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Negasa Fufa
M.Sc, Department of Post-Harvest Technology, Researcher at Ethiopian Institute of Agricultural Research, Ambo Agricultural Research Center, P.O. Box: 37, Ambo, Ethiopia

Corresponding Author:
Negasa Fufa
M.Sc, Department of Post-Harvest Technology, Researcher at Ethiopian Institute of Agricultural Research, Ambo Agricultural Research Center, P.O. Box: 37, Ambo, Ethiopia

Assessment of aflatoxin in groundnut under storage condition of Ethiopia

Negasa Fufa

Abstract
The assessment was conducted in major groundnut producing areas of Ethiopia. The objective of the study was to detect important mycotoxins in stored groundnut and to estimate post-harvest loss due to associated mycotoxigenic fungi and mycotoxins in stored groundnut. Structured questionnaires were directed through personal interviews to obtain primary and other information from farmers, retailers and wholesalers. The data was analyzed using SPSS (version: 26.0) and the mean separated by LSD. 86% of the interviewed farmers shelling pods by their hands and 98% used sack storage. In the result of agar plate methods analysis, five fungi genera, Aspergillus, Fusarium, Penicillium, and Rhizopus & Trichoderma were identified. From these, Aspergillus species had the most dominant 75% incidence after four months of storage. Four aflatoxins, AfB1, AfB2, AfG1 and AfG2 were detected and quantified in all the surveyed areas. Among these, high levels of AFB_1 386.10 and 360.96µg/kg were recorded in Limu Kosa and Limu Shayi areas of Jimma zone with a higher total aflatoxin 542.25µg/kg than the other areas. Therefore, it was concluded that the storage periods, storage methods, methods of shelling, locational differences and farmer's ways of drying and storing favors the development of aflatoxins in the storage.

Keywords: Aflatoxin, chromatogram, food losses, mycotoxin, oil seed, storage

Introduction
Groundnut (*Arachis hypogea* 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 of the world (Surendranatha *et al*., 2011) [1] being cultivated in more than 100 countries in six continents (Sharma and Mathur, 2006) [2]. Despite of its significant the crop is affected by mycotoxins such as aflatoxins (AFs), fumonisins (FUMs), deoxynivalenol (DON), ochratoxin A (OTA), and zearalenone (ZEN) are agriculturally important (Milicevic *et al*., 2010) [3]. Different studies showed that 18 different aflatoxins were identified, with aflatoxin B1, B2, G1, G2, M1, and M2 being the most common. Among these, Aflatoxin B1 and G1 arise frequently (Mishra and Das, 2003) [4]. Ayalew *et al*. (1995) [5] reports the amount of aflatoxin in peanuts was 5 to 250 µg/kg. Also, Eshetu (2010) [6] stated the aflatoxin level was reached up to 447 µg/kg in groundnut seed from his assessment in eastern Ethiopia. Additionally, the finding of Mohammed *et al*. (2016) [7] 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) [8]. Moreover, Liu and Wu (2010) [9] reports about 40% of liver cancer incidences in Africa have been allocated to dietary intake due to aflatoxin exposure. Mycotoxin's infection has strictly affected Africa 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) [10]. The economic losses imposed the rejection of the country's export products in response to dangerous levels of mycotoxins (Firew *et al*., 2020) [11]. Diener and Cole (1982) [12] 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 groundnut 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 groundnut.

2. Materials and Methods

2.1 Survey Areas and Sample Collection

The assessment was conducted in 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 farmers views on the mycotoxin's contamination of groundnut under storage conditions and storage practices used. Structured questionnaires were directed through personal interviews to obtain primary and other information from farmers, retailers and wholesalers. The 250 to 500 grams of samples were taken from the bottom, sides, middle and top of the storage.

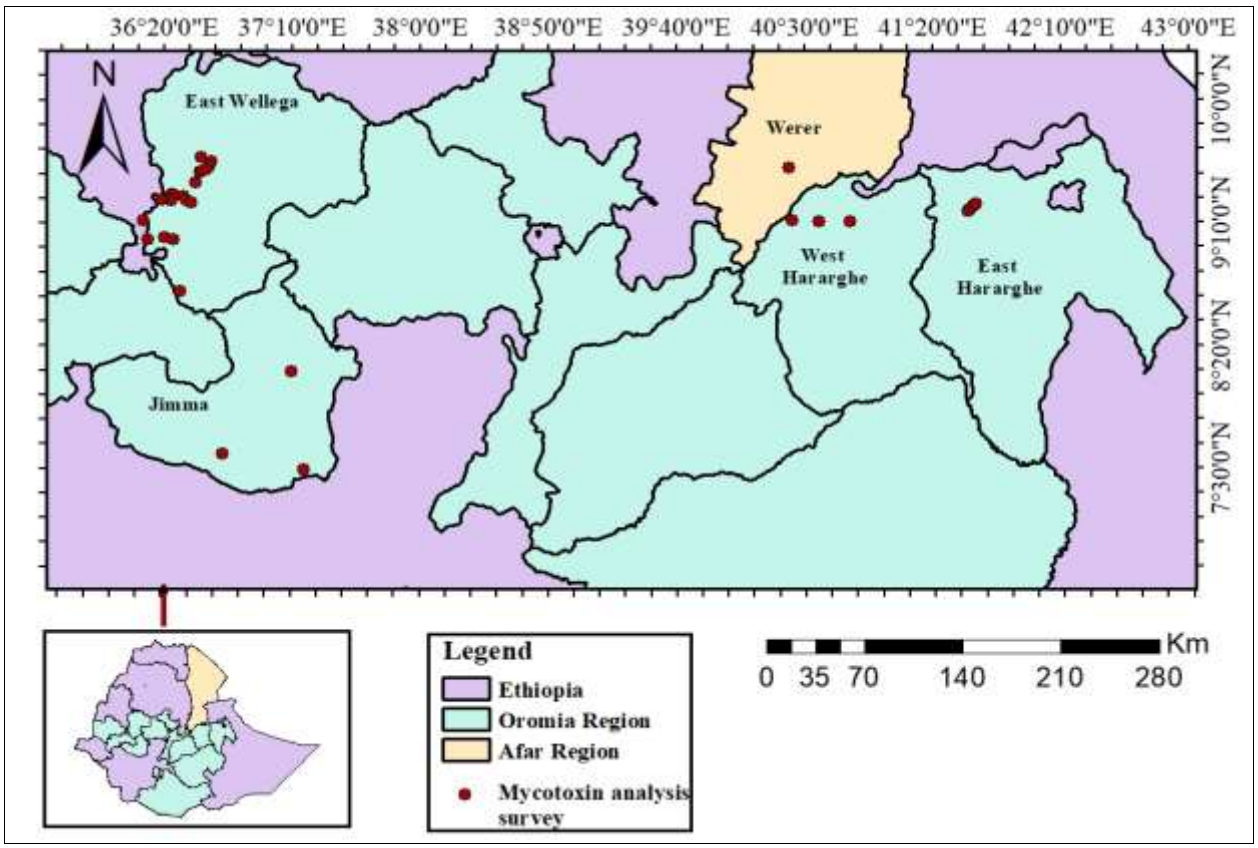


Fig 1: Assessment of groundnut storage methods in Ethiopia (2018/20)

2.2 Isolation and Identification of Mycotoxigenic Fungi

Agar plate method: Samples of commercial maize grains with and without surface disinfection were used and 10 grains of each treatment were aseptically placed on potato dextrose agar (PDA) by the method of agar plate according to the procedures used by Binyam T and Girma (2016) [13]. The laboratory analysis was carried out in the Ambo Plant Protection Research Center mycology laboratory department. Firstly, from each sample, 360 maize grains; in 3 replications of 120 seeds were selected. Initially, freshly harvested seed of BH661 was used and periodically the stored maize grains were used and thoroughly washed with distilled water at each period. 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 plate that contains fungus was incubated at 26°C for 7 days and after 7 days of incubation, the identification of fungi isolates was done based on: septate, growth rate, color, and morphology of mycelia, conidia and sporulation structures. Then, the isolated fungi were subculture after three days of incubation for purification of the isolate. Finally, incidence of isolation fungi (%) and frequency of isolation of fungi (%) were calculated as follows: Incidence of fungi: Incidence of fungi infections on each sample was calculated by using the following formula:

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

2.3 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 were filled into 1000ml of graduating cylinder. Take 230ml of buffer from the prepared and add 20ml polytene 2020. 20gm of samples were weighed and 2gm of NaCl was added into conical flask and Shaked by using mechanical shaker, and then filtered by vacuum pump. The two layers were separated and the bottom layers were used for the analysis. Take 7ml of samples and 43ml of buffer. Elute 50ml of solution in ingenuity affinity column (Afla CLEAN) and wash by distill water. Then add 2ml methanol to degrade the proteins and

wait for 5 minutes and elute. Finally use the preserve glass and take into vial and inject and the analysis undergone. 200 gm of samples were weighed and placed in labeled 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 percentages analysis were used. All the collected data were computed using Microsoft Excel 2010 and SPSS statistical software (Version 26) for the differences among aflatoxin percentage micrograms per kilogram.

3. Results and Discussions

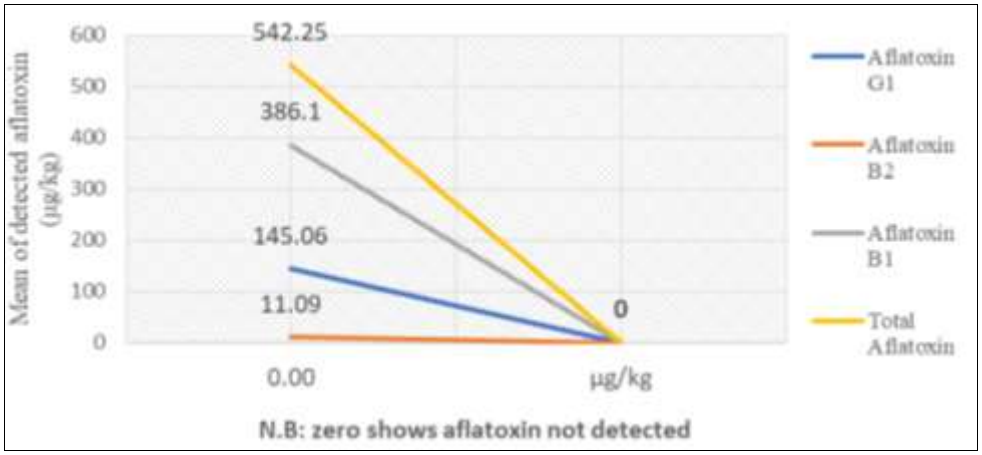
3.1 Shelling and storage methods

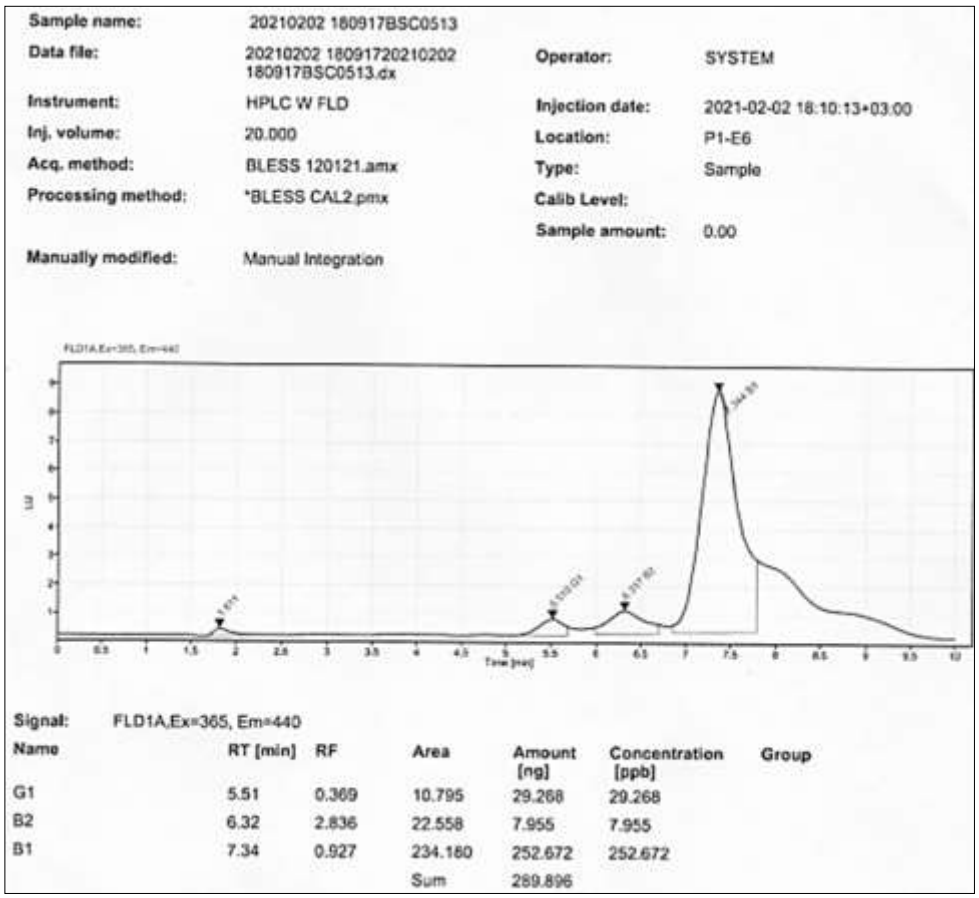
The samples were collected from 4 zones, 11woredas and a total of 77 farmers were covered during the survey. Most of the interviewed farmers 86% (n= 77) in all areas shelling their groundnut pods by their hands and stored in sacks which is labour intensive but it is effective for small scale farmers especially for selection of planting seed for the following season, reducing the contaminant as well moulds development. Few of the interviewed farmers 22% (n = 77) especially in Sasiga areas of east Wollega zone used mechanical rotary types shellers that is important for shelling a number of 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. 98% of the surveyed farmers used sack storages and stored their groundnuts for four to six months of storage periods. Storage periods, storage methods, methods of shelling and farmer's ways of storing their produce favors the development of the moulds during storage. The majority of farmers sell their groundnut to markets which is eaten as kolo in the urban and rural areas and a bitter taste when eaten causes harm to health. The quality and flavor of edible peanuts and peanut products can be affected by the fatty acid composition of the lipids (Ul-Hassan *et al*., 2012) [14].

3.2 Isolation and identification fungi species in the samples using agar plate: The result indicated that five fungi genera, *Aspergillus, Fusarium, Penicillium, Rhizopus & Trichoderma* were identified. Among these, *Aspergillus spp*. was the most dominant 75% incidence in all the collected samples. *Fusarium, Penicillium, Rhizopus and Trichoderma* spp. occurred with low incidence in the samples. Ihejirika *et al*. (2005) [15] reported that *Aspergillus niger* was occurred with the highest incidence 60% followed by *Aspergillus versicolor* 25% whereas, *Aspergillus fumigatus* occurred with the lowest incidence of 15%. Likewise, Aliyu and Kutama (2007) [16] identified six fungal species, *Aspergillus Rhizopus, Penicillium Curvularia, Fusarium and Mucor*. However, Vikas and Mishra (2010) [17] identified nine species of fungi from the seeds of groundnut stored for one year. 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.

3.3 Detection of aflatoxin from the groundnut samples using of HPLC

The samples were composited into five, BSC0509, BSC05010, BSC0511, BSC0512 and BSC0513 and four aflatoxin types, AfB1, AfB2, AfG1 and AfG2 were detected and quantified. However, Mishra and Das (2003) detected 18 different types of aflatoxins with aflatoxin B1, B2, G1, G2, M1, and M2 being the most common. Among the composited samples aflatoxin was not detected in the sample's code of BSC0512. This is due to the samples being collected at the initial storage periods. AFG2 was only detected in the samples collected from Babile woredas of east Hararghe. Aflatoxin B1 was obtained with high concentration levels 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. Similarly, Mutegi *et al*. (2013) [18] explained that the contamination of aflatoxin was increased with increase in storage period in 25% of the samples with polypropylene bags. The finding of Assefa *et al*. (2012) [19] indicated that the incidence of toxigenic fungi species and the rate of aflatoxins contamination showed a fluctuation based on the geographical location. Furthermore, Chala *et al*. (2013) [20] reports the aflatoxin levels of 12,000 µg/kg in groundnut seed sampled from Babile district in Eastern. Bisrat and Gebre (1981) [21] reported that the mean levels of aflatoxin B1 was 34.7 and 105 µg/kg in groundnut samples and peanut butter, respectively, in Ethiopia. Another study of Amare *et al*. (1995) [22] showed aflatoxin levels of 5-250 µg/kg in groundnut seed from eastern Ethiopia. Recently, Alemayehu *et al*. (2012) [23] reported that total aflatoxin levels ranged between 15 and 11865 µg/kg in groundnut seed. Minimum levels of AFB1 19.18 and 163.92µg/kg were recorded in the samples analyzed from BSC0510 and BSC0509, respectively. This is due to in Werer the samples were collected from agricultural research center which the storage was appropriately kept with recommended moisture content, well aerated and clean. In Hararghe, the farmers dry on the well stoned ground with no moisture absorbance of the produce.



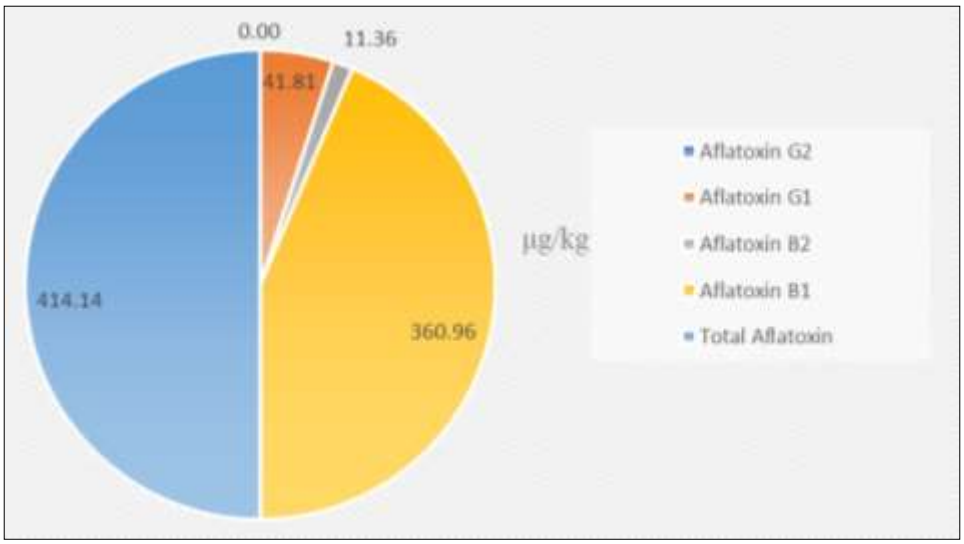


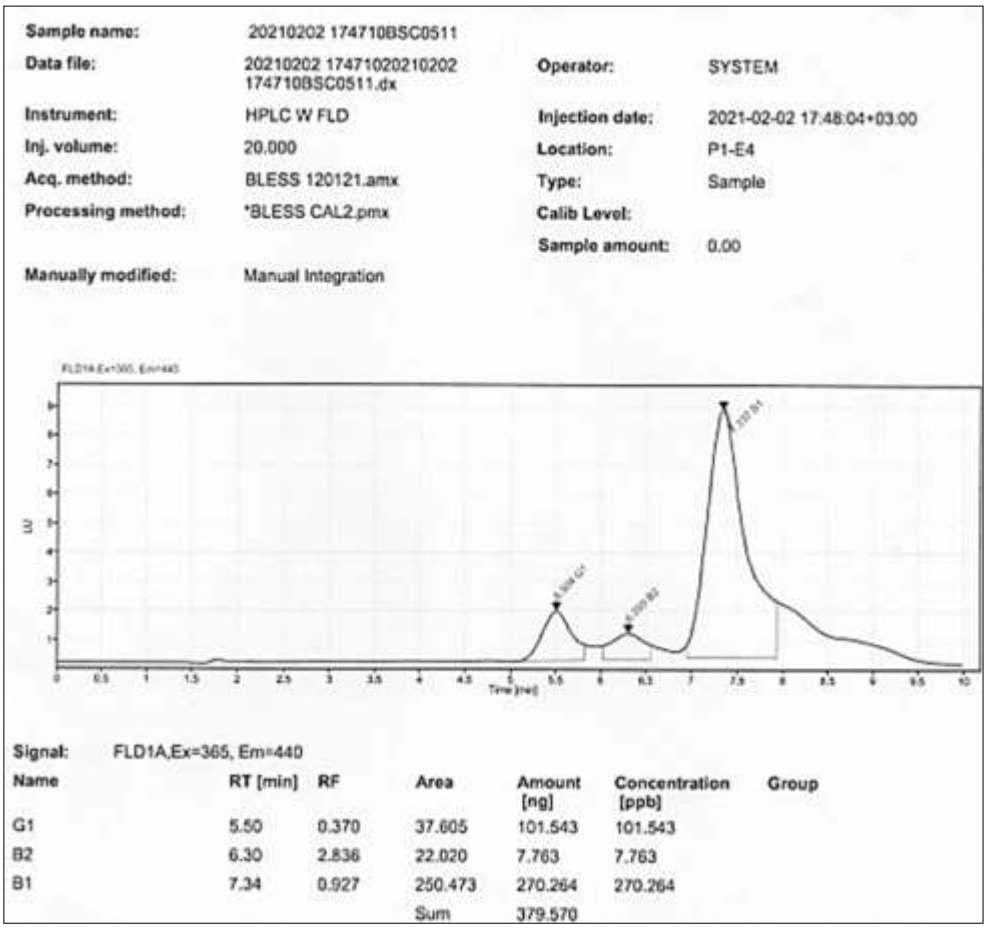
Sample name:	20210202 180917BSC0513				
Data file:	20210202 18091720210202 180917BSC0513.dx	**Operator:**	SYSTEM		
Instrument:	HPLC W FLD	**Injection date:**	2021-02-02 18:10:13+03:00		
Inj. volume:	20.000	**Location:**	P1-E6		
Acq. method:	BLESS 120121.amx	**Type:**	Sample		
Processing method:	*BLESS CAL2.pmx	**Calib Level:**			
		Sample amount:	0.00		
Manually modified:	Manual Integration				

Signal: FLD1A,Ex=365, Em=440

Name	RT [min]	RF	Area	Amount [ng]	Concentration [ppb]	Group
G1	5.51	0.369	10.795	29.268	29.268	
B2	6.32	2.836	22.558	7.955	7.955	
B1	7.34	0.927	234.180	252.672	252.672	
			Sum	289.896		

Fig 2: Aflatoxin detected in the groundnut samples & single injection reports of BSC0513



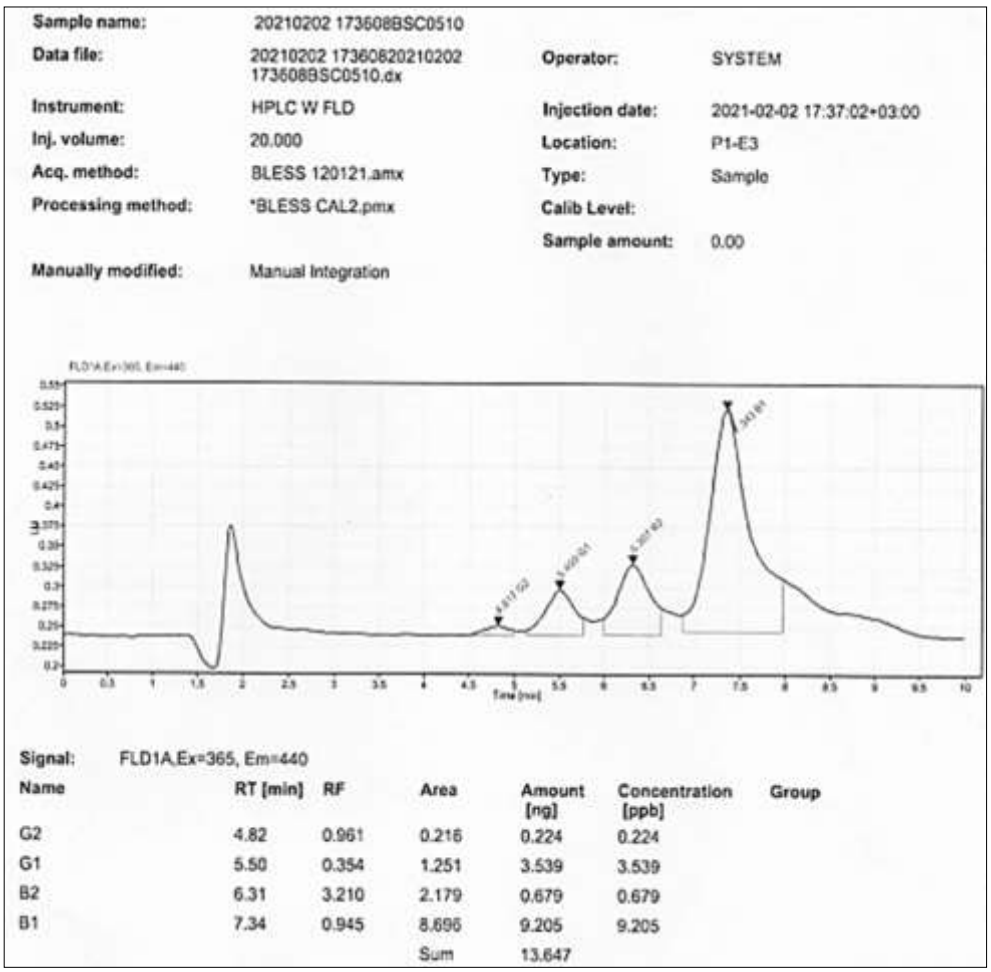


Sample name:	20210202 174710BSC0511		
Data file:	20210202 17471020210202 174710BSC0511.dx	**Operator:**	SYSTEM
Instrument:	HPLC W FLD	**Injection date:**	2021-02-02 17:48:04+03:00
Inj. volume:	20.000	**Location:**	P1-E4
Acq. method:	BLESS 120121.amx	**Type:**	Sample
Processing method:	*BLESS CAL2.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	Manual Integration		

Signal: FLD1A,Ex=365, Em=440

Name	RT [min]	RF	Area	Amount [ng]	Concentration [ppb]	Group
G1	5.50	0.370	37.605	101.543	101.543	
B2	6.30	2.836	22.020	7.763	7.763	
B1	7.34	0.927	250.473	270.264	270.264	
			Sum	379.570		

Fig 3: Aflatoxin detected in the groundnut samples & single injection reports of BSC0511



Sample name:	20210202 173608BSC0510		
Data file:	20210202 17360820210202 173608BSC0510.dx	**Operator:**	SYSTEM
Instrument:	HPLC W FLD	**Injection date:**	2021-02-02 17:37:02+03:00
Inj. volume:	20.000	**Location:**	P1-E3
Acq. method:	BLESS 120121.amx	**Type:**	Sample
Processing method:	*BLESS CAL2.pmx	**Calib Level:**	
		Sample amount:	0.00
Manually modified:	Manual Integration		

Signal: FLD1A,Ex=365, Em=440

Name	RT [min]	RF	Area	Amount [ng]	Concentration [ppb]	Group
G2	4.82	0.961	0.216	0.224	0.224	
G1	5.50	0.354	1.251	3.539	3.539	
B2	6.31	3.210	2.179	0.679	0.679	
B1	7.34	0.945	8.696	9.205	9.205	
			Sum	13.647		

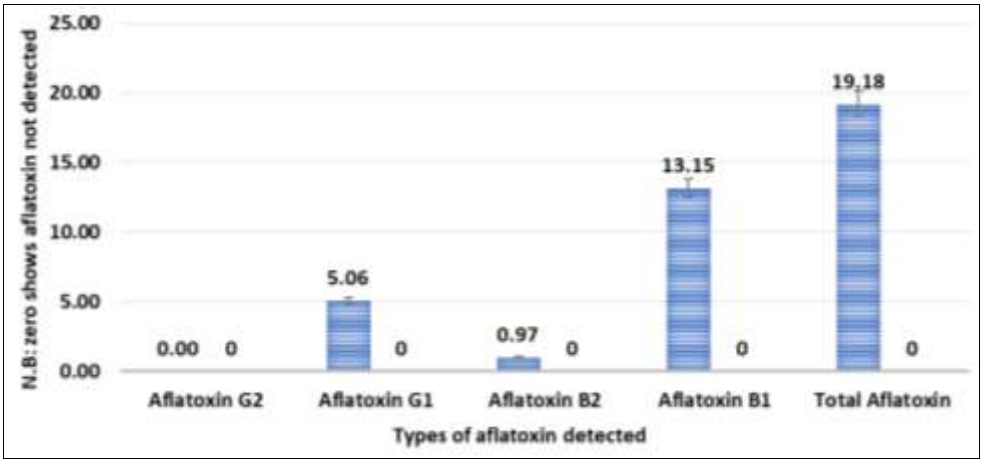
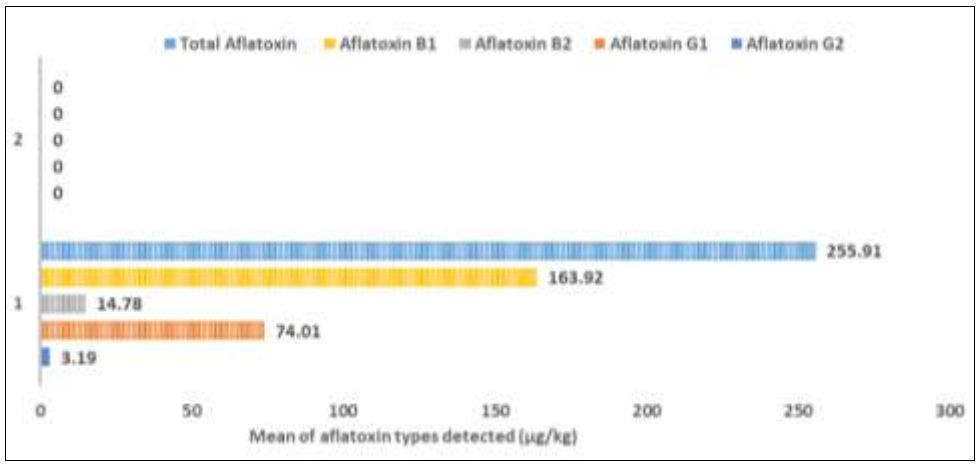
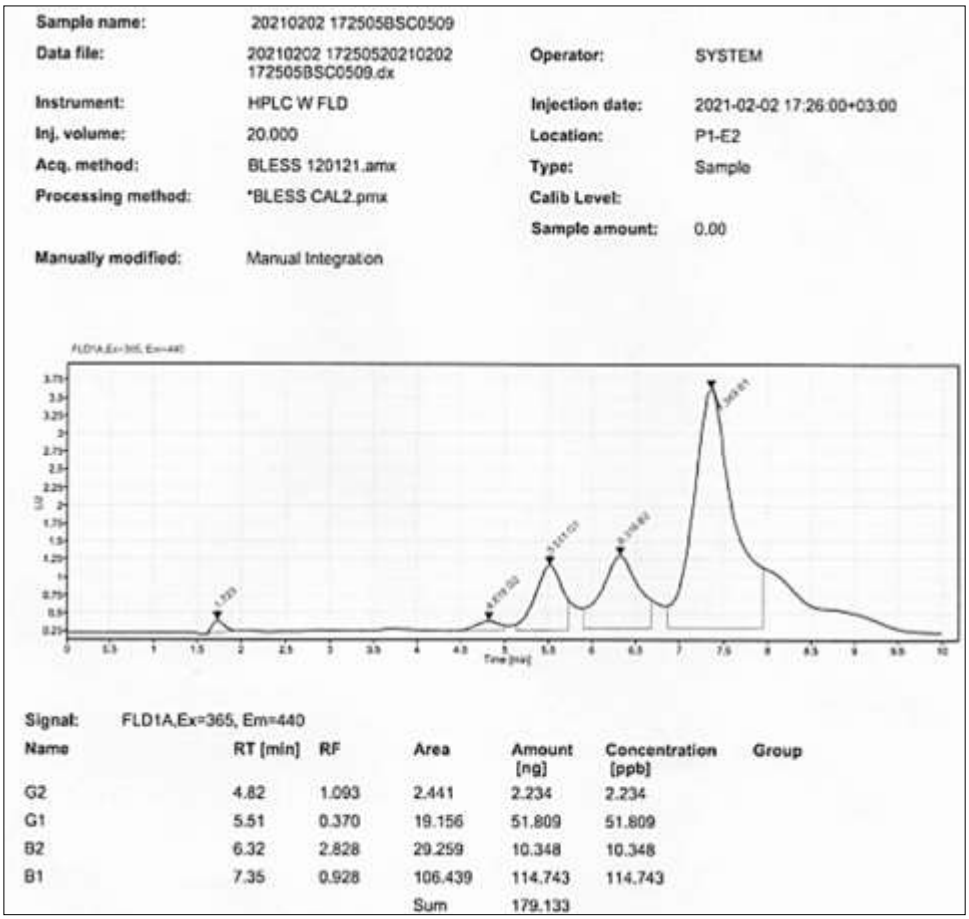


Fig 4: Aflatoxin detected in the groundnut samples & single injection reports of BSC0510





Sample name:	20210202 172505BSC0509		
Data file:	20210202 172505202102021725050BSC0509.dx	Operator:	SYSTEM
Instrument:	HPLC W FLD	Injection date:	2021-02-02 17:26:00+03:00
Inj. volume:	20.000	Location:	P1-E2
Acq. method:	BLESS 120121.amx	Type:	Sample
Processing method:	*BLESS CAL2.pmx	Calib Level:	
		Sample amount:	0.00
Manually modified:	Manual Integration		

Signal: FLD1A,Ex=365, Em=440

Name	RT [min]	RF	Area	Amount [ng]	Concentration [ppb]	Group
G2	4.82	1.093	2.441	2.234	2.234	
G1	5.51	0.370	19.156	51.809	51.809	
B2	6.32	2.828	29.259	10.348	10.348	
B1	7.35	0.928	106.439	114.743	114.743	
			Sum	179.133		

Fig 5: Aflatoxin detected in the groundnut samples & single injection reports of BSC0509

4. Conclusions and Recommendations

Among the four types of aflatoxin detected aflatoxin B_1 was occurred with high concentration in the sample of Limu Kosa and Limu Shayi of Jimma zone with high total aflatoxin because of improper storage of the produce, poor storage management and the farmers, put/laying of the sacks one over the other. The aflatoxin was not detected in the sample collected from east Wollega. This is due to the sample being taken at shelling/initial storage and most of the farmers used hand shelling of the pods. The concentration of aflatoxins shows increasing trends as the storage period increases. This is due to storage periods, storage methods, methods of shelling and farmer's ways of storing their produce that favors the development of the moulds during storage. The majority of farmers sell their groundnut to markets which is eaten as kolo in the urban and rural areas and a bitter taste when eaten causes harm to health. The farmers pool the pods with soils, shell immature, drying on the ground with the soil contamination, using improper storage methods and laying the sacks one over the other during storage which increase moisture content and temperature of the produce and support the development of moulds. Multidisciplinary intervention of government, stakeholders of must require for reducing of the aflatoxin level in the produce. Extension work participation is more advisable during storage for the control of impact of aflatoxins from producers to consumers.

5. Conflict of Interests

The authors have not declared any conflict of interests regarding the materials.

6. Acknowledgements

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