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EVALUATION OF GROWTH, YIELD, SEED QUALITY AND AFLATOXIN BUILD-UP IN SELECTED LOCAL AND IMPROVED GROUNDNUT (*Arachis hypogaea* L.) VARIETIES

A THESIS SUBMITTED TO THE DEPARTMENT OF HORTICULTURE, FACULTY OF AGRICULTURE, COLLEGE OF AGRICULTURE AND NATURAL RESOURCES, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF M. Sc. DEGREE IN SEED SCIENCE AND TECHNOLOGY

BY

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CERTIFICATION

I hereby declare that this submission is my own work towards the Master of Science (Seed Science and Technology), and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except for references where due acknowledgement has been given in the text.

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DEDICATION

This work is dedicated to all the teachers, children, parents and all those who are passionate for the Children's Ministry of the Church of Pentecost, Ayeduase Central and English Assemblies.

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ABSTRACT

A field trial was conducted on a sandy loam soil at the research fields of the CSIR-Crops Research Institute, during the major season of 2011 to study the growth, yield and seed quality of ten groundnut (*Arachis hypogaea* L.) varieties, in a 3 × 10 randomized complete block design (RCBD). Following the field trial, samples of the ten groundnut varieties were dried and afterwards, germination, vigour and dormancy tests were carried out in the laboratory in four replicates of hundreds seeds per replicate. Samples of the seed were also stored in two storage receptacles: storage in jute bag and storage in jute bag interlaced with polyethylene, all at ambient conditions. Factors were studied in 2 × 2 × 10 factorial in completely randomized design (CRD). Pod and seed yields were generally higher for both improved and local varieties while germination rates were significantly ($p \leq 0.05$) higher for *fastigiata* than the *hypogaea*. Other agronomic and yield attributes were generally influenced by varieties and differed significantly among and between the *hypogaea* and *fastigiata* sub-species. A further microbial examination revealed that the various seed samples were susceptible to various species of saprophytic and pathogenic fungi. Aflatoxin levels detected were generally not above the critical maximum though some varieties had aflatoxin levels well over 100% above recommended levels for acceptable groundnut seed.

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LIST OF ABBREVIATIONS

CGR	Crop growth rate
WAE	Weeks after emergence
FAO	Food and Agricultural Organization
ppb	parts per billion
FSW	fresh shoot weight
AOAC	Association of Official Analytical Chemists
KNUST	Kwame Nkrumah University of Science and Technology
HCC	hepatocarcinogenic
EU	European Union
UN	United Nations
MAD	University of Madras, Agricultural Department
PAC	Preharvest aflatoxin contamination
IVSCAF	*in vitro* seed colonization by *A. flavus*
FSCAF	field resistance to seed colonization by *A. flavus*

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The cultivated groundnut (*Arachis hypogaea* L.) is an ancient crop of the New World, which originated in South America (Southern Bolivia/ North West Argentina region) where it was cultivated as early as 1000 BC. Dissemination of the crop to Africa, Asia, Europe and the Pacific Islands occurred presumably in the 16th century and 17th century with the discovery voyages of the Spanish, Portuguese, British and Dutch (Smartt, 1969; Hammons, 1982). Currently, it is grown in the areas between 40 ^{o}S and 40 ^{o}N of the equator where average rainfall is 500 to 1200 mm and mean daily temperatures are higher than 20 ^{o}C (ICRISAT, 1990).

Groundnuts are also known as peanuts and are the edible seed obtained from the plant, *Arachis hypogaea.* It is a widely grown crop in developing countries and it has various uses and ways of preparation and consumption. Groundnuts may be eaten as roasted, peanut butter, chocolate based products and other supplementary and confectionery uses for infants. It is also one of the most important oilseeds in world agricultural trade (International Trade Forum, 1999). It is particularly an important crop because of its role in mitigating the protein gap in the diets of most households who cannot afford to obtain it from animal sources. Groundnut is also a rich source of essential dietary oil, being an important oilseed crop with about 30-50 % oil. It is also able to fix atmospheric nitrogen in the soil through a symbiotic association with soil bacteria found in the soil. It is therefore a crop of choice for resource-poor farmers in particular who do not have the means to enrich the soil through soil amendments (MAD, 1949).

Groundnuts are grown in Ghana mainly in the Guinea Savanna and Transitional agro-ecological areas (Jolly *et al.,* 2008). It has a growing period of about 90-120 days depending on the variety. Most farmers engaged in the production of the crop are resource-poor and have no good treatments for the pods once they are harvested from the field. The production, harvesting, drying and storage of the crop therefore are largely associated with generally poor agricultural practices (Nigam *et al*., 2004). As a result, many infections are transmitted from known and unknown sources to the pods even before they are shelled for planting at the start of the next season. Some of the major parasites that infect the crop in store include fungi species such as *Aspergillus flavus* and *A. parasiticus*. These two fungi are known to transmit toxic substance known as aflatoxin to the seed when conditions of storage are poorly managed (Wilson *et al*., 1977).

1.2 PROBLEM STATEMENT

Adams (1977) reported that storage fungi especially *Aspergillus, Penicillium, Rhizopus* and *Mucor* species infect seed after harvest and can grow on them during storage. These species of fungi, when associated with the crop cause various infections and damage to the crop including toxins which they release as by-products of their metabolism.

Aflatoxin is about the most popular and widespread mycotoxin. Its name is derived from the fact that it was originally found to be produced by *Aspergillus flavus* (Agrios, 1978), but is now known to be produced by other species of *Aspergillus*. Generally, mycotoxins have been implicated as causative agents of different human and animal health disorders. Both the toxigenic fungi and the mycotoxins they produce are potential problems from both health and economic perspectives.

Traditional storage structures used by farmers for on-farm storage include containers made of plant materials (woods, bamboo, thatch) or mud placed on raised platforms and covered with thatch or metal sheet. These widely contrasting storage practices may explain the range of storage losses in the developing countries. The type of storage plays a fundamental role in storage efficiency (Asafo-Adjei *et al.*, 1998).

The adoption of high yielding varieties (mostly with poor storability) by farmers has made the traditional storage systems to become inadequate.

However, it has been very difficult to promote the new storage technologies such as the use of metal bins by small scale farmers due to their high cost.

1.3 JUSTIFICATION

In several groundnut producing areas, studies of the farmers stock of groundnut seed show that improvement in seed quality and farmers' seed management require immediate attention to maintain healthy seed stock. It is also noteworthy that availability of quality seed at the time of sowing is sometimes a problem. If storage facilities are created at the farmers' level or village level and farmers are made aware regarding the benefits of the quality seed this problem could be solved to a large extent. Thus, seed production activity at village level may be advantageous over existing centralized, large-scale production and procurement by state-owned organizations in various developing countries (communities) while at the same time reducing cost of production (ICRISAT, 1990).Research is needed to develop and find suitable storage practices that maintain the integrity of seed for use at the appropriate time and also economical (Atuahene-Amankwa *et al.*, 1990).

1.4 OBJECTIVES OF THE STUDY

The general objective of this study was to determine whether the type and duration of storage have effects on the presence of *Aspergillus* spp. and aflatoxins contamination on groundnut (*Arachis hypogaea* L) and to assess the aflatoxins accumulation at different intervals.

Specific objectives of the study were to;

1. study the performance of varieties for seed yield and quality
2. determine aflatoxin levels of groundnut in relation to storage practices
3. examine the accumulative rate of aflatoxins with respect to period of storage
4. detect presence of *Aspergillus* spp. and other mycotoxin causing fungi from stored samples
5. examine whether *Aspergillus* seed infection levels and aflatoxin build-up can occur due to varietal differences.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and Distribution of Groundnut

`Groundnut is believed to have originated from Bolivia and North Western Argentina. It is an ancient crop of the New World widely cultivated in Southern American countries such as Mexico. It is thought to have been introduced into other parts of the world by the discovery voyages of the Spanish and Portuguese during the sixteenth and seventeenth centuries (Smartt, 1969; Hammons, 1982). Currently, the crop is cultivated mostly in the tropical, subtropical and temperate regions of the world. It is a very important crop in West Africa (Nigam *et al*., 2004) and Ghana in particular where it is a source of livelihood for a number of subsistence, resource-poor farmers. Average yields worldwide have also risen significantly from a previously known figure of less than 1000 kg/hectare to more than 1500 kg/hectare and in some cases over 2000 kg/hectare (FAOSTAT, 2010).

2.2 Utilization of Groundnuts

The uses of groundnut are diverse between countries and among ethnic groups. All parts of the plant can be used. The nut (kernel) is a rich source of edible oil, which contains about 36-54 % oil and 20-31 % protein (Asibuo *et al.*, 2008b). The oil is used primarily for cooking, for manufacture of margarine, shortening and soaps. Seeds are consumed directly either raw or roasted, chopped in confectioneries or ground into peanut powder and butter. In traditional medicine, groundnut is used for aphrodisiac purposes, inflammation, chloecystosis, nephritis and anticoagulant (Duke and Ayensu, 1985). In China, the oil is taken with milk for the treatment of

gonorrhoea, and used externally for rheumatism, while in East Africa groundnut is used in traditional medicine (Duke, 1981).

2.3 Botany of Groundnuts

The cultivated groundnut belongs to the family *Leguminoesae*, sub-family *Papillionoinaceae*, tribe *Aeschnomeneae*, sub-tribe *Stylosanthineae*, genus *Arachis* and species *hypogaea*. The genus name *Arachis* stems from a-rachis (Greek, meaning without spine) in reference to the absence of erect branches. The species name *hypogaea* also comes from hupo-ge (Greek, meaning below earth) and relates to the gynophores (flower stalk or peg) that grow downward into the earth so that the pod develops underground (Smartt, 1969).

There are two major subspecies of *A. hypogaea* that mainly differ in their branching pattern (Gibbons *et al*., 1972): subspecies *hypogaea* with alternate branching and subspecies *fastigiata* with sequential branching. Within the sub-species *hypogaea* are two botanical varieties; var. *hypogaea* (Virginia and runner types) and var. hirsuta (Peruvian humpback and Chinese dragon). Subspecies *fastigiata* is also divided into botanical varieties *fastigiata* (Valencia type) and vulgaris (Spanish type). The two main groups may be runners or bunched types in growth habit. The Spanish types usually have better flavour and are grown for nut consumption while the Virginia types are grown mainly for the oil (Krapovickas and Gregory, 1994).

2.4 Morphology and Development of Groundnuts

Groundnut seed consists of two cotyledons, stem axis and leaf primordial, hypocotyls and primary root. The function of the hypocotyls is to push the soil surface during germination and its length is determined by the planting depth. The hypocotyl stops elongating as soon as light strikes the emerging cotyledon. Thus,

groundnut is intermediate between the epigeal and hypogeal types. The taproot grows very fast, reaching a mean length of 10-12 cm within 4-5 days (Stalker and Simpson, 1995).

Lateral roots appear about three days after germination (Gregory *et al*., 1980). Initial plant growth is slow, with more rapid growth being observed between 40 and 100 days after emergence (Ramanatha Rao, 1988).Groundnut is a self pollinated, annual herbaceous legume, growing upright or prostrate and has an indeterminate growth habit. Natural cross pollination occurs at rates less than 10 % due to atypical flowers (Duke, 1981). The plant is sparsely hairy and generally grows 12-65 cm high.

Plants develop three major stems; the main stem develops from the terminal bud on the epicotyls while the two lateral stems equal in size to the central stem develop from the cotyledonary auxiliary buds. Groundnut develops a well developed taproot with many lateral roots. The flowers are self pollinated around sunrise and within 5-6 hours. Within one week of fertilization, the tip of the ovary bearing about one to five ovules grows out from between the floral bracts bearing with it dried petals, calyx lobes and hypanthia; creating a unique structure called the carpophore, commonly known as peg or gynophore (Ramanatha Rao, 1988). The peg quickly elongates with positive geotropism until it penetrates several centimetres (5-6) into the soil when the tip becomes diageotrophic and the ovary starts developing into a pod (Ramanatha Rao, 1988). Because flowering continues over a long period and because of the relationship between the number of pods per plant and rainfall pattern, pods are in all stages of development at harvest. Pods reach maximum size after two to three weeks in the soil, maximum oil content in six to seven weeks and maximum protein content after five to eight weeks (Ramanatha Rao, 1988).

Considerable variability exists in the groundnut morphological traits; seed size (0.15 to more than 1.3 g/seed), seed colour (white, light rose, rose, red, purple, white, blotched with purple red); number of seeds per pod (1-5), pod length (11-83 mm), and pod breadth (9-27 mm) (Krapovickas and Gregory, 1994, Stalker and Simpson, 1995).

2.5 Ecology of Growth of Groundnuts

Groundnut grows in regions with an average rainfall of 500-1200 mm; thrives best when more than 500 mm of rain is evenly distributed during the growing season Moisture stress during reproductive growth causes embryo abortion, reduces seed set by restricting calcium uptake by the pods and increases aflatoxin contamination of the seeds (Tallury *et al.*, 1995).

Seed is the basis for agricultural productivity and continuity and high levels of field performance (seed quality) are essential for predicting seedling establishment. High seed quality and seedling establishment are considered as prerequisites for profitable, efficient and sustainable crop production (Nigam *et al*., 2004).

Dormancy is an important component of physiological seed quality. It is generally the case that plants with a long history of domestication and plant breeding generally have lower seed dormancy than wild or more recently domesticated species (Gibbons *et al*., 1972). However, dormancy can increase when germination takes place under stress (i.e. poor field conditions).

In practice, dormancy not only affects the number of seeds which germinate, but also their rate of germination especially under sub-optimal conditions. A rapid germination rate is a widely accepted measure of seed vigour; which is a key component of seed quality. Thus, factors that determine both seed dormancy and vigour may overlap.

In cereal crops, a certain degree of dormancy at harvest is a desirable trait because it prevents viviparous germination of grains in the head as result of exposure to cool moist conditions that favour germination (Benech-Arnold *et al.*, 1990). This pre-harvest sprouting in many wheat cultivars, due to low harvest dormancy, has led to reduced seed and grain quality at harvest and therefore serious economic losses.

On the other hand, high harvest dormancy of some cereals such as barley results in extra storage costs because the grain requires after-ripening to achieve the rapid and uniform germination required for the malting process. Thus, a defined level of seed dormancy is an essential component of seed quality (Baskin and Baskin, 1998).

The family *Leguminosae* has been found to have fully developed embryos (Baskin and Baskin, 1998), with some having impermeable seed coat. They include the three subfamilies: *Caesalpinioideae*, *Mimosoideae* and *Papilionoideae*. The hardness and impermeability of the dried testa of seeds of *Leguminosae* is caused mainly by the contraction of the walls of the palisade layer as the seed ripens. For seeds with physical dormancy to germinate, the water-impermeable layer(s) must become permeable, thereby allowing passage of water to the embryo (Baskin and Baskin, 1973). A hard seed coat contributes to the viability of stored seeds. Dormancy and viability can be maintained for long periods in hard-seeded soybean accessions because their seed coats are impermeable to water.

On the other hand, black coated soybean seeds have slower initial imbibition rates higher resistance to field deterioration (Tully *et al.*, 1981) and tougher testa with higher lignin contents and fungicidal properties in comparison with non-black seed coated cultivars. In legumes, white seeds imbibe water more rapidly than coloured seeds and then germinate earlier.

Seeds with unpigmented seed coat deteriorate more rapidly and are more susceptible to imbibition damage (Abdullah *et al.*, 2011; Asiedu and Powell, 1998). An association between rapid imbibition and white or partially white-coated seeds has been observed in cultivars of a large number of legume species; seeds of other colours tend to absorb slowly. This property has been attributed to seed coat permeability, adherence of the seed coat to cotyledons and thickness of the testa.

2.6 Seed quality of groundnuts

Speed of germination or germination rate can be used as a tool for evaluating seedling vigour as seed lots with similar total germination often vary in rate of seedling emergence and rate of growth. It is calculated by summing the number of normal seedlings counted on each day divided by respective number of days after sowing (Macguirie, 1962).

Germination percentage of groundnut seeds can drastically reduce when subjected to accelerated ageing with high relative humidity and temperature as the stress factors and catalysts of the ageing process (Bewley and Black, 1994).

2.7 Development of resistant variety against Mycotoxins

Development of commercially acceptable varieties of groundnut that would resist toxin-producing moulds or completely inhibit production of toxin would be an ideal solution. It has been reported that impermeable seed coat cottonseed have less tendency to allow *Aspergillus flavus* to grow and produce aflatoxins than seed without this 'hard coat' trait, and that possibility exists for mould invasion and hence production of aflatoxin to be controlled by genetic means (Agrios, 1978). A varietal difference in the production of aflatoxins in groundnuts inoculated with atoxigenic strain of *A. flavus* has been reported, but this has not been confirmed. Elimination of

aflatoxin from the human food chain is a goal of many countries but management of aflatoxin in groundnuts is complex. Besides adopting certain cultural and storage practices, resistant cultivars should be an effective and low-cost part of an integrated aflatoxin management program. Four types of resistance to *Aspergillus* have been defined: resistance to *in vitro* seed colonization by *A. flavus* (IVSCAF), field resistance to seed colonization by *A. flavus* (FSCAF), pre-harvest resistance to aflatoxin contamination (PAC), and resistance to aflatoxin production. (Waliyar *et al.*, 1994

Although researchers have not been able to identify germplasm combining all types of resistance mechanisms, it is expected that stable high-level resistance will be achieved by accumulating different resistance genes from different sources into one genotype.

Research directed towards identifying groundnut lines with resistance to toxin-producing moulds is continuing, and appears to show some promise, but this is a long-term approach and no lines have yet been released. Results of these efforts at genetic control of aflatoxin will surely guide analogous efforts with other mycotoxins (Waliyar *et al.,* 1994)

2.8 *Aspergillus* spp. and mycotoxins in seeds

Adams (1977) reported that storage fungi especially *Aspergillus, Penicillium, Rhizopus* and *Mucor* species infect seed after harvest and can grow on them during storage.

Aflatoxin B1 is produced by *Aspergillus terreus,* though it may also be produced by *Aspergillus flavus* as well as *Aspergillus oryzae* (Ellis *et al.,* 1991). It is the most toxic, carcinogenic and most prevalent of the different aflatoxins. Generally, mycotoxins have been implicated as causative agents of different human and animal

health disorders. Both the toxigenic fungi and the mycotoxins they produce are potential problems from both health and economic perspectives (Raffi *et al*., 2006).

Storage fungi are usually not present in large quantities before harvest but are widely distributed and almost always present. Contamination occurs through small quantities of spores contaminating the seed as it is going into storage from the harvest in handling and storage equipment or from spores already present in storage structures (IRRI, 2006). Under high temperatures and moisture this small amount of inoculum can increase rapidly.

2.9 Impact of Storage on Seed quality

Quality characters of seed, such as germination, moisture content, vigour, seed discolouration and seed-borne fungal prevalence are known to be influenced by various factors during storage (Neergaard, 1977). Field fungi like *Alternaria, Aspergillus, Cladosporium*, *Curvularia*, *Fusarium* and *Helminthosporium* invade seeds as they develop on the plants in the field or after they have matured, but before they are harvested (Christensen and Kaufmann, 1969). These fungi usually do not continue to develop on seed after harvest, but may remain alive for years when stored at low moisture content and low temperature (Christensen, 1973).

In general, the damage caused by field fungi is done by the time the crop is harvested, although invasion of the developing or mature embryos of seeds by *Aspergillus* may result in development of discoloured embryos during storage.

The storage fungi, mainly comprising several species of *Aspergillus* and *Penicillium*, do not invade seed to any appreciable degree before harvest (Tuite, 1961), but they can cause severe discolouration of seed in storage resulting in germination failure, discoloured or otherwise damaged embryos or entire seeds and production of mycotoxins that constitute a health hazard for man and animals (Christensen and

Kaufmann, 1969). Each species or group of species of *Aspergillus* has its own minimum limit of moisture content usually between 13 and 18 % for invasion of stored seed. Generally, storage fungi grow rapidly on moist seed at a temperature of about 30 ^{o}C. Therefore, any method of storage, which is aimed at preventing or retarding the invasion of these fungi and creating unfavourable conditions for their growth and multiplication will help in improving the quality of seed. This should include, among other things, the reduction of moisture content and the temperature of the storage receptacle (Malaker *et al*., 2008).

Seeds in storage produce a large pool of fungi, of which *Aspergillus* and *Rhizopus* are found to be the major constituents. *Curvularia* spp., which is common in outdoor air, contributes only a few spores to the airspora in the store.

On the other hand, *Rhizopus* spp. occurs only occasionally. Their study reveals some distinct trends in the frequency of fungal population in seeds. Further, high fungal infection was at the start of the storage experiment due to the prevailing high relative humidity during harvest time. During harvest, infection was mostly induced by the field fungi (miscellaneous fungi), including *Alternaria* spp., *Curvularia* sp., etc. The numbers of field fungi is said to decrease gradually during storage probably because they are replaced by storage fungi, mainly by different species of *Aspergillus,* as found by earlier works (Neergaard, 1977). Seed moisture generally depends on the chemical nature of the seeds. According to Neergaard (1977), seeds with high oil content possess lower moisture than those with high protein or starch. It is well known that water availability, defined as Water Activity (Aw) plays an important role in the deterioration of stored seeds. Water activity may increase as a result of absorption of water from the inter seed atmosphere in order to reach an equilibrium

with the prevailing high RH of the storage atmosphere, particularly during rainy months (Lee and Magan, 2000).

2.10 Aflatoxigenic Fungi

Aspergillus is a large genus comprising more than 180 accepted anamorphic species (Pitt and Samson, 2000), with teleomorphs described in nine different genera. There are seven subdivisions of the genus, which are further divided into two sections. *Aspergillus* subgenus *Circumdati* section *Flavi*, also called the *Aspergillus flavus* group, has attracted attention of the scientific community for its industrial use and toxigenic potential. Section *Flavi* is divided in two groups of species. One of them is the aflatoxigenic species *A. flavus, A. Parasitics* and more recently *A. nomius* which cause serious problems worldwide in agricultural commodities, and the other includes the non-aflatoxigenic species *A. oryzae*, *A. sojae* and *A.tamari*, traditionally utilised for production of fermented foods in Asia (IRRI, 2006).

2.11 Physicochemical Properties of Aflatoxins

Aflatoxins are produced as secondary metabolites by the fungus *A. flavus* and *A. paraciticus* and they contaminate various foods and feeding products. Aflatoxins dissolve in various polar organic solvents including methanol, aqueous acetone and aqueous hexane-acetone-water azeotrope that develop for extraction procedures of natural products. It precipitates in petroleum ether or hexane (Andrallos *et al.*, 1967)

The major members are aflatoxin B1, B2, G1and G2 among the 18 different types of aflatoxins identified. Aflatoxins are fluorescent under the ultra violet light. Aflatoxin B1 and B2 emit blue fluorescence whereas aflatoxin G1 and G2 emit green fluorescence. The quantity and relative proportion of these four compounds in culture extracts vary depending on mould strain, medium composition and culture

conditions. Normally, aflatoxin B1 is present in largest amounts whereas B2 and G2 are produced in small yield (Wogan, 1966).

The molecular formula of aflatoxin B1 and G1 were established as $C_{17}H_{12}O_6$ and $C_{17}H_{12}O_7$ respectively. Aflatoxins B2 and G2 are the dihydro derivatives of the parent compounds, molecular formula of these are $C_{17}H_{14}O_6$ and $C_{17}H_{14}O_7$, respectively. All four aflatoxins have high melting points. The chemical structure of aflatoxin B1, B2, G1 and G2 were proposed in 1963 (Asao *et al.*, 1963).

2.12 Toxicity of Aflatoxins

Evidence as to the acute susceptibility of man to the aflatoxins is very scanty. However, there has been a report from Uganda of a 14 year old boy who died of acute hepatic necrosis. It was found on subsequent examination that the diet (cassava) he consumed contained large amounts of aflatoxins (Hanssen, 1970). Attempts have been made by Shank *et al*. (1972) and Wogan (1966), in Thailand to describe an acute lesion of encephalopathy in children which has a seasonal incidence and is associated with damage to both liver and kidney. Although they did not have direct evidence that this was indeed induced by aflatoxin, the compound was found in the viscera of the children (Shank *et al*., 1972).

One of the first major documented reports of aflatoxins in humans occurred in western India in1974 where 397 persons were affected and 108 persons died. More than 150 villages were involved (Krishnamachari *et al.*, 1975). Recently in Kenya, an incidence of aflatoxin poisoning occurred involving 317 cases and 125 deaths due to consumption of aflatoxins contaminated maize, the largest and most severe outbreak of acute aflatoxicosis documented worldwide (Lewis *et al.*, 2005).

Regulations do little to help reduce aflatoxin and its related health effects in less developed countries. This is because the general focus of rural people is on

availability, and not quality of food. Promoting the adoption of strategies that can control aflatoxin and its associated health risks, in the field, and in postharvest conditions would be laudable (Bankole and Adebanjo, 2003).

2.13 Biocontrol of aflatoxin producing fungi and aflatoxin production

Aflatoxin contamination of crops can be minimized by early harvest, prevention of insect damage, and proper storage (Cotty, 1997). However, under careful management, unacceptable aflatoxin levels may occur from unpreventable insect damage to the developing crop, excessive exposure of the mature crop to moisture either prior to harvest or during storage, handling, transportation or even use (Cotty and Lee, 1989).

Aflatoxins cannot be readily removed from contaminated foods by currently investigated detoxification methods. There is current interest in developing a biological control method hat can increase crop safety by decreasing toxin content which is based on the displacement of toxigenic isolates using atoxigenic isolates of the same species. It has been reported that aflatoxin production is inhibited by lactic acid bacteria, *Bacillus subtilis* and many moulds (Dorothy, 2002). The inhibition may occur from many factors including competition for space and nutrients in general for competition of nutrient required for aflatoxin production but not for growth and production of anti-aflatoxigenic metabolites by co-existing microorganisms.

The bacteria belonging to *Stenotrophomonas maltophilia* are identified for the degradation of aflatoxin B1 by 82.5 % after incubation in the liquid medium at 37 ^{o}C for 72 h. This study indicates that the culture supernatant of isolate was able to degrade Aflatoxin B1effectively whereas the viable cells and cell extracts were less effective. The investigators suspected the enzyme(s) in culture supernatant of the

isolate might be responsible for degrading but recommend further investigation to confirm their findings (Davis *et al*., 1987)**.**

2.14 Health Impacts of aflatoxin

Food safety is an issue which is more often downplayed or overlooked entirely in most African countries since the most important issue of food is not one of quality but of availability. In areas where food shortages are caused by recurrent natural weather phenomena such as drought, safety of food is frequently secondary to issues of food security (Bankole and Adebanjo, 2003). In addition, many subsistence farming communities in Africa rely on the consumption of home-grown crops irrespective of the quality considerations normally applied in the developed world. It is therefore necessary to handle seed so well that it's infection by toxin producing microorganisms would be minimised, if not, prevented (Nautiyal and Joshi, 1991). Nevertheless, some African governments have instituted safety regulations to control mycotoxin, especially aflatoxin contamination of the national food supply and research into natural occurrence of aflatoxins in a range of local foods is widely conducted.

Aflatoxins, fumonisins, and ergot alkaloids have been implicated in acute mycotoxicosis (the result of consumption of high levels of mycotoxins over a short period of time) in both human and farm animals. A number of studies have revealed an association between liver cancer incidence and the aflatoxin content of the diet. These studies have not established a cause and effect relationship but rather suggest an association (Otsuki *et al.,* 2001a). Outbreaks of aflatoxic hepatitis in humans have been reported in India, Kenya, and Malaysia.

The effect of these toxins on the health of organisms can be evaluated under two groups: biochemical and biological effects. From biochemical point of view; they

have effects on energy metabolism, carbohydrate and lipid metabolism, nucleic acid and protein metabolisms. On the biological front, they are highly hepatocarcinogenic (HCC), tetratogenic and mutagenic compounds.

Epidemiological studies carried out in several parts of Africa and Asia indicates a correlation between exposure to aflatoxins and primary liver cancer (Ramesh and Siriguri, 2003). The risks associated with exposure to aflatoxins are enhanced by simultaneous exposure to hepatitis B and possibly hepatitis C viruses.

2.15 Economic impacts of aflatoxin

Mycotoxin contamination in agricultural commodities has considerable economic implication. Losses from rejected shipments and lower prices for inferior quality can devastate developing country export markets. Costs to farmers include reduced income from outright crop losses and lower selling prices for contaminated commodities. The economic impact on livestock production includes mortality as well as reduction in productivity, loss in weight gain, feed efficiency, fertility, and ability to resist disease (Otsuki *et al.*, 2001a).

It is estimated that in Indonesia, the Philippines, and Thailand, 5 percent of the maize and groundnut produced are discarded because of aflatoxin contamination (Ramesh, and Siruguri, 2003).

These figures could even be higher in Ghana where there are generally no verification mechanisms. The annual cost of contamination due to aflatoxin and other moulds in these countries in terms of product spoilage, human health effects, and losses in the poultry and pork sectors was calculated to be 477 million Australian dollars over a decade ago (Otsuki *et al*., 2001a)

On the other hand, one study conducted in University of Hohenheim, Stuttgart, Germany on ‘quantification of the economic impact of EU aflatoxins standards on

developing and transition countries' shows that, 1 % tightening of the standards would reduce trade flows from these countries by 1.07 % (Khachatryan *et al.,* 2005). It estimated that reducing the standard from 20 ppb to 10 ppb in countries where percentage of carriers of hepatitis B1 is around one percent (e.g. members of the European community) would result in a drop in the population risk of approximately 2 cancer deaths a year per billion people (Otsuki *et al.,* 2001a). Based on this study, former UN Secretary General, Kofi Annan, bemoaned the strictness of the standards and the little achievement it makes and called for a more realistic alternative which will help improve trade between countries (Otsuki *et al*, 2001b).

2.16 Prevention and regulation of aflatoxin

At community level, mycotoxin formation in crops can be limited before harvest through good agricultural practices such as rotating crops, irrigating to eliminate drought stress, controlling weeds, cultivating mould-resistant stocks, and introducing bio-controls such as non-mycotoxigenic fungal strains. Post-harvest measures include drying rapidly by mechanical means and keeping crops dry, sorting out contaminated nuts by physical means, sorting by colour, and washing with water to reduce mycotoxins (Nautiyal and Joshi, 1991).

Apart from prevention measures, most importing countries and international and regional organization regulate mycotoxins by setting maximum allowable concentration of mycotoxins, thus affecting international trade. Notably, the European commission has set a total aflatoxin standard of 4 ppb in food and an aflatoxin B1 standard of 2 ppb, considerably more precautionary than any national or international standards currently existing. However, Ghana has not yet adopted such standards for mycotoxins including aflatoxins though the task required to measure aflatoxin and set regulatory control are difficult. Accredited national quality control

laboratories with well trained staff are needed to determine the levels of aflatoxin contamination throughout the cultivation, harvesting and post-harvesting stages, as well as certify the quality of export products at origin.

CHAPTER THREE

3.0 MATERIALS AND METHODS

The field experiment was set up during the major season of 2011 to study the performance of varieties for seed yield and quality and afterwards in the laboratory to examine the effect of two storage practices on the fungi pathogens infection levels as well as aflatoxin content of seeds of the different varieties.

3.1 Field Trial

3.1.1 Soil and climate of experimental site

The field was located at the research fields of The Crops Research Institute of the Council for Scientific and Industrial Research in the semi-deciduous forest zone with elevation of 186 m above sea level and has bimodal rainfall distribution. The soil at the experimental site at Fumesua is Asuansi series, a ferric Acrisol (FAO/UNESCO Legend, 1986). The site was cropped to tomato the previous season.

3.1.2 Meteorological data of experimental site

Meteorological data for the area during entire growing season was obtained from the weather station at the Institute.

3.2 Soil Analysis

Soil samples were taken from different locations of the field and composited to obtain a representative sample which was used for analysis. Two soil depths were sampled for the analysis, which were 0-15 cm and 15-30 cm. Among the various properties that were analyzed include pH, total nitrogen, available phosphorus, exchangeable ions and organic matter.

3.3 Experimental Design

The field was laid out in Randomized Complete Block Design (RCBD). It consists of 10 varieties of groundnut, obtained from the Legumes Division of the Crops Research Institute of the Council for Scientific and Industrial Research (CSIR-CRI). The varieties were Shitaochi, Nkatebroni , Mireku, Kumawu Local, Kwame Danso and Konkoma (local accessions) and Nkosour, Jenkaah, Azizivi , Adepa (improved). The treatments were replicated three times. and in three blocks of Plots which measure 2 m by 4 m. The plant spacing was 30 × 20 cm thereby giving a plant population of 166,660 plants per hectare.

3.4 Cultural Practices

Weed control was carried out as and when necessary. Earthing-up was undertaken on the plants to secure the roots firmly grounded and also to ensure proper peg formation in the soil.

3.5 Data Collection

The following parameters were assessed for on the growth of the crop on the field: percentage field emergence, days to 50 % flowering, number of leaves per plant, days to maturity, plant height, fresh and dry shoot weights.

3.5.1 Percentage field emergence

Percentage field emergence was assessed at 14 days after planting by counting the number of seedlings that have emerged in the two middle rows. The outcome was expressed as a percentage of the total expected from the two rows.

3.5.2 Days to 50 % flowering

A visual assessment which involved the number of days it took for 50 % of the plants from the experimental plots to reach anthesis, was undertaken.

3.5.3 Days to Maturity

The days to maturity was assessed when 70 % or more of the pods were mature. In each plot, 2 plants were uprooted and the number of the mature pods examined by checking the internal blackening of the kernels and also the colour of the kernels in accordance with the suggestions of Williams and Drexler (1981).

3.5.4 Number of Leaves per Plant

This was obtained by counting the number of leaves on five tagged plants in the two middle rows of each plot, at weekly intervals starting from two weeks after planting.

3.5.5 Plant Height

Plant height was measured on five tagged plants with the help of a meter rule. This measurement was taken weekly from the ground level to the highest leaf axils of the main stem.

3.5.6 Shoot fresh and Dry Weight

This was determined from five plants that were cut at ground level weekly from the plots for destructive sampling. The plants were taken from the two penultimate rows; hence the 2^{nd} and 7^{th} rows of each plot. Fresh shoots were weighed and recorded before being dried at a temperature of 80 ^{0}C for 48 hours and weighed using an electronic scale.

3.5.7 Relative Growth Rate (RGR)

The relative growth rate was determined using the formula of Hunt (1978) to provide more informative comparison of relative performance of the plants. The RGR is expressed as:

$$RGR = \frac{InW_2 - InW_1}{T_2 - T_1}$$

Where W_1= the dry mass of initial sampling

W_2=dry mass of the sampling that followed the initial sampling.

The corresponding times of sampling are designated as T_1 and T_2 respectively. In is the natural log of the expression.

3.6 Yield Data

3.6.1 Number of filled Pods per plant

This parameter was estimated at harvest by counting the number of filled pods on the five tagged plants from the two middle rows.

3.6.2 Number of unfilled pods at harvest

The number of empty pods from the same five tagged plants was also taken. Unfilled pods are evidenced with shrivelled seeds when pressed with the fore finger and the thumb. They usually produce a 'pop' sound when pressed.

3.6.3 100 Seed weight

Samples were taken at random from the dried seed lots of the harvest and weighed. The weight was taken for one hundred seeds for each variety.

3.6.4 Shelling percentage

This was calculated as the ratio of seed weight to the pod weight expressed as a percentage.

3.6.5 Pod Yield

Plants from the two middle rows were harvested together, pods collected and sundried to the safe moisture content of about 9 percent.

The pods were then weighed and then the outcome extrapolated to obtain the pod yield in kg/ha using the formula:

$$\text{Pod yield (kg/ha)} = \frac{\text{pod yield (kg)}}{\text{Harvested area (m}^2\text{)}} \times 10000\ \text{m}^2$$

3.6.6 Seed Yield

The pods of the harvested plants from the two middle rows were shelled, and the weight recorded at safe moisture content and also converted using the formula:

$$\text{Seed yield (kg/ha)} = \frac{\text{seed yield (kg)}}{\text{Harvested area (m}^2\text{)}} \times 10000\ \text{m}^2$$

3.7 Laboratory Trials

The laboratory trials were set up at the Department of Biochemistry laboratories at the Kwame Nkrumah University of Science and Technology, Kumasi. Seeds of the ten groundnut varieties were used in the laboratory to test for aflatoxin content after harvesting. The laboratory design was a 2 × 2 × 10 factorial in Completely Randomized Design. This comprised the ten varieties (unshelled) two storage treatments and two different time intervals. The two seed storage treatments were storage in a jute sack without any internal lining and storage in a jute sack with interior polyethylene lining. There were two samplings for analyses at 2 and 4 months following storage. The storage environment was ambient.

3.7.1 Seed quality testing

3.7.2 Germination percentage, seed vigour and dormancy testing

The germination percent of the seeds was tested concurrently with aflatoxin analysis using recommended ISTA rules (ISTA 2007). The first count of seedlings at 10 days was used as an indicator of vigour and the following count at 14 days used for germinability. Also, how long it takes the seed to emerge was recorded in days and the differences among the varieties tested using the formula:

$$SGI = \frac{N1}{D1} + \frac{N2}{D2} + \frac{N3}{D3} + \ldots + \frac{Nn}{Dn}$$

Where,
N : Number of seedlings germinated
D : Number of days taken to germinate the seeds

3.7.3 Post-harvest Seed Pathology

3.7.4 Seed Sampling

At 2 and 4 months after storage, seed of each variety was sampled to obtain a representative sample using the manual hand halving method. In this procedure, the seed lot from which the sample was to be taken was sub-divided into eight different subsamples. Subsample one and seven were combined while subsample two and eight were also combined. The same procedure was repeated for subsamples three and five and four and six respectively.

3.8 AflatoxinAnalysis

3.8.1 Extraction of aflatoxins

Extraction of aflatoxin from the test samples was done according to the official methods of the Association of Official Analytical Chemists (AOAC, 2005).

3.8.2 Running Aflatoxin standards

Aflatoxins standard (AFB1, AFB2, AFG1 and AFG2) was purchased from Sigma Aldrich, Germany. Standard stock solution was prepared according to the Association of Official Analytical Chemists (AOAC) official methods (AOAC, 2005) (0.3 µg/ml for B1 and G1 and 0.1 µg/ml for B2 and G2). Accordingly, a standard calibration curve of five solutions was prepared (5 µg/kg, 10 µg/kg, 15 µg/kg, 30 µg/kg and 50 µg/kg) to calculate aflatoxin contents of each kind of B1, B2,

G1, G2 using chrompass computer software (Calibration curves can be found in the appendix section).

3.8.3 HPLC (High performance liquid chromatography) conditions for aflatoxin analysis

Mobile Phase consists of Water: Acetronitrile: Methanol (60:20:20) with addition of 120 mg of potassium bromide and 350 ul of nitric acid per litre of mobile phase

Column specifications: 30 cm long x 4.6 mm wide, 5 um Supelco C-18

Detector: Fluorescence Detector with Excitation Wavelength of 365 nm and Emission Wavelength of 435nm

Flow rate: 0.8 ml/min

Injection Volume: 100 ul per injection

Table 3.1 Standard Preparation for Calibration Curve

STANDARD	CONCENTRATION (NG/G)	G2 (NG/G)	G1 (NG/G)	B2 (NG/G)	B1 (NG/G)
1	104	14	40	14	40
2	208	24	80	24	80
3	312	36	120	36	120

3.8.4 Seed Health Testing

The Blotter Method of seed health testing was used for the detection of all fungal seed microflora on the seeds used in the investigations. Four hundred untreated pure seeds from each of the samples were plated on 3 moistened blotters (Whatman No. 1) in a 9 cm diameter Petri dish at the rate of 20 seeds per dish and the seeds were incubated for 7 days at 20-25 ^{o}C under alternating cycles of 12 hours near ultraviolet light and 12 hour darkness. Seeds were examined under a stereomicroscope for the presence and absence of fungi. Identification was confirmed by examining for the presence of mycelium and/or conidia under a compound microscope.

3.9 Data Analysis and Presentation of results

Data collected was subjected to analysis of variance (ANOVA) using Genstat Statistical Package. Least significant difference (LSD) test was used to compare the differences among the means.

CHAPTER FOUR

4.0 RESULTS

4.1 Soil analysis and weather data of experimental site

Organic carbon was higher in the topsoil (0 -15 cm depth) than in the 15-30 cm depth for the soil sample analysed. The same was true for organic matter, and all the major nutrients comprising total nitrogen, calcium and phosphorus. Of all the exchangeable ions tested, only Magnesium was found to be higher in the 15-30 cm depth than in the 0-15 cm depth. A textural survey of the area revealed the soil was a sandy loam, and the effective cation exchange capacity was 8.18 mol/kg in the 0-15 cm depth while that of the 15-30 cm depth was 5.86 mol/kg.

Table 4.1 a Soil physical and chemical characteristics of experimental field

Soil Depth (cm)	Organic C %	Organic Matter %	Total Nitrogen	K	Na	Ca	Mg	Al	H
				(cmol/kg)					
0-15	0.98	1.69	0.13	2.26	0.32	4.3	1.3	0.5	2.7
15-30	0.92	1.59	0.11	0.24	0.22	4	1.4	0.4	2.4

Table 4.1 b Soil chemical and textural properties of the experimental field

Soil depth (cm)	Available P (mg/kg)	pH	% Sand	% Clay	% Silt	ECEC mol/kg
0 - 15	135.14	6.56	72.55	15.69	11.76	8.18
15 - 30	120.05	6.55	74.51	11.76	13.73	5.86

A total of 596.20 mm of rain was recorded over the growing period with the month of August receiving the least amount of rainfall and June receiving the highest amount. Relative humidity ranged from a low of 88.23 % during the month of June to the highest of 90.63 % in September. The highest average maximum temperature recorded for the area was 32.25 ^{o}C in September while the least minimum temperature was 18.81 ^{o}C in August. A total of 56 rainy days was experienced with the month of August recording the least (8) number of days with rainfall activity.

Table 4.1c Weather Data of the growing period

Month	Temperature (^{o}C) Max	Min	Mean	Relative Humidity (%)	Total Rain (mm)	Rainy Days
June	32.98	19.62	25.4	88.23	283.03	18
July	31.74	18.98	24.35	89.91	146.6	12
August	31.05	18.81	24	90.32	41.2	8
September	32.25	20.08	24.51	90.63	121.77	18
Total					592.6	56

4.2. Field emergence, flowering and maturity of the varieties evaluated

The field emergence of the ten varieties ranged from 7 to 9 days with all the improved varieties emerging later than the local types. Fifty percent of all the local varieties had emerged at the seventh day's count. There were however significant differences between all the improved varieties and the local varieties in respect of

emergence. The number of days to flowering, however, differed significantly ($p \leq 0.05$) only between the local variety Mireku and the improved variety Jenkaah with 24 and 28 days respectively to flowering. The remaining varieties within the range are from 25 to 27 days with the improved varieties still taking more days to flower than the local varieties.

Mireku had the lowest days to maturity of 85 days while Kumawu Local had the highest of 105 days. Significant differences were observed in mean days to maturity between the tested varieties. Kumawu Local variety and Azizivi exceeded 100 days while Mireku, Konkoma and Nkosour took just under 90 days to reach maturity. Significant differences ($p \leq 0.05$) were observed between all but one of the improved varieties and all except one of the local varieties.

Table 4.2 Field emergence, flowering and maturity of the varieties evaluated

Variety	Days to 50 % field emergence	Days to 50 % flowering	Mean days to maturity
Mireku	7	24	85
Kwame Danso	7	26	92
Konkoma	7	25	86
Nkate Broni	7	25	99
Kumawu Local	7	26	105
Shitaochi	7	25	91
Adepa	9	27	99
Azizivi	9	27	102
Nkosour	9	27	88
Jenkaah	9	28	100
Mean	8	26	95
L.S.D (0.05)	2	3	12
C.V (%)	9.5	6.50	7.50

4.3 Plant Height of the varieties evaluated

Significant differences were observed between the varieties at the various sampling periods. At one week after emergence (WAE), the local varieties were taller than the improved varieties. Mireku was progressively the shortest variety across the entire sampling period, finishing as the shortest with just 18.27 cm average height. This trend generally continued throughout the sampling period. Significant differences were observed between the local varieties and the improved varieties at first WAE. Except for Nkosour and Jenkaah, there were significant differences ($p \leq 0.05$) between Konkoma and all the remaining 7 of the 10 varieties evaluated. There were significant differences ($p \leq 0.05$) between Mireku and Nkate Broni, Shitaochi, Konkoma and Kwame Danso.

Of the varieties used in this study, Mireku, Kwame Danso, Konkoma, Shitaochi, Nkate Broni and Jenkaah are of the sub-species *fastitiata* while Adepa, Azizivi, Nkosour and Kumawu Local are *hypogaea* sub-species. Among the varieties belonging to the species of *hypogaea*, Adepa and Kumawu Local were each found to have significant ($p \leq 0.05$) differences between Shitaochi, Mireku, Nkate Broni and Konkoma. On the contrary, Nkate Broni and Kwame Danso recorded the tallest and second taller plants respectively to make the *fastigiata* dominate over the *hypogaea* as the taller of the two sub-species.

Table 4.3 Mean plant height (cm) of the varieties at different weeks after planting

Variety	1WAE	2WAE	3WAE	4WAE	5WAE
Mireku	10.20	15.60	17.93	17.67	18.27
Kwame Danso	16.40	25.13	29.93	34.70	35.73
Konkoma	12.90	19.60	22.63	25.73	27.67
Nkate Broni	18.20	28.13	34.40	38.53	38.93
Kumawu Local	15.47	23.53	28.80	32.60	35.27
Shitaochi	10.50	15.97	18.40	21.57	20.40
Adepa	10.80	15.13	17.87	21.03	24.13
Azizivi	9.43	14.30	15.97	18.48	20.40
Nkosour	11.80	17.30	20.67	22.83	23.80
Jenkaah	11.80	16.90	19.50	22.80	24.47
Mean	12.75	19.16	22.61	25.59	26.91
L.S.D (0.05)	1.70	2.50	3.16	4.47	3.93
CV (%)	18.50	18.10	19.30	24.20	20.20

4.4. Number of leaves per plant of the varieties evaluated

Mean leaf number increased gradually from 1WAE to 4 WAE and decreased marginally at 5WAE. Average leaf number increased 35 % from 1 WAE to 2 WAE, 25.9 % from 2 WAE to 3 WAE, 55.9 % from 3 WAE to 4 WAE

The varieties differed significantly ($p \leq 0.05$) at all sampling stages. At 1WAE (Table 4.4) significant differences were observed among the varieties with Adepa, Nkosour, Jenkaah, Azizivi and Nkate Broni being significantly ($p \leq 0.05$) lower than the other varieties.

The *fastigiata* species Mireku, Kwame Danso, Konkoma, Shitaochi, Nkate Broni and Jenkaah were found to have less leaves as opposed to the *hypogaea* which seemed to have more leaves. Nkosour had the highest number of leave at the end of the 5 week sampling period with the local variety Shitaochi recording the least number of leaves.

Table 4.4 Number of leaves per plant at different weeks after planting

Variety	1WAE	2WAE	3WAE	4WAE	5WAE
Mireku	27	42	45	74	70
Kwame Danso	34	45	53	76	89
Konkoma	31	63	54	71	66
Nkate Broni	42	59	61	86	88
Kumawu Local	30	38	43	68	84
Shitaochi	28	35	41	57	65
Adepa	50	65	125	143	156
Azizivi	49	64	91	111	171
Nkosour	48	62	83	130	143
Jenkaah	59	71	88	244	119
Mean	40	54	68	106	105
C.V (%)	23.30	43.30	7.50	11.8	32.20
L.s.d (0.05)	7.00	17.00	37.00	85.00	24.00

4.5 Fresh shoot weight of varieties over five week period (kg/ha)

There was rapid increase in the fresh shoot yield from the first week to the fourth week and then there was a gradual increase afterwards (Table 4.5). Nkate Broni recorded the highest shoot yield for the first and second samplings while Nkosour and Kumawu local followed the same trend at the lower end of the comparison respectively. Nkate Broni was significantly higher than all the varieties during the first week. Also, Kwame Danso was found to be significantly ($p \leq 0.05$) higher than Azizivi and Nkosour during the same period. During the penultimate week of sampling, Adepa and Jenkaah were found to be significantly ($p \leq 0.05$) higher than Azizivi and generally higher than all the other varieties in the trial. Among the local varieties, Kwame Danso and Nkate Broni were also significantly ($p \leq 0.05$) higher than Azizivi. Similar trend was observed during the final week of sampling with the two improved varieties Adepa and Jenkaah and two local varieties, Kumawu local and Kwame Danso recording significant yield over Azizivi, an improved variety.

When compared in the light of *hypoggaea* and *fastigiata*, the trend is the same. The two improved varieties which were significant over Azizivi are *hypogaea* while the other two local varieties were also members of the *fastigiata*.

Table 4.5 Fresh shoot weight (FSW) of varieties over five week period (kg/ha)

Variety	FSW 1	FSW 2	FSW 3	FSW 4	FSW 5
Mireku	1473	1798	2259	3372	3851
Kwame Danso	1525	2226	3138	4912	5477
Konkoma	1298	2205	2864	3767	4122
Nkate Broni	2126	2796	3535	4780	5335
Kumawu Local	1299	1621	1898	3546	3966
Shitaochi	1349	1936	2298	3792	4192
Adepa	1255	2186	3114	5044	5417
Azizivi	1071	1634	2238	2450	2648
Nkosour	1065	1810	2859	4116	4483
Jenkaah	1327	2208	3203	5050	5404
Mean	1379	2042	2741	4083	4490
L.S.D (0.05)	388	661	1372	1911	2041
C.V (%)	16.50	19.00	29.40	27.50	26.70

4.6 Shoot dry weight and relative growth rate of the varieties evaluated

Dry weight of the varieties increased from the first week of sampling at 1WAE until the final sampling week at 5 WAE. The increase, however, was highest between the third and fourth week of sampling (Table 4.6) and the rate of increase decreased in the following week. The mean dry weight of Nkate Broni was 63.8 g, the highest in the first week showing significant ($p \leq 0.05$) differences with all the varieties but it finished as the fourth highest at the end of the sampling period of 5 WAE. Two improved varieties, Adepa and Jenkaah were among four varieties with the highest dry shoot weight; the remaining two being Kwame Danso and Nkate Broni. In all, the *hypogaea* sub-species were averagely higher ($p \leq 0.05$) in terms of dry shoot weight compared to those of the *hypogaea*.

Table 4.6 Effect of variety on shoot dry weight (g) at different weeks of sampling

Variety	1WAE	2WAE	WAE	4WAE	5WAE
Mireku	44.20	53.90	67.80	101.20	115.50
Kwame Danso	45.80	66.80	94.20	147.40	164.30
Konkoma	38.90	66.20	85.90	113.00	123.60
Nkate Broni	63.80	83.90	106.10	143.40	160.10
Kumawu Local	39.00	58.10	92.50	106.40	119.00
Shitaochi	40.50	48.60	69.00	113.80	125.80
Adepa	37.60	65.60	93.40	151.30	162.50
Azizivi	32.10	49.00	67.10	115.70	124.70
Nkosour	32.00	54.30	85.80	123.50	134.50
Jenkaah	39.80	66.20	96.10	151.50	162.10
Mean	41.40	61.30	85.80	126.70	139.20
L.S.D ((0.05)	12.01	18.35	23.23	43.38	45.61
C.V (%)	16.90	17.50	15.80	20.00	19.10

4.7 Number of Pods per plant, filled and unfilled pods of the varieties evaluated

Among the varieties, Azizivi had the highest number of pods per plant (38) while Mireku had the least number (27) of pods per plant. There were significant differences in the number of pods per plant between Konkoma and Mireku, a local variety and Azizivi, an improved variety. Most of the varieties including improved ones were within the range of 28-36 pods per plant. Azizivi (38) was significantly higher ($p \leq 0.05$) than Shitaochi, Nkosour, Nkate Broni and Kwame Danso.

Azizivi, an improved variety had the highest number of filled pods (29) while Nkate Broni, a local variety and Nkosour (improved) recorded the least (17). In terms of filled pods, Azizivi was significantly higher than Nkate Broni and Nkosour. With the number of unfilled pods, Konkoma had the highest while Mireku had the least. There were significant differences ($p \leq 0.05$) between these two varieties. Most of the improved varieties had less number of unfilled in pods contrast with those of the

local varieties. Generally, species of *hypogaea* also had less number of empty pods than those belonging to the *fastigiata* sub-species. All the varieties had more number of filled pods than unfilled pods.

Table 4.7 Average number of pods, filled and unfilled pods per plant of the varieties

Variety	Pods/plant	Filled Pods	Unfilled Pods
Mireku	27	19	8
Kwame Danso	30	18	12
Konkoma	36	20	16
Nkate Broni	29	17	12
Kumawu Local	33	19	14
Shitaochi	30	18	12
Adepa	35	23	12
Azizivi	38	29	9
Nkosour	28	17	11
Jenkaah	32	22	10
Mean	32	20	12
L.S.D (0.05)	8	12	7
C.V (%)	15	36	36

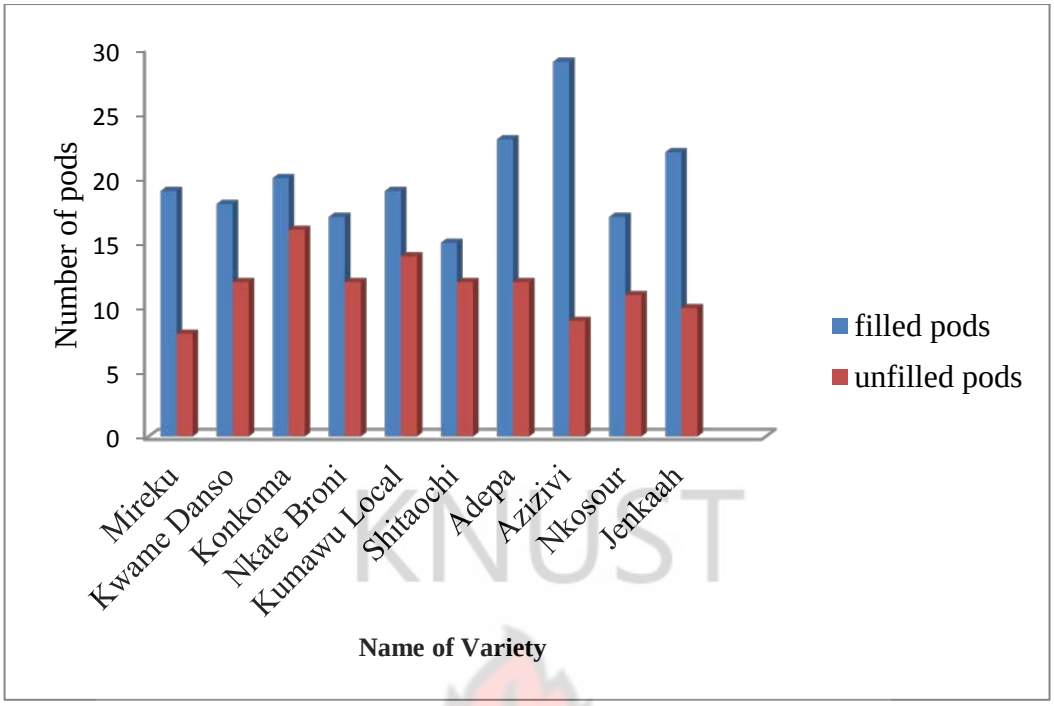

Figure 4.1 Comparison of filled and unfilled pods of the varieties

4.8 Pod and seed yield as influenced by variety

The means of the pod and seed yield of the varieties are displayed in Table 4.8. The results of the analysis showed significant differences ($p \leq 0.05$) in the varieties in terms of pod and seed yield. The pod yield of Mireku (1635 kg/ha) was significantly lower than Nkate Broni, Kwame Danso, Shitaochi and Konkoma.Nkate Broni had the highest yield of pods (2399 kg/ha) among the local varieties while Mireku had the least (1635 kg/ha). Among the improved varieties, Adepa had the highest pod yield (2317 kg/ha) while Azizivi had the least (1883 kg/ha). The percentage difference between the two high yielding varieties was approximately four.

The seed yield of the varieties differed significantly. Nkate Broni had the highest with 1500 kg/ha of seed while Mireku again recorded the least weight of seed (1022 kg/ha) yield. The seed yield of Nkate Broni was significantly ($p \leq 0.05$) higher than

Mireku, Azizivi and Kumawu Local. Adepa and Azizivi had the highest (1448 kg/ha) and the least (1177 kg/ha) amount of seed respectively among the improved varieties while that of the local varieties were Nkate Broni (1500 kg/ha) and Mireku (1022 kg/ha) respectively. Figure 4.2 shows the degree of relationship between pod yield and shoot dry weight. The degree of relationship as shown on the figure was $r^2 = 0.51$.

Table 4.8 Influence of variety on pod and seed yield of groundnut

Variety	Pod yield (kg/ha)	Seed yield (kg/ha)
Mireku	1635	1022
Kwame Danso	2294	1434
Konkoma	2240	1400
Nkate Broni	2399	1500
Kumawu Local	1833	1145
Shitaochi	2241	1400
Adepa	2317	1448
Azizivi	1883	1177
Nkosour	2036	1273
Jenkaah	2140	1337
Mean	2102	1314
L.S.D (0.05)	545	345
C.V (%)	31	32

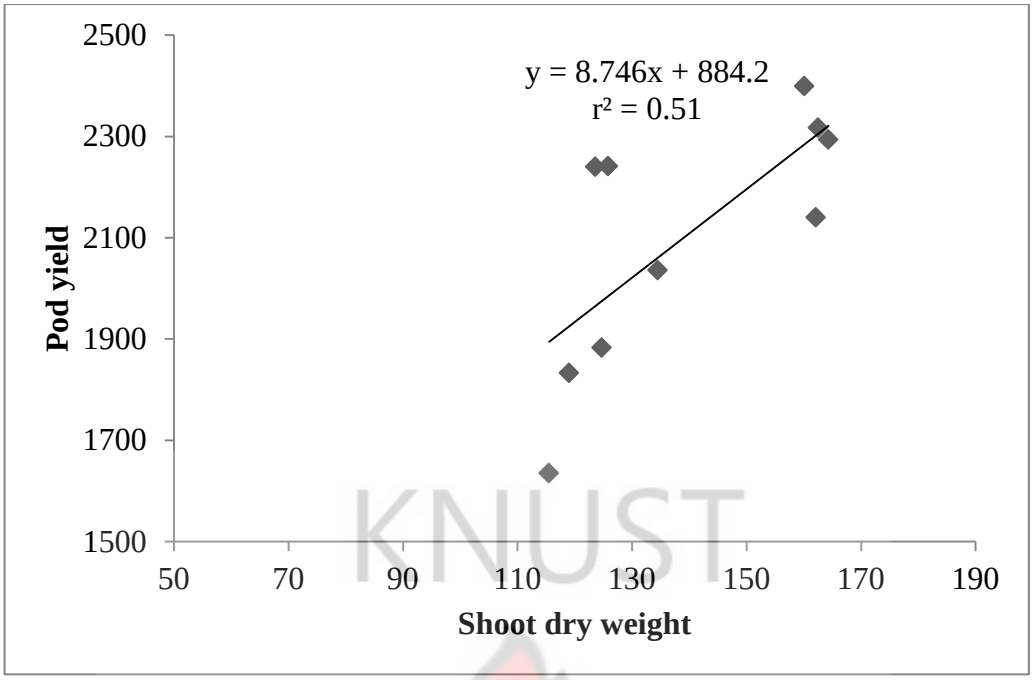

Figure 4.2 Relationship between pod yield and shoot dry weight

4.9. Hundred seed weight, shelling percentage and harvest index as affected by variety

Table 4.9 shows the means of the different varieties in terms of shelling percentage, 100 seed weight and harvest index. Konkoma and Azizivi had higher 100 seed weights of 64.8 g and 64.1 g compared with all the other varieties and same were significant ($p \leq 0.05$) compared with all the local varieties. There were no significant differences among the improved varieties in terms of 100 seed weight. Mireku and Azizivi had the highest and least shelling percentage of 76.8 and 58.4 respectively.

The harvest index of these two varieties also followed the same trend. The shelling percentage of Mireku was significantly ($p \leq 0.05$) higher than the two improved varieties namely Azizivi and Adepa.

Table 4.9 Effect of variety on hundred seed weight, shelling percentage and harvest index

Variety	100 Seed Weight (g)	Shelling Percentage	Harvest index
Mireku	47.5	76.80	0.32
Kwame Danso	51.8	64.40	0.31
Konkoma	47.7	72.40	0.29
Nkate Broni	51.3	63.90	0.21
Kumawu Local	64.8	67.90	0.20
Shitaochi	47.5	63.60	0.35
Adepa	59.3	62.60	0.15
Azizivi	64.1	58.40	0.10
Nkosour	57.0	63.80	0.17
Jenkaah	56.2	65.40	0.16
Mean	54.7	65.90	0.23
L.S.D (0.05)	10.7	14.60	0.15
CV (%)	14	12.90	4

4.10 Vigour, germination and speed of germination

Nkate Broni had the highest germination percentage (86), vigour (73.67) and speed of germination (18.73) while Adepa recorded the lowest of 66 %, 49 and 13.38 respectively in all three parameters (Table 4.10). In terms of germination percent, significant difference ($p \leq 0.05$) was observed between Adepa, an improved variety and Jenkaah and all the local varieties except Kumawu Local. The same trend was observed in the vigour and speed of germination parameters. Generally, the local varieties had higher germination percentage, vigour and speed of germination than the improved varieties. The *fastigiata* species mainly germinated more than their *hypogaea* counterparts.

Table 4.10 Effect of variety on vigour, germination and speed of germination

Variety	Germination %	Vigour	speed of germination
Mireku	79	64.00	16.61
Kwame Danso	79	67.00	17.15
Konkoma	79	67.33	17.14
Nkate Broni	86	73.67	18.73
Kumawu local	67	52.00	13.95
Shitaochi	79	66.00	17.08
Adepa	66	49.33	13.38
Azizivi	69	56.00	14.66
Nkosour	73	60.00	15.64
Jenkaah	81	68.67	17.60
Mean	75.80	62.40	16.19
LSD (0.05)	10.30	8.04	1.90
C.V (%)	7.90	7.50	6.80

4.11 Dead seeds, hard seeds and seed moisture content

The results from Table 4.11 show that Nkosour and Jenkaah, both improved varieties had fewer number of dead seeds just like Kwame Danso, Nkate Broni and Shitaochi (local varieties). Azizivi had the highest number of dead seeds (7) while Nkate Broni had the least (2). There was significant difference ($p \leq 0.05$) between Azizivi and

Jenkaah, Nkate Broni and Kwame Danso. There was also a significant difference between Nkate Broni and Konkoma, Mireku, Kumawu local and Adepa. For hard seeds, Shitaochi had the least (4) while Kumawu Local, Adepa and Azizivi had nine each. Shitaochi was significantly ($p \leq 0.05$) lower than Kumawu Local, Adepa and Azizivi. The range of moisture content was highest for Nkate Broni (9.25 %) and lowest for Adepa (8.45 %). There were significant ($p \leq 0.05$) differences between only these two varieties.

Table 4.11 Number of dead seeds, hard seeds and seed moisture content before storage

Variety	Dead seeds	Hard seeds	Seed Moisture content
Mireku	6	6	8.88
Kwame Danso	3	6	8.83
Konkoma	6	6	8.62
Nkate Broni	2	4	9.23
Kumawu Local	6	9	9.25
Shitaochi	4	4	8.73
Adepa	6	9	8.45
Azizivi	7	9	8.99
Nkosour	4	8	8.70
Jenkaah	3	5	8.61
Mean	5	7	8.83
LSD (0.05)	4	5	0.77
CV (%)	5.30	15	5.10

4.12 Description of the storage environment

The range of temperature was between 16.44 ^{o}C and 34.89 ^{0}C recorded for the months of October and December and 34.89 ^{o}C during the same period. Relative humidity ranged from 60.90 % in November to being saturated at 100 % both in November and December. The least rain was recorded in December while the highest amount of rainfall was received in October as shown in Table 4.12.

Table 4.12. Average relative humidity, temperature and rainfall in the storage environment

MONTHS	ENVIRONMENT	AVERAGE	MAXIMUM	MINIMUM
SEPTEMBER	RELATIVE HUMIDITY (%)	92.33	99.90	63.27
	TEMPERATURE (^{o}C)	28.67	31.25	20.10
	RAINFALL (MM)	35.80		
OCTOBER	RELATIVE HUMIDITY (%)	81.78	98.20	60.00
	TEMPERATURE (^{o}C)	25.72	34.89	16.44
	RAINFALL (MM)	296.62		
NOVEMBER	RELATIVE HUMIDITY (%)	60.90	100	52.60
	TEMPERATURE (^{o}C)	26.17	33.89	20.06
	RAINFALL (MM)	44.61		
DECEMBER	RELATIVE HUMIDITY (%)	73.25	100	18.20
	TEMPERATURE (^{o}C)	25.81	34.89	16.44
	RAINFALL (MM)	0.2		

4.13. Aflatoxin levels (ppb) in fresh seed samples before storage of groundnuts

Table 4.13 shows the level of aflatoxin in seeds before storage was imposed. Four varieties, namely Mireku, Konkoma, Adepa and Azizivi recorded some levels of aflatoxin. The amount recorded ranged from as low as 0.013 ppb for Adepa to just

about 0.070 ppb for Konkoma. All six remaining varieties did not have any amount recorded for their analyses.

Table 4.13.Aflatoxin levels (ppb) in seed samples before storage

Variety	Aflatoxin content (ppb)	Remarks
Mireku	0.023	Very low
Kwame Danso	0	Absent
Konkoma	0.07	Very low
Nkate Broni	0	Absent
Kumawu Local	0	Absent
Shitaochi	0	Absent
Nkosour	0	Absent
Adepa	0.013	Very low
Azizivi	0.023	Very low
Jenkaah	0	Absent

4.14. Aflatoxin levels (ppb) in seed samples two months after storage of groundnuts

The levels of aflatoxin in Table 4.14 show that, Jenkaah, Azizivi, Kumawu Local, Mireku and Shitaochi had no aflatoxin recorded irrespective of the storage method used. Nkosour recorded the highest aflatoxin amount under jute bag storage only. There were no aflatoxins detected in Kumawu Local, Shitaochi, Adepa, Azizivi and Jenkaah under jute bag only while under polyethylene and jute bag combination, Mireku, Konkoma, Nkate Broni, Kumawu Local, Shitaochi and Azizivi had no aflatoxins.

Table 4.14. Aflatoxin levels (ppb) after two months in stored samples of groundnuts

	AFLATOXIN CONTENT (PPB)		AFLATOXIN CONTENT (PPB)	
VARIETY	JUTE BAG STORAGE	REMARKS	JUTE BAG WITH POLYETHYLENE LINING	REMARKS
MIREKU	0	ABSENT	0	ABSENT
KWAME DANSO	0.025	VERY LOW	0.147	VERY LOW
KONKOMA	0.010	VERY LOW	0	ABSENT
NKATE BRONI	0.040	VERY LOW	0	ABSENT
KUMAWU LOCAL	0	ABSENT	0	ABSENT
SHITAOCHI	0	ABSENT	0	ABSENT
NKOSOUR	148.21	VERY HIGH	0	ABSENT
ADEPA	0	ABSENT	0.049	VERY LOW
AZIZIVI	0	ABSENT	0	ABSENT
JENKAAH	0	ABSENT	0	ABSENT

4.15. Aflatoxin levels in seed samples four months after storage of groundnuts

The levels of aflatoxin ranged from zero parts per billion for Mireku, Adepa, Azizivi and Jenkaah with Jute bag storage and Konkoma and Nkate Broni under polyethylene and jute bag combination to as high as 410.974 ppb for Kwame Danso, also under polyethylene and jute bag storage (Table 4.15). Only Kwame Danso, Nkosour and Adepa had some amount detected in them in the jute bag storage. Four varieties, namely Mireku, Konkoma, Nkate Broni and Jenkaah had no levels of aflatoxin detected irrespective of the storage method used. The aflatoxin level of Kwame Danso in polyethylene and jute bag combination was 410.974 ppb, several hundred folds more than those in other varieties.

Nkosour and Adepa, under jute bag storage at 2 months and four months respectively recorded very high levels of aflatoxin B1 and B2. While Nkosour had 148.304 ppb

for aflatoxin B1 and 53.459 ppb for aflatoxin B2, Adepa had 24.029 ppb of aflatoxin B2 but no aflatoxin B1. On the other hand, Kwame Danso, under jute bag and polyethylene storage combination recorded the highest levels of aflatoxin B1 (337.870 ppb) and B2 (72.614 ppb) in this trial.

Table 4.15. Aflatoxin levels (ppb) after four months in stored samples of groundnuts

	AFLATOXIN CONTENT (PPB)		AFLATOXIN CONTENT (PPB)	
VARIETY	JUTE BAG STORAGE	REMARKS	JUTE BAG WITH POLYETHYLENE LINING	REMARKS
MIREKU	0	ABSENT	0	ABSENT
KWAME DANSO	0.025	VERY LOW	410.974	VERY HIGH
KONKOMA	0	ABSENT	0	ABSENT
NKATE BRONI	0	ABSENT	0	ABSENT
KUMAWU LOCAL	0	ABSENT	0.023	VERY LOW
SHITAOCHI	0	ABSENT	0.057	VERY LOW
NKOSOUR	0.040	VERY LOW	0.035	VERY LOW
ADEPA	45.918	VERY HIGH	0.146	VERY LOW
AZIZIVI	0	ABSENT	0.021	VERY LOW
JENKAAH	0.003	VERY LOW	0	ABSENT

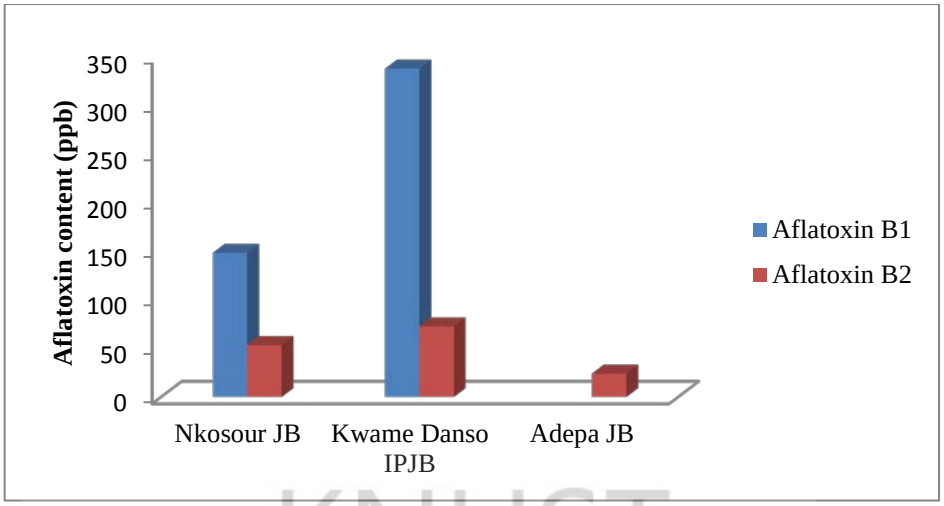

Figure 4.3. Comparison of varieties with high aflatoxin B1 and B2 contents

JB - Jute bag, IPJB - Interlaced polyethylene jute bag

4.16 Fungi pathogens detected from seed samples under the study

Various fungi pathogens associated with the crop were detected on the seed before the start of storage and also during storage. Major pathogens detected are fungi of the genus *Aspergillus, Rhizopus* and *Fusarium* (Table 4.16). Very moderate to high percentages of *A. spp, Fusarium, Rhizopus* and other undefined species were found on the seed surface when examined after storage. The fungi were ubiquitous in all the samples irrespective of the storage method employed. Initial percentage seed infection averaged about 20 % but increased over the first two months. Between the first two months and the fourth month, there was a marginal increase in the seed infection rates compared with the increase between start of storage and two months into storage.

Table 4.16. Percentage infection of fungal pathogens detected in the groundnut seed samples

Variety	*A. flavus*	*A. parasiticus*	*Fusarium* spp.	*Rhizopus* spp.	*Undefined* spp.
Mireku	30.00	16.67	36.67	26.67	20.00
Kwame Danso	26.67	20.00	33.33	30.00	6.67
Konkoma	23.33	23.33	26.67	36.67	16.67
Nkate Broni	26.67	20.00	40.00	23.33	10.00
Kumawu Local	20.00	16.67	50.00	20.00	20.00
Shitaochi	46.67	23.33	40.00	30.00	10.00
Nkosour	23.33	13.33	20.00	36.67	13.33
Adepa	43.33	23.33	30.00	30.00	20.00
Azizivi	33.33	23.33	36.67	23.33	23.33
Jenkaah	33.33	16.67	43.33	26.67	10.00

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 The physical and chemical properties of soil at the experimental site

The study area was a sandy loam preferred for groundnut production in the tropics and sub-tropics. There was high amount of phosphorus because the area had been previously amended for crops that preceded the trial. Low pH aggravates the problem of Ca deficiency in the pod-forming region of the groundnut for good groundnut yields. However, as the Ca concentration increases, the negative effect of pH on seedlings lowers while germination increases. When Ca concentration is less than 1.0mM, there is higher negative effect of low pH causing survival of less numbers of seedlings. This could be the liming effect of Ca in raising pH for suitable substrate that supports crop growth (Zharare *et al*., 1998)

Exchangeable bases were generally low but they did not have any significant effects on the pH since the pH level was acceptable. Organic matter valuation was low humic in both depths of soil.

An optimal pH range of 5.62-6.69 is recommended for improved yield for groundnuts. This falls within the pH range of 5.5 – 6.5 widely reported to be good for groundnut production (Murata *et al*., 2007). Calcium requirement for groundnut is greatest at start of gynophores swelling and early pod stages.

Murata *et al*. (2003) observed that pod set, growth and development were enhanced at pH levels above 5 and declined rapidly when the pH was less than 5. They also reported that pod and seed production were severely retarded at pH of 3 resulting in almost no pod production.

5.2 The seed yield and quality of the varieties

Mireku flowered earliest and also matured earlier than all the other varieties in this study. Although Nkosour took longer time to flower, it matured earlier. The maturity days were generally shorter for the *fastigiata* than those of the *hypogaea*. Upadhaya *et al*. (2009) observed among other parameters, that it took an average of 8 days for seeds to germinate, between 19 and 25 days to flower and between 110 and 120 days to mature. Nigam *et al*. (1983) reported that these differences are due to the different market types, and that Valencia and Spanish types flower earlier and mature faster than Virginia types that flower later and mature late. These findings also agree with those of Kamara *et al*. (2011) who observed that, Shitaochi, a Spanish market type flowered faster and matured earlier than Nkosour, a member of the Virginia group. Omokanye *et al*. (2001) observed that, RMP, one of the varieties studied took more than 127 days to mature, going beyond the actual duration of the season. This, they said was not unexpected because of its inherent capacity for dormancy as a member of the subspecies *hypogaea* which generally took more days than the *fastigiata* species to mature. Kamara *et al*. (2011) noted that, differences in days to maturity were due to the different market types and subspecies. Given the maturity days in the current study, the materials could be said to have early maturity.

In the current study, the plant dry weight increased rapidly initially until the rate of increase decreased from the fourth week. This could be due to the onset of pod formation and the partitioning of more assimilates towards the sink than any other part of the plant during the reproductive stage. It could also be due to the fact that most leaves had got their photosynthetic ability reduced and so did not contribute significantly to the dry matter build-up. This was due to the decline in leaf area characterised by senescence and consequently less photosynthesis due to same. The

improved varieties began with low shoot dry weight but their average increase of 45 % ensured that they matched with the local varieties at 5 WAE. Adepa, an improved variety, finished at the last sampling (5 WAE), in second place with a shoot dry weight of 162.5 g with an average relative crop growth rate of 0.09 g/g day, only second to Kwame Danso, a local variety which finished first with a relative crop growth rate of 0.15 g/g day and shoot dry weight of 164.30 g.

Members of the *fastigiata* were generally taller than those of the *hypogaea*. This could be as a result of their generally erect growth habit. The *hypogaea* were generally shorter possibly due to their spreading growth habit. The *hypogaea* plants were generally about 4-10 % shorter than the *fastigiata* in this study. On the other hand, local varieties were generally 26 % taller than the improved varieties.

The 100 seeds weight ranged from 47.5 g to 64.8 g for the varieties handled under this trial. Kumawu local (64.8 g) and Azizivi, (64.1 g) both *hypogaea,* were significantly higher than all the *fastigiata* subspecies. Pod yields of cultivars had an average of 2.59 t/ha. Vichai and Suteera (2006) observed differences among local accessions than advanced breeding lines that were evaluated. The minimum values obtained for the cultivars in this trial (13.38 for Adepa) and 13.95 for Kumawu Local) were higher than the upper limit of that found by Vichai and Suteera (2006) for reason of good soil and uniform rainfall distribution at the early stages of crop growth.

Kale *et al*. (2010) observed that, advanced breeding lines out yielded the local check in all qualities assessed. Kajgopal and Chandran (2000) reported significant differences in pod yield, shelling percentage and haulm yield in addition to other yield attributes for groundnut.

In the present study, a short dry spell in August may be responsible for the poor pod filling which resulted in generally low pod yield. This is because the crop was in the pod filling stage when the dry spell occurred.

Four varieties, Jenkaah, Adepa (improved) and Kwame Danso and Nkate Broni yielded forage in excess of 5 t/ha. This can be attributed to the good rainfall distribution obtained during the early growing period. This may be a good reason to combine varieties for dual purpose since the forage demand by livestock during the rainy season may be met (Larbi *et al*., 2001).

M-Blummel *et al*. (2005) found that haulm quantity (yields) and pod yields were directly related. The degree of relationship, however, was weak with an $r^2 = 0.21$. They, however, suggest that haulm yields in groundnut should be considered in breeding programmes in their own right because pod and seed yields tend to exhibit some degree of independence. However, the degree of relationship between haulm and pod yield in this trial was $r^2 = 0.51$, thus, pod and haulm yield may be related in groundnut breeding programmes.

Average shelling percent in the current study was above 65 %, the highest being 76.80 % for Mireku, a local variety and a member of the *fastigiata subspecies* while the least was recorded by Azizivi, an improved variety and a *hypogaea subspecies.* Azizivi was also the only variety which did not attain 60 % shelling percentage though it had the highest number of filled pods. This could be as a result of the short dry spell which occurred during the pod filling stage and the thick pods taking a good chunk of the entire pod biomass. Konkoma performed well in the number of pods per plant but the number of unfilled pods ensured the ratio of filled to unfilled pods was less than two. Azizivi had the widest ratio (3:1) of filled to unfilled pods,

possibly due to the efficient utilisation of available phosphorus during the pod filling stage of the crop. It is also likely that Azizivi is tolerant to short dry spells during the pod filling stages.

Variations in crop seed responses in germination, vigour and speed of germination might be attributed to the differences in chemical composition. Genetic makeup, as well as environment may be implicated in determining seed germination. Also, relative effects of temperature, relative humidity, and moisture content at the time of sowing may affect germination of various varieties.

Tekrony and Egli (1991) noted that irrespective of the treatment method before storing, seed vigour and vigour index could decrease with length of storage. Varietal differences did not show any significant differences for the trial.

The results from the study showed moderate delay in germination for *hypogaea* subspecies while those of the *fastigiata* recorded higher germinations exceeding 75 % suitable to be certified as seed. Jenkaah, an improved variety and also a *fastigiate,* had the highest germination percentage while Adepa, also an improved variety and belonging to sub-species *hypogaea* had the least germination percent. Toole *et al.* (1964) observed that dormant seeds fully imbibed water and remained firm and free of micro-organisms though removal of the seed coat significantly increased germination. Asibuo *et al.* (2008a) reported that over 90 % of freshly harvested seeds of Shitaochi, also a *fastigiata,* germinated on or before 14 days. Other non-genetic factors may have influenced germination in groundnut (Dharmaputra *et al*., 2010). Thompson and Ooi (2010) opined that lack of germination is a genotypic (inherent) rather than an environmental problem and that dormancy aims at modifying the sensitivity of seeds to signals that trigger a switch from its state of rest to

germination. This characteristic, they suggested, differed from seed to seed and may vary between 100 % and nothing.

In the current study, the germination achieved by Adepa, Kumawu Local, Azizivi and Jenkaah were less than 75 percent. This falls short of the minimum germination of 75 % required for quality certified seed in groundnut (MacRobert, 2009). Jenkaah (81 %) and Nkate Broni (88 %), however, had fairly high levels of germination. Adepa, Azizivi, Nkosour and Kumawu Local which recorded low germination also were found to be less vigorous.

5.3 The presence of *Aspergillus spp*. and other mycotoxin-causing fungi in stored groundnut seed samples

Under ambient conditions, groundnut seeds stored in impermeable material retained quality for more than a year. Amoako-Atta *et al*. (2011) observed that seeds stored in jute bags were predisposed to fungal activity as opposed to those stored in impermeable polypropylene bags interlaced in jute bags. This contradicted the observations of this study where seeds stored in jute bag with polyethylene were found to be infected by pathogens. This could also be as a result of the fact that insect activity which predisposed the seed to fungal attack was not curtailed by the polyethylene receptacle. Irrespective of the packaging method used, there was no protection of the seed for most of the varieties. This was unexpected because of the intact testa and pods acting both as physical (pod) and chemical barriers (testa) to ward off fungal infection with pathogens and mycotoxins (Awuah and Ellis, 2001). Wounded seed and pod surfaces when left unprotected could also act as easy points of entry for fungi. This may account for why some of the samples had higher aflatoxin levels.

5.4 Aflatoxin contents of the varieties in relation to storage practices

Initial storage moisture content of the seeds was within the recommended safe zone of (9 %), (MacRobert, 2009) suitable enough to limit growth of fungus to thrive and produce aflatoxins. However, with the use of jute bags, drying could proceed gradually to bring down the moisture content of the seeds during the storage period. This possibly ensured that the initial seed moisture content which was averagely 8.5 % and generally known to be effective against fungal invasion was maintained. By reducing the moisture content of the seed lot and consequently the water activity of the seed, fungal activity also reduced, if not, inhibited thereby making the seed partly unaffected by aflatoxins. Awuah and Ellis (2001) reported that the moisture content of seeds packaged in jute bag and polyethylene bag decreased from the start of the storage to an average of 5.8 % after 6 months. The use of jute bag only however, resulted in more pathogenic infection possibly due to the ability of room humidity to raise the moisture content of the seed slightly above the safe zone.

Kernels infection by *Aspergillus flavus* is a function of moisture content of kernels while kernel moisture content is strongly correlated with the relative humidity of the storage environment. *Aspergillus spp.* from various seeds also produced aflatoxins (Dharmaputra *et al*., 2010). Malaker *et al*. (2008) found that the moisture content and black point incidence of seeds stored in different containers increased with the progress of storage and attributed the increase in moisture content and black point incidence of the stored seed to the activity of storage fungi.

In the current study, Mireku, Konkoma, Nkate Broni, Kumawu Local, Shitaochi, Azizivi and Jenkaah seemed to have tolerated the pathogen infections. This was because even though they were infected, they failed to produce aflatoxins beyond a certain threshold.

5.5 Accumulation of aflatoxins with respect to period of storage

Raffi *et al*. (2006) observed after just a month of storage that groundnut seeds stored in hermetically sealed and vacuum containers recorded lower aflatoxin levels. This was because those containers may have avoided gaseous exchange with the environment, and ensured a stable environment devoid of microbe activity.

Prior to storage, aflatoxin contamination was detected in only four of the varieties and there was presence of aflatoxin-producing fungi such as *A. flavus* and *A. parasiticus* in the seed lot. Awuah and Ellis (2001) made the same observation when mouldy samples as well as pathologically clean samples were both found to have some amount of aflatoxins despite the suspicion that the polyethylene storage could inhibit aflatoxin production compared to jute bag storage. Dorner *et al* (2003) suggested that infection of groundnuts by *A. flavus, A. parasiticus* and other mycotoxin-causing mould was a poor indicator of aflatoxin contamination because there is no indication of the amount of fungal growth that had occurred.

This is because it is possible to detect aflatoxins in pathologically clean seeds while a similar looking seed may not contain any amount of aflatoxin. It is also difficult to use percentage pathogen infections as a guide to aflatoxin build-up since even disinfected seeds may still carry the fungal propagules and continue to sporulate in storage. Thus, *Aspergillus* infection and growth are separate events, which is why *Aspergillus* spp. may be on the seed after plating and incubation but no degree of growth may have occurred. Cole *et al*. (1983) established poor correlations between infection percentages and aflatoxin contamination.

The length of time taken for aflatoxins to be detected in biologically significant quantities was about 5 months (Atehnkeng *et al*., 2008). Thus, the length of time used in this study may just be short for the same observation to be made.

Aspergillus spp. was more frequent than other species of mycotoxin-causing fungi. This was due to the fact that *A.* spp. is more invasive than most other species and often dominanted in groundnut seeds (Malaker *et al*., 2008). *A* spp. are able to grow and establish within a very short time on their substrate. In terms of competition, *A. spp.* is more competitive and its allelopathic relationship with other fungi is very strong. Guo *et al*. (1999) reported that plant seeds contained other proteins which may act as inhibitors of fungi infection and growth during storage and germination. When these proteins are concentrated in the seed, it may prevent fungal invasion and reproduction and also exhibit bioactivity against growth of *A. flavus*, and other mycotoxin-causing fungi. In the current study, some of the varieties may have recorded no aflatoxin due to the interaction effects of the seed inhibiting proteins (phytoalexins) and competition among the fungi. When this happens, attention is focused on capturing resources instead of producing metabolites to contaminate their substrate (Guo *et al*., 1999). Therefore, there could be a decline in the aflatoxin content as the storage period prolongs because of the enzymes released by competing pathogens to degrade already released fungal metabolites.

CHAPTER SIX

6.0. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

The study was carried out to study the seed yield, seed quality characteristics and the pathogen infection and aflatoxin build-up in ten selected varieties. Findings from the field evaluation and laboratory analysis suggest that:

1. Genetic make up could be implicated for the variations in flowering, maturity and germination percentages.
2. There was a possibility of improving Kwame Danso, Nkate Broni and Adepa for dual purposes i.e. growing them both for seed and fodder to feed livestock.
3. Kwame Danso, Nkate Broni and Shitaochi (all local varieties) are good germplasm which can be improved and added to the pool of improved groundnut varieties already being used by farmers.
4. Groundnut seed, when carefully taken care of during growth, harvesting and storage, though may be subject to fungal infection, could limit pathogen infection and mycotoxin production.
5. None of the varieties screened had stable resistance to pathogen build up though there was some degree of resistance against aflatoxin in varieties such as Konkoma, Jenkaah and Shitaochi.

6.2. Recommendations

1. Multi-location trials would be needed to confirm the field results.
2. Time of storage should be prolonged beyond 4 months to assess the effects of long term storage on the *Aspergillus* infection and aflatoxin build-up in the stored seed.
3. Future research must be extended to capture seeds stored during the humid season to examine which of the two seasons are well conditioned for seed storage.
4. Because small groundnut seed (3 kg) lots were used in the present study, trials with large seed lots (≥50 kg) need to be conducted to ascertain the activity of fungi in bulky seed lots.
5. Haulms should be analysed to ascertain their quality before selecting among the varieties which one is suitable as a fodder for feeding livestock.
6. Socio-economic studies should be conducted on the use of the jute bags and interlaced polyethylene jute bags as packaging receptacles especially by the small scale farmers.
7. Research should continue on the possible use of plant extracts (botanicals) as alternative means (preservatives) for storing the seed over a prolonged period to ensure it's quality for subsequent season's planting.

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APPENDIX

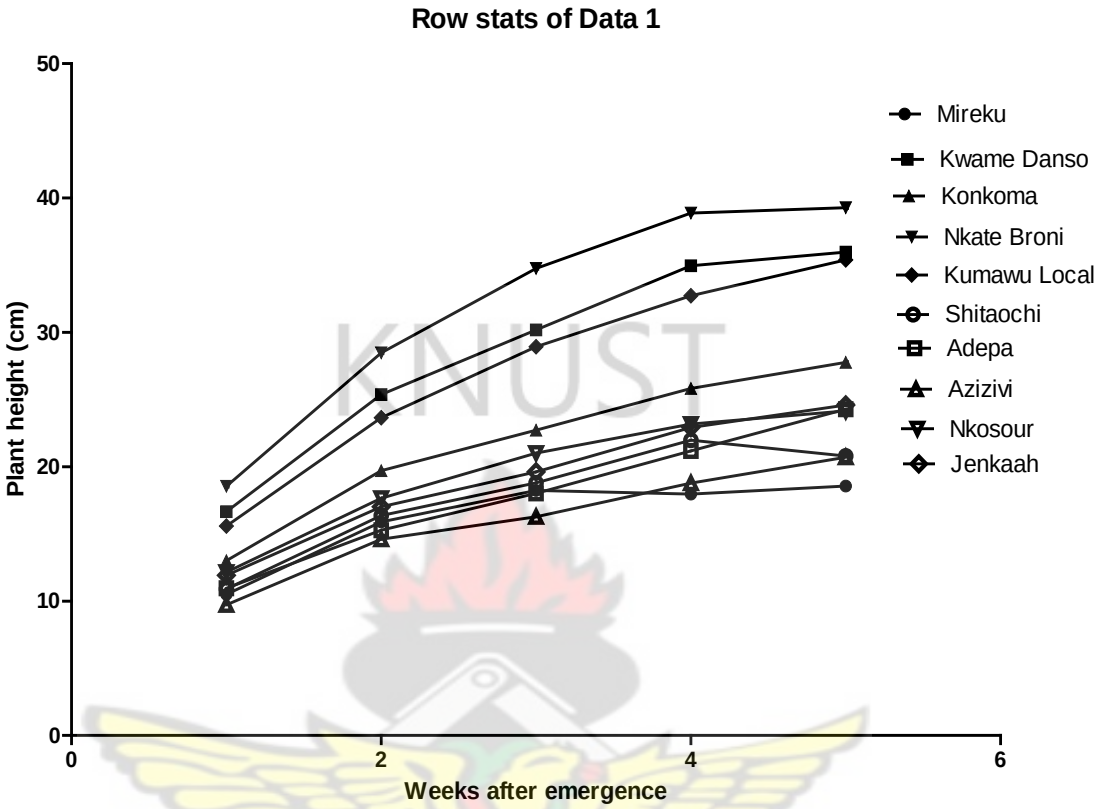


Fig. 1. Plant height progress over five sampling periods

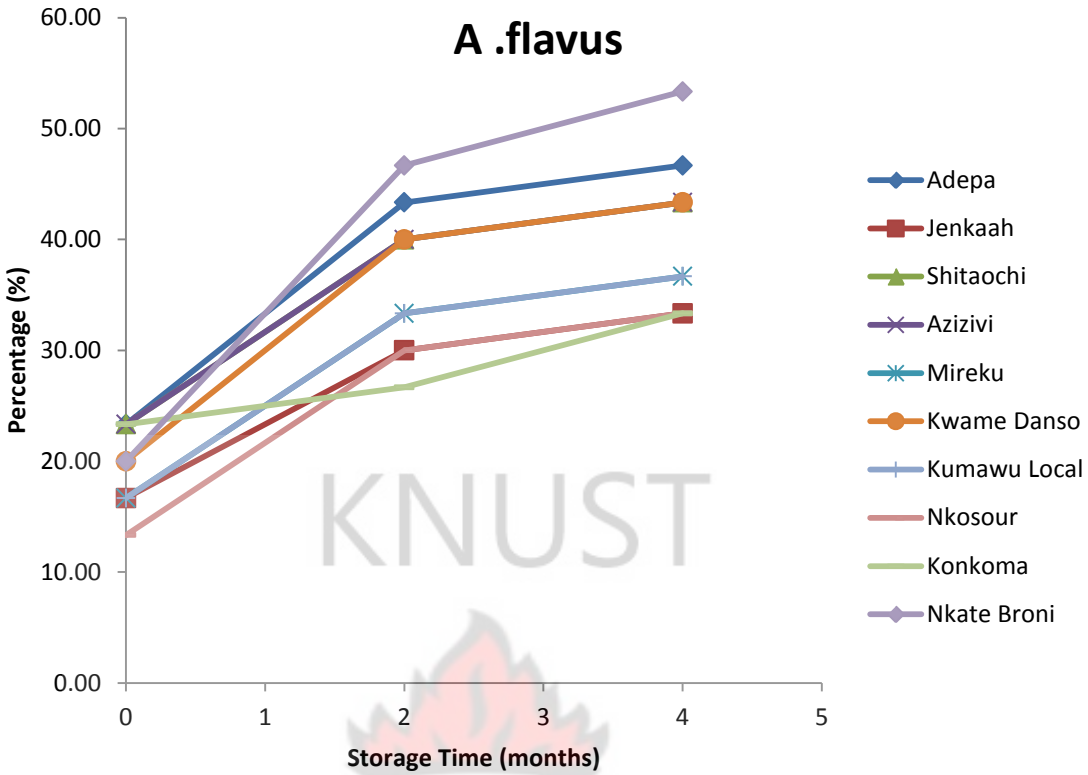


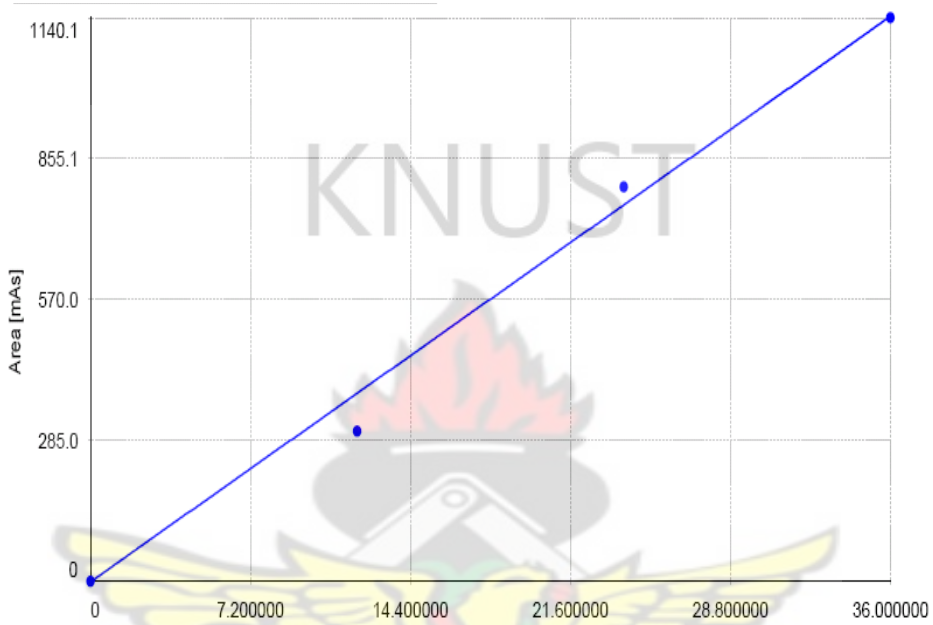
Fig 2. Growth of *Aspergillus flavus* in the seed lot during storage

Fig. 3. Calibration Curves for Quantifying Aflatoxin in the Groundnut Samples

Calibration Curve for G2

Quantity [ng/g] = 0 + 31.4891 * Area [As]

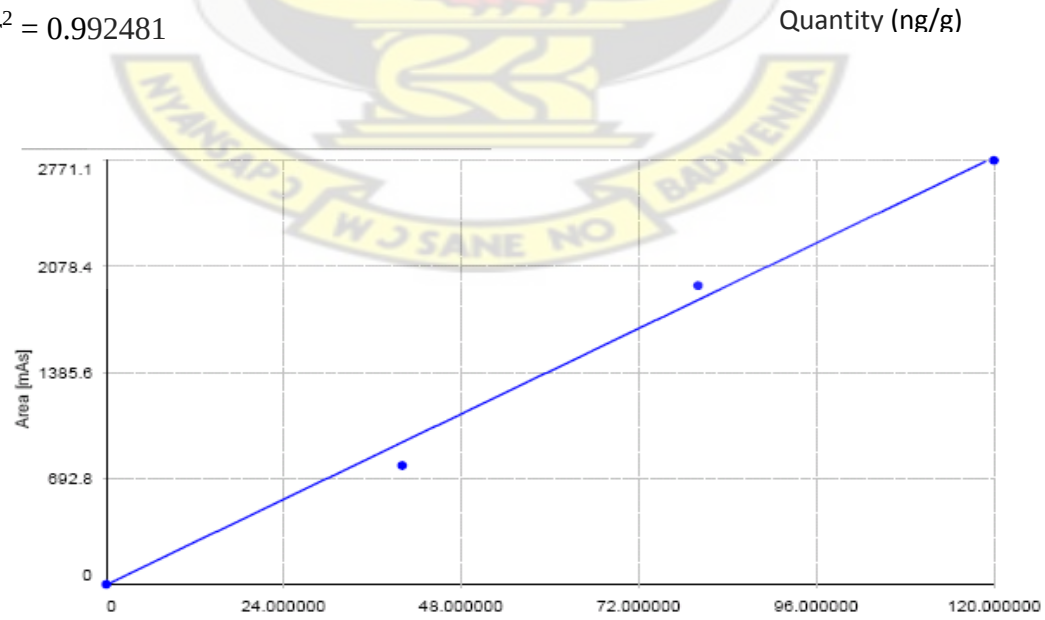
r^2 = 0.989933



Calibration Curve G1

Quantity [ng/g] = 0 + 42.9806* Area [As]

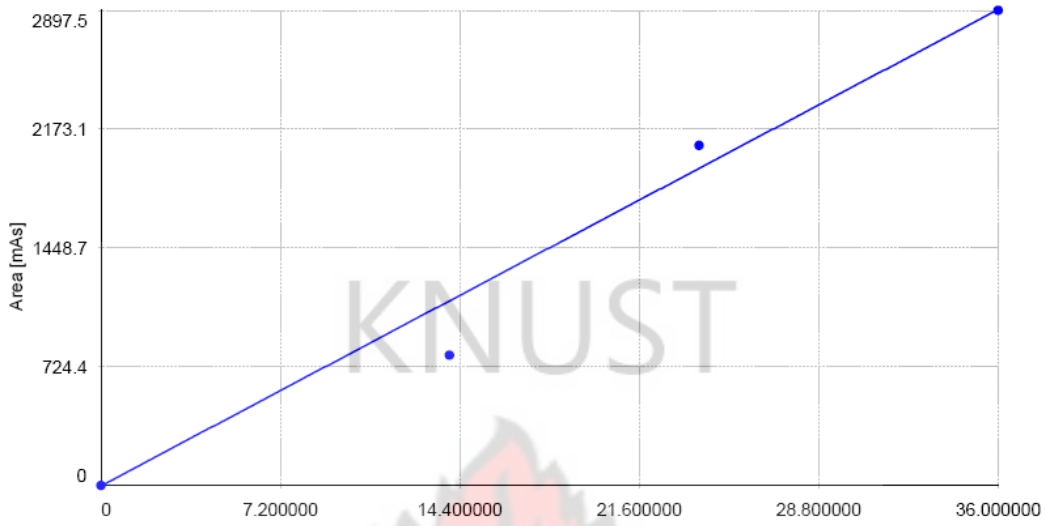
r^2 = 0.992481



Calibration Curve B2

Quantity [ng/g] = 0 + 12.3973 * Area [As]

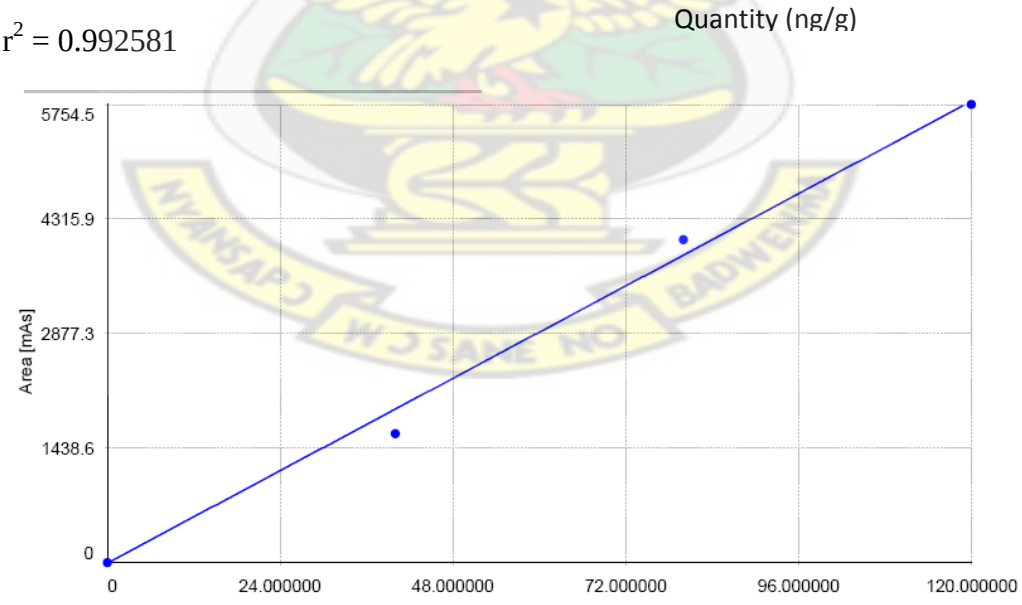
r^2 = 0.970891



Calibration Curve B2

Quantity [ng/g] = 0 + 20.6967 * Area [As]

r^2 = 0.992581



Quantity (ng/g)