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Effect of temperature, relative humidity and moisture on aflatoxin contamination of stored maize kernels

Francis Collins Muga[1*], Moses Okoth Marenya[2,3], Tilahun Seyoum Workneh[1***]**

[1]*Department of Bioresources Engineering, School of Engineering, University of KwaZulu-Natal, Private Bag X01, Pietermaritzburg, South Africa*
[2]*Department of Agricultural and Rural Engineering, School of Agriculture, University of Venda, Private Bag X5050, Thohoyandou, South Africa*
[3]*Institute for Agricultural Engineering, Agricultural Research Council, Private Bag X519, Silverton, Pretoria, South Africa*
[*]*Corresponding author:* 215081106@stu.ukzn.ac.za; mugafc@gmail.com

Abstract

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This study investigated the aflatoxin contamination of maize kernels for selected temperature, relative humidity and moisture content levels. Samples of maize kernels at moisture content levels of 14, 15, 16, 18, and 20% (wb) were inoculated with *Aspergillus flavus* spores and incubated in a climatic test chamber for ten days at 20°C and 30°C, and relative humidity of 60% and 90%. The results indicated that aflatoxin contamination was significantly ($p \leq 0.05$) affected by temperature and relative humidity whereas moisture content had no significant ($p > 0.05$) effect. Aflatoxin contamination occurred at both 20°C and 30°C. The production of aflatoxin was pronounced at 30°C, ranging between 0.3 $\mu g.kg^{-1}$ – 11179.7 $\mu g.kg^{-1}$, compared to 20°C that ranged between 0.8 $\mu g.kg^{-1}$ – 733.7 $\mu g.kg^{-1}$. Relative humidity of 90% had higher levels of aflatoxin contamination of between 3.9 $\mu g.kg^{-1}$ – 11179.7 $\mu g.kg^{-1}$, while a relative humidity of 60% had levels of aflatoxin contamination of between 0.3 $\mu g.kg^{-1}$ – 2.4 $\mu g.kg^{-1}$. The interaction between temperature and relative humidity significantly ($p \leq 0.05$) influenced aflatoxin contamination of maize. However, the interaction between temperature and moisture content, moisture content and relative humidity, as well as the combined interaction of temperature, moisture content, and relative humidity had no effect on the level of aflatoxin contamination. The results indicate that the level of aflatoxin contamination at a relative humidity of 60% was lower than 5 $\mu g.kg^{-1}$. Consequently, maintaining storage conditions at a relative humidity level of less than 60% results in minimal aflatoxin contamination of maize kernels, thus assuring its safety for consumption.

Keywords: *Aspergillus flavus*; aflatoxin; maize; storage

Introduction

Sub-Saharan Africa (SSA) experiences severe yield losses in maize. Insufficient post-harvest practices exacerbate these losses. Losses of up to 50% have been reported across many countries in Africa, most of which are experienced during storage (Hodges et al., 2011). Insects and fungi collectively account for more than 50% of grain lost during storage (Udoh et al., 2000). Fungal spoilage of maize is a grave concern due to the mycotoxins associated with it.

Maize is very susceptible to fungal degradation, particularly *Aspergillus* and *Fusarium* which cause aflatoxins and fumonisins respectively (Tefera, 2012). These mycotoxins impact human and animal health and lower the economic

value of produce (Gnonlonfin et al., 2013). Aflatoxin contamination of maize causes significant grain losses in SSA (Dwivedi and Singh, 2011; Wagacha et al., 2013). *Aspergillus flavus*, the primary cause of aflatoxin, attacks maize in the field and its effects are compounded by inappropriate post-harvest practices (Marin et al., 2004).

Inadequate storage techniques and environmental conditions fuel fungal growth and aflatoxin contamination of maize. The complex interaction of the biotic and abiotic factors within the grain storage ecosystem determines the severity of aflatoxin contamination of stored maize (Magan et al., 2010). The primary factors that promote contamination of stored maize by *A. flavus* are high temperature, grain moisture content and relative humidity of the surrounding air (Alborch et al., 2011; Mrema et al., 2012; Tefera, 2012). Freshly harvested maize usually has a moisture content (MC) of between 18% – 125% which necessitates for rapid drying to reduce the moisture content to below 14% to prevent fungal growth (Magan and Aldred, 2007). Majority of small-scale farmers in SSA depend on sun-drying. Sun-drying is largely based on the local weather conditions and often does not dry maize adequately or quickly to limit fungal attack (Wagacha et al., 2013; Womack et al., 2014). The prevailing conditions in large parts of SSA predispose the stored maize to attack by *A. flavus*, contaminating it with aflatoxins.

A. flavus is a mesophilic fungus that thrives in the temperature range of 10°C – 143°C and relative humidity levels greater than 85% (Giorni et al., 2012; Al-Shikli et al., 2017). The traditional storage methods used by maize farmers in SSA do not offer environmental control of the storage environment. This lack of control exposes the stored maize to conditions that promote the growth of fungi and mycotoxin production (Ngamo et al., 2007; Giorni et al., 2012). Inadequate ventilation that characterises most of the storage structures used by the resource-poor small-scale farmers leads to moist hot spots that exacerbate *A. flavus* and aflatoxin contamination.

Studies have established how moisture content and temperature (Giorni et al., 2012), as well as relative humidity and temperature (Pratiwi et al., 2015; Al-Shikli et al., 2017), affect *A. flavus* growth and aflatoxin production. This study investigated the combined effect of grain moisture content and the ambient storage conditions, viz., temperature and relative humidity, on the aflatoxin contamination.

Materials and Methods

Inoculum preparation

A. flavus fungal strain was obtained from the Department of Plant Pathology, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, South Africa. The fungus was placed on potato dextrose agar (Merk, Darmstadt, Germany) at 25°C for five days after which the conidia was harvested by flooding a single culture with distilled water and scraping the surface mycelia with a sterile scraper. The resulting suspension was filtered through a cheesecloth to obtain pure spore suspension. The spore suspension was then adjusted to 4×10^6 cells.ml^{-1} using a Neubauer hemocytometer to make the inoculum (Hruska et al., 2014).

Preparation of maize samples

White maize variety SC411 was obtained from the Seed Co Pty Ltd (South Africa). The initial moisture content of the maize was 12.19% ±0.10 (w.b). The maize kernels were surface sterilised by immersing in 5% (v/v) sodium hypochlorite (NaClO) and stirred for one minute then rinsed twice with distilled water (Reese et al., 2011). The maize kernels were soaked in distilled water for a predetermined period of time to adjust the moisture content to five different levels, viz., 14%, 15%, 16%, 18% and 20% (w.b). The rehydrated samples were sealed in Ziploc bags and refrigerated for 3 days at 4°C. The samples were periodically shaken manually to ensure a uniform moisture distribution within the bags.

Experimental design and data analysis

A completely randomised design with three replicates was employed in the experiment. A three-factor full-factorial design was used with the first two factors at two levels and the third factor at five levels. The factors studied were temperature (20°C and 30°C); relative humidity (60% and 90%) and moisture content (14%, 15%, 16%, 18%, and 20%).

The data obtained from the study was subjected to analysis of variance (ANOVA) at 5% significance level to determine the effect of the studied storage environmental parameters on maize kernel attack by *A. flavus* and the subsequent aflatoxin contamination. Where a significant ANOVA result was obtained, the mean comparison was done using Duncan's Multiple Range Test. GenStat® 17th Edition (VSN International Ltd, Hemel Hempstead, United Kingdom) was used for the statistical data analysis.

Inoculation and incubation of maize

The rehydrated maize kernels were retrieved from the cold storage and allowed to equilibrate to room temperature. 500 g of maize kernels at a moisture content of 14%, 15%, 16%, 18% and 20%, were weighed into autoclaved plastic containers. 2 ml of the inoculum was sprinkled on each sample and mixed by hand. The samples were then randomly placed into the climatic test chamber (*CTS GmbH, Hechingen, Germany*). The climatic test chamber was used to regulate both tempera-

ture (T) and relative humidity (RH) with an error margin of ±1°C and ±5% respectively. The samples were incubated for 10 days and fungal growth terminated by transferring the samples to a 70°C forced-air drying oven for 72 hours.

Data collection

Sampling was done immediately prior to incubation (day zero) and at the end of incubation period (day ten). The samples were analysed for the presence and level of aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2) and moisture content (MC). Aflatoxin analysis was done using a liquid chromatography-tandem mass spectroscopy (LC-MS/MS) as outlined in De Kok et al. (2007). The liquid chromatography (LC) had an acquity, ultra-performance liquid chromatography, ethyle bridge hybrid column (UPLC BEH C_{18} 1.7 µm; 2.1×100 mm column). The mobile phase A and mobile phase B was 0.1% formic acid in water and 0.1% formic acid in acetonitrile respectively.

The samples were ground using a retsch rotor mill (*SK 1, Germany*). 25 g of each sample of the ground maize was mixed with 80 ml of acetonitrile and 20 ml of distilled water for 2 hours. The extract was filtered and diluted four-times with distilled water. 20 µL of the diluted extract was fed into the LC-MS/MS. The LC flow rate was 0.4 $ml.min^{-1}$. The eluent from the LC column was directed to the mass spectrometer. The electrospray source was operated in a positive ionisation multiple reaction monitoring (MRM) mode. The data acquired was analysed using Waters Masslynx™ software. The limit of detection for the LC/MS/MS was 0.5 $\mu g.kg^{-1}$, whereas the quantification limit was 2 $\mu g.kg^{-1}$.

Authentic standards for AFB1, AFB2, AFG1, and AFG2 obtained from Sigma-Aldrich (Carlsbad, California, USA), were used to produce reference chromatogram for the four types of aflatoxins, as well as the standard calibration curves from which the aflatoxin content of the test samples was determined by interpolation.

Results

Fungal growth was evident from visual examination at all the MC levels for samples stored at 30°C and 90% RH. Aflatoxin levels were, therefore, highest at these conditions ranging from 4998.97 $\mu g.kg^{-1}$ – 11179.67 $\mu g.kg^{-1}$ for AFB1, 451.39 $\mu g.kg^{-1}$ – 1404.27 $\mu g.kg^{-1}$ for AFB2, 32030.47 $\mu g.kg^{-1}$ – 53630 $\mu g.kg^{-1}$ for AFG1, and 2043.13 $\mu g.kg^{-1}$ – 5826.46 $\mu g.kg^{-1}$ for AFG2. Samples for all the other T and RH combination showed no fungal growth from visual examination despite being contaminated with aflatoxin.

AFB1 was detected in all treatments. AFG1 levels were the highest in all treatments except at MC levels of 15%, 16% and 18% at 30°C and 60% RH where it was not detected. AFB2 levels were the lowest across all treatments. AFB1, AFB2 and AFG2 had low/undetectable (nd) levels at 60% RH across all T and MC levels (undetectable – 2.81 $\mu g.kg^{-1}$). AFG1 showed relatively higher levels of between 11.16 $\mu g.kg^{-1}$ – 117.08 $\mu g.kg^{-1}$ at 20°C and 60% RH. Both AFB2 and AFG2 were not detected at 30°C and 60% RH at all MC levels. Higher aflatoxin levels were observed at 90% RH although AFB2 was not detected at

Table 1. Mean aflatoxin contamination levels for all treatments ($\mu g.kg^{-1}$)

T °C	RH %	Type of aflatoxin	MC %				
			14.0	15.0	16.0	18.0	20.0
20	60	AFB1	0.8	1.23	1.17	1.53	1.9
30	60		0.3	1.07	2.13	2.1	2.43
20	90		3.93	37.3	80.1	400.53	733.7
30	90		4998.97	7540.0	8338.23	11013.9	11179.67
20	60	AFB2	nd	0.12	0.66	2.81	0.14
30	60		nd	nd	nd	nd	nd
20	90		nd	nd	2.21	14.08	30.01
30	90		451.39	798.02	852.89	1031.51	1404.27
20	60	AFG1	12.88	11.16	14.17	17.08	16.23
30	60		1.49	nd	nd	nd	20.74
20	90		36.91	384.69	1180.8	7520.91	7739.77
30	90		32030.47	47459.79	50247.94	46588.12	53690.78
20	60	AFG2	nd	nd	1.59	nd	0.6
30	60		nd	nd	nd	nd	nd
20	90		nd	1.75	13.06	26.35	45.22
30	90		2043.13	3444.17	4075.16	5172.18	5826.46

*nd indicates not detected

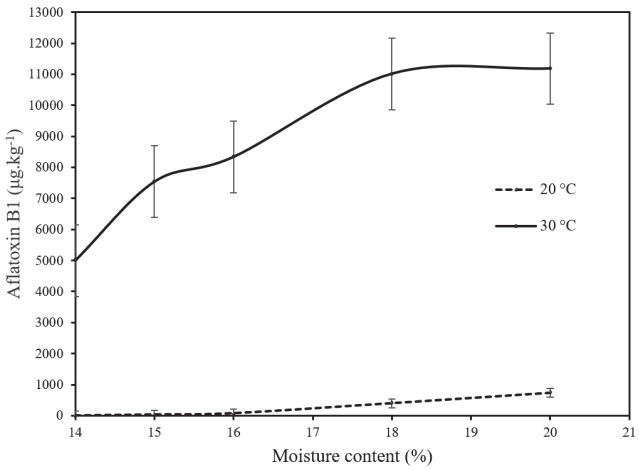

Fig. 1. Aflatoxin levels at 90% RH at both 20°C and 30°C (LSD p < 0.05 = 2634.891, CV = 23.6)

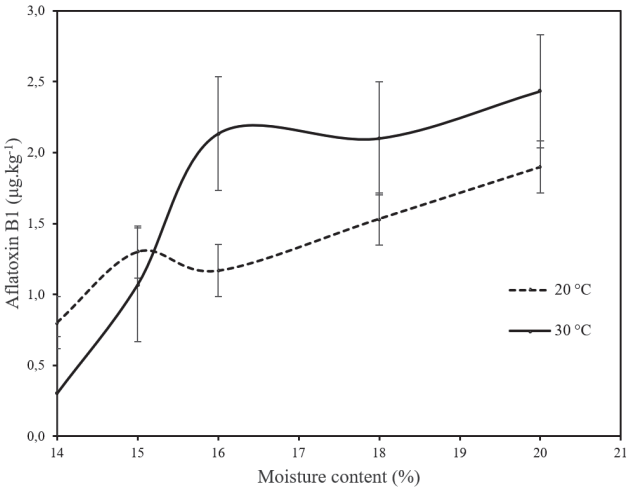

Fig. 2. Aflatoxin levels at 60% RH at both 20°C and 30°C (LSD p < 0.05 = 2634.891, CV = 23.6)

14% and 15% MC whereas AFG1 was not detected at 14% MC, all at 20°C. The mean aflatoxin contamination levels for all the treatments are shown in Table 1.

The storage temperature significantly ($p \leq 0.05$) affected aflatoxin production in maize at 60% and 90% RH. Aflatoxin levels were greater at 30°C than at 20°C particularly at 90% RH (Fig. 1).

Aflatoxin production was also significantly ($p \leq 0.05$) affected by relative humidity (RH). Aflatoxin levels were higher at 90% RH (3.9 $\mu g.kg^{-1}$ – 11179.7 $\mu g.kg^{-1}$) than at 60% RH (0.3 $\mu g.kg^{-1}$ – 2.4 $\mu g.kg^{-1}$). At 60% RH, 30°C still resulted in higher levels of aflatoxin than 20°C except at a MC of 14% and 15% as shown in Fig. 2.

Moisture content (MC) was the only experimental factor that did not significantly ($p > 0.05$) affect the levels of aflatoxin contamination in the maize kernels. T and RH regulated the MC of the maize kernels setting up an equilibrium moisture content (EMC). The EMC at the end of day ten was: 10.23%, 11.42%, 14.12% and 15.98% for the samples stored at 20°C and 60%; 30°C and 60%; 20°C and 90% and 30°C and 90% respectively. High EMC corresponded to high levels of aflatoxin and vice versa. The interaction between T and RH significantly ($p \leq 0.05$) influenced the level of aflatoxin contamination in the maize kernels. However, the interaction between other factors such as T×MC, RH×MC and T×RH×MC, had no significant ($p > 0.05$) effect on the level of aflatoxin contamination. The level of significance of various factors, as well as their interaction, is shown in Table 2.

Table 2. Mean AFB1 levels ($\mu g.kg^{-1}$) and the significance level for each experimental factor

T °C	RH %	MC %				
		14	15	16	18	20
20	60	0.8^{d}	1.23^{d}	1.17^{d}	1.53^{d}	1.9^{d}
30	60	0.3^{d}	1.07^{d}	2.13^{d}	2.1^{d}	2.43^{d}
20	90	3.93^{d}	37.3^{d}	80.1^{d}	400.53^{d}	733.7^{d}
30	90	4998.97^{c}	7540.0^{bc}	8338.23^{b}	11013.9^{a}	11179.67^{a}
Significance levels						
T		≤ 0.05				
RH		≤ 0.05				
MC		≥ 0.05				
T × RH		≤ 0.05				
T × MC		≥ 0.05				
RH × MC		≥ 0.05				
T × RH × MC		≥ 0.05				

Means within a row followed by the same letter(s) are not significantly different according to Duncan's multiple range test (P < 0.05).

There were no significant differences in the mean aflatoxin levels for all treatments, apart from those treatments at 30°C and 90% RH. However, at 30°C and 90% RH, the difference between the means at MC of 18% and 20% were also not significant (Table 2). The same phenomenon is exemplified by the overlapping error bars at these treatments as shown in Fig. 1.

Discussion

Environmental factors such as temperature and relative humidity influence the growth of *A. flavus* and aflatoxin production (Alborch et al., 2011; Giorni et al., 2012). Aflatoxin production in maize was detectable at 20°C and 30°C at 90% RH, with maximum aflatoxin levels observed at 30°C and 90% RH. The results are in agreement with the findings of Das et al. (2012) and Al-Shikli et al. (2017). These previous studies reported that *A. flavus* grew well at 30°C and relative humidity values greater than 85%.

Aflatoxin contamination of maize was pronounced at 90% RH compared to 60% RH implying that RH significantly ($p \leq 0.05$) affects aflatoxin contamination in maize. These results are consistent with the findings by Cotty and Jaime-Garcia (2007) and Kusumaningrum et al. (2010). A 90% RH provides sufficient water activity for the growth of *A. flavus* which then attacks the maize kernels. The result of this attack is the production of aflatoxins. At 60% RH, the water activity is below 0.65, which is the minimum water activity level necessary for microbial or fungal growth (Giorni et al., 2012). The growth of *A. flavus* is, therefore, impeded at 60% RH and the low aflatoxin content observed could have been caused by the high initial moisture content before the EMC is reached.

MC was found to have no significant effect on aflatoxin contamination of maize. T and RH influence EMC of the grain (Cotty and Jaime-Garcia, 2007; Al-Shikli et al., 2017). Consequently, the interaction between T and RH significantly ($p \leq 0.05$) affected the level of aflatoxin in maize. The EMC for the maize kernels at 90% RH was 15.98% and 14.12% for 30°C and 20°C respectively. High RH within the grain storage encourages moisture absorption by the grains, resulting in elevated levels of aflatoxin as reported by Kaaya and Kyamuhangire (2006) and Atehnkeng et al. (2008). At 60% RH, the EMC was 10.23% at 20°C and 11.42% at 30°C. At such low EMC level, the fungal growth was limited resulting in low levels of aflatoxin recorded in this study.

The low levels of aflatoxin in maize kernels samples stored at 60% RH at both 20°C and 30°C suggest that these conditions can be used to store maize without severe aflatoxin contamination occurring. AFB1 contamination at 60% RH was below 5 $\mu g.kg^{-1}$ (0.3 $\mu g.kg^{-1}$ – 2.4 $\mu g.kg^{-1}$), which is the acceptable limit for AFB1 residue in food in South Africa, Kenya, Tanzania and Malawi (Kimanya et al., 2008; Rheeder et al., 2009; Kilonzo et al., 2014). AFB1 contamination at 60% RH was way below the international standard of 15 $\mu g.kg^{-1}$, set by the Codex Alimentarius Commision, and 20 $\mu g.kg^{-1}$ set by the United States Department of Agriculture (Wu and Guclu, 2012).

Some techniques for reducing and controlling the moisture content of grain in storage have been reported. Mixing food grade super absorbent polymers (SAPs) with grain can help lower the MC of the grain (Mbuge et al., 2016). Desiccants such as silica gel, quick lime, calcium chloride and zeolite seed drying beads have also been used in drying seeds for storage (Kiburi et al., 2014). Both SAPs and desiccants absorb moisture from the air thus lowering the RH, and consequently, the EMC of the grains. Incorporating such techniques in storage structures can help lower the RH and MC of the grains within the storage structure. Low relative humidity below 70% creates a dry environment that stifles the growth of *A. flavus* and consequently inhibits aflatoxin production (Pratiwi et al., 2015).

Conclusion

Aflatoxin contamination of maize is associated with inadequate post-harvest and storage practices. Typical traditional storage techniques used by small-scale farmers offer little or no control of the storage environment, thus no protection against factors that promote aflatoxin contamination. Aflatoxin contamination of maize can be aggravated at high relative humidity (90%) at typical ambient conditions. Maize kernels stored at these conditions have extremely high levels of aflatoxin and are, therefore, not suitable for human or animal consumption. This study has shown that maize kernels stored at a relative humidity of 60% had AFB1 levels below 5 $\mu g.kg^{-1}$, hence they are safe for human consumption. Controlling aflatoxin contamination of maize during storage necessitates the development of simple storage facilities that can maintain the level of relative humidity below 60%. This study, therefore, recommends research into appropriate technologies that can be used to regulate the relative humidity in storage structures such as the inclusion of super absorbent polymers and desiccants in the design of such structures.

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