MODELING PRE-HARVEST AFLATOXIN INCIDENCE IN GROUNDNUT (*Arachis hypogaea* L.) USING SELECTED SOIL PROPERTIES AND AMBIENT TEMPERATURE

By

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DECLARATION

I, **Hendrix M. Chalwe,** hereby declare that all the work presented in this thesis is my own original work and has never been submitted for a degree award in any other university.

…….. ……

Hendrix M. Chalwe **Date**

CERTIFICATE OF APPROVAL

This thesis of **Hendrix M. Chalwe** was approved as fulfilling the requirements for the award of the degree of **Doctor of Philosophy in Soil Science** by the University of Zambia.

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ABSTRACT

Aflatoxin contamination of groundnut (*Arachis hypogaea* L.) and its products is a worldwide food safety concern. Contamination of kernels during crop growth is associated with adverse soil factors and weather conditions. Therefore, good agricultural practices are an important strategy to minimize the pre-harvest aflatoxin contamination risk in groundnut. The main objective of this study was to formulate regression models to predict pre-harvest aflatoxin contamination risk in groundnut using selected soil properties and ambient temperature. Field experiments were conducted in Lusaka and Chongwe districts of Zambia to evaluate the effects of soil amendments, namely, gypsum (15.6 % soluble calcium) and compost on pre-harvest aflatoxin incidence in groundnut. Additionally, the effects of soil moisture content and soil temperature during groundnut pod development stage on aflatoxin concentration were evaluated. The data generated from these experiments were analysed using appropriate tests at 5 % level of significance. Treatment effects were evaluated using the one or two-way ANOVA test as appropriate. The Tukey test was used to separate significantly different treatment means. Pearson correlation analysis was performed on the data to evaluate relationships between continuous variables. Simple and multiple linear regression analysis was performed on significantly correlated variables. All these tests were performed using the R-statistical software. Results showed that higher levels of compost were associated with lower aflatoxin contamination. The gypsum amendment did not have a significant effect on aflatoxin contamination of groundnut kernels. Further, regardless of the treatments applied, ambient temperature and soil temperature were positively correlated with aflatoxin contamination whereas soil moisture content was negatively correlated. Simple linear regression models gave R^2 values of 0.30 for maximum ambient temperature, 0.24 for soil temperature and 0.38 for soil moisture content. Combining soil moisture content and soil temperature in a multivariate regression model explained 54 % of the variation in aflatoxin contamination. Therefore, soil moisture and soil temperature can be used to predict aflatoxin contamination risk in groundnut. The two predictive variables, soil moisture and soil temperature, can be manipulated through agronomic practices to reduce the risk of pre-harvest aflatoxin contamination in groundnut.

DEDICATION

To my wife Angela Botha, son Walusungu Nathanael Chalwe, dad Henry Zitambuli Chalwe, mum Violet Bwalya and my siblings, thank you all for your love and support.

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Thank you.

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ABBREVIATIONS AND ACRONYMS

ATDC	Agricultural Technology Demonstration Centre
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IITA	International Institute of Tropical Agriculture
KATC	Kasisi Agricultural Training Centre
KKIA	Kenneth Kaunda International Airport
NISIR	National Institute for Scientific and Industrial Research
USDA	United States Department of Agriculture
UNZA	University of Zambia
ZABS	Zambia Bureau of Standards
ZARI	Zambia Agricultural Research Institute
CSO	Central Statistical Office

CHAPTER ONE

1 INTRODUCTION

1.1 Background

Groundnut (*Arachis hypogaea* L.) is an important food and cash crop of Zambia. It is grown throughout the country and is rated the second most widely cultivated crop among small-holder farmers (Ross and Klerk, 2012; Mukuka and Shipekesa, 2013). According to the post-harvest survey of the 2014/15 cropping season conducted by the Zambian Central Statistical Office, 49.8% of all the agricultural households in Zambia planted groundnuts (CSO, 2015).

However, one of the critical food safety concerns in the Zambian groundnut value chain is the documented high concentrations (>20 ppb AFB_1, which is greater than the allowable limit of 15 ppb) of aflatoxins in some of the groundnut-based food products such as groundnut flour and peanut butter (Njoroge *et al*., 2017; Banda *et al*., 2018). Aflatoxins are naturally-occurring toxic metabolic substances produced by certain *Aspergilli* fungi predominantly, *Aspergillus flavus* and *A. parasiticus*. These fungal species are ubiquitous and are adapted to soil environments that are prohibitive to other soil-borne microorganisms such as very dry and hot soil conditions (Richard and Payne, 2003).

Long-term aflatoxin exposure in humans has been associated with stunting in children, suppression of the immune system, liver cancer, and genetic mutations (Williams *et al*., 2004; Richard, 2007). In Zambia, stunting among children under the age of 5 years has been associated with exposure to aflatoxin through consumption of contaminated groundnut-based foods (Ismail *et al*., 2014). These and other health issues have resulted in the formulation of tolerable limits and monitoring of aflatoxin levels in commercial groundnut products. For instance, in Zambia, the tolerable limit of total aflatoxin content in peanut butter is 15 ppb (ZABS Regulatory Standard number ZS 723: 2008). It is worth noting that the permissible limits for AFB_1 concentration in groundnuts meant for direct consumption on the more lucrative EU market is 2 ppb (EC 2010). Therefore, products that do not meet such legal requirements are rejected and removed

from the market resulting in huge loss of income for the traders/farmers whose produce is rejected.

Given that aflatoxin contamination in groundnuts can occur before, during and after harvesting (Pitt *et al*., 2013), appropriate interventions at field level (before and during harvesting) can minimize aflatoxin content in harvested kernels. Soil factors such as soil moisture content and soil temperature during pod development (Hill *et al*., 1983; Rachaputi *et al*., 2002; Torres *et al*., 2014), exchangeable calcium content during pod development (Cox *et al*., 1976; Jain *et al*., 2011; Jordan *et al*., 2014) and soil organic matter content (Waliyar *et al*., 2013) have a strong influence on pre-harvest aflatoxin contamination of groundnut kernels. Other equally important factors include weather parameters such as ambient temperature and rainfall (Bowen and Hagan, 2015). The relationships between environmental factors and aflatoxin content in harvested kernels can form a basis for predicting the risk of aflatoxin contamination.

In the past 2 decades, there have been concerted efforts to develop models to predict the risk of aflatoxin contamination of groundnut during pod development using relationships between environmental conditions and aflatoxin content in harvested kernels (Henderson *et al*., 2000; Craufurd *et al*., 2005; Chauhan *et al*., 2010; Bowen and Hagan, 2015). The most critical period for pre-harvest aflatoxin contamination in groundnut growth is the pod development stage which occurs in the last six weeks of the growing cycle (Blankenship *et al*., 1984; Hill *et al*., 1983).

The ability to predict pre-harvest aflatoxin contamination risk offers an opportunity for timely and appropriate interventions when the risk is high. Except for the work of Bowen and Hagan (2015), most of the models cited above are relatively complex and heavily depend on computer simulations to calculate model parameters. There is, therefore, need for simpler alternatives that are adapted to the local conditions and circumstances.

This study aimed to formulate a less complex, easy-to-use statistical model to predict pre-harvest aflatoxin contamination risk in groundnut using soil and weather parameters that affect pod development and infection by *Aspergilli* spp. and subsequent aflatoxin contamination of kernels. The parameters that were selected include ambient

temperature, soil temperature and moisture, exchangeable calcium and soil organic matter. Therefore, field experiments were designed to assess the effect of each of these parameters either individually or in combination on pre-harvest aflatoxin contamination. The experiments were conducted for two successive cropping seasons and in multiple locations under rain-fed conditions. At harvest, aflatoxin contamination was determined and used to quantify relationships between the aforementioned parameters during pod development and aflatoxin contamination.

1.2 Statement of the problem

The prevalence of higher than acceptable aflatoxin concentration in the Zambian groundnut value chain has important health and market implications. In order to design appropriate interventions for safe and marketable locally-grown produce, there is need to understand contributing factors at every level of the value chain. According to literature, factors with a high influence on pre-harvest aflatoxin contamination in groundnut include soil moisture, soil temperature and ambient temperature during the pod-development stage. To this end, researchers such as Craufurd *et al*., 2005; Chauhan *et al*., 2010 and Bowen and Hagan, 2015 have developed predictive models using combinations of such risk factors. However, the application of these models in a tropical climate requires adapting them to local conditions. Additionally, the heavy reliance on computer simulations and the broad crop and weather data requirement makes some of these models too complex to be adapted to climatic conditions of Zambia. Simpler models based on fewer and easily-measurable, but very critical aflatoxin risk factors are needed in order to abate the challenge. The current work aimed to first identify and evaluate these critical risk factors and then using them formulate aflatoxin predictive models. .

1.3 Objectives and hypotheses

1.3.1 Main objective

The main objective of this study was to formulate statistical models to predict pre-harvest aflatoxin contamination risk in groundnuts using selected soil properties and ambient temperature.

1.3.2 Specific objectives

The specific objectives of this study were:

1. To evaluate the effect of soil temperature and moisture during pod development on the risk of pre-harvest aflatoxin contamination in groundnut,
2. To assess the role of soil organic matter on the risk of pre-harvest aflatoxin contamination in groundnut,
3. To evaluate the contribution of exchangeable calcium to the risk of pre-harvest aflatoxin contamination in groundnut, and
4. To model relationships between soil temperature, soil moisture, soil organic matter, exchangeable calcium and ambient temperature and the risk of pre-harvest aflatoxin contamination in groundnut.

1.3.3 Hypotheses

The research hypotheses were:

1. Elevated soil temperature and low moisture content during pod-development increases the risk of pre-harvest aflatoxin contamination in groundnut.
2. High levels of soil organic matter content reduce the risk pre-harvest aflatoxin contamination in groundnut.
3. Exchangeable calcium levels in the soil influences pre-harvest aflatoxin contamination in groundnut.
4. The risk of pre-harvest aflatoxin contamination in groundnut can be modelled using soil temperature, soil moisture, soil organic matter, exchangeable calcium and ambient temperature during growth.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Overview on aflatoxins

Aflatoxins constitute the most potent class of mycotoxins produced by *Aspergilli* fungi. Of the four species known to produce aflatoxins, the most predominant include *Aspergillus flavus* and *A. parasiticus*. The discovery of aflatoxins came as a result of research on an outbreak of an unknown disease of turkeys in England in the early 1960s. The disease was eventually linked to aflatoxin contamination of groundnut feed imported from Brazil. Since then, preventing aflatoxin contamination of feed and food has been of world-wide concern due to its carcinogenic effect on human beings (Richard, 2008).

Although large populations of these saprophytic soil-inhabiting fungi can occur in diverse environments, the occurrence of aflatoxin contamination is mostly associated with warm and dry climates such as the sub-tropical and warm temperate climates. The ability of *Aspergillus* to grow on a wide range of substrates even under very dry and hot environments gives them capacity to colonise a wide range of grains and nuts. Among the most susceptible field crops are maize and groundnut (Richard and Payne, 2003; Richard, 2007).

2.2 Types of aflatoxins

The four major types of aflatoxins include B_1, B_2, G_1 and G_2. This classification is based on fluorescence under ultraviolet light. Aflatoxin B_1 and B_2 have a blue fluorescence, while aflatoxin G_1 and G_2 have a green fluorescence. Two intermediate types of aflatoxin that give a blue-violet fluorescence under ultraviolet light are referred to as M_1 and M_2. The subscripts on all the types denote the relative chromatographic mobility. The types with 1s have longer retention time than those with 2s (Richard and Payne, 2003).

2.3 Prevalence of aflatoxin contamination in groundnut and groundnut products in Zambia

The problem of aflatoxin contamination in agricultural commodities such as groundnut is widespread and highly recurrent in sub-Saharan Africa including Zambia. This is partly because most of this region of Africa has favourable climatic conditions for fungal growth and subsequent formation of aflatoxins. Thus, these favourable climatic conditions present a major challenge to the control of aflatoxins in the tropics. Additionally, groundnut is also one of the most susceptible legume crops to infection by *Aspergillus*. With groundnut being a widely consumed legume crop, the risk of poisoning in humans is also very high (Hell and Mutegi, 2011).

Although research in aflatoxin contamination of food crops in Zambia has only received concerted effort in the recent years, a study by Sibanda *et al*. (1997) revealed that Zambia first recorded cases of aflatoxin poisoning in animals consuming contaminated feedstuffs in 1972. The same study also reported that consumption of aflatoxin contaminated food and animal feed was associated with stunting and mortalities. In recent years, studies have reported high aflatoxin contamination of groundnut kernels and groundnut products. For instance, a three-year survey on aflatoxin B_1 in groundnut kernels and milled powder from major groundnut producing districts and selected urban areas of Zambia revealed that whole kernels had mean AFB_1 concentrations higher than 20 ppb (higher than the ZABS threshold of 15 ppb for peanut butter) in the majority of samples from all the research sites during the research period (Njoroge *et al*., 2017).

Similarly, a comprehensive analysis of aflatoxin concentrations in selected local and international (Malawi, Zimbabwe and South Africa) brands of peanut butter on the Zambian market, revealed that about 69 percent of the tested samples had AFB_1 content more than 20 ppb (Njoroge *et al*., 2016). In a related study, Banda *et al*. (2018) conducted a survey aflatoxin contamination of locally-sourced local and international brands of peanut butter, and the results showed that 23.9% of the 109 samples analysed had total aflatoxin content greater than 15 ppb. Results from these studies indicate that there is need for interventions to minimize aflatoxin contamination in consumable groundnut products.

2.4 Effects of aflatoxins on health of consumers and international trade

2.4.1 Health of consumers

Aflatoxins affect a wide range of animal species including birds, fish and mammals such as human beings (Richard and Payne, 2003). Aflatoxins have been associated with reduced production of milk in cattle and eggs in poultry, stunting in children, suppression of the immune system, liver cancer and genetic mutations in humans (Richard, 2007). In cases of acute exposure, deaths have been reported. For instance, in 2004, the republic of Kenya recorded deaths due to acute aflatoxicosis associated with the consumption of contaminated maize (Probst *et al*., 2007). Similar deaths were reported in neighbouring Tanzania in 2016 (Kamala *et al*., 2018).

2.4.2 International trade

The presence of mycotoxins in agricultural commodities can affect international trade due to stringent trade and food safety standards (Murphy *et al*., 2006). For instance, according to the ZABS regulatory standard number ZS 753: 2008, the maximum acceptable concentration of total aflatoxin in peanut butter in Zambia is 15 ppb. This implies that peanut butter with total aflatoxin contamination higher than the regulatory standard would be removed from the market. This has potential to affect growth of groundnut-based economies as it prevents the sale of the affected commodities. For instance, in September 2016, ZABS confiscated over 11 000 1 kg containers of a named brand of peanut butter from major grocery stores in the capital city Lusaka, due to non-compliance with the ZABS regulatory standard (Lusaka Times Newspaper, 7 September, 2016). The estimated cost of the confiscated product was $ 29 750 US Dollar. Thus, the economic loss associated with mycotoxin contamination can be huge when large quantities of contaminated crops are destroyed (Kolossova *et al*., 2009).

2.5 Environmental factors affecting pre-harvest aflatoxin contamination of groundnut kernels

Optimal environmental conditions for the growth of fungi and subsequent mycotoxin contamination are mostly prevalent in the tropics including Zambia. They include among others, climatic factors such as drought stress often associated with high soil temperature, low soil moisture content and high ambient temperatures, low

exchangeable calcium in the soil, low soil organic matter content and low clay content (Hell and Mutegi, 2011).

In groundnut, fungal infection and subsequent aflatoxin contamination is usually associated with elevated soil temperature and moisture stress during pod development (Blankenship *et al*., 1984, Craufurd, 2005; Waliyar *et al*., 2013). According to Hill *et al*. (1983) there was very minimal colonisation of undamaged pods and contamination of kernels with aflatoxins in groundnut grown under either cool-dry conditions or hot-wet conditions during pod-development.

Although there exist several pathways for pre-harvest fungal infection and subsequent aflatoxin contamination in groundnut, the major entry point is the soil directly in contact with the developing pod also known as geocarposphere (Pitt *et al*., 2013). Thus, as documented by Waliyar *et al*. (2013) mechanical damage to pods by soil borne-insects, nematodes or by cultural operations such as weeding promote fungal infection and subsequent aflatoxin contamination even under less severe temperature and moisture conditions. This is partly because once kernels are exposed; they may dry down to moisture content that promotes fungal growth (Wagacha and Muthomi, 2008).

2.5.1 Soil and ambient temperature

Aflatoxin contamination in groundnut is often associated with increased soil temperature and ambient temperature during pod development. Studies have shown that extensive colonisation of undamaged groundnut pods by *A. flavus* and the subsequent aflatoxin contamination of kernels during drought stress only occurred at temperatures ranging from 26.0 to 29.6 °C (Cole *et al*., 1985). However, a study by Chauhan *et al*. (2010) reported that a lower threshold of soil temperature, 22 °C, was needed for aflatoxin contamination to occur, when groundnut was grown under water deficit. The common factor in these studies is that soil temperature only had an effect under dry soil conditions. This suggests that *A. flavus* is thermopillic in nature and thrive in hot and dry soil conditions. Thus, as reported by Hill *et al*. (1983), cooling the soil to less than 25 °C, with supplemental irrigation in the final 4 to 6 weeks of the groundnut growth cycle was associated with very low aflatoxin contamination in undamaged mature kernels.

Similarly, warmer ambient temperatures tend to encourage the growth of *A. flavus* and aflatoxin formation during storage of groundnut kernels (Mutegi *et al*., 2009). As reported by Achar and Sanchez (2006), maximum growth of *A. flavus* and the subsequent formation of aflatoxins in freshly harvested mature and undamaged groundnut pods occurred at ambient temperatures ranging from 27 to 30 °C, while fungal growth was limited at temperatures less than 10 °C and above 37 °C. It, therefore, follows that the range of 10 to 27 °C has less effect on fungal growth and aflatoxin formation than the range of 27 to 30 °C.

2.5.2 Soil moisture

Prolonged soil moisture deficit usually coupled with elevated soil temperatures during pod development is among the important factors contributing to the pre-harvest aflatoxin contamination of groundnut kernels (Torres *et al*. 2014). Drought stressed plants have a reduced natural defence against fungal infection due to reduced metabolism (Dorner *et al*., 1989; Craufurd *et al*., 2005). Additionally, drought stress tends to reduce water activity in the soil, resulting in reduced microbial activity, while promoting the growth of the adapted organisms such as, *A. fl*avus and *A. parasiticus* (Pitt *et al*., 2013). In studies involving fungal-resistant maize varieties, it was reported that drought stress negatively affected expression of genes for resistance against aflatoxins (Guo *et al*., 2008).

Wotton and Strange (1987) showed decreased resistance to aflatoxin contamination as a result of low water activity in kernels. This can be attributed to a decreased capacity of kernels to produce phytoalexins, which are antimicrobial substances that inhibit fungal growth and thus offer resistance to aflatoxin contamination in groundnut (Dorner *et al*., 1989). When growing under normal soil moisture conditions, groundnuts exhibit a strong negative association between the kernel's capacity to produce phytoalexins and invasion of kernels by *A. flavus*. Thus, as documented by Okello *et al*. (2010), it is very important to supplement poorly distributed rainfall and the late-season-drought with irrigation water in order to minimize water deficits and elevated soil temperatures.

2.5.3 Exchangeable calcium and potassium

Calcium is a critical element in the development of healthy groundnut pods and kernels (Cox *et al*., 1976; Jain *et al*., 2011; Jordan *et al*., 2014). In general, calcium deficiency has a detrimental effect on seed-filling leading to seed abortion, which may result in empty pods or fruits with severely underdeveloped seed also known as "pops" and increased rotting of weak pods and skin. Thus, because of its role in ensuring proper pod development, calcium deficiency is associated with higher fungal infection and subsequent aflatoxin contamination. This is primarily because weak immature pods are easily colonized by *A. flavus* (Blankenship *et al*., 1984). On the other hand, fully-developed mature pods are not easily infected by vectors such as insects and this tends to minimize the infection of seed tissue by fungi. It is on this principle that calcium-containing soil amendments are reported to reduce pre-harvest aflatoxin contamination in groundnut.

Additionally, sufficient calcium fertilization enhances drought tolerance in groundnut. When growing under soil moisture deficit, groundnut plants with double the recommended calcium level exhibited a reduced loss of water than plants growing under calcium deficiency (<100 mg/kg). Plants that received supplemental calcium also exhibited less damage to leaf membranes than plants grown without calcium supplements (Chari *et al*., 1986). Low exchangeable calcium in the soil is, therefore, strongly linked to low pod yield and higher pre-harvest aflatoxin contamination of kernels. However, cultivar response to calcium inputs partly depends on kernel size. Large-seeded cultivars require higher inputs of calcium than small-seeded kernels (Jordan *et al.*, 2014). Further, the soil's chemical characteristics such as the inherent exchangeable calcium content also determine calcium fertilizer recommendation for a particular cultivar (Cox *et al*., 1976).

Although potassium plays an important role on growth and yield of groundnut, there are no reported effects of potassium on pre-harvest aflatoxin contamination risk in groundnuts.

2.5.4 Soil organic matter

Soils with high organic matter content are often associated with high populations of *A. flavus* and *A. parasiticus* (Zablotowicz *et al*., 2007). Therefore, it is logical to expect soils that are rich in soil organic matter to promote fungal proliferation and subsequently high aflatoxin contamination of kernels when other conditions are favourable. However, studies by Waliyar *et al*. (2013) suggested a 42 % decrease in aflatoxin contamination in groundnut kernels following an application of farm yard manure at the rate of 2.5 t/ha. This could be attributed to improved water holding capacity and higher nutrient status of the soil usually associated with the addition of manure (Chalwe *et al*., 2019). These effects of soil organic matter on soil can reduce aflatoxin contamination risk even in soils with a high population of *Aspergillus*.

2.5.5 Soil texture

Torres *et al*. (2014) reported that mould infection of groundnut pods can be significantly influenced by soil texture, with light sands favouring the proliferation of fungi. This can be attributed to the fact that light textured soils have poor water holding capacities, making the plants more susceptible to water stress than in heavier soils with higher moisture retention capacities.

2.6 Production practices affecting pre-harvest aflatoxin contamination of groundnut

2.6.1 Seed quality and genotype

Seed factors that may influence pre-harvest aflatoxin contamination in groundnut include seed size, which influences calcium requirement (Jordan *et al*., 2014), its aflatoxin resistance status and mechanical damage by harvesting equipment or insects (Cole *et al*., 1995). Mechanically- damaged seed may contain spores of *A. flavus* that germinate and begin growing when conditions are favourable for growth. Cultivars that are resistant to pests and diseases are often associated with lower susceptibility to aflatoxin contamination (Mutegi *et al*., 2009). According to Okello *et al*. (2010) drought-tolerant groundnut cultivars also have lesser susceptibility to pre-harvest aflatoxin contamination. On the contrary, results of a study by Hamidou *et al*. (2013)

did not show a direct relationship between drought tolerance and pre-harvest aflatoxin contamination.

2.6.2 Disease and pest management during production

Appropriate crop management during field production is important to minimize mycotoxin contamination risk in susceptible crops. During growth, it is important to minimise crop stresses that predispose crops to disease, attack by rodents and insects or injury due to herbicides. Similarly, soil moisture and plant nutrient levels, especially calcium (Cox *et al*., 1976), should be monitored to minimize plant stress. Recommended seed rates should be followed to reduce competition for soil moisture and plant nutrients which predisposes crops to fungal attack. Suitable crop rotations with disease-resistant crops should be practiced to minimize diseases and mycotoxins. Further, plant residues from the previous crop should be properly managed to avoid carrying over of toxigenic fungi (Hell *et al*., 2000; Munkvold, 2003; Wagacha and Muthomi, 2008; Mutegi *et al*., 2009; Kendra, 2009). These practices enhance plant vigour and give the crop capacity to resist fungal infection, pests and diseases. Although aflatoxin contamination can be reduced through soil moisture conservation by means of residue retention, care must be taken to avoid *Aspergilli*- infected residues. In general, a good and healthy crop stand has lower risk of pre-harvest aflatoxin contamination than a diseased crop (Kendra, 2009).

2.7 Management practices to mitigate pre-harvest aflatoxin contamination in groundnut

2.7.1 Traditional pre-harvest aflatoxin management practices

Traditional measures to control mycotoxin contamination are heavily focused on methods that prevent fungal infection during crop growth. This includes mechanisms that inhibit mould growth and the destruction of present mycotoxins (Murphy *et al*., 2006). According to Cole *et al*. (1995) agronomic practices that reduce plant stress to maximize crop growth will in turn decrease aflatoxin contamination of groundnut kernels. As discussed before, the most critical factors that encourage pre-harvest aflatoxin contamination in groundnut include soil moisture stress in combination with high soil temperature. Therefore, most of the agronomic practices that target effective management of soil moisture in order to avoid moisture stress and elevated soil

temperatures especially during pod development can help to minimize pre-harvest aflatoxin infection of kernels. According to Richard and Payne (2003), good agronomic practices such as suitable rotations, timely planting of suitable varieties, tillage using clean equipment, adequate fertilization, weed and pest control and supplemental irrigation during droughts are important to minimize aflatoxin incidence in susceptible crops.

Additionally, planting cultivars that are resistant to *Aspergillus* fungus has also shown potential to minimize pre-harvest aflatoxin contamination of groundnut kernels. In terms of breeding for aflatoxin resistance, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has in the past decade produced groundnut varieties that have shown low (< 4 ppb total aflatoxin content) aflatoxin contamination. With emphasis on breeding for early maturity so as to avoid drought stress at the end of the rainy season, research efforts have targeted the farmer-preferred lines that are resistant or less susceptible to *Aspergillus* (Waliyar *et al*., 2013). Similar research in Zambia has shown that very low aflatoxin contamination could be achieved in cross-breeds between selected susceptible Zambian groundnut cultivars and imported resistant cultivars (Mudenda, 2013).

2.7.2 Current pre-harvest aflatoxin management practices

Recently, there has been growing interest in the development of bio-control agents to minimize pre-harvest aflatoxin risk in maize and groundnut. The technology uses non-toxin producing (atoxigenic) strains of *Aspergillus* to supress the toxin-producing (toxigenic) strains of the same fungus. In most countries of sub-Saharan Africa, including Zambia, research in this technology is spearheaded by the International Institute of Tropical Agriculture (IITA) in partnership with local research institutions. At present, IITA has produced a bio product known as Aflasafe^{TM} which is made of a sterile substrate inoculated with spores of atoxigenic strains of *Aspergillus* (www.aflasafe.com).

The product is broadcast in a field before the grain filling stage of the growing crop to allow the spores to germinate and start growing ahead of the toxigenic strains and other fungi resident in the soil, thereby giving the former the capacity to outcompete the

latter. Boiled sorghum grain is used as a source of food and as a means to spread the friendly fungal strains across the field (www.aflasafe.com). The rationale for the use of atoxigenic strains of the same fungi is that they are better adapted bio-competitors as both classes live in the same environment and subsist on the same substrate (Richard and Payne, 2003).

To enhance the efficacy of the product, the current strategy is for each country to use local atoxigenic strains of *Aspergillus*. In Zambia, IITA in collaboration with Zambia Agricultural Research Institute (ZARI) and the National Institute for Scientific and Industrial Research (NISIR) have produced two registered brands of aflasafe^{TM}, namely Aflasafe^{TM} ZM01 and Aflasafe^{TM} ZM02 by isolating four local strains of *A. flavus* for each product. The products have been tested and proved effective in reducing pre-harvest aflatoxin contamination of groundnut and maize in the three agro-ecological regions of Zambia (www.aflasafe.com). Test results from fields supplied with the aflasafe^{TM} product over two cropping seasons showed consistent reduction in aflatoxin contamination by 86 % and 92 % in maize and groundnuts, respectively. These results are only achievable with strict adherence to good agronomic crop management practices including regular weeding, optimal fertilization and supplemental irrigation where necessary (www.aflasafe.com).

2.8 Use of models to forecast aflatoxin contamination risk in groundnuts

Environmental risk-based crop models have been used successfully to predict the occurrence of crop damage from adverse environmental conditions. For groundnut, relationships between environmental conditions during the pod development stage of growth have been used to develop pre-harvest aflatoxin contamination risk models (Henderson *et al*., 2000; Craufurd *et al*., 2005; Chauhan *et al*., 2010; Bowen and Hagan, 2015). Prolonged moisture deficits and high soil temperatures during pod development are strongly associated with elevated pre-harvest aflatoxin contamination risk in groundnut kernels (Hill *et al*., 1983; Rachaputi *et al*., 2002; Torres *et al*., 2014).

However, as previously observed by Bowen and Hagan (2015), most of the existing models for predicting pre-harvest aflatoxin contamination risk in groundnuts are relatively complex such as the CROPGRO-peanut model (Craufurd *et al*., 2005) and the

Agricultural Production Systems Simulator (APSIM) peanut module (Chauhan *et al*., 2010). The other downside is that some of the current models require non-routine weather data such as pan evapotranspiration that are not available in most developing nations such as Zambia. There is, therefore, need for simpler alternatives that are adapted to a particular environment with own set of local characteristics. Suffice to mention that notwithstanding the observed extensive crop data requirements used in the existing models, they all have a component of soil moisture as an important input variable. The current study is an effort to formulate simpler regression models using easily measurable data such as soil moisture under the prevailing economic and climatic conditions of Zambia.

2.9 Summary of review and conclusions

This review has established that aflatoxin contamination in groundnuts is widespread in Zambia and has both food safety and economic concerns on the crop. In order to effectively deal with these concerns, there is need for interventions before, during and after harvesting the crop. Pre-harvest interventions contribute to minimizing the aflatoxin load at harvest. It has also been established that both soil and climatic factors, especially during pod-development significantly contribute to pre-harvest aflatoxin contamination. Some of the key soil factors include moisture, temperature, organic matter content and exchangeable calcium. Among climatic factors, ambient temperature is frequently cited by many authors. There is, therefore, need to identify and assess which of these factors or factor combinations have a greater capacity to explain trends in pre-harvest aflatoxin contamination in groundnut. Once identified, factors or factor combinations with greater influence on aflatoxin contamination should be targeted when designing effective measures to minimise pre-harvest aflatoxin incidence.

CHAPTER THREE

3 MATERIALS AND METHODS

3.1 Model formulation

3.1.1 Selection of input variables

Linear regression analysis was used to formulate statistical models to predict the pre-harvest aflatoxin contamination risk in groundnut kernels. Pearson's correlation analysis was used to determine factors that were significantly correlated with pre-harvest aflatoxin contamination. Based on the correlation results, the following factors were selected: maximum ambient temperature, soil temperature and soil moisture content during the last 30 days of pod development. These variables were evaluated through a backward elimination stepwise linear regression process to select the most important variables in relation to the output variable. Variable inflation factors were also computed in order to screen closely related input variables in the model. It is important to note that authors of similar studies such as Craufurd *et al*., 2005; Chauhan *et al*., 2010; Bowen and Hagan, 2015, successfully employed linear regression analysis to build useful models for prediction of pre-harvest aflatoxin contamination.

3.2 Data collection and analysis

3.2.1 Description of study sites

Data for model formulation were generated from field experiments that were conducted at selected locations in two districts of Zambia (Figure 1). The selected research sites fall within the agro-ecological region IIa of Zambia (Soil Survey Branch, 2002), which is the most important farming region of the country. This region is characterized by mean annual rainfall of between 800 - 1000 mm. The rain-fed crop-growing season runs from mid-December to late April. All the sites were located in close proximity to functional weather stations and all the fields had not been used for growing groundnuts in the immediate past growing season. The soil type at the University of Zambia Field Station (UNZA) located at 28°20.278' E and 15°28.646' S, is classified in Soil Taxonomy as Typic Isohyperthemic Paleustalfs (Banda and Chabala, unpublished) and as Ferric Luvisols in WRB (2014). At the Agriculture Technology Demonstration Centre of the University (ATDC) located at 28°27.419' E and 15°21.404' S, the soil type

is classified in Soil Taxonomy as Fine-loamy mixed Hyperthemic Arenic Paleustalfs (Chinene, 1988) and as Eutric Planosol in WRB (2014). The soil type at Kasisi Agricultural Training Centre (KATC) research site located at 28° 29.013' E and 15°14.989' S, is classified in the Soil Taxonomy as an Acrisol according to the Soil Map of Zambia (1991).

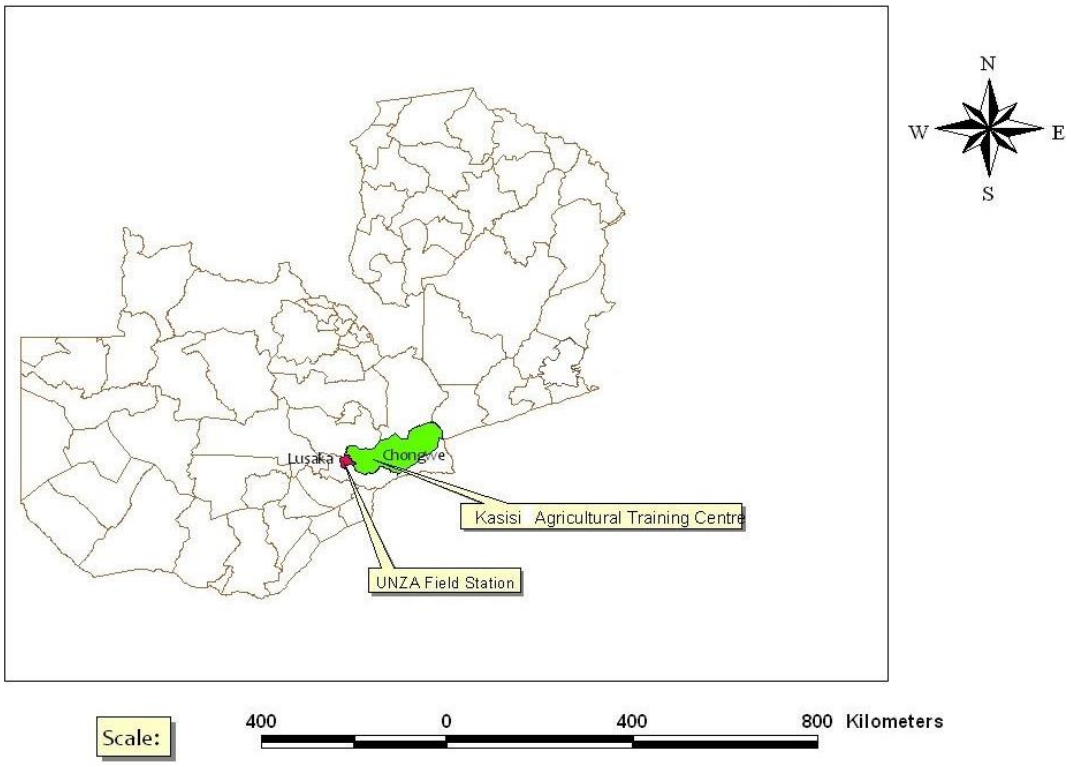

Figure 1: Location of study sites on the Map of Zambia.

The average monthly weather conditions at the research sites located at UNZA in Lusaka district and at KATC and ATDC in Chongwe district are presented below (Figures 2 and 3). The weather conditions at the three sites were typical of the wet and dry tropical climate of Zambia with three distinct seasons, namely the hot-wet season (December to April), cold-dry season (May to August) and the hot-dry season (September to November). The plotted data were compiled from UNZA and Kenneth Kaunda International Airport (KKIA) weather stations between October 2013 and September 2018 as recorded by the SASSCAL weather data service (http://www.sasscalweathernet.org). The data service is available online and the data were accessed on 24 September, 2018.

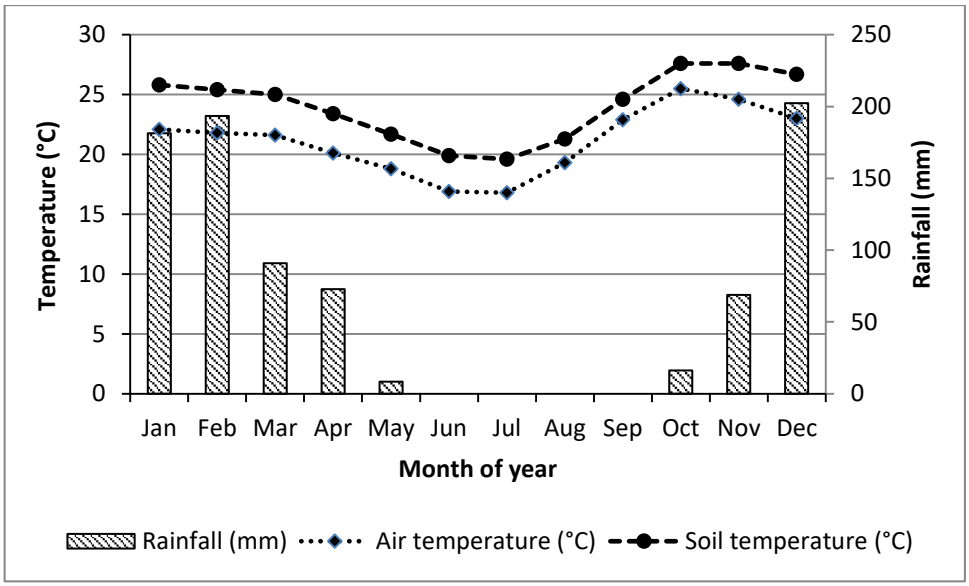

Figure 2: Monthly average rainfall, air temperature and soil temperature over a five-year period (2013 to 2018) at the University of Zambia

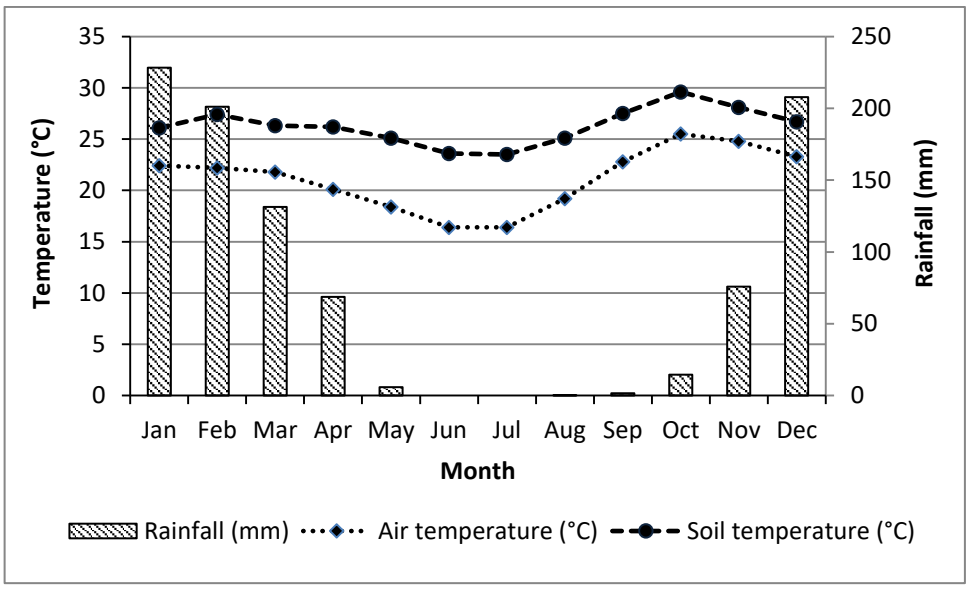

Figure 3: Monthly average rainfall, air temperature and soil temperature over a five-year period (2013 to 2018) at Kenneth Kaunda International Airport[a]

[a]Weather data from KKIA, which is close proximity to KATC and ATDC were used to estimate weather conditions at the two locations.

3.2.2 Soil sampling and characterization

Before laying out field experiments, composite soil samples were collected from each study site. For each site, the disturbed composite soil sample was constituted by combining 4-8 sub-samples randomly sampled from the top 20 cm depth using a soil auger. The actual number of sub-samples was determined based on the size of the experimental plot (the biggest being 0.12 ha) and 20 cm was considered to be the plough layer within which most plant roots for most crops are found. The duly constituted composite sample was air-dried, passed through a 2 mm sieve and then analysed in triplicate for pH, organic matter, texture, available phosphorus, exchangeable potassium and calcium and total nitrogen. At least four undisturbed core samples per experimental plot were also collected using standard core rings. These were used to determine the bulk density of the soil.

3.2.2.1 Determination of soil pH

The soil reaction (pH) was determined using a pH glass electrode according to van Reeuwijk (1992). Ten grams of air-dried soil samples was equilibrated in 25 mL of 0.01 *M* $CaCl_2$ for 30 minutes. The pH was measured in the supernatant solution using a glass electrode fitted to a pH meter (pH 3110, WTW 82362, Weilheim, Germany).

3.2.2.2 Determination of soil organic matter

Soil organic matter content was determined using the wet oxidation method of Walkley and Black (1934). One gram of air-dried soil was completely oxidized in 10 mL of 1N $K_2Cr_20_7$ in an acid medium containing 20 mL of concentrated H_2SO_4. The digest was equilibrated for 30 minutes, after which 150 mL of distilled water and 10 mL concentrated H_3PO_4 were added successively. The digest was then titrated with $FeSO_4$ solution to a green end point using 10 mL diphenylamine indicator.

3.2.2.3 Determination of particle size distribution

The particle size distribution was determined using the hydrometer method (Day, 1965). Fifty grams of air-dried soil was placed into a dispersing cup to which 50 mL of sodium hexametaphosphate (calgon) was added as a soil dispersing agent. The cup was then half filled with tap water and continuously stirred for 5 minutes. The suspension was quantitatively transferred to the sedimentation cylinder using a stream of distilled water

and then filled the cylinder to the 1000 mL mark. The temperature of the suspension was measured using an alcohol thermometer with a temperature range of -20 to 100 ºC. A plunger was inserted and moved up and down to stir the suspension thoroughly. After 20 seconds, a hydrometer was lowered into the soil suspension and the density reading was taken at 40 seconds to determine the silt and clay content. This was repeated three times to obtain an average value. The suspension was left to stand for 2 hours and then took the density reading for clay content. The percentages of clay, silt and sand were calculated as outlined by van Ranst *et al*. (1999). The soil textural class was determined using the USDA Texture Triangle (Figure 4).

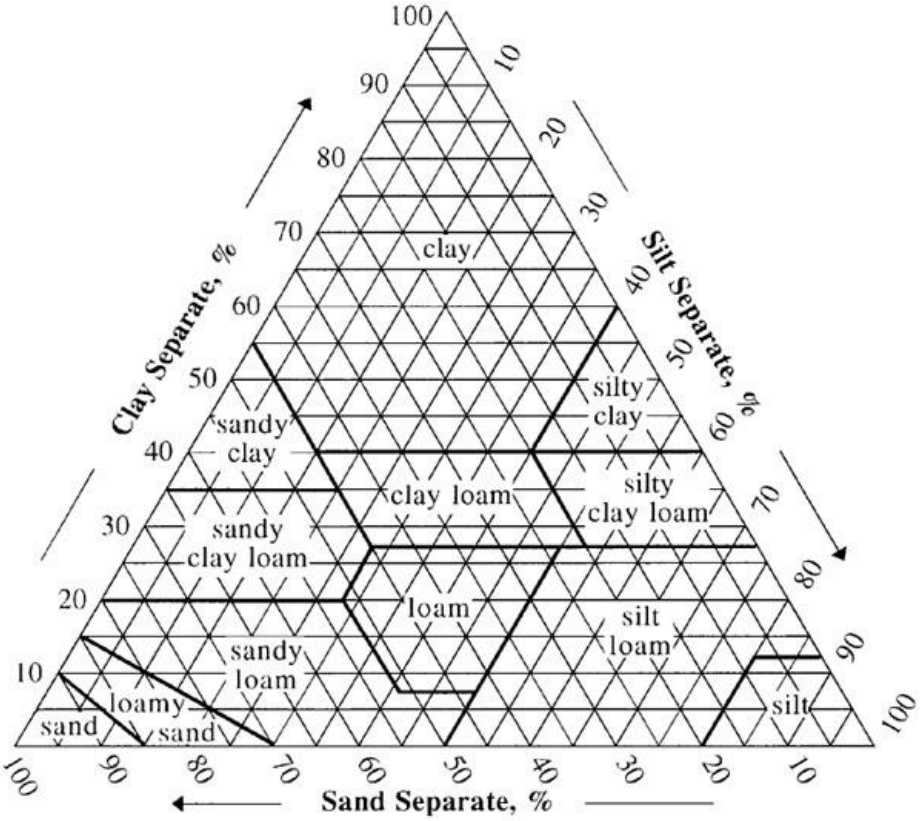

Figure 4: The USDA Soil Texture Triangle

Source: https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey.

3.2.2.4 Determination of plant available phosphorus

Three grams of air-dried soil was equilibrated with 21mL of the Bray 1 solution (Bray and Kurtz, 1945) for 1 minute and then filtered through Whitman No. 42 filter paper. Five millilitres of the filtrate was pipetted into a 25 mL^3 volumetric flask and then diluted with 10 mL of distilled water. Four (4) mL of 2 % ascorbic acid was also added and then filled up the flask to the mark with distilled water. The mixture was allowed to stand for 15 minutes in order to develop colour. Phosphorus content was determined using spectrophotometry at 882 nm wavelength.

3.2.2.5 Determination of exchangeable potassium and calcium

The leaching method was used to extract potassium (K) and calcium (Ca) from the soil using 1 M ammonium acetate buffered at pH 7.0. Ten grams of soil was equilibrated in 50 mL of ammonium acetate at pH 7.0 for 30 minutes. After shaking for 30 minutes, the mixture was filtered through Whitman No. 42 filter paper after which the concentration of K was read directly from the filtrate on the Atomic Absorption Spectrophotometer (Analyst 400, PerkinElmer Life and Analytical Sciences, Shelton, USA). To determine the concentration of calcium, 2 mL of the filtrate was added to a 25 mL volumetric flask to which 10 mL of 5000 mg/L strontium chloride was added. The volumetric flask was then filled to the mark with 1 M ammonium acetate and thereafter determined the concentration of calcium using spectrophotometry (Van Ranst *et al.*, 1999).

3.2.2.6 Determination of total nitrogen

One gram of air-dried soil was placed in a 500 mL Kjeldahl flask and digested in 10 mL of concentrated sulphuric acid and 3 g of the catalyst mixture. After digestion, the mixture was allowed to cool and then diluted with 100 mL of distilled water. Ten millilitres of 10 M NaOH was added to 10 mL of the digested sample and the ammonia produced from the reaction was trapped in 20 mL^3 of H_3BO_3 indicator solution. The boric acid indicator mixture was then titrated with 0.05 M HCl to a pink end point (Bremner and Mulvaney, 1982).

3.2.2.7 Bulk density

Soil bulk density was determined using the core ring method of Blake (1965). Undisturbed soil samples were collected from the top 15 cm depth using standard core rings. The samples in each core ring were weighed and then oven-dried for 24 hours at 105 °C to a constant weight. Bulk density was calculated by dividing the oven dry mass of the soil by the bulk volume of the undisturbed sample estimated from the volume of the steel rings.

3.2.3 Field experimental set up and treatments

The field experiments were designed to evaluate the effect of selected soil properties on pre-harvest aflatoxin contamination of groundnut under rain-fed conditions. To evaluate the effect of ambient temperature, observational data was used. The selected soil factors were exchangeable calcium, soil organic matter, soil moisture and temperature. Treatments to assess the effects of exchangeable calcium and soil organic matter comprised use of gypsum (15.6 % soluble calcium) and compost manure, respectively. Soil moisture and temperature were controlled indirectly using phased- planting (varying the planting dates from the on-set of the rainy season sometimes referred to as staggered plating), altering the planting spacing and cultivar selection. The aim of staggered planting dates and cultivar selection was to expose the crop to naturally different soil temperature, soil moisture and ambient temperature during the pod-development phase of growth. Similarly, planting different aflatoxin- susceptible cultivars ensured that the pod-development stage of each of cultivar occurred at different soil temperature, moisture and ambient temperature conditions.

As noted earlier, the pod-development stage of the crop is the most crucial stage for pre-harvest aflatoxin development. Except for the cultivar selection experiment, a medium maturing Virginia bunch type groundnut cultivar, MGV 4 (Table 1) was planted in all the field experiments. The chosen variety is among the most widely grown and most available commercial groundnut seed in Zambia. Each experiment was conducted for two successive cropping seasons. In addition to these purposely designed experiments, this study used observational weather data (ambient temperature) during the last 30 days of pod development to model its relationship with pre-harvest aflatoxin contamination.

3.2.3.1 Effect of soil temperature and soil moisture content on pre-harvest aflatoxin contamination in groundnut

As described above, soil moisture and temperature were controlled indirectly using phased planting, plant spacing (plant population density) and cultivar selection. These sub-experiments were conducted to evaluate the effect of soil moisture content and soil temperature during pod-development on pre-harvest aflatoxin contamination in groundnut grown under rain-fed conditions. The experiments were conducted at the University of Zambia, Field Research Station during the 2016/17 cropping season and repeated during the 2017/18 season.

Each experimental plot measured 16 m^2 with a 1 m border around each plot. Initial land preparation was done by ploughing to a depth of 20 cm using a tractor-mounted disc plough. Seed beds were prepared by levelling and breaking of clods using a hand hoe. Certified and treated groundnut seed was sown at a depth of 5 cm in flat seed beds. There were 5 plant rows per plot and all the data were collected from the three middle rows, making up the net plot. Crop management practices included regular weeding and application of pesticides to control pests, mostly the black legume aphid (*Aphis craccivora*). Soil moisture content and day-time soil temperature were measured during the last 30 days of pod development as described in the next sub-section.

In the phased planting date experiment, the aim was to expose the groundnut crop to different soil moisture and soil temperature conditions during pod development. Therefore, in this experiment, the effects of selected planting dates on pre-harvest aflatoxin incidence under rain-fed conditions were evaluated. Groundnut variety MGV 4 was planted successively at 5-10 day intervals from the on-set of the planting season in each of the two seasons. During the 2016/17 season, groundnut seed was sown on five different planting dates. During the 2017/18 season, the crop was planted on seven different planting dates. Groundnut seed was sown on dates when the soil was visibly moist for optimal germination. Treatments were laid out in a randomized complete block design (RCBD) and replicated four times.

In the experiment on plant population, the crop was planted at the recommended inter-row spacing of 75 cm and three intra-row plant spacing of 5, 10 (recommended

practice) and 15 cm were used to achieve three plant densities of 266,667, 133,333 and 66,667 plants per hectare, respectively. Thus, the effects of the three plant population densities on soil moisture content and soil temperature under the canopy were evaluated and then related to pre-harvest aflatoxin contamination in kernels. Treatments were laid out in a randomized complete block design (RCBD) and replicated four times.

In the experiment on cultivar selection, the aim was to expose the selected cultivars to the effects of different conditions of soil moisture and soil temperature under the canopy and subsequent pre-harvest aflatoxin contamination based on the canopy structures of each cultivar. Four aflatoxin- susceptible Virginia market type medium- kernelled cultivars with a bunch growth habit were evaluated. The evaluated cultivars were MGV 4, MGV 6, MGV 7, and Chishango (Table 1). These treatments were laid out in a Latin Square experimental design and replicated four times. Soil moisture content and day-time soil temperature were measured daily during the last 30 days of pod development to evaluate the effect of cultivars on the two parameters and subsequently the pre-harvest aflatoxin contamination risk in kernels.

Table 1: Characteristics of selected groundnut cultivars

Cultivar	Type	Growth habit	Seed size	Skin color	Days to maturity	Potential yield
MGV 4	Virginia	Bunch	Medium	Red	120-140	2.0 t/ha
MGV 6	Virginia	Bunch	Medium	Red	115-125	2.5-3 t/ha
MGV 7	Virginia	Bunch	Medium	Red	120-130	2.5-3 t/ha
Chishango	Virginia	Runner	Medium	Tan	120	2.0 t/ha

(Sources: www.ehinga.org; appsazambia.org)

3.2.3.1.1 Determination of soil temperature and moisture content

In-situ soil moisture content and temperature in the pod zone (top 0-5 cm soil depth) were measured daily in the last 30 days of pod development. Six randomly selected points under the crop canopy within the net plot were chosen as measurement points. Soil temperature was measured using a soil temperature probe (HI98331, Hanna Instruments, Kungsbacka, Sweden). Soil moisture content under the canopy was measured using the SM150 Soil Moisture Kit (SM150T, Delta-T Devices, Cambridge,

England). Soil temperature and moisture readings were averaged to represent an entire experimental unit. Although time of day was not considered for soil moisture, soil temperature was measured during the hottest part of the day between 12:00 and 3:00 pm.

3.2.3.2 Effect of soil organic matter on pre-harvest aflatoxin contamination in groundnut

The field experiment was conducted at Kasisi Agricultural Training Centre in Chongwe district. Treatments were 6 rates of composted cattle manure applied to each experimental plot before planting. The rates of application were equivalent to 0, 4.5, 12.0, 19.5, 27.0 and 34.5 metric tons/ha. Except for the zero (control) treatment, the applied rates were meant to increase the soil organic matter content from the inherent 0.7 % to equivalent values of 1.0, 1.5, 2.0, 2.5, and 3.0%, respectively. The manure was uniformly spread on the soil surface by hand and then incorporated into the top 10 cm depth using a hand hoe. Planting was done one week after amending soil with the manure. Treatments were replicated 6 times and laid out in a Latin Square design to control for the fertility gradient observed at the site. Each experimental plot measured 25 m^2 with a 1 m border around. Thus, the experiment covered an area of about 0.12 hectares.

The experiment was conducted for two successive cropping seasons, December 2015 to April 2016 and December 2016 to April 2017. The compost used in the study was prepared in compost heaps and consisted of cattle manure mixed with wheat straw arranged in windrows. These windrows were moistened when necessary and turned regularly until the materials were decomposed to stable compost. During the course of this experiment, soil respiration and plant available water were determined as key soil parameters with an effect on microbial activity, which in turn would have an effect on aflatoxin producing capacity of *A. flavus*.

3.2.3.2.1 Determination of soil respiration and plant-available-water

Soil respiration and plant-available-water (PAW) were determined at 90 days after planting (during pod-development stage) as once-off soil health indicators. To determine soil respiration, composite soil samples each weighing 2 kg per plot were

collected from 4 random sampling points in the top 10 cm of soil using a bucket soil auger. The samples were transported in air-tight plastic jars stored in a cooler box filled with ice between jars containing soil samples. To determine carbon evolution due to microbial respiration, the evolved carbon dioxide was trapped in 1 M KOH (Landa and Fang, 1978) and quantified by back titrating samples with 1 M HCl.

To determine PAW, three undisturbed soil samples per plot were collected from the top 10 cm depth of soil using standard core rings. The samples were then placed in the pressure plate apparatus to determine water content at field capacity (FC) and permanent wilting point (PWP). The samples were, therefore, subjected to pressures of -10 kPa and -1500 kPa for FC and PWP, respectively.

3.2.3.3 Effect of exchangeable calcium on pre-harvest aflatoxin contamination in groundnut

Two field experiments were set up in Chongwe and Lusaka districts to evaluate the effect of exchangeable calcium on aflatoxin contamination in groundnut. The experiments were conducted during the 2015/16 and 2016/17 growing seasons. In both experiments, a granulated commercial gypsum (15.6% soluble calcium) fertilizer was applied on the soil surface under the plant canopy at flowering stage.

In the first experiment, the effect of gypsum amendment on pre-harvest aflatoxin contamination in groundnut was evaluated. This experiment was conducted at the Agricultural Technology Demonstration Centre in Chongwe district. The soil was ploughed to a depth of about 20 cm using a tractor-mounted disc plough followed by harrowing to ensure a fine tilth. Gypsum was applied at five levels of 0, 100, 150, 200 and 300 kg/ha which is equivalent to 0, 15.6, 31.2 and 46.8 kg soluble calcium per hectare, respectively. The treatments were arranged in a randomised complete block design (RCBD) design with three replicates. The experimental plots measured 5 m x 5 m with 1 m border around. Groundnut variety MGV 4 was sown in all the experimental units at the recommended spacing of 75 by 10 cm, inter-row by intra-row spacing, respectively. Planting was done on 22nd December and 25th December 2015 and 2016, respectively.

In the second experiment, the effect of gypsum amendment on pre-harvest aflatoxin contamination in three aflatoxin-susceptible groundnut cultivars under acid and neutral soil conditions was evaluated. The study was conducted at the University of Zambia, Field Research Station in Lusaka district and at Kasisi Agricultural Training Centre in Chongwe district. The soil at UNZA was high in exchangeable calcium while that at KATC was deficient in exchangeable calcium. The aim of the experiment was partly to evaluate whether the initial calcium content had an effect on crop response to gypsum. It is important to note that the soil at KATC had similar characteristics with the soil at ATDC, the site for the initial experiment.

The treatments in the experiment were two rates of gypsum and three cultivars. Gypsum was applied at rates of 0 and 400 kg/ha at the flowering stage. The cultivars were: MGV 5, MGV 4 and Kadononga (Table 2). These treatments were laid out in Split-plot design with gypsum as the main plot factor and cultivar as the sub-plot factor, respectively. The time of application and the rate of 400 kg/ha of gypsum were based on the recommendation by Waliyar *et al*. (2013). Each treatment was replicated three times. The experimental plots measured 4 m by 4 m with a 1 m border around each plot. The field experiment was conducted from December 2016 to April 2017.

Table 2: Characteristics of selected groundnut cultivars for experiment two

Cultivar	Type	Growth habit	Seed size	Skin color	Days to maturity	Potential yield
MGV5	Virginia	Runner	Large	Tan	120-130	2.0 t/ha
MGV 4	Virginia	Bunch	Medium	Red	120-140	2.0 t/ha
Kadononga*	Spanish	Bunch	Small	Tan	90-100	1-1.5 t/ha

(Source: www.ehinga.org)

*Not a released groundnut cultivar

3.2.4 Harvesting, drying and shelling of pods

For all the experiments in the study, harvesting was done when pods were physiologically mature and dug out using a hand hoe. Pod yield was determined by counting and then weighing grain-filled pods from representative plants in each

experimental unit. The pods were then dried to 10 % gravimetric (w/w) moisture content in two steps. The first step was sun-drying the pods in a single layer on a polythene tent laid on a concrete floor for 24 hours. The pods were then transferred into in an electric convectional food drier (D-6450 Hanau, Heraeus Instruments, Germany) and dried continuously at 45 ºC for 3 to 4 days. All the pods from the net plot were hand-shelled and the kernels constituted the bulk sample from which laboratory samples were obtained.

3.2.5 Sampling and preparation of groundnut samples for aflatoxin analysis

The bulk sample per plot had an average weight of 0.8 kg and rarely exceeded 1 kg. To obtain samples for laboratory analysis , the bulk sample was placed in a basin and then sub-samples drawn by repeatedly scooping six 50 g sub-samples (300 g) of shelled kernels using a 120 mL plastic cup (1/2 standard cup). The bulk sample was shaken after every scoop to ensure homogeneity. A laboratory sample was constituted by aggregating the 50 g scoops from each bulk sample. Duly constituted laboratory samples were mixed by shaking them together and then grinding them into fine flour using an ordinary kitchen grinder (LM2211BM, Moulinex, China). Ground samples were homogenized by thorough shaking. The flour was passed through a 1 mm sieve before analysis for total aflatoxin content.

3.2.6 Procedure for aflatoxin analysis

Total aflatoxin content in samples was determined using the Neogen Afla Reveal® Q+ aflatoxin kit (Neogen Corporation, USA). For each treatment, three sub-samples each weighing 10.0 g were taken and analyzed for total aflatoxin content. Thirty milliliters (30 mL) of 65% ethanol obtained by diluting 95% ethanol (UN1170, Xilong Scientific Co., Shantou City, China) was added to the peanut powder. The mixture was shaken on a rotary shaker (ISO-9001-2000, Navyug, India) at 120 rpm for 3 minutes and the suspension then filtered through Whitman No. 42 filter paper. Five hundred microliters of the diluent buffer solution were added into a sample dilution cup using a standard 500 µL micro pipette and then thoroughly mixed with 100 µL of each sample extract using a clean, sterile micro pipette. One hundred microliters of the sample/diluent solution mixture was transferred into a measuring cup. One Afla Reveal® Q+ test strip was placed in the cup and allowed to react with the mixture for 6 minutes. After 6

minutes, the strip was placed into a strip holder of a computer tablet (K011, ASUS Corporation, USA). An aflatoxin reader (mReader) application installed on the tablet was used to measure the aflatoxin contamination of each sample. Aflatoxin standard solutions for groundnuts were used to calibrate that the aflatoxin reader. According to the App used, the lower and upper limits of detection of aflatoxin were 1 and 50 µg/kg, respectively and all the samples had aflatoxin content within detection limits.

3.2.7 Data management and analysis

Statistical analysis of the data was done using version 3.4.4 of the R-statistical software (R Core Team, 2017). The data were checked for outliers and observations with studentized residuals of magnitude |3| were classified as extreme outliers and were removed from the data set. The removed outliers were replaced by the group means of the particular treatment. After replacing the outliers, the data were tested for normality of distribution and the equality of variances as preliminary statistical tests preceding the analysis of variance (ANOVA) test that assumes normally-distributed data with equal variances. Normal Q-Q plots were used to assess normality in the distribution of the data. The central limit theorem was applied when data points were 30 and above. The Levene's test of homogeneity of variance was conducted to test for equality of variances. All the data sets in this study met the two assumptions for the ANOVA test. Statistical significance of all the tests was judged at 5% level of significance. In case of significant treatment effects, means were separated using the Tukey Test at 95% confidence interval.

The data that were generated from individual experiments in the study were pooled together in order to formulate regression models on the effects of measured soil and observational weather data during the last 30 days of pod development on pre-harvest aflatoxin contamination in groundnut. Data pooling was necessary due to small sizes of data sets obtained from individual experiments. Pearson's correlation analysis was performed to identify variables that significantly correlated with the output variable. Significantly correlated input variables were then analyzed through backward elimination multiple regression analysis. Both linear and logistic regression models were formulated to predict pre-harvest aflatoxin contamination in kernels for given soil

and weather conditions. Model validation was done by applying two cross-validation methods, namely, the leave-one-out cross-validation and the k-fold cross-validation.

CHAPTER FOUR

4 RESULTS AND DISCUSSION

4.1 Soil characteristics at experimental sites

Soils at the three experimental sites were generally suitable for groundnut production (Table 3). Nonetheless, soils at KATC and ATDC were characterized by low pH coupled with deficiencies in calcium and phosphorus. These sites characterized by calcium deficiency were considered suitable to evaluate crop response to calcium in form of gypsum whereas the soil at KATC with low soil organic matter content was ideal for the organic matter amendment trial. In general, groundnuts require deep, well-drained sandy loam or loamy sand soil with pH ranging from 5.3 to 7.3 with no nutrient imbalances in the other nutrients (Singh and Oswalt, 1995; Okello *et al*., 2010; Jordan *et al*., 2014). In this study, no correctional measures to the observed soil constraints were taken so that the experiments could be conducted under the prevailing soil constraints experienced by small-holder farmers. Soil amendments were added only where they served as treatments in the study.

Table 3: Selected initial soil physical and chemical properties

	Research site			
	UNZA	**KATC**	**ATDC**	**Critical level/ Ideal texture**
pH ($CaCl_2$)	6.92	4.22	4.25	5.3 to 7.3
Bulk density (g/cm^3)	1.47	1.47	1.40	1.40
Organic matter (%)	0.80	0.71	1.00	2.00
Total N (%)	0.12	0.06	0.90	0.20
Available P (mg/kg)	7.04	0.56	12.3	10.00
Exchangeable K (mg/kg)	249.6	42.90	0.16	90.00.
Exchangeable Ca (mg/kg)	738	12	4	100.00
USDA Textural Class	Sandy Loam	Sandy loam	Sandy loam	Sandy loam or loamy sand

(Sources: Singh and Oswalt, 1995; Okello *et al*., 2010; Jordan *et al*., 2014)

4.2 Effects of soil temperature and moisture during pod development on aflatoxin contamination in groundnut

Results from the study showed that soil temperature and moisture during pod development had significant effects on pre-harvest aflatoxin contamination in groundnut. This was regardless of the actual treatment applied to control soil moisture and temperature. Results from each of the three sub-experiments are presented under different sub-themes of the main experiment.

4.2.1 Effect of plant population density on pre-harvest aflatoxin contamination in groundnut

A higher plant population density was associated with lower pre-harvest aflatoxin contamination of kernels in both seasons. During the 2016 season, the total aflatoxin content in groundnut kernels for the treatment with 66,667 plants per hectare was 23 % and 38 % higher than it was in kernels for treatments with 133,333 and 266,667 plants per hectare, respectively (Table 4). Although, the total aflatoxin content tended to be lower in kernels from the treatment with 266,667 than with 133,333 plants per hectare, the difference was not statistically significant (Table 4). During the 2017 season, the mean total aflatoxin content in the kernels from treatments with 66,667 and 133,333 plants per hectare was respectively 1.54 and 1.56 times higher than aflatoxin content in kernels from 266,667 plants per hectare treatment (Table 4).

The results also showed that higher soil temperatures were associated with higher aflatoxin contamination while higher soil moisture content was associated with lower aflatoxin contamination (Table 4). This result is consistent with the findings of Chalwe *et al*. (2016) who observed lower aflatoxin contamination at higher soil moisture content during pod development. Pitt *et al.* (2013) showed that higher soil moisture content was linked to greater soil microbial activity, which tends to increase competition among soil fauna. This minimizes the dominant tendencies of *A. flavus* and *A. parasiticus* against other soil microorganisms.

Table 4: Effects of plant population density on soil temperature and soil moisture content and total aflatoxin content in groundnut kernels

Planting season	Population density (plants/ha)	Total aflatoxin (ppb)	Mean daily soil moisture content (%)	Mean daily soil temperature (ºC)
2016	66,667	15.5 a	8.1 a	23.6 a
	133,333	12.6 b	9.7 b	22.7 b
	266,667	11.2 b	11.5 c	21.8 c
2017	66,667	8.2 a	8.3 a	23.4 a
	133,337	8.3 a	8.3 a	23.5 a
	266,667	5.3 b	12.7 b	21.4 b

Treatments followed by the same letter in the same column did not show significant differences in aflatoxin content per season.

It is also noteworthy that higher soil temperatures were associated with lower plant population densities (wider plant spacing). Conversely, higher moisture content was associated with higher plant population densities (narrower plant spacing). At higher plant population density the crop canopy is wider, resulting in more shade under the canopy. Shading reduces the soil temperature under the plant canopy thereby minimizing water loss through evaporation. This is because plant exposure to direct sunlight is reduced.

4.2.2 Effect of phased-planting on pre-harvest aflatoxin contamination in groundnut

Planting groundnut seed in phases (different planting dates from the onset of the rainy season) did not show a consistent relationship with pre-harvest aflatoxin contamination in groundnut (Table 5). During the 2016 season, there were no significant differences in aflatoxin content attributable to the variation in planting dates. During the 2017 season, the total aflatoxin content decreased by 62 % between the fifth and seventh planting dates. The earlier planting dates were associated with higher aflatoxin contamination than the latter planting dates. This could be partly because of cooler soil temperatures associated with the latter planting dates. Generally, aflatoxin contamination in kernels was 59 % lower in the first season than in the second season (Table 5). The lower

aflatoxin level during the 2016 season was associated with higher moisture content during the last 30 days of pod development which was 2.3 times more than in 2017 season. Nevertheless, there was a strong negative correlation (r = -0.642) between total aflatoxin content in kernels and the soil moisture content. On the contrary, soil temperature did not significantly correlate with the total aflatoxin content in kernels.

Table 5: Total aflatoxin content associated with selected plating dates during a season

Year	Planting date	Date of harvesting	Total aflatoxin (ppb)
2016	17 Dec 2016	17 April 2017	3.9 a
	27 Dec 2016	27 April 2017	3.0 a
	02 Jan 2017	02 May 2017	3.5 a
	10 Jan 2017	10 May 2017	4.8 a
	15 Jan 2017	15 May 2017	3.4 a
2017	15 Dec 2017	15 April 2018	10.4 a
	20 Dec 2017	20 April 2018	9.2 a
	25 Dec 2017	25 April 2018	9.5 a
	29 Dec 2018	29 April 2018	10.6 a
	04 Jan 2018	04 May 2018	10.0 a
	12 Jan 2018	12 May 2018	7.9 b
	22 Jan 2018	23 May 2018	3.8 c

Treatments followed by the same letter in the same column did not show significant differences in aflatoxin content.

The use of phased planting to estimate aflatoxin contamination risk is based on the effect of crop exposure to end-of-season drought often associated with high temperatures and low soil moisture content. As documented by other authors (Hill *et al*., 1983; Craufurd *et al*., 2005), soil temperature and soil moisture content are among the most important soil parameters affecting the activity of *Aspergillus* fungi and their capacity to produce aflatoxins in the field. In this study, it was observed that aflatoxin contamination in kernels was a function of actual soil moisture and temperature regardless of the planting dates (Table 6).

One of the challenges of using duration of crop exposure to end-of-season drought stress to predict aflatoxin contamination risk in groundnut kernels is that drought stress is not always associated with high soil and ambient temperatures. In Zambia, both ambient and soil temperature show a decreasing trend during the growing season (figures 2 and 3), signifying the need to determine actual temperatures during pod development.

Table 6: Pre-harvest aflatoxin contamination and the associated soil moisture content and temperature during pod development

Year	Total aflatoxin (ppb)	Mean daily soil moisture content (%)	Mean daily soil temperature (ºC)
2016	3.9 a	17.9 a	23.3 a
	3.0 a	20.1 b	23.1 a
	3.5 a	21.1 b	24.4 b
	4.8 a	21.7 b	24.0 b
	3.4 a	18.8 a	22.9 a
2017	10.4 a	9.3 a	23.1 a
	9.2 a	9.8 b	22.9 a
	9.5 a	6.7 c	23.6 a
	10.6 a	9.7 b	23.6 a
	10.0 a	9.5 b	23.5 a
	7.9 b	7.9 d	23.3 a
	3.8 c	6.8 c	24.3 b

Treatments followed by the same letter in the same column did not show significant differences in the measured parameter.

4.2.3 Effect of cultivar selection on aflatoxin contamination in groundnut

There were no significant differences in aflatoxin contamination between different cultivars (Table 7). This result could be partly explained by the fact that none of the tested cultivars was resistant to aflatoxin contamination. Additionally, there were no significant differences in mean daily soil temperature and the daily soil moisture content associated with the tested cultivars in the last 30 days of pod development (Table 7).

Table 7: Effect of cultivar selection on pre-harvest aflatoxin contamination in groundnut

Year	Cultivar	Total aflatoxin (ppb)	Mean daily soil moisture content (%)	Mean daily soil temperature (ºC)
2016	MGV 4	3.5 a	16.2 a	22.2 a
	MGV 6	5.9 a	14.1 a	23.0 a
	MGV 7	3.9 a	16.6 a	22.2 a
	Chishango	4.4 a	15.5 a	21.6 a
2017	MGV 4	11.7 a	13.5 a	23.4 a
	MGV 6	13.0 a	12.8 a	24.4 a
	MGV 7	11.1 a	12.5 a	23.2 a
	Chishango	12.6 a	13.1 a	23.6 a

Treatments followed by the same letter in the same column and season did not show statistical differences.

As earlier noted, soil temperature and soil moisture content significantly influenced pre-harvest aflatoxin contamination. It is, therefore, expected that there should be no significant differences in aflatoxin incidence for equally aflatoxin- susceptible cultivars growing under similar soil temperature and moisture conditions. Further, it is important to also note that several other factors such as the presence of the causal fungus, the *A. flavus* can also affect aflatoxin incidence in the crop. Additionally, it is possible that the prevailing soil moisture and temperature conditions during the study were not suitable for the existing toxigenic fungal species to discriminate among cultivars. The soil temperatures recorded in the study were generally lower than those reported by other authors such Cole *et al*. (1985) who reported elevated aflatoxin levels as a function of temperature.

4.3 Effect of soil organic matter on pre-harvest aflatoxin contamination in groundnut

Pre-harvest aflatoxin contamination varied according to the rate of compost applied (Figure 5). High rates of compost were associated with lower aflatoxin contamination.

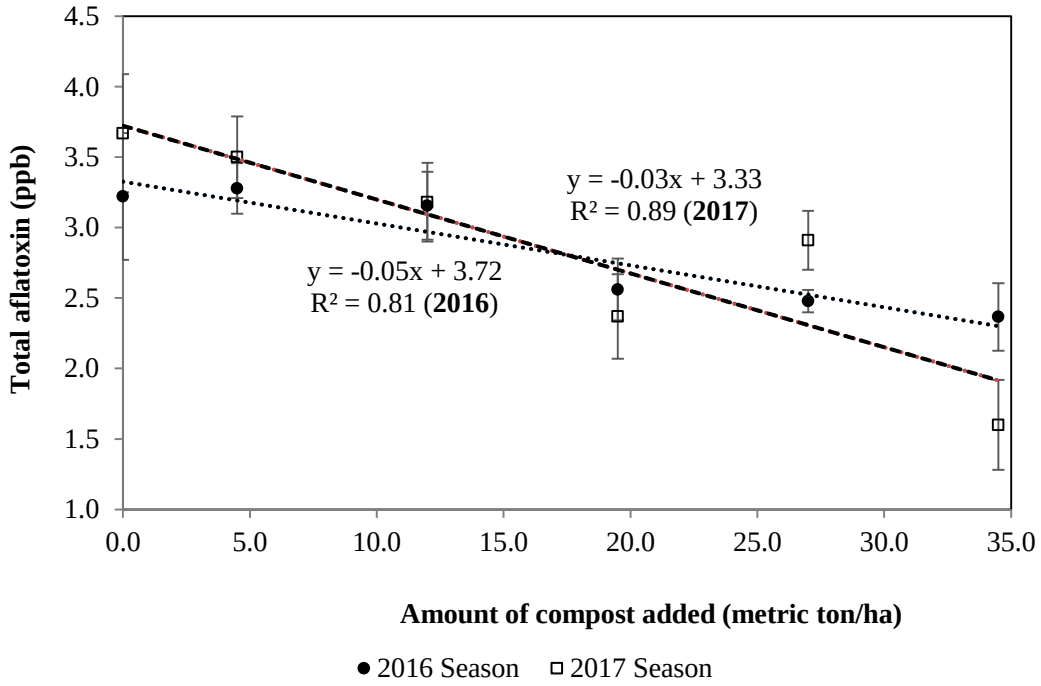

Figure 5: Effect of compost amendment on total aflatoxin contamination in kernels
Error bars represent standard error of the mean. The plotted values are means of 6 replicates.

This result can be attributed to the observed increments in plant-available-water (Figure 6) and soil microbial respiration (Figure 7) associated with higher rates of compost. Moisture availability in the soil during pod development in groundnut is not only critical in determining pod colonization by *A. flavus* (Blankenship *et al*., 1984) but also plays a critical role in the development of healthy kernels. According to Blankenship *et al.* (1984) higher pod colonization by *A. flavus* was associated with higher aflatoxin content in groundnut that were formed in poorly-developed or immature pods. This partly explains why moisture deficit during pod development is strongly associated with higher aflatoxin contamination (Hill *et al.*, 1983; Cole *et al.*, 1985; Pitt *et al*., 2014; Sibakwe *et al*., 2017).

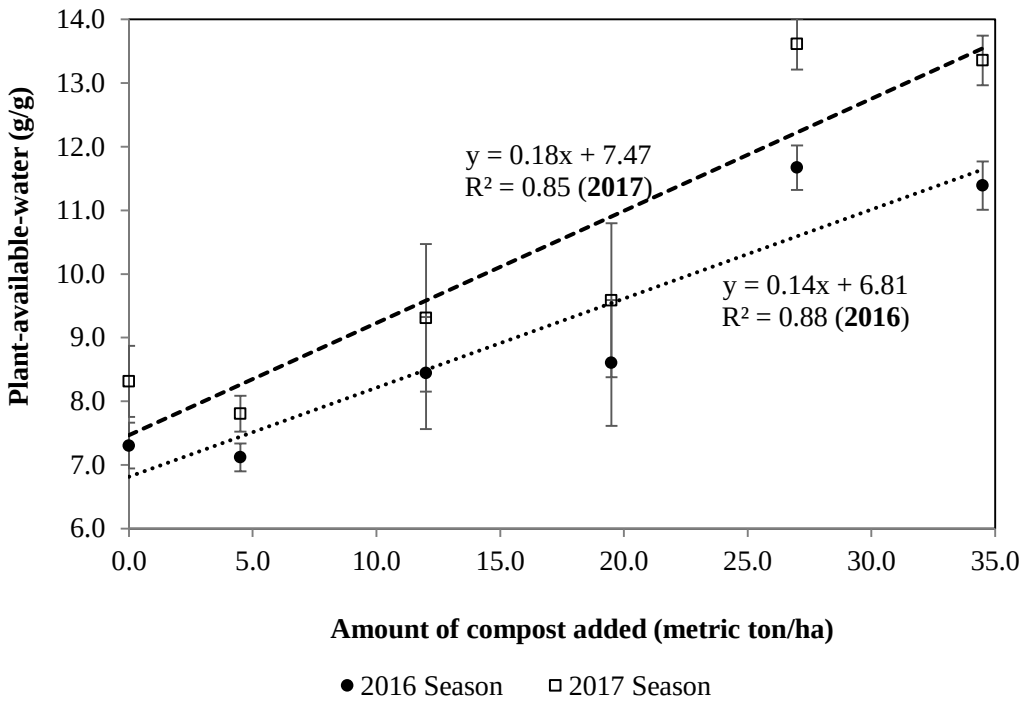

Figure 6: Effect of compost amendment on plant-available-water

Error bars represent standard error of the mean. The plotted values represent means of 6 replicates.

Although *A. flavus* and *A. parasiticus*, the two major toxigenic fungi are soil-borne and saprophytic (Richard and Payne, 2003), their capacity to produce aflatoxins is dependent on the soil moisture and temperature conditions (Cole *et al*., 1985). Being xerophytic in nature, *Aspergillus* become active and produce aflatoxins under severe moisture deficits often associated with elevated soil temperature (Bowen and Hagan, 2015). Adequate soil moisture is also important to minimize soil temperature, an equally important factor influencing pre-harvest aflatoxin contamination in groundnut (Hill *et al*., 1983; Dorner *et al*., 1989; Bowen and Hagan, 2015). As such, enhancing soil moisture retention capacity using organic amendments, which often keeps the soil temperatures low, seems to override the effect of the high presence of *A. flavus* in soils that are rich in organic matter on aflatoxin contamination (Zablotowicz *et al*., 2007).

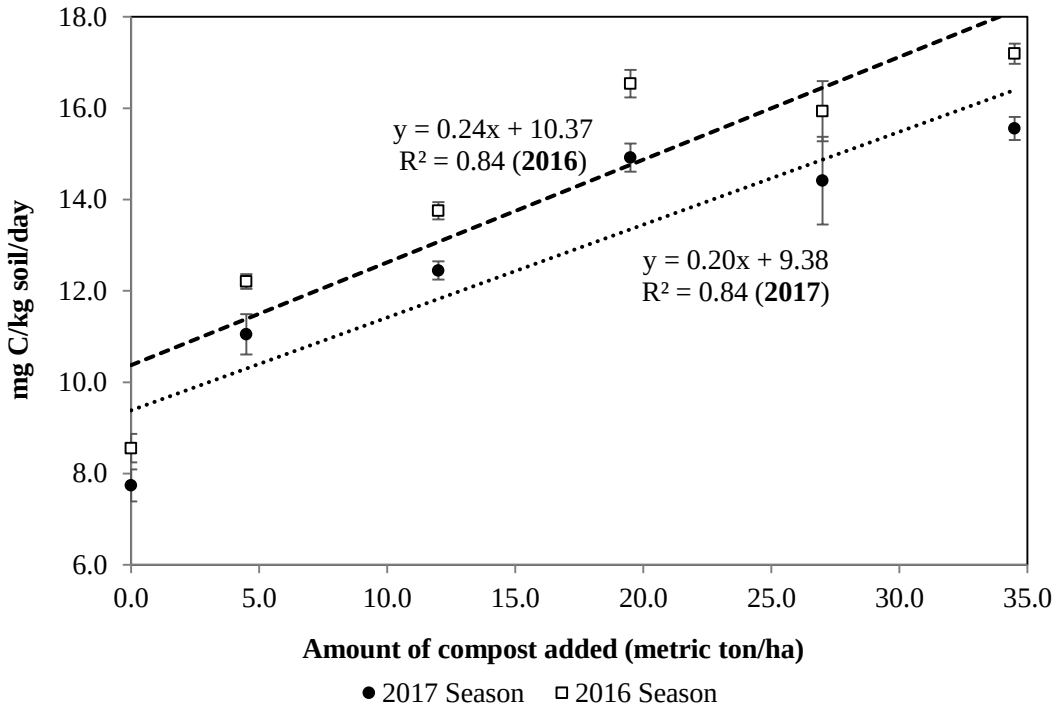

Figure 7: Effects of compost amendment on soil microbial respiration

Error bars represent standard error of the mean. The plotted values represent means of 6 replicates.

An increase in soil microbial respiration is indicative of improved microbial activity in the soil. Compost manure inoculates the soil with microorganisms and adds nutrients to the soil (Gaiottia *et al*., 2017). Adequate moisture and nutrients are essential for microbial activity. As reported by Cole *et al.* (1985) high microbial activity minimized pod colonization by *A. flavus*. These results are consisted with studies by Waliyar *et al.* (2013) who reported 42% reduction in total aflatoxin levels following an application of farmyard manure at the rate of 2.5 metric ton/ha.

It is noteworthy that although there were significant differences in aflatoxin contamination, the observed concentrations were comparatively low (<4 ppb) for the warm climatic conditions under which the experiment was conducted. Soil temperature data from a weather station 8 km southeast of the study site showed average soil temperature of 23.2 ºC during the last 6 weeks to harvesting of the groundnuts coupled with a fairly well-distributed average annual rainfall of 905 mm during the two growing seasons (SASSCAL Weather data, KKIA). The weather conditions were more favourable for plant growth and development than for pre-harvest aflatoxin

development and hence the low levels of aflatoxin observed across all treatments. According to Cole *et al.* (1995), agronomic practices that reduce plant stress and meant to maximize crop growth would in turn reduce mycotoxin occurrence. In both planting seasons, the field was free of weeds and diseases throughout the growing period and there were no physical signs of water stress such as wilting during the pod development stage of the crop.

Given that the aflatoxin contamination levels measured in this experiment were all very low (<4 ppb), no attempt was made to build regression models to predict pre-harvest aflatoxin contamination using soil organic matter content as an input variable.

4.4 Effect of exchangeable calcium on pre-harvest aflatoxin contamination in groundnut

There was no significant effect of gypsum (calcium input) on pre-harvest aflatoxin contamination of groundnut kernels during the two growing seasons (Table 8, Appendix 2). Total aflatoxin content at all the five levels of gypsum was low and ranged from 2.8 to 3.8 ppb. This narrow range of aflatoxin concentration is within the acceptable limit based on the Zambia Bureau of Standards (ZABS) maximum limit of 15 ppb in groundnut kernels. The narrowness of the range obtained could partly explain the absence of statistical differences in the data. The other possible explanation for lack of response to gypsum could be that the applied gypsum levels could not meet the calcium requirement for the groundnut cultivar tested in the study. The applied rates of gypsum were based on the recommendation of 200 kg gypsum per hectare on soil with less than 100 mg exchangeable calcium per kilogram of soil as documented by Cilliers, (nd[a]). Thus, considering the inherently low exchangeable calcium content of 12 mg/kg of soil at the study site, it was logical to expect a response to gypsum at the recommended rate of 200 kg gypsum/ha. Therefore, the results of this study suggest that soil calcium content was not an important determinant of aflatoxin contamination under the prevailing soil and weather conditions during the study.

[a]Publication not dated, but available online at http//:ww.arc.agric.za/arc-gci/Fact%20Sheets%20Library/Groundnut%20Production.pdf

Table 8: Effects of gypsum on total aflatoxin content in groundnut kernels

	Mean total aflatoxin concentration (ppb) in kernels	
	2015/16 Season	**2016/17 Season**
Gypsum (kg/ha)		
0	3.7 a	3.2 a
100	3.2 a	3.1 a
150	3.1 a	2.9 a
200	3.8 a	2.8 a
300	3.6 a	3.3 a

Treatments followed by the same letter in the same column did not show significant differences in total aflatoxin content.

4.4.1 Effect of gypsum on pre-harvest aflatoxin contamination in selected cultivars

Gypsum applied at the rate of 400 kg/ha had no significant effect on total aflatoxin content in kernels in the three cultivars that were evaluated (p-value = 0.61, Appendix 3). Nevertheless, there was a strong interaction between gypsum levels and soil type (Figure 8). Mean total aflatoxin content in groundnuts grown without an input of gypsum was higher in acid sandy soil at KATC than neutral sandy loam soil at UNZA. This result implies that groundnut grown in acid sandy soil may be more susceptible to aflatoxin contamination than when grown in a less acid soil conditions. This is partly because fungi are generally more tolerant to acid conditions than bacteria and therefore, dominate under acid soil. Thus, the growth of *Aspergillus* and subsequent colonization of pods would be higher under acid than neutral soil condition. According to Sibakwe *et al*. (2017), higher population of *Aspergillus* was associated with higher pre-harvest aflatoxin contamination of groundnut pods. Additionally, acid soils are inherently lower in exchangeable calcium content than less acid soils (Brady and Weil, 2010). Thus, a groundnut crop grown in acid soil is more likely to respond to calcium input than that grown in neutral soil.

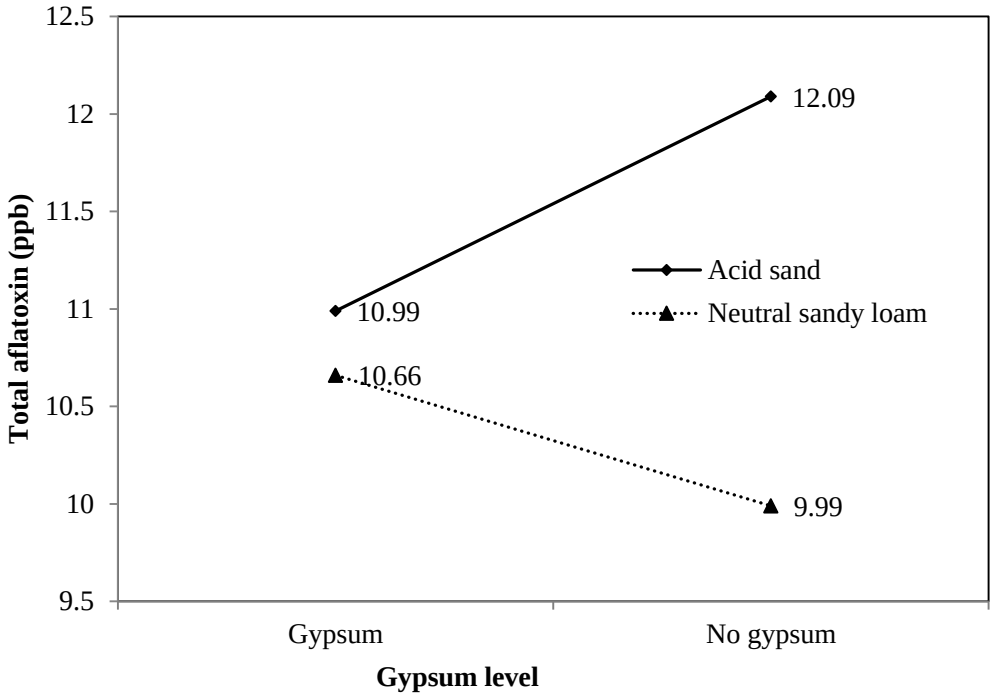

Figure 8: Interaction plot between gypsum level and soil type

According to Cox *et al.*, (1976), groundnut response to calcium inputs is dependent on soil characteristics such as the exchangeable calcium content. Recommendations must, therefore, consider soil type and in particular the native calcium content at which additional amounts would trigger a response. The poor response to gypsum observed in the current study suggests that the chosen rate of gypsum application may not be applicable to the soil types at the two sites. The choice of 400 kilograms per hectare in this field study was based on the recommendation by Waliyar *et al*, (2013).

4.4.2 Effect of cultivar on pre-harvest aflatoxin contamination in groundnut

The cultivars evaluated in this study (Kadononga, MGV 4 and MGV 5) were chosen according to their popularity among small-holder farmers and the ready availability of seed on the market. Groundnut varieties MGV 5 and MGV 4 were among the most common commercial cultivars while Kadononga was among the most common local landrace mostly preferred for its early maturity and 'tasty' kernels. All the three cultivars are susceptible to aflatoxin contamination and have a bunch type growth habit. MGV 5 and MGV 4 are virginia market types taking between 120-130 days to physiological maturity with potential yields of 2.5 to 3.0 metric ton/ha and 1.5 to 2.5 metric ton/ha, respectively. Kadononga is a Spanish type that takes between 90 -100 days to maturity with a yield potential of 1.0 to 1.5 metric ton/ha. In terms of seed size,

MGV 5 is large-seeded while MGV 4 is medium-seeded and Kadononga is a small-seeded cultivar.

Results from the study showed that there were significant differences ($p = 0.0025$, Appendix 3) in pre-harvest aflatoxin contamination of the three groundnut cultivars (Table 9). The aflatoxin content varied according to seed size whereby the larger the seed, the higher the corresponding total aflatoxin content measured. The overall mean total aflatoxin content across the two sites were 10.1, 10.8 and 12.0 ppb for Kadononga (small-seeded), MGV 4 (medium-seeded) and MGV 5 (large- seeded), respectively. The cultivars, Kadononga and MGV 4 had lower aflatoxin content than MGV 5. Mean total aflatoxin concentration in the cultivars at the two research sites followed a similar pattern. Although the aflatoxin contamination in all the tested cultivars was below the maximum permissible limit of 15 ppb according to ZABS safety standard, results from this experiment suggest that more effort is needed to manage aflatoxin contamination in larger-kernelled cultivars than in smaller ones.

Soil moisture status assessed in terms of mean daily rainfall received during the pod development stage did not show significant differences between sites. On the other hand, the mean soil temperature in the pod-zone during the last 30 days of pod-development was significantly lower than the temperature reported to be associated with elevated aflatoxin contamination. According to Hill *et al*. (1983), soil temperatures lower than 25 °C in the geocarposphere did not encourage aflatoxin contamination even under low soil moisture conditions. In addition, the difference in mean temperature between cultivars was minimal (0.8 °C), which may not be significant from the biological perspective. Therefore, the observed differences in aflatoxin contamination could not be attributed to rainfall and temperature in the pod-zone during pod development.

Therefore, results from the current study can be attributed to differences in plant nutrient and moisture requirements of each cultivar. For example, small-seeded groundnut cultivars have a lower calcium nutrient requirement (Jordan *et al.*, 2014) and most likely low soil moisture requirement than larger ones. Thus, if there is a limited supply of nutrients, especially exchangeable calcium and plant-available-water in the soil, it would be easier to meet the crop requirements for the small-seeded Kadononga

than the larger-seeded MGV 4 and MGV 5 that may need external inputs. Well-developed pods are less susceptible to fungal penetration and subsequent aflatoxin contamination than immature and weak pods (Waliyar *et al.*, 2013). This could explain the lower aflatoxin contamination in Kadononga than the other cultivars.

Table 9: Effect of cultivar on pre-harvest aflatoxin contamination in groundnut

Site	Cultivar	Total aflatoxin (ppb)	Mean soil temperature during pod development (ºC)	Mean daily rainfall during pod-development (mm)
UNZA	Kadononga	9.9 ± 0.4[a]	24.5 ± 0.1[a]	4.9 ± 1.3[a]
	MGV 4	9.9 ± 0.3[a]	24.1 ± 0.1[b]	3.2 ± 1.0[a]
	MGV 5	11.1 ± 0.5[b]	23.7 ± 0.1[c]	2.9 ± 0.8[a]
KATC	Kadononga	10.2 ± 0.6[a]	24.3 ± 0.1[a]	4.8 ± 1.4[a]
	MGV 4	11.6 ± 0.3[b]	24.0 ± 0.1[b]	2.6 ± 0.8[a]
	MGV 5	12.8 ± 0.9[b]	24.0 ± 0.1[b]	2.6 ± 0.8[a]

[a]Treatments followed by the same letter in the same column and site did not show significant differences according to Tukey test at $p \leq 0.05$. Results were entered as treatment means ± the standard error of the mean.

4.5 Modeling total aflatoxin content in groundnut kernels using ambient temperature, soil temperature and soil moisture content in the pod zone during pod development

4.5.1 Predicting aflatoxin contamination in groundnut using ambient temperature

Aflatoxin contamination in groundnut was greater for plants exposed to higher average maximum ambient temperatures during pod development than those exposed to lower temperature (Figure 9). Aflatoxin content in kernels was predicted according to the equation:

$$\boldsymbol{y = -73.42 + 3.15x} \qquad \textbf{Model 1}$$

where: y = Total aflatoxin content (ppb),

x = Mean maximum ambient temperature (°C) during pod development

According to the model, maximum ambient temperature explained 30. 6 % (R^2= 0.306) the observed variation in total aflatoxin content in groundnut kernels. Similar results were reported by Bowen and Hagan (2015) who reported a strong positive correlation between aflatoxin B_1 contamination in kernels and the maximum ambient temperature in the final 6 weeks of the growing season. This observation can be partly explained by a strong link between maximum ambient temperature and day-time soil temperature. This relationship could be because in the lower atmosphere, ambient temperature is a function of upward heat transfer from the soil surface to the air above it (Ahrens, 2009). Thus, higher soil temperatures are associated with elevated ambient temperatures. Similarly, Achar and Sanchez (2006) reported that the maximum growth of *A. flavus* and the subsequent formation of aflatoxins in freshly harvested mature undamaged groundnut pods occurred at ambient temperatures ranging from 27 to 30 °C, while fungal growth and aflatoxin contamination was limited at temperatures less than 10 and above 37 °C.

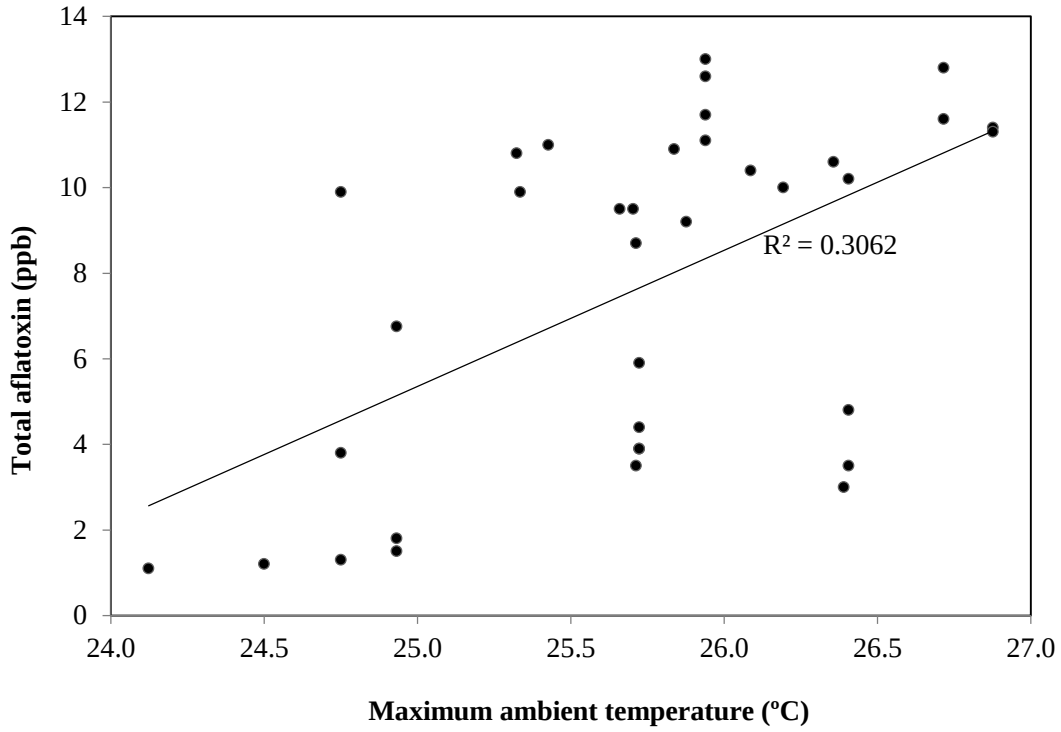

Figure 9: Total aflatoxin content as a function of maximum ambient temperature.

Ambient temperature data are relatively easy to access from most online weather data services. It is also one of the most frequently measured data parameters in almost all modern weather stations. In Zambia, a free weather data service is provided by the Southern African Science Service for Climate Change and Adaptive Land Management (SASSCAL) WeatherNet. This data service is accessible online at http://www.sasscalweathernet.org. Farmers and/or agricultural extension staff can use these data and determine the aflatoxin contamination risk in their region using the proposed model. High risk areas require appropriate agronomic interventions to minimize the risk of contamination.

4.5.2 Predicting aflatoxin contamination in groundnut using soil temperature

There was a significant positive linear relationship between pre-harvest aflatoxin contamination and soil temperature at 5 cm soil depth under the crop canopy during the last 30 days to physiological maturity (Figure 10). Total aflatoxin content was greater at higher mean daily soil temperature in the pod zone during pod development than at lower soil temperature.

Based on soil temperature, total aflatoxin content in harvested kernels could be predicted according to the model:

$$y = -32.83 + 1.76x \qquad \textbf{Model 2}$$

where: y = Total aflatoxin content (ppb)

x = Mean soil temperature (°C) at 5 cm depth under the crop canopy

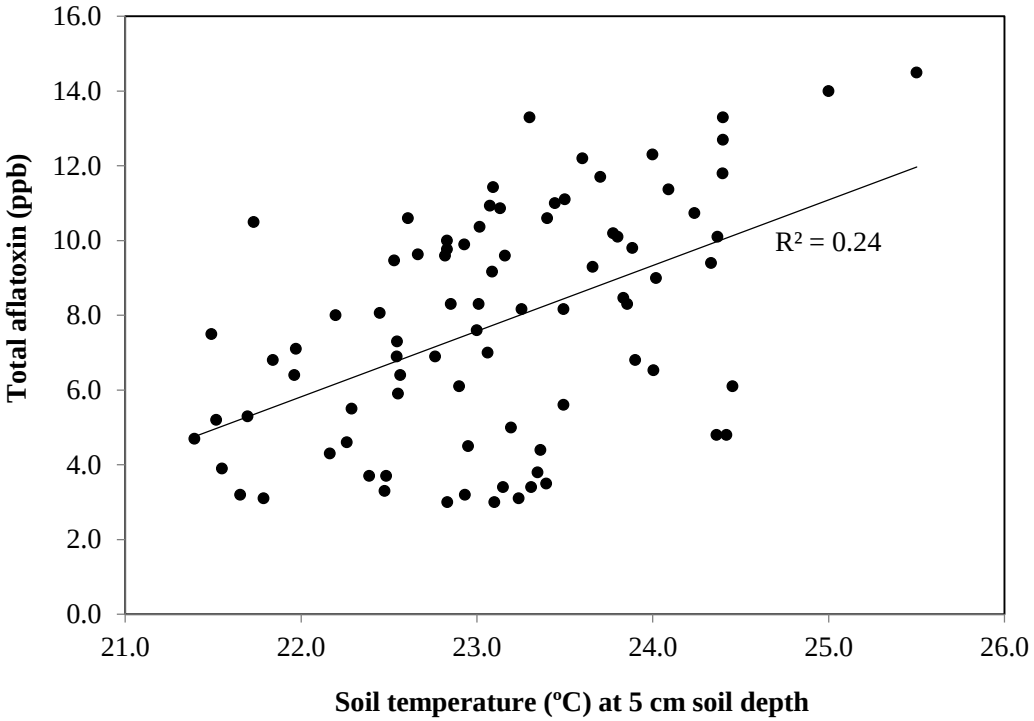

Figure 10: Total aflatoxin content in kernels as a function of day-time soil temperature at 5 cm depth under the groundnut crop canopy

In the model presented above, soil temperature accounted for 24 % (R^2 = 0.24) of the observed variation in total aflatoxin content in groundnut kernels. According to Blankenship *et al*. (1984), the mean threshold geocarposphere temperature of 25.7 to 27 ºC during the last 60 days of the growing season was necessary for aflatoxin development. In the same study, there was 95.7 % colonization of undamaged mature kernels by *A. flavus* due to heat treatment raising the mean soil temperature to 30.5 ºC. This was proportionate to aflatoxin content in harvested kernels, which was higher with greater colonization of pods. Although Blankenship *et al*. (1984) suggested non-existence of aflatoxin contamination in treatments with geocarposphere temperatures of 23.6 ºC or less, a study by Chauhan *et al*. (2010) reported that the formation of

aflatoxins also occurred at soil temperatures ranging from 22 to 35 ºC under water deficit conditions. According to Chauhan *et al*. (2010), only soil temperatures below 22 ºC were assigned weight of zero when calculating the soil temperature function of the model.

In the current study, the pre-harvest aflatoxin contamination level of 4.7 ppb was recorded at low mean daily soil temperature of 21.4 ºC. This result could be attributed to other confounding crop growth factors such as soil moisture content. Another possible explanation is that there could have been days of higher than the recorded average soil temperature. Such periods of high temperature could have occasioned the pre-harvest aflatoxin contamination reported in this study. As observed in the current study, ignoring soil temperatures of less than 22 ºC could therefore, lead to an underestimation of the pre-harvest aflatoxin contamination risk.

4.5.3 Predicting aflatoxin contamination in groundnut using soil moisture content

Aflatoxin contamination in groundnut was higher at lower mean daily volumetric soil moisture content during the final 30 days of pod development (Figure 11). According to the fitted regression line (unstandardized $R^2 = 0.38$), moisture alone accounted for 38 % of the observed variations in aflatoxin contamination. The aflatoxin content in kernels followed the model:

$$\boldsymbol{y = 11.90 - 0.36x} \qquad \textbf{Model 3}$$

where: y = Total aflatoxin content (ppb),

x = Mean volumetric soil moisture content (%) in the top 5 cm soil layer under the crop canopy.

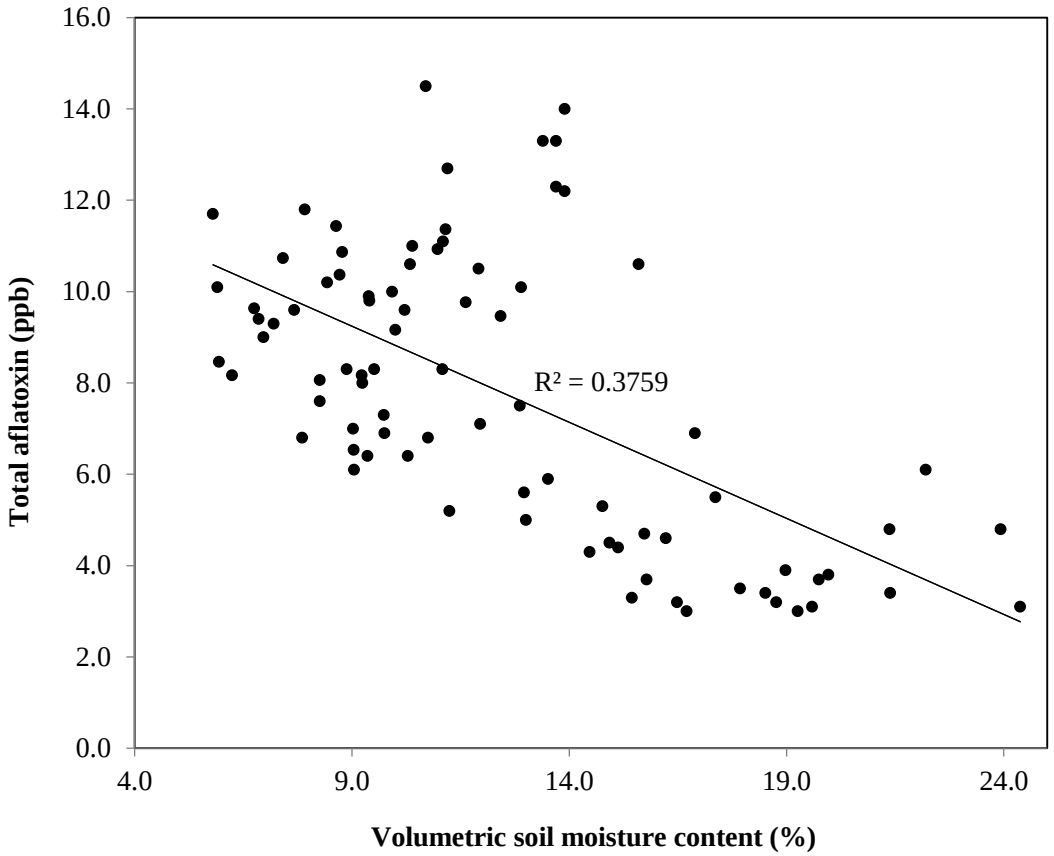

Figure 11: Total aflatoxin content in harvested kernels as a function of volumetric moisture content in the top 5 cm soil layer under the crop canopy during the last 30 days of pod development

Soil moisture content is an important variable influencing the colonization of pods by *A. flavus* and the subsequent aflatoxin contamination of kernels (Hill *et al*., 1983). Craufurd *et al*. (2005) reported a strong negative linear relationship between aflatoxin contamination and the average simulated fraction of extractable soil water (FESW) from flowering until harvesting of mature kernels. The FESW was calculated using simulated daily soil water content in the root zone during the last 40-25 days of the growing season. According to Dorner *et al*. (1989), groundnut kernels developing under low soil moisture content have reduced capacity to produce phytoalexin, an antimicrobial substance that inhibits fungal growth and thus offer resistance to the invasion of kernels by *A. flavus*. This observation implies that higher soil moisture content promotes resistance of pods to fungal colonization and subsequent aflatoxin contamination of kernels, suggesting that soil moisture conservation is an essential factor in minimizing pre-harvest aflatoxin contamination. For instance, results from the current study showed

that higher soil moisture content at higher planting density was associated with lower total aflatoxin content in kernels.

According to Pitt *et al*. (2013) drought-stressed plants show a reduced natural defence against infection due to reduced metabolism as plants wilt. Additionally, drought stress tends to reduce water activity (a_w; the ratio of the vapour pressure of soil to that of pure water) in the soil, resulting in reduced microbial activity while at the same time promoting the growth of the adapted organisms such as, *A. fl*avus and *A. parasiticus* (Dorner *et al*., 1989) . In a study involving aflatoxin-resistant groundnut cultivars, Hamidou *et al*. (2013) showed that moisture stress resulted in higher aflatoxin contamination, while drought tolerance was not always correlated with aflatoxin resistance.

4.5.4 Predicting aflatoxin contamination in groundnut using combined factors of soil moisture content, soil temperature and maximum ambient temperature

Using a multivariate linear regression model, soil moisture content, soil temperature and maximum ambient temperature were used as input variables to predict total aflatoxin contamination in groundnut. However, maximum ambient temperature was excluded from the model because it was judged to be a non-significant variable (p value = 0.09, Appendix 4) in the model. Nonetheless, maximum ambient temperature alone in the absence of soil temperature had a significant value (p value = 0.00, Appendix 6) to the model. Therefore, both combinations of volumetric soil moisture content with either soil temperature or maximum ambient temperature were evaluated in the study. Between soil and ambient temperature, soil temperature is directly linked to pre-harvest aflatoxin contamination. As earlier noted, ambient temperature is mostly a function of soil surface temperature (Ahrens, 2009).

Using volumetric soil moisture content and soil temperature to predict total aflatoxin content in groundnut kernels yielded the following regression model:

$$\boldsymbol{y = -21.03 - 0.38x_1 + 1.45x_2} \qquad \textbf{Model 4}$$

where: y = Total aflatoxin content (ppb),

x_1 = Mean volumetric soil moisture content (%) during pod development, and

x_2 = Mean soil temperature in the pod zone (°C) during pod development.

According to **Model 4**, combining volumetric soil moisture content and soil temperature together explained 54 % (Unstandardized R^2 = 0.54) of the variation in aflatoxin content.

Although soil temperature is related to soil moisture content, these two variables showed independence in the current study. This result could be because soil temperature is also a function of other factors such as soil surface cover by plants and also other soil properties such as color and texture (Brady and Weil, 2010). For example, the soil under shade can be very dry and yet record a low temperature.

As earlier noted in the simple linear regression models, soil moisture and soil temperature play important specific roles in the activity of *A. flavus* as individual factors. Based on partial correlations of r = -0.628 and r = 0.508 for soil moisture and soil temperature respectively, it was shown that each of the two factors had significant impact on aflatoxin content as individual parameters. This result implies that neither soil temperature nor soil moisture content alone could sufficiently account for the pre-harvest aflatoxin contamination in groundnut. Similar results were reported by other researchers. For instance, Craufurd *et al*. (2005) reported that soil moisture content in the pod zone could be used to predict *Aspergillus* infection and pre-harvest aflatoxin contamination in groundnut only when soil temperature was high (28-34 ºC). Similarly, Hill *et al*. (1983) reported elevated *Aspergillus* infection and subsequent pre-harvest aflatoxin contamination in kernels when high pod zone soil temperatures (28.4-29.6 ºC) were coupled with very low soil moisture (tension of 18 bars at 5 cm under plant rows) content. On the other hand, very low soil moisture content was not associated with higher aflatoxin content in groundnut kernels as long as the soil temperature was lower than 23.6 ºC (Blankenship *et al*., 1984).

In the second multivariate model, soil moisture content and the mean maximum ambient temperature were used as input variables to predict pre-harvest aflatoxin contamination.

According to the model, aflatoxin contamination could be predicted as follows:

$$\boldsymbol{y = -34.09 - 0.51x_1 + 1.88x_2} \qquad \textbf{Model 5}$$

where: y = Total aflatoxin content (ppb),

x_1 = Mean volumetric soil moisture content (%) during pod development, and

x_2 = Mean maximum ambient temperature (°C) during pod development.

The above regression model had an R^2 value of 0.46, implying that the model could explain 46 % of the variation in aflatoxin contamination. As noted above, this study showed that multivariate models produced better predictive ability than simple linear models **1**, **2** and **3** (Table 10)**.** In particular, combining volumetric soil moisture content and soil temperature in model 4 gave higher predictive ability (R^2 = 0.54) than all the other models.

Table 10: Comparison of the predictive ability of the fitted models

Model	**Predictor variable(s)**	**R^2**	**p-value (Regression ANOVA)**
1	Ambient temperature	0.31	0.01
2	Soil temperature	0.24	<0.001
3	Soil moisture	0.38	<0.001
4	**Soil temperature, soil moisture**	**0.54**	**< 0.001**
5	Ambient temperature, soil moisture	0.46	< 0.001

The current work showed notable similarities with published results such as those from Craufurd *et al*. 2005 (hereafter referred to as Craufurd's model), Chauhan *et al*. 2010 (hereafter referred to as Chauhan's model); Bowen and Hagan, 2015 (hereafter referred to as Bowen's model). One of the most important similarities is that all the models were essentially regression models using at least one of the following parameters; soil moisture content, soil temperature and ambient temperature as input data variables. For example, **Model 4** in the current study can be considered as a modification of Bowen's model. Notable similarities include the use of stepwise regression analysis as the applied statistical method and the inclusion of maximum ambient temperature as an

important input data variable. The cumulative 3-day-dry period in Bowen's model is equivalent to volumetric soil moisture content in the proposed model from this study.

Nonetheless, there were also significant differences in input data requirements especially with the other cited models. For instance, Craufurd's and Chauhan's models involved the use of computer simulations to generate certain model input parameters which required a wider data base to calculate. In Craufurd's model, the computer based CROPGRO peanut-growth simulation model was used to simulate the fraction of extractable soil water (FESW) in the last 25 days of pod development as an input variable in the regression model to predict aflatoxin content in groundnut kernels. Although the FESW was calculated using simulated daily soil water content and available water content, the overall CROPGRO model had a wider input data requirement as follows: basic crop data (flowering, podding and maturity dates), agronomic data (sowing date, soil water holding capacity and soil type) and weather data (daily rainfall, air and soil temperature, radiation and pan evaporation).

Similarly, the Agricultural Production Systems Simulator (APSIM) peanut module applied by Chauhan *et al*. (2010) heavily relied on computer simulation to calculate the aflatoxin risk index (ARI). The reliance on computer-simulated data may not be very practical for small-holder farmers in developing countries such as Zambia because this category of farmers may not have the required computer skills. The other downside is the non-availability of the input data as some of the data variables such as solar radiation and pan evaporation are not usually provided by most weather monitoring services. Therefore, similar to Bowen's model, the model from the current study has an advantage of using fewer and easily measurable parameters such as soil moisture content, soil temperature and ambient temperature.

In terms of predictive ability, **Model 4** (R^2 = 0.54) of the current study gave similar predictive ability to Craufurd's model (R^2 = 0.54) and Bowen's model (R^2 = 0.55). It is noteworthy that all the three existing models discussed in this thesis applied only at temperatures not very common in the wet and dry tropical conditions of Zambia. The models in the current study were not restricted to temperature conditions. The other major concern with application of the existing models is that their application is

climate-specific. There is, therefore, need to adapt them to the climatic conditions prevalent in the region of application.

Nonetheless, the application of all predictive models discussed in this thesis requires adherence to sound crop management practices. For instance, results presented in this study were based on groundnuts grown on soil with only minor constraints and under weed-free conditions and pest management using pesticides as essential crop management practices. Growing conditions other than these may negatively influence the accuracy of the proposed statistical models.

4.5.5 Application of logistic regression analysis to estimate the probability of having toxic pre-harvest aflatoxin contamination in groundnut

4.5.5.1 Estimating the probability of aflatoxin contamination at given volumetric soil moisture and temperature conditions

Given that all the aflatoxin levels measured in this study ranged from 3.0 - 14.5 ppb, which is within the allowable limit of 15 ppb according to the ZABS Standards, a critical limit of 10 ppb was adopted to demonstrate the application of logistic regression analysis to estimate the probability (π) of having aflatoxin contamination higher than this critical limit. Aflatoxin concentration of 10 ppb in groundnuts is the maximum limit for most African countries (FAO, 2004). Based on the critical limit of 10 ppb, the following logistic regression models were formulated as described below.

Let $y = 0$ if total aflatoxin content is less than the critical limit (< 10 ppb),
and $y = 1$ if total aflatoxin content exceeds 10 ppb,

x_1 = volumetric soil moisture content (%) in top 5 cm soil depth,

x_2 = soil temperature (°C) at 5 cm soil depth.

The general logistic regression model takes the form:

$$\boldsymbol{P(y = 1) = logit(\pi(x_1, x_2) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \varepsilon}$$ **Equation 1**

or, $$\boldsymbol{\pi(x_1, x_2) = \frac{e^{\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \varepsilon}}{1 + e^{\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \varepsilon}}}$$ **Equation 2**

where;

β_0 is the y-intercept (the probability of having total aflatoxin content higher than the critical limit when the volumetric moisture content and soil temperature equal to zero),

β_1 is the increase in the probability of having total aflatoxin content higher than the critical limit when the volumetric moisture content increases by 1 % while the soil temperature is kept constant,

β_2 is the increase in the probability of having total aflatoxin content higher than the critical limit when the mean soil temperature increases by 1°C while the soil volumetric moisture content is kept constant, and

ε the error terms are independent and normally distributed with mean equal to zero and variance equal to σ^2 : ($\varepsilon \sim N(0, \sigma^2)$.

From the model output in R (Appendix 7) the following logistic model was fitted:

$$\hat{\pi}(x_1, x_2) = \frac{e^{-28.69-0.12x_1+1.25x_2}}{1+e^{-28.69-0.12x_1+1.25x_2}} \qquad \textbf{Model 6}$$

4.5.5.2 Estimating the probability of toxic aflatoxin levels using soil temperature during pod development

Using soil temperature as a single predictor variable (x) yielded a model that takes the general form:

$$P(y = 1) = \pi(x) = \frac{e^{\beta_0+\beta_1 x+\varepsilon}}{1+e^{\beta_0+\beta_1 x+\varepsilon}} \qquad \textbf{Equation 3}$$

From **Equation 3** above and using the output from R (Appendix 7), the fitted model was:

$$\hat{\pi}(x) = \frac{e^{-30.42+1.27x}}{1+e^{-30.42+1.27x}} \qquad \textbf{Model 7}$$

Assuming a 50% probability of y = 1:

$\hat{\pi}(x) = 0.5$: $x = -\frac{\beta_0}{\beta_1} = \frac{30.42}{1.27}$ = **23.95 °C**.

It can be concluded that there would be a 50% chance that the total aflatoxin content in groundnut grown in soil with mean soil temperature of 23.95 °C during pod development would exceed 10 ppb.

4.5.5.3 Estimating the probability of toxic aflatoxin levels using soil moisture content during pod development

Using soil moisture content as a single predictor variable (x) yields a model that takes the general form:

$$P(y = 1) = \pi(x) = \frac{e^{\beta_0 + \beta_1 x + \varepsilon}}{1 + e^{\beta_0 + \beta_1 x + \varepsilon}}$$

Based on Equation 3 above and using the output from R (Appendix 8) the fitted model was:

$$\hat{\pi}(x) = \frac{e^{0.84 - 0.15x}}{1 + e^{0.84 - 0.15x}}$$

Assuming a 50% probability of y = 1:

$\hat{\pi}(x) = 0.5$: $x = -\frac{\beta_0}{\beta_1} = \frac{0.84}{0.15}$ = **5.6 %.**

Based on the above calculation, it can be concluded that there would be 50 % chance that the pre-harvest aflatoxin contamination in groundnut grown in soil with mean soil moisture content of 5.6 % during pod development would exceed 10 ppb.

The implication of these findings is that soil conditions with mean moisture content lower than 5.6 % and soil temperature above 23.95 °C during pod development, would result in more than 50 % chance of having groundnut kernels with total aflatoxin content exceeding 10 ppb. For example, using the fitted values of volumetric soil moisture content (5.6%) combined with soil temperature of 23.95 °C would result in 64 % chance of having total aflatoxin content exceeding 10 ppb (**Model 6**). Based on **Model 4** and using the fitted values of the two predictor variables, the predicted total aflatoxin content would be 11.6 ppb, which exceeds 10 ppb.

4.5.6 Application of cross-validation to estimate the predictive performance of the proposed model

Cross-validation procedures were conducted to assess whether the proposed model's predictive performance deteriorated substantially when applied to data that were **not** used in building the model. In this study, **Model 4** was formulated using the mean soil temperature and soil moisture content during the last 30 days of pod-development. The

model validation approaches discussed in this section were based on Kassambara (2018). The principle of cross validation is that a model is used to make predictions of a reserved small sample (subset) of the dataset used to build the model. The predicted values are compared with the true (observed) values by computing the prediction error.

One of the commonest measures of prediction error is the **M**ean **S**quared **E**rror (**MSE**). A model with good performance gives similar **MSE** values when applied to data that were not used in building the model, while a model with bad predictive performance gives very different MSE values when applied to other subsets. A variance of the **MSE** known as the **R**oot **M**ean **S**quare **E**rror of **P**rediction (**RMSEP**) measures the average difference between the observed values and the predicted values. The other commonly used model performance indicator is the prediction R-squared value **(R^2)** also known as the coefficient of determination. The **R^2** is a measure of the proportion of the variation in the predicted values of the outcome variable that can be explained by the model.

Two methods were applied and these were; the leave-one-out cross validation (LOOCV) and the k-fold cross validation. In both techniques, two model performance measures (metrics) were assessed and these were; **R^2** and **RMSEP**, which are calculated as follows:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2} \qquad \textbf{Equation 4}$$

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n}} \qquad \textbf{Equation 5}$$

where; y_i is the observed (true) i^{th} value of y,

$\hat{y}$ is the predicted (fitted) value of y,

$\bar{y}$ is mean value of y, and

n is the total number of data points in the dataset.

4.5.6.1 Leave one out cross validation-LOOCV

The LOOCV method can be explained in the following five major steps. In step one, the first observation was removed from the dataset and fit a model using the remaining (n-1) data points. The second step is to predict the removed data point and compute the prediction error (MSE) using the model fitted in step one. In step three, steps one and two were repeated on the remaining observations in the dataset. The fourth step was to

calculate the average prediction error by dividing the sum of all the computed MSEs for all data sets by the total number of observations. In the fifth step, a one sample t-test was performed to compute the mean difference in the MSEs computed in step 4, which should be equal to zero for a good model.

Results from this study showed that there were no significant differences in the mean values of the computed **RMSEPs** (p-value = 0.85) and $\mathbf{R^2}$ (p-value = 0.99) at 5% level of significance (Appendices 10 and 11, respectively). Further, results of an independent two sample t-test conducted to determine the mean difference between the observed and predicted total aflatoxin content at the given values of the predictor variables showed that there was no significant difference (p-value = 0.96) between the predicted and the observed data (Appendix 12). In addition, a plot of the predicted total aflatoxin content versus the observed total aflatoxin content (Figure 12) had significant correlation (r = 0.70, p-value < 0.01). These results showed consistency in the two model performance metrics, indicating good predictive performance of the model.

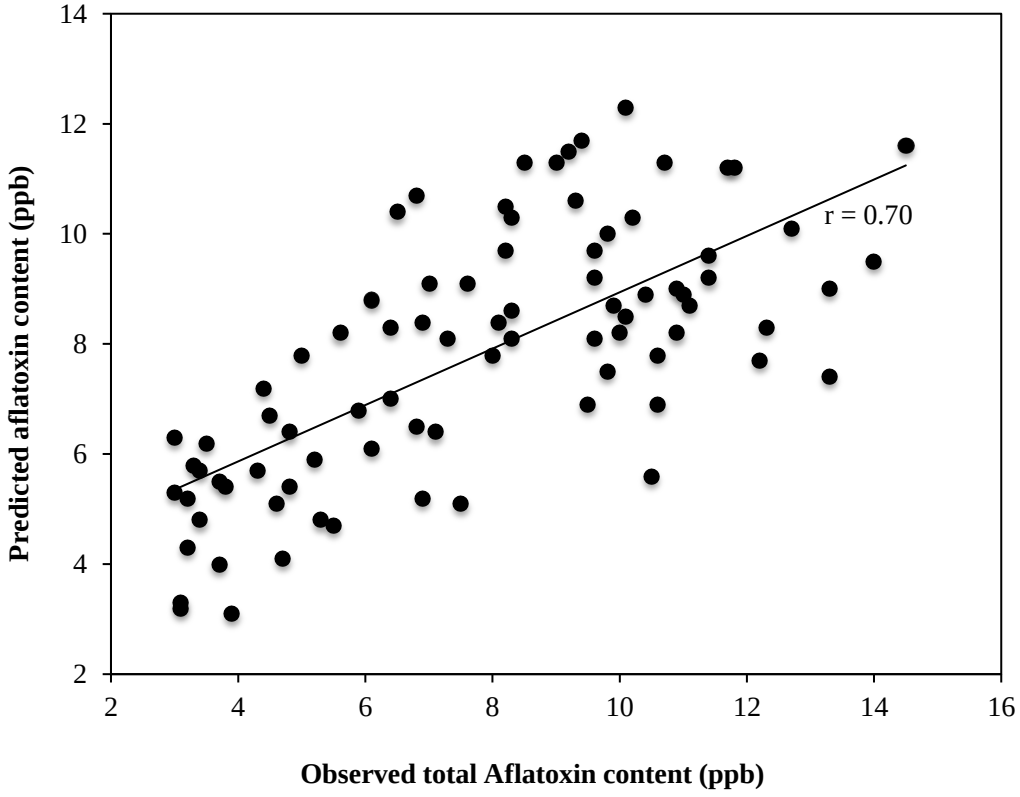

Figure 12: Plot of the predicted total aflatoxin content against the observed values

4.5.6.2 K-fold cross validation

The k-fold cross-validation method is based on a similar approach to the LOOCV. As with the LOOCV, the k-fold cross-validation method evaluates model performance using the average prediction error rate. The major difference is in the size of subsets (test sets) which is larger than the single-observation subsets in LOOCV. Thus, the procedure involves splitting a given dataset into k-subsets (k-fold). If k equals 5, then the method becomes a 5-fold cross-validation.

In this study, a 10-fold cross-validation test was conducted according to the following steps: The first step involved randomly splitting the dataset into 10 subsets. In the second step, models were formulated using 9 subsets and then tested them on one subset and computed the MSE for each model. Step two was repeated until each of the 10 subsets served as a test dataset. In the fourth step, the average MSE of the 10-recorded MSEs was determined. In the final step, a One Sample t-test was performed to assess if there were significant differences in the computed prediction errors.
Statistical results showed that there were no significant differences (p-value = 0.99) in the recorded **MSE**s (Figure 13 and Appendix 13). As with the LOOCV method, the consistency in the calculated prediction errors is an indication of good model prediction performance.

```
set.seed (17)
cv.error.10 = rep (0 ,10)
for (i in 1:10) {
glm.fit <- glm(Aflatoxin ~ poly(Moisture + Soiltemp ,i),data=data)
cv.error.10[i] <- cv.glm(data ,glm.fit ,K=10) $delta [1]
}
cv.error.10
```

```
##  [1] 6.973409 7.107324 6.854035 6.961964 6.795772 7.185018 6.972181
##  [8] 8.919627 8.790451 8.102459
```

Figure 13: Screen shot of the 10-fold cross-validation procedure and the computed MSEs

CHAPTER FIVE

5 CONCLUSIONS AND RECOMMENDATIONS

This study has showed that soil organic matter content, soil temperature, maximum ambient temperature and volumetric soil moisture content are important variables for managing aflatoxin contamination risk in groundnut. These variables can be used either singly or in combination to predict pre-harvest aflatoxin contamination risk in groundnut kernels.

In general, simple linear regression models had weaker predictive ability than multivariate models. From the simple linear regression models, it has been established that soil moisture was the single most important factor contributing to pre-harvest aflatoxin contamination in groundnuts. This, therefore, implies that soil moisture conservation during pod-development plays a significant role to minimizing pre-harvest aflatoxin levels in groundnuts. The other important factors during pod-development include maximum ambient temperature and soil temperature in that order of significance.

With multivariate analysis, this study has established that ambient temperature has a non-significant contribution to the model when soil temperature is included as an input variable. From the two possible multivariate models, the results showed that combining volumetric soil moisture content and soil temperature gave the highest predictive ability and explained 54 % of the variation in pre-harvest aflatoxin contamination of groundnut. Based on logistic regression models, agronomic practices such as supplemental irrigation during late-season-drought to maintain soil moisture content at >5.6 % and to keep the soil temperature below 24 ºC could reduce the risk of pre-harvest aflatoxin contamination to less than the 10 ppb limit considered safe in groundnut and groundnut products in most African countries.

Given that few relatively easy-to-collect data variables were used to predict pre-harvest aflatoxin contamination suggests a possibility of using these data to map the aflatoxin contamination risk associated with various geographical locations at country or regional levels. It is also very important to note that the application of the proposed models requires adherence to sound crop management practices. For instance, results presented

in this study were based on groundnut grown on a fertile soil under weed-free conditions and good pest management using pesticides as essential crop management practices. Growing conditions other than these may negatively influence the accuracy of the proposed statistical models.

Considering the overall mean total aflatoxin content of 7.8 ppb recorded in raw unsorted groundnut kernels from this study, it can also be concluded that the environmental conditions under which the current study was conducted do not pose a high risk of aflatoxin contamination. The high toxic aflatoxin contamination levels reported by other authors could, therefore, be due to severe environmental conditions during experimentation. Therefore, management of aflatoxin contamination in groundnut should consider appropriate agronomic interventions in the field as well as adherence to appropriate handling of mature pods during and after harvesting.

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5.2 Appendices

Appendix 1: Analysis of Variance (ANOVA) output table on the effect of compost manure on aflatoxin contamination in groundnut

```
Anova Table (Type II tests)

Response: Total.aflatoxin.(ppb)
               Sum Sq Df F value      Pr(>F)
Compost        20.9333  5  8.9489 0.000002276 ***
Season          1.2952  2  1.3842      0.2585
Compost:Season  3.8034  5  1.6259      0.1673
Residuals      27.6025 59
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1


Tukey's HSD
Alpha: 0.05

             Mean     G1 G2 G3
No compost     3.427778  A
1.0% Compost 3.380556  A
1.5% Compost 3.156944  A  B
2.0% Compost 2.684722  A  B  C
2.5% Compost 2.455556     B  C
3.0% Compost 1.975000        C
```

Appendix 2: Analysis of Variance (ANOVA) output table on the effect of gypsum on aflatoxin contamination in groundnut

```
Anova Table (Type II tests)

Response: Total.aflatoxin.(ppb)
               Sum Sq Df F value Pr(>F)
Gypsum          2.412  4  0.3245 0.8607
Season          4.109  1  2.2115 0.1414
Gypsum:Season   1.562  4  0.2101 0.9320
Residuals     131.923 71
```

Appendix 3: Analysis of variance (ANOVA) output table on the combined and interactive effects of gypsum, soil type and cultivar on aflatoxin contamination in groundnut

```
Anova Table (Type II tests)

Response: Total.aflatoxin.(ppb)
                          Sum Sq Df F value   Pr(>F)
Gypsum                     0.845  1  0.2510 0.618212
Variety                   43.498  2  6.4601 0.002878 **
Soil.type                 26.645  1  7.9144 0.006621 **
Gypsum:Variety             0.503  2  0.0748 0.928059
Gypsum:Soil.type          14.045  1  4.1718 0.045504 *
Variety:Soil.type          7.290  2  1.0827 0.345213
Gypsum:Variety:Soil.type   1.560  2  0.2317 0.793904
Residuals                202.000 60
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1


Tukey's HSD
Alpha: 0.05

          Mean  G1 G2
MGV 5     11.95833  A
MGV 4     10.77500  A  B
Kadononga 10.07500     B


Tukey's HSD
Alpha: 0.05

                   Mean      G1 G2
Acidic sand        11.54444  A
Neutral loamy clay 10.32778     B
```

Appendix 4: Linear regression model output on the combined effect of soil moisture, soil temperature and ambient temperature on pre-harvest aflatoxin contamination in groundnut

```
> summary(LinearModel.1)

Call:
lm(formula = Aflatoxin ~ Airtemp + Moisture + Soiltemp, data =
Aflatoxin)

Residuals:
    Min      1Q  Median      3Q     Max
-3.8967 -1.5149 -0.2644  1.3685  5.6103

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -39.4390    12.4706  -3.163  0.00221 **
Airtemp       0.9358     0.5438   1.721  0.08914 .
Moisture     -0.4317     0.0596  -7.243 2.42e-10 ***
Soiltemp      1.2362     0.2983   4.144 8.41e-05 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

s: 2.11 on 80 degrees of freedom
Multiple R-squared: 0.5534,
Adjusted R-squared: 0.5367
F-statistic: 33.05 on 3 and 80 DF,  p-value: 5.349e-14


> anova_reg(LinearModel.1)
Analysis of Variance Table
           Df Sum Sq Mean Sq F value    Pr(>F)
Regression  3 441.47 147.156   33.05 5.349e-14 ***
Residuals  80 356.20   4.453
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> vif(LinearModel.1)
 Airtemp Moisture Soiltemp
1.466992 1.349033 1.238804

> round(cov2cor(vcov(LinearModel.1)), 3) # Correlations of parameter
estimates
            (Intercept) Airtemp Moisture Soiltemp
(Intercept)       1.000  -0.859    0.314   -0.103
Airtemp          -0.859   1.000   -0.493   -0.419
Moisture          0.314  -0.493    1.000    0.321
Soiltemp         -0.103  -0.419    0.321    1.000

> PRESS(LinearModel.1)
[1] 387.1774

> R2pred(LinearModel.1)
[1] 0.5146143

> rmsep(LinearModel.1)
[1] 2.146918
```

Appendix 5: Linear regression model output on the combined effect of soil moisture and temperature on pre-harvest aflatoxin contamination in groundnut

```
> summary(LinearModel.2)

Call:
lm(formula = Aflatoxin ~ Moisture + Soiltemp, data = Aflatoxin)

Residuals:
    Min      1Q  Median      3Q     Max
-3.8847 -1.8138 -0.2419  1.5439  5.7224

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept) -21.01302    6.47010  -3.248  0.00169 **
Moisture     -0.38114    0.05249  -7.261  2.1e-10 ***
Soiltemp      1.45117    0.27415   5.293  1.0e-06 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

s: 2.135 on 81 degrees of freedom
Multiple R-squared: 0.5369,
Adjusted R-squared: 0.5255
F-statistic: 46.96 on 2 and 81 DF,  p-value: 2.879e-14


> anova_reg(LinearModel.2)
Analysis of Variance Table
           Df Sum Sq Mean Sq F value    Pr(>F)
Regression  2 428.28  214.14  46.957 2.879e-14 ***
Residuals  81 369.39    4.56
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> vif(LinearModel.2)
Moisture Soiltemp
1.021626 1.021626

> round(cov2cor(vcov(LinearModel.2)), 3) # Correlations of parameter
estimates
            (Intercept) Moisture Soiltemp
(Intercept)       1.000   -0.244   -0.994
Moisture         -0.244    1.000    0.145
Soiltemp         -0.994    0.145    1.000

> PRESS(LinearModel.2)
[1] 393.7945

> R2pred(LinearModel.2)
[1] 0.5063187

> rmsep(LinearModel.2)
[1] 2.165186
```

Appendix 6: Linear regression model output on the combined effect of soil moisture content and ambient temperature aflatoxin contamination in groundnut

```
> summary(LinearModel.3)

Call:
lm(formula = Aflatoxin ~ Airtemp + Moisture, data = AflatoxinDataset)

Residuals:
    Min      1Q  Median      3Q     Max
-3.9902 -1.5045 -0.4137  1.3343  6.5187

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept) -34.09428   13.58594  -2.510 0.014082 *
Airtemp       1.87949    0.54092   3.475 0.000824 ***
Moisture     -0.51102    0.06182  -8.266 2.24e-12 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

s: 2.311 on 81 degrees of freedom
Multiple R-squared: 0.4576,
Adjusted R-squared: 0.4442
F-statistic: 34.16 on 2 and 81 DF,  p-value: 1.741e-11


> anova_reg(LinearModel.2)
Analysis of Variance Table
           Df Sum Sq Mean Sq F value    Pr(>F)
Regression  2 364.99 182.496  34.164 1.741e-11 ***
Residuals  81 432.68   5.342
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> vif(LinearModel.2)
 Airtemp Moisture
1.209809 1.209809

> round(cov2cor(vcov(LinearModel.2)), 3) # Correlations of parameter
estimates
            (Intercept) Airtemp Moisture
(Intercept)       1.000  -0.998    0.369
Airtemp          -0.998   1.000   -0.416
Moisture          0.369  -0.416    1.000

> PRESS(LinearModel.2)
[1] 454.7727

> R2pred(LinearModel.2)
[1] 0.4298733

> rmsep(LinearModel.2)
[1] 2.326792
```

Appendix 7: Logistic regression model estimating the probability of having >10 ppb total aflatoxin content at known soil moisture content and soil temperature

```
glm(formula = Safety ~ Moisture + Soil temperature, family =
binomial(logit),
    data = Aflatoxin)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.5991  -0.7524  -0.4382   0.8007   2.4373

Coefficients:
             Estimate Std. Error z value Pr(>|z|)
(Intercept) -28.69143    9.00925  -3.185  0.00145 **
Moisture     -0.12162    0.06393  -1.902  0.05714 .
Soiltemp      1.25444    0.38425   3.265  0.00110 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

    Null deviance: 100.509  on 83  degrees of freedom
Residual deviance:  80.976  on 81  degrees of freedom
AIC: 86.976

Analysis of Deviance Table (Type III tests)

Response: Safety
         LR Chisq Df Pr(>Chisq)
Moisture   4.0621  1  0.0438548 *
Soiltemp  13.5720  1  0.0002296 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Appendix 8: Logistic regression model estimating the probability of having >10 ppb total aflatoxin content under set soil temperature

```
glm(formula = Safety ~ Soiltemp, family = binomial(logit),
    data = Aflatoxin)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.4498  -0.7826  -0.5247   0.9760   2.4410

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept) -30.4225     8.6509  -3.517 0.000437 ***
Soiltemp      1.2671     0.3693   3.431 0.000602 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

    Null deviance: 100.509  on 83  degrees of freedom
Residual deviance:  85.038  on 82  degrees of freedom
AIC: 89.038

Analysis of Deviance Table (Type III tests)

Response: Safety
         LR Chisq Df Pr(>Chisq)
Soiltemp   15.471  1 0.00008377 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Appendix 9: Logistic regression model estimating the probability of having > 10 ppb total aflatoxin content under given volumetric soil moisture content

```
glm(Safety ~ Moisture, family=binomial(logit), data=Aflatoxin)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.1624  -0.9313  -0.6246   1.2970   1.8341

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)   0.8410     0.7857   1.070   0.2844
Moisture     -0.1485     0.0664  -2.237   0.0253 *

Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 100.509  on 83  degrees of freedom
Residual deviance:  94.548  on 82  degrees of freedom
AIC: 98.548

Analysis of Deviance Table (Type III tests)

Response: Safety
         LR Chisq Df Pr(>Chisq)
Moisture   5.9614  1    0.01462 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Appendix 10: R results of the one sample t-test on mean difference in RMSEP values across models fitted using the LOOCV method

```
One Sample t-test

data:  CVDATA$RMSEP
t = 0.18534, df = 83, p-value = 0.8534
alternative hypothesis: true mean is not equal to 2.165
95 percent confidence interval:
 2.161524 2.169190
sample estimates:
     mean of x  std.dev. of x
    2.16535714     0.01766123
```

Appendix 11: R results of the one sample t-test on the mean difference in R^2 of prediction models fitted using the LOOCV method

```
One Sample t-test

data:  CVDATA$R2 PRED
t = 0.010482, df = 83, p-value = 0.9917
alternative hypothesis: true mean is not equal to 0.5063
95 percent confidence interval:
 0.5045023 0.5081167
sample estimates:
     mean of x  std.dev. of x
   0.506309524    0.008327594
```

Appendix 12: R results of the two sample t-test on the mean difference between the observed and predicted total aflatoxin contents for the given predictor variables

```
Two Sample t-test

data:  CVDATA$Obs and CVDATA$Fitted

t = -0.051058, df = 166, p-value = 0.9593

alternative hypothesis: true difference in means is not equal to 0

95 percent confidence interval:

 -0.8500478  0.8071907

sample estimates:

       mean of x        mean of y  pooled std.dev.

        7.780952         7.802381         2.719905
```

Appendix 13: Results of the one sample t-test on the mean difference in the MSE values across models fitted using the 10-fold cross-validation method

```
One Sample t-test

data:  Dataset$MSE
t = 0.0008609, df = 9, p-value = 0.9993
alternative hypothesis: true mean is not equal to 7.466
95 percent confidence interval:
 6.879992 8.052455
sample estimates:
     mean of x  std.dev. of x
     7.4662231      0.8194952
```

Appendix 14: Journal publications

Chalwe, H. M., Lungu, O. I., Mweetwa, A. M., Phiri, E., Yengwe, J., Njoroge, S. M. C., Brandenburg, R. L., and Jordan, D. 2019. **Predicting aflatoxin content in peanuts using ambient temperature, soil temperature and soil moisture content during pod development.** *African Journal of Plant Science,* 13(3):59-69.

Abstract

Higher than acceptable aflatoxin levels in peanut kernels (*Arachis hypogaea* L.) and related products is a worldwide food safety concern. Strict regulatory standards by major importers of peanuts limit the marketability of peanuts for many developing tropical countries including Zambia. The incidence of pre-harvest aflatoxins is strongly linked to soil and weather conditions during pod-development. This study aimed to formulate statistical models to predict total aflatoxin content in peanut kernels using selected environmental factors during pod development. Field experiments were conducted for two years during which the peanut crop was exposed to 84 combinations of ambient temperature, soil temperature and soil moisture content measured during in the last 30 days of pod development. These data were used to formulate regression models to predict total aflatoxin content in peanut kernels. Simple linear regression models had R^2 values of 0.30 for maximum ambient temperature, 0.24 for soil temperature and 0.38 for soil moisture content. Combining soil moisture content and soil temperature in a multivariate regression model could explain 54% of the variation in total aflatoxin content while a combination of soil moisture content and maximum ambient temperature could only explain 46% of the variation in total aflatoxin content.

Key words: Aflatoxin, groundnut, linear regression, statistical model, Zambia.

Chalwe, H. M., Lungu, O. I., Mweetwa, A. M., Phiri, E., Njoroge, S. M. C., Brandenburg, R. L., and Jordan, D. 2019. **Effects of compost manure on soil microbial respiration, plant-available-water, peanut (*Arachis hypogaea* L.) yield and pre-harvest aflatoxin contamination.** *Peanut Science*, 46(1): 42-49.

Abstract

Peanut production in Zambia is often characterized by low yields and high aflatoxin incidence in harvested kernels. Soil amendments such as farmyard manure have shown potential to increase yields and reduce pre-harvest aflatoxin incidence. The aim of the current study was to evaluate the effects of composted cattle manure on soil properties that relate to yield and pre-harvest aflatoxin contamination of peanut kernels. We evaluated the effects of composted cattle manure on soil respiration, plant-available water (PAW), peanut yield and pre-harvest aflatoxin contamination in a field experiment conducted in two successive rain-fed cropping seasons starting in December, 2015 and ending in April 2017, in Chongwe district, Zambia. Six (6) levels of compost were incorporated into the top 10 cm of the soil at rates of 0, 4.5, 12.0, 19.5, 27.0, and 34.5 metric tons/ha 1 wk before planting. Treatments were replicated 6 times and laid out in a Latin Square design. There was a strong positive correlation between levels of compost and soil microbial respiration (R^2=0.84) and PAW (R^2=0.86). Secondly, compost manure was associated with increases in pod (R^2=0.65) and kernel (R^2=0.61) yield. The kernel yield potential of the planted cultivar was achieved at the rate of 12 metric tons per hectare. Thirdly, there was a reduction in total aflatoxin levels with increasing levels of compost (R^2=0.85). The improvement in peanut yield and the decrease in aflatoxin concentrations in kernels can be attributed to the improvement in soil moisture retention capacity and soil microbial activity arising from manure amendments. This study demonstrated the potential of compost manure to increase soil microbial activity, PAW, peanut yield and minimize aflatoxin contamination at field level.

Keywords: *Aspergillus* spp, peanut, pod yield, soil health, Zambia

Chalwe, H. M., Lungu, O. I., Mweetwa, A. M., Phiri, E., Njoroge, S. M. C., Brandenburg, R. L., and Yengwe, J. 2020. **The effects of gypsum on pod-yield and pre-harvest aflatoxin contamination in selected peanut cultivars of Zambia.** *African Journal of Plant Science*, 14(3):134-138.

Abstract

Good agricultural practices are an effective means of minimizing pre-harvest aflatoxin contamination in peanuts. A field experiment was conducted to evaluate the effect of gypsum on pod yield and aflatoxin contamination in three peanut cultivars (Kadononga, MGV 4 and MGV 5) in Zambia. The experiment was conducted in Chongwe and Lusaka Districts. Gypsum (15.6 % calcium) was applied at rates of 0 and 400 kg/ha at flowering stage. Although gypsum had no significant effect on aflatoxin contamination, there were significant differences ($p = 0.009$) in cultivar susceptibility to aflatoxin contamination. The cultivar with the smallest kernels had 18.8 % lower aflatoxin content than the large-kernelled cultivar. Additionally, gypsum did not have a clear effect on pod yield. For instance, gypsum was associated with 44.8 % more grain-filled pods in Kadononga ($p = 0.005$) at the site in Lusaka, but this result did not apply to the other two cultivars. At the site in Chongwe, gypsum was associated with 34.6 % higher pod yield of MGV 5 only ($p = 0.006$). These results further suggest that plant factors such as kernel size may have an influence on natural resistance to aflatoxin contamination in peanuts.

Keywords: aflatoxin, gypsum, peanut cultivar, pod-yield, Zambia