Journal of Scientific Research & Reports
9(6): 1-12, 2016; Article no.JSRR.17836
ISSN: 2320-0227

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Incidence of Aflatoxigenic Fungi and Aflatoxins in Maize Cultivated in Highland and Mid-altitude Agro-ecological Zones in Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SC, WW, CB and JG designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors SC, JG and DO managed the literature searches, analyses of the study and performed the ELISA for Aflatoxin quantification. Authors SC and CB identified the fungal species. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JSRR/2016/17836
Editor(s):
(1) Da-Long Guodalong, Department of Forestry, Henan University of Science and Technolgoy, China.
(2) Luis H. Alvarez Valencia, Laboratory of Environmental Biotechnology and Microbiology, CIIBAA, Instituto Tecnológico de Sonora (ITSON), Obregon Sonora, México.
Reviewers:
(1) Amina M. Ahmed El-Imam, University of Nottingham, UK.
(2) Abdullahi, Isa Obansa, Ahmadu Bello University, Nigeria.
(3) Anonymous, Anadolu University, Turkey.
Complete Peer review History: http://sciencedomain.org/review-history/12426

Original Research Article

Received 28th March 2015
Accepted 27th May 2015
Published 25th November 2015

ABSTRACT

Kenya has had recurrent aflatoxicoses of regional epidemiological pattern spanning over three decades now. A comparative study was therefore undertaken aimed at establishing factors contributing to fungal and aflatoxin contamination differences in maize cultivated in two Agro-Ecological Zones.
Methods: A total of 309 maize samples obtained from the Highland Agro-Ecological Zone and

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Mid-Altitude Agro-Ecological Zone of Kenya were analyzed in a three year study, 2008-2010. A purposeful sampling technique was used. Aflatoxigenic fungal contamination involved culture on modified Potato dextrose agar supplemented with Yeast Extract Sucrose agar and 0.3% β-cyclodextrin. Aflatoxin quantification was by a commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit. The findings from these two variables were compared according to the agroecological zone. Similarly, correlation analysis between the grains' moisture content, fungal and aflatoxin contamination was undertaken.

Results: Significant differences existed both in the mean grains' moisture content and mean fungal load for maize samples from the two Agroecological zones (P < 0.001) with 12.8% and 3.0 CFU/g, respectively for Highland samples while Mid-Altitude samples had 13.2% M.C. and 83.5 CFU/g, respectively. A significant agro-ecological relationship was established regarding frequency of aflatoxigenic fungal species, with *Aspergillus flavus* in only 8.4% of Highland samples but predominant in 54.3% of Mid-Altitude samples samples(X^2=16.764, p=0.05). Significant regional differences (P< 0.001) also existed regarding aflatoxin contamination, occurring in only 18% of Highland samples at 2.0 µg/kg mean contamination level and 73.7% of Mid-altitude samples at 14.0 µg/kg mean aflatoxin level. However, weak correlation existed between fungal and aflatoxin contamination in both Agro-Ecological Zones. Use of certified maize seed was a common practice among farmers in Highland Agro-Ecological Zone.

Conclusion: The observed regional differences in both the fungal and aflatoxin load could be attributed to agro-ecological conditions and farming practices. The cool humid agro-ecological conditions prevalent in the Highland Agro-Ecological Zone together with sound farming practices particularly use of certified maize seed could have accounted for the lower contamination levels. Serious research on development of maize varieties that are not only drought resistant but also high yielding and resistant against pathogenic fungal infections including aflatoxigenic fungi is urgently needed so as to encourage farmers in the Mid-altitude Agro-Ecological Zone to adopt modern maize cultivars over the indigenous types.

Keywords: Aflatoxigenic fungi; aflatoxins; ELISA kit; maize; agro-ecological zones.

1. INTRODUCTION

Maize is staple food in Kenya with an average consumption rate of 0.5 Kg/person/day with the Rift-Valley province contributing about 90 % of the annual national production. Trans-Nzoia, Uasin-Gishu and Nandi counties are the major maize producing zones [1,2]. Due to the high consumption and the spectrum of use of maize in Kenya, mycological and aflatoxin safety is of great public health concern. Among the fungi and mycotoxins that have had epidemiological significance with regard to Kenyas' public health is aflatoxigenic fungi, particularly, *Aspergillus flavus* and aflatoxins. Ingestion of aflatoxin contaminated foods evokes acute and chronic pathological human illnesses collectively known as aflatoxicoses [3,4,5].

Aflatoxigenic fungi and aflatoxins are the primary focus of this study due to their prevalent contamination of dietary maize at levels that have caused recurrent fatal aflatoxicosis outbreaks thereby posing a significant hazard to the National food security and public health in Kenya. Kenya, to date, is the only nation worldwide that has repeatedly experienced epidemics of fatally acute aflatoxicosis over the past three decades including 1982, 2001, 2004, 2005 [6,7,8]. The most fatal outbreak was where 150 deaths occurred in several districts of Eastern Province including Machakos, Makueni, Kitui and Embeere between 2004 and 2006 [9,10]. The largest documented aflatoxicoses outbreak in Kenya was in April 2004 involving 317 hospitalized cases and 125 deaths [11,12]. Aflatoxins occurred in 55 % of maize meals at levels greater than the then statutory limit of 20 parts per billion (ppb), 35 % had more than 100 ppb and 7 % had more than 1000 ppb [13].

Other than these recent fatal aflatoxicosis episodes, by virtue of the dietary preferences of maize among Kenyans and the carcinogenic nature of aflatoxins, long-term consumption of sub-optimally aflatoxin contaminated maize meals poses even greater public health hazard relative to the acute cases that is given high publicity sporadically. Recent evidence suggests that mycotoxins may affect families for one or two generations beyond initial exposure [14]. The recurrent aflatoxin incidence in maize harvest including 2.3 million bags in 2010 and the recent reports of detection of aflatoxins in milk implies aflatoxin remains a serious threat to national food safety and security in Kenya [15,16]. In addition to the health concerns, aflatoxins can restrict regional trade on major agricultural crops and crop products including maize and groundnuts.

Market regulations especially for aflatoxins have been strict, with Kenya's tolerance levels downgraded to 10 µg/kg for total aflatoxins in foodstuffs by Kenya Bureau of standards (KEBS) the statutory agency mandated to uphold food quality in 2009 [17]. The high costs of aflatoxin tests is however a deterrent among most of stakeholders in the maize industry. However, continued educational awareness and national surveys on aflatoxin contamination will ultimately result in prevention of contamination along the maize production chain.

Studies undertaken in Kenya on aflatoxigenic fungi and aflatoxins in maize in the past have had little focus on the influence of farming practices and agro-ecological conditions prevailing in the regions historically associated with frequent aflatoxin outbreaks compared to areas that have hardly had these outbreaks. Except for previous findings where the high temperature and periodic drought prevalence in the semi-arid regions were attributed to the higher levels of *A. flavus* and Aflatoxin B1(AFB1) contamination in the lower region of Eastern Province compared to those in North-Rift Province [8,12,18], recent studies have focused on aflatoxin contamination in market maize samples [19].

In the last major aflatoxin outbreak in Kenya, investigation showed that the lower parts of Eastern Province with less than 1500 mm of rain per year and low maize output was greatly affected [20]. A comparative study to establish fungal and aflatoxin contamination of maize cultivated in Highland agroecological zone with characteristic ample rainfall throughout the year and high maize output was therefore necessary. Similarly, no studies have attempted to establish if maize varieties preferred by farmers in these two agro-ecological zones could account for differences in contamination of maize by both aflatoxigenic fungi and aflatoxins. Maize varieties that have a good husk cover with ears that droop at physiological maturity have been found to hinder pre-harvest fungal and aflatoxin contamination. These varieties avoid moisture penetration while in the field before the crop is harvested [2,21]. Information on susceptibility of various maize varieties grown in Kenya in the various agroecological zones to aflatoxigenic strains of *A. flavus* and *A. parasiticus* is still greatly lacking. This study therefore aims to identify these factors and also establish whether contamination of maize by aflatoxigenic fungi is cultivar-specific.

2. MATERIALS AND METHODS

2.1 Sampling Sites and Sample Collection

The study was conducted in Uasin-Gishu County in Rift-Valley Province and two districts historically associated with aflatoxin outbreaks in from lower Eastern namely Kitui and Kibwezi districts. The two study regions were chosen purposefully on the basis of their agro-ecological and levels of maize production differences. Uasin-Gishu district is among the High altitude maize cultivation agro-ecological zone in Kenya with altitude ranges of 2100-2700 metres above sea (7000- 9000) feet. It has a cool and temperate climate with temperature range of 8.4℃ to 27℃ and annual rainfall ranging from 900mm to 1,200 mm. The maize varieties cultivated are high yielding and the region is ranked second in maize output in Kenya. On the other hand, Kitui and Kibwezi districts have low maize output and are under the Mid Altitude maize cultivation agro-ecological zone. The region has comparatively higher temperature ranging 16℃-32℃ and low, unreliable biannual rainfall pattern with a mean of 712 mm. While Uasin-Gishu has no documented aflatoxin outbreaks, Kitui and Kibwezi districts have had a long history of aflatoxicosis over the last three decades.

Sampling of maize was done twice from each of these agro-ecological zones during 2008-2010 growing seasons whereby a total of 215 samples and 94 samples were obtained from the high altitude and low altitude of Uasin-Gishu and Kitui/Kibwezi districts, respectively. The preferred maize varieties were established in each zone. A representative minimum working sample of 2-kg was obtained at each sampling stage. The samples were packed in paper khaki bags and stored at 4ºC until further analysis which was carried out within 72 hours. Fungal isolation was done at Centre for Biotechnology and Bioinformatics, University of Nairobi, Kenya while Aflatoxin analysis was done at BORA Biotech Ltd laboratories, Nairobi.

2.2 Determination of Grains' Moisture Content

The 2 Kg of each representative sample maize grain was ground under aseptic condition into fine flour using Wiley Mill No.1 at National Public Health Laboratories, Nairobi. Precaution to safeguard against cross-contamination between samples during milling was achieved by opening up the milling section of machine, dusting it using sterile brush then spraying off the remaining minute particulate flour using 70% ethanol and wiping dry with sterile cotton wool. The moisture content of each sample was determined using the hot-air oven method [22].

2.3 Isolation and Identification of Aflatoxigenic Fungal Species

The isolation of *Aspergillus* from the maize samples was undertaken using modified Potato Dextrose Agar (PDA) enriched with Yeast Extract Sucrose (YES) agar containing 0.3% β-cyclodextrin. This media is ideal for simultaneous enumeration and detection of afllatoxigenic fungal isolates [23,24]. In the present study, the target aflatoxigenic species were *A.flavus* and *A.parasiticus.* Fungi isolation was by dilution plating technique described for isolation of fungi from powdered foods [25]. Triplicate sub-samples of 10 gm powdered per sample were diluted in 100 ml of sterile double distilled water and vortexed for 1 min. Dilutions of 1ml mixture suspension were inoculated using a sterile glass spreader on a set of triplicate agar plates. Plates were then incubated without illumination at 30ºC for 3 days. After incubation, isolates were identified according colony color and conidia morphology. Isolates having yellowish-green colonies and smooth conidia were classified as *A. flavus* whereas dark-green colonies with rough conidia were classified as *A.parasiticus* [25,26]. Fungal load in each maize sample was expressed as Colony Forming Units per Unit weight in grams (CFU/ g).

2.4 Determination of Aflatoxin Contamination in Maize Samples

Aflatoxin levels in the maize samples were determined by direct competitive enzyme linked ELISA commercial kit, Boratest®, from BORA Biotech, Kenya. The method has a lower detection limit of 2 µg/kg [27,28]. Briefly, the method involved aflatoxin extraction from portion of 5 g finely ground sub-sample using methanol/water solution. The extract was then defatted with hexane and centrifuged followed by supernatant recovery and dilution with buffered saline (PBS). The resulting solution was further diluted with methanol-PBS mixture before aflatoxin quantification on ELISA microtiter plates. The wells of ELISA plate were prior coated with anti-aflatoxin antibody, incubated overnight in a moist chamber then emptied. Any free protein binding sites were blocked using bovine serum albumin in PBS followed by plate washing with Tween 20 solution and semi-dried. Volumes of sample extract and equal volumes of AFB1 standards were added into separate wells. Solution of AFB1-enzyme conjugate was simultaneously added to all wells before 2 hour incubation in darkness followed by plate washing and allowing wells semi-dry. A solution of enzyme substrate was added to all wells so as to establish the extent of binding between anti-aflatoxin antibody and aflatoxin-enzyme conjugate whereby upon incubation color develops. The intensity of resulting color both in the sample extracts and standards was determined by reading absorbance at 450 nm using a spectrophotometer ELISA plate reader (Uniskan II- Labsystems, Finland) [29]. Aflatoxin levels was expressed in µg/kg, equivalent to ppb.

2.5 Data Analysis

Data analysis was by General Lineal Model (GLM) suitable for unbalanced data using *PASW statistics* 18.0 for Windows software (SPSS Inc.) according to Payne et al. [30]. Analysis of variance was performed on mean of variables including Moisture content, fungal and aflatoxin load at 5% (α = 0.05) significance level. Pearson's Chi-square test was used to compare fungal and aflatoxin contamination frequencies according to agroecological zone while Pearson's correlation established the relationship between ecological / independent variable and biological / dependent variables. Fungal and aflatoxin contamination constituted dependent variables while independent variables were moisture content and maize variety/agrological zone.

3. RESULTS

3.1 Regional Variation in Grain Moisture Content of Highland Agro-ecological Zone (HAEZ) and Mid-altitude Agro-ecological (MAEZ) Maize Samples

The moisture content of the maize samples from the HAEZ had comparatively lower moisture content and also narrower range compared to MAEZ samples (Table 1). An analysis of mean moisture content (M.C) according to agro-ecological region showed that a significant difference existed (*$P < 0.001$*) with mean M.C. of

12.8% for HAEZ samples and 13.2% for MAEZ samples. However, the proportion of samples with M.C ≤ 13.5%, the recommended moisture content for safe storage of maize grain was only marginally higher in HAEZ maize samples than MAEZ samples, having been 92.6% and 90%, respectively (Table 1).

Univariate Analysis of variance (ANOVA) among HAEZ maize varieties established that H614 variety had the lowest mean moisture content of 12.6% whereas H628 had the highest mean M.C 13.2% (Fig. 2). This implied that the highest mean M. content among the samples HAEZ maize varieties was similar the mean M.C. among MAEZ maize samples.

3.2 Regional variation in Aflatoxigenic Fungal Contamination of Highland Agro-ecological Zone (HAEZ) and Mid-Altitude Agro-ecological (MAEZ) Maize Samples

The frequency of total aflatoxigenic fungal spp. was comparatively lower among HAEZ than MAEZ samples with only 9.8% and 71.3% of the samples having been contaminated in the two agro-ecological zones, respectively. Similarly, maize from HAEZ had 8.4% of all its samples contaminated by *A. flavus* while *A. parasiticus* occurred in only 1.4% of the samples. On the hand, MAEZ maize had 54.3% of all the samples contaminated by *A. flavus* while *A. parasiticus* was isolated in 17% of the samples. However, the co-occurrence of both aflatoxigenic spp. was rare, having occurred in only 2 out of 94 (0.021%) MAEZ samples whereas this phenomena was not observed in HAEZ samples (Table 2).

An analysis of the regional frequency of the two aflatoxigenic fungal species established a significant relationship between the two agro-ecological zones and the various aflatoxigenic fungal species ($X^2=16.764,\ P=0.05$). The prevalence of *A. flavus* was comparatively higher than *A. parasiticus* in both regions having occurred in 85.7% of the contaminated samples from HAEZ while for MAEZ it was 76.1% of the contaminated samples. In contrast the frequency of *A. parasiticus* among the contaminated samples in the two regions was only 14.3% and 23.9%, respectively (Table 3).

The contamination levels by the aflatoxigenic fungi on account of total fungal load had the highest proportion of samples from both the two study regions within the range CFU≥10<100, whereby HAEZ and MAEZ had 90.5% and 59.7% of their samples within this range, respectively (Table 4). Similarly, *A. flavus* occurred predominantly across all contamination levels in both agroecological zones. However, considering the samples that had ≥500 cfu/g, MAEZ had a higher proportion of its samples at this contamination level compared HAEZ having been 9.0% and 4.8%, respectively.

Table 1. Moisture content profiles of maize grain samples from highland agro-ecological zone (HAEZ) and mid-altitude agro-ecological zone (MAEZ)

Region	Highest M.C. (%)	Lowest M.C. (%)	Moisture content range	Mean M. content (%)	Proportion (%) of samples M.C ≤ 13.5%
HAEZ 215 samples	14.0	10.9	3.1	12.8	92.6
MAEZ 94 samples	14.8	11.1	3.7	13.2±0.1	90

Table 2. Frequency of aflatoxigenic fungi in maize grain samples from highland agro-ecological zone (HAEZ) and mid-altitude agro-ecological zone (MAEZ)

Region	Samples (%)							
	Total aflatoxigenic fungi		*A. flavus*		*A. parasiticus*		Both species	
	AF[a]	RF[b]	AF[a]	RF[b]	RF[b]	RF[b]	AF[a]	RF[b]
HAEZ 215 samples	21	9.8%	18	8.4%	3	1.4%	0	
MAEZ 94 samples	67	71.3%	51	54.3%	16	17.0%	2	0. 021%

[a] Absolute frequency, [b] Relative frequency

A statistical analysis in the variation of means of total fungal load according to agro-ecological region established that strongly significant differences existed ($P < 0.001$). The mean total fungal load was significantly lower in maize samples from HAEZ compared to MAEZ having been 3.0 CFU/g and 83.5 CFU/g, respectively. However, all the 309 maize samples from the two agro-ecological zones had contamination levels within the statutory limits of 1.0×10^4CFU/g recommended by KEBS [16].

3.3 Regional Variation in Aflatoxin Contamination of Highland Agro-ecological Zone (HAEZ) and Mid-Altitude Agro-ecological Zone (MEAZ) Maize Samples

The incidence of aflatoxins was analyzed in a total of 232 maize samples from the two agro-ecological zones showed that 76 out of 156 samples (18 %) and 56 out of 76 samples (73.7%) from HAEZ and MAEZ, respectively had aflatoxins. Similarly, a regional variation was observed where all the contaminated samples from HAEZ had aflatoxin levels within the range > 4.0 ≤ 10.0 ppb and >10.0 ≤ 100.0 ppb while the MAEZ samples had contamination level spread over all the five ranges. The proportion of samples that had more than 10 ppb of total aflatoxins, the statutory maximum contamination level recommended by KEBS was 6.4 % and 17.1% in HAEZ and MAEZ, respectively (Fig. 1).

A statistical analysis by ANOVA established that significant differences existed in the incidence of aflatoxins on agro-ecological basis ($P< 0.001$), with a comparatively lower mean aflatoxin level of 2.0 ppb for HAEZ samples compared to 14.0 ppb for MAEZ samples. Similarly, the highest contamination level was 16.0 ppb for HAEZ samples while for MAEZ samples it was 111 ppb.

3.4 Relationship between Maize Grains Moisture Content, Fungal Load and Aflatoxin Contamination

The relationship between the grains' moisture content and the fungal load in the maize samples on one hand, and also between fungal and aflatoxin contamination on the other hand in the two agro-ecological regions was both positive. However, a positively stronger correlation existed for MAEZ samples than HAEZ samples with a correlation coefficient of R^2=0.0192 and R^2= 0.0044, respectively for the former relationship while for the latter it was R^2=0.0416 and R^2= 0.0004 for samples from MAEZ and HAEZ, respectively.

Table 3. Frequency of *Aspergillus flavus* and *A. parasiticus* in contaminated maize grain samples from highland agro-ecological zone (HAEZ) and mid-altitude agro-ecological zone (MAEZ)

Region	Aflatoxigenic fungal species	
	A. flavus	***A. parasiticus***
HAEZ 21 samples	18 samples: 85.7%	3 samples: 14.3%
MAEZ 67 samples	51 samples: 76.1%	16 samples: 23.9%

Table 4. Absolute and relative frequencies (%) of aflatoxigenic fungi isolated in contaminated maize grain samples from highland agro-ecological zone (HAEZ) and mid-altitude agro-ecological zones (MAEZ)

Region	Mean CFU	Contamination range (CFU/g)	Total Aflato. fungi		*A. flavus*		*A. parasiticus*	
			AF[a]	RF[b]	AF[a]	RF[b]	AF[a]	RF[b]
HAEZ Samples	3.0±1.5	≥500	1	4.8	1	4.8	0	0.0
		≥100<500	1	4.8	1	4.8	0	0.0
		≥10<100	19	90.5	16	76.2	3	14.3
MAEZ Samples	83.5±15.0	≥500	6	9.0	5	7.5	1	1.5
		≥100<500	21	31.3	17	25.4	5	7.4
		≥10<100	40	59.7	30	44.8	10	14.9

[a] Absolute frequency, [b] Relative frequency calculated from contaminated samples: HAEZ= 21: MAEZ= 67

Regarding the relationship between various maize varieties in the two agro-ecological zones as an independent variable and each of the dependable variables, all HAEZ maize varieties except H628 lower mean M.C. compared to the *Kikamba* variety of MAEZ. However, the mean fungal load for all the six HAEZ maize varieties were significantly lower compared to *Kikamba* variety. A similar phenomena was observed regarding the incidence of aflatoxins in the two agroecological zones (Fig. 2).

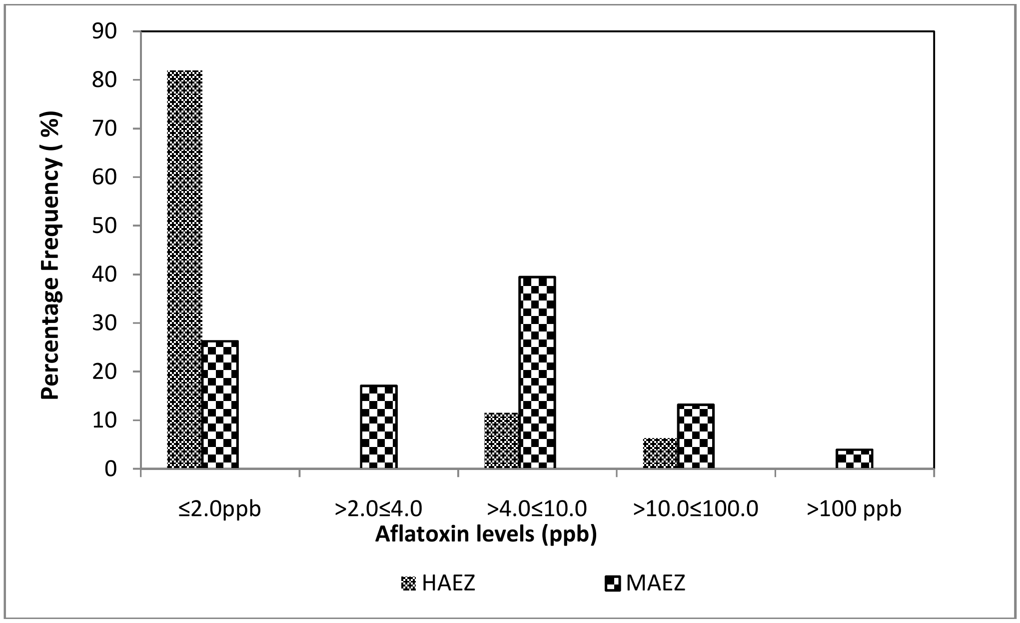

Fig. 1. Aflatoxin contamination profiles in maize grain samples from highland agro-ecological zone (HAEZ) and mid-altitude agro-ecological zone (MAEZ)

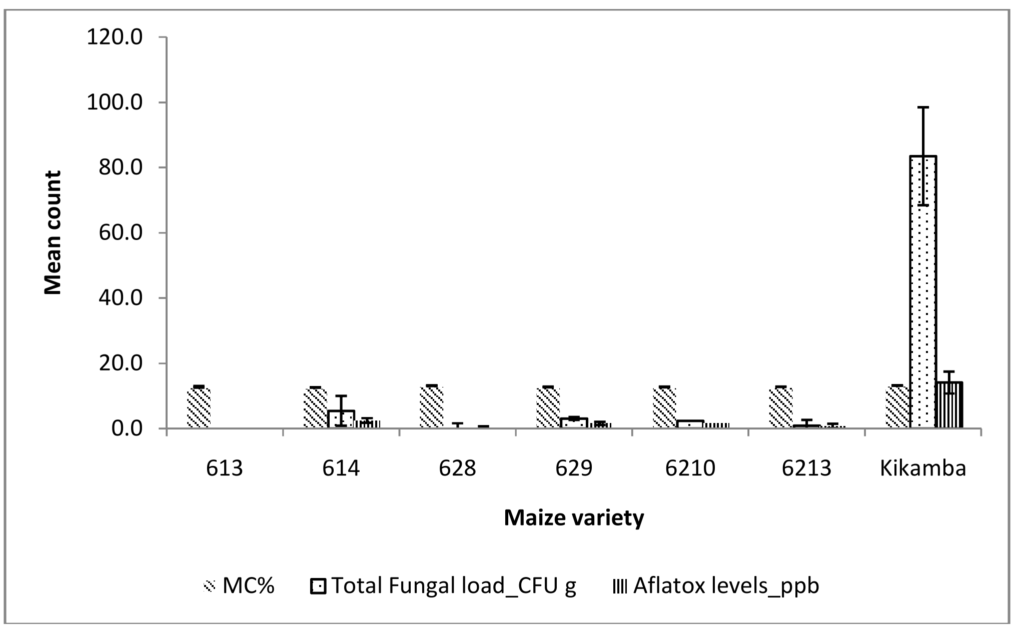

Fig. 2. Relationship between the mean moisture content, fungal load and aflatoxin levels and the maize varieties cultivated in two agro-ecological zones (Highland and mid-altitude agro-ecological zones)

4. DISCUSSION OF RESULTS

Maize samples from High altitude agro-ecological Zone had comparatively lower mean levels for all the three parameters under this study including moisture content, fungal load and aflatoxin levels than samples from Mid-Altitude Agro-ecological maize growing zone. These findings clearly demonstrate that fungal food spoilage of maize is more serious in Mid-Altitude Agro-ecological zones of Kenya compared to the High altitude agro-ecological Zone. The agro-ecological variation in fungal contamination whereby maize samples from Mid-Altitude Agro-ecological zone had significantly higher contamination levels compared to those from High altitude agro-ecological Zone corroborates earlier findings where maize samples from semi-arid regions of Kenya had higher fungal and aflatoxin contamination compared to those from humid regions [18]. Similarly, the observed general dominance of *A. flavus* is in agreement with previous studies on aflatoxin-producing fungi both in maize and peanuts from Kenya [7,8,12,31,32,33]. In other major maize producing agro-ecological zones West Africa [34,35,36] and also in Latin America [37,38,39] maize has been found to have *A. flavus* prevalence.

During the 2004 acute aflatoxicosis outbreak in Kenya, Mid-Altitude Agro-ecological zones including Machakos, Makueni and Kitui districts were greatly affected, *A. flavus* was identified as the causal agent having been isolated in all the analyzed maize samples while *A. parasiticus* was isolated in only 14.4% of the samples [7]. In another study related to the current study on the regional distribution of toxigenic strains of aflatoxigenic fungal spp., *A. flavus* was also more prevalent than *A.parasticus* in maize samples from Mid-Altitude Agro-ecological and High altitude agro-ecological Zone, the latter having had no *A. parasiticus* contamination et al. [12]. In the current study, unlike previous studies undertaken in regions that have had a long history of aflatoxicoses, the approach was identification of factors leading to the predominance of aflatoxigenic fungi thereby resulting in the identification of good management practices effective in controlling the recurrent aflatoxicoses outbreaks in Kenya.

The observed regional difference in the maize grain moisture content and fungal contamination could be attributed to the prevailing agro-ecological conditions and farming practices in the two regions under this study. The combination of drought, windy conditions and high ambient temperatures is the primary environmental factor leading to proliferation of aflatoxigenic fungi, conidia dispersal and subsequent kernel infection in the growing crop [5,40,41,42,43]. Soils from the semi-arid regions of Kenya including Kitui, Machakos and Makueni districts were found to have comparatively higher *A. flavus* levels than soils from the humid zones including Trans-Zoia and Uasin-Gishu districts [18]. The findings of this research where maize samples from drier Mid-Altitude Agro-ecological zone had higher fungal and aflatoxin levels than samples from High altitude agro-ecological Zone which have ample rainfall and humid conditions are in agreement with other studies on aflatoxin contamination of maize in Africa including Senegal [35], Benin [44,45,46] Ghana [47], and Nigeria [34,36,48]. However, a contrasting phenomenon was observed in Uganda where maize from high altitude agro-ecological zone recorded higher mycotoxin levels than those from mid altitude-moist and mid-altitude-dry zones [49].

The farming practices undertaken by farmers in these two agro-ecologically diverse regions under this study could also account for the observed regional variation in the level of maize moisture content, fungal and aflatoxin contamination. The two regions of Mid-Altitude Agro-ecological zone under this study have a bimodal pattern of rainfall therefore maize is grown biannually. However, while this practice is critical in enhancing food security in the region, its main drawback is it leaves little time for proper land preparation and sufficient maize drying. For instance, in 2010, about 2.3 million 90 bags of maize from Eastern province in Kenya was found to have aflatoxin level beyond the statutory levels of 10 ppb whereby the early onset the long rains in February 2010 caught farmers unprepared and the poor storage facilities further exposed maize stocks to the contamination [15]. The rapid turn-over of maize cultivation in the same farm provides ready inoculums infecting subsequent season crop [5,21,50]. In contrast, maize is grown only annually in the High altitude agro-ecological Zone under this study, thereby providing ample window period of about 4 months for land preparation including discarding of maize residues through burning and feeding on livestock.

In this study, an attempt was made to establish whether maize varieties grown in the regions in Kenya considered as high aflatoxin risk would be a contributing factor to the recurrent aflatoxicosis in these areas compared to areas considered low aflatoxin risk. An interesting observation was established whereby while all the farmers in High altitude agro-ecological Zone used commercially certified seeds of Kenya Seed Company, use of indigenous seed varieties especially *Kikamba* that are locally sourced was a popular farming practice among farmers in Mid-Altitude Agro-ecological zone. This finding is corroborated by previous findings on popular maize varieties in the region [51]. The highland seed varieties established popular with farmers in this study included H614D, H629, H6210, H6213, H613, H624, and H628. These Highland Zone varieties are high yielding and have a good husk cover with ears that droop at physiological maturity hence avoiding pre-harvest fungal contamination [14,21,52]. The H614D was found to have the mean lowest moisture from findings of this study. In contrast, while the indigenous Mid-Altitude Agro-ecological zone maize varieties are drought resistant, they not droop at maturity instead they remain erect thereby predisposing it to birds' damage, water accumulation and subsequent fungal infection. Further, the practice of using old grain stock as seeds together with mixed crop farming system commonly practiced among Mid-Altitude Agro-ecological zone farmers could be the main factor accounting for the comparatively high fungal and aflatoxin contamination in Mid-Altitude Agro-ecological maize samples. This practice has been found to encourage cross-transfer of toxigenic strains from one infected crop to another in the maize farm ecosystem in studies from Nigeria and Uganda [48,49].

It is noteworthy that though farmers in Kenya are now aware of the association between aflatoxin contaminations with inadequate drying, the importance of avoiding contact between the maize and the soil is still unclear [14]. This accounts for the drying of maize grain on road surfaces, a practice common among commercial maize traders especially in Rift-Valley province. It is important to promote not just awareness of aflatoxins among maize farmers and traders but also an in-depth knowledge of the problem along the entire food chain. This would educate farmers even in areas of high maize productivity zones of the health risks associated with feeding domestic animals including poultry, fish and dairy cows on aflatoxin contaminated feeds as this will eventually result in transfer of the toxins to animal products including meat, eggs and milk. This concern has recent been raised by International Livestock Research Institute [16]. The overall effect of reduced aflatoxin contamination in maize is enhanced national food safety and food security.

5. CONCLUSION

The findings from this research demonstrate that agro-ecological conditions and farming practices, particularly use of certified maize seeds accounted for the lower fungal and aflatoxin contamination levels in the High altitude agro-ecological Zone compared to Mid-Altitude Agro-ecological zone maize samples. Therefore provision of subsidized commercial high yielding and fungal resistant Mid Altitude Agro-ecological zone maize varieties together with continuous educational awareness and national surveys on aflatoxin contamination in Kenyas' major staple food crops will ultimately result in reduced aflatoxins along the entire food production chain. Similarly, farmers in the aflatoxin hot spots of Kenya should be encouraged to diversify farming, particularly in view of the fact that maize cultivation in the region compared to the High altitude zones, ought to be for domestic rather than commercial purposes. Instead, adoption of drought resistant, quick maturing food crops including sorghum, millet and cassava would act as ideal alternatives both as staple dietary food crops and commercial crops particularly under the prevalent semi-arid agroecological conditions. This will ultimately contribute to enhanced public health in Kenya through improved national food safety and food security.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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