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# The Role of Drought Stress on Aflatoxin Contamination in Groundnuts (*Arachis hypogea L.*) and *Aspergillus flavus* Population in the Soil

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**Abstract:** Aflatoxins are naturally occurring toxic chemical substances that are produced by fungal species called *Aspergillus flavus.* The toxic substances are secondary metabolites, which contaminate groundnut while growing in the field and also post-harvest. Drought stress is one of the factors that contribute to increased aflatoxin levels in groundnut during field production. This study was conducted in a screen house at ICRISAT-Chitedze Agricultural Research Station, Malawi to investigate the effects of drought on aflatoxin contamination and *A.flavus* population in the soil. Four drought stress levels; prolonged (4 weeks), min (3 weeks), mild (2 weeks) and no drought were imposed on five groundnut varieties at pod filling stage. Soil samples were collected from each plot four times; at planting, beginning of drought, end of drought and at harvest. Aflatoxin levels in groundnut grain samples were estimated by use of neogenstrips read with mobile assay tablet reader. Population densities of *A.flavus* in soil samples collected from the plots were estimated using serial dilutions plated on the selective media, modified dichloran Rose Bengal (MDRB) and quantify *A.flavus* within 3 days after incubation at 37°C.The results showed that there were significant differences in aflatoxin contamination between drought stress levels ($p = 0.011$). High aflatoxin contamination was observed under prolonged drought (22.0 ppb) compared to and no drought treatment (1.5 ppb). None of the varieties used in the study showed either resistance or susceptibility to aflatoxin contamination under drought or adequate soil moisture. The results also showed that there were significant differences in *A.flavus* population at drought period and harvesting time and the mean population of *A.flavus* in prolonged drought at end of stress and harvesting were 8511 and 6044 cfu/g of soil respectively. It was concluded that drought contribute to aflatoxin contamination in groundnut, and also increased the *A.flavus* population in soil and also at harvesting.

**Key words:** groundnut, aflatoxin, drought, *A. Flavus*, contamination, CFU

## 1. Introduction

Groundnut (*Arachishypogaea*) is one of the major important legumes grown in Malawi and the entire world. It is ranked 13th important food crop and also 4th important crop for oil production in the world [1]. Groundnut is used to improve human nutrition, soil fertility and economic status of many people [2]. One of major challenges that affect groundnut production in Malawi as well as other countries is aflatoxin contamination. Aflatoxin are substances produced by fungus *Aspergillus flavus* and *Aspergillus parasiticus* as secondary metabolites and are known with negative effect on human health as well on economic wellbeing of individuals and nations at large [3]. They are also known to be carcinogenic, toxic, and

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immunosuppressive and cause death to both human and animals [3]. The contamination of groundnut by aflatoxin occurs during pre-harvest (in field) and post-harvest. Agricultural products such as groundnut are being denied at international market due to high levels of the aflatoxin contamination beyond the acceptable standard level. The acceptable level of aflatoxin by (European Union) EU and Malawi bureau of standards (MBS) is 4 ppb while for World Health Organization (WHO) is 20 ppb [4, 5]. One of the contributing factors to the contamination in groundnut during pre-harvest is drought. Drought is a deficit in precipitation which creates a deficit in soil moisture [6]. In this study, four levels of drought were imposed to test their effects on aflatoxin contamination on five groundnut varieties and also on populations of *A.flavus*. The drought levels assessed were i) prolonged, ii) minimal, iii) mild and iv) No-drought and these were induced at the pod filling stage of the crop. There are several ways in which drought contribute to aflatoxin contamination. Drought condition increases the *A.flavus* population as groundnut roots and pods during drought produce more sucrose which is the growth substrate of *A.flavus* hence increasing the risk of contamination [7]. Drought condition is associated with poor pod filling and development, this result into shrived seeds with small seed size and usually they have small cracks which allow easy penetration of *A.flavus* [8, 9]. Drought stress increases susceptibility of plants to insects and diseases because it reduces the accumulation of phytoalexinsin plants hence increasing the risk of contamination [10, 11]. Much of the studies has been conducted on the effect of drought on aflatoxin contamination in groundnut. Drought stress can vary from mild to severe. However, no or little has been done to evaluate the effects of different drought levels on aflatoxin contamination and *A.flavus* population focusing at pod filling stage. Therefore, this study was conducted to quantify the aflatoxin contamination in the soil and groundnut at different drought durations at pod filling stage.

## 2. Materials and Methods

The experiment was conducted in a screenhouse from October 2015 to February 2016, at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Malawi, hosted at Chitedze Agricultural Research Station in Lilongwe district. The Agricultural Research Station lies at 1097 m above sea level 13°59'S latitude and 33°38'E longitude.

### *2.1 Treatments and Experimental Design*

The experiment had two factors which were drought at four levels and variety at five levels. The four levels of drought were imposed at pod filling. The four drought stress levels were; prolonged (4 weeks' drought), minimal (3 weeks' drought), mild (2 weeks' drought) and no drought (control). Normal Watering resumed in all treatment after the end of each drought period up to physiological maturity. The varieties included five Spanish varieties commonly grown in Malawi were JL24 (*Kakoma*) [1], ICGV-SM99568 (*Chitala*), ICG 12991 (*Baka*), ICGV-SM99566 and ICGV-SM01514. These were selected because they are widely grown by most farmers in Malawi and also ICGV-SM99566 and ICGV-SM01514 are newly released varieties in Malawi.

The experiment was laid in 4×5 split plot block design replicated three times which means that there were 60 subplots. Replicates also acted as blocks. The drought acted as main plot while varieties acted as subplots. The main plot was 2 m long and 0.6 m wide, (1.2 $m^2$) and sub plot was 0.6 m long and 0.4m wide (0.24 $m^2$). The whole subplot also acted as net plot where by the plants harvested from it were used for measurement of yield, aflatoxin level and seed size. Soil samples were collected four times at a depth of 0 to 10 cm and 3 sub-samples per sub plot; planting (zero days after planting (0 DAP), beginning of stress (58

[1]All the variety names in blankets and italics are local names of the varieties and are named after the places where they perform best.

DAP), end of stress (86 DAP) and harvesting (111 DAP). Timber boxes were used as planting containers. They were filled with soils sourced from fields around Chitedze Research Station not previously used for growing groundnuts. Soil samples collected before planting were analyzed for nutrient status and *A.flavus* population while subsequent samples were analyzed for *A flavu* sonly.

*2.2 Estimation of Populations of A.flavus*

The populations of *A.flavus* in soil were estimated by enumerating (through plating) on selective medium called Modified Dichloran Rose Bengal (MDRB) as described by Horn B. and Dorner J. (1998) [12]. 3.3 g of soil was suspended in 9 ml water agar (0.2% agar) and vortexed. Serial diluted up to $10^{-5}$ and then plated on 90 mm diameter Petri dishes with MDRB medium [13]. Petri-dishes were then incubated at 37°C for 3 days and bright yellow-green colonies were counted as Aspergilli colony with the Jenko dissecting microscope at 2x-10x magnification. Colony forming units (CFU) were computed using the following formula as documented by[14]

$$\text{CFU/g of soil} = A \times 10^n / V$$

where A = number of colonies;

$10^n$ = level of dilution at which the counting was carried out;

V = Volume of inoculum.

All harvested grain samples from each sub plot were sun dried (up to ≤7% moisture level), hand shelled and taken to the laboratory for aflatoxin estimation using neogen strips read with a mobile assay tablet reader[2]. The whole sample from the subplot was blended in a Waring Commercial blender and sieved (0.5 mm sieve). From each blended sample, 10 g was weighed. Thirty ml of 65% ethanol was added to 10 g blended sample and blended further to homogenize the mixture. The mixture was shaken at 300 rpm for 5 minutes using Gallenkamp Orbital Shaker and finally filtered into filter cup (conical flask) through Whatman filter paper. 100µl of the filtered liquid was pipetted into red sample cup[3] and then 500µl of dilutant was added and mixed by pipetting 3 times. 100µl was pipetted from red sample cup into transparent sample cup[4]. The neogen test strip (arrow down) was inserted into the transparent sample cup and left for 6 minutes. Finally, after 6 minutes the strip was removed and placed in strip holder of mReader tablet for aflatoxin readings.

## 3. Results and Discussion

*3.1 Effect of Moisture Stress Levels on Aspergillus Flavus Populations in Soil*

Figure 1 shows population of *A.flavus* at different drought stress levels. The results show that) there was a decrease in populations of *A.flavus* towards the beginning of the drought stress at 58 DAP. However, there were no significant differences in populations of *A.flavu*at different drought stress levels. However, at the end of the drought stress at 82 DAP there were significant differences observed in *A.flavus* populations. The control (no drought) had significantly lower populations (1,922 cfu/g of soil) mild drought followed second (3,511 cfu/g of soil), while min drought (7,556 cfu/g of soil) and prolonged drought (8,511 cfu/g of soil) had significantly higher populations (P =< 0.001). It was also observed that there was an increase in populations of *A.flavus*in all the treatments except in plots under no drought where there was continuous decrease in populations. There was also a decrease in populations of *A.flavus* after the end of drought stress or at the resumption of irrigation or prior to harvesting time. The populations were higher than the preceding stages before the beginning of drought stress. High populations were attributed to the hot and dry conditions experienced in the month of October, these two factors favour *A.flavus* population buildup [15]. *Aspergillus flavus* populations decreased with time in all treatments until at the drought

[2] The whole analysis based on the Protocol for Use of mReader Application Neogen Reveal Q+.

[3] Small cup used d for mixing the sample and dilutant.

[4] Small cup used for inserting the test strip.

induction stage, this was attributed to watering all plots with same watering interval and volume. This cooled the soil in all the plots and eventually decreased soil temperature, low temperature and presence of enough moisture in the soil affected the *A.flavus* populations [16]. Results from other studies also show a similar trend that, *A.flavus* populations and aflatoxin contaminations are high during hot-dry season and a bit lower during the rainy season [15, 17].

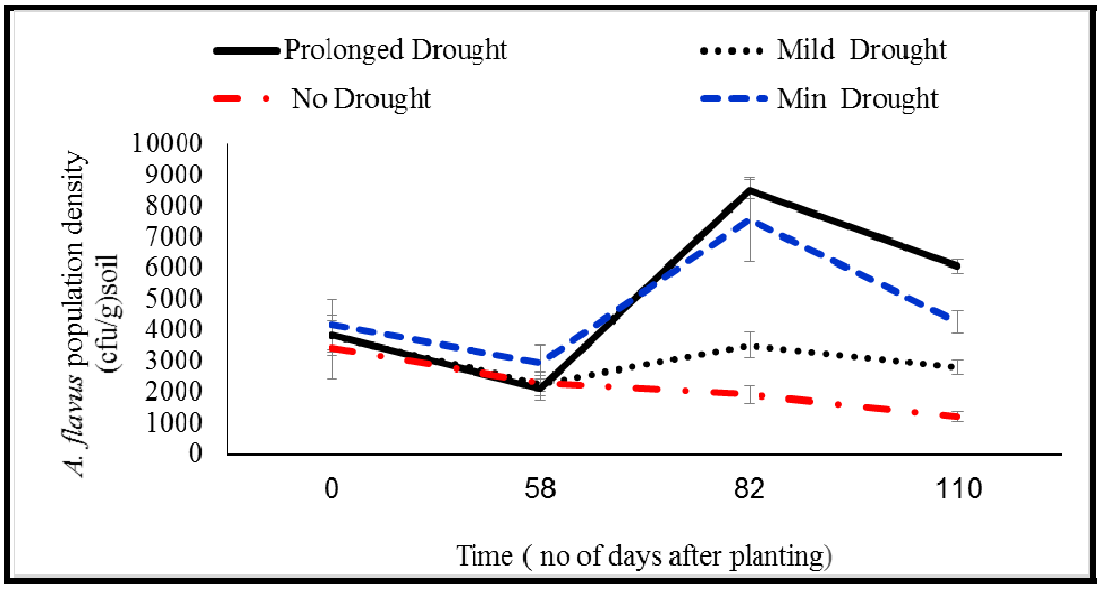


**Fig. 1** ***Aspergillus flavus*** **population densities under different drought stress levels.**

### *3.2 Effects of Drought on Aflatoxin B1 Contamination in Groundnuts*

The results in Table 1 show that there were significant differences (P = 0.011) in aflatoxin levels among drought stress levels. Highest levels were observed in prolonged drought (22.0 ppb) while the lowest levels were observed in no drought (1.5 ppb). There were no significant differences (P = 0.492) in aflatoxin levels among varieties. Interaction between drought stress level and variety was also not significant (P = 0.337). Exposing groundnut plants at pod filling stage (58 DAP) to drought has an effect on aflatoxin levels. The findings showed that the longer the period the plants are exposed to drought, the higher the risk of groundnut getting contaminated and this has been suggested [18-20]. Severe drought especially at critical stages of plant growth and development is associated with poor pod growth and development hence making them more susceptible to aflatoxin contamination [10]. Severe drought stress is linked with high exudation of sucrose by groundnuts pods and roots which support the growth of *A.flavus* hence increasing risk of high aflatoxin levels [7]. Another possible reason for high aflatoxin levels in prolonged drought; since there was poor pod filling and development, this resulted into shriveled seeds which tend to have small cracks hence increasing risk of being penetrated by *A.flavus* [8, 9].

Low aflatoxin levels in no drought could be attributed to adequate soil moisture which is associated with high production of phytoalexins by plants, these boost the defensive mechanisms against pathogens [21]. In plants under normal soil moisture as discussed earlier, the pods and roots produce less sucrose hence less *A.flavus* colonies in soil as well as low aflatoxin levels [7, 22].

### *3.3 Effects of Drought Duration Imposed at Pod Filling Stage on Aflatoxin Levels*

The results in Fig. 2 show that there was strong positive correlation between drought duration and aflatoxin levels as regression coefficient was high ($R^2$ =

0.8551). This means drought duration can be used to predict possible amount of aflatoxin levels in groundnut. Exposing groundnut plants to drought stress for long time increases aflatoxin as well as reduces yield [8, 23-25]. Therefore, irrigation should be used whenever there is drought in older to reduce the possibility of aflatoxin levels especially during rain fed farming.

**Table 1 Aflatoxin B1 levels (ppb) in groundnut varieties under different drought levels.**

| Drought stress level | Variety | | | | | |
|---|---|---|---|---|---|---|
| | Baka | Chitala | Kakoma | ICGV-SM01514 | ICGV-SM99566 | Mean |
| None | 1.5 | 1.6 | 2.1 | 1.1 | 1.3 | $1.5^{a}$ |
| Mild | 3.0 | 3.7 | 7.1 | 3.9 | 4.0 | $4.3^{ab}$ |
| Min | 14.1 | 5.7 | 5.1 | 15.0 | 27.0 | $13.4^{bc}$ |
| Prolonged | 18.4 | 19.9 | 37.6 | 10.2 | 23.8 | $22.0^{cd}$ |
| Mean | 9.3 | 7.7 | 13.0 | 7.6 | 14.0 | |
| Trt (Frob) | 0.011 | | | | | |
| Variety (Frob) | 0.492 | | | | | |
| Trt*variety (Frob) | 0.337 | | | | | |
| Trt (LSD) 0.05 | 10.4 | | | | | |

Means with different superscripts are significantly different ($p < 0.05$); Trt = Treatment; LSD (0.05), Least significant difference at 5%

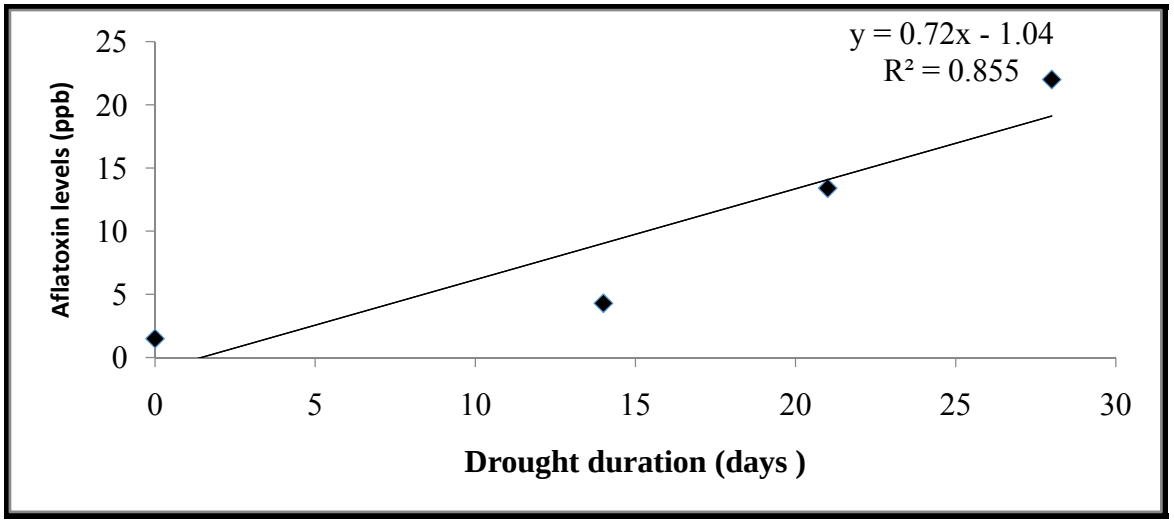


**Fig. 2 Relationship between drought stress and aflatoxin levels.**

### *3.4 Correlation Coefficients of Aflatoxin Levels and Other Parameters*

In Table 2, Aflatoxin levels were positively correlated with drought duration ($P$ =< 0.01, $r$ = 0.53), soil temperature ($P$ =< 0.01, $r$ = 0.54) and CFU/g of soil ($P$ = 0.05, $r$ = *0.45)* and negatively correlated with seed size ($P$ =< 0.01, $r$ = 0.50), yield/ha ($P$ =< 0.01, $r$ = 0.51) and soil moisture ($P$ =< 0.01, $r$ = 0.54). CFU/g of soil was positively correlated with drought duration ($P$ =< 0.01, $r$ = 0.87), soil temperature ($P$ = <0.01, $r$= 0.75) and negatively correlated with seed size ($P$ = < 0.01, $r$ = 0.70) yield/ha ($P$ =< 0.01, $r$ = 0.75) and soil moisture ($P$ <0.01, $r$ = 0.67). Aflatoxin levels correlated with drought duration; lack of moisture provided a conducive environment for *A.flavus* to start producing aflatoxins hence increasing risk of high aflatoxin levels [10, 18, 24]. Aflatoxin levels also correlated with seed size, yield/ha, *A.flavus* population density, soil temperature and soil moisture. This means there is high possibility of high aflatoxin levels in groundnut samples with shrivelled. Small seed size is mostly associated with stresses such as drought, nutrients and other factors which affect growth and

development of the pods [26-28]. The results also showed that yield/ha can also be used to predict the possibility of aflatoxin levels in groundnut.

**Table 2 Correlation coefficients of different parameters.**

| | Aflatoxin levels | Drought duration | CFU/g of soil | Seed size | Grain yield | Soil moisture | Soil temperature |
|---|---|---|---|---|---|---|---|
| Aflatoxin levels | - | | | | | | |
| Drought duration | 0.53** | - | | | | | |
| CFU/g of soil | 0.45* | 0.87** | - | | | | |
| Seed size | -0.50** | -0.80** | -0.70** | - | | | |
| Grain yield | -0.51** | -0.88** | -0.75** | 0.90** | - | | |
| Soil moisture | -0.54** | -0.75** | -0.67** | 0.67** | 0.69** | - | |
| Soil temperature | 0.53** | 0.80** | 0.75** | -0.69** | -0.72** | -0.76** | - |

*, ** denotes significant at $P < 0.05$ and $P < 0.01$, respectively

## 4. Conclusions

The study on role of abiotic (drought) stress showed that there were high aflatoxin contamination levels in the prolonged drought and lower levels in no drought. This means that drought stress has effect on aflatoxin levels and the levels depend on the duration of the drought stress. The longer the drought duration the higher the aflatoxin levels should be expected. The study also revealed that, the varieties used played no role on aflatoxin levels in any of the drought levels, meaning that none of the varieties was more resistant or susceptible to aflatoxin levels under no drought or drought situation. Higher *A.flavus* populations were observed in prolonged drought and lower in no drought. There was also positive correlation between the *A.flavus* populations and aflatoxin levels. It was also noted that prolonged drought did not only increase aflatoxin levels but also contributed to low yields of groundnuts. It was recommended that whenever there is drought in groundnut fields, farmers should be advised to supplement water to crops using irrigation as it will minimize the risk of high aflatoxin levels, increase yield as well as reduce *A.flavus* population in the soil. This will be applicable to farmers who have access to water reservoirs for irrigation. Proper water requirement should be well calculated and available to plants depending on soil type and vegetative stage to avoid stress especially for irrigation farming as this will reduce aflatoxin levels. Farmers should also be practicing technologies that conserve water in soil to avoid moisture stress to groundnut plants. Some of the practices could be mulching and box or tie ridges to retain water in the soil.

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