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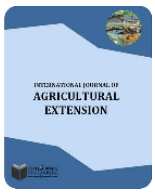

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ASSESSMENT OF AFLATOXIN IN GROUNDNUT UNDER STORAGE CONDITION OF ETHIOPIA

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ABSTRACT

The assessment was conducted in major groundnut-producing areas of Ethiopia. The study was to detect important mycotoxins and to estimate post-harvest losses in stored groundnut due to associated mycotoxigenic fungi. Structured questionnaires were used to obtain primary and other information from farmers, retailers and wholesalers. The samples were detected using agar plate and HPLC methods. The data were analyzed using SPSS statistical software (version: 26.0) and the mean was separated by LSD. 86% of the interviewed farmers shelled groundnut pods with their hands and 98% of them used sack storage. According to the laboratory results of agar plate methods, five fungi genera, Aspergillus, Fusarium, Penicillium, Rhizopus & Trichoderma were identified from the stored groundnut samples. Among these, Aspergillus species had the most dominant 75% incidence after four months of storage. Four aflatoxin types, AfB1, AfB2, AfG1 and AfG2, were detected and quantified in all the surveyed areas. The concentration of AFB1 was high at 386.10 and 360.96µg/kg was recorded in the samples collected from Limu Kosa and Limu Shayi areas of the Jimma zone with higher total aflatoxin of 542.25µg/kg. At the initial stage of the storage periods, the concentration of aflatoxin in the samples was minimal, but it increased as the storage periods increased in different locations. Therefore, it was concluded that the storage periods and methods, harvesting time, moisture content, locational differences and farmer's ways of shelling, drying and storing favour the development of aflatoxins in the storage.

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INTRODUCTION

Groundnut (*Arachis hypogaea* L.), which is also known as peanut, earthnut, monkey nut and goobers, is an annual legume. It ranks the 13th most important food crop and 4th most important oilseed crop in the world (Reddy *et al.*, 2011) because it is cultivated in more than 100 countries on six continents (Sharma and Bhatnagar-Mathur). Despite its significance, the crop is affected by mycotoxins such as aflatoxins (AFs), fumonisins (FUMs), deoxynivalenol (DON), ochratoxin A (OTA), and zearalenone (ZEN) are agriculturally important (Milićević *et al.*, 2010). Different studies showed that 18 different aflatoxins were identified, with aflatoxin B1, B2, G1, G2, M1, and M2 is the most common. Among these, Aflatoxin B1 and G1 arise frequently (Mishra and Das, 2003). Amare *et al.* (1995) report the amount of aflatoxin in peanuts was 5 to 250 µg/kg. Also, Eshetu and Gedif (2007) stated the aflatoxin level reached up to 447 µg/kg in groundnut seed from his assessment in eastern Ethiopia. Additionally, the finding of Mohammed *et al.* (2016) showed the aflatoxin B1 level was up to 2,526 and 158 µg/kg, in groundnut seed and groundnut

cake from the local "Halawa", respectively, of eastern Ethiopia. Aflatoxins are highly harmful, immunosuppressive, mutagenic, teratogenic and carcinogenic chemicals that increase liver disease and carcinogenicity (Peraica *et al.*, 1999). Moreover, Liu and Wu (2010) report about 40% of liver cancer incidences in Africa have been allocated to dietary intake due to aflatoxin exposure. Mycotoxin's infection has directly affected Africa, and is consequential in huge economic loss; for example, AFs contamination of crops alone has been reported to cause an annual loss of more than USD 750 million (Udomkun *et al.*, 2017).

The economic losses imposed the rejection of the country's export products in response to dangerous levels of mycotoxins (Mamo *et al.*, 2020). Diener *et al.* (1982) observed that when the seed moisture exceeds 9% at the equilibrium humidity of 80% and 30 °C temperature, the chances of invasion by Aspergillus flavus increase drastically. The development of mycotoxigenic fungi associated with stored groundnuts in Ethiopia and its correlations to the country's vast agro-ecological disparity, farmers' poor storage practices, and its impact have not been documented. Therefore, the objectives of this research are to detect and quantify important mycotoxins and to estimate post-harvest losses due to associated mycotoxigenic fungi and mycotoxins in stored groundnuts.

MATERIALS AND METHODS

Survey Areas and Sample Collection

The assessment was conducted from east Wollega, Werer agricultural research center, Limu Shayi and Limu Kosa, Bable, Fedis, Chalanko and Miesso woredas based on their production potential together with woreda agricultural experts to understand farmer's views on the mycotoxin's contamination of groundnut under storage conditions and storage practices used. Three woredas were selected based on the production potential. From each woredas, three kebele, each kebele, and three farmers were selected randomly ten meters apart from each. Structured questionnaires were directed through personal interviews to obtain primary and other information from farmers, retailers and wholesalers. The 250 grams of samples per farmer, retailer and wholesaler were taken by inserting the spear into the grain mass straight to the maximum depth from the bottom, sides, middle and top of the storage. The initial groundnut samples from each storage structure were taken as a control at the beginning of the storage.

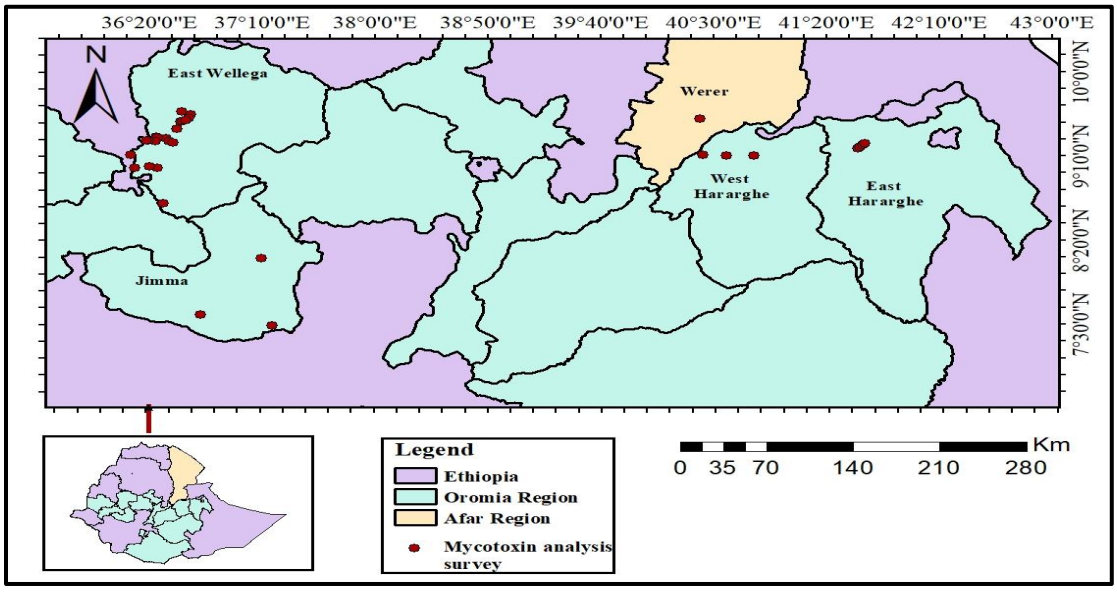

Figure 1. Assessment of groundnut storage methods in Ethiopia (2018/20).

Isolation and Identification of Mycotoxigenic Fungi

Agar plate method: Samples of groundnut grains with and without surface disinfection were used and 10 seeds/grains/Petri dishes of each sample were aseptically placed on potato dextrose agar (PDA) using an agar plate method according to the procedures used

by Tsedaley and Adugna (2016). The laboratory analysis was carried out in the Ambo Agricultural Research Center, the pathology department and the mycology section laboratory. Firstly, from each sample, 360 groundnut grains, in 3 replications of 120 seeds/grains were selected randomly. Initially, farmer's freshly harvested seeds/grains of groundnut were used and periodically the stored groundnut seeds/grains were sampled and thoroughly washed with distilled water surface disinfected and non-disinfected. From surface disinfected and non-disinfected samples, 10 grains/Petri-dish/plate (9 cm diameter plates) containing potato dextrose agar (PDA) were aseptically placed. The seeds without surface disinfection were used as a control. The plate that contains the fungus was incubated at 26°C for 7 days and after 7 days of incubation, the identification of fungus isolates was done based on: septate, growth rate, color, and morphology of mycelia, conidia and sporulation structures. Then, the isolated fungi were subcultured after three days of incubation for purification of the isolate. Finally, the percentage of incidence and frequency of isolation of fungi were calculated as follows: Incidence of fungi:

$$\text{Incidence } (\%) = \frac{\text{Number of cultured grains}}{\text{Total number of grains used}} \times 100$$

Detection and Quantification of Mycotoxins Using HPLC Method

50.14gm of disodium phosphate (DSP) was dissolved with 700ml of distilled water in a 1000ml flask. 42.50gm of Sodium phosphate monobasic was dissolved in 350ml of distilled water. The two dissolved solutions were mixed to adjust it to 7.4 PH. 200ml of buffer was filled into 1000ml of graduating cylinder. Take 230ml of the buffer from the prepared and add 20ml polytene 2020. 20gm of samples were weighed and 2gm of NaCl was added into a conical flask and Shaked by using a mechanical shaker and then filtered by the vacuum pump. The two layers were separated and the bottom layers were used for the analysis. Take 7ml of samples and 43ml of the buffer. Elute 50ml of the solution in the ingenuity affinity column (AflaCLEAN) and wash it with distilled water. Then add 2ml of methanol to degrade the proteins and wait for 5 minutes and elute. Finally, use the preserved glass and take it into the vial and inject the analysis undergone. 200 gm of samples were weighed and placed in labelled paper bags before they were sent to the Bless Agri-food laboratories services PLC (ISO/IEC 17025:2017 Accredited) which was established by the joint venture of Ethiopia and French investors.

Data Analysis

Descriptive statistics such as frequency distribution and percentage analysis were used. All the collected data were computed using Microsoft Excel 2010 and SPSS statistical software (Version 26), and the differences between the means were separated by the least significant difference (LSD).

RESULTS

Shelling and storage methods

The samples were collected from 4 zones, 11woredas and a total of 77 farmers were covered during the survey. 86% (n=77) of the interviewed farmers are shelling their groundnut pods with their hands, which is labour intensive, but it is effective for small-scale farmers, especially for the selection of planting the seed for the next season and reduces the contamination of the products as well as mould growth and 98% of them used sacks storage which the produce stayed not more than four to six months of storage periods. 22% (n = 77) of the interviewed farmers especially in Sasiga areas of east Wollega used mechanical rotary types shellers that shell several sacks at a time in a continuous operation (rather than shelling in batches) and new designs produce very little wastage in terms of damaged seed. Generally, storage methods and periods, farmers' time of harvesting, ways of shelling and storing and storage structures used created favorable conditions for the development of moulds during storage. The majority of farmers sell their groundnuts to markets, which are eaten as kolo in the urban and rural areas and a bitter taste when eaten causes harm to health.

Isolation and identification of fungi species in the samples using agar plate

From the results of this study, it was indicated that five fungi genera; Aspergillus, Fusarium, Penicillium, Rhizopus & Trichoderma were identified. Among these, the percentage incidence of Aspergillus spp. was the most dominant at 75% in all the collected samples. Fusarium, Penicillium, Rhizopus and Trichoderma spp. occurred with low incidence in the samples. The samples collected from Limu Shayi and Limu Kosa of Jimma zone from the wholesalers store the mould growth was high

because it was bought with high moisture content, not properly sorted and improper storage. Since groundnut is the major export oil crop for Ethiopia, it needs more research and multidisciplinary work. The fungal development was highly obtained as the storage period increased because of the metabolic activity of the produce, inappropriate storage conditions and moisture increment due to microbial activities.

Detection of aflatoxin from the groundnut samples using HPLC

Five into composited samples; BSC0509, BSC05010, BSC0511, BSC0512 and BSC0513 were used for the laboratory analysis and four types of aflatoxin, AfB1, AfB2, AfG1 and AfG2 were detected and quantified except in the samples of BSC0512. This is due to the samples being collected during the initial storage periods. AFG2 was only detected in the samples collected from Bable woredas of east Hararghe. Aflatoxin B1 was obtained with high concentration levels of 386.10 and 360.96µg/kg in the samples of BSC0513 and BSC0511. The levels of aflatoxins in the collected samples showed increasing trends as the storage periods increased with the differences in the locations.

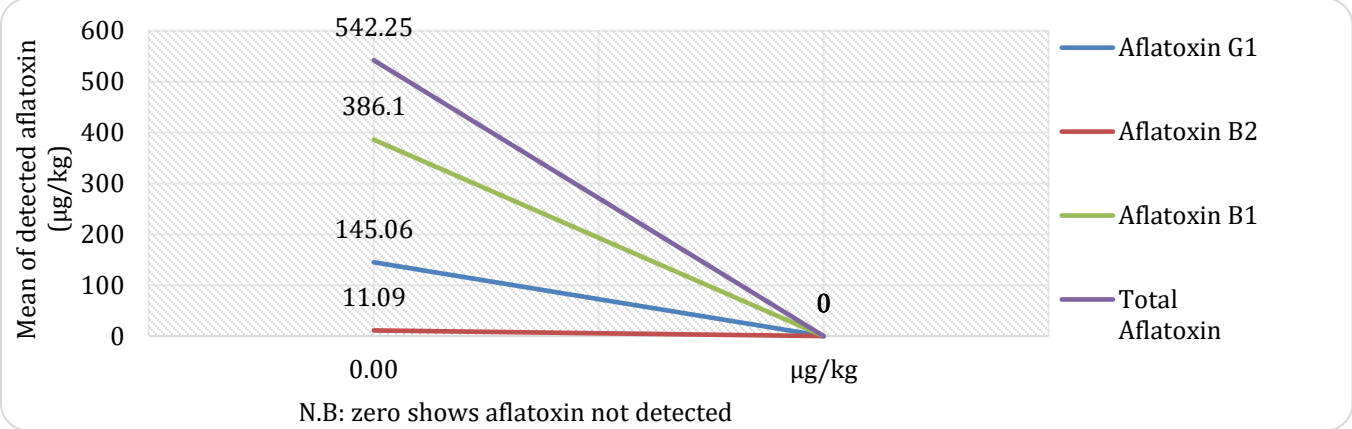

Figure 2. Aflatoxin was detected in the groundnut samples & single injection reports of BSC0513.

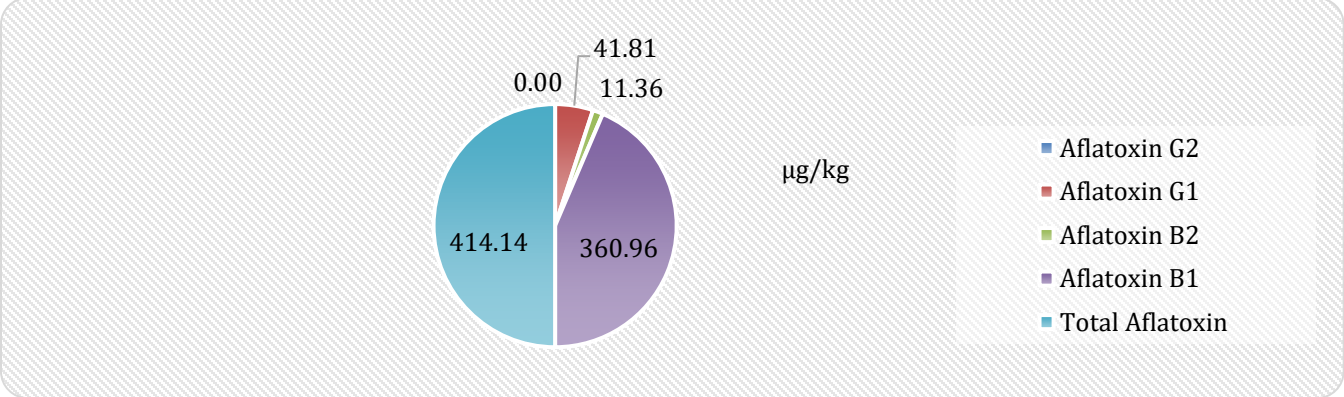

Figure 3. Aflatoxin was detected in the groundnut samples & single injection reports of BSC0511.

Minimum levels of AFB1 19.18 and 163.92µg/kg were recorded in the samples analyzed from BSC0510 and BSC0509, respectively. This is because, in Werer, since it is a research center, there is good storage management, well aerated and the produce is harvested with recommended moisture content and stored. In Hararghe, the farmers dry the groundnuts on the well-stoned ground with no moisture absorbance of t he produce. Levels of aflatoxins in the samples showed increasing trends as the storage periods increased because of the metabolic activity of the produce, which increased the respiration rate and moisture content and increased infestation of weevils and development of mou lds.

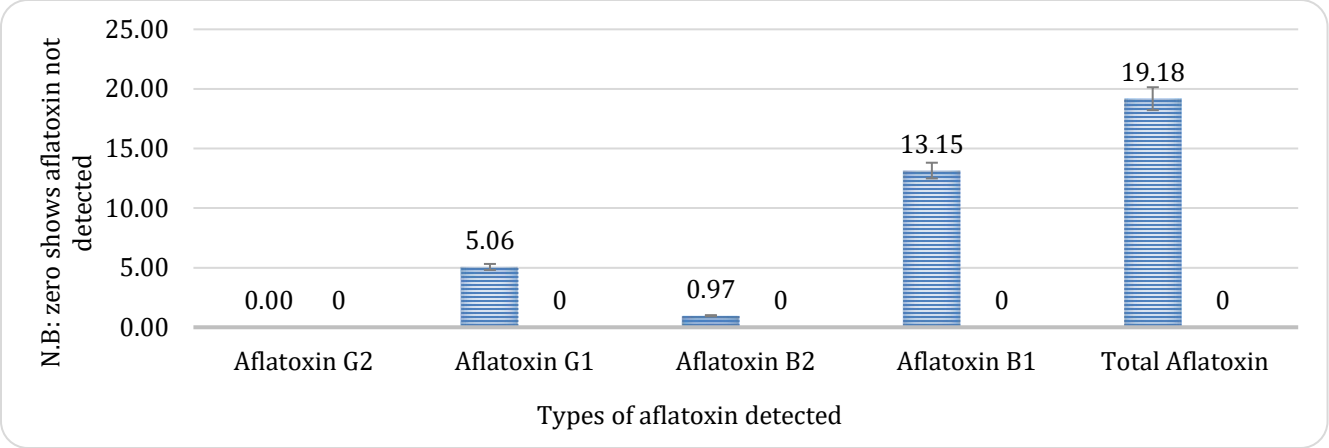

Figure 4. Aflatoxin was detected in the groundnut samples & single injection reports of BSC0510.

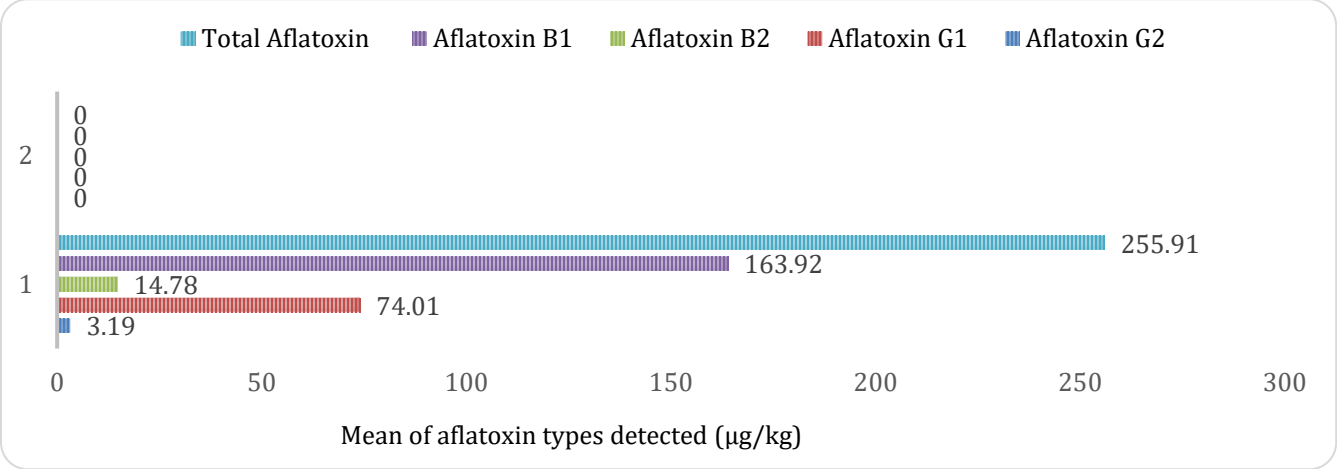

Figure 5. Aflatoxin was detected in the groundnut samples & single injection reports of BSC0509.

DISCUSSIONS

In this study, important types of aflatoxin in stored groundnuts were detected and quantified. Six fungal species, Aspergillus Rhizopus, Penicillium Curvularia, Fusarium and Mucor were identified from groundnut samples (Aliyu and Kutama, 2010). However, Patel and Mishra (2010) identified nine species of fungi from the seeds of groundnut stored for one year. In similar studies, Ihejirika *et al.* (2005) reported that Aspergillus niger occurred with the highest incidence of 60% followed by Aspergillus versicolor at 25%, whereas, Aspergillus fumigatus occurred with the lowest incidence of 15%.

According to this study, five fungi genera, Aspergillus, Fusarium, Penicillium, Rhizopus & Trichoderma were identified using the agar plate method and the incidence of Aspergillus spp. was high at 75% in all the samples. Eighteen different types of aflatoxins, with aflatoxin B1, B2, G1, G2, M1, and M2 is the most common (Mishra and Das, 2003). In this finding, four aflatoxin types, AfB1, AfB2, AfG1 and AfG2 were detected and quantified in all the samples collected except in the samples collected from east Wollega (Uke, Angar Gute, Mandar 10, Angar), which the aflatoxin was not detected and quantified. High levels of 386.10 and 360.96µg/kg of Aflatoxin B1 were obtained within the samples of BSC0513 and BSC0511. Minimum levels of AFB1 19.18 and 163.92µg/kg were recorded in the samples analyzed from BSC0510 and BSC0509, respectively. Chala *et al.* (2013) report the 12,000 µg/kg aflatoxin levels in groundnut seed sampled from Bable district in the Eastern. Besrat and Gebre (1981) reported that the mean levels of aflatoxin B1 were 34.7 and 105 µg/kg in groundnut samples and peanut butter, respectively, in Ethiopia. Another study by Amare *et al.* (1995) showed aflatoxin levels of 5-250 µg/kg in groundnut seeds from

eastern Ethiopia. Latterly, Chala *et al.* (2013) reported that total aflatoxin levels ranged between 15 and 11865 µg/kg in groundnut seed. According to Wagacha *et al.* (2013), the contamination of aflatoxin was increased in the storage period in 25% of the samples with polypropylene bags. Kebede *et al.* (2016) indicated that the incidence of toxigenic fungi species and the rate of aflatoxins contamination showed a fluctuation based on the geographical location.

CONCLUSIONS AND RECOMMENDATIONS

Of the four types of aflatoxin detected, the concentration of aflatoxin B1 was high in the sample collected from Limu Kosa and Limu Shayi of Jimma zone with high total aflatoxin. This is because of improper storage of the produce, poor storage management and the farmers and wholesalers of the areas putting/laying the sacks one over the other. This increases the respiration and the metabolic activity of the product. Any types of aflatoxin were not detected in the sample collected from east Wollega. This is because the sample was taken at shelling/initial storage and most of the farmers used hand shelling of the pods. From the study, it was observed that the concentration of aflatoxins shows increasing trends as the storage period increases. This is due to improper storage methods, and farmers' ways of shelling and storing their produce that favour the increment of respiration and metabolic activity in the product which results in the growth and development of the moulds during storage. The farmers pool the pods with soil, shell immature pods, dry them on the ground with soil contamination, improper storage methods and lay the sacks one over the other during storage, which increases moisture content and temperature in the products and supports the development of moulds. Multidisciplinary interventions are more advisable in the supply from producer to consumers for controlling the concentrations of aflatoxins and for exporting safe products and reducing health risks.

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