MYCOGENERA OF STORED GRAIN IN OGBETE MAIN MARKET

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ESUT/2009/99536

A PROJECT PRESENTED TO THE
DEPARTMENT OF APPLIED MICROBIOLOGY AND BREWING
FACULTY OF APPLIED NATURAL SCIENCES
ENUGU STATE UNIVERSITY OF SCIENCE AND TECHNOLOGY
(ESUT) ENUGU

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN APPLIED MICROBIOLOGY AND BREWING

APRIL, 2014.

APPROVAL PAGE

This is to certify that this report on Mycogenera of stored grain in Ogbete main market was carried out by Nwachukwu Ogochukwu Gloria with registration number, ESUT/2009/99536, under the supervision of the department of Applied Microbiology and Brewing, Enugu State University of Science and Technology Agbani, Enugu State. This project is original and meets the rules and regulations governing the award of the degree of Bachelor of Science (B.Sc) in the institution.

DEDICATION

This project work is dedicated to Almighty God for giving me the wisdom, knowledge and understanding throughout the years of my study and to my beloved parents Mr. and Mrs. Charles Nwachukwu whom I am greatly indebted to, for their support and encouragement to pursue my interests.

ACKNOWLEDGEMENT

This is to thank all those who supported and helped me throughout the commencement of this project report. I would have never succeeded in completing my task without the cooperation, encouragement and help provided to me by various people; I therefore take this opportunity to express my profound sense of gratitude and respect to all those who helped me through the duration of this project.

The head of department (HOD) Lady Dr. (Mrs). F.O. Tasie who is a mother and a good teacher. I thank her most sincerely, my other lecturers, Dr.(Mrs.) G.O. Okafor, Dr. S.O. Anozie, I appreciate the imparted knowledge and most especially; I acknowledge with gratitude and humility my indebtedness to my project supervisor Dr J.I. Nwankwo (Rev) under whose guidance I had the privilege to complete this project. I wish to express my deep gratitude towards him for providing individual guidance and support throughout this project work.

I would also like to thank my parents Mr. and Mrs. Charles Nwachukwu for whom words alone cannot express how grateful I am for their love, support, prayers and encouragement. I thank you mum and dad so much for being there for me all the way and to all the special persons in my life; Anuty Ginika, Kene, Chinwuba, Nonso, Uche, Olisa and all my dear friends. I love you all and thanks for your love, support and encouragement. May God bless you all.

ABSTRACT

A study of fungi associated with rice, maize, millet, Bambaranut, pigeon pea, beans and cowpea cereal grains found in Ogbete main market, Enugu state was carried out in other to determine the prevalence of fungi. These samples were cultured on sabouraud dextrose agar with species of *Aspergillus* being the most predominant fungi throughout the cereal grains. Species of *Penicillium*, and *Fusarium* were also found. These fungi species produce mycotoxin in stored cereal grains which can increase if storage conditions are poorly managed and if ingested or inhaled can lead to a life-threatening condition in humans and animals. Preventive methods together with careful inspection are by far, the most important tools for safe storage.

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INTRODUCTION

Grains are major sources of food for animals and millions of humans in Nigeria. Most of these grains are exposed to varieties of microorganisms, rodents, insects and pest invasion, with fungi invasion being the majors concern. The invasion of these grains by various fungi not only results in reduction in crop yield and quality with significant economic losses but also contamination of grains with poisonous fungal secondary metabolites called mycotoxin. The ingestion of mycotoxin-contaminated grains by animals and human beings has enormous public health significance, because these toxins are capable of causing diseases in man and animals (Bhaat and Miller, 2010).

Apparently, the harvesting of more and more cereal grain at moisture level is too high for safe storage and has contributed to the mycotoxin problem. Often fungal organisms also enter store grains on different foreign materials. They may be present on farm machinery, vehicle for transportation or found already in the stores (Siegel and Feist, 2010).In the situation where the cereal grains has several of these mycotoxins, their effect may accumulate and cause disease symptoms in the form of burning eyes, shortness of breath, chest pain, fever and dry cough, high risk exposure to inhaled mycotoxins occurs due to insufficient storage and lack of air circulation in the store. Although not all aspects of the impact of mycotoxins on humans by inhalation are known, there is reason to believe that long-term inhalation of even small doses of these compounds not only can cause mycotoxicosis, which is disease caused by the harmful effects of these mycotoxins but may also involve the risk of cancer (Rola and Prabhu, 2011).

Usually, these deteriorative changes in grains are prevented by reducing the moisture content to a level too low for fungi to grow. High-temperature drying has been feasible in the past, but as fuel becomes more expensive and less available, alternative methods to control fungi invasion in cereal grains will be required (Ostry, 2008). In view of the negative public health and economic impacts of fungi in store cereal grains, this work looks at the isolation of micogenera from stored grains in Ogbete main market.

LITERATURE REVIEW

Cereal grain

Cereal grain also called ``grain” is a type of grass grown for their edible grain. With the advent of agriculture in the early history of human civilization, grain consumption became prominent, especially in developing countries (Logrieco and Bottalico, 2008). Cereal grains are grown in greater quantities and provide more food energy worldwide than any other type of crop. These grains constitute a major source of energy in most households today. When combined with a variety of both animal and plant based foods, grains are a reliable source of cheap energy, capable of sustaining and promoting human life. Examples of cereal grains are maize, cowpea, millet, rice, etc. Like all food groups, over-consumption of grains may disrupt health and well being in virtually all people. Therefore, grains should be consumed moderately in a mixed diet (Bottalico, 2008).

Cowpea as cereal grain

Cowpea is an annual legume and is also commonly known as blackeye pea. Cowpea originated in Africa and is chiefly used as a grain crop, for animal fodder and as a vegetable (Akaaajime, 20011). It is popularly called `Akidi’ in Nigeria and also known as *vigna unguiculata*

L .walp. Cowpea seed is a nutritious component in the human diet, as well as a nutritious livestock feed and the green cowpea seeds are boiled as a fresh vegetable, or maybe frozen or canned. The tender green leaves are an important food source in Africa and are also prepared as a pot herb (Shimoni, 2012). Cowpea is a warm-season crop well adapted to many areas of the humid tropics and temperature zones. It tolerates heat and dry conditions**,** but is intolerant of frost (Yoshizawa, 2010). Cowpea performs well on a wide variety of soils and soil conditions, but performs best on well-drained sandy loams or sandy soils**.** Post- harvest provides shade and adequate ventilation is necessary. Dry cowpea is cleaned and packed in small plastic bags for sale to consumers (Magan and Olsen, 2013).

Millet as cereal grain

Millets are some of the oldest cultivated crops. Millet originated in the African savannah and it is grown as a food grain. It is also called *pennisetum glaucum*. Millet is used for wild bird feeds and also consumed as food grain by humans. Millets require warm temperatures for germination and development. They are efficient users of water. Millets grow well on well-drained loamy soils. They will not tolerate water- logged soils or extreme drought (Chiejina and Ulobo, 2009).

Bambaranut as cereal grain

Bambaranut also known as `okpa‘ in Ibo language originated in sub-Saharan Africa (Amadioha, 2009). It is also called *vigna subterranean*. Due to the high protein value of Bambaranut, it is a very important crop for poorer people in Africa who cannot afford expensive animal protein and is used as a beverage (Taiga, 2009). Optimal soil for bambaranut production is sandy soil to prevent water-logging.

Pigeon pea as cereal grain

Pigeon pea (*cajanus cajan)* also known as `fio-fio' is the most essential ingredient in animal feed used in west Africa, most especially in Nigeria, where it is grown (Suleiman, 2011). Pigeon pea makes a well-balanced human food and is an important crop for manure.

Rice as cereal grain

Rice *(oryza sativa)* is a grain which forms an important part of the diet in many countries. It is grown in wet or humid conditions, near very wet soil thus resulting in attacks by a great number of fungi (Vckoric and Bagi, 2012). Rice straw is used as roofing and packing material. The seeds are used in folk medicine for breast cancers, and other tumors (Fernadez, 2009).

Beans as cereal grain

A bean is an important grain which serves as food to thousand of Nigerians. It‘s large attractive flowers make insect pollination easy (Niessen, 2008).

Maize as cereal grain

Maize (*zea mays)* is a cereal for human food and is believed to be originated Mexico. Maize is used as animal feed, fodder, human food. Corn is used as vegetable, folk medicine and as currency in Peru (Asam and Konitzer, 2011).

Fungi and cereal grain

Fungi have been isolated from cereal grains over the past 20 years (Christensen and Kaufmann, 2010). They are among the most widely distributed organisms on earth. Fungi are everywhere in very large numbers and many of its species produce compounds called mycotoxins that are very toxic to animals including humans (Kaufmann, 2010).

A fungus is known as a eukaryote that digests food externally and absorbs nutrients directly through its cell wall. This fungus is very useful to humans and at the same time harmful. Fungi can become significant pathogens of humans and other animals by invasion of these cereal grains which can have a large impact on human health, food supplies and local economies (Azcarate and Terminiello, 2008). These fungi that can invade and cause damage to grains were divided into field fungi and storage fungi

Storage fungi

Storage fungi invade cereal grains during storage. These storage fungi are usually not present to any serious extent before harvest. Small quantities of spores of storage fungi may be present in handling and storage equipments or structures.

Under improper storage conditions, this small amount of inoculum can increase rapidly leading to significant problems. The development of storage fungi in stored cereal grains is influenced by moisture content of the stored grain, the temperature of the stored grain, the condition of the cereal grain going into storage, the length of time the cereal grain is stored and the amount of insect and mice activity in the grain. The most common storage fungi are species of Aspergillus, Fusarium and Penicillium. These fungi are widely distributed and almost always present (Rychlik, 2011).

<u>Aspergillus species</u>

This is one of the oldest named genera of fungi. It had also become one f the best-known and most studied fungi. Their prevalence in the natural environment, their ease of cultivation in laboratory media and the economic importance of several of its species ensured that many microbiologists were attracted to their study (Andrew and Pitt, 2009). Several species of this *Aspergillus* also contaminate cereal grains and other foods with toxic metabolites which is a threat to the health of humans and other animals. Food utilized by humans and our domestic animals are good nutritional sources for *Aspergillus*. Words like `decay', `rot' and `spoilage' are used to describe this fungi utilization of our foodstuffs, which can occur in the field prior to harvest, during storage, and coking in the home (Mims, 2007). The defining characteristic of Aspergillus is the spore- bearing structure. It is the most important microscopic character used in *Aspergillus* identification (Karim, 2010).

<u>Penicillium species</u>

This is comparable to *Aspergillus* spp. The name *Penicillium* comes from the word `brush' this refers to the appearance of spores in *Penicillium spp*. This is post-harvest pathogens and is one of the most common causes of fungal spoilage in cereal grains. They live a long time and they are quiet durable, even under adverse conditions (Nachman and Pinto, 2008). *Penicillium* colonies are usually green, blue-green, or grey-green but can also be white, yellow or pinkish. This *Penicillium* also produces mycotoxins which can cause allergic and asthmatic reaction in susceptible individuals (Barkai-Golan, 2008).

Fusarium species

Grains are well known to be invaded by *Fusarium spp*. under both field and storage conditions. This fungus is responsible for many economically important cereal grain diseases resulting in severe reductions in crop yield (Chou and Wu, 2008). Symptoms of *Fusarium* in grains are usually sparse, making it difficult to tell if the cereal grains have been infected. Some *Fusarium spp*. produce mycotoxins in invaded cereal grains which can cause disease in human and animals if infected grains are consumed (Chou, 2008).

In these cases, the major concern is therefore the production of mycotoxins by these fungi.

Mycotoxin

Fungi produce a large number of mycotoxins in stored cereal grains. They can be found in a range of food crops in the field or in cereal grains during storage (Bottalico, 2008). These mycotoxins are toxic substances produced by fungi growing on foodstuffs or animal feeds under certain conditions. Because of their potent toxic nature and fairly common occurrence under natural conditions, mycotoxins have attracted worldwide attention in the recent years. The reason for production of mycotoxins is not yet known, they are necessary for neither growth nor the development of the fungi (Logrieco, 2008).

Natural occurrence of mycotoxin in cereal grains

Grains constitute the most important food and feed sources which are affected by various mycotoxic fungi. Mycotoxins seem to pose great problem in the tropics than in the temperate regions but no part of the world can be considered to be mycotoxin free zone due to the movement of various foodstuffs from one part of the globe to the other (Magan and Olsen, 2013).

Human and animal implications of mycotoxin

Mycotoxicosis is the disease outbreak resulting due to the ingestion or inhalation of mycotoxins produced by fungi. When this happens, the implications are wide and span from health to economic (Magan and Olsen, 2013).

Human

The appearance of mycotoxicosis symptoms depends mainly on the level of contamination, length of exposure and pre-existing pathological status of the victim. Mycotoxins have the potential for both acute and chronic health effects through ingestion, skin contact and inhalation. These toxins can enter the blood stream and lymphatic system and inhibit protein synthesis, damage macrophage system particle clearance of the lung and increase sensitivity to opportunistic infections (yoshizawa, 2010).

If symptoms appear within a short period of less than 7 days of contamination, it is termed `acute mycotoxicosis' but if the interval between contamination and appearance of the symptoms persist longer, it is termed ``chronic mycotoxicosis" . In acute cases, victim may die if adequate treatment measures are not taken whereas in chronic cases, the victim may live longer through the protracted illness (Li and Yoshizawa, 2010).

Livestocks

Mycotoxins are known to be consumed by livestock through contaminated feed ingredients. They are probably the causative agents or suspected contributing factors in farm animal diseases that cause great economic losses. Young and pregnant animals are generally the most susceptible to mycotoxicosis. Under some conditions, the fungi may produce potent mycotoxins at levels that may adversely affect livestock production. At moderate levels, effects may appear initially with more obvious symptoms within a few days to several weeks of ingestion of the contaminated foods or feeds (Khanzada and Jamil, 2011).

Economic impact of mycotoxin in Nigeria

Estimating the economic impact of mycotoxins require good data sets and expertise, which are missing in Nigeria (Suleiman and Taiga, 2009). The economic losses associated with mycotoxin contamination are difficult to assess in a consistent and uniform way. Thus, the baseline against which economic impacts should be defined is at best fuzzy and often lacking.

Economic losses and impact of mycotoxins on International trade

The adverse economic effects attributed to mycotoxin contamination and losses are widely felt in all sectors of food production and particularly in grains (Hill, 2011).Mycotoxins contamination of cereal grains has considerable economic implications ‘ losses from rejected shipments and lower prices for inferior quality can devastate developing country export markets. Consumers who are primary target in this chain end up paying higher prices due to increased monitoring at all levels of handling and in extreme cases, health problems due to consumption of contaminated products (Hill and Waller, 2011).

Contamination of food by mycotoxins has economic consequences due to rejection of exports and loss of credibility as trading partners; for example, importing countries frequently require guarantees that minimum standards of hygiene been applied in the manufacture of a food product and that food do not have excessive mycotoxin contamination (Ostry, 2008). Africa loses an estimate of sixty seven ($67) million dollars annually from export rejects due to high levels of mycotoxins in food and agricultural produce coming to developed countries (Webley and Jackson, 2009).

Storage pest infestation

Controlling storage pests is a necessity for reducing losses in stored grains and maintaining grain quality. Cereal grains damaged by insects are always open to fungal attack. Insect pests have the capacity to multiply rapidly, so that in a very short time, you can easily have thousands of them attacking your cereal grains. This rapid population growth makes them the major cause of food loses in stored cereal grain (Chou and Wu, 2009). Storage pests are well adapted to darkness, and to movement in confined spaces and amongst stored cereal grains. The moisture content in grain is sufficient for stored product insect survival and reproduction; this is the main reason why heavily infested grains tend to become moist and hot (Ulobo, 2009).

Stores that allow insects like weevil, moth, termites, etc to build large populations are producing problems for themselves and other stores in their area. Insect populations should be controlled while the numbers are still low to avoid fungal invasion.

Rodent infestation

Rodents, mainly rats and mice, cause some of the heaviest losses to stored cereal grains. A rat can eat an amount of cereal grain equivalent to about 7% of its body weight daily thereby leading to losses of approximately 7kg of grain per year (Patriarca and Fernandez, 2009). Rodents contaminate grains with urine, feaces and other pathogens such as fleas. It is usually impossible to remove these contaminants and infested grains become spoiled and unfit for human consumption. But it is easier to keep them away by a good structural design of the store building because they are much larger (Caddick, 2011).

Factors affecting incidence of fungi in cereal grains

Various classifications are used in categorizing the factors that affect the incidence of mycotoxigenic fungi. Some classification categorize these factors as extrinsic and intrinsic, some as physical, chemical and biological factors while other classify them as ecological, environment and storage factors (Suleiman and Akaajime, 2011). Irrespective of the form of classification, Patriarca (2009) identified the key elements involved in stating that the type and amount of mycotoxin produced is always determined by the fungi and environmental factors.

Climatic conditions

Probably the two most important environmental conditions favoring fungi growth and mycotoxin production are hot and humid conditions. Mycotoxins occur more frequently in areas with a hot and humid climate, favourable for the growth of moulds. Although they can also be found in temperature zones, tropical climates such as those existing in Ogbete main market have been found to be quite conducive for fungi growth and mycotoxin production. Mycotoxigenic fungi are most abundant in tropics and as such are major food spoilage agents in these warmer climates (Darby and Caddick, 2011).

Availability of nutrients and conditions for fungi growth

The fact that a strain of fungi has the genetic potential to produce a particular mycotoxin is not enough for it to do so. There must be enough nutrients to encourage fungi growth and the level of mycotoxin production would in part be influenced by the nutrients available to the mould. Typically, mould requires a source of energy in the form of carbohydrates in addition to a source of nitrogen either organic or inorganic, trace elements and available moisture for growth and toxin production. Substrate may also play a role in selecting for or against toxin producing strains of a given species.

Farming systems and agricultural techniques

A number of farming techniques have been shown in various reports as a stimulating fungi growth in agricultural produce. Previously fungicide treated soil has been shown to reduce incidence of *A. flavus*.

Soil type and soil conditions

Soil is a natural factor that exerts a powerful influence on the incidence of fungi. Cereal grains grown in different soil types may have significantly different levels of mycotoxin contamination.

Pre-harvest condition

Drought, soil type, fertilization level and insect activities are important components in determing the likelihood of pre-harvest contamination (Chiejina and Ulobo, 2009). However, the most important factor appears to be high night time temperatures, which favours fungal growth and toxin production at a time when the plant is deprived of its usual energy source and thus least able to resist fungal attack.

Time of harvesting

Harvest is the first stage in the production chain where moisture content becomes the most important parameter in terms of the management and protection of the cereal grains. It also marks a shift from problems caused by plant pathogenic fungi, like *Fusarium*, to problems caused by storage fungi, like *Penicillium.* Ideally, grains will always be harvested after a spell of dry weather when it is at safe' moisture

Early harvesting reduces fungal infection of grains in the field and consequent contamination of harvested produce. Even though majority of farmers in Africa are well aware of the need for earl harvesting, lack of storage space, unpredictable weather, labour constraint, need for cash, threat of thieves, rodents and other animals compel farmers to harvest at inappropriate time (Bhatti and Soomii, 2009).

Post-harvest handling

The post-harvest stages are those stages following harvest and leading up to primary processing. This will typically involve drying, storage and transportation steps. Post-harvest movement of food or feed commodities can be complex, passing as it may between a number of intermediaries such as traders and intermediate processors, who may be suited at different geographical locations. In the simplest case, produce may remain on-farm is store or buffer storage for short periods of time before being passed directly onto the processor. In more complex cases it may pass through the hands of merchants or third party drying facilities and held in storage for periods of time before finally arriving at the processors, at all times the produce can become susceptible to fungal contamination and mycotoxin production if the storage conditions are not strictly controlled.

Drying conditions and duration

Rapid drying of agricultural products to low moisture level is critical as it creates less favourable conditions for fungal growth and insect infestation. The general recommendation is that harvested commodities should be dried as quickly as possible to a very low moisture level. This can be achieved through simple sun-drying under the high humidity conditions of many parts of Africa, such as the humid southern Nigeria is very difficult. Even when drying is done in the dry season, it is not completed before loading grains into stores as observed by Amadioha (2009) and these cereal grains can be easily contaminated.

Storage Factors

Mycotoxin contamination of cereal grains may result from inadequate storage and handling of harvested products. To preserve quality in storage, it is necessary to prevent biological activity through adequate drying (Azcarate and Terminiello, 2008). Several storage fungi have been reported in Nigeria and post-harvest contamination is normally characterized by the activities of the `storage' fungi typically *Aspergillus* and *Penicillium* species that are able to grow in relatively dry conditions.

Lack of awareness

Lack of awareness of the dangers posed by mycotoxin contamination of grains is a major factor responsible for its high incidence in Nigeria. Majority of farmers, food handlers and processors are illiterate with virtually no knowledge of the implications of fungi growth.

Broken cereal grains and foreign materials

Broken grains and foreign materials are likely to be contaminated with fungi going into storage and more likely to be invaded once they are in storage. Foreign materials may restrict air movement through the grains mass leading to temperature and moisture problems which may favour storage fungi development.

Amount of insect and mice activity

Insects and mice may carry fungal spores on their bodies thus introducing fungi into the grain. Insects and mice activity in cereal grains tends to lead to an increase in both temperature and moisture content of the cereal grain surrounding the insect.

Cereal grain spoilage causes;

Fungi do a tremendous amount of damage to cereal grains by casing poisoning in man and animals due to the production of mycotoxins, lungs disease in humans, reduction in germination capacity of the cereal grain, direct loss when the grain is too mouldy to eat, deterioration of store fabrics especially wood, causing rotting, deterioration and weakening of fibres used for packaging or protecting the cereal grains which leads to spillage or water entry and deterioration in flavour, colour and texture of the cereal grains.

What then should be done with mouldy cereal grains?

This is a vexed question as no one wishes to waste food. However, since the discovery over the decade that a wide range of storage fungi can produce substances that can cause disease when fed to animals, the old maxim that fungi contaminated cereal grains can be safely fed to animals therefore no longer applies (Rola and Prabhu, 2011).

If fungi invaded grain is used for animal feed, it may cause death and at best is likely to give poor results like a reduction in expected weight increase. sIt is worth noting here that cleaning grain to remove surface fungal growth practiced mainly in some rural communities; is unlikely to remove any toxin present within the cereal grains and therefore is not recommended. Similarly, cooking does not necessarily destroy mycotoxins produced by these fungi. Preventive methods together with careful inspection and sorting to remove visibly damaged cereal grains are therefore highly recommended.

Possible intervention strategies

The climatic conditions and agricultural practices affect fungi prevalence greatly in the different parts of Nigeria. The complete elimination of mycotoxin contaminated commodities is not achievable, hence good agricultural practices represent a primary line of defense against contamination of cereal grains with mycotoxin followed by the implementation of good manufacturing practices during the handling, storage and distribution of cereal grains for human food and animal feed (Darby and Caddick, 2011). The achievement of mycotoxin reduction and control is dependent on the concerted actions of all actors along the food production and distribution chain. Any possible intervention strategies to reduce fungi in cereal grains must begin from good agricultural practices and prevention.

Prevention and control

Little can be done to prevent or reduce the invasion of cereal grains by fungi. However the following practices to minimize damage from stored grain fungi should help prevent storage problems (Suleiman and Akaajime, 2011).

- Sanitation in the stores is essential. Cleaning up cereal grains and debris that can harbor insects is part of any good control program.
- Bags of old cereal grain from previous sells should be disposed of immediately.
- Avoid physical damage to the grains at all stages of handling.
- Check the moisture content of all cereal grains before storage. Make sure that it is quite dry first. If there is any doubt, or if drying has proved to be a problem, do not store the cereal grains in solid walled containers. `Pigeon hole' can be used for bagged cereal grains suspected of being damp as it allows air to circulate through the stack.
- Avoid storing warm cereal grains as the heat will be retained and encourage rapid fungi and insect multiplication.
- Ensure that all stores are in good repair before use.
- When storing bagged cereal grains, keep it away from the walls of the store and use dunnage to raise the sacks away from floor.
- Allow newly constructed concrete stores or floors dry out thoroughly before use.

- If possible, cover all metal stores to prevent direct sunlight from falling on the walls, if this is not possible, a coat of white paint will help to reflect the heat and keep the cereal grains cool.
- Apply adequate pest control measures to prevent insect `hot spots' from developing.
- Do not load or unload cereal grains in the open if it is raining and avoid placing sacks of cereal grains on wet ground.
- Ensure that all wagons, Lorries and other mobile containers are adequately covered and in good repair, especially if movement during the rainy season is likely.
- Clean bins to reduce contamination.

- Remove all broken cereal grains, dust and foreign materials that can produce contamination.
- Check and repair storage bins to prevent moisture leaks from faulty joints or other problems.
- Mow around the stores to discourage insect and rodent activity.

MATERIAL AND METHODS

Study Area

The study area of this project was cereal grain stores at Ogbete main market Enugu state, Nigeria. Ogbete main market serves as a medium by distribution of cereal grains to most towns and markets within and outside Enugu due to her proximity to the region famous for the production of these products commercial quantities (Northern region of Nigeria).

Sample Collection

The cereal grain samples which consist of brown and white beans, yellow and white maize, rice, okpa(bambaranut), akidi(black beans), white and red Sorghum was collected from a major distributor on 28th of August 2013 at Ogbete main market. Most of the grain were actually bought in jutes bags and Jumbo polythene bags from the producers in the northern region/state (Nigeria). The sample were remold by shifting open the bags with a sterile commercial grain collector while sterile dried Durham were used to collect the grain samples which were taken to the laboratory for analysis.

Sterilizing Techniques

In the course of this studies, secured sterilized method were adapted, the method described by Cruickshank and Harrisan (2005) were adopted for the sterilization of media at 121^{o}C for 15 minutes and glass were at 170^{o}C for 2 hours using autoclave and heatoven respectively. The method of (Winter, 2009), as described by Okpokwasili and Ogbulic (2005) were adopted for the sterilization of polythene bags used for sample collection. This was done (Christensen and Kaufmann, 2010) by soaking the polythene bag in 2.5% acid alcohol over-night. Glass rods and inoculating needle, were then sterilized by flaming.

Media Preparation

200mls of water was weighed and poured into a calibrated flask containing 13g of sabouraud dextrose agar containing water. The mixture was allowed to stand for 10mins before swirling and heated under the flame to dissolve the agar thoroughly before autoclaving. The mixture was autoclaved (sterilized) at 121^{o}C for 15mins at the end of the sterilization period. The medium was allowed to cool to about 47^{o}C before the aseptic pouring of the medium into plated 5mls of methanol which was mixed with 0.10g of powdered chloramphenicol and a drop of it was added into already prepared medium and swirled. This was aimed at inhibiting the growth of other flora and allowing only the growth of only fungal genera. The medium was poured into the petri dishes and allowed to set.

Mycological analysis

The isolation of fungal mycoflora of the cereal grain was carried out using the direct plating method of Petter (2005).

Direct plating method

Each samples collected from different stores in Ogbete main market was placed on the prepared medium in the petri dishes using forcep. These petri dishes were then properly labeled and then incubated at room temperature at about 37^0c for 5-7 days.

Isolation of Fungal Species

The isolation of fungi species was carried out as described by Allexopolous and Mims, (2007). The fungal spp. observed to be growing on the sabouraud dextrose agar medium after 5-6days of growth, was sub-cultured to isolate pure culture for easy identification. Different organisms were picked and with the help of forcep which was first sterilized by passing it through bunsen burner and allowed to cool. The picked organism were carefully stamped onto the new plate and allowed for 3-5days at room temperature, pure culture were put on slant.

Slant Culture

A medium was prepared and poured into universal bottle, it was then autoclaved and chloramphenicol was added. It was slant to obtain a slant shape organism were picked from the subculture plate and cultivated into the slant bottle. It was then kept for growth.

Slide Culture

Slide culture was cleaned using cotton wool and glass rods were placed inside a sterile petri dish dumped cotton wool was placed between the glass rods to aid the growth of fungi. The organisms were collected from the slant and placed directly on the slide which is place on top of the glass rod. The prepared medium in the petri dish were cut into blocks were the organisms were placed on the slide. The slide was covered with cover slip and left for 3-5days.

Staining of Organism

Cotton wool was used to clean the slide to be used a drop of lactophenol was placed on the surface of the slide with the help of calibrated micropipette. The organism were carefully picked with collecting needle and placed on the pool of the picked organism were carefully tasted to avoid breakage of the hyphae of the fungi, cleaned cover slip was placed on the slide containing the organism. The slides was viewed under the microscope using x10 and x40 objective lense respectively.

RESULTS

Result of the isolated cereal grains in Ogbete Main Market Enugu State revealed different mycogenera from different grains in Ogbete main market stores. The general description of mycogenera isolated from cereal grains is shown in the table and figures below. The album of fungi isolated also from the cereal grains in Ogbete main market is also shown in the appendix.

Table 1.

Description of general mycogenera isolated in Ogbete main market

Grain	Slant	Microscopy	Organism
1. Rice	White colour	Spore head	*Aspergillus flavus*
2. White maize	Black	Non-septate	*Penicillium*
3. Brown bean	Black colour part	Non-septate and spore head	*Aspergillus niger*
4. White bean	Black part	Spore head/Non	*Mucor*
5. Red fio fio	White colour	Spore head nonseptate	*Rhyzopus*
6. Okpa(bambara nut)	Gray colour part	Non-septate Spore head	*Aspergillus niger*
7. Foreign rice	White colour Green colour	Spore head/non-septate	*Mucor*
8. Millet	Brown colour Black colour	Spore head/non-septate non-septate	*Aspergillus niger*
9. Akidi (black bean)	Brown part	Spore head	*Fusarium*

10.	Wheat	Black part	Spore head/non-septate	*Aspergillus niger*
11.	Yellow maize	Black part	Sporehead/ non-septate	*Penicillium*
12.	White fio fio	Black part	Sporehead/non-septate	*Sporotrichum*
13.	Red sorghum	Brown part	Spore head and non-septate	*Rhyzopus*
14.	White sorghum	Gray part	Spore head and non septate	*Rhyzoctonia*

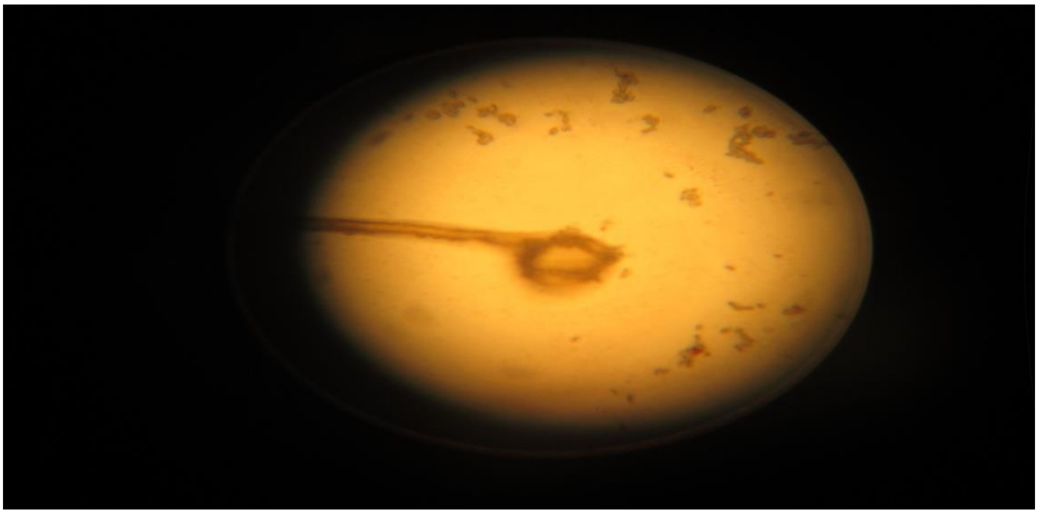

Figure 1: Sporehead and mycelia of *Aspergillus flavus* isolated from rice (Microscopic characteristics: Non-Branched condiospore with bulb end carries conidia. Macroscopic characteristics: pin like green growth).

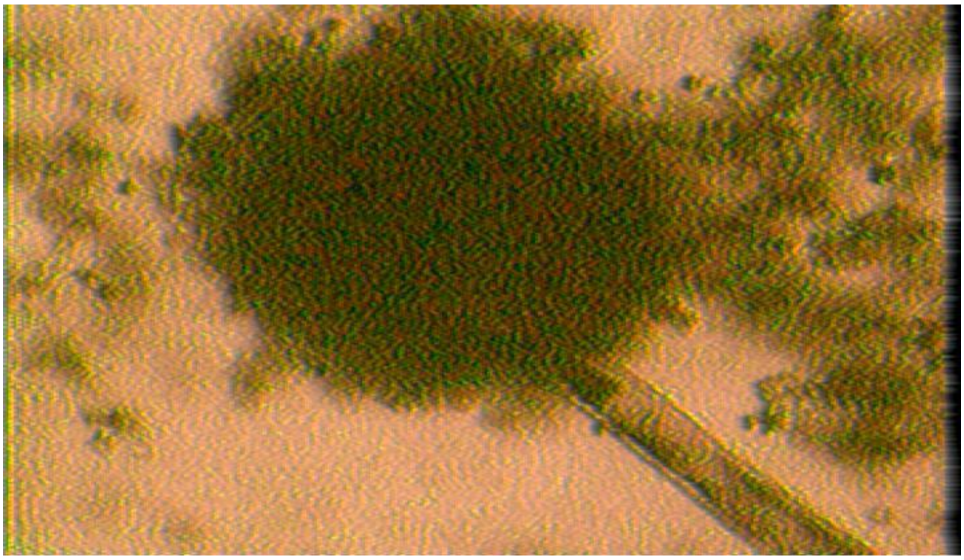

Figure 2: Sporehead and mycelia of *Penicillium* spp. from white maize(Microscopic characteristics: Brush-like conidiospore carries conidia. Macroscopic characteristics: Colour-green or green-greyish colour and colonies grows over fruits especially citrus).

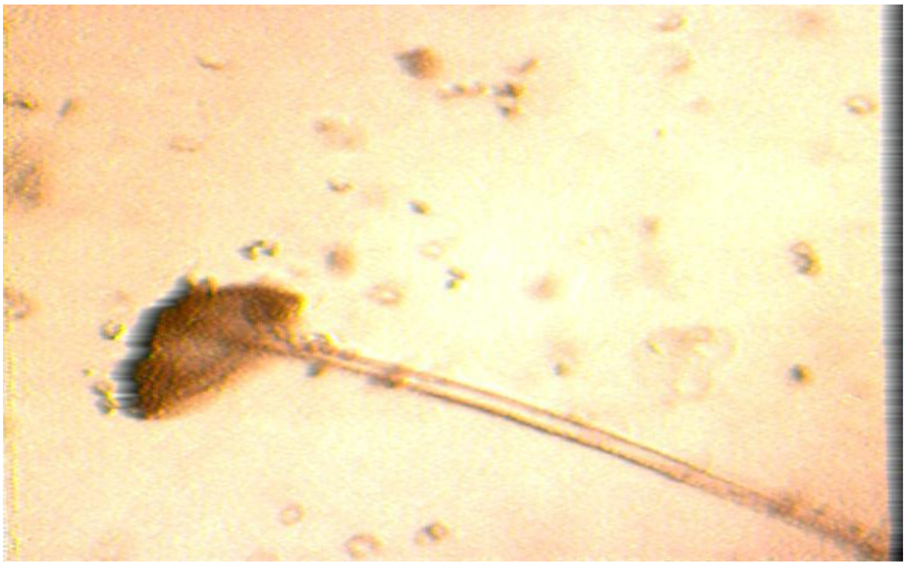

Figure 3: Sporehead of *Aspergillus niger* isolated from brown bean (Microscopic characteristic: Non-Branched conidiospore with bulb end carriesconidia like sun rays. Macroscopic characteristics: pin like black growth).

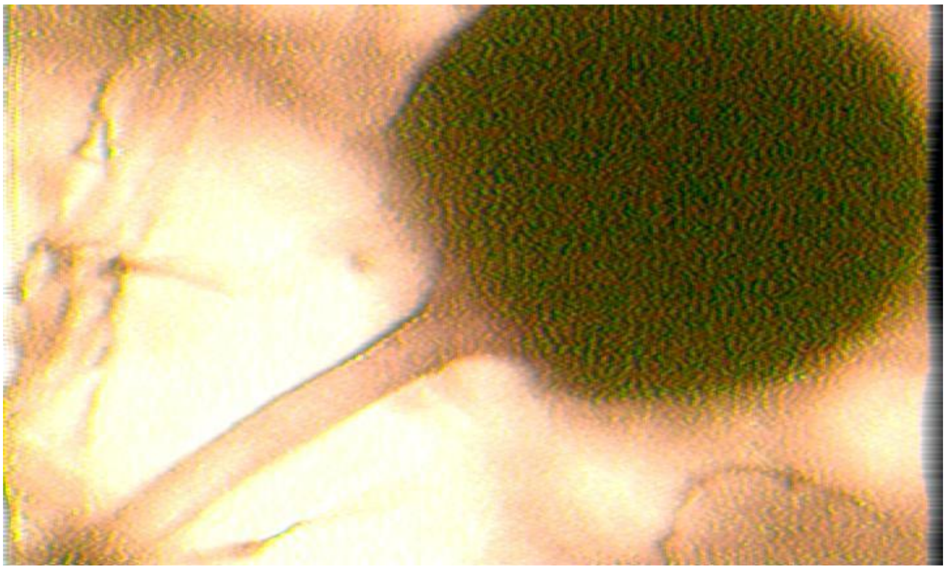

Figure 4: Sporehead and mycelia of *Rhyzopus* spp. isolated from fio-fio (Microscopic characteristic: contain spores and cotton like growth spotted with black colour).

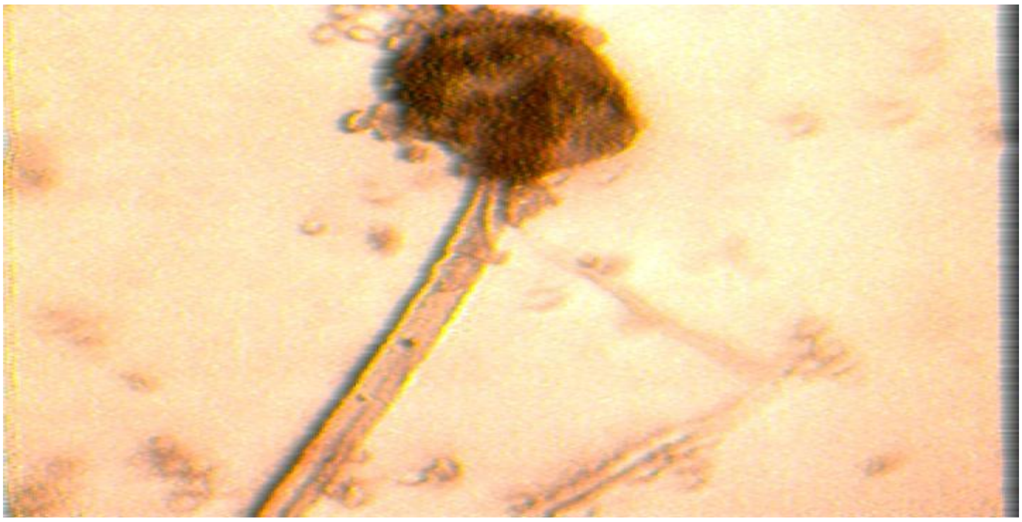

Figure 5: Sporehead and mycelia of *Aspergillus niger* isolated from Okpa (Microscopic characteristic: Non Branched with bulb end carries conidia like sun rays. Macroscopic characteristic: Pin like black growth).

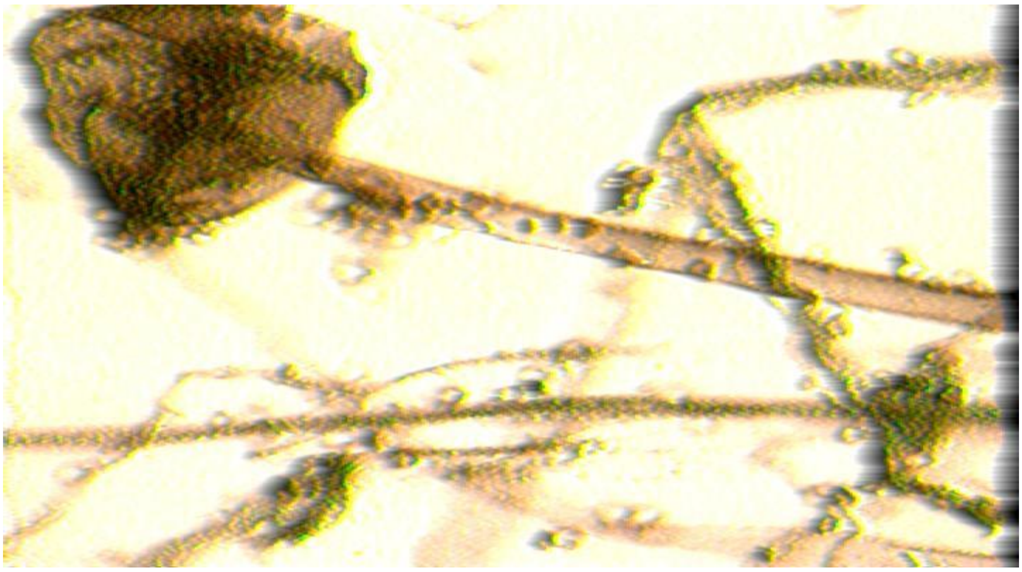

Figure 6: Sporehead and mycelia of *Mucor* spp. isolated from foreign rice (Microscopic characteristic: Contain spores, do not have rhizoids, cotton like white spotted with black colour).

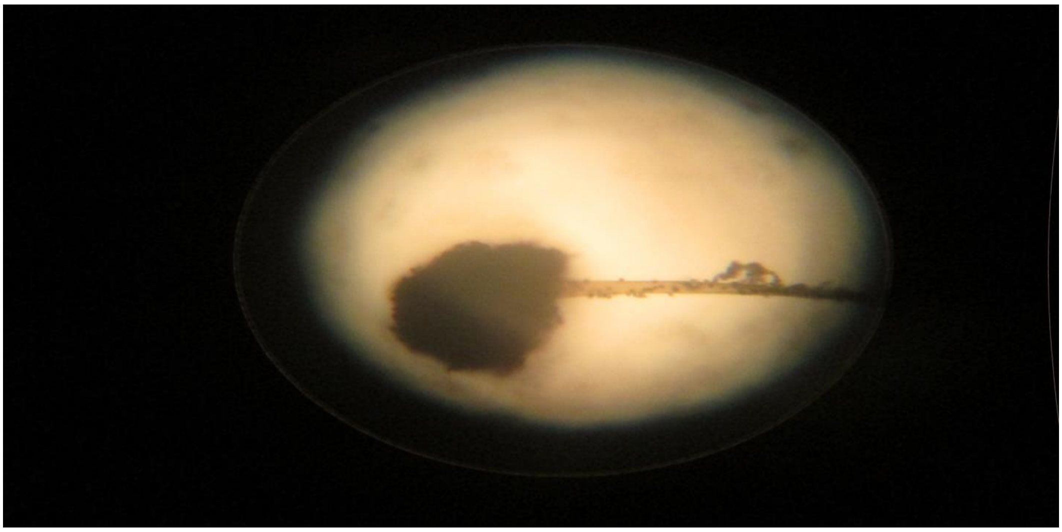

Figure 7: Sporehead of *Aspergillus niger* isolated from millet (Microscopic characteristics: Non-Branched conidiophores with bulb end carries conidia like sun rays. Macroscopic characteristic: Pin like black growth).

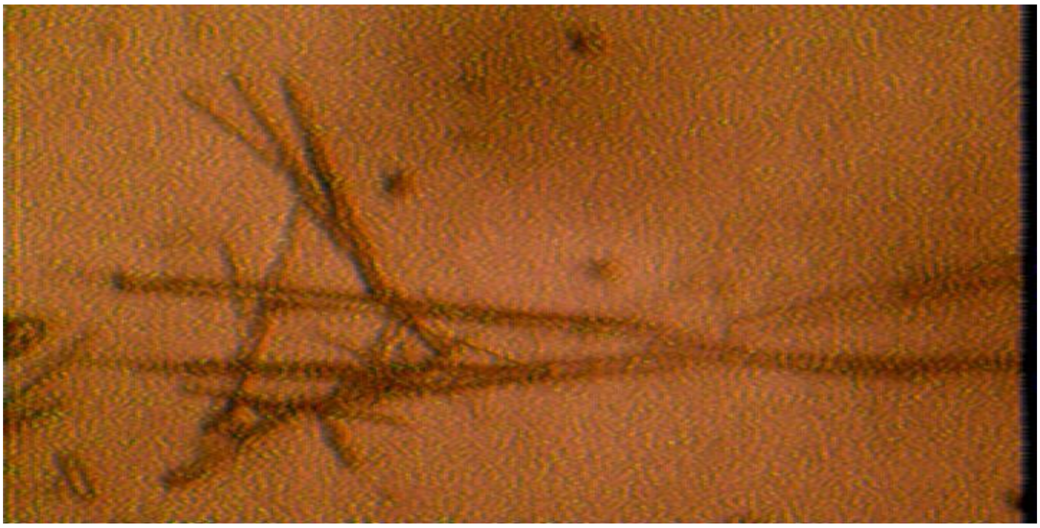

Figure 8: Sporehead and mycelia of *Fusarium* spp. isolated from Akidi (black bean). (Microscopic characteristic: spindle-like conidia, multi-cellular. Macroscopic characteristics: Colonies appear brown or pink in center and with white edges).

Figure 9: *Aspergillus niger* isolated from wheat (Microscopic characteristics: Non-branched conidiophore with bulb end carries conidia like sun rays. Macroscopic characteristic: Pin like black growth).

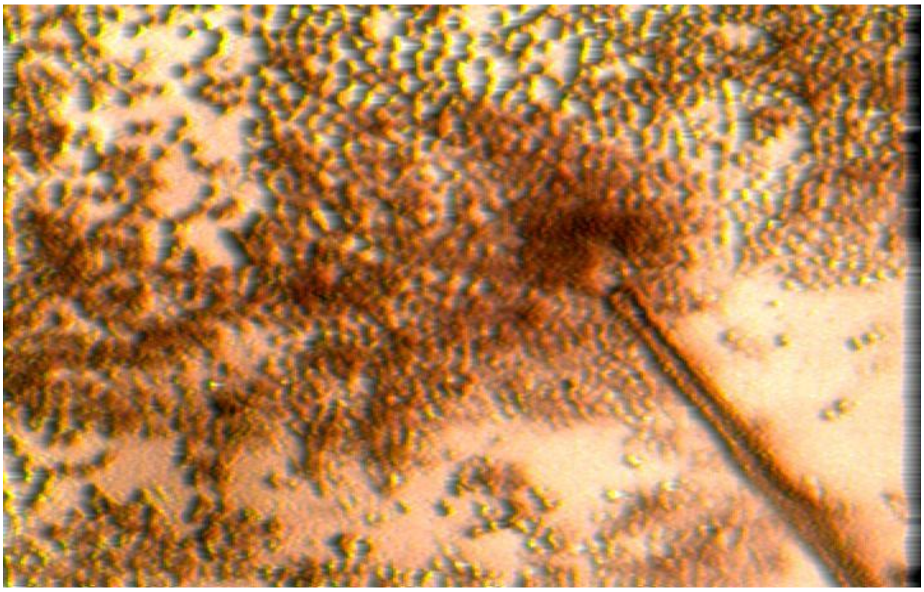

Figure 10: Spore head of *Penicillium spp.* (Microscopic characteristic: Brush-like conidiospore carries conidia. Macroscopic characteristic: Green or green-greyish colour colonies grows over fruits especially citrus).

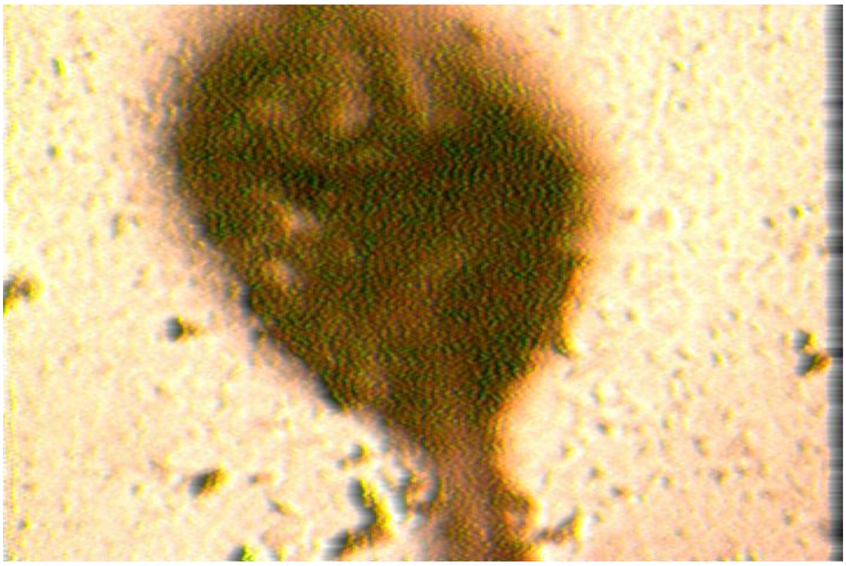

Figure 11: Mycelia and spore head of *Rhyzopus* spp. isolated from red sorghum (Microscopic characteristic: contain spores and cotton like growth spotted with black colour).

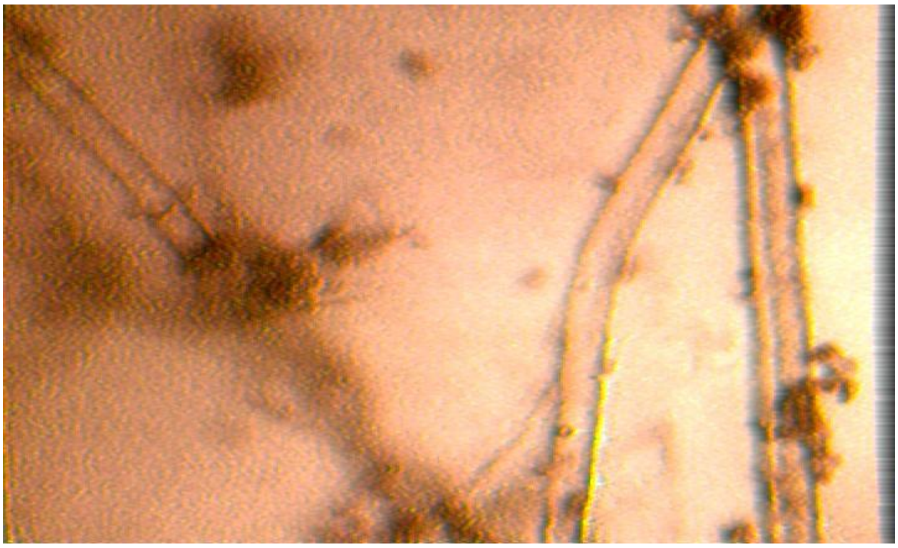

Figure 14: *Rhizoctonia* spp. isolated from white sorgum.

DISCUSSION

Cereal grains are the most important stable food crop in Nigeria, which favor fungal contamination and mycotoxin production. The high viable fungi count recorded in store grains in Ogbete main market may be associated with inadequate post processing handling practices such as the open display of grains bowls and basins in the market, measurement with the aid of bare-hand, coughing and sneezing while selling and the use of non-microbiologically determined hessian bags for packaging and haulage. These may be responsible for the vast array of fungi detected and isolated (li and Youshizawa, 2010).

During storage, the development of fungi, especially *Aspergillus*, *Fusarium* and *Penicillium* species, is an unresolved problem. They are responsible for quantitative and qualitative used. These groups of fungi have also been variously linked with the production of various types of mycotoxins under various conditions. Exposures to mycotoxins through ingestion of contaminated grains and inhalation of this toxin have been linked to acute and chronic toxicity in animals (Christensen and Kaufmann, 2010).

Several studies in Nigeria (Suleiman and Kaufmann, 2010), have reported toxin levels far above the limits allowed by international regulation agencies in food and agriculture product. Effect such as acute liver cirrhosis, induction off tumor and other and other genetic effect in animals and human are well documented. Furthermore, no single Governmental or private organization has the resource in personal, expertise, money or time to fight against mycotoxin contamination .Therefore, collaboration, in project involving multidisciplinary team is needed for effective research, documentation, monitoring, evaluation and control of mycotoxin in Nigeria.

CONCLUSION AND RECOMMENDATION

Conclusion

Contamination of store grains by various types of toxigenic fungi is a serious and a widely neglected problem. High bioload of *Aspergillus*, *Fusarium* and *Penicillium spp.* which are well- known pathogenic storage fungi were found in almost all the stored grains examined. This is a very alarming and threatening and therefore warrants renewed vigilance on the efficacies of stored cereal grains conditions, handling technique and handlers know-how.

Recommendations

Prevention of fungal invasion on stored cereal grains is by far the most effective method highly recommended for avoiding fungal problem in stored cereal. Integrated management program, focus on good agricultural practices, food quality from the field to the consumers, hygiene practices and safety of finished products should also be considered. In addition, strict application and implementation of quality control, quality assurance and good manufacturing practice principles will help to ensure of grains consumed by several millions of Nigeria.

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APPENDIX

Album of micogenea of cereal grains found in Ogbete main market, Enugu state.

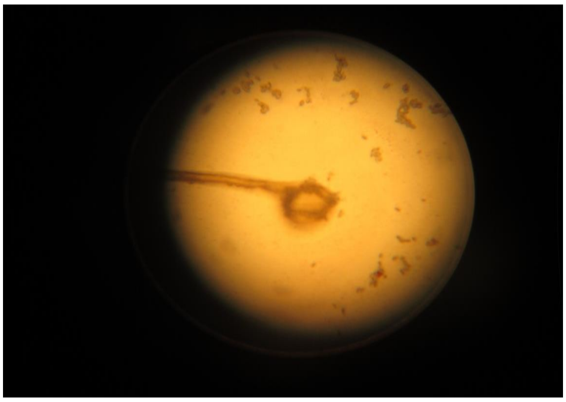


Figure 1: *Aspergillus flavus* spp

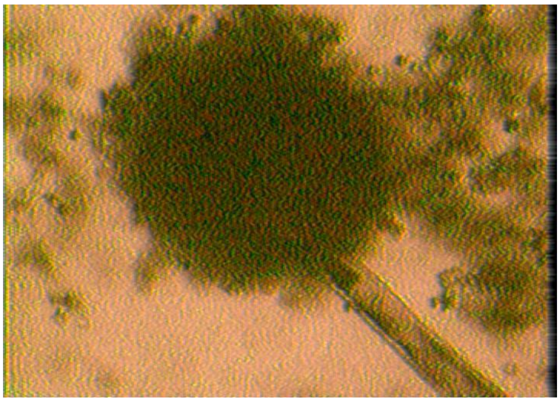


Figure 2: *Penicillium* spp.

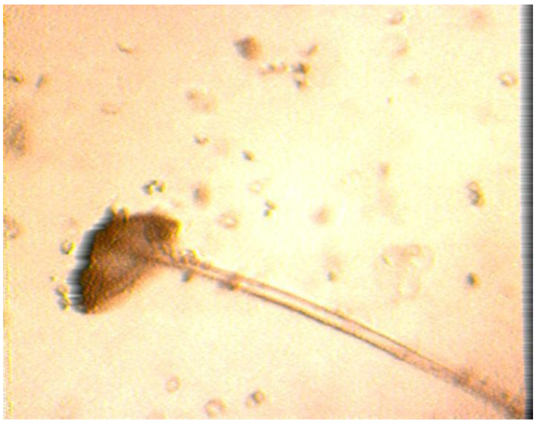


Figure 3: *Aspegillus niger* spp.

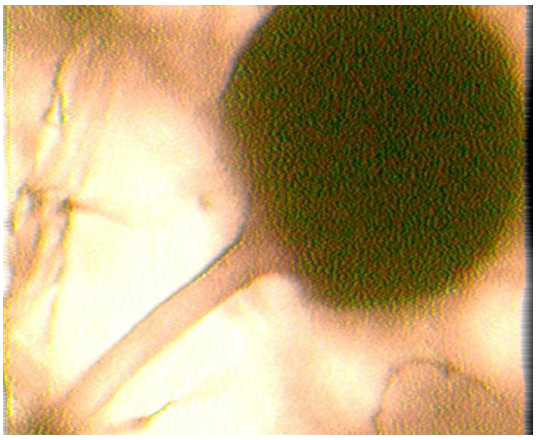


Figure 4: *Rhyzopus* spp.

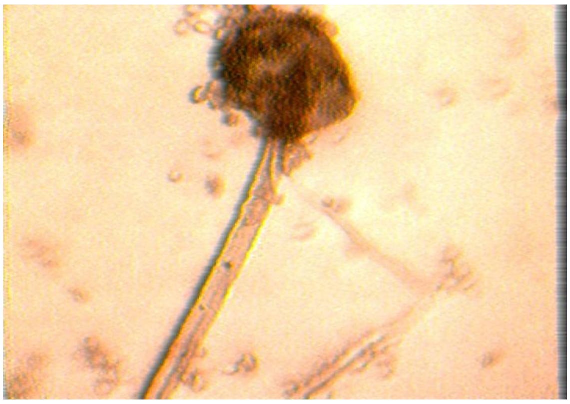


Figure 5: ***Aspergillus niger*** **spp.**

Figure 6: ***Mucor*** **spp.**

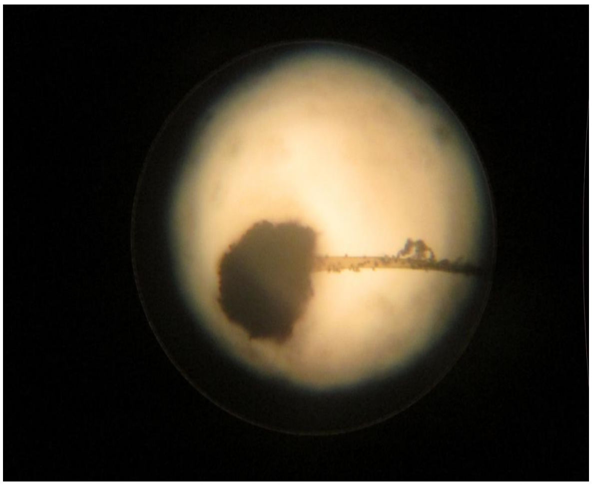


Figure 7: ***Aspergillus niger*** **spp.**

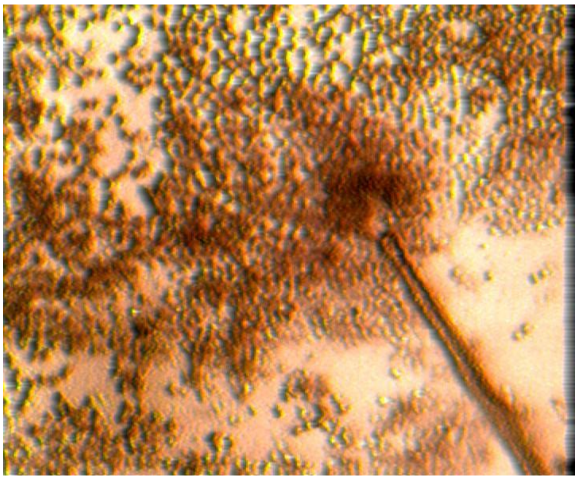


Figure 8: ***Fusarium*** **spp*.***

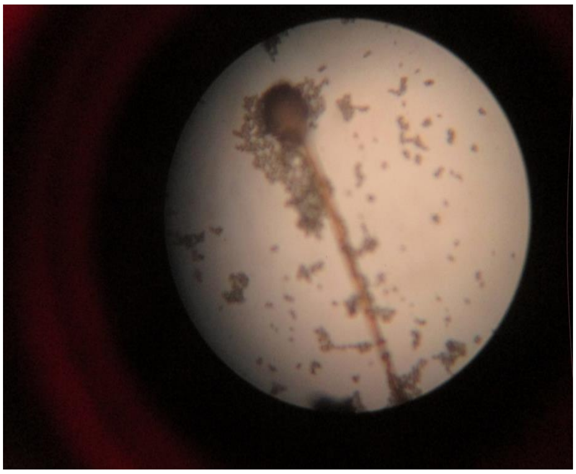


Figure 9: ***Aspergillus niger*** **spp.**

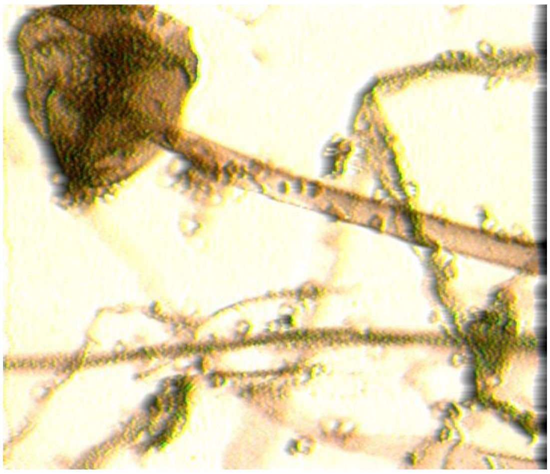
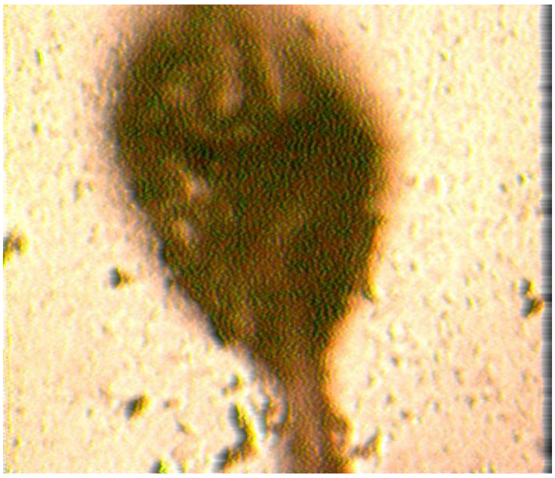


Figure 10: ***Penicillium spp.***



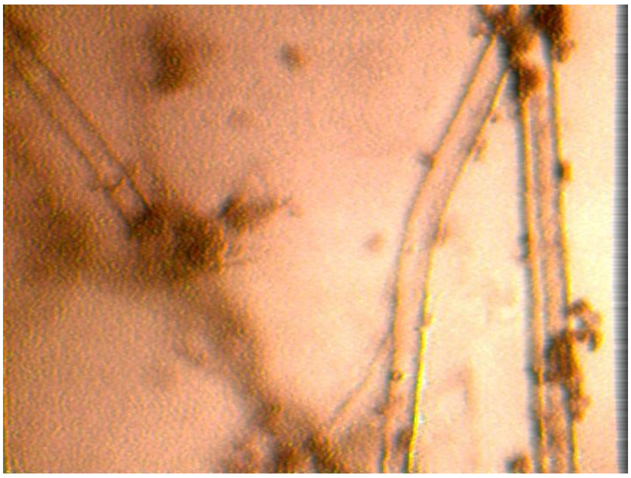


Figure 11: ***Rhyzopus*** **spp.**

Figure 12: ***Rhizoctonia*** **spp.**