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OCCURRENCE OF AFLATOXIGENIC FUNGI AND AFLATOXINS IN MAIZE GRAINS AND ASSOCIATED AWARENESS AND HANDLING PRACTICES AMONG FARMERS AND TRADERS IN SOUTH SUDAN

Bilal EK[1]*, Owaga EE[1] and DM Njoroge[1]

Emmanuel Kenyi Bilal

*Corresponding author email: emmanuelkenyi2@gmail.com

[1]Institute of Food Bio-resources Technology, Dedan Kimathi University of Technology (DeKUT), Private Bag - 10143, Dedan Kimathi, Nyeri, Kenya

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ABSTRACT

Maize is a popular staple food among the urban population of South Sudan. However, due to inadequate food safety surveillance and enforcement systems, there is limited information on the aflatoxin safety of maize grains at farm and market levels. Therefore, this study aimed at assessing aflatoxins awareness and handling practices of maize grains among randomly selected farmers (n=30) and traders (n=30) as well as determining the moisture content, Aspergillus species counts and aflatoxins contamination. A cross-sectional descriptive study was carried out in Magwi maize producing areas, Juba retail/wholesale markets and Nimule border points of South Sudan. Moisture content, fungal and aflatoxins contamination in maize grain samples were determined using dry air oven method, dilution plating technique, and High-Performance Liquid Chromatography (HPLC), respectively. The respondent results showed that farmers (97%) and traders (83%) were unaware of aflatoxins. About 83% of the farmers dried their maize grains on tarpaulins, for 4 - 5 days (77%). In addition, most farmers (77%) stored their maize grain bags on raised platforms, whereas most traders (73%) stored grains on bare ground. All the maize grains met the required moisture content limit (below 13.5%). Maize from Nimule main park had the highest levels of contamination with Aspergillus flavus (9 log CFU/g), *Aspergillus parasiticus* (12 log CFU/g), aflatoxin B_1 (505.56 µg/kg) and total aflatoxins (1,032.19 µg/kg). Maize from Gudele market was contaminated with *Aspergillus parasiticus* (12 log CFU/g), and aflatoxin B_1 (76.55 µg/kg), and had a total aflatoxin content of 94.09 µg/kg. Omeo farmers' maize grains had the least levels of contamination of *Aspergillus parasiticus* (6 log CFU/g), aflatoxin B_1 (4.39 µg/kg), and total aflatoxins (7.83 µg/kg). In addition, Aspergillus flavus was not detected from Omeo farmers' maize grains and no aflatoxins were detected from Agoro and Paluonganyi farmers' grains. This study recommends wider aflatoxins awareness and regular aflatoxin screening of maize grains by the relevant stakeholders in South Sudan.

Key words: aflatoxins, aflatoxigenic fungi, awareness, farmers, handling practices, maize, South Sudan, traders

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INTRODUCTION

Maize is an important staple food for more than 1.2 billion people in Sub-Saharan Africa and Latin America [1]. In South Sudan, sorghum and maize are the major cereals consumed with higher per capita consumption of maize in urban areas (3.70 kg per capita per month) than in the rural areas (2.63 kg per capita per month) [2]. Maize is susceptible to fungal growth especially *Aspergillus spp.* and subsequent contamination by aflatoxins (B_1, B_2, G_1, G_2). Aflatoxins are associated with various health effects such as immunosuppressive, carcinogenic, mutagenic and teratogenic [3]. South Sudan has recorded 5,900 deaths in relation to cancer in 2014, and the prevalence rate is on the rise, which is a great concern to the health and medical community [4]. In recent past, multiple maize related outbreaks of acute aflatoxin exposure have occurred in Kenya and Tanzania [5].

Aflatoxins are persistent and their contamination can occur pre- or post-harvest, and at various points along the maize supply chain [6]. The extent of food and feed contamination with aflatoxins varies with geographical location, agricultural and agronomic practices [7]. South Sudan is situated in a temperate climatic region that is conducive for the growth of several fungi, such as *Aspergillus spp.* in foods [8]. Poor handling practices are factors commonly associated with aflatoxin contamination in developing countries [9]. Other factors include inadequate aflatoxin awareness, and inefficient regulatory enforcement [10]. In South Sudan, there is limited data on the aflatoxin safety of the maize, largely because of the inadequate food safety surveillance and enforcement systems. Therefore, there is a need to obtain information on knowledge and handling practices among the traders and the farmers in relation to prevalence of fungi and aflatoxins contamination in maize grains. Subsequently, this information could be used to guide the relevant authorities and stakeholders on the interventions required for prevention of aflatoxins contamination in maize. Therefore, the main objective of this study was to assess aflatoxins awareness and handling practices of maize grains among farmers and traders, as well as to determine regulatory compliance with regards to the moisture content levels, occurrence of Aspergillus species and aflatoxins contamination in maize grains from selected maize producing areas, markets and import border points of South Sudan.

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MATERIALS AND METHOD

Study areas

The study was carried out in selected maize producing areas of Magwi County (Agoro, Omeo, Paluonganyi), retail / wholesale markets of Juba (Konyo-Konyo, Customs, Gudele), and Uganda-South Sudan transit border point (Nimule). Juba was chosen because of its strategic location as the Capital City, and most of the national trading activities for agricultural crops such as maize are based in Juba. Magwi County is located East North of Juba (Figure 1) and is an important maize producer and supply source to Juba City. Nimule is the main border area between Uganda and South Sudan and is the key entry point for most of the food and non-food items from neighboring countries such as Uganda, Kenya and Tanzania.

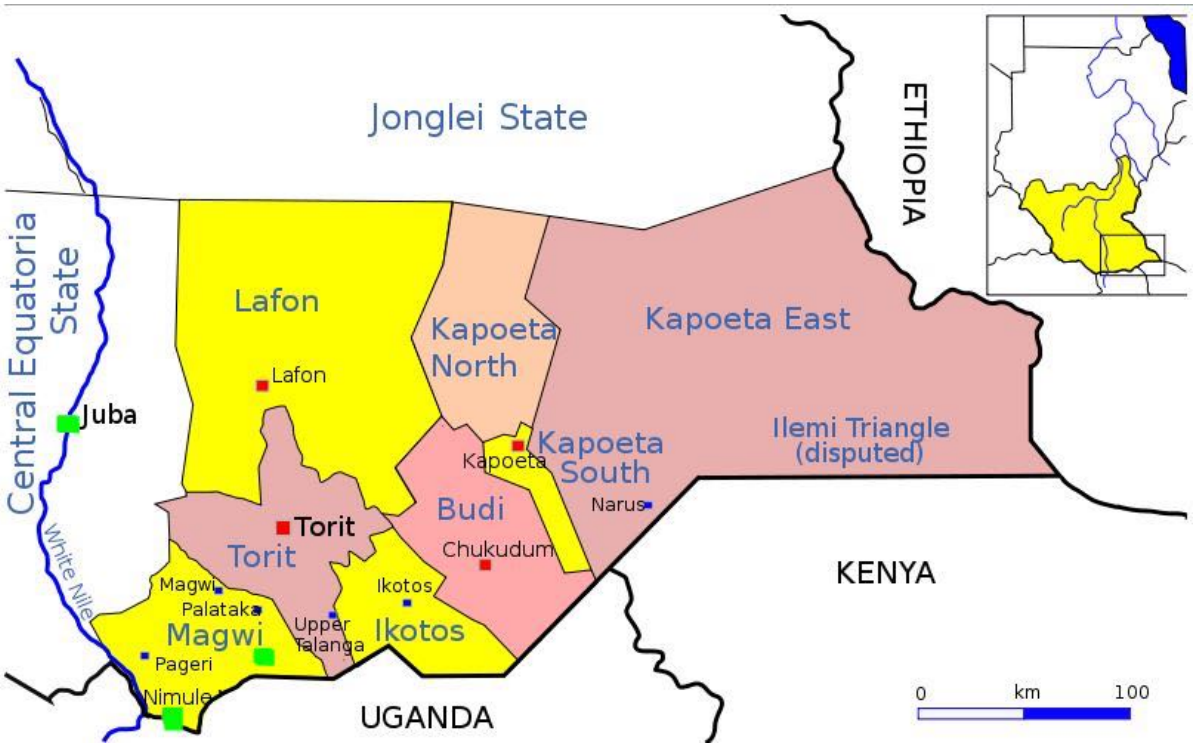

Figure 1: Map showing the location of Juba, Magwi County and Nimule border in South Sudan https://en.wikipedia.org/wiki/Magwi_County

Sample size estimation

At the point of carrying out the study, the exact population of maize traders and farmers were not known, therefore, the sample size was estimated using the Kothari equation [11]:

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$$n = Z^2 P \frac{(1-P)}{e^2}$$

where n = sample size, Z = standard variate at 95% confidence level = 1.96, P = Probability is set at 5% (0.05) and e = acceptable error (the precision/ estimation error) set at 8% (0.08). Thus, the sample size of the study was derived as shown:

$n = 1.96^2 \times 0.05\ (1 - 0.05)/0.08^2 = 30.$

Therefore, 30 farmers and 30 traders were randomly selected for the study in order to give equal chances to all the study respondents in the population selected.

Study design and population

A cross-sectional descriptive study was carried out in selected maize producing areas of Magwi County (Agoro, Omeo, Paluonganyi), retail/wholesale markets of Juba (Konyo-Konyo, Customs, Gudele), and Nimule transit border points (Nimule main park, Rock city park and Jebel park). Traders from Konyokonyo (n=10), Custom (n=10) and Gudele (n=10) markets of Juba and Farmers from Agoro (n=10), Omeo (n=10) and Paluonganyi (n=10) farming areas of Magwi County were randomly selected for this study using a simple random sampling technique.

Data on awareness and handling practices

Structured questionnaires were administered to the respective farmers and traders after getting their written consent to participate in the study. The questionnaires were prepared in English and further translated into simple Arabic during the interview, whenever there was such a need. The questionnaires were pre-tested on a random sample of 5 maize traders from Libya market in Juba and 5 farmers from Pajok maize producing area of Magwi County.

Maize Sampling and procedures

A simple random sampling technique was applied among the selected 30 farmers, 30 traders and 30 importers to collect maize samples. Upon completion of respective interviews on aflatoxin awareness and maize handling practices, maize sampling was done in accordance to the procedure of ISO 13690 [12], about 100g maize samples collected from each of the 10 traders of Konyo-konyo, 10 traders of Customs and 10 traders of Gudele markets of Juba were mixed to form 1kg maize sample to represent each of the respective market, thereby resulting in 3 market samples from Konyo-konyo, Customs and Gudele markets, respectively. Similarly, 100g maize samples were collected from each of the 10 farmers from Agoro, 10 farmers from Omeo and 10 farmers from Paluonganyi in Magwi County and mixed to form 1kg maize sample for each farming area, resulting in 3 farmers' samples

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from Agoro, Omeo and Paluonganyi farming areas, respectively. About 100g maize samples were also collected from each of the 10 maize importers from Rock city, 10 importers from Jebel and 10 importers from Nimule main parking areas of Nimule border and mixed to form 1kg maize sample from respective parking areas (Rock city, Jebel and Nimule main). Sampling was done from the top, middle, and bottom of each bag. After the collection of maize samples in plastic containers, the samples were coded and temporarily stored at South Sudan National Bureau of Standards (Central Laboratory) while awaiting transportation to Food Microbiology Laboratory at Dedan Kimathi University of Technology (DeKUT), Nyeri, Kenya. Samples were stored in plastic containers at 4°C until the time of analysis. Moisture and microbial tests were done at DeKUT, whereas aflatoxin tests were conducted at Chemistry Laboratory of International Livestock Institute of Research (ILRI), Nairobi, Kenya.

Determination of moisture content of the maize obtained from farmers and traders

The moisture content was determined using dry air oven method in accordance with the Association of Official Analytical Chemists Standard Method No. 930.15 [13], as describe below:

About 5g of ground maize were weighed into crucibles of a known weight. The crucibles were first dried in an oven at 105 °C for 1 hour and kept in a desiccator. The samples were placed in the crucibles and transferred to hot dry-air oven (MRC, MUNRO – Laboratory Equipment, UK) at 105 °C for about four hours. The samples were then allowed to cool in a desiccator and weighed again. All the samples were tested in triplicate. The percentage moisture contents of the maize samples were calculated using the formula below:

$$\% \text{ moisture content} = \frac{\text{Change in weight}}{\text{initial weight of sample before drying}} \times 100$$

Isolation and identification of Aspergillus species in maize samples

Aspergillus species were isolated by the dilution plating technique on Rose Bengal Chloramphenicol Agar medium (RBCA) (HIMEDIA, India), and the media was prepared in accordance with the procedure of Kortei *et al.* [14]. Ten grams of grounded maize were aseptically transferred into a conical flask containing 90 ml of prepared peptone water. The mixture was thoroughly mixed to form a stock solution and held overnight to allow resuscitation of the moulds from the effects of freezer storage. Then, 1 ml of the stock solution was transferred into a test tube containing 9 ml of peptone water and serially diluted to 10^{-4}. Then 0.1 ml aliquot of dilutions 10^{-3} and 10^{-4} were drawn and cultured on RBCA media plates by a spread

plate technique, using a sterilized stainless-steel spreader. All samples were done in triplicate and the plates incubated at 25 ^{0}C for 7 days. After incubation period, the plates containing 10 - 100 colony-forming units (CFU) were considered for the calculation of fungal population. The colonies were counted using a colony counter and were expressed as colony forming units per gram of sample (CFU/g) as described in the equation below and later converted to log CFU/g.

$$\text{CFU}/10\text{g} = \frac{\text{number of colonies} \times \text{reciprocal of the dilution factor}}{\text{plating volume (ml)}}$$

The isolated mould colonies were enumerated and sub-cultured using sterilized inoculating wire loop on Rose Bengal Chloramphenicol Agar (RBCA) media, then, the plates were incubated again at 25 ^{0}C for 7 days. Colonies of Aspergillus flavus were identified by their greenish-yellow appearance and powdery texture with golden to red brown or pale to yellow reverse side whereas *Aspergillus parasiticus* were identified by their blue-green appearance, and the white to yellow appearance reverse side [15]. After sub-culturing, the microscopic features of the isolates were studied in a light microscope (OPTIKA, Italy) using cello-tape method as per the procedure of Olee *et al.* [16], in which a small piece of clear tape with the sticky surface was gently placed on the mould colony so that mycelial fragments and some spores were transferred on to the tape. Sticky tape culture was placed on a glass slide and then the slide was mounted on the light microscope and observed under X40 objective lens. Aspergillus species were identified by their colony characteristics such as color and texture of the mycelium, characteristics of conidiophores such as shape of conidial heads and color of the stipes.

Determination of aflatoxin B_1, B_2, G_1 and G_2 in the maize samples

Extraction of aflatoxins from the maize samples

The Aflatoxins B_1, B_2, G_1 and G_2 were analyzed in accordance with AOAC Method No. 2005.08 [17] using Ultra high performance liquid chromatography – Fluorescence detector (UHPLC-FLD) as follows: about five gram (5g) of grounded maize were weighed accurately, using sterilized spatula, into a 50 ml falcon tube (BD, Franklin Lakes, NJ, USA). Subsequently, 25 ml of 70% methanol was added and the mixture vortexed for 1 min and shaken in a mechanical orbital shaker (New Brunswick, NJ, USA) at 250 rpm for 30 min at room temperature (21 – 25 ^{0}C). The mixture was further centrifuged at 3500 rpm for 10 min. The recovered extract was diluted with 1% acetic acid at the ratio of 1:1 (700 µl: 700 µl) in a 2 ml Eppendorf tubes and vortexed for at least 10 seconds. The extract was filtered through a 0.2µm Polytetrafluoroethylene (PTFE) syringe into vials. Then, the vials were

capped and loaded into the Ultra High performance Liquid Chromatography (UHPLC) auto-sampler for subsequent analysis.

Analysis of aflatoxins using ultra high-performance liquid chromatography

Chromatographic separation was performed using Nexera Ultra High Performance Liquid Chromatography (UHPLC) system (Shimadzu Corporation, Kyoto, Japan) fitted with Auto sampler (SIL-30AC), prominence pumps (LC-20AD) and fluorescence detector (RF-20AXS). A synergi hydro-RP analytical column (2.5 µm particle size, 100 mm x 3.00 mm), (Phenomenex, Torrance, CA, USA) operating at flow rate of 0.4 ml/min was used for the separation of aflatoxins. A binary mobile phase, consisting of mobile phase A (methanol (40%)) and mobile phase B (1% acetic acid (60%)), was utilized to achieve this separation. The injection volume was 10 µl and the column oven temperature was set at 50 ^{0}C. The liquid chromatography program was set at 8 min per run and 60% methanol was used as the flushing solution of the column. Fluorescence detection was carried out at wavelengths of λ_{ex} = 365 nm and λ_{em} = 435 nm. A standard calibration curve consisting of a plot of peak areas against the known concentration of the injected series of standards for aflatoxin B_1, B_2, G_1 and G_2 was established and used for estimation of the concentrations of the samples in the Lab Solutions software version 5.89 (Shimadzu Corporation, Kyoto, Japan). Individual types of aflatoxin were identified by comparing the retention time of the chromatographic peak of the target aflatoxin in the test sample to that of the corresponding standard chromatographic peak. Samples with values above the linear range of the standard curve were diluted and re-tested. The limit of detection (LOD) for aflatoxins B_1, B_2, G_1 and G_2 during the time of analysis were 0.360, 0.086, 0.223 and 0.072 µg/kg, respectively. The concentration of individual aflatoxins in the test samples was calculated as follows:

$$\mathrm{X\ (ng/g)} = \frac{\mathrm{C \times V \times F \times 100}}{\mathrm{W \times R}}$$

Where
X – total content of individual aflatoxin in the test sample, ng/g
C – concentration of aflatoxin in the test sample, ng/ml after calibration using Lab Solutions software.
V – extraction volume used in ml
F – dilution factor after extraction with 1 % acetic acid
100 - Percentage for recovery
W – weight used of the test sample, g
R - experimentally determined recovery factor from spike recovery experiment

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Statistical analysis

The data from questionnaires were analyzed using Statistical Package for Social Sciences (IBM SPSS version 29, Armonk, NY, U.S.A). Data on Aspergillus species were converted from colony Forming Units per gram (CFU/g) into log CFU/g using Microsoft Excel 2016. Data on moisture contents and aflatoxins were analyzed using Graph Pad Prism (Graph Pad Prism version 9.5.0, Boston, U.S.A) and the results were presented as means and standard deviations. One-way analysis of variance (ANOVA) was used for the data on moisture contents and aflatoxins levels and Tukey's multiple comparisons test were used to test significant differences at 95% confidence interval (CI).

Ethical consideration

The study was approved for data collection by the Institute of Food Bio-resources Technology, Dedan Kimathi University of Technology, Nyeri, Kenya. Further, the study design and the research tools were approved and licensed by the Ministry of Higher Education Republic of South Sudan (RSS/MoHEST/USO/J/IM). All participants willingly participated in the study by signing a letter of consent. All the names of the participants were withheld and kept confidential.

RESULTS AND DISCUSSION

Socio-demographic characteristics of the farmers and traders

Among the respondents, majority of the farmers (70%) and traders (77%) were female, and most of the farmers (43%) and traders (63%) age ranged between 30 - 45 years (Table 1). Most of the farmers in the current study had attained Primary Education (43%), whereas 30% of them indicated not having any formal education (Table 1).

Aflatoxin awareness among the farmers and traders

The present study revealed that the majority of the farmers (97%) and traders (83%) had no prior knowledge on aflatoxins (Table 2). This could be attributed to the low levels of education among the respondents (Table 1) and consequently minimal aflatoxins awareness. In Ethiopia, 99% of farmers, 97% of traders and 70% of consumers were unaware of aflatoxins and its health consequences [18]. The majority of the farmers (77%) and traders (90%) were unaware of the prevention measures for aflatoxins contamination (Table 2). About 10% of the traders and 16% of the farmers concurred that proper drying of maize grains is an effective measure toward prevention of aflatoxin contamination in maize (Table 2).

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Proper drying of crops before storage minimizes chances of mould infestation and hence prevents aflatoxins contamination [19].

Handling practices of maize among the farmers and traders

The present study found that 87% of the farmers harvest their maize at physiological maturity (Table 3). Timely harvesting of grains upon maturity in dry conditions and early removal of any damaged maize kernels or cobs has been reported as a feasible aflatoxins reduction strategy, because delayed harvest increases the chances of fungi infection of the maize kernels [20]. Most of the farmers (63%) reported that they harvest their maize during wet conditions (Table 3). This practice is possibly due to lack of knowledge among farmers on the role of moisture accumulation on mould growth and aflatoxins contamination. It is recommended to dry maize grains while on the cob before shelling because the cobs are tougher and can overcome physical damages commonly associated with the shelling maize having low moisture content [21]. Most farmers (93%) dried their maize in both forms of cobs and shelled grains (Table 3). Drying of maize grains by heaping up or spreading out on bare ground for a few days can create favorable conditions for mould growth and mycotoxin contamination [22]. The current study found most farmers (83%) dried their maize on a tarpaulin or mat (Table 3), an observation that is similar to the report in Morogoro municipality and Makambako district in Tanzania where farmers (97.8%) dried their maize on tarpaulin or mats [23]. This observation implies that despite lack of awareness about aflatoxins, farmers are aware of the other potential food contamination and quality risks upon placing maize directly onto the ground. The present study revealed that most farmers (77%) dried their maize under hot sunny conditions for a period of 4 - 5 days (Table 3). Similarly, in Southern and West Shewa districts of Ethiopia, majority of the farmers (78%) dried their maize grains for a minimum of 3 - 5 days [20]. Proper drying of crops for more than 4 days prior to storage minimizes chances of mould growth and hence prevents subsequent aflatoxins contamination [19]. Most farmers (93%) and traders (77%) disclosed that they checked the dryness of maize by biting with their teeth (Table 4). In Ghana, farmers also checked for maize drying level by biting using their teeth [24].

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Figure 2: Storage condition for maize grains in one of the stores in Gudele market

Most farmers (77%) kept their bags of maize on a raised platform, but this was not the case with most traders (73%), who kept their maize bags on bare ground surface (Table 4). The current study observed that most farmers (67%) stored their maize for a period between 1 - 4 months whereas most traders stored for 1 - 6 months (Table 4). Similar observations have been reported in Kenya, indicating that subsistence farmers stock maize under various sub-optimal conditions for more than 3 months prior to use or sale [25]. In Tanzania, 62.8% of traders stored their maize for 3 - 6 months [23]. The majority of the traders in the current study reported presence of rats (53%) and weevils (13%) in their stores (Table 5). The high levels of insect infestation reported in our study could be due to the poor storage conditions of the maize stores (Figure 2). Aflatoxin contaminations can occur if rodents and other pest attack and damage maize grain over long periods of storage [26].

Moisture content of the maize samples collected from the traders and farmers

Moisture content of produce is an important factor which influences fungal contamination and subsequent aflatoxin production in cereals [27]. The present study found that all the maize samples from both farmers and traders met the moisture content regulatory limit of below 13.5% [28]. Nimule border samples (Rock City, Jebel, Nimule Main Park) recorded significantly ($P \leq 0.05$) higher moisture content (12.3%, 12.2% and 12.2 %, respectively) compared to those of market samples from Juba City (Konyo-Konyo, Customs and Gudele markets) (11.8%, 11.9% and 12.3%, respectively) and Magwi farmers' samples (Agoro, Omeo and Paluonganyi) (11.1%, 11.0% and 10.9 %, respectively) (Table 6). The lower moisture content of maize grains in this study is attributed to the loss of moisture from the grain through transpiration during extended storage [29]. These findings are comparable to the report from Benue State, Nigeria, where they

reported 11.7% and 11.9% moisture content in maize samples stored for four months in Makurdi and Gbajimba markets, respectively [30]. The farmers' maize grain samples (Agoro, Omeo and Paluonganyi farmers) had significantly ($P \leq 0.05$) lower moisture content (11.1, 11.0 and 10.9%, respectively) compared to those from importers in Nimule border and the traders from various markets in Juba (Table 6). This could be partly due to drying of maize grains on tarpaulin under hot sunny conditions for a period of 4 – 5 days by most farmers (77%) (Table 3).

Occurrence of Aspergillus Species contamination in maize from farmers and traders

The present study found that most maize grains collected from the farmers, traders and importers had varied counts of Aspergillus species (Aspergillus flavus and *Aspergillus parasiticus*) (Table 6), which are the main producers of aflatoxin [31]. According to East African standards for maize grains, maize grains should be practically free from moulds [28]. Among the several Aspergillus species isolated from maize samples in Ethiopia, Aspergillus flavus and Aspergillus niger are widely distributed across the storage, field, and market samples [20]. In our study, maize samples with highest count of Aspergillus flavus (9 log CFU/g) and *Aspergillus parasiticus* (12 log CFU/g,) were obtained from Nimule Main Park, followed by the samples collected from Gudele market (Aspergillus flavus 3 log CFU/g) and *Aspergillus Parasiticus* (12 log CFU/g) (Table 6). Aspergillus flavus was not detected from the samples collected from Agoro and Omeo farmers hence could be considered as meeting the requirement of East African Standard for maize [28]. Samples from Paluonganyi farmers recorded less counts of Aspergillus flavus (6 log CFU/g). Although *Aspergillus parasiticus* was not detected from maize samples obtained from Agoro farmers, it was detected in maize samples from Omeo (6 log CFU/g) and Paluonganyi (3 log CFU/g) (Table 6). Good handling practices among the farmers particularly proper sun - drying of maize grains on a tarpaulin or mat (Figure 3) could be the reason for the reduction of mould contamination in the maize grains. The present study also revealed that the maize grain samples from Konyo-konyo market were contaminated with Aspergillus niger (9 log CFU/g) (Table 6). The lower Aspergillus flavus and *Aspergillus parasiticus* contamination levels in the maize collected from farmers compared to the maize collected from traders could be due to the good practices in handling maize grains among the farmers, particularly adequate sun - drying of maize grains on a tarpaulin for a period of 4 – 5 days by 77% of the farmers (Table 3).

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Figure 3: Farmers' maize drying practices using tarpaulin in Magwi county of South Sudan

Maize grains tend to be contaminated with moulds from the farm during pre - harvest and increase with poor post-harvest handling [32]. The mould contamination in traders' maize grain could be due to the poor storage conditions particularly placing maize grains bags direct on the ground floors of the stores (73%) (Table 4).

Aflatoxins contamination of the maize grain

The present study revealed that all the maize grains collected from farmers, traders, and importers were contaminated with aflatoxins at varied levels ranging from 0.51 - 505.56 µg/kg (Table 7). In addition, most of the levels were above the acceptable safety limit as prescribed in the East African Standard for aflatoxin B1 (5µg/kg) and total aflatoxins (10 µg/kg) [28]. In the current study, the grains from Nimule Main Park were contaminated with significant ($P \leq 0.05$) levels of aflatoxin B_1 (505.56 µg/kg), B_2 (108.29 µg/kg), G_1 (336.96 µg/kg), and G_2 (81.38 µg/kg) (Table 7). Similar observations on high levels of aflatoxin B1 have been reported in Egypt whereby the highest aflatoxin B_1 concentration recorded in maize was 440 µg/kg [33]. The samples from Gudele market were also contaminated with significant ($P \leq 0.05$) high levels of aflatoxin B_1 (76.55 µg/kg), and total aflatoxins (94.08 µg/kg), respectively (Table 7) when compared with levels in samples from the farmers. This could possibly be due to the extended period of storage of maize grains in the markets for more than one month under poor storage conditions by traders (Table 4). The comparatively higher aflatoxin levels in the samples from Nimule Border and Juba markets when compared to those from Magwi farmers could be attributed to the differences in the level of Aspergillus flavus and *Aspergillus parasiticus* in the samples (Table 6), low levels of education (Tables 1) and inadequate awareness programmes among the traders in the markets (Table 2). In contrast, significant ($P \leq 0.05$) lower levels of aflatoxin B_2 were recorded in

the samples from Konyo-Konyo and Customs market (2.64 and 3.42 µg/kg, respectively) with relatively significant ($P \leq 0.05$) difference in the samples from Gudele market (17.06 µg/kg). Similarly, significant ($P \leq 0.05$) levels of aflatoxin G_1 was recorded in the samples collected from Konyo-Konyo, Customs and Gudele markets (13.47, 2.47 and 0.012 µg/kg, respectively) as well as aflatoxin G_2 (3.01,0.64, and 0.47 µg/kg, respectively) (Table 8). These significant ($P \leq 0.05$) levels of aflatoxin B_2, G_1 and G_2 in the grains from Juba markets could be associated with the level of contamination of the maize with *Aspergillus Parasiticus* (Table 6). The significant ($P \leq 0.05$) levels of aflatoxins contamination in market samples could be attributed to the poor handling and storage of these maize grains (Figure 2), which in turn contributed to contamination of the maize with aflatoxigenic fungi (Table 6). Similarly, the significant ($P \leq 0.05$) high levels of aflatoxins contamination in the Nimule border samples could be due to the contamination levels of the respective samples with aflatoxigenic fungi (Table 6), under the prevailing favorable climatic and environmental conditions such as heavy rains, sudden droughts, high humidity, average temperature of 25°C, and occasional floods commonly experienced in Uganda [34]. Despite the significant ($P \leq 0.05$) levels of aflatoxins contamination of the maize grains from the border and the markets, the current study found significant ($p \leq 0.05$) lower levels of aflatoxins contamination in maize grains collected from the farmers. The samples from Omeo farmers had significant ($P \leq 0.05$) low levels of Aflatoxins B_1 (4.39µg/kg), B_2 (0.75 µg/kg), G_1 (2.18 µg/kg), and G_2 (0.51 µg/kg) (Table 7), all below the set limits of aflatoxins contamination by the East African Standard. No aflatoxins were detected from Agoro and Paluonganyi farmers' samples of Magwi county (Table 7). The significant ($P \leq 0.05$) low safety levels of aflatoxins contamination in maize grains of the farmers could be partly due to proper drying of maize grains on a tarpaulin under hot sunny conditions for a period of 4 – 5 days by 77% of the farmers (Table 3), and the low levels of contamination with aflatoxigenic fungi (Table 6). The aflatoxin contamination in the farmers' samples were also an indication that aflatoxin occurrence in maize starts from the farm where the kernels can be infected with the aflatoxigenic moulds [32].

CONCLUSION, AND RECOMMENDATIONS FOR DEVELOPMENT

Most farmers and traders had limited knowledge on aflatoxins and poor handling practices of maize, therefore strategic public sensitization on aflatoxins in maize and maize good handling practices is necessary among maize farmers and traders. Although all the maize grains from all the farmers and traders met the regulatory moisture content limit (below 13.5%), there are high chances of maize grains not meeting the required moisture content limit during trade because

majority of the farmers and traders use their teeth to test the dryness of maize grains. Thus, there is need for the relevant authorities to provide the farmers and traders with moisture meters in order for them to effectively monitor the moisture content level of their maize grains. All the maize grain samples from the traders were contaminated with significant ($P \leq 0.05$) high levels of aflatoxins, which indicate that most consumers of maize grains in the study areas are at risk of aflatoxins health effects. Therefore, it is necessary to conduct regular aflatoxins screening of all maize grains, both imported and locally produced.

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Conflict of interest

The authors declare no conflict of interest.

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Table 1: Socio-demographic characteristics of the farmers and traders

Variables	Sub-variables	Farmers (n=30)	Traders (n=30)
a) Gender	male	9 (30%)	7 (23%)
	female	21 (70%)	23 (77%)
b) Age group (years)	18-30	11 (37%)	1 (3%)
	30-45	13(43%)	19 (63%)
	>45	6 (20%)	10 (33%)
c) Education background	Primary	13(43%)	15(50%)
	Secondary	7(23%)	12(40%)
	Vocational	0 (0%)	0(0%)
	University	1(3%)	0 (0%)
	None	9 (30%)	3(10%)

Table 2: Aflatoxin awareness among the farmers and traders

Variables	Sub-variable	Farmers (n=30)	Traders (n=30)
a) Have you ever heard of aflatoxin?	Yes	1(3%)	5 (17%)
	No	29 (97%)	25 (83%)
b) Do you know the measures to avoid aflatoxin ?	Yes	7 (23%)	3 (10%)
	No	23 (77%)	27(90%)
c) What measures needs to be taken ?	Drying maize properly	5 (16%)	3 (10%)
	Sorting maize	2 (7%)	0 (0%)

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Table 3: Handling practices of maize by farmers in relation to stage of harvesting, environmental conditions during harvesting, and drying period

Variables	Sub-variables	Farmers (n=30)
a) Stage of harvesting maize	At physiological maturity	26 (87%)
	Completely dry maize	4 (13%)
b) Environmental conditions under which maize is harvested	Dry conditions	11 (37%)
	Wet Conditions	19 (63%)
c) Forms of drying maize	Shelled grains	2 (7%)
	Drying in both conditions	28 (93%)
d) Drying places for the maize	On a tarpaulin/mat	25 (83%)
	On bare ground	1 (4%)
	On cemented floor	4(13%)
e) Drying period of the maize grains	2-3 days	5(17%)
	4-5 days	23(77%)
	6-8 days	2 (6%)

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Table 4: Handling practices of maize grains among the farmers and traders

Variables	Sub-variable	Farmers (n=30)	Traders (n=30)
a) techniques of knowing that the maize grains are well dried	Moisture meter	0(0%)	3(10%)
	Biting the maize	28(93%)	23(77%)
	Hand shaking of maize	2(7%)	3(10%)
	No idea	0(0%)	1(3%)
b) Materials of the walls for the stores	Mud	29(97%)	0(0%)
	Stone/brick	1(3%)	4(13%)
	Iron sheets	0(0%)	25(83%)
	Trailer container	0(0%)	1(3%)
c) Roofing materials for maize grain stores	Grass-thatched	28(93%)	0(0%)
	Iron sheets	2(7%)	29(97%)
	Trailer container	0(0%)	1(3%)
d) Surfaces where maize grains are placed in the store	On bare earth floor	2(7%)	22(73%)
	On the wooden racks	5(17%)	0(0%)
	On raised platform	23(77%)	3(10%)
	On cemented floor	0(0%)	5(17%)
e) Length of storage for maize grains in the store	<7 days	0(0%)	4(13%)
	< 4 weeks	0(0%)	11(37%)
	1 months	7(23%)	0(0%)
	1-4 months	20(67%)	0(0%)
	1-6 months	3(10%)	14(45%)
	> 6 months	0(0%)	1(3%)

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Table 5: Pest and control measures of pest among traders

variable	Sub-variable	Traders (n=30)
a) Pest present in the stores	Weevils	4(13%)
	Rats	16(53%)
	Both rats and weevils	2(7%)
	Not present	8(27%)
b) Control of the pests	Trapping	13(43%)
	Dusting	2(7%)
	Spraying	4(13%)
	No control	3(10%)

Table 6: Moisture content (MC) and Occurrence of Aspergillus *species* contamination for maize grain samples collected from the farmers and traders

Clusters	Moisture content (MC)	Aspergillus Species counts (log CFU/g)		
	Mean (%) ± SD	*A. flavus*	*A. parasiticus*	*A. niger*
Agoro farmers	11.1 ± 0.06^{ab}	ND	ND	ND
Omeo farmers	11.0 ± 0.10^{ab}	ND	6	ND
Paluonganyi farmers	10.9 ± 0.10^{ab}	6	3	ND
Konyo-Konyo market	11.8 ± 0.23^{c}	3	ND	9
Customs market	11.9 ± 0.10^{d}	3	6	6
Gudele market	12.3 ± 0.12^{e}	3	12	ND
Rock city park	12.3 ± 0.36^{a}	3	6	ND
Jebel park	12.2 ± 0.21^{a}	3	3	ND
Nimule main park	12.2 ± 0.00^{b}	9	12	ND

SD – standard deviation, Values within the column for moisture content marked with different superscript letters are significantly different (P ≤ 0.05). **ND –** Not detected, **CFU/g** – Colony Forming Units per gram**, Log =** Log base 10

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Table 7: Aflatoxins content of maize grains collected from the farmers, traders and importers

Clusters	*Aflatoxins concentration (µg/kg)*			
	AFB1	**AFB2**	**AFG1**	**AFG2**
Agoro farmers	< LOD	< LOD	< LOD	< LOD
Omeo farmers	4.39 ± 0.54^{ab}	0.75 ± 0.12^{bcd}	2.18 ± 0.25^{aef}	0.51 ± 0.26^{afd}
Paluonganyi farmers	< LOD	< LOD	< LOD	< LOD
Konyo-Konyo market	20.03 ± 1.38^{d}	2.64 ± 0.31^{de}	13.47 ± 0.72^{cfa}	3.01 ± 0.35^{bbc}
Customs market	18.87 ± 2.63^{e}	3.42 ± 0.28^{abc}	$2.4\ 7 \pm 0.89^{edc}$	13.11 ± 0.68^{acd}
Gudele market	76.55 ± 0.75^{f}	17.06 ± 0.71^{abd}	0.012 ± 0.01^{efc}	0.47 ± 0.09^{cad}
Rock city park	282.74 ± 3.62^{a}	54.27 ± 4.54^{bc}	28.75 ± 3.33^{cdf}	16.79 ± 0.66^{acf}
Jebel Park	65.76 ± 1.46^{b}	15.83 ± 0.56^{cd}	89.57 ± 1.03^{fcd}	0.64 ± 0.04^{eac}
Nimule main park	505.56 ± 0.70^{c}	108.29 ± 0.61^{df}	336.96 ± 0.99^{cdc}	81.38 ± 0.52^{edf}

AFB1= aflatoxin B1, AFB2 = aflatoxin B2, AFG1 = aflatoxin G1, AFG2 = aflatoxin G2, < LOD = less than the limit of detection. Values within the column marked with different superscript letters are significantly different ($P \leq 0.05$)

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