. Res.* 17(1):71-78.

Leong, S. L., Lantz, H., Petterson, O. V., Frisvad, J. C., Thrane, U., Heipieper, H. J., (2014). Genome and physiology of the ascomycete filamentous fungus *Xeromycesbisporus*, the most xerophilic organism isolated to date. *Environ. Microbiol.*doi: 10.1111/1462-2920.12596 [Epub ahead of print].

Lev- Yadun,S., Gopher, A and Aboo, S.(2000).The Cardle of Agriculture. Science288:1602- 1603.

Malone, J.P. and Muskett, A.E. (1964). Seed-borne fungi ISTA, Proc. Vol. 29(2): 200-378.

MAPAQ (Ministère de l'Agriculture, des Pécherieset de l'Alimentation du Quebec). (1984). Agdex 111/20, 200-A Chemin Sainte-Foy, Quebec

Mathur SK, SB Mathur, P. Neergaard (1975).Detection of seed borne fungi in sorghum.Pear millet and groundnut. Seed Science Techology, 3:683-690.

Neergard, P. (1977). Seed pathology vol. 1 and II. The Macmillan Press Ltd. London 49-60.

Oka, H.I. (1988). Origin of cultivated rice. Elsevier, Amsterdam.

OMAF (Ontario Ministry of Agriculture and Food). (1994). Field Crop Recommendations. Publication 296.Queen's Printer for Ontario, Toronto, Ontario.

Rifai M.A (1969) Revision of the genus Fusarium and Alternaria. Mycological papers 116:40-95.

Sauer, D.B., Meronuck, R.A. and C.M. Christensen, 1992.Microflora.*In* Storage of grains and their products, Sauer, D. B. ed., American Association of Cereal chemists, pp.313-340.

Second, G. (1986). Isozymes and phylogenetic relationships in *Oryza*. In IRRI (ed.), *Rice Genetics*, 27-39, IRRI.

Slack, G., Puniani, E., Frisvad, J. C., Samson, R. A., and Miller, J. D. (2009). Secondary metabolites from *Eurotium*species, *A. calidoustus*and*A. insuetus*common in Canadian homes with a review of their chemistry and biological activities.*Mycol. Res.* 113, 480–490. doi: 10.1016/j.mycres.2008.12.002

Sprague, G.F. and S.A. Eberhart. (1977). Maize Breeding pp. 312-313 In Corn and Corn Improvement. Agronomy Monographs No. 18. American Society of Agronomy, Madison, Wisconsin.

Tanno, KW and Wilox,G.(2006). How fast Way Wild Wheat domesticated. Science 311:1886.

Van der Straat, L., Vernooij, M., Lammers, M., vand en Berg, W., Schoneville, T., Cordewener, J., et al. (2014). Expression of the *Aspergillusterreus*itaconic acid biosynthesis gene cluster in *Aspergillusniger*.*Microb.Cell Fact.*13:11. doi:10.1186/1475-2859-13-11.

Wendorf, F., Schild, R., Hadidi, N.E., Close, A.E., Kabusıewicz, M., Wieckowska, H., Issawı, B. and Haas, H., (1979). Use of barley in the Egyptian late paleolithic.*Science*, 205: 1341-04 1347.

Wych, R.D. (1988). Production of hybrid seed corn.In G.F Sprague and J.W. Dudley, Eds. Maize and Maize Improvement. Agronomy Monographs No.18; pp. 565-605. American Society of Agronomy: Madison, Wisconsin.