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Research Application Summary

The role of drought stress on aflatoxin contamination in groundnuts and *Aspergillus flavus* population in the soil during field production

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Abstract

Drought stress is one of the factors that contribute to aflatoxin contamination in groundnuts during field production. A study was conducted aimed at investigating the effects of drought on aflatoxin contamination and *A. fluvus* population in Malawi. 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. The groundnut grain samples were prepared and analyzed for aflatoxin contamination using Mobile assay tablet reader and neogen strips. *A. flavus* population densities in soil samples collected from the plots were estimated using serial dilutions plated on *A. Aspergilli* medium (selective media called dichloronitroaniline Rose Bengal (MDRB). There were significant differences (p=0.011) in aflatoxin contamination among drought stress levels. High contamination was observed in treatments under prolonged drought (22.0 ppb) while low contamination was observed in samples under no drought (1.5 ppb). None of the varieties used in the study were resistant or susceptible to aflatoxin contamination under drought or adequate moisture. The results also showed that there were significant effect of drought period and harvesting time for *A. flavus* population. There was high population of *A.flavus* in prolonged drought at end of stress and harvesting, 8511 and 6044 cfu/g of soil, respectively. It was concluded that drought contributed to aflatoxin contamination in groundnuts and also increased *A.flavus* population in soil during drought period and also at harvesting.

Key words*:* Aflatoxin, *Arachis hypogea*, drought, Malawi

Résumé

Le stress hydrique est l'un des facteurs favorisant la contamination de l'arachide par l'aflatoxine au champ. La présente étude a été menée pour examiner les effets de la sécheresse sur la contamination par l'aflatoxine et les populations d'*A. Fluvus* au Malawi. Quatre niveaux de stress de sécheresse prolongée (4 semaines), 3 semaines, 2 semaines et

« aucune sécheresse » ont été soumis à cinq variétés d'arachides. Des échantillons de sol ont été prélevés de chaque parcelle à quatre reprises: lors du semis, au début de la sécheresse, à la fin de la sécheresse et à la récolte. Les échantillons de grains d'arachide ont été préparés et analysés pour détecter la contamination due à l'aflatoxine. Les densités de population de *A. flavus* dans les échantillons de sol prélevés sur les parcelles ont été estimées en utilisant des dilutions en série sur milieu à *A. aspergilli* (milieu sélectif appelé dichloronitroaniline Rose Bengal. Des différences significatives (p = 0,011) de contamination due l'aflatoxine ont été observées au sein des traitements. Une contamination élevée a été observée pour les traitements de sécheresse prolongée (22,0 ppb) contre une faible contamination dans les échantillons sans sécheresse (1,5 ppb). Aucune des variétés utilisées n'était résistante ou susceptible à la contamination par l'aflatoxine en période de sécheresse ou d'humidité. Les résultats ont également montré que la durée de la sécheresse et celle de la récolte ont des effets significatifs pour les populations de *A. flavus*. Il y avait une forte population de *A. flavus* en période de la sécheresse prolongée, à la fin du stress et de la récolte, respectivement 8511 et 6044 cfu / g de sol. Il a été conclu que la sécheresse a favorisé la contamination des arachides par l'aflatoxine et a également augmenté la densité des populations de *A. flavus* dans le sol pendant la sécheresse tout comme à la récolte.

Mots clés : Aflatoxine, *Arachis hypogea*, sécheresse, Malawi

Introduction

Groundnut (*Arachis hypogaea*) is one of the major important legumes grown in Malawi. Groundnut is used to improve human nutrition, soil fertility and economic status of many people (Monyo and Gowda, 2014). One of major challenges that affect groundnut production in Malawi is aflatoxin contamination. Aflatoxin are substances produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* as secondary metabolites and are known to be toxic to humans and thus have a negative impact on the economic wellbeing of individuals and nations (Hell *et al*., 2010). The contamination of groundnut by aflatoxin occurs during pre-harvest (in field) and post-harvest. Agricultural products with high levels of aflatoxin contamination are being denied access to international markets. The acceptable level of aflatoxin by the European Union (EU) and Malawi Bureau of Standards (MBS) is 4 ppb while for the World Health Organization (WHO) it is 20 ppb (Monyo *et al*., 2012). Drought is thought to be a contributing factor to the contamination in groundnut as it increases aflatoxin contamination. Drought is reported to favour increased production of sucrose in groundnut roots and and pods which is a growth substrate for *A. flavus* (Puntanse *et al*., 2004). Drought is also associated with poor pod filling and development, resulting into small shrived seeds which usually develop small cracks thus allowing easy penetration of *A. flavus* (Okello *et al.*, 2010; Hamidou *et al.,* 2013). Drought also makes a plant more susceptible to insects and diseases because it causes a reduction of phytoalexins accumulation hence increasing the risk of contamination (Wotton and Strange, 1987; Balota, 2012). This study was carried out to quantify aflatoxin contamination as influenced by different drought duration at pod filling stage.

Methodology

The study was conducted in a screen house at ICRISAT-Malawi from October 2015 to February 2016.The experiment was laid down as a split plot, with drought as main plot factors and variety as subplot factors. Planting was done in wooden boxes filled with soil sources from Chitedze research station. Main plots measured 2 m x 0.6 m while sub plots measured 0.6 m x 0.4m. Four levels of drought were imposed at pod filling on five Spanish varieties commonly grown in Malawi (Baka, Kakoma, Chitala, ICGV-SM01514 and ICGV-SM99566) . The four drought stress levels were; prolonged (4 weeks' drought), min (3 weeks' drought), mild (2 weeks' drought) and no drought (control). Watering resumed in all treatment after the end of each drought period up to harvesting period. Soil samples were collected four times at a depth of 0 to 10 cm. From each sub plot, soil samples were collected at planting, beginning of stress, end of stress and harvesting. The population of *A. flavus* in soil was determined using the modified dichloronitroaniline Rose Bengal (MDRB) selective media. Petri-dishes were then incubated at 37 ^{o}C for 3 days, colony counts done and expressed as colony forming units (CFU). The groundnut samples were prepared and analyzed for aflatoxin contamination using Mobile assay tablet reader and neogen strips.

Results and discussions

There were significant differences (p=0.011) in aflatoxin contamination among the different drought stress levels. Contamination was highy in plots under prolonged drought (22.0 ppb) and lowest from samples from plots with no drought (1.5 ppb) (Table 1). 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 infection (Balota, 2012).

Figure 1 shows the population of *A. flavus* in soil from planting to harvesting under different drought conditions. Higher population trends were observed in plots that were under prolonged drought than other droughts. There was also general increase in *A. flavus* population during

Table 1. Aflatoxin contamination in different varieties under different drought levels (ppb)

Drought stress level	Variety					Mean
	Baka	Chitala	Kakoma	ICGV-SM01514	ICGV-SM99566	
No drought	1.5	1.6	2.1	1.1	1.3	1.5
Mild drought	3.0	3.7	7.1	3.9	4.0	4.3
Min drought	14.1	5.7	5.1	15.0	27.0	13.4
Prolonged drought	18.4	19.9	37.6	10.2	23.8	22.0
Mean	9.3	7.7	13.0	7.6	14.0	
Trt (Fsg)	0.011					
Fp Trt*variety (Fsg)	0.337					
Variety (Fsg)	0.492					

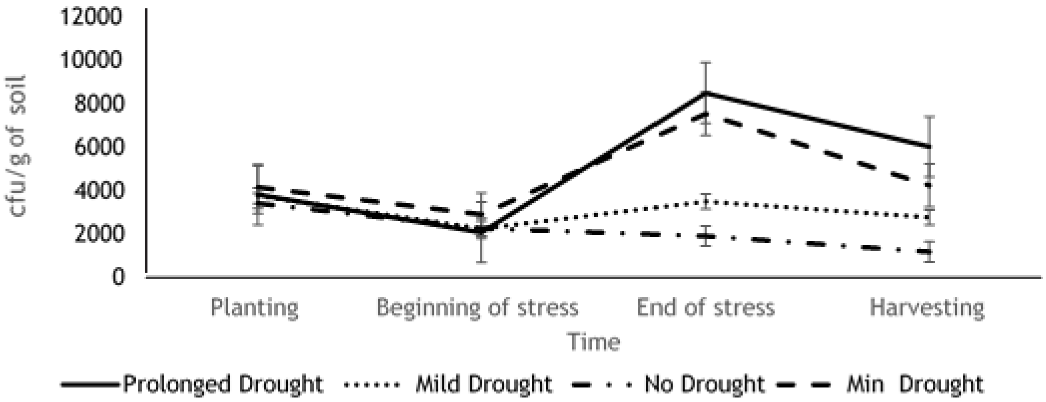

Figure 1. Trends of *Aspergillus flavus* population under different drought stress levels

drought period except under no drought conditions where the population kept on decreasing. Groundnut roots and pods under drought stress condition exudes more sucrose than under non-drought conditions, and these exudes released provide growth substrate which enhance *A. flavus* population increase(Puntanse *et al*., 2004).

Conclusions and recommendations

The study showed that high contamination was observed in prolonged drought and lower contamination in no drought conditions. This means different drought stress scenarios play a significant role in aflatoxin contamination, with long drought periods encouraging higher aflatoxin contamination. The study also revealed that the varieties used in the study had no influence on aflatoxin contamination irrespective of length of drought. There was also a positive correlation between *A. flavus* population and aflatoxin contamination. It is recommended that whenever there is drought, fields should be irrigated. In addition, good agronomic practices such as good time of planting, correct plant population, insects and diseases control, weed control and proper time of harvesting should be enforced.

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