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Screening for the Occurrence of Aflatoxin Producing Fungi in Drought affected Groundnut seeds in Katsina State, Nigeria

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ABSTRACT

Drought stress is thought to increase the susceptibility of pods and seeds to aflatoxin producing *A. parasiticus* and *A. flavus* invasion in pre-harvest groundnut. In this research, two drought stressed groundnut varieties namely; SAM-NUT 22 and Ex-Dakar were obtained from Katsina state Agricultural and Rural Development Authority (KTARDA) and examined for fungal invasion and subsequent aflatoxin contamination. A total of 23 fungal isolates were obtained all of which were identified to be *Mucor*, *Fusariumoxysporum*, *Penicillium* sp *Aspergillus parasiticus*, *A. flavus* and *A. niger* with *A. niger* as the most frequently occurring with 12 of the 23 isolates (52.2% relative abundance) recovered. The results of the isolation test revealed that SAM-NUT 22 was most associated with *A. parasiticus* and *A. flavus* with only one and two isolates of *A. parasiticus* and *A. flavus* respectively which were the only aflatoxin producing fungi discovered in this study. None of the aflatoxigenic fungus occurred in Ex-Dakar. Aflatoxin using thin layer chromatography test revealed the absence of this mycotoxin in the two test groundnut seeds probably because there were no much of both *A. parasiticus* and *A. flavus* recovered from the two samples. It appeared that drought alone is not the only factor that single handedly leads to the growth of aflatoxin contaminating fungi in groundnut seed.

Introduction

Aflatoxins are naturally occurring mycotoxins that are produced by many species of the genus *Aspergillus*, notably *A. flavus* and *A. parasiticus*. It has been shown that *A. flavus* is the most common producer of aflatoxin (Buoraima *et al.*, 1993). Aflatoxins are toxic and among the carcinogenic substances known to man (Hudler, 1998). Carcinogenic aflatoxins produced by *Aspergillus flavus* and *A. parasiticus* are common contaminants of groundnut seed, cottonseed, corn and tree nuts (Dorneret *al.*, 1998; Payne, 1998).

Several factors have been found to be responsible for the contamination and colonization of groundnut by aflatoxins producing fungi all over the world (Mohammed and Kutama 2007). Drought stress is an important factor in the contamination and subsequent colonization of groundnut by aflatoxin fungi before harvest. Drought stress is thought to increase the susceptibility of the pods and seeds to fungal invasion by decreasing the moisture content of the pods thereby lowering the physiological activities in the kernel (Donald and Harkness, 1964).

In Nigeria, Bampton (1963) and in South Africa, Abrams *et al.* (1967) had earlier linked drought stress to aflatoxin contamination. In the United States earlier researches conducted by Dickens *et al.* (1968) and Pettit *et al.* (1971) showed a positive correlation between drought stress and aflatoxin producing *A. parasiticus* and *A. flavus* invasion in pre-harvest groundnuts. In irrigated fields however, although groundnuts were infested by *A. flavus* (60%), no contamination by aflatoxin was reported indicating that drought is related to the growth of aflatoxin producing fungi in groundnut seed. Drought stress has a vital role in accelerating *A. flavus* infection and aflatoxins production in sound mature kernels. According to Lillehoj *et al.* (1976) late-season drought stress particularly in the semi-arid and tropics is a major factor associated with aflatoxin contamination in peanuts. In the same vein, drought during pod formation has also been found to substantially increase the level of aflatoxin contamination. It has also been suggested that the highest levels of *A. flavus* and *A. parasiticus* infection and aflatoxin contamination are associated with seed damage (Blakenship *et al.*, 1984; Dowd, 1995; Hill *et al.*, 1983; Sanders *et al.*, 1985).

Conversely, it was reported that pre-harvest infection requires a drought of 30-50 days and a mean soil temperature of 29-31°C in the podding zone (Cole *et al.*, 1984). Wilson and Stansell (1999) demonstrated that the susceptibility of groundnut to aflatoxin contamination is related to lower water holding capacity of 0.80 to 0.90 inches in the kernel and a favorable temperature of 25-32°C for the growth of *A. flavus*. This is probably because as the kernel moisture content decreases at the end of the season, natural mechanism for drought protection is lost and the kernel become vulnerable to

colonization by *A. flavus* and aflatoxin contamination.

In Africa and South eastern Asia, aflatoxins have been shown to increase the incidence of human liver cancer by acting synergistically with hepatitis viruses (Wild and Turner, 2002). In addition to the health concerns related to aflatoxins, the substance has been found to cause marked deterioration in the pod and kernel quality there by making the seed unfit for consumption and greatly reducing grower profit for the farmers (Wikipedia, 2009). Snowdon (1992), demonstrated that post-harvest contamination of food crops are threat to the year round availability of quality food in the United State. Because of the high correlation between seed moisture and pre-harvest aflatoxin contamination it is possible to select for reduced pre-harvest aflatoxin contamination by identifying germplasm with capacity for high kernel moisture activity during severe drought. The aim of this investigation was to screen some drought affected groundnut varieties for aflatoxin producing fungi.

Materials and Methods

Collection of Seeds

Two varieties of drought stressed groundnut seed samples identified as SAM-NUT 22 and Ex-Dakar were collected from crop production unit, Katsina State Agricultural and Rural Development Authority separately in sterile polythene bags in December, 2009. The samples were brought to microbiology laboratory, Bayero, University, Kano and kept in cupboard before being screened.

Isolation and identification of aflatoxigenic fungi

Portions of the groundnut seeds were sterilized separately in 1% (v/w) sodium hypochloride for two minutes and later washed three times in sterile distilled water.

Cultivation of fungi was done on Petri dishes using the agar plate technique described by Jha (1995) and incubated for 7 days at 28 ±1°C. Purified fungal isolates were identified according to John (1986) and Bayman *et al.* (2002).

Determination of Aflatoxin

Groundnut samples were ground into powder and 15.0 g of the powder was defatted after which n-hexane in a Soxhlet extraction chamber was used for the extraction. The residual solvent was later dried up by heating in an oven at

temperature of 105°C for 30 minutes. Ten grammes of the defatted material was weighed into a 250 ml wide mouthed bottle and 10 ml of distilled water was added and mixed thoroughly. The bottle was covered and shaken on wrist action shaker for 30 minutes to extract toxin after which it was filtered. The filtrate was later concentrated to recover the extract and the extract was characterized by using thin layer chromatography as described by Shroeder and Ashiront (1965).

Table 1. Fungal isolates obtained from two drought stressed Groundnut varieties

Groundnut variety	Fungal species recovered
SAM-NUT 22	<i>Mucor</i> , <i>Penicillium</i> , <i>Fusarium</i> , <i>Aspergillus flavus</i> , <i>A. parasiticus</i> , <i>A. niger</i>
Ex-Dakar	<i>Mucor</i>

Table 2. Distribution and relative abundance of fungal isolates recovered from two groundnut varieties

Groundnut variety	Name and Number of isolate identified						Percentage abundance
	<i>Aspergillus niger</i>	<i>A. flavus</i>	<i>A. parasiticus</i>	<i>Fusarium</i>	<i>Penicillium</i>	<i>Mucor</i>	
SAM-NUT 22	12	2	1	5	1	1	88.0
Ex-Dakar	ND	ND	ND	ND	ND	3	12.0
Total	12	2	1	5	1	4	100.0

ND= Not detected

Table 3. Result of aflatoxin test on SAM-NUT 22 groundnut variety

Groundnut variety	Type of aflatoxin detected	Nature of aflatoxin
SAM-NUT 22	N.D	Negative

Results and Discussion

A total of 23 fungal isolates were recovered from the two groundnut varieties examined. The results showed that SAM-NUT 22 was the most affected groundnut variety with about 23 fungal isolates belonging to four fungal genera.

These include *Mucor*, *Penicillium* sp, *Fusarium* sp, *Aspergillus niger* and *A. parasiticus* (Table 1.), with *A. niger* being the most abundant (52.2 %) and the least occurring were *Penicillium* sp and *A. parasiticus* each with 4.3% abundance. On the other hand, *Mucor* was recovered from Ex-Dakar groundnut variety (Table 2.).

The high frequency of *Aspergillus* in the present study clearly explains the nature of the fungus; they are food contaminants usually associated with grain and seed in stores (Muhammed and Kutama, 2007; Usman *et al.*, 2010). Their association with groundnut as observed in this study might be because during shelling, a lot of groundnut seeds are inadvertently wounded which consequently exposes the cotyledon containing nutritious food substances to the fungi or that the moisture content of the SAM-NUT 22 seeds was high during storage to facilitate for easy colonization by fungi such as *Aspergillus*. Horn (2005) reported that the extent of seed colonization by *Aspergillus* increased with increasing water activity and temperature. The high occurrence of *A. niger* in the groundnut variety SAM-NUT 22 was probably because this species is predominantly part of the air spora as such they easily attack and colonize the seeds. John (1986) reported that *A. niger* is essentially a contaminant that is predominant in the air and most food substrates. *A. niger* was the predominant colonist because the species has moisture and temperature requirements which are slightly broader than those of *A. flavus* (Martin *et al.*, 1999). In this study, *A. flavus* and *A. parasiticus* only occurred sparsely but were not in a significant quantity. According to Bayman *et al.* (2002) *A. niger* and *A. flavus* commonly co-occur on agricultural commodities such as groundnut, corn, cotton seeds and tree nuts.

Similarly, the appearance of *Fusarium* and *Penicillium* in the groundnut variety SAM-NUT 22 might be due to the fact that both fungi were also a toxin producing fungi which are usually found dwelling in the soil (Usman *et al.*, 2010).

The result of the study had also indicated the absence of aflatoxins in the two groundnut varieties tested. The absence

of aflatoxin in the groundnut samples was probably due to the absence of the major aflatoxin producing fungi; *A. flavus* and *A. parasiticus* in a quantity enough to facilitate for the production of this toxin within the seeds. It is very pertinent that *A. flavus* and *A. parasiticus* are the most common aflatoxin producing fungi belonging to the genus *Aspergillus*. Bhat and Vashanti (1999) therefore confirmed that the presence of *Aspergillus* in food does not always indicate harmful level of aflatoxin. In the same vein, the study had shown that drought alone was not responsible for aflatoxin contamination of groundnut seeds as reported by Doster and Michaihdas (1994). Earlier reports had shown that drought and temperature were together responsible for the incidence of aflatoxin in pre-harvest groundnut (Cole *et al.*, 1984). It seems both factors act synergistically in the enhancement of aflatoxin formation mature kernel. Cole *et al.* (1984) reported that pre-harvest infection by *A. flavus* requires a drought period of 30 – 50 days and a mean soil temperature of 29 - 30° C in the podding zone. In a similar report, aflatoxin formation in groundnut is favoured by prolonged end of season drought and associated elevated temperature (Rachaputiet *al.*, 2002; Wikipedia, 2009).

Conclusion

The present study has shown that there are varieties of fungi associated with drought stressed groundnut at post harvest. However, drought stress seemed not to be the only factor responsible for aflatoxin formation in groundnut. It is likely that aflatoxin formation is due to several interacting factors in both pre and post-harvest conditions. Therefore research should be intensified on the combine causes of aflatoxin formation in pre-harvest groundnut.

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