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The effect of harvest, sorting and drying practices on aflatoxin contamination of maize

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Abstract

Aflatoxins are the result of fungal metabolites that contaminate agricultural produce and can cause death to both humans and animals. The risks of using contaminated food and feed with aflatoxins have increased due to environmental factors, pre-harvest, post-harvest and socio-economic factors. This study revealed on harvesting, drying, and sorting practices that can reduce aflatoxin contamination. Experiments were designed in three districts; Kilosa, Gairo, and Mvomero with five (5) treatments replicated nine (9) times under farmers' conditions. Samples were collected during harvesting, drying, and sorting; and analyzed for aflatoxin B1 using High Performance Liquid Chromatography (HPLC) at Tanzania Bureau of Standards (TBS) laboratory. Analysis of variance and comparison of means for moisture content, mold levels, grain damage, and aflatoxin levels were performed using GenStat® Executable release 16 Statistical Analysis Software. Results indicated that aflatoxin contamination levels were lower at maize grain harvested into bags (456.9µg/kg) compared to maize harvesting onto the ground (889.1µg/kg). It was also observed that maize dried on tarpaulin and raised platform had significantly ($p<0.05$) low aflatoxin levels (65.5 µg/kg, 67.1µg/kg respectively) while maize dried on the ground had higher aflatoxin infestation (179 µg/kg). Again, sorting maize by color significantly ($p<0.05$) reduces aflatoxin contamination. These results indicate that proper postharvest management of maize, such as harvesting maize on bags, drying maize on tarpaulin, raised platform and sorting maize by color gives the lowest aflatoxin contamination levels. Hence, proper education to farmers on harvesting maize using container/bags and drying maize on raised platform to be established in farmer level.

Keywords: Aflatoxin; Contamination; Drying; Harvesting

1. Introduction

Aflatoxins are secondary metabolites primarily produced by fungi including *Aspergillus flavus* . The fungus is widely distributed in nature and is mostly found in peanuts, corn, and rice crop [1]. *Aspergillus flavus* can grow on crops before harvest, during harvest, drying, sorting, and storage [1]. The main supporting conditions for A.flavus growth are relative humidity that is higher than 85%, and temperature above 27 °C [2]. Aflatoxin contamination is more common in the tropics and sub-tropic regions including Morogoro region in Tanzania. This region is characterized by the rainy season that favors the growth of *A. flavus* [1].

Formerly, more than 18 different types of aflatoxin have been discovered, and the most common and important ones are aflatoxin B1, B2, G1, and G2. These names were given due to their characteristic of absorbing and emitting light. Aflatoxin B1 and B2 show blue fluorescence under ultraviolet light at 425 nm, while G1 and G2 appear green under UV ultraviolet light at 540 nm [3]. The International Agency for Research on Cancer (IARC) classifies aflatoxin B1 as a

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member of the group I carcinogens whose toxicity is the most dangerous to human health [2]. Aflatoxin B1 has been reported to bind with DNA and alters its structure causing genotoxicity [4].

The incidence of aflatoxin in food will significantly affect quality and safety of the food. Moreover, it will result in economic loss, and death for both humans and animals. The outbreak of aflatoxin in Tanzania that occurred between May and November 2016, affected 68 people, of which 20 died, making a case fatality rate of 30% [5]. The health problems caused by aflatoxin are immune suppression, low birth weight, stunted growth in children and liver cancer diseases. Post-harvest management's practices are key factor to be considered in reducing aflatoxin contamination. Many studies reported in different post-harvest managent practices used in controlling aflatoxin yet not showing those exactly measured used in farmer level. Therefore, in this study, the effects of harvest, sorting, and drying practices under different conditions on the growth of A.flavus and the production of aflatoxin were investigated.

2. Material and methods

The main materials used in this research were polythene bags for maize harvest, a tarpaulin sheet, and a raised platform for drying maize. Also, moisture meter was used for checking the moisture percentage for maize grain, while temperature, relative humidity, and sunshine were recorded in the nearby meteorological stations. Aflatoxin B1 and total aflatoxin analysis were conducted using high-performance liquid chromatography (HPLC) with fluorescence detection.

2.1 Study area

The study was conducted in three districts in the Morogoro region, namely Kilosa, Gairo, and Mvomero. Kilosa district is located about 76 kilometers from the Morogoro region headquarters (latitude 6° and 42´ South, and longitude 36° and 48´ East) and is elevated at 489 m above sea level (Figure 1). The district is characterized by sandy loam soil and predominantly grassland vegetation. The average annual rainfall ranges between 800 and 1400 mm [6] distributed in two rain seasons; the short rains between November and January and the long rains between March and early June. Regardless of the two rain seasons, the pattern and amount of rainfall in the district allow for only one harvest of the main staples per cropping season [6]. Also, the climatic conditions of Kilosa district favor maize production.

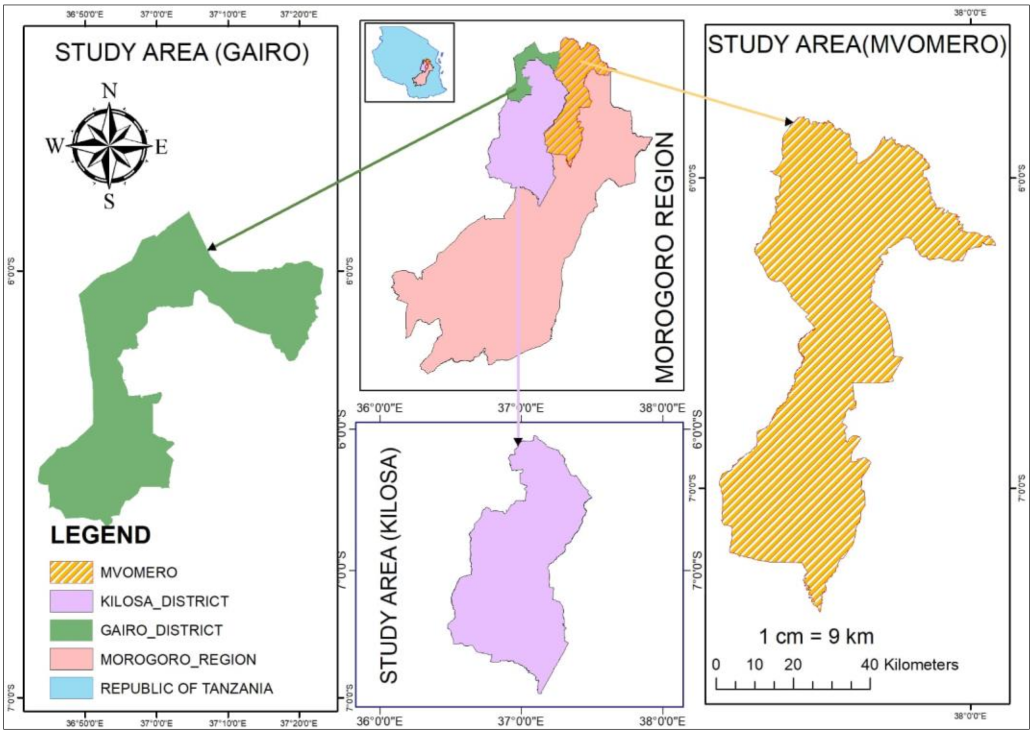

Figure 1 Morogoro region map showing Kilosa, Mvomero, and Gairo Districts where the effect of maize harvesting, sorting and drying practices were conducted among the respondent farmers

Gairo district is located at 36° 45' E and 6° 30' S and an altitude of about 1000 m above sea level (Figure 1). The district receives an average rainfall of 499 mm per year. The main crops grown include maize, cassava, sweet potatoes, cotton, lablab, soya beans, and pigeon peas.

Mvomero district is located between latitudes 05° 80′ and 07° 40′S and between longitudes 37°20′ and 38° 05′E and altitude of 300 - 400m above the sea level. The annual rainfall is variable depending on the altitude; about 800 to 1000 millimeters of rainfall are received near the coast, while in the inland areas towards Dodoma and north of the Wami Sub-Basin, the average rainfall is 500 to 600 millimeters per year [7]. The common crops grown are maize, rice, and sorghum.

These districts were purposefully selected due to their different agro-ecological zones with temperatures above 27°C, atmospheric humidity levels above 62%, and annual rainfall ranging from 800 mm to 1600 mm, which are the main predisposing factors of aflatoxin contamination [8].

2.2 Field establishment

The study was carried out from April to June 2022 during the maize harvesting seasons in Kilosa, Gairo, and Mvomero districts. The test materials were evaluated using a randomized complete block design arrangement with five replications in nine (9) selected villages. No fertilizer, pesticides, or supplementary water were applied, and also no seed treatment before planting was applied.

The assessment of the effect of harvest and drying practices on aflatoxin contamination was divided into two harvesting practices:

- Manual harvesting of maize followed by collection in bags.
- Manual harvesting of maize followed by collection on the ground.

Also drying treatments were divided into three practices:

- Drying maize on the ground,
- Drying maize on a tarpaulin sheet
- Drying maize on a raised platform.

The maize grain was dried up to the recommended dry basis moisture content of 12-13%. The samples were later subjected to aflatoxin testing using high-performance liquid chromatography (HPLC).

2.3 Data Collection

2.3.1 Weather data

Ambient temperature, relative humidity, sunshine duration, evaporation, and rainfall data were collected and recorded at the Tari-Ilonga weather stations center in Morogoro region.

2.3.2 Moisture content determination

Moisture content of maize samples was measured using moisture meters, which were calibrated to ensure accuracy. To determine the moisture content, maize samples were initially shelled. Then, a total of 0.5 kg maize sample was filled in the moisture meter loader, after which the loader was emptied into the analyzer. The results were read using the display window on the moisture meter.

2.4 Aflatoxin analysis under high-performance liquid chromatography (HPLC)

2.4.1 Sample Extraction

Five grams of sample are taken into erlymeyer flask and then 100 mL (70:30 Methanol:Water) of extraction solvent are added into the flask 250 mL containing the sample. The mouth of the flask are been covered with aluminium foil and Shake using gyratory shaker for 30 minutes at 250 rpm. Funnel are been placed into the funnel holder, filter extract into a sample container using filter paper (whatman no1) [9]. The top layer was filtered through Whatman no 1 filter paper into clean and separately labeled 50 mL plastic centrifuge tubes.

2.4.2 Clean-up

During clean-up, 6 mL of extracted sample was passed through a solid phase extraction (SPE) column by gravity which had 1.5g of 50:50 (w/w) Alumina Neutral/Octadecyl (C18) that was sandwiched between two filter discs. The filtrate of each sample was collected in a labeled 7 mL vial and 4 mL of the filtrate was taken from each vial separately and dried under a gentle nitrogen stream at room temperature.

2.4.3 Derivatization

The residue obtained in the previous step was reconstituted in 400μL acetic acid solution, vortexed for 10 s at maximum speed, and then heated at 65 °C in a heating block for 15 min. The solutions obtained from this step were incubated for at least 20 h at room temperature before the HPLC analysis.

2.5 HPLC and its conditions

HPLC (Waters 2695 separation module) fitted with a vacuum degasser, a quaternary pump, an automatic sample injector, a Waters 2475 Multi-wavelength fluorescence detector, and software to control the instrument, data acquisition, and data analysis was used for separation and quantification of aflatoxin B1. The prepared sample was injected automatically and an injection volume of 20 μL was used. The mobile phase consisting of water and acetonitrile was pumped at a flow rate of 1.0 mL/min. The total run time in the HPLC was 33 min.

2.6 Data analysis

Analysis of variance and comparison of means for moisture content, mold levels, grain damage, and aflatoxin level was performed using GenStat® Executable release 16 Statistical Analysis Software to compare harvesting and drying treatments. The means were compared by Duncan's multiple range test (DMRT) at 5% probability. Regression (R^2) between weather factors and aflatoxin infestation was established.

3. Results

3.1 The effect of maize harvesting and drying practices in aflatoxin contamination

3.1.1 Moisture content

Statistical analysis justified significant difference ($p<0.001$) between drying and harvesting techniques adopted by farmers. Maize dried on raised platform and tarpaulin lost moisture significantly and were effectively dried to 12% dry moisture contents. Maize dried on the ground reached the moisture of 13.22%, while the maize harvested in bag was dried to 15.22% and that harvested on the ground had the highest moisture content of 16.22%. (Table 1). Again**,** Drying and harvesting treatments had no significant variability on mold infestation ($p>0.05$) (Table 1)

3.1.2 Grain damage

Harvesting techniques and drying methods had significant effect on grain damage ($p<0.001$). Maize harvested into bags and the maize dried on raised platforms had minimal damage of 30.22% and 31.11%, respectively. On the other hand, the maize dried on the ground, dried on a tarpaulin and that harvested onto the ground had higher damage of 40.67%, 41.22% and 42%, respectively (Table 1)

3.1.3 Aflatoxin B1 level

The level of aflatoxin infection was significantly higher for maize harvested onto the ground (730.4 μg/kg), followed by maize harvested into bags (519.6 μg/kg) ($p<0.001$). The maize dried on a tarpaulin, a raised platform and the ground had significantly low aflatoxin levels (65.5 μg/kg, 67.1 μg/kg and 179 μg/kg, respectively) (Table 1)

Table 1 The effect of drying and harvesting practices on moisture content, mold infestation, grain damage, and aflatoxin level

Post-harvest practices	Moisture content (%)	Mold (%)	Damaged grains (%)	Aflatoxin B1 level (μg/kg)
Dry maize on ground	13.22^b	71.56^a	40.67^b	179^a
Dry maize on raised platform	12^a	59.11^a	31.11^a	67.1^a

Dry maize on tarpaulin	12[a]	59[a]	41.22[b]	65.5[a]
Harvest maize into bags	15.22[c]	68[a]	30.22[a]	519.6[b]
Harvest maize onto ground	16.22[d]	69.89[a]	42[b]	730.4[c]
Grand mean	13.37	65.5	37.04	312
Cv%	3.9	9	8.7	30.3
P-value	<.001	0.084	<.001	<.001

Means carrying the same letter along the column were not significantly different under Duncan's Multiple Range Test (DMRT) at p<0.05

3.1.4 Effect of moisture content, mold infestation, and grain damage on aflatoxin level

There was a significant ($p<0.001$) positive relationship between moisture content and aflatoxin level ($r=0.777$, $R^2=0.605$). Mold infestation had a positive relationship also, but was not significant similar to the level of damaged grains with a negative relationship, which was not significant as shown in Figure 2 and Table 2.

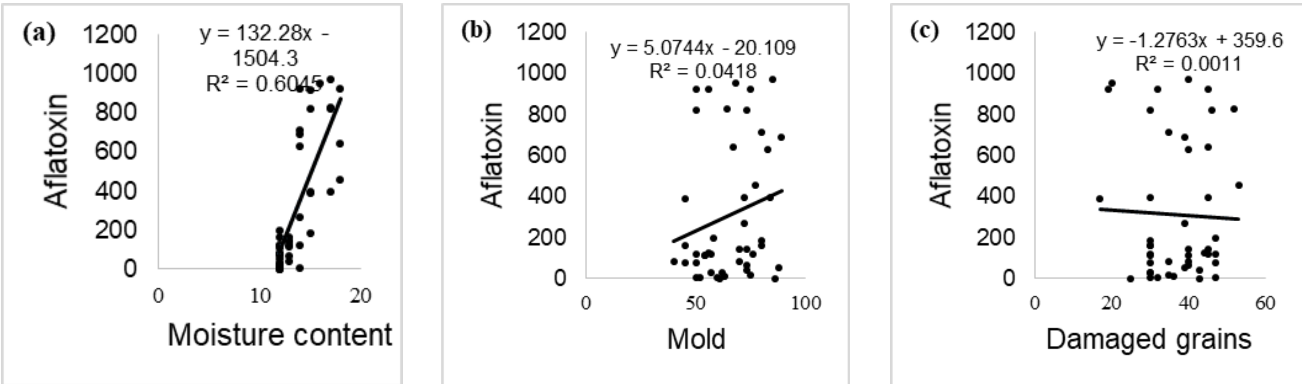

Figure 2 Regression relationships between aflatoxin level with (a) moisture content, (b) mold infestation and (c) grain damage

Table 1 The correlation and regression analysis shown only moisture content had a significant effect on aflatoxin

	Moisture content	**Mold**	**Grain damage**
p-value	<0.001	0.177936	0.830609
R-squared	0.604488	0.041797	0.001076
R	0.777488	0.204444	-0.032804

3.1.5 Weather parameters and their effects to aflatoxin level

There was significant positive relationship between aflatoxin infestation with temperature ($p<0.001$, $R^2=0.36$, $r=0.55$) and relative humidity ($p<0.001$, $R^2=0.35$, $r=0.58$). Rainfall also had positive relationship with aflatoxin infestation ($p<0.05$, $R^2=0.13$, $r=0.35$) (Table 3). There were negative relationships between sunshine and evaporation with aflatoxin. However, their relationships were not statistically significant (Figure 3, Table 3).

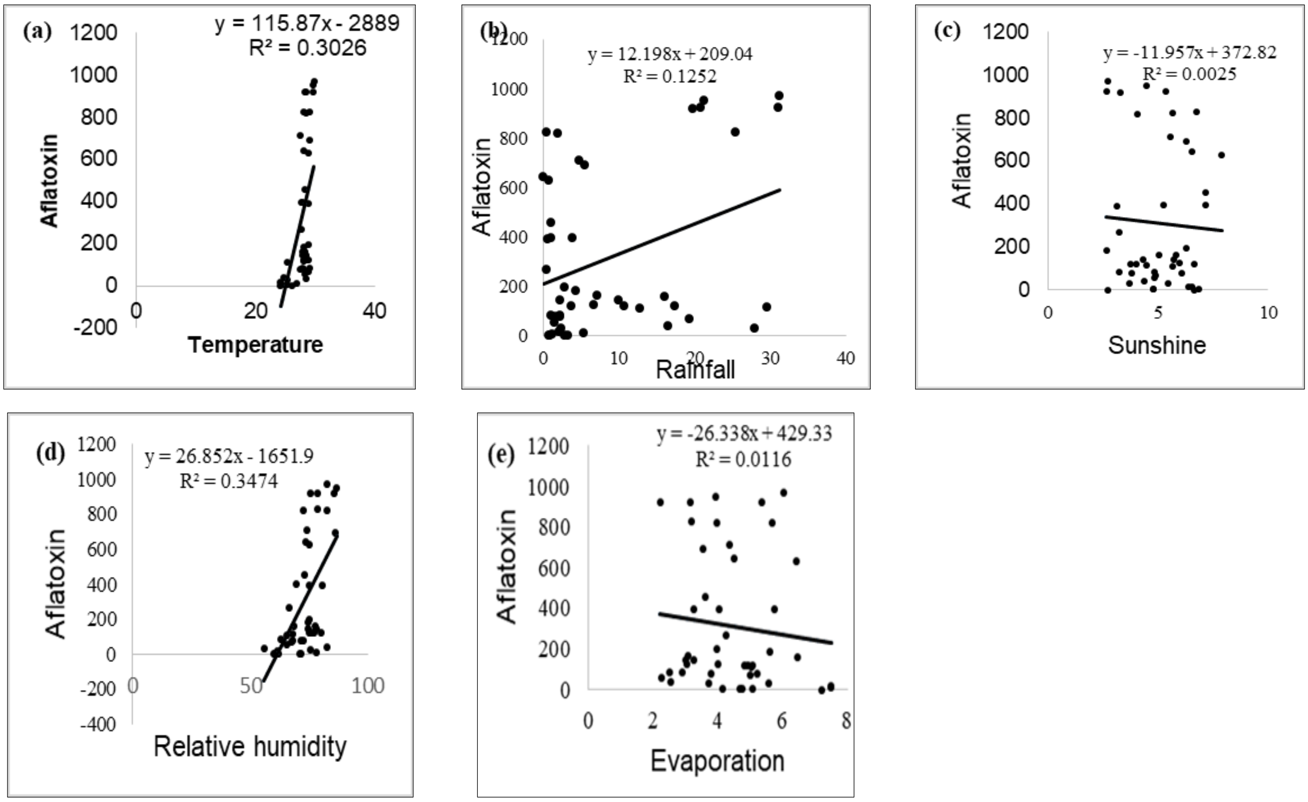

Figure 3 The regression relationship between weather parameters and aflatoxin levels

As shown (Figure 3), temperature, rainfall and relative humidity had strong positive relationship with aflatoxin growth while other weather parameters had weak relationship (Table 3).

Table 3 Regression and correlation relationship between weather parameters and their effects on aflatoxin infestation

	Temp	**Rainfall**	**Sunshine**	**RH**	**Evaporation**
p-value	<0.001	0.017123287	0.743040254	<0.001	0.481787609
R-square	0.30263459	0.125153811	0.002525389	0.347410919	0.011574247
R	0.550122341	0.353770845	0.050253244	0.589415744	0.107583673

3.1.6 Effect of sorting on aflatoxin infestation

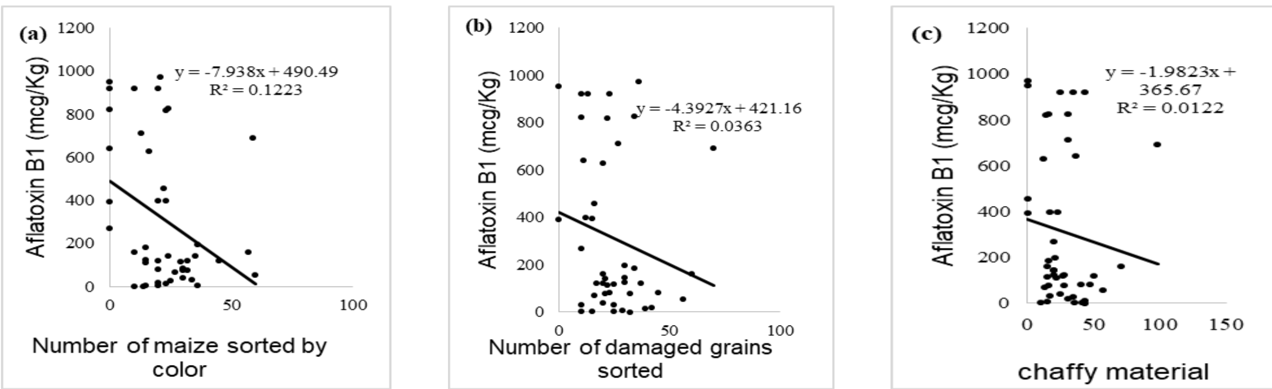

Figure 4 Relationship between aflatoxin level with sorting (a) by color, (b) grain damage, and (c) chaffy materials

Numerous practices were conducted to reduce the number of colored maize grains, damaged grains, and chaffy materials from sampled maize. The intensity of sorting proved to reduce the aflatoxin intensity (Table 4 and Figure 4). For any sorting practice, the increased intensity of sorting reduced the level of aflatoxin after sorting.

Sorting maize by color significantly reduces the aflatoxin ($p<0.05$). The fact is, that sorting maize by color removes infected maize (Table 4).

Table 4 Regression and correlation relationship between aflatoxin levels with sorting practices

	Sorted by color	Sorted by damaged grain	Sorted by chaffy materials
p-value	0.018507	0.210228649	0.469612215
R2	0.122346	0.036260047	0.012226891
R	-0.34978	-0.190420711	-0.110575273

Figure 5 shows the variability of each sorting technique to the resulting aflatoxin level identified after sorting.

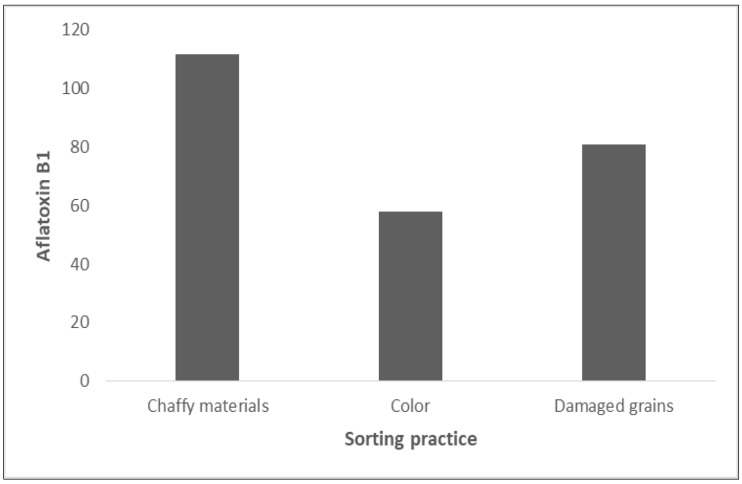

Figure 5 Aflatoxin level identified after sorting against sorting practice

4. Discussions

4.1 The effect of maize harvesting and drying practices on aflatoxin contamination

Field observations have shown that on average, aflatoxin contamination levels were lower in maize harvested into bags compared to maize harvesting onto the ground. The high aflatoxin levels in maize harvested onto the ground were attributed to high moisture content and adverse conditions of wet and humid weather, which provided conducive conditions for fungal invasion and consequently aflatoxin production. This confirmed the findings of [10] who reported that harvesting maize onto the ground when it is rainy and moist resulted in high aflatoxin levels in maize grain. The findings were also consistent with the findings by [3] who found that aflatoxin contamination was positively correlated with wet weather during harvest (rainfall). It has also been shown that harvesting maize into bags lowers the level of aflatoxin contamination. This concurs with the study results by [11] that when farmers harvest their maize into containers it will result in lower levels of aflatoxin contamination.

The correct drying of harvested maize is very important, as the use of appropriate drying material can help to lower fungal growth. At harvesting stage, maize grains have higher moisture content (15-17%) and must be dried to 12-13% to prevent the growth of fungi. This is in agreement with the study by [12] that drying methods greatly influence the resistance of maize to fungal attack. It has been established from the results of this study that both drying maize on raised platforms and on tarpaulin were effective in reducing the moisture content of maize to the recommended level of 12-13%, thereby reducing the chances of heavy aflatoxin contamination on the maize grains. The tarpaulin drying method was more rapid in reducing moisture levels compared to the raised platforms. Nevertheless, the advantage of the raised platform drying method over tarpaulin drying was that exposure of the maize grain to sunlight provided increased air circulation, which led to efficient and effective drying, resulting in the lower fungal invasion. This confirmed the findings that if drying is too rapid, there are alterations in the maize grain that favor fungal infection [13]

High aflatoxin contamination levels with the tarpaulin drying method could also be a result of weather conditions. Postharvest abrupt rainfall during the drying period resulted in wetting of the grain and prevented drying of the grain during sun drying in the open space on some days when it rained all day. This resulted in the creation of moist conditions conducive for aflatoxin production by the fungi. This was not the case with the raised platform since the grain was covered with leaves and thereby preventing water from reaching the grain and ensuring exposure to air circulation all the time. One of the disadvantages of drying maize on tarpaulins is the time and effort required to gather the maize grain together and cover them during rain showers and spread the grain as soon as possible to continue drying. This is difficult and the adverse moist conditions favor fungal invasion and aflatoxin production. However, in general, it has been observed that both the raised platform and the tarpaulin drying methods were more effective in the prevention of aflatoxin contamination on maize grain than drying maize on the ground. Moreover, the raised platform and tarpaulin drying methods ensured that the maize grain attained the recommended moisture content (12-13%). Also, crop not in direct contact with the soil, thereby preventing easy access of fungi to the grain and thus ensuring minimum fungal invasion.

4.2 Effect of moisture content, mold infestation, and grain damage on aflatoxin level

There was a significant ($p<0.001$) positive relationship between moisture content and aflatoxin level. This implies that as moisture content increases fungal growth is favored. This study is supported by [14] who revealed a decrease in moisture level of grain results in low level of aflatoxin infestation. Reduction of moisture content of maize during field drying depends on the weather, and may vary from place to place. Field drying helps to reduce the moisture of the grain, thus reducing the time required to dry the grains at home. Furthermore, it reduces the weight of maize grain to be transported to the farm yard and inccuse few problems during drying.

Again, the other factors that can lead to fungal growth are mold infestation as well as damaged grain. The study found that mold infestation had a positive relationship with aflatoxin level, but damaged grain show negative relationship with aflatoxin level. High temperature and humidity favor the growth of molds growth and this results into high aflatoxin contamination. Study by [15] reaved that most of sample analyzed for aflatoxin has high level of contamination which caused by high rise in mold growth.

4.3 Weather parameters and their effects on aflatoxin level

The effect of temperature, rainfall, and relative humidity have positive relationship to fungal growth that are highly resulted to aflatoxin contamination. Again, Most the studies have shown that weather conditions directly influenced host susceptibility to aflatoxin contamination [16]. The differences in the intensity of aflatoxin contamination between Kilosa, Gairo, and Mvomero districts could be attributed to temperature, rainfall, and relative humidity. In general, Mvomero district had significantly higher aflatoxin contamination levels compared to Kilosa and Gairo districts. This was correlated to higher than normal temperatures (≥30 °C) and late season rainfall, which created warm and moist conditions suitable for fungal growth, and subsequent higher aflatoxin contamination levels on the maize grain. These outcomes are similar to earlier accounts that temperature, rainfall, and more humid conditions tend to aggravate aflatoxin levels as they enhance the growth of *Aspergillus* species and production of aflatoxins in maize compared to drier climatic conditions [15]. Furthermore, studies by [17] reported that the optimal temperature range for the production of aflatoxin is approximately 25–30 °C, which is in agreement with this study. The study also recorded higher aflatoxin B1 contamination levels in the maize grain above the recommended 10 μg/kg (US standards) in Kilosa, Gairo, and Mvomero districts. This could be a result of higher air temperatures (≥30 °C) along with elevated relative humidity (≥70%), which provided optimum conditions for fungal invasion, especially for the *Aspergillus* section *Flavi* and later production of aflatoxins. This was consistent with the findings by [18] who reported that environmental conditions that favor the *Aspergillus* group of fungi included high soil or air temperature (25–30 °C), and high relative humidity (70–85%).

4.4 Effect of sorting on aflatoxin infestation

Sorting reduces aflatoxin intensity, especially sorting by color. The study found that sorting of maize by color reduce aflatoxin level ($p<0.05$). The fact is, sorting maize by color removes infected maize that can highly favor the growth of insects/pests which results in fungal growth. Similar findings were reported from other studies conducted in various parts of Tanzania by [19,5] reported that sorting of maize by color reduces the rate of aflatoxin contamination. Additionally, [20] reported that, in Nigeria there was a higher level of aflatoxin contamination in unsorted maize compared to sorted one. Furthermore,[21] reported that the sorting practice reduced the levels of aflatoxin contamination in grains by 40%-80%

5. Conclusion and recommendations

The results of the assessment of different harvesting practices and different drying methods conclude that harvesting maize onto the ground results in the highest levels of aflatoxin, while harvesting maize into bags lowers levels of aflatoxin contamination. Also, drying maize on the ground results into high levels of aflatoxin but drying maize on raised platforms and tarpaulin sheet are more effective in reducing aflatoxin contamination. However, the implementation of such good postharvest handling practices requires close monitoring at the farmer level. It may be interesting to research into best methods of intervention to make farmers adopt good harvesting and post-harvest practices. Besides, it is difficult to avoid operating in the optimal temperature range for the production of aflatoxin (between 25 and 30 °C) in the study area. Wet and humid conditions quite evidently aggravate aflatoxin levels. Scenarios may be useful to better understand the necessary trade-offs to be made by the farmer to optimize harvesting times and drying methods depending on the local context, more specifically on the availability of tarpaulin sheets, making raised platforms, harvesting maize into bags or containers and weather forecasts. Therefore, farmers are advised to dry their maize on tarpaulin sheets or raised platforms since they have shown to have good efficacy in lowering moisture level and aflatoxin infestation. Moreover, proper maize sorting before storage should be advocated. Also, more research is required to investigate and design the color sorter machine that could be used to reduce aflatoxin exposure to farmers and grain consumers.

Compliance with ethical standards

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Authour Contribution

Counceptualization up to developments of the manuscript: Christina Wuiya, review, editing, supervison of the work: Prof.Valerian. C. K. Silayo, Dk. Frida Nyamete and Dk. Ramadhani Omari Majubwa.

Disclosure of conflict of interest

No competing interest.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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