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The Effects of Water Stress and Exchangeable Calcium on Pre-Harvest Aflatoxin Concentrations in Groundnut Kernels

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Abstract

Zambia is one of the African countries failing to export groundnuts to restricted markets because of high aflatoxin concentrations. Currently, the volume of groundnuts being exported has significantly reduced compared to the period between 1960 and 1970. Since aflatoxin formation starts in the field, aflatoxin mitigation measures must also begin at field-level. A greenhouse experiment was conducted to determine the interactive effects of water stress and exchangeable calcium on pre-harvest aflatoxin content in kernels. Four water moisture levels combined with four levels of gypsum application were applied from flowering to full maturity. The groundnuts were harvested at full maturity and the total biomass and aflatoxin content in kernels were determined. Treatment effects were determined by Analysis of Variance and the non-parametric Kruskal Wallis test at 95% Confidence Interval. Water stress resulted in about 10 folds increase in aflatoxin content at the least level of water supply ($p = 0.032$). There were no significant differences in the distribution of mean pre-harvest total aflatoxin concentrations due to gypsum application ($p = 0.274$). Similarly, there were no significant differences in the distribution of mean total biomass attributed to both water stress and gypsum application. These results suggest that adequate water supply can help to minimize pre-harvest aflatoxin incidences, while the time and the rates of gypsum applied as a source of calcium may also influence the aflatoxin response to the treatment.

Keywords: *Water Stress; Exchangeable Calcium; Harvest*

Introduction

Groundnut (*Arachis hypogaea L.*), which is also known as peanut, earthnut, monkeynut and goobers, is the second most important food crop in Zambia. It is an annual legume that is well-adapted to different agro-ecological regions. Groundnut kernels contain 40 - 50% oil, 20 - 50% protein and 10 - 20% carbohydrate and are rich in vitamin E, niacin, riboflavin, thiamine, falacin, calcium, phosphorus, magnesium, zinc, iron and potassium. Groundnut kernels are consumed directly as raw, roasted, or boiled kernels, or the oil extracted from the kernel is used as culinary oil. Oil pressings, seeds and the hulls of groundnut are used as animal feed, while the seed cake is used as industrial raw material and fertilizer. These multiple uses of the groundnut plant make it an excellent cash crop for domestic markets as well as foreign markets in both developing and developed countries. As a legume crop, it is an important nitrogen fixer in soils and thus contributes to the improvement of soil fertility [1].

Groundnut production in Zambia is constrained by different factors including weed control (67%), disease such as fungal infestations during dry periods (47%), labour intensity (26%), long dry periods leading to low yields and the limited access to markets due to high aflatoxin content (21%) [2,3]. Until a few decades ago, groundnuts were a major export crop and a foreign exchange earner for Zambia.

Between 1960 and 1970, Zambia, through the then Eastern Province Cooperative Marketing Union exported over 8000 metric tons of groundnuts to the United Kingdom. However, the export market has since collapsed due to concerns over toxic concentrations of aflatoxins [4].

Aflatoxins were discovered in 1960 when more than 100,000 young turkeys died in England over the course of a few months from an apparently new disease that was termed "Turkey-X disease." It was soon found that the mortality was not limited to turkeys but also ducklings and young pheasants. After some careful survey of the outbreaks, the disease was found to be associated with the Brazilian groundnut meal. Intensive studies on groundnut meal revealed its toxic nature as it produced typical symptoms of Turkey-X disease when consumed by poultry and ducklings. It was suggested that the toxin originated from the fungus *Aspergillus flavus*. It was then that the toxin was named "aflatoxin" by virtue of its origin from *A. flavus* (Guo., *et al*. 2008). This was the event that stimulated scientific interest and gave rise to modern mycotoxicology. Research on aflatoxins led to a "golden age" of mycotoxin research, during which several new mycotoxins were discovered. Other important mycotoxins produced by *Aspergillus, Fusarium and Penicillium* include ochratoxin, patulin and fumonisins. Among all mycotoxins and polyketide compounds synthesized by fungal species, aflatoxins (the most potent hepatotoxic and carcinogenic metabolites) continue to receive significant attention and are the most intensely studied (Guo., *et al*. 2008).

Human exposure to aflatoxins usually occurs by consuming contaminated food products such as grains and animal products. Numerous epidemiological studies have established the connection between aflatoxin consumption and the incidence of liver cancer in humans. Acute aflatoxin poisoning is rare, with about 25% of these cases being fatal, while chronic exposure rates are high, especially in developing countries [5].

Aflatoxin exposure can suppress the immune system of susceptible populations in humans, such as young children and HIV/AIDS patients. In a study in the Gambia, it was found that secretory immunoglobulin-A in saliva may be reduced by dietary levels of aflatoxin in children resulting in reduced levels of antibodies [6]. In Ghana, changes in the percentages of immune cell subsets following aflatoxin B_1 exposure reduced cell immunity, decreased human resistance to infections and reduced immune responses to vaccinations [7]. Aflatoxins can also cause oxidative stress, liver necrosis, hemorrhage and death in broiler chickens, pigs and cattle.

Developing countries have unusually high incidences of aflatoxicosis because of relaxed regulations and the prohibitive cost of aflatoxin testing and the non-availability of uncontaminated food sources [8]. As such, many countries have established legal limits for aflatoxin concentrations allowed in foods, such as groundnuts intended for both direct and indirect human consumption.

As in human beings, animals get exposed to aflatoxin through the ingestion of contaminated feed or pasture. The carcinogenic effects of aflatoxin in animals are well established and highly species-dependent. Some of the impact aflatoxin contamination in livestock and poultry include reduced production of milk and eggs, respectively [6].

In view of the foregoing, several Governments, through their agencies have formulated standards on aflatoxins. For instance, the threshold concentration of total aflatoxin in groundnuts intended for direct consumption according to different organizations is as follows: 4 µg/kg, 20 µg/kg 15 µg/kg, for the European Union (EU), the US Food and Drug Administration (FDA) and the World Health Organization (WHO), respectively.

In groundnuts, fungal infection and subsequent aflatoxin contamination have been strongly associated with plant exposure to heat and moisture stress during pod-formation (Waliyar., *et al*. 2013). Under severe and prolonged drought conditions the soil surface near the growing plants may be exposed to direct sunlight resulting in elevated temperatures in the geocarposphere. Water stress makes the kernels susceptible to fungal infestation due to cracking of pods of which the cracks became the entry point for the bacteria and fungi. Water is an important factor in avoiding the cracking of the pods, especially during pod-formation.

Soil calcium is another significant factor in the growth of groundnuts. The availability of exchangeable calcium contributes to the growth and development of groundnuts by making the kernels strong to resist fungal attacks and also minimizing pod rot and seed abortion. Several studies [9] have shown that soil calcium availability status plays a critical role in the development of pods and seed tissues. Thus, because of its role in ensuring proper pod development, low levels of exchangeable calcium have been associated with high rates of fungal infestation and subsequent aflatoxin contamination. This is primarily because poorly developed pods are easily infested by *Aspergillus flavus*. Chari., *et al*. [10] demonstrated that sufficient calcium fertilization could also enhance drought tolerance in groundnuts.

Problem statement

In Zambia, high levels of aflatoxin concentrations in groundnuts have compromised the safety of groundnuts for consumers. Additionally, the crop's access to international markets is limited, thereby leading to a loss of income to farmers and the country's potential foreign exchange income. There is, therefore, a need to evaluate interventions that have shown potential to reduce pre-harvest aflatoxin contamination, such as prudent soil water management and the application of exchangeable calcium.

Objectives

General Objective

The main aim of this study was to evaluate the interactive effects of water stress and exchangeable calcium on pre-harvest aflatoxin content in groundnuts.

Specific Objectives

The specific objectives included:

1. To evaluate the effect of water stress on groundnut yields and pre-harvest aflatoxin content.
2. To evaluate the effect of calcium levels on groundnut yields and pre-harvest aflatoxin content.

Hypotheses

1. Water stress can reduce yields and increase the incidence of pre-harvest aflatoxin in groundnut kernels
2. Low plant- exchangeable calcium can reduce yields and increase the prevalence of pre-harvest of aflatoxin in groundnuts.

Literature Review

The control of elevated pre-harvest aflatoxin incidences in groundnuts must take into consideration all the varied environmental and agronomic factors that influence pod and seed infection by the aflatoxin-producing fungi and the consequent formation of aflatoxins. These factors can fluctuate considerably from one location to another and between seasons in the same location. However, using good agricultural practices, including crop rotations, tillage, planting dates and management of irrigation and fertilization, can reduce aflatoxin concentrations in groundnuts [11].

Soil moisture status

While many factors are known to influence the production of mycotoxins in the field of these, drought stress during plant growth is among the most important. The fungi penetrate into the pods through small cracks that develop during pod maturation and drying (Robert., *et al.* 1971; Sanders., *et al.* 1984). Aflatoxin contamination increases under drought stress (Girdthai., *et al.* 2010) because of a decrease in the water activity that creates cracks in pod wall and allows the penetration of the *Aspergillus flavus*. Damaged pods are likely to contain more aflatoxin than pods with undamaged shells (Sudhakar., *et al*. 2007). In general, prolonged moisture deficit during pod formation enhances aflatoxin production [6,12-14].

Under prolonged drought conditions, groundnut genotypes, which maintained high kernel moisture, showed enhanced resistance and produced low aflatoxins (Cole., *et al*. 1993). Other findings demonstrated that the decrease of kernel water activity reduced phytoalexin production leading to increased aflatoxin contamination [15]. Although the relationship between seed infection percentage and aflatoxin production is not consistent (Sudhakar., *et al*. 2007), the above authors showed that aflatoxin production in kernels is mitigated when the soil maintained high relative water content which allows phytoalexin production. Phytoalexins are antimicrobial and antioxidative substances synthesized by plants that accumulate rapidly in areas of pathogen infection. Phytoalexins produced in plants act as toxin to the attacking microorganisms. Under drought conditions, phytoalexin production is inhibited by soil moisture deficits, which favors *A. flavus* growth [15]. Thus, drought is a predisposing factor for aflatoxin production in groundnut (Waliyar., *et al*. 2003).

Cole., *et al*. [12] suggested that even after kernels are infected by *Aspergillus flavus* or *Aspergillus parasiticus*, aflatoxin production does not occur in the kernel until the natural resistance mechanism of the plant is broke down as a result of water deficits and elevated soil temperature. For this reason, late-season irrigation is recommended to help combat drought stress, but this cultural practice seems to be impractical in some areas, especially in semi-arid and arid areas where water supplies are limited and may be uneconomical.

The effect of exchangeable calcium

Calcium plays an important role in cellular structural functions, regulating membrane permeability and strengthening cell walls [16]. In groundnut, the calcium requirement varies with the stage of pod-development and is high at the start of gynophore swelling. Deficiencies at this stage result in failure of the gynophore to expand into the pod [17]. An increase in the number of empty pods and decrease in the quality of seeds was reported in calcium-deficient soils [18]. Bledsoe., *et al*. [19] studied absorption of radioactive calcium by the groundnut fruit by growing a runner groundnut variety in sand culture with the root and fruiting zones separated from each other. This study revealed an enhanced absorption of labelled calcium by the gynophores.

Calcium is involved in regulating a myriad of plant functions, including cell elongation and division, membrane fluidity and permeability, ion fluxes, cellular pH, source-sink translocation of carbohydrates, N-metabolism, reproductive development, stress responses and apoptosis (Hepler 2005). Calcium (Ca^{2+}) deficiency has a noticeably deleterious effect on seed filling and significantly increased the incidence of pod rot and seed abortion, resulting in "pops" or empty fruits or fruits with severely underdeveloped seeds [16]. These increase the incidences high of aflatoxin concentrations in groundnuts

Calcium is reported to affect the water status and membrane permeability of groundnut leaves [10]. Under moisture stress conditions, the loss of water in leaves of groundnut plants treated with twice the recommended level of calcium was lower compared to leaves of plants grown in a calcium-deficient medium. The extent of membrane damage was also lower in leaves of plants fed with higher levels of calcium, compared to leaves of plants grown with no calcium supplementation [10]. Drought has also been reported to affect calcium uptake by peanut kernels.

As such, groundnut yield and quality is highly sensitive to calcium (Ca^{2+}) availability in the soil pegging zone either due to calcium (Ca^{2+}) deficiency or reduced uptake from the soil solution due to water deficit [20]. Calcium (Ca^{2+}) deficiency adversely affects seed viability and germination the following season (Wright., *et al*. 2009). Calcium cannot be repartitioned from older to younger tissues via the phloem route. Therefore, actively growing organs must utilize readily available xylem-mobile calcium (Ca^{2+}), thus posing an additional burden on the hypogeal groundnut fruit since it lacks significant transpirational pull.

Materials and Methods

Soil sampling and characterization

A greenhouse experiment was conducted on soil collected from the top 15 cm depth at the University of Zambia Farm located at $13^0$21.404'S and $028^0$27.419' E. Undisturbed soil samples were characterized for bulk density, while disturbed soil samples were characterized for soil reaction (pH), texture, soil organic matter, nitrogen, phosphorus, potassium, calcium and magnesium.

Bulk density

Soil bulk density was determined using the core ring method. 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 at 105°C to constant weight for about 24 hours. Bulk density was determined by dividing the oven-dry mass of the soil by the bulk volume of the undisturbed sample.

Determination of soil reaction

The reaction (pH) of the soil was determined by equilibrating 10 g of air-dried disturbed soil samples in 25 cm^3 distilled water. The pH value of the suspension was read using a pH meter (pH 3110, WTW 82362, Weilheim, Germany).

Determination of particle size distribution

The particle size distribution was determined using the hydrometer method. 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 the cylinder filled to the 1 dm^3 mark. The temperature of the suspension was measured using an alcohol thermometer with a temperature range of -20 to 100°C. A plunger was inserted and moved up and down to stir the suspension thoroughly. After 20 seconds a hydrometer was then lowered into the soil suspension and the density reading taken at 40 seconds to determine the silt and clay content. This was repeated three times to obtain an average reading. The suspension was then left for 2 hours and then the density reading was taken for the clay content. The percentage of clay, silt and sand were calculated as outlined in the Practical Manual for Soil Science by Van Ranst., *et al*. (1999). The soil textural class was determined by plotting the percentages of sand, silt and clay on the USDA Texture Triangle.

Determination of soil organic matter

Soil organic matter content was determined using the wet oxidation extraction method of Walkley and Black. 1gram of air-dried soil was completely oxidized in 10 ml of 1N $K_2Cr_2O_7$ in an acid medium containing 20 ml of concentrated H_2SO_4. The suspension was equilibrated for 30 minutes and then added 150 ml of distilled water and 10 ml concentrated H_3PO_4. The suspension was then titrated with $FeSO_4$ solution to a green endpoint using 10 ml diphenylamine indicator.

Determination of total nitrogen

One gram of air-dried soil was placed in a 500 cm^3 kjeldahl flask and digested using 10 cm^3 of concentrated sulphuric acid and 3 g of the catalyst mixture. After digestion, the mixture was allowed to cool and then diluted with 100 cm^3 of distilled water. 10 cm^3 of 10 M NaOH and was added to 10 cm^3 of the digested sample and the ammonia produced from the reaction was the trapped in 20 cm^3 of the H_3BO_3 indicator solution. The boric acid indicator mixture was then titrated with 0.05 M HCl to a pink endpoint.

Determination of available phosphorus

About 3 g of air-dried soil was equilibrated with 21 cm^3 of the Bray 1 solution for 1 minute and then filtered through Whitman 42 filter paper. 5 cm^3 of the filtrate was pipetted into a 25 cm^3 volumetric flask and diluted with 10 cm^3 of distilled water. 4 cm^3 of reagent B was added and filled up to the mark with distilled water. The mixture was allowed to stand for 15 minutes in order to develop color. Phosphorus content was determined using spectrophotometry at 882 nm.

Determination of exchangeable bases (K, Ca and Mg)

Ten grams of air-dried soil were equilibrated with 50 cm^3 of NH4OAc at pH 7.0 for 30 minutes. The suspension was filtered through Whitman 42 filter paper. The concentration of potassium was determined by Atomic Absorption Spectrophotometry. For calcium and magnesium, 5 ml of 500 mg/dm^3 strontium chloride and 15 ml of distilled water were mixed in a 25 ml plastic container and then their concentration was determined by Atomic Absorption Spectrophotometry.

Experimental design

The experiment was carried out as a two factorial design consisting of four gypsum application rates applied on four moisture levels. The experimental units were planting pots laid out in a completely randomized design. The selected four (4) moisture levels were 25%, 50%, 75% and 100% of the crop water requirement from flowering to full maturity were combined with four levels of gypsum application, these being 0, 100, 200 and 300 kg $CaSO_4.H_2O$/ha. This resulted in a total of 16 treatment combinations, which were replicated thrice resulting in 48 experimental units.

Planting and management

A pot experiment was set up in a greenhouse at the University of Zambia, Great East Road Campus, located at $15^0$23.642'S, $28^0$20.057'E. Each planting pot contained about 7.6 kg of soil and was planted with a Virginia type groundnut variety MGV 4 with a growth cycle of 120 to 140 days. One seed per pot was sown at a depth of about 5 cm. They were planted on the same date and watered with good quality harvested rainwater. At about 5 weeks of growth, the groundnuts showed signs of yellowing with necrotic leaf edges, a visual symptom of potassium deficiency. This was controlled by applying some Compound D fertilizer. The plants were attacked by black aphids and were controlled by an acaricide pesticide called boxer (active ingredient pryrethriod thyroid). On the 68^{th} day of growth, about 0.8 kg of dry soil was added to the pots as an earthing to the visible pegs.

The amount of water applied to each pot from flowering through to full maturity was estimated from the crop water requirements for groundnuts for the said phase of growth. Accordingly, 500 mm was used as an ideal amount for the calculations. This is because the rainfall requirement for the groundnuts from flowering to pod development is between 400 to 500 mm [21]. Thus, 125, 250, 375 and 500 mm were used for 25, 50, 75 and 100% of the crop water requirements, respectively. Gypsum applied in each pot was 0, 0.4, 0.8 and 1.2 $CaSO_4$ g/8.4 kg soil for 0, 100, 200 and 300 kg $CaSO_4$/ha respectively.

Harvesting and drying

The groundnuts were harvested at full maturity by uprooting plants from the pots. Total biomass was obtained by determining the fresh weight of whole plants. The pods were then stripped by hand and rapidly dried, first in the sun for two (2) days, then transferred into an oven set at 30^oC for 24 hours. The pods were shelled and the kernels were further dried to a constant weight in an oven set at the same temperature.

Determination of aflatoxin concentrations

Dried groundnut kernels were ground into a fine flour using an electric blender. A 2 g sample was weighed into a 50 ml plastic container to which 6 ml of 65% ethanol was then added. The mixture was put on the mechanical shaker for 1 minute and then filtered through Whitman No. 2 filter paper. 100 µl of the sample was mixed with 500 µl of diluent in the sample dilution cup. 100 µl of the solution was transferred into the measuring cup where an aflatoxin measuring strip (Neogen Afla Reveal Q+ test strip) was put standing upright. After allowing to stand for 5 minutes, the strip was then inserted into a strip holder, which was then mounted on an aflatoxin reader, a computer tablet installed with a mycotoxin reader application. The aflatoxin concentration was read within a minute.

Statistical analysis

Statistical tests on the collected data were determined using the Statistical Package for the Social Sciences (SPSS) version 20. Analysis of Variance (ANOVA) or the non-parametric Kruskal Wallis Test was used to determine the treatment effects on aflatoxin concentrations and total biomass yield at a 95% Confidence Interval. Multiple comparisons of means were made using Fisher's Protected Least Significant Difference.

Results and Discussion

Soil characterization

Results in table 1 below shows that the soil had no major challenges in the selected physical parameters, such as bulk density and texture as both parameters were within acceptable thresholds. However, there were major soil chemical fertility challenges due to high acidity, resulting in very low levels of plant-available calcium, magnesium, potassium and phosphorus. The soil organic matter content was equally low probably due to the sandy texture and low acidity that may affect decomposition of soil organic matter. The soil pH of 4.2 also presents major challenges with nodulation and nitrogen fixation in legumes. The additional challenge of phosphorus fixation is also likely and may explain the low available phosphorus in this soil. Liming of this soil could have improved its biological and chemical fertility, but no lime was added to avoid additional calcium from the lime.

Texture	pH	Organic matter (%)	N (%)	Available P (mg/ kg)	Ca (cmol+/kg)	Mg (cmol+/kg)	K (cmol+/kg)	Bulk density (g/cm^3)
SL	4.24	0.98	0.91	0.56	0.02	0.01	0.16	1.4

Table 1: *Selected physical and chemical soil characteristics.*

Effects of water stress on pre-harvest aflatoxin in groundnuts

As shown in figure 1 below, there were significant differences in the distribution of mean total pre-harvest aflatoxin levels due to water stress. Results indicate a reduction in pre-harvest aflatoxin levels with increasing water application ($p = 0.032$). The aflatoxin levels ranged from 2.2 to 30.1 ppb with mean values of 30.1, 23.9, 24.3 and 2.2 for 25, 50, 75 and 100% crop water requirements, respectively. Considering the threshold of 15 ppb (Zambia Bureau of Standards, ZS 723, 2008) for aflatoxin concentrations in peanut butter in Zambia, would mean only kernels produced at 100% moisture supply would meet the Zambian Standard.

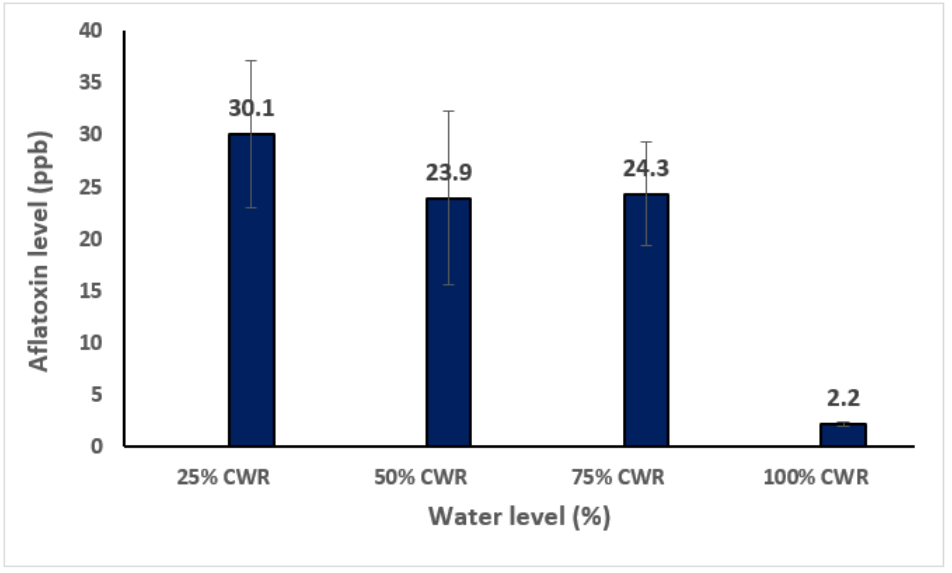

Figure 1: *Effects of water stress on pre-harvest aflatoxin content in groundnuts. Error bars represent standard errors. CRW is the crop water requirement in percentages.*

Drought stress influences the production of aflatoxins at field level mainly during pod-formation. Cole., *et al*. [12] suggested that even after kernels have been infected by fungi *A. flavus* or *A. parasiticus*, aflatoxin production in the kernel does not occur until the natural resistance mechanism is broken down due to water deficits and elevated soil temperature. The reason is that drought stress induces a great increase in proline in plants, which can enhance aflatoxin production [22]. Proline is a proteinogenic amino acid with exceptional conformational rigidity and is important for primary metabolism. Proline accumulation is a common metabolic response of higher plants to water deficits and salinity stress and allows plants to increase cellular osmoregulation during water deficits and make the plant vulnerable to fungal attacks.

At 25% of the crop water requirement, the groundnuts were stressed, making the kernels more vulnerable to fungal infection leading to high pre-harvest aflatoxin levels. 50% and 75% crop water requirement had a similar effect of increasing the pre-harvest aflatoxin contamination in groundnuts but less than that of 25%. This may suggest that there was insufficient soil moisture for plants to have a natural resistance mechanism against fungal infection. There was a 92.8% reduction in aflatoxin concentrations at 100% crop water requirement in comparison with 25%.Maintaining high kernel water activity until the time of harvest preserves the natural defense mechanism of phytoalexin production, which acts against the growth and formation of aflatoxins by aflatoxigenic fungi, even if fungal invasion occurs [15,23]. Adequate water can control or reduce aflatoxin production in groundnuts.

Effect of Calcium on pre-harvest aflatoxin in Groundnuts

From figure 2 below, there were no significant differences (P = 0.274) in the distribution of mean pre-harvest total aflatoxin levels attributed to gypsum application. Total aflatoxin concentrations ranged from 12.2 ppb to 31.3 ppb with mean values of 26.9 ppb, 12.2 ppb 31.2 ppb 19.6 ppb and for, 0 kg/ha, 100 kg/ha, 200 kg/ha and 300 kg/ha, respectively.

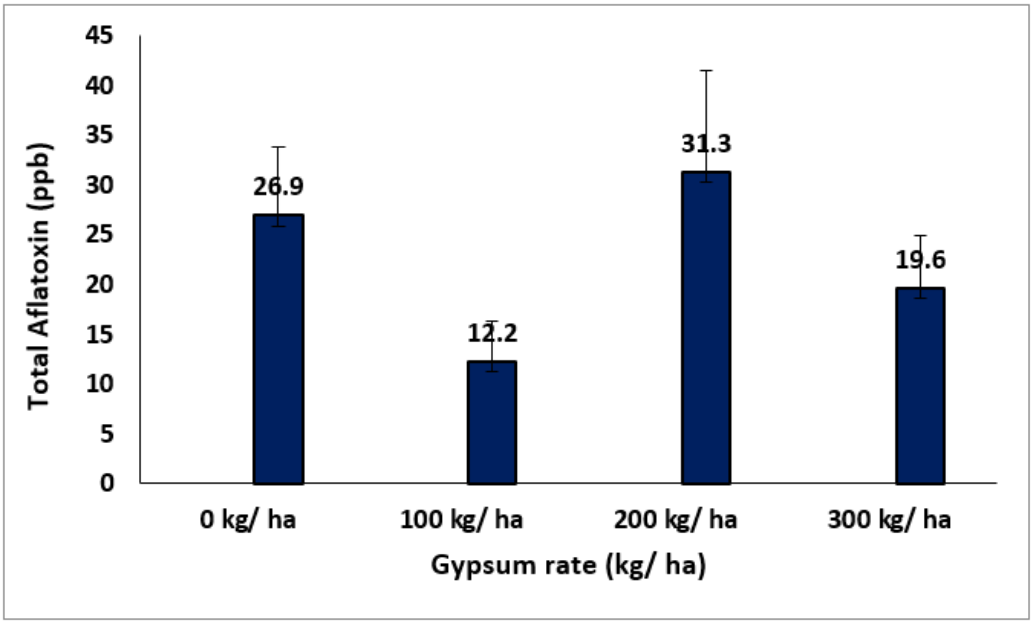

Figure 2: *Effects of calcium on pre-harvest aflatoxin content in groundnuts. Error bars represent standard errors.*

These results do not agree with most literature and this can be attributed to some factors such as the low rates and time of gypsum application. According to Guchi [24] soil amendments with gypsum, applied either singly or in various combinations at different cropping stages would contribute to reduction in the pre-harvest *A. flavus* infection and aflatoxin contamination in groundnut. Application of gypsum at the time of sowing was found to be most effective in reducing seed infection and aflatoxin contamination with a mean reduction of 80% compared to controls. In the current study, gypsum was applied at the onset of pegging. Early application of gypsum may allow for complete dissolution and availability for plant uptake.

Wilson and Walker [25] conducted an experiment with six peanut cultivars and four levels of gypsum: 0, 560, 1120 and 1680 kg/ha (0, 133, 265 and 398 kg/ha of supplemental soil calcium, respectively) applied at early bloom. They did not observe significant differences in aflatoxin concentrations among the six cultivars or among the gypsum treatments, but greater amounts of aflatoxins (130 µg/kg) were observed in the control treatments. In a greenhouse trial with three different levels of added soil calcium (0, 50 and 200 mg/kg), an inverse relationship was observed between soil calcium level and seed invasion by *A. flavus* [26]. There was reduction of 80% in aflatoxin levels in groundnut kernels from the 200 mg/kg calcium treatment compared to kernels from the 50 mg/kg soil calcium treatment. These two experiments suggested that soil calcium may play a role in peanuts for protecting against aflatoxin contamination.

Effects water stress on total biomass yield in groundnuts

As shown in table 2 below, there were no significant differences in the distribution of mean total biomass attributed to the applied water levels ($p = 0.882$). The total biomass yield ranged from 25 to 185g with means of 102.2, 102.9, 106.5 and 113.7 g for 25, 50, 75 and 100% of crop water requirements, respectively. Although these results do not show any particular trend in the distribution of total biomass of groundnuts relating to different water levels, the treatment with 100% crop water requirement had the highest mean total biomass of 113.7g. This shows that adequate water supply is essential for better yields.

Treatment	Mean total biomass per plant (g)	Standard Error
25% CWR	106.5	11.9
50% CWR	102.1	9.7
75% CWR	102.9	13.3
100% CWR	113.7	10.9

Table 2: *Effects of water stress on total biomass yield in groundnuts.*

Different studies have shown a positive relationship between rates of water supply and crop yield. For instance, Koksal., *et al*. [27] assessed the effects of different irrigation levels on groundnut crop yield and quality. Their findings showed that the highest irrigation level had highest total crop yield compared to the one that had the lowest irrigation level. Although water stress significantly increases the production of oleic acid, it reduces plant protein, linoleic acid and oil content, which affects the physiological growth of plants.

Results from the current study can be attributed to some external factors outside the scope of the study. These may include the acidic nature and low chemical soil fertility of the soils used for the study (Table 1).

Effects of gypsum on total biomass yield of groundnuts

Results from this study did not show significant differences in the distribution of mean total biomass of groundnuts due to gypsum application ($p = 0.836$). There was also no particular trend in the total biomass yield with respect to gypsum application rates. The mean total biomass per plant ranged from 96.8 to 111 g with means of 111, 108.6, 96.8 and 109.2 g for 0 kg/ha, 100 kg/ha, 200 kg/ha and 300 kg/ha, respectively. The gypsum application rate of 0 kg/ha had the highest total biomass of 111 kg while 200 kg/ha had the lowest total biomass.

Treatment	Mean total biomass per plant (g)	Standard. Error
0 kg Gypsum/ha	110.0	11.7
100 kg Gypsum/ha	108.6	13.0
200 kg Gypsum/ha	96.8	12.5
300 kg Gypsum/ha	109.2	8.9

Table 3: *Effects of gypsum on total biomass of groundnuts.*

These results are contrary to reports by other authors. Ying [28-31] evaluated the effect of applying different concentrations of calcium (Ca^{2+}) on vegetative growth, leaf chlorophyll content, photosynthetic rate and protective enzyme activities, as well as the yield and kernel quality of the cultivars under calcium (Ca^{2+}) stress.

Application of calcium increased pod and kernel yields. This is because of the increase in the number of pods per plant and of the number of kernels per pod. It also promotes the transformation of soluble sugar into fat and protein in groundnut kernel, increases the kernel fat and protein contents and improves the kernel quality under cadmium stress. The difference in results with the literature can be attributed the factors like not applying gypsum before planting in order for the calcium to be absorbed in good time for the groundnuts to use it.

Conclusion

The results of the study have shown that applying adequate water supply from flowering through to harvest can significantly reduce the incidence of pre-harvest aflatoxin contamination in groundnuts. Therefore water stress should be avoided to ensure good quality groundnut kernels. Gypsum application did not show any significance in reducing pre-harvest aflatoxin content in groundnuts when it was applied at pod- formation. Water stress and calcium did not show the effects of reducing the total biomass yield in groundnuts.

Recommendations

A similar experiment should be conducted in the near future focusing on the effects of calcium on pre-harvest aflatoxin in groundnuts, but this time applying the gypsum before planting the groundnuts and also considering the effects of temperature.

Appendices

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Total_Aflatoxin	Between Groups	4215.885	3	1405.295	3.246	.032
	Within Groups	16453.211	38	432.979		
	Total	20669.096	41			
Total_Biomass	Between Groups	1017.156	3	339.052	.220	.882
	Within Groups	64704.583	42	1540.585		
	Total	65721.739	45			

Appendix 1: *ANOVA Table showing the effect of water stress on pre-harvest aflatoxin content.*

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Total_Aflatoxin	Between Groups	2218.408	3	739.469	1.342	.274
	Within Groups	22038.361	40	550.959		
	Total	24256.770	43			
Total_Biomass	Between Groups	1311.891	3	437.297	.285	.836
	Within Groups	64409.848	42	1533.568		
	Total	65721.739	45			

Appendix 2: *ANOVA Table showing the effect of gypsum on pre-harvest aflatoxin content.*

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