FACULTY OF AGRICULTURE AND FORESTRY ENGINEERING

DEPARTMENT OF CROP PROTECTION

YIELD ASSESSMENT AND AFLATOXIN CONTAMINATION IN GROUNDNUT IN MOZAMBIQUE

BY

EMMANUEL ZUZA JUNIOR

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DECLARATION

I do hereby declare that this Thesis entitled "Yield assessment and aflatoxin contamination in groundnut in Mozambique" is my original research work and has not been submitted for a degree to any other university. Where any material could be construed as the work of others, it is fully cited and referenced, and/or with appropriate acknowledgement given. This thesis does not contain other persons' data, photos, graphs or other evidence, unless specifically acknowledged as being sourced from them.

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THESIS ABSTRACT

Groundnut (Arachis hypogaea L*) is an important crop, both in subsistence and commercial agriculture in Mozambique. However, its underground nature of fruiting and its indeterminate growth habit makes it difficult to determine the time of maximum maturity of pods. This results into either harvesting the crop too early or too late, which in-turn results into reduced crop yields and exposes the crop to fungal invasion and subsequent aflatoxin contamination. The objectives of the study were therefore to evaluate the effect of (i) harvesting time on yield and yield components of groundnut and (ii) harvesting time and drying methods on aflatoxin contamination of the crop at two locations namely; Nampula Research Station (PAN) and Mapupulo Agricultural Research Center (CIAM) in Northern Mozambique.*

In order to assess the effect of harvesting time on yield and yield components of groundnut a randomized complete block design in a split plot arrangement was used. The varieties (ICGV-SM-99568, ICGV-SM-01514 and JL-24) were the main factor and three harvesting dates (10 days before physiological maturity, at physiological maturity and 10 days after physiological maturity) were the sub-plots. Results from the study showed that harvesting at physiological maturity resulted into higher groundnut yields (1390.22 Kg/ha) compared to harvesting 10 days before (927.3 Kg/ha) and 10 days after (938 Kg/ha) physiological maturity. Furthermore, yield losses ranging from (16-25 %) and (30-40 %) were incurred as a result of harvesting groundnut 10 days before and 10 days after physiological maturity respectively.

Evaluation of the effect of harvesting time and drying method on aflatoxin contamination of groundnut involved experimental trials arranged in a randomized complete block design in a split-split plot arrangement with four replications. Three groundnut varieties (ICGV-SM-99568, JL-24 and ICGV-SM-01514), were considered as main plots and two drying methods (A-frame and tarpaulin) and three harvesting dates (10 days before physiological maturity, at physiological maturity and 10 days after physiological maturity) as the sub-plots. Aflatoxin contamination of groundnut kernels was lower at physiological maturity (≤ 10) compared to harvesting 10 days before (≤ 15 ppb) and 10 days after (≥ 20 ppb). It was also observed from this study that both the A-frame and tarpaulin drying methods were effective in reducing groundnut kernel moisture to the recommended level of ≤ 7 % which is ideal to prevent growth of fungi including aflatoxigenic strains.

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who's Portuguese is even not a third language. Through their wisdom and guidance, the language barrier was overcome.

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DEDICATION

THE FEAR OF THE LORD IS THE BEGINNING OF WISDOM, AND KNOWLEDGE OF THE HOLY ONE IS UNDERSTANDING.

This thesis is dedicated to the people closest to me who have always held in me: my parents (Emmanuel K.S. and Beata M. Zuza), my beloved brother (Tawonga J. Zuza), my sisters (Brenda and Francisca Zuza), Temwa and Beauty Movete, Gift, Pemphelo, Rose, Yamika and Kinsley Lungu and my late uncle and cousin (Rev. Bishop. Joseph Mukasa Zuza and Eric Aaron Movete).

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LIST OF ACRONYMS AND ABBREVIATIONS

CO_2 : Carbon Dioxide

CIAM : Mapupulo Agricultural Research Center

cm : Centimetres

EU : European Union

FAO : Food and Agricultural Organization of the United Nations

FAS : Foreign Agricultural Service

^oC : Degree Celsius

GDP : Gross Domestic Product

IIAM : Agricultural Research Institute of Mozambique

MPPL : Mapupulo

m : Metres

ml : Millilitres

PAN : Nampula Research Station

% : Percentage

PICS : Purdue Improved Crop Storage

ppb : Parts per billion

RH : Relative Humidity

SSA : Sub Saharan Africa

USA : United States of America

USDA : United States Department of Agriculture

WHO : World Health Organization

CHAPTER ONE: GENERAL OVERVIEW

1.1. INTRODUCTION

Agriculture forms the mainstay of the economy in Mozambique, accounting for 29 % of the Gross Domestic Product (GDP) and is one of the main export earners along with fisheries (Mozambique Government, 2012). Groundnut (*Arachis hypogea* L.) is an important legume crop for most parts of the world including Mozambique. In Mozambique, groundnut plays an important role both as a food and cash crop for smallholder farmers (Jeffrey, 2011). Furthermore, it is an important component of rural diet and also provides supplementary cash income to women farmers in the country who support their families, especially children's education and health. Additionally, groundnut fixes atmospheric nitrogen in soils and thereby improving the soil fertility and saves fertilizer costs in subsequent crops. This is particularly important when considered in the context of the rising prices for chemical fertilizers that makes it difficult for smallholder farmers to purchase them (Jeffrey, 2011).

In the world, Mozambique is ranked number eleven as a major producer of groundnuts (USDA-FAS, 2010). Furthermore, groundnut takes 2 % share of the total exports in the country. Groundnut from Mozambique is mainly exported to the European Union (EU) countries and India (Jeffrey, 2011). However, groundnut export from the country have recently reduced due to regulations on the total amount of aflatoxins in the crop by importing countries (Almeida *et al*., 2013).

Aflatoxins are secondary metabolites produced by various microorganisms during pre and post-harvest handling and storage of groundnuts. Post-harvest deterioration in groundnut is largely due to mould development and subsequent aflatoxin contamination caused by fungi, especially *Aspergillus Flavi* group (Waliyar *et al*., 2015). Contamination of aflatoxins is perceived to be dangerous both to humans and livestock because these are considered to have, teratogenic, carcinogenic, estrogenic and immunosuppressive effects (Klich *et al*., 2009). Additionally, aflatoxin contamination of groundnut is high during post-harvest than during pre-harvest conditions (Wild and Hall, 2000).

Poor management practices and adverse climatic conditions at harvest and post-harvest are some of the prompting factors for post-harvest aflatoxin contamination. The timing of harvesting greatly influences mould production (Okello *et al*., 2010). Harvesting should take place as soon as the crop is fully grown, this is because crops left in the field for longer periods

of time present higher levels of aflatoxin contamination (Guo *et al*., 2003; Cotty and Lee, 1990).

Substantial grain losses caused by fungi also occur during storage because of prevailing ambient conditions (Waliyar *et al*., 2015). Conditions e.g. (excessive heat, high humidity, lack of aeration in stores and insect and rodent damage), which are common in the tropics and subtropics, including Sub-Saharan Africa (SSA) intensify toxin accumulation (Hell and Mutegi, 2011; Bhat and Vasanthi, 2003). Liu and Wu (2010) reported that of 550,000 to 600,000 new hepatocellular carcinoma cases reported worldwide annually, approximately 25,200 to 155,000 cases are attributed to aflatoxin exposure of which most are in SSA, China and Southern Asia. These are mainly as a result of uncontrolled aflatoxin contamination in food.

Apart from its impact on health, aflatoxin contamination denies these countries access to export markets for their crops that are most susceptible to aflatoxin contamination for example maize, groundnuts and sorghum. African countries are estimated to lose approximately $ 670 million annually due to the inability of African farmers to meet the aflatoxin standards of the EU (4 parts per billion, ppb) and the United States (US) (20 ppb) for the crops that they produce (Moss, 2002; Creepy, 2002; Otsuki *et al*., 2001).

Aflatoxin contamination stops groundnuts from entering the major import markets more than any other factor. Importers are required by law to systematically test incoming shipments for the total amount of aflatoxins and reject those exceeding the permitted maximum levels. Exporters unaware of aflatoxin contamination issues, limits, regulations and standards risk costly rejections, claims, downgrading of shipments or the banning of the export. In Mozambique, there is no existing data on the levels of aflatoxin in the countries' groundnut exports or in domestically consumed groundnuts. However, the importance of aflatoxins in the country is illustrated by the high levels of certain types of cancer, the strong links between HIV infection rates and aflatoxin intake, and negative correlations between aflatoxin in the diet and development in children (Almeida *et al.*, 2013).

Increased food production together with reduced post-harvest losses is an ideal strategy for overcoming worldwide hunger (Kimatu *et al*., 2012). Sub-Saharan Africa is the only region in the world where food production continues to decline. Reduction in post-harvest losses is one of the solutions to improving profit and in addition post-harvest management is important for increasing food availability without the need for additional resources (Waliyar *et al*., 2015;

Kimatu *et al*., 2012). Moreover, the cost effectiveness, sustainability, and technical feasibility of pod-handling methods need to be assessed with regard to local context and practices before devising strategies for post-harvest aflatoxin contamination (Waliyar *et al*., 2015). Post-harvest stages include drying, cleaning, grading, transportation, storage, processing, packaging and retailing at the market (Kimatu *et al*., 2012). Some of the factors affecting aflatoxin contamination in food grains are; harvesting, drying and storage methods as well as moisture content, physical and insect damage (Kaaya and Warren, 2005).

In general, adopting proper practices, for example, harvesting at the right crop maturity stage followed by pod stripping after harvest, rapid drying, and cleaning of any extraneous matter including damaged pods and gynophores reduce aflatoxins after harvest prior to storage (Rahmianna *et al*., 2007).

1.2. PROBLEM STATEMENT AND JUSTIFICATION

Aflatoxin contamination in groundnuts, caused by *Aspergillus* species, is a major pre- and post-harvest constraint in Sub-Saharan Africa (SSA), causing kernel quality loss. Additionally, once aflatoxins develop, it is very difficult to eliminate them completely. Furthermore, adoption of proper post-harvest handling measures by smallholder farmers and small retailers is a big challenge in part due to high costs associated with improved practices, such as; timely harvesting of groundnuts, proper drying using solar dryers, decontamination and ammonification and storage of pods in hermetic bags (Hell and Mutegi, 2011).

Groundnuts need to be harvested at the correct physiological time, which is when the crop is fully grown. Delay in harvesting results in over maturity leading to mould infections and subsequent aflatoxin contamination (Wright *et al.*, 2005; Cotty and Lee, 1990). Proper drying of groundnuts is crucial in prevention of mould development. However, the traditional groundnut drying techniques in developing countries, such as in Mozambique, involve field and bare ground drying that result into high moisture levels. High moisture exacerbates post-harvest moulding and aflatoxin contamination (Heathcote and Hibbert, 1978). However, adequate drying of grains after harvest to less than 7 % moisture content is ideal to prevent fungal growth, including aflatoxigenic strains (Dick, 1987).

One way to achieve effective drying of groundnuts to moisture levels less than 7 % is through the use of improved drying methods such as; invented windrowing, A-frames and tarpaulin methods. These enable increased air circulation and protect groundnut kernels from harsh environmental conditions such as; showers and excessive sunlight, thereby facilitating rapid and efficient drying and also preventing mould growth and aflatoxin contamination (Kaaya *et al*., 2007).

Aflatoxin contamination is believed to be a serious quality problem of groundnut among Mozambican farmers, which has resulted into low quality of the nuts, and thus the loss of international and regional export markets (Monyo, 2013).

Since contamination of groundnuts by aflatoxins is greatest during or after post-harvest handling, this study was aimed at assessing post-harvest handling technologies of groundnuts being used by smallholder farmers in Mozambique. It was hoped that best practices would be identified through the results and would be recommended for post-harvest aflatoxin

management options and enhance the use of good agricultural practices for mitigating the problem.

1.3. RESEARCH OBJECTIVES AND HYPOTHESES

1.3.1. General objective

To evaluate the effect of harvesting time and drying methods on groundnut yield and aflatoxin contamination in Mozambique.

1.3.2. Specific objectives

- To evaluate the effect of harvesting time on groundnut yield and yield components.
- To evaluate the effect of harvesting time on groundnut aflatoxin contamination.
- To compare the effectiveness of "A-frame" and Tarpaulin drying methods in reducing aflatoxin contamination of groundnut.

1.3.3. Hypotheses

- Harvesting time does not have an effect on groundnut yield and yield components.
- Harvesting time does not have an effect on groundnut aflatoxin contamination.
- The use of A-Frame and Tarpaulin drying technologies compared to traditional means does not reduce groundnut aflatoxin levels.

1.4. LITERATURE REVIEW

1.4.1. Origin and distribution of groundnuts

Archaeological evidence suggests that groundnut has been cultivated for more than 3500 years, and was undoubtedly first domesticated in northern Argentina and eastern Bolivia (Singh and Simpson, 1994). It is believed that the cultivated type, *Arachis hypogaea*, originated in this region, since *Arachis monticola*, the only wild tetraploid species that is cross compatible with it, is found in this area (Singh and Simpson, 1994). The crop was introduced to other parts of the world through various routes and reasons. At present, groundnut is grown worldwide with China, India and the United States of America (USA) being the largest producers.

1.4.2. Groundnut botany and Taxonomy

The botanical term of groundnut is *Arachis hypogaea*. The name is derived from the Greek word *arachis* meaning 'legume' and *hypogaea* meaning 'below ground', referring to the formation of pods in the soil (Pattee and Stalker, 1995). Groundnut is a member of the family Leguminosae, tribe Aeschynomeneae, sub-tribe Stylosanthinae of genus *Arachis*. *Arachis hypogaea* is an annual herb of indeterminate growth habit which has been divided into two subspecies, *hypogaea* and *fastigiata*, each with several botanical cultivars (Holbrook and Stalker, 2003). Sub-specific and varietal classifications are mostly based on location of flowers on the plant, patterns of reproductive nodes on branches, numbers of trichomes and pod morphology (Muitia, 2013).

1.4.3. Importance of groundnuts

Groundnut is one of the most important legume crops for several millions of people in the world and is a valuable cash crop for small-scale farmers in developing countries. It is an annual legume and grown primarily for its high quality edible oil and easily digestible protein in its seeds (Upadhyaya *et al*., 2006). Groundnuts have different types of uses, including, food for humans (roasted, boiled and cooking oil), animal feed (pressings, straw and seeds), and industrial raw materials (soap, detergent and cosmetics) (Maiti and Wesche-Ebeling, 2002). A large percentage of the world production of groundnuts is used for edible oil, whereas in the USA, approximately 60 % of total groundnut production is used for human consumption (Moss and Rao, 1995). The principal uses are; groundnut butter and groundnut candy. In some places, the vines with leaves are used as source of protein hay for horses and ruminant livestock. In

addition, groundnuts fix nitrogen so that they are used in agricultural systems to improve soil fertility.

1.4.4. Worldwide Production of Groundnuts

Groundnuts are grown on approximately 42 million acres globally. The crop is grown by 108 countries worldwide with 90 % being developing countries (Kaiser and Ernst, 2012). It is the third major oilseed of the world next to soybean and cotton. China is the leading producer of groundnuts, having the greatest share of overall world production, followed by India and then the United States (Table 1) (Nigam, 2014). In contrast to these large producers, in southern Africa, Mozambique is the biggest producer of groundnuts (Nautiyal, 2002; Putnam *et al*., 1991).

Table 1: Top 10 groundnut growing countries in the world.

Country	**Percentage (%) Share in world production**
China	40.7
India	14.0
USA	7.4
Nigeria	5.8
Myanmar	3.3
Sudan	2.5
Argentina	1.9
Indonesia	1.7
Senegal	1.6
Cameroon	1.3
Mozambique	0.10

Source: Nigam, 2014

Although groundnut is a common crop in many developing nations of which Mozambique is a part, the productivity levels are lower than in developed nations. The low productivity is attributed to various production constraints, such as; cultivation of the crop on marginal lands under rain fed conditions, low adoption of improved technologies for example: improved varieties, low soil fertility, poor pest and disease control, lack of organized markets for groundnuts and its products, frequent drought stress, poor weed control, limited availability of

good quality seed and related to socio-economic infrastructure (Jeffrey, 2011; Simtowe *et al.*, 2009).

1.4.5. Groundnut production in Mozambique

In Mozambique about 90 % of rural households are engaged in agriculture, and this equates to 80 % of the total population (approximately 21 million). The main agricultural products grown in the country by smallholder farmers are; maize, cassava, groundnuts, pulses, sorghum, millet, sweet potatoes and cotton, of which the main agricultural exports are tea, sugar, cotton, groundnuts and cashew nuts (Mucavele, 2006).

The groundnut, also known as peanut, earth-nut, amendoim, Manilla and poor man's nut is an important crop for Mozambique. It is important in terms of being an essential source of livestock feed and a component of both rural and urban diets. The crop is consumed in different forms including; consumption of the pods, roasted, or boiled. In addition, peanut butter is incorporated in traditional African dishes. Once considered a food crop, today the groundnut is considered as a cash crop due to its economic importance and ability to generate income for Mozambican farmers (Jeffrey, 2011).

There are four types of groundnut cultivars that are cultivated (Ramas, Virginia, Spanish, and Valencia) throughout Mozambique and a number of factors are responsible for low production yields. In general, farmers lack high-quality farm inputs for example; improved seed, inoculum and fertilizers and training in good agricultural practices. Soil fertility, scarcity of rainfall, diseases, and local pests also contribute to low productivity (Jeffrey, 2011). Figure 1 shows the groundnut production trends of Mozambique.

Most groundnut farmers are smallholders who use traditional approaches of cultivation and farm less than 1 hectares (ha). These farmers struggle with having to sow seed varieties that are not uniform in size or color, have limited technical knowledge to improve productivity, and their post-harvest practices encourage rather than prevent or limit aflatoxin contamination (Muindi & Bernardo, 2010).

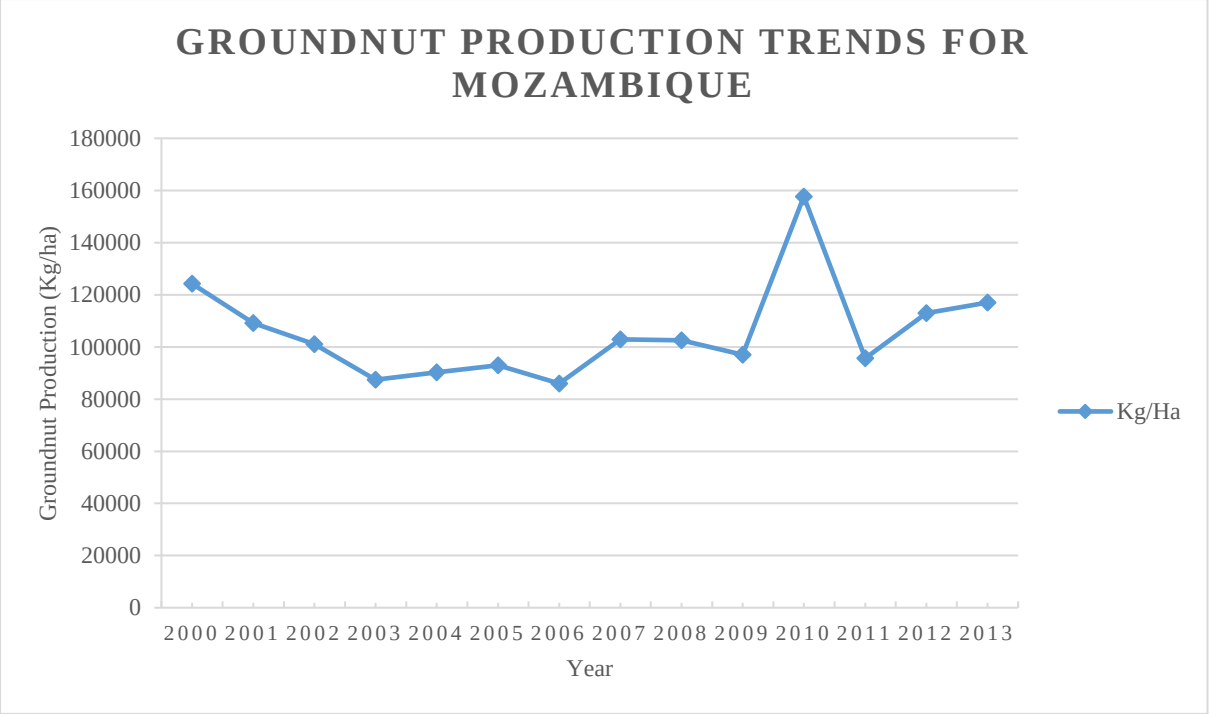

Figure 1: Groundnut production trends in Mozambique (FAO-STAT, 2015).

1.4.6. Groundnut production constraints in Mozambique

Groundnut yields obtained by small scale farmers in Mozambique are quite low (400-600 kg ha-1). The low yields have been attributed to several constraints. Some of the major groundnut production constraints include; poor cultural practices, pests, weeds, drought, and diseases (Muitia, 2013). The poor cultural practices include; low plant population, and delays in planting due to uncertainty of rainfall. Farmers plant groundnut in wide spacing leading to very low plant density (Muitia, 2005). The low plant density may be attributed to lack of seed and to the mixed cropping systems practiced by the farmers. Most of farmers use their own seed for sowing in the following season because groundnut prices at the beginning of growing season are quite high and most of the farmers do not afford (Muitia, 2013). Mixed cropping system is common for many farmers in Mozambique. The system reduces the risk of crop loss due to adverse conditions thereby ensuring substantial yield advantages and harvests as compared to sole cropping.

Other major reasons behind the low groundnut yields in the country include; insect pests, diseases and weeds. The major pests include; termites, aphids (*Aphis craccivora*), thrips (*Frankliniella fusca*) and foliage feeding pests (Ramanaiah, 1988). Termites are a major pest at all stages of crop growth and they feed on pods, seeds and plant foliage (Figure 2). Aphids are a major pest at seedling stage and they suck plant sap. Thrips attack flower buds and

consequently contribute to low seed set. Foliage feeding pests attack the crop during vegetative growth and thereby reducing the photosynthetic area. Some of these pests such as; aphids are vectors of the most destructive virus diseases in Sub-Saharan Africa, such as groundnut rosette disease. Besides groundnut rosette disease, aphids are also vectors of groundnut mottle, groundnut stripe, groundnut stunt and groundnut chlorotic streak (Kokalis-Burelle *et al*., 1997). The control measures applied by farmers to reduce insect pest infestation include; cultural practices and insecticide application. Cultural practices include; early planting, such that the crop matures before the period of peak pest population, and mixed cropping. Insecticides are effective in killing insects. However, they should be applied only if economically sustainable since they are expensive.

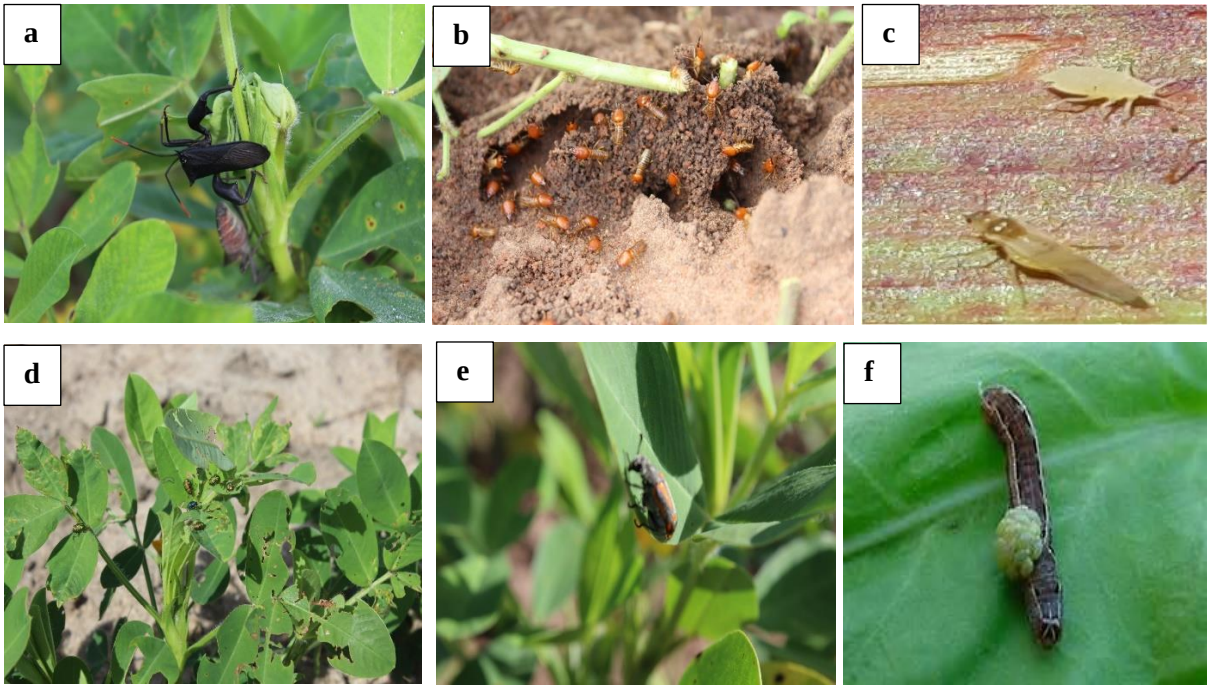

Figure 2: Common pests of groundnuts found at PAN and CIAM: (a); Groundnut hopper (*Hilda patruelis Stal*), (b); Termites (*Odontotermes badius*), (c); (*Aphis craccivora*), (d) Groundnut green leaf eating beetles (*Colaspis favosa*), (e); Groundnut leaf sucking weevil and (f); Fall Armyworm (*Spodoptera frugiperda*).

Weeds constitute another major problem for groundnut during the first few weeks after planting and at harvesting. Failure to control weeds can result in reduced crop yields since they compete with the groundnut crop for nutrients and water. In addition, they interfere with the harvesting process. Furthermore, they harbor pests and disease vectors. Cultural practices such as good land preparation and crop rotation are the most recommended control measures for weeds. In addition, herbicide application, when available, is also recommended for weed control (Kokalis-Burelle *et al*., 1997).

Diseases are also another major constraint to groundnut production. Early leaf spot (*Cercospora arachidicola* Hori) and late leaf spot (*Cercosporidium personatum* Berk and Curt), rust (*Puccinia arachidis* Speg.) and groundnut rosette disease virus are very common and can cause significant losses to the crop (Figure 3). Leaf spots and rust damage the crop by reducing the photosynthetic area through lesion formation and stimulating leaflet abscission. The shedding of the leaflets results in premature ageing of the crop, and therefore, yield losses. Crop rotation, use of tolerant cultivars and use of fungicides are some control measures for these diseases. Groundnut rosette disease alone can cause up to 100 % crop loss (Adamu *et al*., 2008). When the disease occurs, rural economies that depend on groundnuts are completely disrupted since smallholder farmers in Sub-Saharan Africa, grow groundnut for both subsistence and as cash crop (Naidu *et al*., 1999). When a disaster such as groundnut rosette disease strikes, rural farmers lose a very important source of protein, a valuable source of income and substantial part of seed for next planting leading to food insecurity (Naidu *et al*., 1999). Consequently, it is suggested that cultivars with resistance to the pathogens would be needed to suppress the two leaf spot diseases even if fungicides control the diseases (Holbrook and Stalker, 2003).

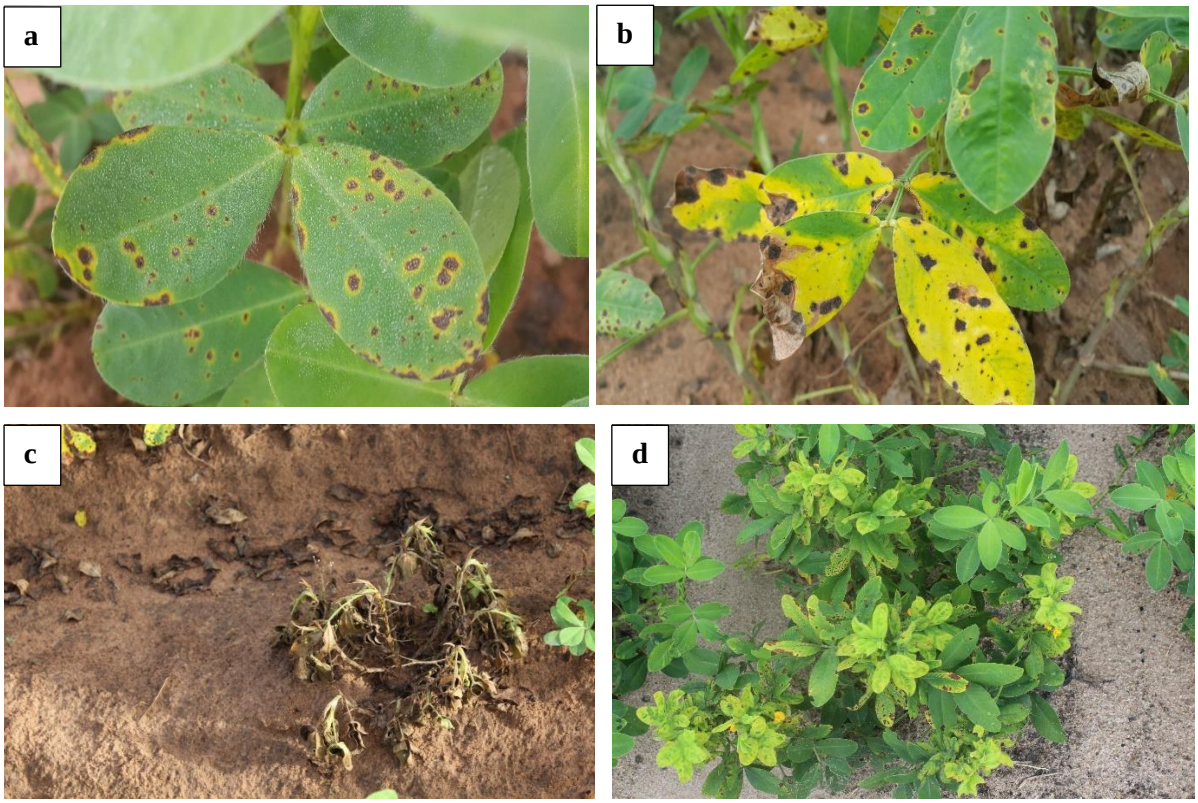

Figure 3: Groundnut diseases at PAN and CIAM; (a) early leaf spot, (b) late leaf spot, (c) defoliation and wilting of plants as a result of severe late leaf spot and (d) rosette virus disease.

In Mozambique, groundnuts are produced under dryland farming, and some groundnut producing regions in the country are characterized by droughts at the end of the season. Drought stress may affect the crop at different stages during the growing season. In groundnut, drought stress during flowering and pod filling stage is critical for yield and agronomic characters. Drought at these stages leads to reduction in crop yield by affecting the number of pods per plant (Muitia, 2005), and irregular and scarce rainfall at pod filling reduces the yield greatly (Malithano, 1980). Not only the yield of groundnut but also the quality of products decreases under drought stress (Rucker *et al*., 1995). When drought occurs in the last 20-40 days of the season, pre-harvest infection by *A. flavus* is increased and consequently, aflatoxin concentration increases. However, genotype selection for drought tolerance may improve aflatoxins resistance and under drought stress conditions, drought tolerant cultivars yield more than susceptible ones (Arunyanarka *et al*., 2010).

Groundnuts are also prone to fungal invasion when in the field and in storage, mainly because the crop produces pods in the soil where microorganisms such as fungi reside. This makes the pods more susceptible to attacks by these soil inhabiting microorganisms (Cotty *et al.*, 2007). Fungi produce various metabolites as a result of respiration of which the majority are mycotoxins (Muitia, 2013). Furthermore, in storage high humidity, high temperature and moisture conditions facilitate growth and development of such fungi.

1.4.7. Mycotoxins

Mycotoxins are secondary metabolites produced by filamentous fungi, which can develop on food crops for example: maize, wheat, groundnut, rice and sorghum and in some cases on commodities of animal origin (meat products, sausages and milk) (Milicevic *et al.*, 2010). Mycotoxins are detrimental to vertebrates when they are absorbed through ingestion, inhalation, or dermal absorption. Studies have shown that ingestion of contaminated food or feed is the main source of mycotoxin exposure to both humans and animals (Milicevic *et al*., 2010).

The word mycotoxin comes from a Greek word ‘mykes’, meaning mould, and ‘toxicum’ meaning poison, and the diseases caused by them are called mycotoxicoses (Brera *et al*., 2008; Viljoen, 2003). Historically, mycotoxins have been present in food and feed since early in the history of humanity and some of their effects have been documented for centuries (Viljoen, 2003). The first occurrence of mycotoxicosis, gangrenous ergotism, known since the middle

ages, is a human disease resulting from consuming rye contaminated with *Claviceps purpurea*. There have been many mycotoxin-related outbreaks in the past century which have led to many deaths, for example 'yellow rice disease' in Japan and Alimentary Toxic Aleukia (ATA) which has claimed many Russian lives.

Currently, there are more than 300 mycotoxins known, but the scientific interest has been concentrated simply on less than 10 compounds that present known toxicological effects on human and animal health (Wu *et al*., 2011). Among them, *aflatoxins, ochratoxins, fumonisins, trichothecenes, deoxynivalenol* and *zearalenone* are the major groups of mycotoxins mostly studied. These mycotoxins have been shown to be associated with immunotoxic, mutagenic, genotoxic, carcinogenic, nephrotoxic and teratogenic effects in livestock and human health (Brera *et al*., 2008).

Mycotoxins are generally produced by fungal species that belong to the genera *Aspergillus, Fusarium, Penicillium, Alternaria, Cladosporium* and *Nigrospora*, which are abundant in the environment (Klich, 2007). Under conducive environmental conditions and also depending on the species and strain, specific fungi produce a particular mycotoxin. The nature of the substrate and environmental conditions determine the type of fungi that dominates in particular food crops, and in some cases the type of mycotoxins produced (Marquardt, 1996).

Environmental conditions, particularly humidity and temperature, influence fungal production of mycotoxins, thus the presence of fungi even at high infection rates does not necessarily imply that mycotoxins are present. In addition, different strains of a given fungal species differ in their ability to produce mycotoxins. In most cases, mycotoxins produced can remain within the infected material long after signs of fungal infection have disappeared (Viljoen, 2003).

The type and level of mycotoxin production results from the interaction between fungi, the host and the environment (Pitt, 2000). It has been estimated that 25 % of crops (maize, groundnuts, rice, cotton, sorghum and millet) produced globally are contaminated each year with 'unacceptable' levels of mycotoxins during food production, processing, transport and storage (Kamika and Takoy, 2011), of these, the economic, health and environmental impacts of these fungal toxins have pushed the understanding of food safety and food poisoning (Marquardt, 1996). Since aflatoxins represent the most widespread risk to food safety in tropical and subtropical Africa and because of the interest and objectives of this study, aflatoxins will be discussed further in the following sections.

1.4.8. Aflatoxins

Aflatoxins are biochemical metabolites naturally produced by the soil-borne saprophytic fungi; *A. flavus* and *A. parasiticus* and less commonly by *A. nomius* that contaminate groundnuts and other crops in the field and during post-harvest handling and storage. Contamination varies from year to year, as well as within the field and is predominantly high when plants are exposed to stresses towards the end of the growing season. Pre-harvest infection and aflatoxin contamination frequently occur when the plant is exposed to moisture and heat stress during pod development, when pods are damaged by insects or nematodes and when they are mechanically damaged during cultural actions (Kimatu *et al.,* 2012). Due to the dependence on rainfall for watering crops and the recent variations experienced with weather patterns, these conditions commonly occur (Bandyopadhyay *et al*., 2007).

Post-harvest infection in groundnuts is influenced by; the method of shelling, relative humidity, temperature, and insect damage. Abbas *et al*., (2011) reported that strains of *A. flavus* also produce Cyclopiazonic Acid; a harmful mycotoxin that is currently not regulated.

In most developing nations the levels of aflatoxin contamination is remarkably very high. For example, results from recent studies in Mali have shown levels of contamination in groundnuts in excess of 300 ppb with a mean contamination of 164 ppb (Waliyar *et al*., 2015). These levels are much higher than international standards by Food Agricultural Organization (FAO) of 20 ppb, allowed for human consumption. In addition, such high levels are subsequent to rejection of groundnut trade for example in the EU, where the allowable level is 4 ppb and in the US 20 ppb total aflatoxin in consignments (Emmott, 2013).

According to the Food Agricultural Organization (FAO), 25 % of the world's population is affected with consumption of contaminated crops that have the potential of causing cancer, immune-system suppression, growth retardation, liver disease, and death in both humans and domestic animals. Countries situated between 40 °N and 40 °S (Figure 4) are thought to be at greatest risk from aflatoxin contamination (Wu *et al.,* 2011). The tropical and sub-tropical nature of these countries results into suitable environmental conditions such as; high temperature and humidity prevail, thereby favouring the growth of fungi and production of aflatoxins on the crops (Klich, 2007).

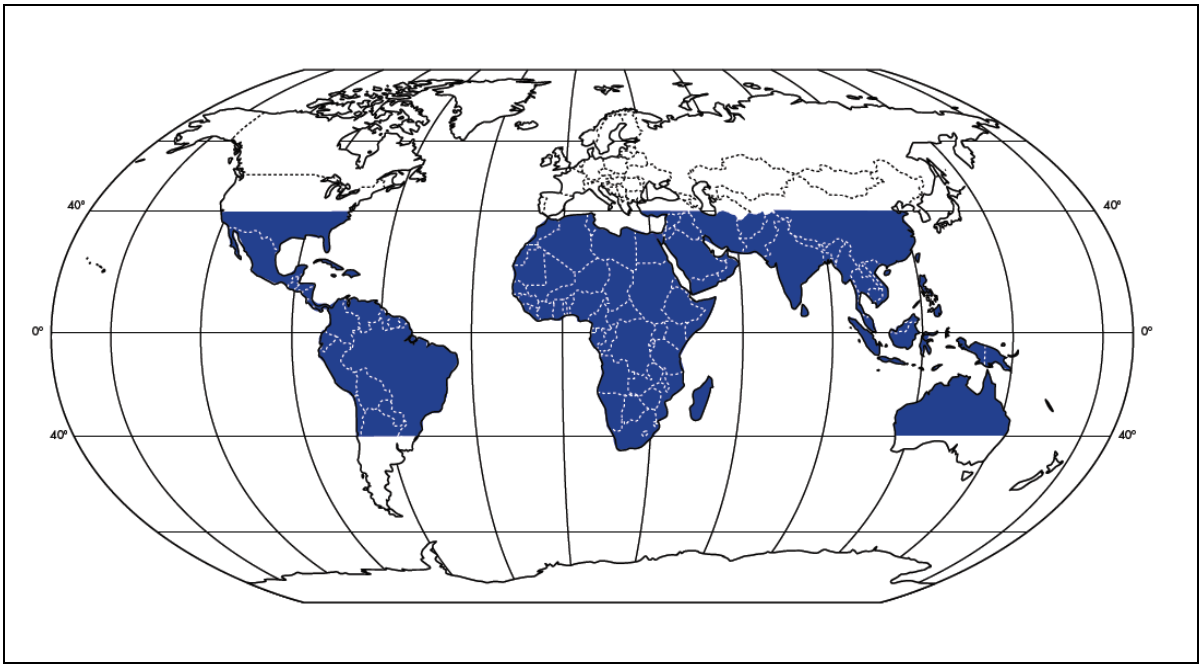

Figure 4: Areas and populations at risk of aflatoxin contamination (Wu *et al*., 2011).

The Gates's Foundation (2011), reported that aflatoxin induced live cancer (hepatocellular carcinoma) in Africa accounts 130, 000 to 500, 000 Disability Adjusted Life Years (DALYS) per year. This was defined by World Health Organization, (2011) as the sum of years of potential life lost due to premature mortality and the years of productive life lost due to disability. Furthermore, aflatoxin induced live cancer is the third-leading cause of cancer death globally, with about 550,000-600,000 new cases each year. Eighty-three percent of these deaths occur in East Asia and sub-Saharan Africa (Khlangwiset *et al*., 2011).

1.4.8.1. Chemistry of Aflatoxins

The major aflatoxins have been classified into B and G series due to their fluorescence being blue and green in Ultra Violet light on thin layer chromatography plates, respectively (Wu and Khlangwiset 2010; Pavao *et al*., 1995). The subscript numbers 1 and 2 indicate major and minor compounds, respectively. The B series (AFB1 and AFB2) are chemically known as difurocoumarocyclopentenones and the G series (AFG1 and AFG2) are difurocoumarolactone series (Figure 5). Structurally the dihydrofuran moiety, containing a double bond, and the constituents linked to the coumarin moiety are of importance in producing biological effects. However, for the B series, cyclopentenone has been reported to be responsible for the major toxicity observed (Fung and Clark, 2004). AFB1 is the most toxic of the aflatoxins, and is the most potent naturally occurring chemical liver carcinogen known, and is seconded by AFG1.

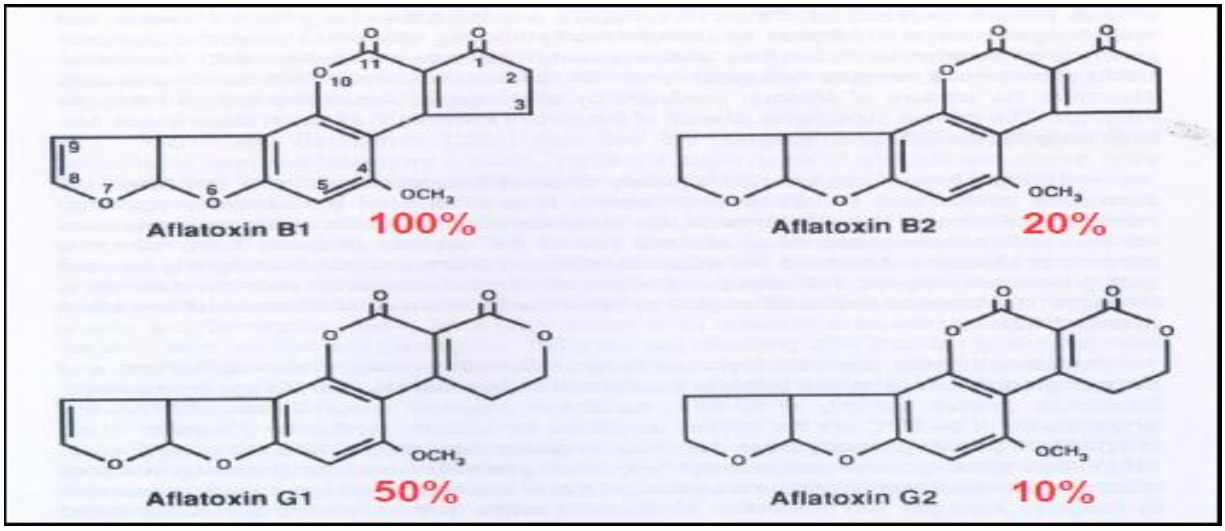

Figure 5: Chemical structures of the four major aflatoxins (Wu and Khlangwiset, 2010).

Studies have shown that aflatoxins are potent liver toxins and their effects vary with concentration, duration of exposure, species, breed and nutritional status. Considerable quantities generate acute toxicity and chronic exposure to low levels may result in cancer (Marquardt, 1996). Aflatoxins bind to albumin and other proteins in circulation found in the liver, kidney, bone marrow and lungs. As a result of excretion, specific P450 enzymes in the liver metabolize aflatoxin into a reactive oxygen species (aflatoxin-8, 9-epoxide), which may then bind to proteins and cause acute toxicity or to DNA and induce liver cancer (Figure 6) (Wild and Gong, 2010).

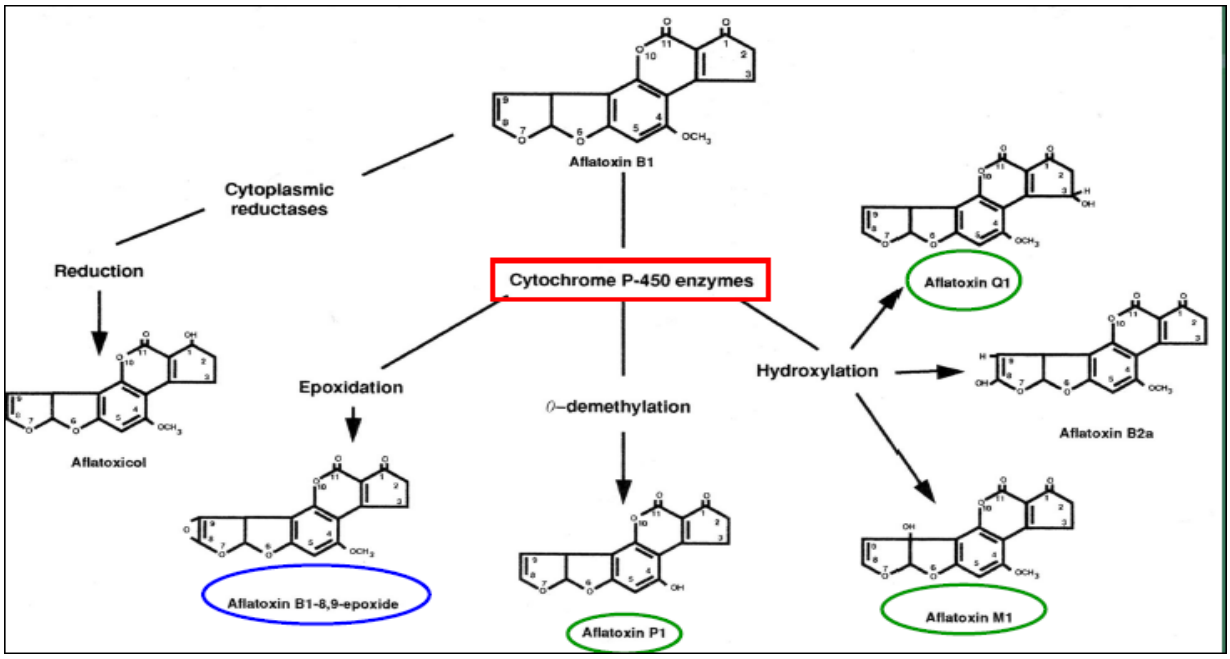

Figure 6: Metabolic pathway of aflatoxins (Wild and Gong, 2010).

14.8.2. Aflatoxin Producing Fungi

Fungi are microscopic organisms that contaminate food and are spread worldwide, and produce effects that are life-threatening (Newberne, 1974). The *Aspergillus* groups, are widespread in nature and are regarded as soil fungi (Gourama and Bullerman, 1995). As a member of a large phylum of *Ascomycota*, the *Aspergillus* genus contains roughly 185 species within 18 groups with morphological, genetic and physiological similarity (Roquebert, 1998). In addition, around 20 species are human and animal pathogens (Barkai-Golan and Paster, 2008; Sanglard, 2002).

Fungi are adapted to a wide range of environmental conditions and geographical distribution, but are found mostly in the tropical and subtropical regions. They can develop on several substrates such as food commodities of plant origin examples include: groundnut, maize, wheat, millet, rice and sorghum and in some cases also on commodities of animal origin (meat products, sausages and milk) (Sanglard, 2002). Their growth on the substrate can lead to the change of nutritional and dietetic qualities of the products and also to the production of mycotoxins (Barkai-Golan and Paster, 2008).

Aspergillus species contain a large number of mycotoxigenic species such as; *A. alliaceous, A. carbonarius, A. flavipes, A. flavus, A. parasiticus, A. fumigatus, A. nomius, A. tamari, A. versicolor, A. terreus, A. niger, A. bombycis, A. ochraceoroseus, A. pseudotamari*, among them. Some species including *A. fumigatus* and *A. niger* can be directly pathogenic to humans and livestock because these are able to invade the living tissues and stimulate illnesses such as aspergillosis (Judson, 2004).

1.4.9. FUNGAL INVASION AND AFLATOXIN CONTAMINATION OF GROUNDNUTS

Fungi have evolved over the centuries and have specialised to exploit a wide variety of environments and as a result different species require different conditions for optimal growth and development (Klich, 2007). However, studies have shown that there are two distinct groups of fungi that invade groundnuts namely; “field fungi” and “storage fungi”. Field fungi attack groundnut pods and kernels while the plant is still developing in the field and contamination can be aided by mechanical damage, drought stress, insect and bird damage, excessive rainfall and late harvesting.

The second group commonly known as storage fungi, invades groundnuts after harvest and during post-harvest handling, transport and storage. The conditions that promote invasion and contamination of the crop in storage are average high temperature (25-40 ^{o}C) and higher moisture content of the seed which is influenced by the drying method (Van Egmond, 2004).

1.4.9.1. Pre-harvest Aflatoxin contamination of Groundnuts

Aflatoxin contamination is divided into two stages namely; infection of the developing crop in the first phase and contamination after maturation in the second phase (Cotty, 2001). Climate influences contamination of groundnuts while it is still growing in the field by affecting the host's susceptibility to fungal invasion and by providing optimal conditions for fungal growth. As weather changes, so do the complex communities of aflatoxin producing fungi (Cotty *et al*., 2007). Climate predisposes hosts to contamination by altering crop development and by affecting insects that create wounds on which aflatoxin producers proliferate. Changes in climate could be drought stress and fluctuating soil temperatures during the latter parts of the growing season (Augusto, 2004; Parmar *et al.*, 1997).

Drought stress and very hot conditions (25-30 ^{o}C) during the first phase of crop development results into substantial fungi infections (Guo *et al*., 2003). This is because drought stress results into a loss of moisture from the groundnut kernels and thereby reducing the efficiency of the kernels to produce phytoalexins, which allows the toxin producing fungi to grow until the low moisture content becomes limiting for fungal growth (Odvody *et al*., 1997; Cole, 1982). Luis (2014), reported that drought and heat stress enhance aflatoxin contamination of groundnuts, especially when such occurs during the last three to six weeks of the growing season and therefore identifying drought tolerant genotypes may aid in development of aflatoxin resistance in groundnuts. This therefore shows a direct correlation between drought tolerance and aflatoxin resistance.

Belli *et al*., (2004) emphasized that temperature (hot or warm conditions) and water activity (humidity or moisture) are the major factors in the growth of fungi and aflatoxin production. This is because the two facilitate in the mechanical damage of the kernels and thereby enabling the entry of aflatoxin causing fungi from the soil into the seed. Other authors argue that temperatures ranging between 25 to 30 ^{o}C are conducive for aflatoxin development (Cotty and Lee, 1990).

Diverse insects carry aflatoxin producing fungi (Williams *et al*., 2002) and specific insect/crop combinations have been repeatedly linked to aflatoxin contamination (Dowd *et al*., 2005). These include, corn borers on maize, pink bollworm on cotton, lesser corn stalk borer on groundnuts and the navel orange worm on pistachio (Guo *et al*., 2003; Sommer *et al*., 1986; Russell *et al*., 1976). Pods and seeds damaged by insects are directly exposed to fungal invasion. French and Morgan, (1972) found out that drought conditions favour the development of lesser corn stalk borers that damages pods and feeds on kernels.

1.4.9.2. Post-harvest Aflatoxin Contamination of Groundnuts

Moisture and temperature influence the growth of toxigenic fungi in stored commodities (Hell and Mutegi, 2011). Higher than normal temperatures in storage are usually accompanied by insect damage and fungi infection. Sugri *et* al. (2015) reported that the optimal temperature range for fungal invasion and aflatoxin production for *A. flavus* and *A. parasiticus* (25-35 ºC) with (0.95) water activity and (10-40 ºC) with (0.99) water activity, respectively, and neither *Aspergillus* species produce aflatoxins when developed below 7.5 ºC or above 40 ºC (Table 2).

Table 2: Environmental factors for *Aspergillus* growth and aflatoxin production.

		A. Flavus			***A. Parasiticus***		
		Minimum	Optimum	Maximum	Minimum	Optimum	Maximum
Growth	T (°C)	10-12	33	43	12	32	42
	Water Activity*	0.8	0.98	>0.99	0.80-0.83	0.99	>0.99
	pH	2	5.0-8.0	>11	2	5.0-8.0	>11
Aflatoxin Production							
	T (°C)	13	16-31	31-37	2	25	40
	Water Activity*	0.82	0.92-0.99	>0.99	12	0.95	>0.99
	pH	-	-	-	0.86-0.87	6	>0.99

International Commission on Microbiological Specifications for Foods (1996).

Groundnuts exhibits indeterminate growth habit, making it difficult to determine the optimum time of harvesting the crop (Kaba *et al*., 2014). However, it is very important to harvest groundnuts at the correct time. Delays in harvesting will result in poor quality seed due to mould infections and subsequent aflatoxin contamination of the kernels/pods (Okello *et al*., 2010). It is therefore very important to harvest the crop at the right time to prevent aflatoxin contamination.

The correct drying of the harvested groundnuts is very important as poor drying can help induce fungal growth and aflatoxin contamination. The traditional groundnut drying techniques in developing countries like Uganda, Mozambique, Malawi and Tanzania involving field and bare ground drying are a major source of fungal contamination (Okello *et al.*, 2010). These are slow, time consuming and labour intensive, which involve lots of crop handling and due to rains that normally persist at harvesting and drying times, it is difficult to achieve the recommended moisture content for safe storage (which is 8-10 %). In addition, bare ground drying of groundnuts persistently exposes the crop to be in direct contact with the soil which is the major source of fungi leading to aflatoxin contamination (Kaaya *et al*., 2007).

Proper groundnut shelling is very important in aflatoxin management, this is because physical damage to the pods and kernels provides a ready entry of fungi (Kaaya *et al*., 2007). Emmott, (2013), highlighted that African groundnut farmers traditionally shell groundnuts by hand, which is painful and time-consuming. During this process the shells are often softened in water to ease the process, and the shelled nuts are subsequently kept in unsuitable storage conditions on-farm until the crop is taken to market. Moisture introduced during shelling promotes fungal growth on the nuts, and the long storage times in poor conditions further increase the risk of aflatoxin contamination.

Freshly harvested groundnuts should be cleaned and sorted to remove damaged nuts and other foreign matter (Farid *et al*., 2013). Foreign material for example; soil, sticks, weeds and stones during storage, transportation and marketing processes may be a source of fungal inoculum and may result into contamination of groundnuts (Augusto, 2004). In order to improve the quality of groundnuts being marketed by smallholder farmers there is need of creating incentives to improve the processes for aflatoxin management and control, one incentive could be increased price of quality groundnuts sold by smallholder farmers and the promotion of collective marketing (Emmott, 2013).

1.4.10. Aflatoxin prevention strategies during pre and post-harvest times in groundnuts

1.4.10.1. Resistant varieties

One of the possible means of reducing aflatoxin contamination is the use of resistant cultivars. Several studies have identified the presence of field resistance to seed infection by *A. flavus* in some cultivars. Resistance to pre-harvest field infection is particularly important in areas where late-season drought stress is a common occurrence (Zambettakis *et al*., 1981). Some

cultivars such as PI 337394F and 55-437 developed by ICRISAT, have shown stable resistance to *A. flavus* across locations. These sources among others have been used in breeding programs, and several lines have been reported to possess resistance and produce high yields. Several breeding lines from ICRISAT have also been reported to be resistant to seed infection and colonization; these are; ICGVs 87084, 87094, 87110, 91278, and 91284.

1.4.10.2. Irrigation and water conservation

Drought stress is one of the major factors that predisposes the groundnut crop to fungal invasion. The use of irrigation and water conservation technologies could therefore assist in mitigation of aflatoxin contamination of groundnuts. This is because irrigation moderates the risk of aflatoxin contamination of groundnuts in the latter part of the growing season or prior to harvest and may reduce the risk of damage from lesser cornstalk borer and other small animals in the soil (Schuster *et al*., 1975). Wilson *et al*. (1989) in their study on the effect of irrigation on percent fungi recovered from NC-7 groundnut kernels and hulls, found that irrigation significantly reduced the numbers of kernels and hulls from which members of the *A. flavus* group fungi were recovered from 19.5 to 7.8 %. Sufficient moisture can also be made available to groundnuts by the construction of box ridges and mulching the field thereby allowing the water to get trapped within the box ridges and sink into the soil and mulching preventing high evaporation losses from the soil (AICC, 2014).

1.4.10.3. Soil treatments

Soil treatments such as application of lime (0.5 t/ha), manure (10 t/ha) and cereal crop residue (5 t/ha) at the time of sowing are also effective in reducing *A. flavus* seed infection and aflatoxin contamination in groundnuts by 50-90 % (Torres *et al*., 2014). Lime is a good source of Calcium for the formation of a strong groundnut seed coat. Fernandez *et al*. (1997) found out that liming was effective in preventing infection by *Aspergillus* species and its aflatoxigenic isolates.

1.4.10.4. Biological control

Biological agents can also be used to control aflatoxin contamination in groundnuts. Cotty (1990) has done considerable research on the use of non-toxigenic strains of *A. flavus* to control aflatoxin contamination. This approach is based on the substitution of aflatoxin-producing strains of *A. flavus* with non-toxigenic strains. New biological control technology has been developed that can prevent much of the contamination of groundnuts with aflatoxins and CPA

that would otherwise occur. The control is based on competitive exclusion and it is achieved by application of a competitive, non-toxigenic strain of *A. flavus* to the soil of developing groundnuts.

1.4.10.5. Cultural Practices

Cultural practices that reduce the incidence of aflatoxin contamination in the field include; timely planting to take advantage of periods of higher rainfall, maintaining good plant density in the fields, removing prematurely dead plants, managing pests, diseases and weeds and avoidance of pod damage during weeding and harvesting (Waliyar *et al*., 2015).

1.4.10.6. Proper Drying of groundnuts

High grain moisture content increases post-harvest grain moulding and aflatoxin contamination (Waliyar *et al.*, 2015). However, proper drying of groundnuts to a desired moisture content of 7-10 % is ideal to prevent growth of fungi, including the aflatoxigenic strains (Dick, 1987). Higher moisture content of greater than 15 % encourage the growth of fungi and aflatoxin contamination.

1.4.10.7. Proper storage of groundnuts

Groundnut pods and kernels should be stored under dry, well ventilated conditions to ensure that moisture content remains low, thus discouraging fungal growth (Turner *et al*., 2005). If groundnuts are stored incorrectly, that is, in an improperly dried state or under high temperatures and humidities with inadequate protection, fungi will inevitably grow. Furthermore, duration of storage is an important factor when considering aflatoxin formation (Okello *et al*., 2010). The longer the retention in storage the greater will be the possibility of building up environmental conditions conducive to *Aspergillus* proliferation and production of aflatoxin.

1.4.11. GROUNDNUT DRYING PROCEDURES USED IN DIFFERENT PARTS OF THE WORLD

Drying and its interaction with the maturing process comprise the single most critical factor in establishing the basic flavor quality of groundnut after harvest. This involves a process of water removal such that groundnut biochemistry and physiology are optimum for food quality (Nautiyal, 2002). Proper drying is critical for safe storage, milling quality and flavor quality.

However, extremely high temperatures, while the crop is in windrows, can promote far too rapid drying and may contribute to the development of off-flavors.

Correct and proper drying of harvested groundnuts is also important because poor drying can help induce fungal growth (producing aflatoxin contamination) and may reduce seed quality for consumption, marketing and germination for the following planting season (Waliyar *et al*., 2015). For good storage and germination, the moisture content of the pods should be reduced to 6-8 % (Waliyar *et al*., 2015; Augusto, 2004). Different methods exist for drying the pods, some of which are better than others.

Ideally pods should be dried with sufficient air circulation and in the shade. Blatchford and Hall, (1963) made extensive surveys of the literature on drying methods for groundnut in various countries. Some of the drying methods being followed in the developing countries are mentioned below.

1.4.11.1. Scattered on the ground

In this method plants are placed directly on the ground, foliage facing downwards, so that the pods are exposed to the sun (Figure 7). Conversely, in some areas, foliage is placed upwards with the pods in contact with the moist-soil and protected from the direct sunrays (Nautiyal, 2002). Plants are left in this position for varied periods of time, which often depends on the beliefs of the individual farmer. There is no criterion of moisture content for determining when the plants should be collected from the ground. In Uganda, the moisture content reduced from about 40 to 25 percent in one day and further diminished to 6 percent after 20 days (Okello *et al*., 2010). In South Africa it is recommended that plants should be allowed to lie on the ground surface for a period of three days to allow leaves to dry out before stacking (Nautiyal, 2002). Drying by this method locally called “sun curing” and has an adverse effects on the quality of kernels, makes it difficult to sell the crop and may result into fungal growth and subsequent aflatoxin contamination of the crop. Additionally, groundnuts are left too long on the ground to dry, dew and sunlight tend to discolour the nuts.

Figure 7: Groundnut drying on bare soil direct to sunlight (Nigam, 2014).

1.4.11.2. Windrows

This method is used for drying groundnuts prior to further drying in stacks (Figure 8). After harvest plants are dried in inverted windrows for 2 to 3 days (AICC, 2014). In these windrows, the pods on top may be exposed to the weather or they may be underneath, next to the ground covered by the foliage. Loose fluffy windrows permit good air circulation, which ensure uniform and fast drying of the pods. Groundnuts 'dried' in fairly large windrows with the pods protected from full sunlight by the haulms have been shown to lose moisture more slowly and suffer no apparent damage as compared to the pods exposed to the sun in small, thin windrows (Nautiyal, 2002).

Adverse weather conditions for example: if there are rains before the pods are dry enough to be picked-up, may result into mould damage and consequent blackening of the shell, together with some blackening of kernels. Furthermore, heat and too rapid drying usually damage groundnuts exposed to direct sunlight at the top of windrows for more than two days (Blatchford and Hall, 1963). Consequently, in colder areas freshly dug and windrowed groundnuts, which become frosted, will have impaired viability and reduced vigour of seedlings. It is recommended that when windrowing is used as a preliminary to all drying, this be done for three to four days to attain moisture content between 15 and 20 %.

Figure 8: Groundnut windrow drying technique.

1.4.11.3. Stacks/Corks

Blatchford and Hall (1963) defined the term 'stack' as structures formed by grouping a number of plants together (Figure 9). They described four types of stacks: ordinary, ventilated, poled and ventilated poled stacks. The ordinary stack is the simplest type of stack and is formed by gathering the plants into heaps, the dimensions of which often range from 3 feet in diameter and 2 to 3 feet high to about 12 feet in diameter and 5 feet high. The pods may either be scattered throughout the stacks, which is common in the African countries or lie at the stack centre or around the outside of the stack, depending on the area and the type of groundnut grown.

In several groundnut-growing areas, pods are picked from the plant after drying for about two to four weeks in these stacks and are made ready for selling or storage. The drying period in these stacks also varies, frequently lasting from 10 to 15 days but in a few cases only two to three days. The small stacks are gathered together into large stacks with the haulms towards the outside and the pods towards the inside. These larger stacks are usually built at the edges of fields and some farmers choose shaded sites for stacking to avoid over drying by the sun.

In ventilated stacks the plants are congregated together so that the centre of the stack remains exposed to assist ventilation. The pods, as with ordinary stacks, may be scattered throughout the stack or all lie at the stacks centre or around the periphery of the stack. Farmers in Malawi build stacks of this type locally known as 'cocks' (AICC, 2014). The stacks are built on small mounds of earth and the pods placed at the inside of the stacks. This protects the pods from adverse weather conditions for example showers and excessive sunlight during drying that may affect kernel quality. It is also suggested that pieces of plastic sheeting or grass thatching be used to cover the stack because this would help to shed rain to outer edges.

Figure 9: Drying groundnuts using stacks/Mandela cork.

When stacks are built near farm buildings on an area of cleared ground, 'ventilation tunnels' constructed with the help of poles available locally is recommended. In most humid parts of Kenya plants are often built into a stack called 'poled-stacks'. In this method plants are grouped around a centre pole, which supports the stack and prevents it being blown down by the wind (Nautiyal, 2002). The pods may be scattered throughout the stack or may lie in the stack centre or round the periphery. It is reported that drying in such stacks takes about three to five weeks.

1.4.11.4. Platforms/A-Frame

Another method of drying groundnuts is the use of platforms or A-frames (Figure 10). Wilted plants are gathered and stacked on an A-frame with the pods facing inwards and away from the soil. These A-frames are easy to construct using three thick poles as a base with thin poles attached to either side of the main poles of the A-frame forming shelves on to which the wilted plants can be placed. The lowest shelf is about 30cm above the ground. Excellent air circulation occurs and, if constructed properly, the drying foliage of the plants protects the pods from rainfall (Nautiyal, 2002).

Figure 10: Platform drying of groundnuts.

1.4.11.5. Racks

The drying of pods on racks has been referred to in a number of countries (Figure 11). In Zambia to prevent termite damage to groundnuts during the drying period, a horizontal rack is used. The rack consists of crossed pieces of local wood 36 inches long, 18 inches apart and rose 18 inches off the ground. Plants hung on the rack are protected from termites and could be arranged so that the nuts are shaded from the direct rays of the sun. The moisture content of pods on the rack comes down from 21 percent to 6 percent during the first 7 days of drying (Nautiyal, 2002).

Figure 11: Drying groundnuts using racks (Okello *et al*., 2010).

1.4.11.6. Tarpaulins

This involves the drying of pods by spreading them in a thin layer on the soil or woven matting or tarpaulin material and is a common practice in many parts of the world including SSA (Figure 12). In Uganda where harvesting occurs largely in the wet season a period of four to six weeks is given as the probable time taken for pods to dry to about 10 % moisture content (Okello *et al*., 2010).

Figure 12: Drying groundnuts using tarpaulins.

Though this technology is cheap and easily accessed by farmers it has two major disadvantages. Initially, there is the problem of moisture in the ground in contact with the pods together with restricted air movement within the produce. The second difficulty is the time and effort required to gather the pods together, cover them during rain showers and re-spreading the pods as soon as possible to continue drying (Nautiyal, 2002).

1.4.11.7. Trays

In some countries farmers are encouraged to spread their produce on trays, which they leave exposed to sun-drying during the day and shifts into the house at night. In Uganda for example trays, which hold one hundred kilograms of produce consist of a metal mesh base and wooden sides with handles at both ends (Okello *et al*., 2010). These trays can be raised off the ground by supporting the four corners on sticks (Figure 13).

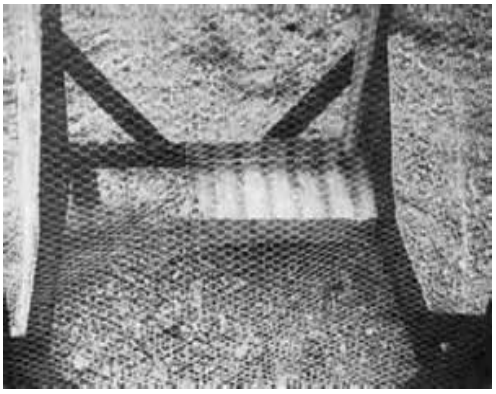

Figure 13: An example of a groundnut tray used for drying (Okello *et al*., 2010).

1.4.12. HUMAN HEALTH IMPACTS OF AFLATOXINS

Considered as the most significant mycotoxin, aflatoxin is a natural potent carcinogen known to affect both humans and livestock (Kamika and Takoy, 2011). Some of the effects of aflatoxins in humans include; impotence in males, under-malnutrition especially in children, liver cancer and sometimes in acute cases death.

1.4.12.1. Acute effects of aflatoxins

Acute toxicity of aflatoxins (aflatoxicosis) is brought about by the intake of a considerable dose of aflatoxin-contaminated food. The ingestion of high-level aflatoxins produces an acute liver failure (hepatic necrosis), which is generally manifested by rupture of blood vessels causing non-stop breeding in the body (haemorrhage), oedema (swelling of ankles), alterations in digestion and changes to the absorption of substances (Williams *et al*., 2004).

An acute outbreak of aflatoxicosis associated with the consumption of aflatoxin-contaminated maize occurred in rural Kenya in 2004, resulting in 317 cases with 125 deaths (Lewis *et al*., 2005). In China, aflatoxicosis caused the deaths of 13 children due to acute hepatic encephalopathy (brain damage) (Brera *et al*., 2008).

1.4.12.2. Chronic effects of aflatoxins

The chronic toxicity of aflatoxins results from long-term exposure to low or moderate levels and does not lead to immediate symptoms as dramatic as acute aflatoxicosis (Moss, 1996). It is stated that chronic exposure to aflatoxin leads to a high risk of developing cancer, especially liver cancer, as well as stunted growth and delayed development in children (Wu *et al*., 2011).

1.4.12.3. Role of aflatoxins in fertility

Aflatoxins were reported to affect the reproduction capacity and fertility of both livestock and humans (IARC, 2002). A study on the effect of AFB1 on sheep epididymal and ejaculatory sperm viability and mortality demonstrated that a group of aflatoxin (AFB1) disrupted the connection tube (epididymis) between the testicles and the male organ of the animal. This affected the ejaculatory and motility of sperms and therefore affecting male fertility (Tajik *et al*., 2007). Another study conducted by Ibeh *et al*. (1991) revealed that when albino rats were exposed to aflatoxins, their spermatozoa were resembling those observed in the semen of infertile men exposed to aflatoxins.

1.4.12.4. Role of aflatoxins in under-malnutrition

Mostly affecting children, under-nutrition has been defined as the outcome of insufficient food intake and repeated infectious diseases. This includes; underweight, dangerously thin and deficient in vitamins and minerals (UNICEF, 2006). It has been reported that in SSA, approximately 50 % of the 4.5 million deaths of children under the age of five are associated with under-nutrition and growth impairment and aflatoxin contamination apparently is the main contributor (Turner *et al*., 2007).

Aflatoxin B1 has also been implicated to the aetiology of kwashiorkor (severe protein deficiency that can cause organ failure and eventually death) and marasmic kwashiorkor (severe acute malnutrition) in humans (Sibanda *et al*., 1997). This association has been reported from several African countries including Sudan, Nigeria, South Africa, Liberia, Rwanda, Mozambique, Malawi, Ghana and the Philippines (Seres and Resurrection, 2003). Oyelami *et al*. (1997) reported the presence of aflatoxins in the lungs of children who died from kwashiorkor and miscellaneous diseases in Nigeria.

1.4.12.5. Role of aflatoxins in development of carcinogenic diseases

Aflatoxins, especially AFB1, are among the most potent naturally occurring carcinogens known and may induce tumour growths in many humans and animals (Moss, 2002). Additionally, aflatoxins have a synergistic effect with other diseases such as kwashiorkor, HIV/AIDS, hepatitis B or C or with other mycotoxins, for example, fumonisins B1, T-2 toxin and zearalenone (Orsi *et al*., 2007) (Figure 14).

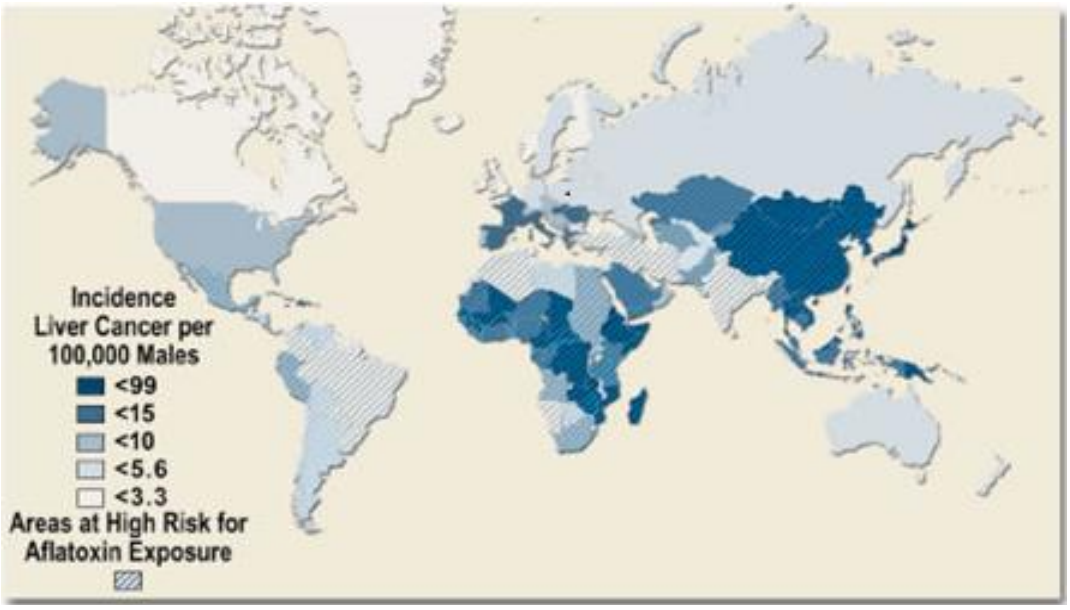

Figure 14: Correlation between populations with high liver-cancer rates and high risk of chronic aflatoxin exposure (National Institute of Environmental Health Sciences, 2011).

1.4.12.6. Role of aflatoxins in development of liver cancer

Chronic aflatoxin exposure results mostly in primary liver cancer of which hepatocellular carcinoma is by far the predominant type (Wild and Hall, 2000). Liver cancer has become a major public health problem being the third most frequent cancer accounting for 695, 000 deaths in 2008 and 550, 000 to 600, 000 new cases each year, with an estimated 42 000 deaths occurring every year in SSA (Wu *et al*., 2011). There is evidence that human exposure to high levels of aflatoxins in food and hepatocellular carcinoma occurs more frequently in developing countries where the incidence of hepatitis B virus is also high (Turner *et al*., 2005).

1.4.12.7. Aflatoxins as immunosuppressant agents

Immunosuppressive agents are substances that prevent activity of the immune system by suppressing the cell-mediated immunity and the humoral immunity (Williams *et al*., 2004). Studies by Jiang *et al*. (2008) have shown that aflatoxins impair DNA-dependent RNA polymerase, which results to inhibiting RNA and protein synthesis, consequently damaging the proliferation and differentiation of immune cells, immunoglobulin, and cytokines, which leads to immune dysfunction. It is therefore hypothesized that aflatoxin exposure may also influence the pattern of infection leading to an immune dysfunction of people living with HIV/AIDS.

1.4.13. ECONOMIC IMPACTS OF AFLATOXINS

Due to the effects of aflatoxin on human and animal health, international trade bodies and health authorities have imposed limits of aflatoxins permissible in various crops. For example, in the European Union (EU), the presence of aflatoxins in groundnuts is strictly monitored and regulated to guarantee their safety with a limit of 2 ppb for AFB1 and 4 ppb for total aflatoxin (van Egmond, 1989). These restrictions cause major agricultural and economic problems since aflatoxins occur in the field, during harvest, storage or during processing (Dorner and Cole, 2002). D'mello (2003) reported that aflatoxin is the most important problem regarding quality of groundnuts worldwide.

Aflatoxins, especially AFB1, can also contaminate many other commodities such as sorghum, pistachio nuts, cottonseed meal, maize, groundnut, rice and millet during growth, harvesting, processing, storage and shipment, thereby causing serious economic losses due to production losses, loss of export markets and rejection of produce at import ports (Kamika and Takoy, 2011). For example, in USA, growers in Texas, Louisiana and Mississippi sustained losses estimated at $85 million to $100 million from maize that could not be utilised for human consumption because of high levels of aflatoxin. It has been estimated that both cattle farming and food packaging/processing industries in North America lose around $5 billion each year because of mycotoxin contamination (Wu, 2004).

African countries are projected to lose approximately $670 million annually due to the inability of African farmers to meet the aflatoxin standards of the EU for the crops that they produce (Otsuki *et al*., 2001). In the 1960/70s Africa used to be the main exporter of groundnuts into international markets accounting for up to 90 % of the global trade, by 2005 this had collapsed to less than 5 % (Table 3). The decrease in exports from Africa is due to tightening controls of aflatoxin levels in commodities by importing countries (Diaz Rios and Jaffee, 2008).

In West African countries such as Senegal where groundnut is an important export crop, the quantities of groundnut exports declined significantly during the period of 1961 (269, 436 Mt) to 2000 (1,792 Mt) because of increasing restrictions on importation of contaminated produce into the EU. This has massive economic implications for African exporters and growers (Boakye-Yiadom, 2003).

Table 3: Share of Leading Global Exports of Raw Groundnuts by Volume.

Year	SSA	USA	Argentina	India	China
	%	%	%	%	%
1962-1971	86.0	4.0	-	1.7	-
1972-1981	37.2	34.7	3.8	6.4	-
1982-1991	4.4	31.1	12.1	2.6	31.2
1992-2001	4.8	19.9	16.2	11.2	30.5
2002-2005	4.5	13.3	8.3	12.2	37.3

Diaz Rios & Jaffee (2008)**.**

It has been reported that the biggest groundnuts-exporting regions which include USA, China, Argentina and Africa would experience economic losses of up to $ 450 million per year if the EU aflatoxin standard of 4 ppb were to be imposed worldwide (Wu, 2004). Table 4 shows the maximum permissible levels of aflatoxin in imported groundnut for human consumption and livestock and poultry feeds in different countries in the world.

Table 4: Maximum permissible levels of aflatoxin in imported groundnut for human consumption and livestock and poultry feeds.

Country	Aflatoxin Type	Maximum Permissible Level ppb	
		Food Stuffs	Livestock feed
Belgium	B_1	5	20
France	B_1	1	20
Ireland	B_1	5	20
The Neverland's	B_1, B_2, G_1, G_2	0	20
Sweden	B_1, B_2, G_1, G_2	5	10
United Kingdom	B_1, B_2, G_1, G_2	4	20
USA	B_1, B_2, G_1, G_2	20	20
Republic of South Africa	B_1, B_2, G_1, G_2	10	20
Malawi	B_1, B_2, G_1, G_2	15	20
Kenya	B_1, B_2, G_1, G_2	15	20
Zambia	B_1, B_2, G_1, G_2	15	20
Nigeria	B_1, B_2, G_1, G_2	4	20
Benin	B_1, B_2, G_1, G_2	4	20

Nautiyal (2002).

1.5. REFERENCE

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CHAPTER TWO

EFFECT OF HARVESTING TIME ON YIELD AND YIELD COMPONENTS OF GROUNDNUT IN NORTHERN MOZAMBIQUE

ABSTRACT

Groundnut (Arachis hypogaea L.*) is one of the most important legume crops in Mozambique grown for food as well as a cash crop. However, it's indeterminate growth habit and below ground nature of fruiting makes it difficult to determine the time of maximum maturity of pods. This results into reduced crop yields if either harvested too earlier or if harvested too late. The objectives of the study were therefore to determine the optimum time to harvest groundnut for optimum yield of pods for three Spanish varieties and to estimate yield losses as a result of harvesting time at two locations namely; Nampula Research Station (PAN) and Mapupulo Agricultural Research Center (CIAM) in Nampula and Cabo Delgado provinces respectively. The experiment was laid out in a randomized complete block design in a split-plot arrangement with four replicates. The varieties (ICGV-SM-99568, ICGV-SM-01514 and JL-24) were the main factor and three harvesting dates (10 days before physiological maturity, at physiological maturity and 10 days after physiological maturity) were the sub-plots. Data was collected on number of pods per plant, pod yields, kernel yields, shelling percentages and late leaf spot incidence and severity. Pod yields on average were between 1276.9 and 1503.6 kg/ha at CIAM and PAN respectively for the three varieties at physiological maturity, which were higher than yields obtained from harvesting the crop 10 days before (904.6 and 950 kg/ha) and 10 days after (826.8 and 1047.4 kg/ha) physiological maturity. Furthermore, yield losses ranged from (16-25 %) and (30-40 %) as a result of harvesting groundnut 10 days before and 10 days after physiological maturity respectively. The results also revealed a negative correlation between late leaf spot severity and pod yield of groundnut. It is therefore advisable that farmers' plant improved varieties, making sure they harvest at physiological maturity, before the onset of the dry season, in order to obtain maximum pod yields of the groundnut.*

Keywords: Groundnut, harvesting time, optimum pod yield, Spanish varieties, late leaf spot.

2.1. INTRODUCTION

Groundnut is one of the most important food legumes in the world and is the third largest oilseed crop after soybean and cottonseed globally. It is an important source of vegetable protein and oil in sub-Saharan Africa (Kaba *et al*., 2014). It also contains good sources of vitamin E, niacin, folate and magnesium (Griel *et al*., 2004). The world production of unshelled groundnut is estimated to be 35.9 million metric tonnes (Kombiok *et al.,* 2012) annually.

In Mozambique groundnut is one of the most important grain legumes. The crop is grown throughout the country with the largest concentration in the Northern provinces of Nampula, Zambezia and Cabo Delgado (Jeffrey, 2011). In the northern region of the country planting is done at the onset of the rains which is between November and December and harvesting is done in April and May. Production is mainly through hand using hoes and no input fertilizer and pesticides whilst using predominantly disease susceptible local varieties.

Yields of groundnut in Africa are much lower than the average world yields, and yields in Mozambique are even much lower (0.4 to 0.8 Mt/ha) (FAO-STAT, 2015). Monyo (2013) attributes this low yield to non-availability of improved seed varieties and lack of organized seed production and delivery systems. However, these constraints are being addressed by IIAM and ICRISAT through their groundnut breeding programs. Currently, several high yielding varieties have been released such as; *ICGV-SM - 99541, ICGV-SM - 99568, ICGV-SM - 01513, ICGV-SM - 01514, CG-7, JL-24, Mamane, Nametil* and *Otitela*.

Despite the release of these high yielding groundnut varieties to farmers to increase productivity, it has been observed that the crop yields on farmers' fields are still lower than expected (Muitia, 2013). Other reasons for the low yields, which have also been identified and are being addressed include pest and disease infestations, and climatic and adverse weather conditions affecting the crop (Muitia, 2005). In addition, farmers' assertiveness and delay in execution of some cultural and agronomic operations on their fields may also be responsible for the current low groundnut yields.

One of the most critical activities not timely executed is harvesting of groundnut. Upadhyaya *et al*. (2006) reported that maintaining groundnut germplasm requires harvesting at optimum maturity to obtain healthy seeds. Timing of harvesting greatly influences the grade and quality of the crop yield. Harvesting at high moisture content exacerbates the chances of fungal

infection on the kernel, while harvesting at low moisture content increases mechanical damage to the kernel (Yadav *et al.*, 2005).

Different methods are used to determine the timing of harvesting groundnut by farmers in different parts of the world. Some farmers determine maturity and harvest their groundnut based on morphological features such as; dropping of older leaves, yellowing of foliage and color changes of the inner groundnut mesocarp (Jordan *et al*., 2008). However, groundnut fruiting is subterranean in nature making it difficult to determine maximum maturity of pods using only morphological features; additionally, groundnut has indeterminate growth habit, which ensures that pods are produced at every stage of its growth (Jordan, 2006a). This, therefore, poses a huge challenge in determining how to balance the continuous production of immature pods and earlier formed pods in terms of when to harvest.

Delay in harvesting may expose the crop to field pests which cause substantial loss. Yield losses due to termites, which predominantly damage harvested kernels was estimated at 10 to 30 % (Umeh *et al*., 2000). Late harvesting also results in leaving many pods in the soil due to weakening of pegs (Singh and Oswalt, 1995). Wright and Porter (1991) indicated that harvesting groundnut too early can reduce yield by 15 % and economic value by 21 %. Furthermore, Kaba *et al*., (2014) reported that premature harvesting of groundnut pods lower's the yield, oil content and seed quality due to immature pods and seeds. As such, harvesting of seeds at the right stage of maturity is most important since harvesting either at early or late stage results in lower yields with poor quality seeds.

2.1.1. JUSTIFICATION

Groundnut is an essential crop in northern Mozambique where it is grown both as a cash and food crop. The crop is grown throughout the country mainly by resource-poor small-scale farmers under rain-fed conditions. However, groundnut yields realized by these small-scale farmers are reasonably low (400-600 kg/ha) and of poor quality (Muitia, 2005). The low yields have been attributed to several constraints. Some of the major groundnut production constraints include; lack of improved cultivars, poor cultural practices, insect pests, diseases, weeds and drought (Muitia, 2013).

The timely execution of cultural and agronomic practices by groundnut farmers is very important as it contributes to kernel yield and quality. However, some activities are not executed on time resulting into reduced crop yield. Among these activities is harvesting of the

crop. It has been observed that groundnut is always harvested several weeks before or after physiological maturity in both Nampula and Cabo Delgado provinces, as farmers are always engaged in both farm and off-farm activities. Furthermore, there is little information on the effect of early and delay of harvesting on the pod and kernel yield of groundnut in Northern Mozambique. It was in the light of this that three Spanish groundnut varieties were subjected to different harvesting dates, starting from 10 days before the actual physiological maturity, to assess the pod and grain yield at each harvest and grain quality with time.

2.1.2. OBJECTIVES

2.1.2.1. General objective

To evaluate the effect of harvesting time on groundnut yield and yield components of three Spanish groundnut varieties and estimate yield losses at different harvesting times.

2.1.2.2. Specific objectives

- To determine the effect of harvesting time on pod and kernel yield of three Spanish groundnut varieties.
- To identify and estimate the losses in groundnut yield due to differences in harvesting time.

2.2. MATERIALS AND METHODS

2.2.1. Description of the Study Area

The study was conducted in two sites namely; Nampula Research Station (PAN) and Mapupulo Agricultural Research Center (CIAM), located in Nampula and Cabo Delgado Provinces respectively (Figure 15). Nampula Research Station (PAN) is located about 7 km east of Nampula city in Northern Mozambique (15º 09' S, 39º 30' E) and is elevated at 432 m above sea level. The soil type is sandy loam and the vegetation is predominantly grassland. The average rainfall is slightly over 1000 mm which starts around November/December up to April/May with its peak in January. The maximum temperature in the region is about 39 ^{o}C and the minimum temperature is 19 ^{o}C (Muitia, 2013).

Mapupulo Agricultural Research Center (CIAM) is located about 18 km south of Montepuez town about 200 km west of Pemba the capital of the province, which lies at (13^{o} 12' S, 38^{o} 53' E) and is elevated at 476.7 m above sea level. The soils are clay loam and deep brown loam. It receives annual precipitation of 1200 mm on average from November/December to April/May, and the average temperature is between 20 and 25 ^{o}C (Muitia, 2013).

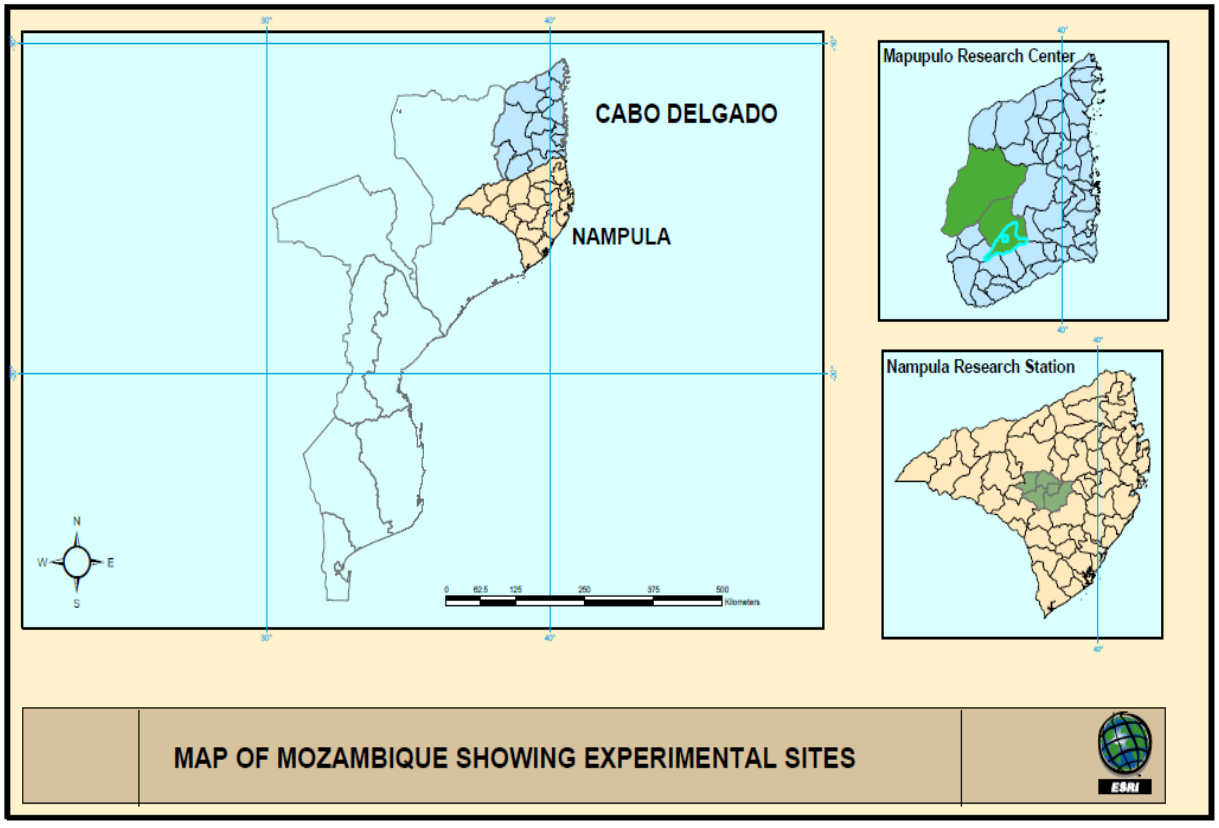

Figure 15: Map of Mozambique showing experimental sites.

2.2.2. Field establishment

The study was conducted during the 2015/2016 growing season at PAN and CIAM research stations in Nampula and Cabo Delgado provinces respectively. The experiments were laid out in a split plot arrangement of treatments in a randomized complete block design with four replications (Appendix 8). The main plot was made up of three groundnut varieties that take 90 days to mature while sub-plots were the three harvesting dates. The harvesting dates were (i) 10 days before physiological maturity indicated as H1; (ii) at physiological maturity indicated as H2 and (iii) 10 days after physiological maturity indicated as H3. The test varieties were Spanish groundnut varieties namely: *ICGV-SM-99568*, *JL-24* and *ICGV-SM-01514*. The net plots were 6 rows by 6 m long with one seed per planting station which were spaced at 50 cm between rows and 10 cm within rows.

Harvesting was carried out at each stage by either digging, using a hand-held hoe, when the soil was dry and by uprooting the plant by hand when the soil was wet. Harvesting was carried out every 10 days with the first harvest at 10 days before the actual physiological maturity.

2.2.3. Data collection

Data collected included; crop initial and final stand, days to 50% flowering, number of pods per plant, weight of dry pods, total weight of kernels, 100-kernel weight, pod yield per ha, kernel yield per ha, shelling percentages (%) and disease incidence and severity.

2.2.3.1. Estimation of groundnut pod yield per hectare

In order to estimate the pod yield per hectare from each net plot, the following expression was used:

$$\textbf{Pod yield per ha} = \frac{\text{Pod weight per net plot (Kg)}}{\text{Area harvested } (18\ m^2)} X\ \ 10000\ \text{m}^2$$

2.2.3.2. Estimation of groundnut kernel yield per hectare

Estimation of kernel yield per hectare involved, drying the pods harvested from each net plot, after which the pods were shelled and the following expression was used:

$$\textbf{Kernel yield per ha} = \frac{\text{Kernel weight per net plot (Kg)}}{\text{Area harvested } (18\ m^2)} X\ \ 10000\ \text{m}^2$$

2.2.3.3. Estimation of groundnut shelling percentage

The groundnut shelling percentage was estimated using the following formula:

$$\textbf{Groundnut shelling \%} = \frac{\text{Kernel weight per net plot (Kg)}}{\text{Pod weight per net plot (Kg)}} X \ 100\ \%$$

2.2.4. Late leaf spot incidence and severity

2.2.4.1. Late leaf spot incidence in groundnut

Incidence of late leaf spot was assessed at 60, 70, 80 and 90 days after sowing (DAS) by counting the number of plants infected and expressing it as a percentage of the total number of plants per plot as given as:

$$\textbf{Incidence} = \frac{\text{Number of plants infected per plot}}{\text{Total number of plants in a plot}} X \ 100\ \%$$

2.2.4.2. Late leaf spot severity

Percentage of leaves infected by late leaf spot per plant was recorded on five middle plants from each plot and averaged for each variety. It was recorded at 60, 75 and 90 days after sowing. The expression below was used:

$$\textbf{Severity} = \frac{\text{Number of leaves infected per plant}}{\text{Total number of leaves per plant}} X \ 100\ \%$$

Disease severity was assessed based on a rating scale of increasing severity of 1-9 (Subrahmanyam *et al*., 1995). Disease score 1 means 0 % foliar infection and 9 for 81-100 % of foliar area infection with plants having almost all leaves defoliated leaving bare stems. Varieties with a disease score 4-6 were considered moderately resistant and 7 were designated as susceptible as reported by Pande and Rao (2001).

2.2.5. Data analysis

The data on yield and yield components were subjected to analysis of variance (ANOVA) to establish treatment and the interactions effect on the parameters measured or calculated. Statistical analyses were performed with GenStat Discovery 4. Groundnut varieties and harvesting dates were treated as fixed effects and replication was treated as a random effect. Main effects and all interactions were considered significant at 0.05 and 0.01 probability levels by the F-test. Means were separated using Tukey's test at 5 % level of probability only, when the F-test showed a significant difference. The following statistical model was used to analyze the data:

$$Y_{ijk} = \mu + H_i + V_j + HV_{ij} + R_{k(ij)} + \varepsilon_{k(ij)}$$

Where:

Y_{ijk} = Observed yield of variety

μ = Overall varietal mean

H_i = Effect of the i^{th} harvesting time

V_j = Effect of the j^{ith} Variety

HV_{ij} = Interaction effect of the i^{th} Harvesting time and j^{th} variety

$R_{k(ij)}$ = Effect of the k^{th} replication in the i^{th} harvesting time

$\varepsilon_{k(ij)}$ = Experimental error

2.3. RESULTS

2.3.1. Effect of harvesting time on number of pods per plant of groundnut varieties

Results of number of pods per plant at different harvesting times are presented in Table 5. Significant differences in the total number of pods per plant were observed in both study locations ($P \leq 0.01$). The highest number of pods per plant was recorded when harvesting was executed at physiological maturity (H2) and the lowest when harvesting was executed 10 days before physiological maturity (H1). The variety *ICGV-SM-01514* produced the highest number of pods per plant (39 and 30) while *JL-24* produced the lowest number of pods per plant (18 and 21) at CIAM and PAN respectively. Additionally, harvesting 10 days after physiological maturity (H3) resulted into reduced number of pods per plant, however, these were to some extent higher than when harvesting was executed 10 days before physiological maturity.

Table 5: Effect of harvesting time on number of pods among groundnut varieties.

	Mapupulo Agricultural Research Center			Nampula Research Station		
	Harvesting time			**Harvesting time**		
Variety	**H1**	**H2**	**H3**	**H1**	**H2**	**H3**
ICGV-SM-99568	20^{d}	32^{ab}	22^{cd}	23^{bc}	25^{b}	24^{bc}
ICGV-SM-01514	20^{d}	39^{a}	28^{bc}	21^{c}	30^{a}	20^{c}
JL-24	18^{d}	31^{bc}	24^{c}	21^{c}	27^{ab}	21^{c}
CV (%)	17.9	9.9	11.9	41.2	29.8	37.5
Mean ± SE		26.0 ± 1.68			23.4 ± 1.40	

Means followed by the same letter in the same column are not significantly different at (**$P \leq 0.01$**).

H1 = Harvesting at 10 days before physiological maturity, H2 = Harvesting at physiological maturity and H3 = Harvesting at 10 days after physiological maturity.

2.3.2. Effect of harvesting time on groundnut pod and kernel yield

Pod yields among the groundnut varieties were directly related to the kernel yields. Significant differences were observed in the total pod yields as a result of harvesting time ($P \leq 0.05$) and ($P \leq 0.001$) at CIAM and PAN respectively, with respect to total pod dry weight. The highest pod yields among the groundnut varieties was recorded at PAN (1166.94 kg/ha) than at CIAM (1002.8 kg/ha). In general, harvesting the groundnut varieties at physiological maturity

produced the highest pod yields than the subsequent dates. The highest pod yields were obtained from *ICGV-SM-01514* (1412.5 kg/ha) and *JL-24* (1596.2 kg/ha) and the lowest pod yields were obtained from *JL-24* (693.1 kg/ha) and *ICGV-SM 01514* (835.4 kg/ha) at CIAM and PAN respectively (Table 6).

Table 6: Effect of harvesting time groundnut pod yield (Kg/Ha).

	Mapupulo Agricultural Research Center			Nampula Research Station		
	Harvesting time			**Harvesting time**		
Variety	**H1**	**H2**	**H3**	**H1**	**H2**	**H3**
ICGV-SM-99568	1043^{abc}	1250^{ab}	786.1^{bc}	1102.1^{ab}	1479.9^{ab}	1150.6^{ab}
ICGV-SM-01514	977.8^{abc}	1412.5^{a}	873.6^{bc}	835.4^{b}	1434.7^{ab}	966.7^{ab}
JL-24	693.1^{c}	1168.1^{b}	820.8^{bc}	912.5^{b}	1596.2^{a}	1025^{ab}
Level of sig.	*	*	NS	**	**	NS
CV (%)	23.6	15.2	16.6	29.1	17.9	28.1
Mean ± SE	1002.8 ± 3.21			1166.9 ± 5.62		

Means followed by * are significant at 5 % level, ** are significant at 1 % level, NS are Not significant.

Kernel yields significantly differed among the groundnut varieties as a result of harvesting time (Figure 16). The highest kernel yields were recorded at physiological maturity for all the groundnut varieties. In addition, kernel yields tended to decline with harvesting 10 days before and 10 days after physiological maturity. However, the kernel yields of harvesting at physiological maturity and harvesting 10 days after physiological maturity were higher than that for harvesting at 10 days before physiological maturity. The kernel yields ranged from 525 kg/ha for *JL-24* and 668.7 kg/ha for *ICGV-SM-01514* for harvesting 10 days before physiological maturity to 1165.3 kg/ha for *ICGV-SM-01514* and 1429.6 kg/ha for *JL-24* for harvesting at physiological maturity at CIAM and PAN respectively.

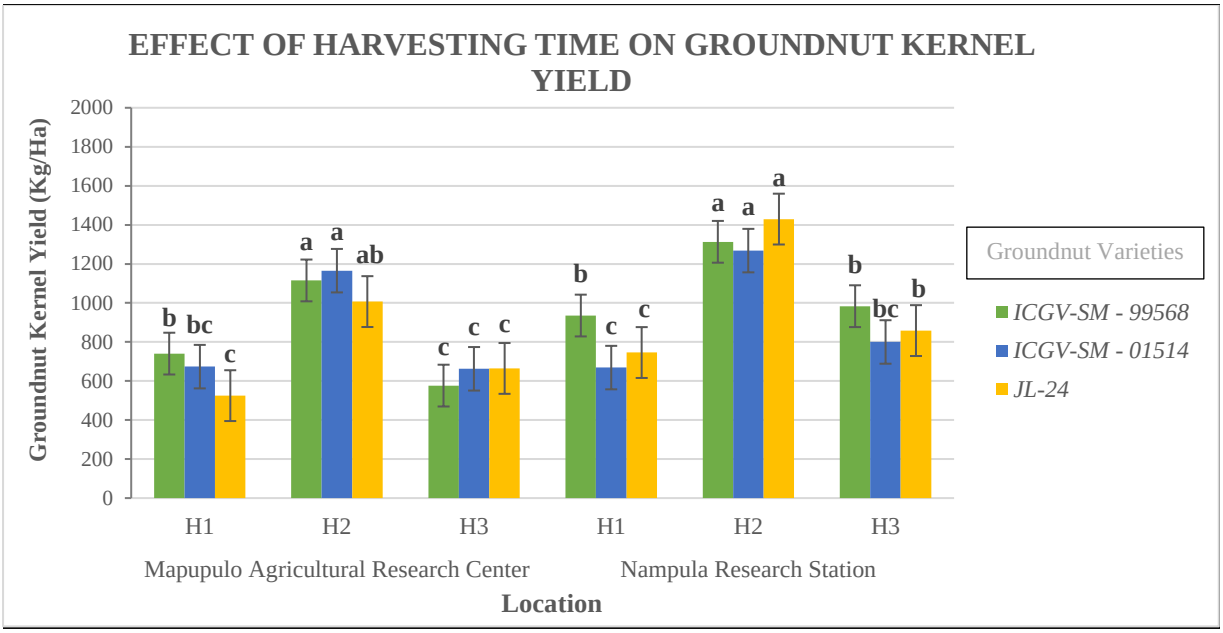

Figure 16: Effect of harvesting time on groundnut kernel yield (Kg/Ha).

Mean values followed by a common letter do not differ significantly according to Tukey's Honestly Significant Difference test (**P ≤ 0.05**).

The interaction between harvesting time and location had significant influences on the total kernel yields of the groundnut varieties (Figure 16). Highest kernel yields were recorded at PAN compared to CIAM. This could be attributed to differences in weather conditions, soil characteristics and incidences of pests and diseases especially leaf spots which were severe at CIAM compared to PAN (Appendix 1 & 2).

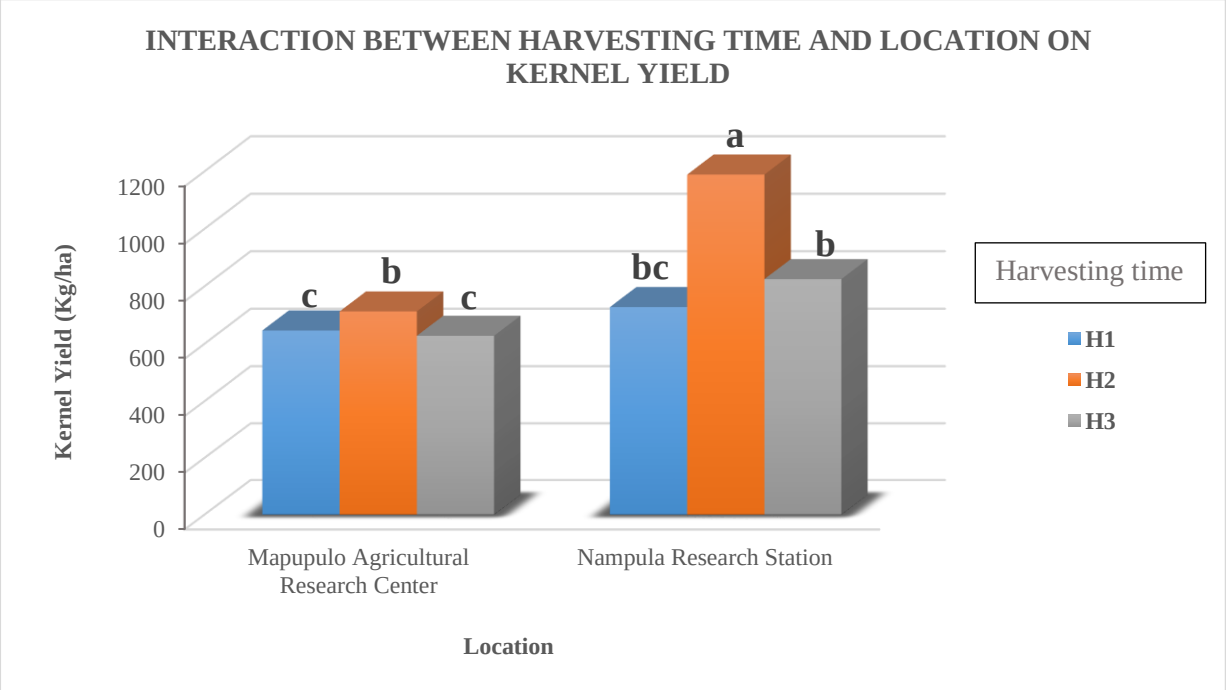

Figure 17: Interaction effect between harvesting time and location on groundnut kernel yield.

2.3.3. Effect of harvesting time on weight of 100-kernels and shelling percentage of groundnut varieties

Significant differences were observed in the weight of 100-kernels among the groundnut varieties as a result of harvesting time (Table 7). The maximum 100-kernel weight (54.9 and 56.9) was obtained from the variety *ICGV-SM-99568* at CIAM and PAN respectively when harvesting was executed at physiological maturity (H2). In addition, the weight of *JL-24* was significantly higher than that of *ICGV-SM-01514* which recorded the lowest kernel weight regardless of harvesting time. The bigger nuts of *ICGV-SM-99568* could be responsible for its higher kernel weight than the *ICGV-SM-01514* variety which had the smallest kernel weight. Additionally, as observed from the results of kernel yields, harvesting 10 days before physiological maturity recorded the lowest 100-kernel weight than the succeeding harvesting times. The 100-kernel weight ranged from 22.4 g for *ICGV-SM-01514* for harvesting 10 days before physiological maturity to 56.9 g for *ICGV-SM-99568* for harvesting at physiological maturity.

Table 7: Effect of harvesting time on 100-kernel weight (g) among groundnut varieties.

	Mapupulo Agricultural Research Center			Nampula Research Station		
	Harvesting time			**Harvesting time**		
Variety	**H1**	**H2**	**H3**	**H1**	**H2**	**H3**
ICGV-SM-99568	42.1^{c}	54.9^{a}	49.9^{b}	44.1^{c}	56.9^{a}	51.9^{b}
ICGV-SM-01514	22.4^{e}	27.4^{d}	26.2^{de}	24.4^{e}	29.4^{d}	28.2^{de}
JL-24	36.7^{bc}	46.9^{ab}	45.1^{ab}	38.2^{c}	48.9^{ab}	47.1^{ab}
CV (%)	4.1	4.3	2.2	4	4.1	2.1
Mean ± SE		49.1 ± 1.03			41.1 ± 1.05	

Mean values followed by a common letter within a column do not differ significantly according to Tukey's Honestly Significant Difference test (**$P \leq 0.01$**).

Visual observations (qualitative) on kernel quality showed that harvesting time had an influence on the kernel quality (Figure 18). The highest quality among the three varieties was obtained at H2 whilst H1 was characterized with poorly formed immature kernels which were shrinked and small while as for H3 the kernels were characterized by damaged kernels as a result of insect activity and some of which had started sprouting.

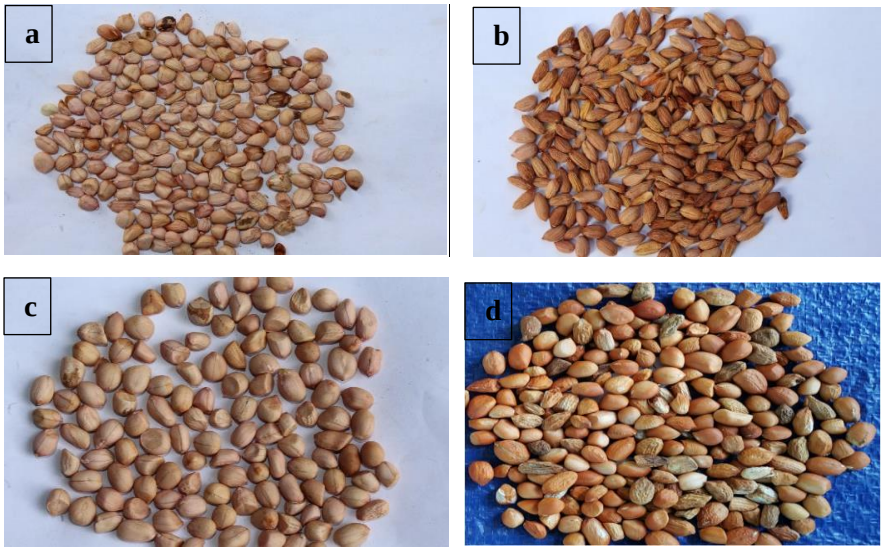

Figure 18: Kernel quality as affected by harvest timing (a & b): poorly formed immature and shrinked nuts, c: physiological mature nuts with variety distinct color and d: nuts attacked by fungi).

2.3.4. Effect of harvesting time on shelling percentage of groundnut varieties

Significant differences were observed in groundnut shelling percentages among the varieties as a result of the influence of harvesting time. Maximum shelling percentages of the three groundnut varieties were observed when the crop was harvested at physiological maturity. Additionally, the study established the influence of the interaction between variety and location on the shelling percentage. This resulted into different performances among the varieties between the two study locations (Figure 19). The varieties *ICGV-SM-99568* and *JL-24* had the highest shelling percentages at PAN and CIAM respectively.

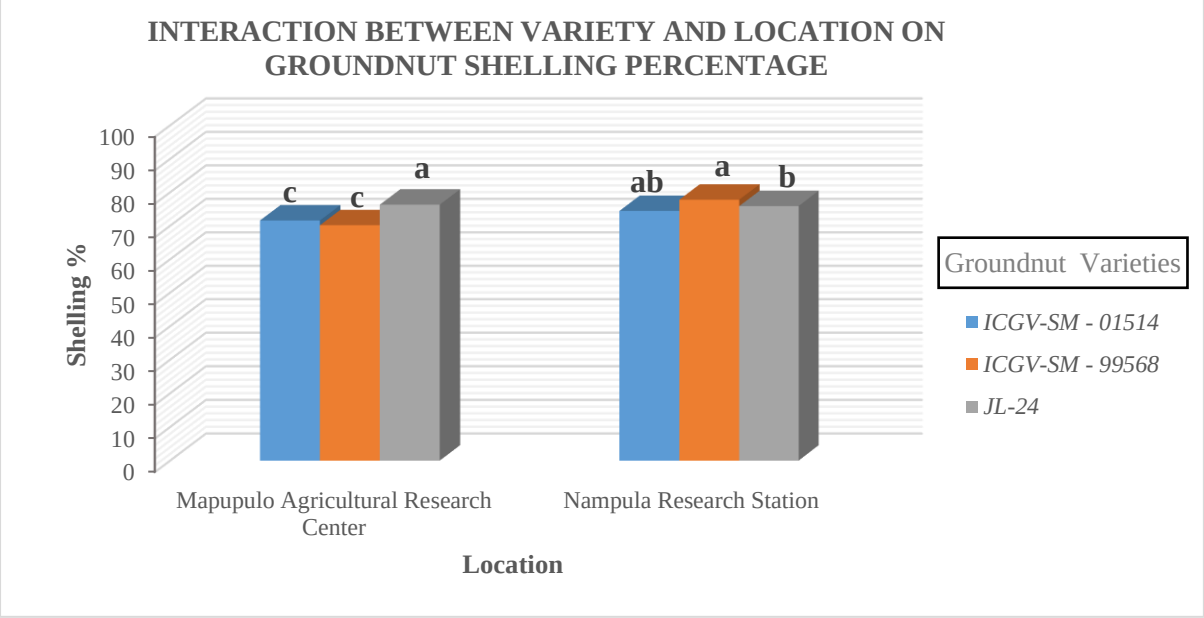

Figure 19: Interaction between variety and location on groundnut shelling percentage.

Harvesting at 10 days before physiological maturity recorded the lowest shelling percentage compared to the subsequent dates, this indicated a low amount of kernel production at the first harvesting time. However, differences among the dates of harvesting was significant only at CIAM ($P \leq 0.001$) (Table 8). But, using the Kruskal-Wallis equality of populations rank test it was found out that at PAN there were significant differences among shelling percentage of the three groundnut varieties at $P \leq 0.05$ (Appendix 3).

Table 8: Effect of harvesting time on shelling percentage (%) among groundnut varieties

	Mapupulo Agricultural Research Center			Nampula Research Station		
	Harvesting time			**Harvesting time**		
Variety	**H1**	**H2**	**H3**	**H1**	**H2**	**H3**
ICGV-SM-99568	72.2^{cd}	89.3^{a}	73.4^{cd}	84.5^{ab}	88.40^{a}	85.2^{ab}
ICGV-SM-01514	70.1^{d}	83.1^{abc}	76.01^{bcd}	72.6^{c}	88.08^{a}	80.5^{bc}
JL-24	76.2^{c}	86.6^{ab}	78.8^{bc}	81.1^{bc}	89.39^{a}	82.4^{b}
CV (%)	9.7	4.6	3.5	12.6	2.5	7
Mean ± SE		78.4 ± 2.57			83.6 ± 4.09	

Means followed by the same letter in a column are not significantly different (**$P \leq 0.001$**) by Tukey's Honestly Significant Difference test.

2.3.5. Effect of late leaf spot on groundnut yield and yield components

Highly significant differences ($P \leq 0.01$) were observed among the groundnut varieties with respect to late leaf spot disease severity at CIAM and PAN respectively (Appendix 1 and Appendix 2). However, the highest late leaf spot severity was recorded at CIAM compared to PAN. This could be one of the reasons CIAM had lower pod and kernel yields compared to PAN. The study has shown that late leaf spot severity had a negative correlation with pod yield, kernel yield, and total number of pods per plant ($P \leq 0.05$) at CIAM (Table 9).

Table 9: Correlations among quantitative traits as a result of the effect of late leaf spot (LLS) severity at CIAM

	# Mature per pods plant	Pod yield/ha	Kernel yield/ha	100-kernel wt	Shelling %	LLS severity %
Number of mature pods per plant	1.0000					
Pod yield/ha	0.7093*	1.0000				
Kernel yield/ha	0.8012*	0.9395*	1.0000			
100-kernel wt	0.0096	0.0263	0.1585	1.0000		
Shelling %	0.4931*	0.1740	0.4961*	0.3685**	1.0000	
LLS severity	-0.1403	-0.0588	-0.0268	0.1616	0.0980	1.0000

Means followed by ** are significant at (**P ≤ 0.001**) and * are significant at (**P ≤ 0.05**).

Late leaf spot severity at PAN was very minimal and therefore no negative correlation existed between the disease and crop quantitative traits (pod yield, kernel yield and number of pods per plant) (Table 10). This phenomenon showed that an increase in late leaf spot severity resulted into a decrease in crop quantitative traits. However, despite the negative correlations between late leaf spot severity and groundnut quantitative traits it was observed that significant positive correlations existed among number of mature pods per plant, pod yield per ha, kernel yield per ha, weight of 100-kernels and shelling percentage. Additionally, 100-kernel weight of the varieties was not affected by the disease.

Table 10: Correlations among quantitative traits as a result of the effect of late leaf spot (LLS) severity at PAN

	# Mature pods/plant	Pod yield/ha	Kernel yield/ha	100-kernel wt	Shelling %	LLS severity %
# Mature pods per plant	1.0000					
Pod yield/ha	0.5782*	1.0000				
Kernel yield/ha	0.4782*	1.0000**	1.0000			
100-kernel wt	0.0654	0.3848*	0.4848*	1.0000		
Shelling %	0.3061*	0.8256**	0.8256**	0.3764*	1.0000	
LLS severity	0.1784	0.0810	0.0810	0.1828	0.1485	1.0000

Means followed by ** are significant at (**P ≤ 0.001)** and * are significant at (**P ≤ 0.05**).

2.4. DISCUSSION

Appropriate harvest timing is critical for optimizing both yield and quality of groundnuts. It has been determined through this study that harvesting time had significant effects on the yield and yield components of groundnut varieties. Harvesting at physiological maturity recorded the highest groundnut pod and kernel yields compared to the other harvesting times. Furthermore, the groundnut quantitative traits (pod and kernel yields, number of pods per plant and 100-kernel weights) significantly decreased with harvesting at 10 days before and 10 days after physiological maturity. This confirmed the results found by Marsalis *et al*. (2009) who found that significant reductions in groundnut yields can occur if harvesting is either executed too early or delayed too long. Additionally, the results of the study are consistent with the findings of RELC (2000) who reported that the timely execution of cultural and agronomic practices especially harvesting time by groundnut farmers is very important as it contributes to kernel yield and quality.

Harvesting groundnut 10 days before physiological maturity resulted in reduced number of pods per plant which in-turn resulted into low pod and kernel yields among the varieties. Yield losses of up to (22.5 %, 20.4 % and 16 %) and (23.3 %, 16.6 % and 18.5 %) for *ICGV-SM-99568, ICGV-SM-01514* and *JL-24* respectively were incurred at CIAM and PAN respectively as a result of harvesting the crop 10 days before physiological maturity. This was attributed to the level of immaturity of pods and some which were empty and shrinked kernels. This is concurrent with the study findings of Wright and Porter (1991) who indicated that harvesting groundnut too early can reduce yield by 15 %. Furthermore, Kombiok *et al*. (2012) indicated that harvesting groundnuts too early resulted in immature nuts, low yields, and off flavors. Additionally, it has been reported by Singh and Oswalt (1995) that premature harvesting of groundnut pods lowered the yield, oil content and seeds quality of groundnuts due to immature pods and seeds.

Field observations from the planting of the groundnut crop to harvesting confirmed the suspicion that significant yield losses occur when harvesting is delayed after physiological maturity. The consequence of this action led to the destruction of the crop by pests especially termites. The results also showed that harvesting at physiological maturity gave the lowest quantities of groundnut pods damaged by termites, than the subsequent harvesting time for all the varieties. Kombiok *et al*. (2012) found that insect damage to pods tended to increase with delay in harvesting due to an increase in insect population with time agreeing with this study.

Delayed harvesting also resulted in sprouting of nuts under the soil due to lack of dormancy of the varieties which resulted into reduced pod and kernel yields. This is concurrent with the study findings of Asibuo *et al*. (2008) who reported that pre-harvest sprouting in groundnut kernels is undesirable since it leads to substantial loss of kernels, both in quantity and quality. Another factor that may have led to lower yields as a result of harvesting 10 days after physiological maturity was adverse effects of dry weather which made uprooting by hand difficult as the soil was too dry and hard. This resulted into harvesting by digging using hand hoes which led to most nuts being left in the soil as a result of weakened pegs due to over maturity and others were physically damaged. This is consistent with the findings of Singh and Oswalt (1995) who indicated that delay in harvesting after physiological maturity resulted in many pods left in the soil due to weakening of pegs.

Yield losses of up to (31.7 %, 35.2 % and 33.1 %) and (36.6 %, 30.7 % and 32.6 %) for *ICGV-SM-99568, ICGV-SM-01514* and *JL-24* respectively were incurred at CIAM and PAN respectively as a result harvesting the crop 10 days after physiological maturity. This phenomenon confirmed the findings of Young *et al.* (1982) who reported that delayed harvesting resulted into groundnut pod losses of up to 40 % depending on the variety and growing conditions. The current study has also shown that there were variations among varietal kernel yields between the two study locations. Mapupulo Agricultural Research Center recorded lowest kernel yields from the variety *ICGV-SM-99568* (576.4 kg/ha), *ICGV-SM-01514* (662.5 kg/ha) and *JL-24* (664.4 kg/ha) and Nampula Research Station recorded lowest kernel yields from the variety *ICGV-SM-01514* (800.0 kg/ha), *JL-24* (858.3 kg/ha) and *ICGV-SM-99568* (983.3 kg/ha). These differences could be attributed to environmental factors such as; rainfall, temperature and relative humidity, soil conditions and severity of late leaf spots between the two study locations. These findings are in accordance to a similar study conducted in Northern Nigeria by Kamara *et al*. (2011) who reported that different agricultural ecologies have different effects on the yield of groundnuts. Moreover, Tindall (1988) indicated that groundnut yield varies depending on the soil, climatic conditions, cultivar characteristics, and level of management.

In both locations, *ICGV-SM-99568* had significantly higher 100-kernel weight (heavier seeds) than *ICGV-SM-01514* but was not significantly different with *JL-24* at each harvesting time. Moreover, the kernel weight of *JL-24* was significantly higher than that of *ICGV-SM-01514*, which recorded the lowest kernel weight regardless of harvesting time. The bigger nuts of

ICGV-SM-99568 could be responsible for its higher 100-kernel weight than *ICGV-SM-01514* which had the smallest kernel size. Mean 100-kernel weight is an expression of the amount of dry matter allocated to the kernel development by treatments which is attributed to plant or varietal factors (Kamara *et al*., 2011). The large kernel nature of *ICGV-SM-99568* and *JL-24* could be the reason farmers prefer to cultivate those varieties in Mapupulo and Nampula.

The shelling percentage (%) of the groundnut varieties varied significantly between the two study locations due to the variation in harvesting time. However, shelling percentages were higher when the crop was harvested at physiological maturity. Furthermore, the shelling percentages were affected by harvesting 10 day before and 10 days after physiological maturity, this reduced the total kernel yields for those harvesting times. This confirmed the findings of Hartmond *et al*. (1996) who found out that kernel yield was directly related to shelling percentage, so that the higher the shelling percentage the higher the kernel yield of that variety.

Leaf spot diseases; early leaf spot (*Cercospora arachidicola*) and late leaf spot (*Cercosporidium personatum*), are economically the most important fungal diseases of groundnut in Mozambique and worldwide. In most areas, both diseases occur together but the incidence and severity of each disease vary with environment and varieties (Pande and Rao 2001). Both early and late leaf spot diseases were observed at CIAM and PAN; however these were more severe at CIAM than at PAN. This could be attributed to the heavy rainfall received at CIAM than that at PAN, which led to the spread of the pathogen spores and subsequent heavy disease severity at CIAM than the later.

The study showed that at CIAM, late leaf spot severity negatively correlated with the total number of pods per plant ($r = -0.1403$), pod yields ($r = -0.0588$) and kernel yields ($r = -0.0268$). However, under low leaf spot severity at PAN no negative correlations among the crop quantitative traits was observed. This was attributed to the low disease pressure at PAN than CIAM. Furthermore, CIAM is considered to be a hot spot of leaf spot diseases compared to PAN. This therefore, confirmed that an increase in late leaf spot severity results into reduced yields of groundnut as a result of falling of leaves and defoliation which reduces the total photosynthetic area of the crop. Muitia (2013) in his study in Northern Mozambique on groundnut reported that Mapupulo Agricultural Research Center had higher late leaf spot incidence and severity than Nampula Research Station, which resulted into heavy defoliation of leaves and subsequent reduced pod and kernel yield. Furthermore, the study found out that

CIAM was a hot spot of leaf spots compared to PAN, agreeing with the current study findings. Additionally, the study findings of Muitia (2013) indicated negative correlations between kernel yield and disease incidence and severity.

2.5. CONCLUSIONS

The results of this study indicated that harvesting at physiological maturity gave the highest groundnut pod and kernel yield than harvesting 10 days before and 10 days after physiological maturity. Indicating that harvesting at physiological maturity, especially when the soil still contains little moisture, will help minimize pod yield losses in groundnut. Among the varieties *ICGV-SM-01514* at CIAM and *JL-24* at PAN had optimum higher yields, making them the highest yielding varieties in those two locations. The study findings have also revealed that premature harvesting of groundnut pods lowered the yield and kernel quality by 16-25 % due immature and empty pods and shrinked kernels. In addition, delayed harvesting after physiological maturity resulted into yield losses ranging from 30-40 % as a result of some pods remaining in the soil due to weakening of pegs, insect (termite) infestation of pods and sprouting of the pods due to lack of seed dormancy.

Additionally, the current study has shown a negative correlation between late leaf spot severity and groundnut quantitative traits. This meant that an increase in late leaf spot severity resulted into reduced total number of pods per plant which in-turn resulted into reduced pod and kernel yields. However, this was only observed at CIAM because the site had higher late leaf spot incidence and severity compared to PAN.

2.6. RECOMMENDATIONS

Based on the findings of this study, it is therefore, recommended that, for farmers to obtain maximum pod yields with high quality kernels, they should cultivate available improved varieties and should make sure to harvest their crop at physiological maturity by keeping the date of planting which can assist in decision making on when to execute harvesting.

Additionally, due to the subterranean nature of groundnuts the government needs to intervene through provision of intensive groundnut farmer trainings on how to determine the time of harvesting the crop. As the falling and yellowing of leaves does not provide sufficient information on the actual maturity of the crop and may also be a sign of disease and drought.

It is also recommended that a different study be conducted to evaluate the effect of late leaf spot on yield stability and resistance of the three groundnut varieties in Northern Mozambique, the resistant variety could be used for breeding purposes.

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CHAPTER THREE

EFFECT OF HARVESTING TIME AND DRYING METHODS ON GROUNDNUT AFLATOXIN CONTAMINATION

ABSTRACT

The production and utilization of groundnut (Arachis hypogea L) *has increased tremendously across all provinces of Mozambique in recent times. However, the presence of mycotoxins, especially aflatoxins has remained a critical food concern in both the human and livestock diet. In this study, the effect of harvesting time and drying methods on aflatoxin contamination were examined at two locations namely; Nampula Research Station (PAN) and Mapupulo Agricultural Research Center in Nampula and Cabo Delgado provinces respectively. A randomized complete split-split block design with four replications was used with three groundnut varieties; (ICGV-SM-99568, ICGV-SM-01514 and JL-24) as the main plot and three harvesting dates (10 days before physiological maturity, at physiological maturity and 10 days after physiological maturity) and two drying methods; (A-frame and tarpaulin) as the sub-plots. In both locations, field observations indicated that on average aflatoxin contamination levels were lower at physiological maturity (H2) (≤ 10 ppb) compared to harvesting 10 days before (H1) (≤ 15 ppb) and 10 days after physiological maturity (H3) (≥ 20 ppb). It was also observed that both the A-frame and tarpaulin drying methods were effective in reducing groundnut kernel moisture to the recommended storage level of ≤ 7 % which is ideal to prevent growth of fungi including aflatoxigenic strains and aflatoxin production. Furthermore, the two drying methods were effective in prevention of aflatoxin contamination on groundnut kernels to levels lower than 20 ppb. However, aflatoxin contamination levels were significantly lower (≤ 12 ppb) as a result of the A-frame than tarpaulin drying (≥ 15 ppb). It is therefore desirable that farmers' harvest at physiological maturity and be encouraged to adopt the A-frame and tarpaulin drying methods in-order to reduce aflatoxin contamination of their groundnut crop.*

Keywords: Groundnut, harvesting time, aflatoxin contamination, drying method.

3.1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is the third most important crop in Mozambique after maize (*Zea mays*) and cassava (*Manihot esculenta*) (Muitia, 2013; Walker *et al*., 2006). It is a major cash crop and the main source of cooking oil for many Mozambican families (Muitia, 2013; Muitia, 2005). In terms of production, groundnut occupies the largest area among the grain legumes in the country (Muitia, 2013; Arias and Libombo, 1994) with the largest concentration in Nampula, Zambezia and Cabo Delgado provinces.

Despite its importance as food, the presence of mycotoxins, especially aflatoxins has the potential to limit its use in both the human and livestock diet (Rahmianna *et al*., 2007). Furthermore, aflatoxin contamination of agricultural crops, such as; groundnut and cereals, causes annual losses of more than US $750 million in Africa and more than US $100 million per year in USA (Kamika and Takoy, 2011). Poor management practices by farmers and adverse climatic conditions at harvest and post-harvest are some of the prompting factors for post-harvest aflatoxin contamination. The timing of harvesting greatly influences mould production at harvest (Guo *et al*., 2003). Wright *et al.* (2005) highlighted that farmers tend to delay in harvesting their crop which results in over maturity leading to mould infections and subsequent aflatoxin contamination.

Correct and proper drying of harvested groundnuts is very essential in prevention of fungal infection of the crop. Additionally, proper drying is critical for maintaining seed quality for consumption and safe storage. However, the traditional groundnut drying techniques in Mozambique, involve field and bare ground drying which rather promote fungal growth and consequent aflatoxin contamination (Jeffrey, 2011). Moreover, these are slow, time consuming and labour intensive, involving lots of crop handling and due to rains that normally persist at harvesting and drying times, it is difficult to achieve the recommended moisture content for safe storage (which is 6-8 %). In addition, the crop is persistently exposed to the soil, which is a major source of contamination by fungi (Okello *et al.*, 2010; Kaaya *et al*., 2007).

Ideally, pods should be dried with sufficient air circulation and in the shade (Okello *et al.*, 2010). This is because excessive exposure to the sun can affect the quality of the seed. Two principal methods are used elsewhere in Africa, both of which can produce good quality seed with reduced levels of fungal infection (AICC, 2014). These drying methods are namely;

Corks and A-Frame methods. However, in the United States windrowing is the accepted method of drying groundnuts (Dickens, 1974).

3.1.1. JUSTIFICATION

The correct drying of the harvested groundnuts is very important, as poor drying can help induce fungal growth (producing aflatoxin contamination) and reduce seed quality for consumption, marketing and germination for the following seasons planting for good storage and germination. The moisture content of the pods should be reduced to 6-8 % in-order to prevent fungal attacks and maintenance of the kernel quality (Okello *et al*., 2010). There are different ways of drying the pods, some of which are better than others. It is particularly important to note that if the pods are exposed to the sun for too long the seed quality can deteriorate considerably, germination can also be affected and as a result of too much heart and microscopic poles can be created thereby aiding the entry of seedborne microorganisms.

Proper drying of groundnuts to a moisture content below 10 % is ideal in as far as aflatoxin contamination management is concerned (Augusto, 2004). However, the traditional drying techniques in Mozambique involve bare ground drying and are a major source of fungal contamination. Furthermore, some farmers do not dry groundnuts immediately after harvest, due to labour constraints needed for plucking (Jeffrey, 2010). Thus, they heap the nuts either in the field or in houses. These practices, coupled with inefficient and slow drying process under the humid conditions enhance aflatoxin contamination greatly.

Although research on the effect of harvesting time and drying method of groundnut on aflatoxin development, has received increasing consideration worldwide, in Mozambique, research on this matter is still very scarce (Almeida *et al*., 2013). However, there is evidence to suggest that aflatoxin contamination is a major food-safety concern in Mozambique where the environmental conditions and socio-economic problems are conducive due to poor post-harvest and storage management and subsequent food spoilage and aflatoxin contamination. This is evident by levels of certain types of cancer and the negative correlations between aflatoxin in the diet and development in children and the declining of groundnut exports from Mozambique since 1998 (FAO-STAT, 2015: Almeida *et al*., 2013).

By assessing different harvesting times and different drying methods it was hoped that the results would enhance the use of good post-harvest handling practices (drying and harvesting time) that would minimize aflatoxin contamination of groundnuts at farmer level.

3.1.2. OBJECTIVES

3.1.2.1. General objective

To evaluate the effect of harvesting time and drying methods in minimizing aflatoxin contamination of groundnut varieties.

3.1.2.2. Specific objectives

- To evaluate the effect of harvesting time on groundnut aflatoxin contamination.
- To compare the effectiveness of "A-frame" and tarpaulin drying methods in ideal reduction of groundnut moisture content and minimizing aflatoxin contamination.

3.2. MATERIALS AND METHODS

The field experiments were conducted in two sites namely; Nampula Research Station (PAN) and Mapupulo Agricultural Research Center (CIAM) in Nampula and Cabo Delgado provinces respectively. Nampula Research Station is located about 7 km east of Nampula in Northern Mozambique (15º 09' S, 39º 30' E) and is elevated at 432 m above sea level. The soil type is sandy loam and the vegetation is predominantly grassland. The average rainfall is slightly over 1000 mm which starts around November/December up to April/May with its peak in January (Muitia, 2013). The maximum temperature in the region is about 39 ºC and the minimum temperature is 19 ºC.

Mapupulo Agricultural Research Center is located about 18 km west of Montepuez town and about 200 km west of Pemba, the capital of Cabo Delgado Province. The research center lies at (13º 12' S, 38º 53' E) and is elevated at 476.7 m above sea level. The soils are clay loam and deep brown loam. It receives annual precipitation of 1200 mm on average from November/December to April/May, and the average temperature is between 20 and 25 ºC (Muitia, 2013).

3.2.1. Field establishment

The study was carried out during the 2015/2016 growing season at PAN and CIAM. The test materials were evaluated using a randomized complete block design in a split-split plot arrangement with four replications (Appendix 9). The main plot was the variety while harvesting time and drying method were sub-plots. The net plots were 6 rows by 6 m long with one seed per planting station which were spaced at 50 cm apart and the planting stations were spaced at 10 cm. Spanish groundnut varieties were used for the study namely: *ICGV-SM-99568, JL-24* and *ICGV-SM-01514*. The experiments were established on 23rd December and 24th December at CIAM and PAN respectively at the onset of the rains. No fertilizer, pesticides or supplementary water were applied, and no seed treatment before planting was applied.

The assessment of the effect of harvesting time and drying method on aflatoxin contamination among the varieties involved dividing the net plots into three harvesting time treatments: (i) 10 days before physiological maturity indicated as H1; (ii) at physiological maturity indicated as H2 and (iii) 10 days after physiological maturity indicated as H3. The following drying treatments were imposed on the plants from each of the plots: (1) pulling and inverted windrowing of plants for 3 days, followed by further drying of the plants with the pods on

constructed “A-Frames” for 4 weeks and (2) pulling and inverted windrowing of plants for 3 days, followed by stripping of the pods and further drying on interlaced tarpaulins mats for 4 weeks (Figure 20). The samples were later subjected to aflatoxin testing using the immunochromatographic method.

Figure 20: A-frame and tarpaulin drying methods respectively.

3.2.2. Data collection

The data that was collected included; weather data (rainfall, maximum and minimum temperature and relative humidity) using the weather stations at the two experimental sites, planting dates, harvesting dates, moisture content of kernels during drying, disease incidence, pest incidence and groundnut aflatoxin levels.

3.2.3. Determination of moisture content

The moisture content of groundnut samples was measured using the Mini GAC and Farmex moisture meters. These were calibrated to ensure the accuracy. To determine the moisture content, groundnut samples were initially shelled. Later, a total of 50 g was filled in the moisture meter loader: after which the loader was emptied into the analyzer. The results were read using the display window on the moisture meters (Figure 21).

Figure 21: Farmex and Mini GAC moisture meters respectively.

3.2.4. Aflatoxin analysis

3.2.4.1. Chemicals and Reagents

A total of 96 groundnut samples from PAN and CIAM were analyzed for aflatoxin contamination levels (48 samples from each location). All solvents used were of analytical grade and purchased from Neogen Corporation (Miami, USA). The Reveal Q^+ test kits included the mReader®, 65 % ethanol solution, reveal dilution vial, reveal sample collection vial, Agri-Grind grinder, measuring scale, timer, graduated cylinder, pipettes of 100 microL (µl) and 500 µl including pipette tips, reveal Q^+ filter papers (24 cm), sample diluent, reveal Q^+ test strips and pink antigenic standard (Figure 22).

3.2.4.2. Principle of immunochromatographic assay for detection of aflatoxins

The method used in this study was described by Vishwanath *et al*. (2009). In this method, antibiotics specific to aflatoxins B1, B2, G1 and G2 are immobilized on a specific reveal Q^+ strip, and a toxin labelled with an enzyme (diluent) is used. The binding of toxin-enzyme conjugate by immobilized antibodies is inhibited by the addition of a free enzyme present in the test sample. Since a fixed number of antibody reaction sites is available, enzyme activity is proportional to the amount of bound toxin-enzyme conjugate. Antibody-toxin-enzyme complex is inversely proportional to concentration of free toxin added. Bound enzyme catalyzes oxidation of substrate thereby changing the color of the strip from green to orange or red. Once the strip is placed in the mReader, presence of aflatoxins in the sample is based on the chromatographic characteristics of the color produced using the camera by the mReader. After which total aflatoxin in the sample is provided by the mReader display window.

3.2.4.3. Validation of immunochromatographic assay analysis

To determine the precision and recovery of the immunochromatographic assay analysis, antigenic standards were used. For high calibration standard procedure, 100 µl of pink antigenic standard was added to 500 µl of sample buffer diluent. Then 100 µl was aliquoted in a separate vial. A reveal Q^+ test strip was placed in the vial and was left to develop for 6 minutes. After 6 minutes the strip was placed in the mReader strip holder and aflatoxin levels were read using the mReader. For the low calibration standard procedure, 35 ml of 65 % ethanol solution was added to a 10 g control groundnut sample which was free of aflatoxins. Then, a 100 µl of the pink antigenic standard solution was added to the 30 ml extracts and mixed for 2 minutes. Later, a 100 µl of the mixture was added to 500 µl of sample buffer

diluent. A mixture of 100 µl was later aliquoted to a separate vial. Finally, the total aflatoxin in the sample was measured by placing the reveal Q^+ test strip in the vial and was left to develop for 6 minutes and aflatoxin reading was done using the mReader.

Figure 22: (a); Diluent, Reveal Q^+ test strips, dilution and sample vials, (b) antigenic standard solution (c); mReader.

3.2.4.4. Sample preparation and aflatoxin determination using immunochromatographic assay analysis

Aflatoxin analysis was carried out using immunochromatographic assay Reveal Q^+ mReader according to the manufacturer's recommendation. Prepared groundnut samples (500 g each) were ground finely using the Agri-Grind grinder until fine particles and homogeneity was obtained. Then, a sub-sample of 10 g was obtained from each of the composite samples. The sub-sample was aliquoting in 35 ml of 65 % ethanol, and the contents were mixed gently by shaking the holding tube manually. After filtration of the blended subsample, 100 µl of the filtrate was mixed with 500 µl diluent solution in a dilution vial. After obtaining a fine mixture, a 100 µl extract of the aliquoted mixture was collected and added to a separate vial. Finally, a reveal Q^+ test strip was placed in the vial containing the aliquoted mixture and was left to develop for 6 minutes. The test strip was later placed in the mReader holder, and aflatoxin contamination levels of the sample was determined using the mReader based on the chromatographic characteristics of the sample in the strip.

3.2.5. Data analysis

The data was statistically analysed using GenStat Discovery 4. An independent Tukey-test was used to compare the means of the aflatoxin results. The tests for relationships was carried out using the Pearson Correlation Index and the interpretation was performed at two-sided 95 % confidence limit. The following statistical model was used to analyze the data:

$$Y_{ijk} = \mu + H_i + V_j + HV_{ij} + D_g + DV_{gj} + DHV_{gij} + R_{k(ij)} + \varepsilon_{k(ij)}$$

Where:

Y_{ijk} = Aflatoxin contamination level of variety

μ = Overall aflatoxin contamination mean

H_i = Effect of the i^{th} harvesting time

V_j = Effect of the j^{ith} Variety

HV_{ij} = Interaction effect of the i^{th} Harvesting time and j^{th} variety

D_g = Effect of the g^{th} drying method

DV_{gj} = Interaction effect of the g^{th} drying method and j^{th} variety

DHV_{gij} = Interaction effect of the g^{th} drying method, i^{th} harvesting time and j^{th} variety

$R_{k(ij)}$ = Effect of the k^{th} replication in the i^{th} harvesting time

$\varepsilon_{k(ij)}$ = Experimental error

3.3. RESULTS

3.3.1. Weather data at CIAM and PAN during the 2015-2016 growing season

Aflatoxin contamination of crops such as; groundnut and maize is influenced by weather and climatic factors. A summary of mean air temperature, relative humidity and rainfall during the 2015-2016 growing season at CIAM is presented in Table 11. The mean daily air temperature during the pod-filling period was about 26.3 ^{o}C up until H1. Although the mean daily temperature declined to around 24.5 ^{o}C by H3. The site received a total rainfall of 684.6 by H1 and 830 mm between H2 and H3 respectively of which 50-65 % fell during the pod-filling period. Additionally, there were also some post-harvest rainfall during the drying period, with 37.2 mm falling between H2 and H3. The average relative humidity was between 80-85 % during the groundnut harvesting and drying periods. However, overall there were generally high temperatures and heavy rainfall during the pod-filling till H2.

Table 11: Weather data during the 2015-2016 growing season at CIAM

Month	**December**	**January**	**February**	**March**	**April**
Average Max Temperature (^{o}C)	34.1	30.5	31.4	31.9	30.8
Average Min Temperature (^{o}C)	21.8	21.6	21.3	22.0	20.3
Cumulative rainfall (mm)	516.6	1300.6	568.7	800.4	859.7
Total number of rainy days	10	20	18	16	22
Relative Humidity (%)	68	83	80	81	79

Nampula Research Station received lower rainfall during the 2015-2016 growing season compared to Mapupulo Agricultural Research Center (Table 12). The site received rainfall of 299.8 mm (for only 11 days) during pod-filling, and the location experienced a mid-season drought (February). However, significant higher rainfall fell during H1, whilst H2 and H3 experienced a prolonged end of season drought. The mean daily air temperatures during the pod-filling period at PAN were higher ranging from 30 to 35 ^{o}C by H1 to H3. Additionally, the location experienced very high relative humidity ranging from 75-85 %.

Table 12: Weather data during the 2015-2016 growing season at PAN

Month	December	January	February	March	April
Average Max Temperature (°C)	35.3	34.8	36.3	35.2	32
Average Min Temperature (°C)	33.2	29.6	32.1	32.3	29.7
Cumulative rainfall (mm)	232.9	469.6	299.8	799.1	43.9
Total number of rainy days	6	12	11	18	4
Relative Humidity (%)	83	87.7	76.3	83	85

3.3.2. Post-Harvest Pod Handling and Kernel Moisture Contents

Moisture content of groundnut kernels greatly influences the growth of toxigenic fungi and subsequent aflatoxin contamination. It is generally recommended that harvested commodities should be dried as quickly as possible to safe moisture levels of less than 7 %. The study has shown that different drying methods had different influences on the total kernel moisture losses at different experimental sites at different harvesting times. Moisture content of kernels from the A-Frame at both sites decreased from an average of 38 % to 7 %, within a 4 week period (Figure 23). These moisture contents were significantly different at ($P \leq 0.05$) from each other. It was observed that kernel moisture loss was rapid just after harvesting compared to the other following weeks. This could be attributed to the high water activity in the seeds just after harvesting than the following weeks, which resulted into increased diffusion rate of water from the seeds to the environment through evapotranspiration and thus leading to rapid loss of water.

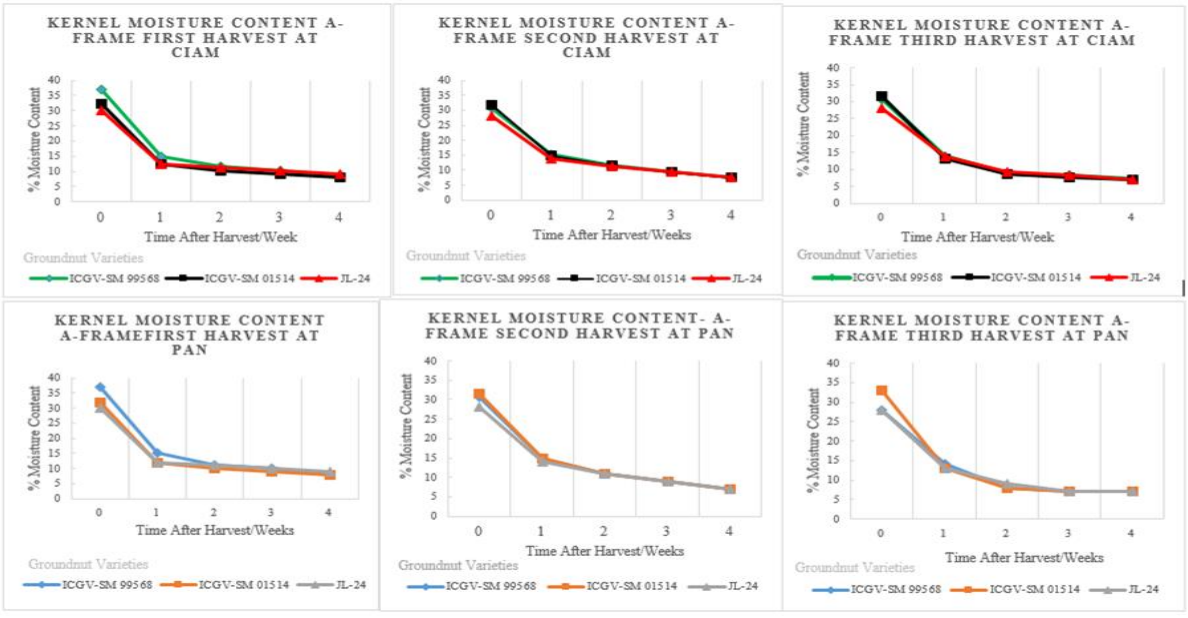

Figure 23: Kernel Moisture losses when using the A-Frame at CIAM and PAN respectively.

Significant differences ($P \leq 0.05$) were also recorded in kernel moisture loss of tarpaulin dried pods. The moisture content decreased from an average of 38 % to 7 %, within a 2 week period (Figure 24). It has been established that, using the tarpaulin drying method kernel moisture loss was more rapid compared to using the A-frame drying method. The reason behind this was that, with tarpaulin drying, pods were exposed to direct sunlight which resulted into rapid losses of kernel moisture within a short period of time, whilst for the A-frame method the kernels took a longer time to dry because the pods were facing inwards and away from the sunlight and soil and were covered by leaves. This ensured a good air circulation and slow but effective drying. The study also revealed that the variety *JL-24* took a shorter period of time to dry compared to the other two varieties irrespective of the drying method. This could be attributed to the lower moisture content of the variety and the thinner layer of the shell. The variety *ICGV-SM-01514* took the longest time to dry irrespective of the drying method and this could be attributed to the thicker shell of the variety which led to slower moisture loss.

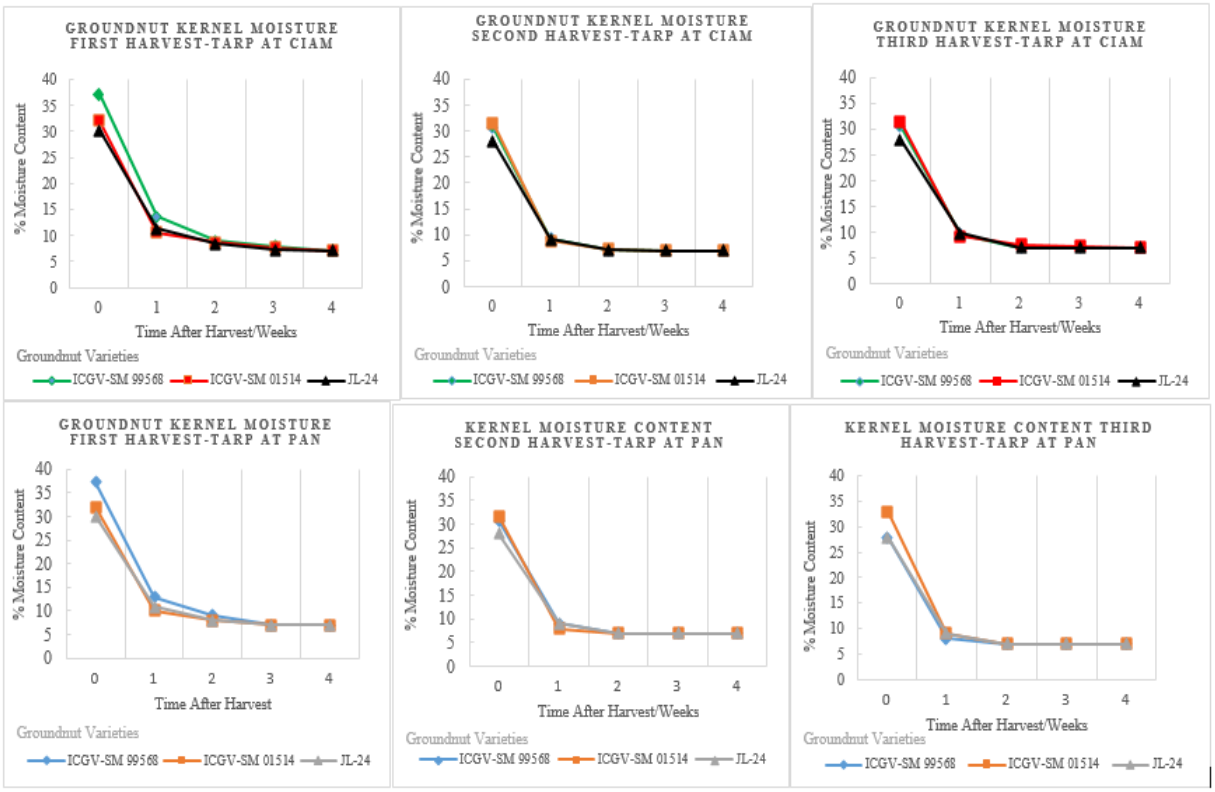

Figure 24: Kernel Moisture losses when using the Tarpaulins at CIAM and PAN.

3.3.3. Effect of harvesting time on groundnut aflatoxin contamination

Aflatoxin contamination levels among groundnut varieties at different harvesting times are presented in Figure 25. Significant differences ($P \leq 0.01$) were observed in the mean aflatoxin contamination levels with physiological maturity (H2) having the lowest aflatoxin

contamination levels (≤ 10 ppb). The highest aflatoxin contamination levels were recorded when harvesting was executed 10 days after physiological maturity (H3) (≥ 20 ppb) compared to when harvesting was executed 10 days before physiological maturity (H1) (≤ 15), which had considerably lower aflatoxin levels.

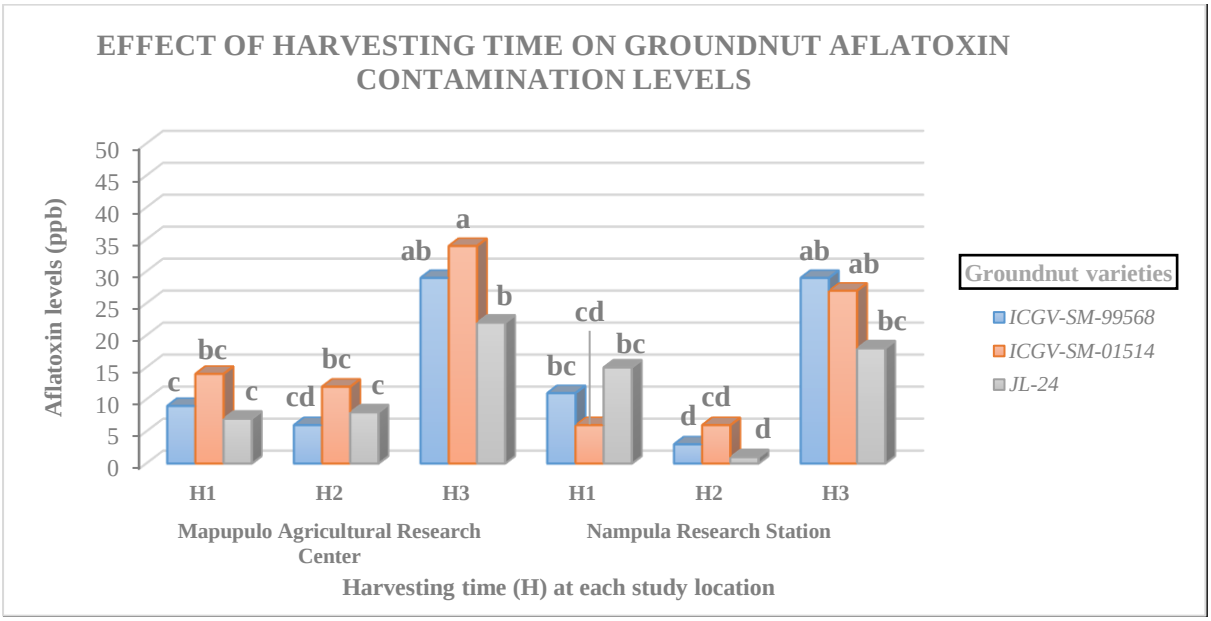

Figure 25: Aflatoxin levels in groundnuts as affected by harvesting time.

The study also revealed significant differences in aflatoxin levels among the three groundnut varieties. The variety *JL-24* had the lowest mean aflatoxin contamination levels compared to the other two varieties. This could be attributed to the lower moisture content of the *JL-24* and the thin shell of the variety which led to rapid drying and minimized fungal invasion and subsequent aflatoxin contamination. Furthermore, it was observed that at CIAM the mean aflatoxin contamination levels of *ICGV-SM-99568* (14.5 ppb) was significantly lower compared to that of *ICGV-SM-01514* (17.9 ppb). A similar trend of results was observed at PAN, however, at this location *ICGV-SM-01514* had the lowest mean aflatoxin contamination levels (12.3 ppb) compared to (14.3 ppb) for the variety *ICGV-SM-99568*.

3.3.4. Effect of drying method on groundnut aflatoxin contamination

Significant differences were observed in aflatoxin contamination levels among the groundnut varieties as a result of drying method. Lower levels of aflatoxin were recorded by the use of the A-Frame compared to the tarpaulin drying method (Figure 26). However, except for the variety *ICGV-SM-01514* (26 ppb) at CIAM, the aflatoxin contamination levels for the groundnut varieties were lower than 20 ppb as a result of both drying methods. Thereby, showing the effectiveness of the two drying methods in prevention of aflatoxin contamination.

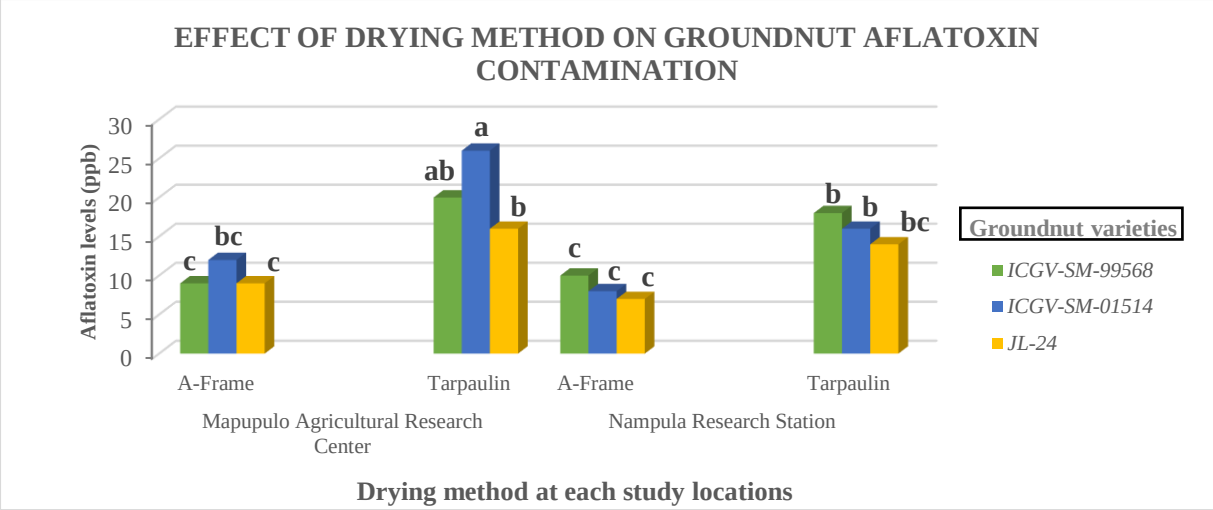

Figure 26: Aflatoxin levels in groundnuts as affected by drying method.

Significant differences in aflatoxin contamination levels were also observed among the groundnut varieties as a result of the interaction between harvesting time and drying methods at the two study locations (Table 13 and Table 14). The results showed that aflatoxin contamination of the nuts started at H1 and significantly increased with delayed harvesting time (H3). At Mapupulo Agricultural Research Center the lowest aflatoxin contamination levels were found to be 3 ppb and 4 ppb for the A-frame and tarpaulin drying methods respectively harvested at physiological maturity. For Nampula Research Station the lowest levels of aflatoxin contamination were found to be 2 ppb for both drying methods harvested at physiological maturity. Higher aflatoxin levels (≥ 25 ppb) were recorded when harvesting was executed 10 days after physiological maturity (H3) with respect to the drying methods.

Table 13: Groundnut aflatoxin levels as affected by the interaction of harvesting time and drying method at CIAM

Drying method	**Variety**	**Harvest timing**		
		H1	**H2**	**H3**
A-Frame	ICGV-SM-99568	3^{c}	7^{bc}	17^{b}
	ICGV-SM-01514	10^{bc}	3^{c}	25^{a}
	JL-24	4^{c}	4^{c}	19^{ab}
Tarpaulin	ICGV-SM-99568	16^{bc}	4^{d}	40^{ab}
	ICGV-SM-01514	17^{bc}	10^{cd}	42^{a}
	JL-24	9^{cd}	13^{c}	25^{b}
Mean ± SE	A-Frame 10 ± 3.77		Tarpaulin	21 ± 5.17

Means followed by the same letters within a column do not differ significantly according to Tukey's honestly significant difference test (**P ≤ 0.01**).

In summary, it has been established that the interaction of delayed harvesting and tarpaulin drying method resulted in higher aflatoxin contamination among the groundnut varieties than the interaction of delayed harvesting and A-frame drying method. Overall, the interaction of harvesting time and A-frame drying method resulted into lower aflatoxin contamination levels than the interaction of harvesting time and tarpaulin drying method.

Table 14: Groundnut aflatoxin levels as affected by the interaction of harvesting time and drying method at PAN

Drying method	**Variety**	**Harvest timing**		
		H1	**H2**	**H3**
A-Frame	ICGV-SM-99568	3^{c}	2^{c}	27^{a}
	ICGV-SM-01514	2^{c}	2^{c}	21^{ab}
	JL-24	10^{bc}	1^{c}	12^{b}
Tarpaulin	ICGV-SM-99568	18^{b}	4^{c}	32^{a}
	ICGV-SM-01514	8^{bc}	8^{bc}	33^{a}
	JL-24	19^{b}	2^{c}	22^{ab}
Mean ± SE	A-Frame 9 ± 4.03		Tarpaulin 16.5 ± 5.6	

Means within a column followed by the same letter are not significantly different based on Tukey's test (**P < 0.01**).

3.3.5. Correlation between late leaf spot severity and aflatoxin contamination

The correlations between late leaf spot severity and aflatoxin contamination are given in Table 15. It has been established that there was a positive correlation between late leaf spot severity and aflatoxin contamination among the groundnut varieties at both study locations. The highest correlation (r = 0.2552) was recorded at CIAM compared to PAN (r = 0.1891). This was attributed to the high disease pressure at CIAM compared to PAN. This therefore has shown that an increase in late leaf spot severity resulted into an increase in aflatoxin contamination of the groundnut.

Table 15: Correlation between late leaf spot severity and aflatoxin contamination

	Late leaf spot severity	Total aflatoxin at CIAM	Total aflatoxin at PAN
Late leaf spot severity	1.0000		
Total aflatoxin at CIAM	0.2552*	1.0000	
Total aflatoxin at PAN	0.1891*	0.7168**	1.0000

Means followed by ** are significant at (**P ≤ 0.01**) and * are significant at (**P ≤ 0.05**).

3.4. DISCUSSION

A number of studies have shown that weather directly influences host susceptibility to aflatoxin contamination (Cotty, 2007). The differences in the intensity of aflatoxin contamination between CIAM and PAN could be attributed to the variability in intensity and duration of rainfall, temperature as well as relative humidity between the two locations. In general, CIAM had significantly higher aflatoxin contamination levels compared to PAN. This was attributed to higher than normal temperatures (≥ 30 ^{o}C) and late season rainfall which created warm, moist conditions suitable for fungal growth and subsequent higher aflatoxin contamination levels on the kernels. These outcomes are similar with earlier accounts that wetter and more humid conditions tend to aggravate aflatoxin levels as it enhances the growth of *Aspergillus* species and production of aflatoxins in groundnuts compared to drier climatic conditions (Menza *et al*., 2015). Previously, Cotty (1991) indicated that warm, moist conditions during harvesting and field drying of cottonseed, was necessary to cause aflatoxin contamination. Widstrom *et al*. (2003) indicated that the optimal temperature range for production of aflatoxin is approximately 25-30 ^{o}C agreeing with the current study.

The study also recorded higher aflatoxin contamination levels in the groundnut kernels above the recommended 20 ppb (US standards) at both CIAM and PAN. This could be as a result higher air temperatures (≥ 30 ^{o}C) along with elevated relative humidity (≥ 70 %) which provided optimum conditions for fungal invasion especially for the *Aspergillus* section *Flavi* and later production of aflatoxins. The was consistent with the findings of Hell and Mutegi (2011) who reported that environmental conditions that favor *Aspergillus* group of fungi included high soil or air temperature (25-30 ^{o}C), high relative humidity (70-85 %) and drought stress. Al- Shikli *et al.* (2010) and Sugri *et al*. (2015) found out that the optimum temperature range for aflatoxin production is 25-35 ^{o}C, although production can occur over a wide range of temperatures (10-40 ^{o}C). Additionally, research results by Kusumaningrum *et al*. (2010) exhibited that relative humidity above 70 % were optimal for growth of *A. flavus* and subsequent aflatoxin contamination. The current study has therefore shown significant positive correlations between local weather conditions and aflatoxin contamination levels, where, high temperature (≥ 25 ^{o}C) and high relative humidity (≥ 70 %) favored growth of *Aspergillus* species and increased the rate of aflatoxin production.

The subterranean nature of fruiting in groundnut and its indeterminate growth habit ensures that pods are produced at every stage of its growth making it difficult to determine the time of

maximum maturity of pods (Kaba *et al*., 2014). Hell and Mutegi (2011) indicated that timing of crop harvest affects kernel yield and extent of aflatoxin contamination in maize and groundnuts. Reports in the literature indicate that maize and groundnut at the time of harvest is typically infected with a variety of fungi; *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus* species are among those reported, (Kaaya *et al*., 2005) agreeing with the findings of this study.

Field observations from this study have shown that on average aflatoxin contamination levels were lower at physiological maturity (H2) compared to harvesting at 10 days after physiological maturity (H3). Furthermore, harvesting the crop at H1 had significantly higher aflatoxin contamination levels than harvesting at H2, with some exceptions. The high aflatoxin levels at H1 could be attributed to immaturity of pods, higher pod and kernel moisture content and adverse conditions of wet and humid weather, which provided conducive conditions for fungal invasion and consequently aflatoxin production. Additionally, most of the pods were small and shriveled, which provided direct access to entry of microorganisms including fungi into the pods and consequently attacking the kernels and later contaminating the crop with aflatoxins. This confirmed the findings of Okello *et al*. (2010) who reported that harvesting groundnuts too early or when the pods are immature results in high aflatoxin levels in the kernels. Furthermore, the findings were consistent with the findings by Cotty and Jaime-Gracia (2007) who found that aflatoxin contamination was positively correlated with wet weather during harvest (rainfall). It has also been shown that as a result of early harvesting, drying concided with some post-harvest rainfall which led into high aflatoxin contamination of the crop since there was excess moisture which provided suitable conditions for fungal growth and development and production of aflatoxins.

Harvesting 10 days after physiological maturity (H3) resulted into highest levels of aflatoxin contamination compared to H1 and H2 among the groundnut varieties in both study locations. Confirming the study findings by Hell *et al*. (2003) who reported that post-harvest contamination with aflatoxin in groundnut increased when harvesting was executed 5 days after physiological maturity. Additionally, the study has shown that delayed harvesting resulted into higher aflatoxin contamination levels greater than the FDA/WHO regulatory levels of 20 ppb (Mphande *et al*., 2004). The high aflatoxin contamination levels at H3 could be attributed to heavy damage of pods by insects especially termites (*Odontotermes badius* and *Odontotermes latericus*) (Appendix 10) which provided ready entry of fungi including *Aspergillus* species and consequently aflatoxin contamination. This confirmed the findings of Dowd (2003) who reported that insects influence the levels of aflatoxin contamination in commodities such as;

maize and groundnut by carrying fungal inoculum and causing damage that provide ready entry of the fungus and thereby increasing the chances of aflatoxin contamination. Furthermore, Kombiok *et al*. (2012) indicated that insects such as termites cause scarification of pods, which weakens the shells and makes them liable to crack during harvesting leading to further insect, microbial and disease infestations.

High aflatoxin contamination levels at H3 could also be attributed to physical damage of pods as a result of digging using hoes. Harvesting groundnut 10 days after physiological maturity coincided with dry weather making it difficult to harvest the groundnuts by hand pulling which led to digging the nuts out of the soil using hand hoes. Similar to the effect of insect damage to pods, physical damage to pods tended to increase with delay in harvesting perhaps due to the dryness of the soil which made pulling and digging out of pods very difficult. As a result many pods of the groundnut varieties got damaged which favored the entry and invasion of the nuts by *Aspergillus* Section *Flavi* that later produced aflatoxins as a result of respiration. These findings are concurrent with the findings of Hell and Mutegi (2011) who indicated that some factors that influence the incidence of fungal infection and subsequent toxin development include: invertebrate vectors (insects), grain damage, inoculum load, substrate composition, fungal infection levels, prevalence of toxigenic strains and microbiological interactions. Moreover, Horn (2005) reported that highest levels of *A. flavus* and *A. parasiticus* infection and aflatoxin contamination are associated with seed damage caused by either insects or physical damage of pods.

It has also been observed that delayed harvesting coincided with high relative humidity (≥ 75 %) and higher air/soil temperatures (30-35 ^{o}C) which provided hot and moist conditions for fungal growth and subsequent aflatoxin contamination. This phenomenal confirmed the findings of Cotty and Jaime-Garcia (2007) who stated that influences of delayed harvesting on aflatoxin contamination are most severe when crops are caught by higher than normal temperatures (25-30 ^{o}C) and high relative humidity just prior to or during harvest (≥ 70 %). Additionally, harvesting groundnut 10 days after physiological maturity coincided with high populations of *Aspergillus* species in the soil which led to high aflatoxin contamination. Vijayasamundeeswari *et al*. (2010) reported that populations of *A. flavus* were significantly higher in the pod-zone than in the field soil and increased with maturation of the crop.

The correct drying of harvested groundnuts is very important, as inappropriate drying can help induce fungal growth and reduce kernel quality for consumption and germination for the

following season. At harvest groundnut fruits have a higher moisture content (38-40 %) and must be dried to (7-8 %) to prevent growth of fungi (Waliyar *et al*., 2015) agreeing with the findings of this study. Moreover, drying method greatly influences the resistance of groundnuts to fungal attack (Rahmianna *et al*., 2007). It has been established from the results of this study that both the A-frame and tarpaulin drying methods were effective in reducing moisture content of groundnut to the recommended level of ≤ 7 % and thereby reduced the chances of heavy aflatoxin contamination on the kernels. However, tarpaulin drying method was more rapid in reducing kernel moisture levels compared to A-frame dying method. This was attributed to the direct exposure of the pods to sunlight compared to the shading of pods with leaves when on the A-frame.

Nevertheless, significant differences were observed in aflatoxin contamination levels between A-frame and tarpaulin drying methods. Lower aflatoxin contamination levels were observed when using the A-frame (≤ 10 ppb) compared to tarpaulin drying (≤ 20 ppb) which had to some extent higher aflatoxin contamination levels. The high aflatoxin contamination levels when using the tarpaulin method was attributed to alterations of the pod and seed coat as a result of direct exposure to sunlight which resulted into creation of microscopic poles and cracks that provided ready entry of fungi and later aflatoxin production. The advantage of the A-frame drying method over tarpaulin drying was that it prevented direct exposure of the pods to sunlight and provided increased air circulation as a result of the pods being on a raised platform which led to efficient and effective drying resulting into lower fungal invasion. This confirmed the findings of Fernandez *et al*. (1997) who reported that if drying is too rapid there are alterations in the seed coat that favor fungal infection. Furthermore, Nautiyal (2002), reported that tarpaulin drying results into restricted air movement within the nuts and thereby providing inefficiency in reducing moisture and providing conditions for fungal growth and consequently aflatoxin contamination. The current study findings are in accordance to the study conducted by Hell *et al*. (2008) who found that drying maize using platforms (A-Frames) reduced contamination of the crop by toxigenic fungi than using tarpaulins.

High aflatoxin contamination levels with the tarpaulin drying method could also be as a result of weather conditions. Post-harvest abrupt rainfall during the drying period resulted into wetting of pods and prevented drying of the pods to the open sun on some days when it rained all day which resulted into creation of moist conditions conducive for aflatoxin production by the fungi. While as for the A-frame this was not the case since the pods were covered with leaves and thereby preventing water from reaching the pods and ensuring exposure to air

circulation all the time. Nautiyal (2002) reported that one of the disadvantages of drying groundnuts on tarpaulins was the time and effort required to gather the pods together and cover them during rain showers and re-spreading the pods as soon as possible in-order to continue drying, this was difficult and the adverse moist conditions as result of the rain provided optimum conditions for fungal invasion and aflatoxin production.

However, in general it has been observed that both the A-frame and the tarpaulin drying methods were effective in prevention of aflatoxin contamination of the groundnut crop than would traditional methods of drying which involve field and bare ground drying. Furthermore, the A-frame and tarpaulin drying methods ensured that the groundnut crop attained the recommended moisture content (≤ 7 %) and ensured that the crop was not in direct contact with the soil thereby preventing easy access of fungi to the pods and thus ensuring minimum fungal invasion. This is similar to the findings of Kaaya *et al*. (2005) who reported that, field and bare drying of maize by traditional methods falls short of attaining moisture levels that are safe for storage; in addition, bare ground drying leads to long-term exposure of the crop to infestation and damage by insects, birds, rodents, wild animals and fungi.

Pre-harvest factors are critical for effective post-harvest prevention of aflatoxins. Some of the pre-harvest factors that may influence the incidence of fungal and subsequent aflatoxin development include; drought stress, grain damage, insect damage, disease stress and environmental stress (Lamboni and Hell, 2009). Moreover, the growing of stressed plants has been linked with a higher infestation of *A. flavus* in crops (Kimatu *et al*., 2012). Results from the current study have shown a strong positive correlation between late leaf spot severity and aflatoxin contamination ($r = 0.2552$, $P \leq 0.05$) and ($r = 0.1891$, $P \leq 0.05$) at CIAM and PAN respectively. This indicated that an increase in late leaf spot severity resulted into an increase in aflatoxin contamination of the groundnut crop. This therefore has shown that late leaf spot severity weakened the plant defence mechanisms which predisposed them to invasions by pathogens such as *Aspergillus* species which subsequently resulted into aflatoxin contamination. This confirmed the findings of Cole *et al*. (1982) who found that under disease stress groundnut production of phytoalexins was reduced thereby exposing the crop to spore germination and hyphae extension of *A. flavus* and in-turn aflatoxin contamination.

3.5. CONCLUSIONS

The study has shown that weather conditions i.e. higher temperatures (≥ 25 ^{o}C) along with elevated relative humidity (≥ 70 %) favored the growth of *Aspergillus* section *Flavi* and subsequent aflatoxin production.

The results of the current study have shown that proper post-harvest management of groundnut such as; harvesting at physiological maturity gave the lowest aflatoxin contamination levels, lower than the FDA/WHO regulatory levels of 20 ppb than harvesting either too early or too late.

The study has made known that drying of groundnut using the A-frame and tarpaulin methods was effective in reducing moisture content of groundnut to ≤ 7%, which is a safer moisture content for prevention of fungal growth. Additionally, the study has demonstrated that drying groundnut using the A-frame and tarpaulin methods was effective in ensuring lower aflatoxin contamination levels of kernels. However, the A-frame was more effective compared to the tarpaulin drying method.

Sufficient evidence from this study has shed light that there was a strong positive correlation between late leaf spot severity and aflatoxin contamination.

3.6. RECOMMENDATIONS

Basing on the findings of this study it is recommended that farmers harvest their groundnut crop at physiological maturity in order to prevent high aflatoxin contamination levels in their groundnut during storage, transport and marketing periods. Additionally, farmers should discard damaged pods as these may contain high levels of aflatoxins.

It is also recommended that farmers be encouraged to try and adopt the A-frame drying method in-order to ensure that the moisture content of the kernels is at the recommended level (≤ 7 %) safe for storage which in-turn prevents further fungal invasion and later aflatoxin contamination.

There is need of the government to implement training and awareness campaigns for both the farmers and the general public on pre and post-harvest management of aflatoxins and their effects on human and livestock health and the export market of groundnut. Knowledge of specific, non-costly aflatoxin management practices such as observing planting and harvesting dates, pest and disease management and proper drying of groundnuts would help to reduce aflatoxin contamination in groundnut and other crops.

In addition, there is need of the government to introduce food safety policies especially on the allowable levels of aflatoxin in groundnut for both human and livestock consumption.

There is need to conduct a similar study in other groundnut growing areas in Mozambique to evaluate the effectiveness of the two drying methods in prevention of aflatoxin contamination. Furthermore, there is need to conduct a study on the effects of disease stress such as; late leaf spot severity on aflatoxin contamination of groundnut. There is also need to conduct characterization studies on the fungal strains responsible for aflatoxin contamination in Mozambique.

3.7. REFERENCES

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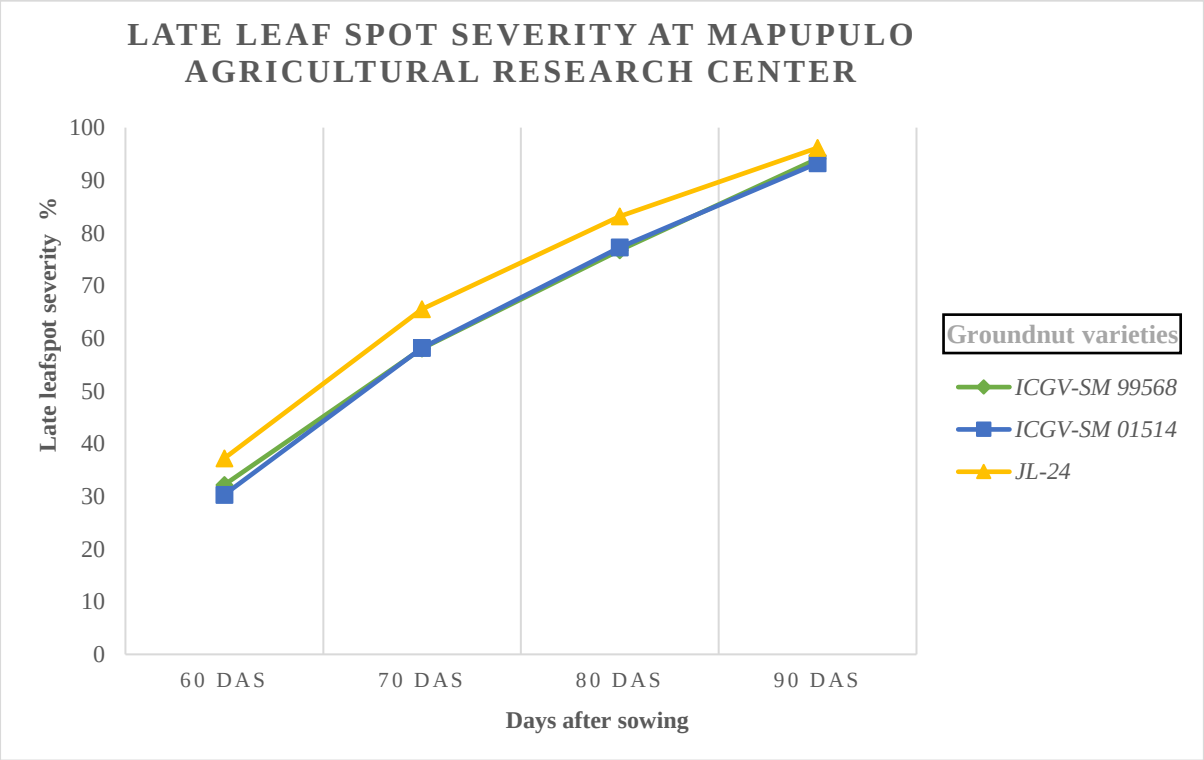
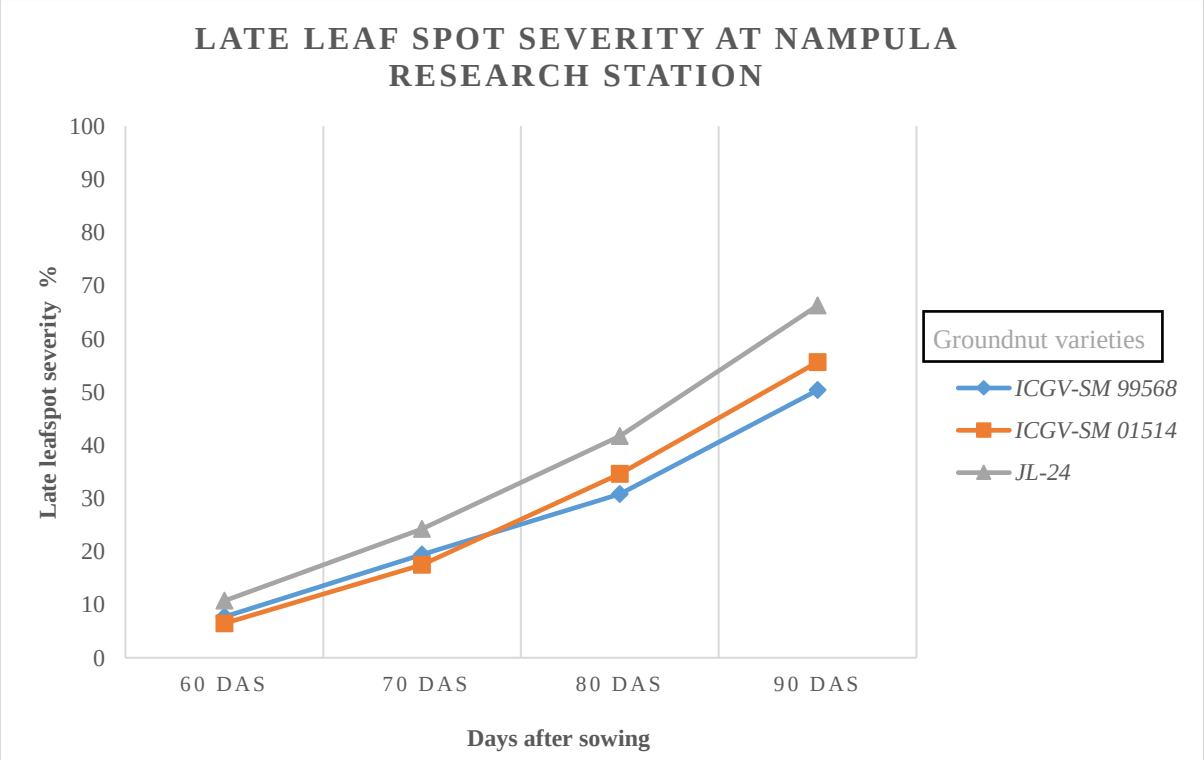
APPENDICES

APPENDIX 1: Late leaf spot severity at Mapupulo Agricultural Research Center



APPENDIX 2: Late leaf spot severity at Nampula Research Station



APPENDIX 3: Kruskal-Wallis Equality of Populations Rank Test for shelling percentage (%) at PAN

Variety	Harvest timing rank test		
	H1	**H2**	**H3**
ICGV-SM 99568	69.00	109.00	67.00
ICGV-SM 01514	43.00	107.50	52.50
JL-24	34.00	122.00	62.00
Chi-square test	**17.776**	**F = 0.0230**	

H1 = Harvest at 10 days before physiological maturity, H2 = Harvest at physiological maturity
H3 = Harvest at 10 days after physiological maturity.

APPENDIX 4: ANOVA TABLE OF EFFECT OF HARVESTING TIME DATA

Source of Variation	d.f.	Mean Squares			
		Aflatoxin	Kernel Yield	100-Kernel weight	Shelling %
Rep	3	64.9	559800	24.08	259.77
Location	1	310.2	2280100*	110.25	404.74
Residual	3	67.3	394576	0	355.87
Variety	2	239.2	66921	7228.5***	120.4
Location.Variety	2	157.3	116849	0	196.43*
Residual	12	105.4	55012	14.82	56.97
Harvesting time	2	5593.2***	970653***	1121.92***	287.68**
Location.Harvesting time	2	105.8	494314***	0	402.31*
Variety.Harvesting time	4	182.7	48245	70.98***	35.28
Location.Variety.Harvesting time	4	94.6	30858	0	45.94
Residual	36	126.7	41702	5.88	72.71
Rep.Location.Variety.Harvesting time	72	106.5	12464700	11.94	87.09
Total	143				

*Data significant at P=0.05; ** Data significant at P = 0.01; ***Data significant at P = 0.001

APPENDIX 5: MEANS, LSD AND COEFFICIENT OF VARIATIONS FOR THE ANOVA TABLE ON EFFECT OF HARVESTING TIME DATA

	Aflatoxin Results (ppb)	Kernel Yield (Kg/ha)	Weight of 100 Kernel (g)	Shelling %
Mapupulo	15.78	662.19	49.09	72.69
Nampula	12.85	913.86	50.84	76.05
Mean	14.32	788.03	49.97	74.37
LSD (5%)	4.35	333.178	2.42	10.01
CV (%)	13.50	18.8	1.6	6.00
Variety				
ICGV-SM 01514	16.35	769.33	36.24	73.01
ICGV-SM 99568	14.66	831.02	59.88	73.98
JL-24	11.93	763.73	53.77	76.11
Mean	14.31	788.03	49.96	74.37
LSD (5%)	4.57	104.314	1.71	3.36
CV (%)	29.30	12.2	3.1	4.10
Harvesting time				
H1	10.38	687.04	44.61	73.68
H2	6.04	950.65	54.00	72.34
H3	26.52	726.39	51.28	77.09
Mean	14.31	788.03	49.96	74.37
LSD (5%)	4.66	84.54	1.00	3.53
CV (%)	55.60	18.3	3.4	8.10

APPENDIX 6: ANOVA TABLE OF EFFECT OF DRYING METHOD DATA

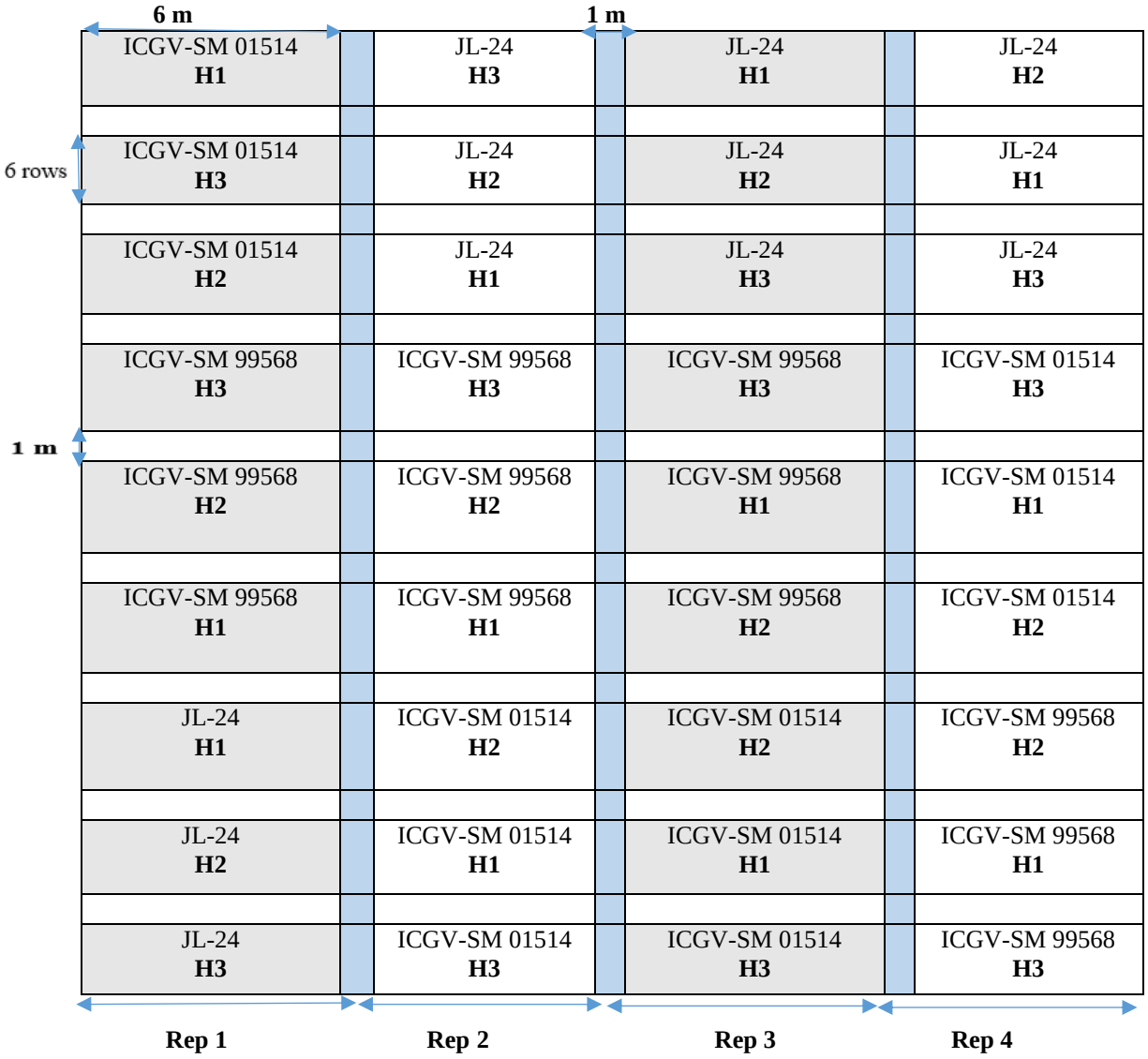
Source of variation	d.f.	Mean Squares			
		Aflatoxin	Kernel Yield	100 Kernel weight	Shelling %
Rep	3	64.9	139950	24.08	259.77
Location	1	310.2	570025*	110.25	404.74
Residual	3	67.3	98644	0	355.87
Variety	2	239.2	16730	7228.5***	120.4
Location.Variety	2	157.3	29212	0	196.43*
Residual	12	105.4	13753	14.82	56.97
Drying method	1	2887.2***	1766930***	38.54***	288.17*
Location.Drying method	1	82.2	736100***	717.79***	1482.09***
Variety.Drying method	2	49	5130	5.38*	73.17
Location.Variety.Drying method	2	31.6	11681	789***	42.86
Residual	18	59.5	13553	1.15	51.88
Rep.Location.Variety.Drying method	96	213.9	15859	29.17	79.76
Total	143				

*Data significant at P=0.05; ** Data significant at P = 0.01; ***Data significant at P = 0.001

APPENDIX 7: MEANS, LSD AND COEFFICIENTS OF VARIATIONS FOR THE ANOVA TABLE ON EFFECT OF DRYING METHOD DATA

	Aflatoxin Results (ppb)	Kernel Yield (Kg/ha)	Weight of 100 Kernels (g)	Shelling %
Mapupulo	15.78	331.10	49.09	72.69
Nampula	12.85	456.93	50.84	76.05
Mean	14.32	394.02	49.97	74.37
LSD (5%)	4.35	166.59	72	10.01
CV (%)	13.50	18.80	1.6	6.00
Variety				
ICGV-SM 01514	16.35	384.66	36.24	73.01
ICGV-SM 99568	14.66	415.51	59.88	73.98
JL-24	11.93	381.86	53.77	76.11
Mean	14.31	394.01	49.96	74.37
LSD (5%)	4.57	52.16	48	3.36
CV (%)	29.30	12.20	3.1	4.10
Drying method				
A-Frame	9.84	504.78	49.45	72.95
Tarpaulin	18.79	283.24	50.48	75.78
Mean	14.32	394.01	49.965	74.37
LSD (5%)	2.70	40.76	72	2.52
CV (%)	31.10	17.10	10.8	5.60

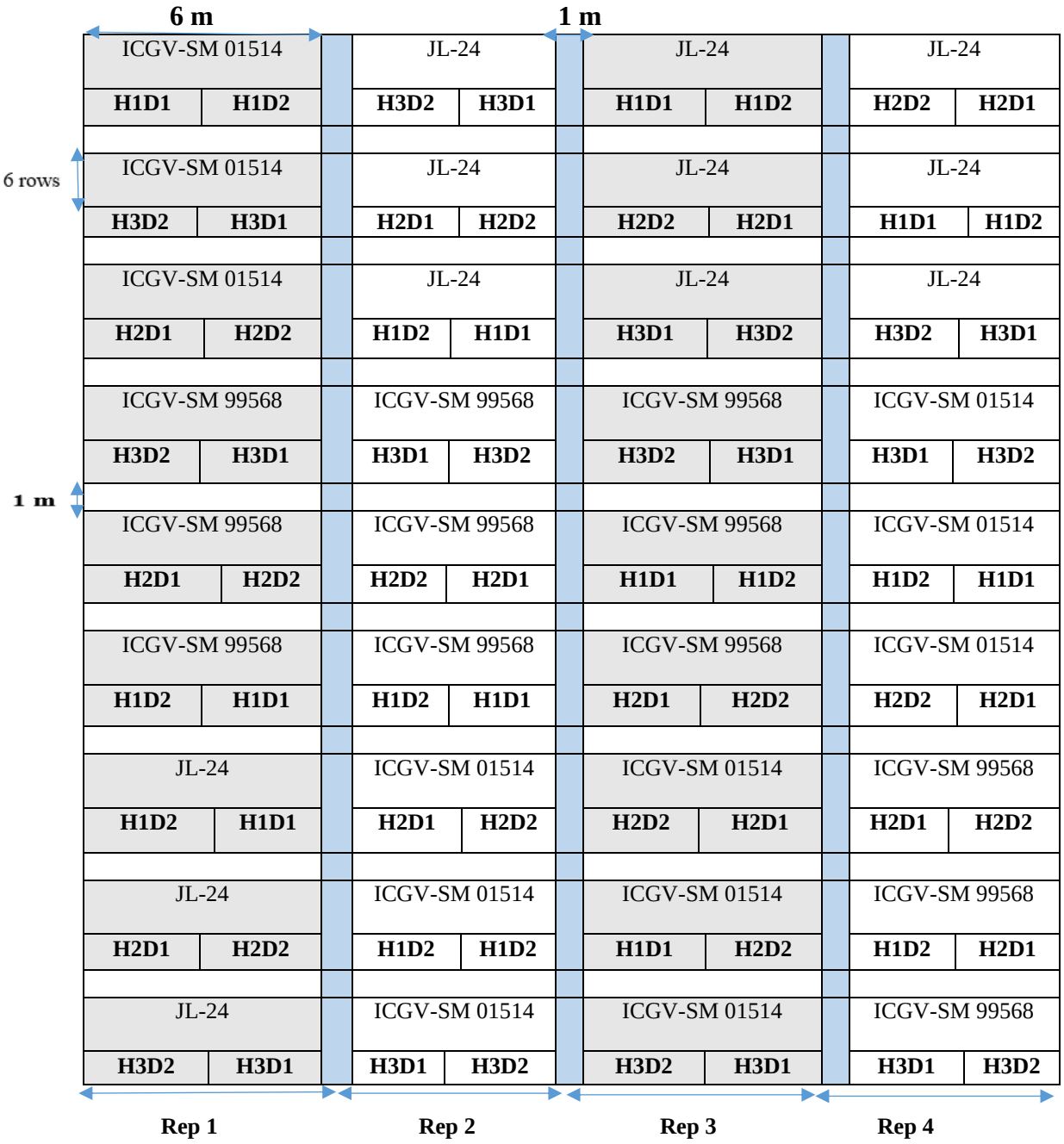
APPENDIX 8: FIELD LAYOUT: EFFECT OF HARVESTING TIME ON GROUNDNUT YIELD AND YIELD COMPONENTS



HARVESTING TIME

1. **H 1 =** Harvesting 10 days before physiological maturity.
2. **H 2** = Harvesting at Physiological maturity.
3. **H 3 =** Harvesting 10 days after physiological maturity.

APPENDIX 9: FIELD LAYOUT FOR EFFECT OF HARVESTING TIME AND DRYING METHOD ON GROUNDNUT AFLATOXIN CONTAMINATION



HARVESTING TIME

1. **H1 =** Harvesting 10 days before physiological maturity.
2. **H2 =** Physiological harvesting maturity.
3. **H3 =** Harvesting 10 days after physiological maturity.

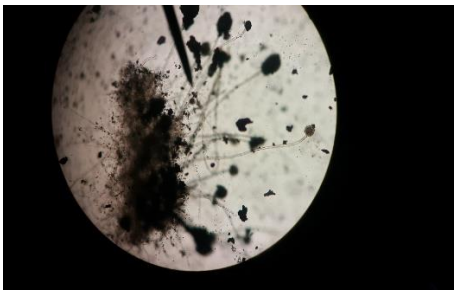
DRYING METHOD

1. **D1** = Tarpaulin drying method.
2. **D2 =** A-Frame drying method.

APPENDIX 10: Photos as illustrative material

	Photo 1: Fallen groundnut crop as a result of termite attack.
	Photo 2: Groundnut crop showing symptoms that it was attacked by thrips (*Frankliniella fusca*).
	Photo 3: Groundnut pods severely attacked by cutworms (*Spodoptera litus Fabricius*) and termites.
	Photo 4: An adult Assassin bug (*Coranus trabeatus*). This is a predator bug commonly found in legumes and pulses.

	Photo 5: Glossy shield bug (*Cermatulus nasalis*). This is a predator that commonly prey's on caterpillars, thrips and aphids.
	Photo 6: Groundnut pods dug and exposed to the sunlight by crows.
	Photo 7: Emmanuel Junior Zuza inspecting one of the trials at PAN.
	Photo 8: Emmanuel Zuza Junior conducting an aflatoxin test at FAEF laboratory.

	Photo 9: Growth of fungi in nutrient media. Black colony (*Aspergillus niger*). Green colony (*Aspergillus flavus*). Grey colony (*Fusallium*).
	Photo 10: *Aspergillus niger* as seen under an electron microscope.