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ASSOCIATION OF FARMERS' SORGHUM GRAIN POSTHARVEST HANDLING PRACTICES WITH AFLATOXIN B1 AND TOTAL FUMONISIN CONTAMINATION IN EAST HARARGHE, ETHIOPIA

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ABSTRACT

Improper farmers' postharvest handling practices of sorghum grain coupled with adverse climatic conditions are conducive for fungal growth and toxin production. The objective of the present study was to investigate the association of farmers' sorghum grain postharvest handling practices with aflatoxin B_1 and total fumonisin contamination in three districts (Babile, Haramaya and Kersa) of East Hararghe Zone, Ethiopia. A total of 90 sorghum grain samples were collected in two phases. Half of the total samples were collected from the threshing yards at the time of threshing and the other half of samples were collected from underground storage pits 5 - 6 months after storage in the year 2013/14. Quantification of the mycotoxins was done using enzyme-linked immunosorbent assay (ELISA). Farmers threshed their sorghum grain either on bare ground, on cow dung smeared ground or on canvas while the underground storage pits were differently managed in all the three study districts. Variation in mycotoxin contamination levels were evidenced from sorghum grain threshed on different grounds. The highest mean (8.52 µg kg^{-1} grain) aflatoxin B_1 and mean total fumonisin (1085.1 µg kg^{-1} grain) were detected from Babile sorghum grain samples threshed on bare ground. The highest total fumonisin (2002.8 µg kg^{-1}grain) was detected from Haramaya sorghum grain samples threshed on bare ground close to the farmers' sorghum fields. The variation was also observed in sorghum grain samples collected from the underground storage pits. High levels of aflatoxin B_1 and total fumonisin were recorded from sorghum grain samples collected from bare underground storage pits in all the three study districts. Use of bare underground storage pits for sorghum grain storage showed a high risk of mycotoxin contamination. The findings of this study call for intervention strategies to be implemented by subsistence farmers to reduce the contamination by aflatoxin B_1 and total fumonisin.

Keywords: Aflatoxin, ELISA, Fumonisin, Grain, Mycotoxins, Postharvest, Sorghum, Toxin

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INTRODUCTION

Mycotoxins are secondary metabolites produced by a wide variety of fungal species that cause nutritional losses and represent a significant hazard to the food chain [1]. Among the food-borne mycotoxins, aflatoxins and fumonisins are likely to be of greatest significance in Africa and other tropical developing countries [2] as they pose various chronic health risks [3]. Aflatoxins have been shown to be a risk factor for cancer of the liver and kidney, a weakened immune system and infectious disease modulation [4]. Aflatoxins are produced in nature by fungi such as *Aspergillus flavus* and *Aspergillus parasiticus* and are recognized as hazardous contaminants of food [5]. Fumonisins, which belong to mycotoxins produced by *Fusarium* species, have also been shown to cause a major risk factor for oesophageal cancer and neural tube defect leading to abortion [6].

The contamination of various foodstuffs and agricultural commodities by aflatoxin and fumonisins is a major problem in the tropics and sub-tropics, where climatic conditions and the pre- and post-harvest practices are conducive for fungal growth and toxin production [7]. Among the post-harvest handling practices, poor drying and threshing practices of grain coupled with improper storage can contribute to fungal growth and increase the risk of mycotoxins production [1].

Traditionally, the majority of Ethiopian farmers thresh their crops on the ground locally known as "Awudema", which is simply level ground smeared with fresh cow dung. For instance, prior to threshing of sorghum, farmers leave the heads of the crop on the bare ground or soil to dry in the sun for some days during which contamination by fungi can possibly occur as most toxigenic fungi are soil borne and ubiquitous. Mashilla [8] indicated that piling and threshing of sorghum heads on non-cemented ground could be a possible source of storage fungi for grain contamination.

Furthermore, Ethiopian farmers, particularly those in the Hararghe region, store their sorghum grain, and sometimes even maize, in traditional underground storage pits [8,9] for one to two seasons [10], until it is consumed or sold. These underground storage pits are mostly neither lined nor plastered with any material that would reduce moisture migration into the stored grain [11]. The direct contact of the grain with wet inner pit walls often leads to moisture ingress from the soil into the inter-granular space elevating both the grain moisture content and the relative humidity inside the pit and ultimately creates favorable condition for mycotoxins producing fungi.

Considering that sorghum is a staple food for the majority of people in East Hararghe Zone, it is necessary to assess the extent of the mycotoxins contamination with respect to farmers' postharvest management practices. No information has been reported so far on the relationship between farmers' postharvest handling practices and the occurrence of aflatoxin and fumonisins in sorghum-producing areas of Ethiopia. Therefore, the objective of this study was to investigate the association of farmers' postharvest handling practices with aflatoxin B_1 and total fumonisin contamination of sorghum grain in East Hararghe Zone, Ethiopia.

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MATERIALS AND METHODS

Study areas

The study was conducted in three districts, namely Babile, Haramaya and Kersa, of East Hararghe Zone, eastern Ethiopia. The districts were purposively selected as they represent different agro-climatic regimes (Table 1).

Sample collection

A total of 90 samples of sorghum grain, intended for direct human consumption, were collected from farmers in two phases. Half of the total samples were collected from the threshing floor at the time of threshing, which is towards the end of December, and the other half of samples were collected in the second round, five to six months later. Farmers selected in the first round sampling were also involved in the second round sampling. Systematic random sampling was carried out and sorghum grain samples were taken at 5 – 6 km intervals within 10 km radius of the respective districts [12].

To have a representative sample, several small sub-samples were taken at random from different spots of each threshing ground floor, and underground storage pits using double-tube sampling spears or sleeves. After thorough mixing of the sub-samples from a single sampling site, 1 kg sorghum grain was taken as a working sample. All samples were properly labelled bearing the name of the respective location and sample collection date and were carried brought in cloth bags to the Haramaya University's plant pathology laboratory, and were kept at 4 °C. Data regarding cropping systems, varieties of sorghum stored, farmers' sorghum threshing and storage methods were recorded.

Mycotoxin analyses

After thoroughly mixing each working sample, a representative subsample of 200 g sorghum grain was ground to fine particle size for mycotoxin analysis. Commercially available enzyme-linked immunosorbent assay (ELISA) kit from HELICA Biosystem (HelicaBiosystemsInc, Santa Ana, CA) was used to analyse the aflatoxin B_1 (AFB_1) and total fumonisin content of the sorghum grain samples. This technique has been used for analyses of mycotoxins such as the fumonisins and aflatoxin B_1[13]. In the process, no clean-up procedure was done [14].

Sample extraction and extract dilution

For the extraction of the mycotoxins, 5 and 10 g ground grain samples were separately diluted with 25 ml of 70% MeOH (1:5 w/v) and with 20 ml of 90% MeOH (1:2 w/v) for aflatoxin B_1 (AFB_1) and total fumonisin extraction, respectively. The diluted samples were shaken for 2-3 minutes, allowed to settle and filtered through Whatman No.1 filter paper. For total fumonisin analysis, the extract was further diluted with sterilized distilled water at the ratio of 1: 20 w/w.

ELISA method for aflatoxin B_1 and total fumonisins

All the ELISA analyses were performed according to the manufacturer's instructions (Helica Biosystems Inc, Santa Ana, CA). The assay procedure for aflatoxin B_1 and total fumonisins was done as explained in Taye *et al.* [15]. All reagents were brought to room

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temperature and the required microwell cartridges and antibody-coated microtiter wells were inserted into the microwell holder separately for each standard and the sample to be tested as follows. For aflatoxin B_1 200 µl conjugate was pipetted into the dilution well and 100 µl aflatoxin B_1 standard solutions and sample extracts were pipetted into the dilution wells containing conjugate and mixed by priming pipette three times. 100 µl mixed solution from each dilution well was transferred to the corresponding antibody-coated microtiter well and incubated at room temperature for 15 minutes. For total fumonisin 100 µl conjugate solution A was pipetted into the dilution well followed by 100 µl conjugate solution B and 100 µl fumonisin total standards solutions and sample extracts were pipetted to the dilution wells containing conjugate and mixed by priming pipettor three times. 100 µl mixed solution from each dilution well was transferred to the antibody-coated microtiter well and incubated for 10 minutes at room temperature.

After incubation, the liquid was discarded out of the microwell and the residual liquid was removed by tapping the microwells holder upside down on a clear filter towel. The wells were filled with distilled water in the case of aflatoxin B_1, whereas for total fumonisin the wells were filled with PBS Tween wash buffer, then emptied and the remaining liquid was removed as before. This washing step was repeated five and three more times for the aflatoxin B_1 and the total fumonisin assay, respectively. The microwell holder was tapped upside down on a clear filter towel to remove the residuals after each washing step. Finally, a 100 µl substrate reagent was added to each well and covered with aluminium foil to avoid direct light and incubated at room temperature for 5 minutes for aflatoxin B_1 and 10 minutes for total fumonisins. After adding 100 µl of the stop solution to each well, the optical density (OD) was recorded with a microtiter plate reader using a 45 nm filter.

Aflatoxin B_1 and total fumonisin content of the sample extracts were calculated from a calibration curve for absorbance data of standards that had concentrations of 0, 1, 2.5, 5.0, 10.0 and 20.0 µg kg^{-1} for AFB1 and 0, 0.1, 0.3, 0.8, 2.0 and 6.0 mg kg^{-1} for total fumonisins. Mean absorbance of standards or samples/ mean absorbance of negative controls (%B/Bo) was calculated by dividing the optical density (OD) of the sample by the OD for the zero standard times 100 to obtain a percentage. The standard concentrations were plotted along the x-axis on a log scale and corresponding %B/Bo values were plotted along the y-axis. Coefficients of correlation (R2) ranged from 0.960 to 0.982. The limit of detection was calculated by taking the mean of 20 replicates of blanks and subtracting 2 times the standard deviation of the blanks to obtain a %B/Bo. The limits of detection were obtained by interpolation from the standard curve and ranged 0.01–0.03 µg kg^{-1}.

Data analysis

To describe and compare different categories of the sample units with respect to the desired characteristics, mean, frequency and percentage of the concentration of the mycotoxins were computed using simple descriptive statistics and Statistical Package for Social Science (SPSS Version 16).

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RESULTS

Farmers' sorghum grain post-harvest management practices

Different sorghum grain postharvest management practices were employed by farmers in the three study districts (Babile, Haramaya and Kersa) (Table 2). Sole sorghum and sorghum mixed with 'chat' (*Catha edulis*) cropping system was observed throughout all the three study districts; however, sole sorghum cropping system was the dominant production system.

Threshing methods

Farmers in the study districts threshed their sorghum grain in three different ways, namely: on bare ground, on cow dung smeared ground floor and on canvas (Figure1). However, sorghum heads were placed on bare ground for a minimum of a week up to a month before threshing. Farmers practiced on-canvas threshing method when their sorghum grains were not more than two quintals. All sorghum grain samples collected from Haramaya district were threshed on bare ground near the sorghum field, whereas the majority of the samples collected from Babile (66.7%) and Kersa (60%) districts were threshed on cow-dung smeared ground locally known in as "*Awudema*". Only three sorghum grain samples (20%) from Babile and one sample (13.3%) from Kersa were threshed on canvas.

Figure 1: Farmers' threshing methods (A) Threshing on bare ground near to sorghum field (B) Threshing on cow dung smeared ground and (C) Threshing on canvas

Storage methods

The underground storage pit structures across the three study districts were differently managed. Sorghum grain storage practices identified in the current study include sorghum grain stored in with plastic bag, sorghum grain stored in bare pits pit, sorghum grain stored in plastic bags in cemented wall pit, sorghum grain stored without plastic bag in cemented wall pit, sorghum grain stored in plastic lined wall pit and sorghum grain stored in mosquito net in bare pit (Fig. 2).

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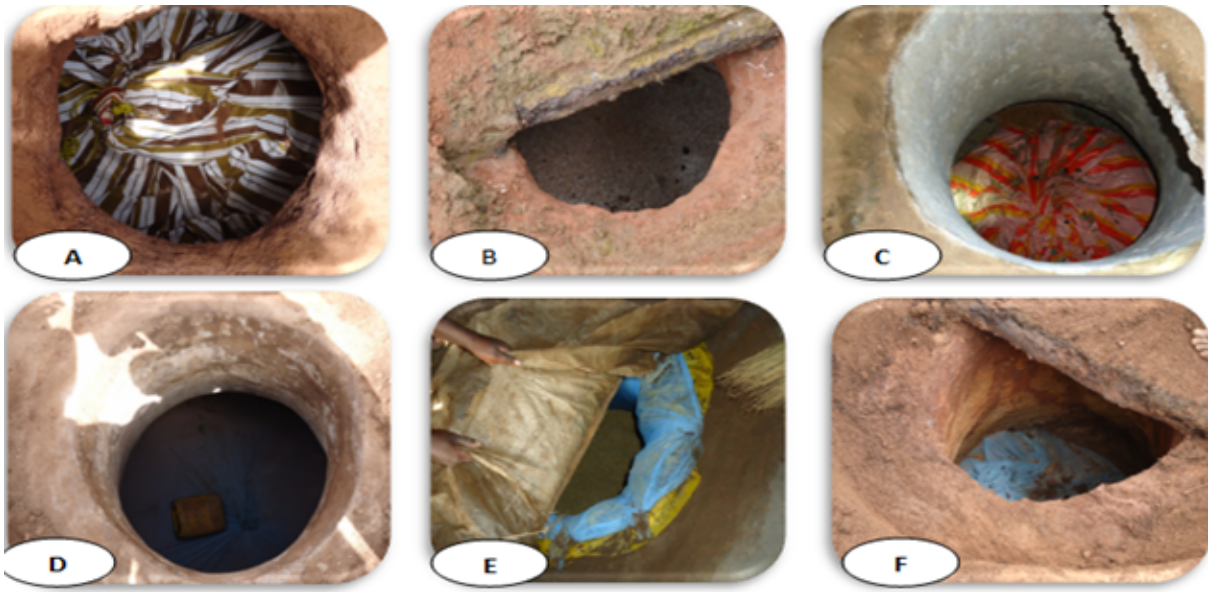

Figure 2: Underground storage pits structures: (A) sorghum grain stored with plastic bag; (B) sorghum grain stored in bare pit; (c) sorghum grain stored with plastic bag in cemented wall pit; (D) sorghum grain stored without plastic bag in cemented wall pit; (E) sorghum grain stored in plastic lined wall pit; and (F) sorghum grain stored with mosquito net in bare pit

About half (53.3%) of the stored sorghum grain samples from Babile were collected from sorghum grain stored in with plastic bag in underground pit. The use of plastic bags in the underground storage pit at Babile and Haramaya districts was more prominent. The Kersa farmers lined the walls of the underground pit with plastic instead of using plastic bags.One-third (15) of the total sorghum grain samples in this study were collected from sorghum grains stored in bare underground pits. The majority (85%) of the sorghum grain samples collected from bare underground pits, irrespective of the study districts, were visibly infested with maize and/or rice weevils (*Sitophilus* spp.) and some beetle species.

Association of threshing and storage methods with aflatoxin B_1 and total fumonisin contamination

Threshing methods

The average aflatoxin B_1 and total fumonisin recorded in the sorghum samples collected from grain threshed on bare ground floor were higher than those in grain samples taken from other threshing floors in all of the three study districts (Table 3). The highest (8.52 µg kg^{-1}) mean aflatoxin B_1 and total (1085.1 µg kg^{-1}) fumonisins were detected from Babile sorghum grain samples threshed on bare ground floor. For both mycotoxins, the lowest mean values were recorded from samples threshed on canvas (Table 3). The highest total fumonisin (2002.8 µg kg^{-1}) was detected from Haramaya sorghum grain samples which were threshed on bare ground near to sorghum field.

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Storage methods

In the current study, high aflatoxin B_1 and total fumonisin levels were recorded from sorghum grain samples collected from bare underground pits across all the three study districts (Table 4). However, the total fumonisin contamination was relatively lower from samples collected from bare ground threshing yards than from other threshing floors. The result revealed that, three sorghum grain samples collected from bare underground storage pits were contaminated with aflatoxin B_1 above the maximum tolerable limit (10 µg kg^{-1}). The highest 33.1 µg kg^{-1} (mean 11.3 µg kg^{-1}) aflatoxin B_1 was recorded from Kersa district.

DISCUSSION

Storage is a critical stage in grain handling, where fungal contamination and mycotoxins accumulation occur. Therefore, care must be taken when storing grains that are wholesome and apparently healthy. The results of this study revealed that farmers in the representative study districts stored their sorghum grains in the traditional underground storage pits in bulk. Previous studies [11] indicated that these underground storage pits were mostly neither lined nor plastered with any material that would reduce moisture migration into the stored grain. Lining of straw and mat, plastic sheets and use of plastic bags in the pit were recommended for prolonged and safe storage of grains [16]. Grain contact with wet inner pit walls leads to moisture ingress from the soil into the inter-granular space elevating both the grain moisture content and the relative humidity inside the pit and these were observed during the study. Such environment leads to Moldings and grain deterioration [17]. The use of plastic bags in the underground storage pit at Babile and Haramaya districts might be due to the intervention of the international non-government organizations that introduced plastic bags in these two districts.

Insect infestation during storage has been associated with storage fungi invasion and aflatoxin contamination [18] that leads to grain deterioration as it predisposes the grain to fungal infection through wounds and bore holes [19]. Kumar *et al.* [20] indicated that *Aspergillus* species are common contaminants in stored rice and their incidence increases with the infestation of rice weevil (*Sitophilus oryzae*). Sorghum samples collected from grains stored in underground storage pits with plastic bags were practically free from insect pest infestation and less corresponding damage was done. For the management of weevil infestation, farmers in the representative study districts used some local tree leaves in their bare underground pits layered on the top of the sorghum grain and a few of the farmers used chemically treated mosquito nets assuming that the chemical could suppress the weevil infestations. However, none of them had been sufficiently tested for their efficacies and efficiencies in managing the insect pests, thereby the aflatoxin in stored crop grains. Use of local plant products for the management of fungi mostly proved their efficacies in *in-vitro* tests [21].

Dejene [8] indicated that piling and threshing of sorghum heads on non-cemented ground could be a possible source of grain contamination by storage fungi. Atukwase *et al.* [22] also reported drying maize on bare ground floor was found to be positively associated with fumonisin contamination, particularly when the harvested maize is allowed to dry

without husks on bare ground. This practice brings maize grains into direct contact with soil, which is a primary source of *Fusarium* [23].

Drying cereal grains on bare ground may cause an increase in water activity of the grains due to absorption of moisture from the soil [24]. Therefore, the difference in mycotoxins contamination in the sorghum grain samples observed in this study could be due to the differences in the direct contact of the grain with the soil. Aflatoxin B_1 detected in this study was below the maximum tolerable level set at 10 µg kg^{-1} for East African Community [25] whereas sorghum grain sample threshed on bare ground floor near to sorghum field collected from Haramaya district was slightly above the maximum tolerable level for total fumonisin set for East African Community, which is 2 mg kg^{-1} (2000 µg g^{-1} grain) [25] this could be due to the direct contact of the grain with that of soil contaminated by *Fusarium* spp.

Grains could be infected by fungi in the field and these field fungi persist in and proliferate with consequent increase in mycotoxin formation during storage when favorable conditions prevail [26]. The environment in the underground storage pits and on bare ground threshing yard might favor the fumonisin producing fungi, *Fusarium* species [27]. Entrance of moisture from the surrounding soil into the grain elevates the grain moisture content and enhances respiration by insect pests and micro-organisms. This situation further increases the granary temperature, relative humidity and grain moisture creating favorable conditions for storage fungi leading to grain spoilage. Storage of harvested grains at >10% moisture content and for prolonged period in poor storage facilities cause proliferation of molds on grains [28]. Therefore, mycotoxin incidence and contents are likely to be higher in sorghum grains stored in bare underground pits than those on the threshing floor. The incidence and concentrations of the two studied mycotoxins were consistently higher in bare underground storage pit than in other types of storage methods. For improvement of underground pits; lining of straw and mat, plastic sheets and use of plastic bags in the pit were recommended by Hell and Mutegi [16] for prolonged and safe storage of grains and in this current study also any improvement made on the underground pit showed low level of contaminations with the two mycotoxins studied.

While storing the sorghum grain in the underground pits, majority of the farmers in the study districts mixed more than two sorghum varieties together. Majority of the farmers in the study districts mixed more than two sorghum varieties together when they store in the underground pits. Such practice of mixing varieties of grains of different grades during storage was considered as a nasty practice by Wagacha and Muthomi [7], especially when one contains a large number of fungal spores that provide inoculum for the good grade and probably contaminate the free grain. In this study, however, mixing of two and more sorghum varieties in the underground storage pit had an advantage over the use of single sorghum variety in reducing the mycotoxin contamination.

CONCLUSION

The study results showed that threshing sorghum grains on bare ground floor and keeping grains in bare underground storage pits, coupled with high moisture content of the grains and high temperature and relative humidity of the pits allowed multiplication and

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invasion by fungi and predisposed the sorghum grains to contamination with aflatoxin B_1 and total fumonisin.

Farmers in the study area and elsewhere that produce sorghum should dry the sorghum grains to safe moisture levels of 10-13% before storing. Threshing and drying the grains should also be done on covered or plastered surfaces, but not on bare grounds as this would lead to an increase invasion by fungi and mycotoxin contamination. In addition, the bare underground storage pit should be improved in ways that prevent the direct contact of the grain with moist inner pit walls, which will lead to moisture ingress elevating both the grain moisture content and the granary relative humidity inside the underground storage pit. Furthermore, there is need for evaluation of the efficacies of those local tree leaves that are used by farmers in their bare underground storage pits to suppress grain infestations by weevils to have a complete picture about their potential in reducing invasion of fungi and thereby mycotoxins contamination.

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Table 1: Characteristics of agro-climatic regimes of the three study districts of East Hararghe, Ethiopia

District	Ecology	Climate	Altitude (m.a.s.l)	Average Annual Temperature (^oC)	Average Annual Rainfall (mm)
Babile	Lowland	Warm semiarid	< 1700	20-27.5	200 - 800
Haramaya	Intermediate	Cool and sub-humid	> 1700 - 2100	17.5 - 20	800 - 1200
Kersa	Highland	Cool and humid	> 2100	11.5 - 17.5	1200 - 2200

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Table 2: Farmers' sorghum grain management in the three study districts, East Hararghe, Ethiopia

Variables	Frequency (n=15)			Percentage (%)		
	B	K	H	B	K	H
Farmers threshing methods						
on bare ground	2	5	15	13.3	26.7	100
on cow dung painted ground	10	9	-	66.7	60	-
on canvas	3	1	-	20	13.3	-
Sorghum cropping system						
Sole sorghum	13	11	10	86.7	73.3	66.7
Mixed with chat	2	4	5	13.3	26.7	33.7
Sorghum grain stored in underground pits						
with big plastic bag	8	1	5	53.3	6.7	33.3
in bare pit	5	5	6	33.3	33.3	40
with plastic bag in cemented wall pit	-	-	1	-	-	6.7
without plastic bag in cemented wall pit	-	-	1	-	-	6.7
in plastic lined wall pit	1	7	2	6.7	46.7	13.3
with mosquito net in bare pit	1	2	-	6.7	13.3	-
Variety of sorghum grain stored						
Mixed variety	3	7	6	20	46.6	40
Muyera	-	4	7	-	26.7	46.7
Fendisha	-	-	2	-	-	13.3
Long muyera	-	4	-	-	26.7	-
Bule local	5	-	-	33.3	-	-
Chame	3	-	-	20	-	-
Duken	1	-	-	6.7	-	-
Teshale	2	-	-	13.3	-	-
Gubiye	1	-	-	6.7	-	-

B= Babile, K= Kersa, H= Haramaya

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Table 3: Level of aflatoxin B_1 and total fumonisin in sorghum grain threshed in different methods in three districts of East Hararghe, Ethiopia

Farmers threshing methods	Aflatoxin B_1 (μgkg^{-1})					
	Babile		Kersa		Haramaya	
	Range	Mean	Range	Mean	Range	Mean
on bare ground	0.04, 17	8.52	0.39 - 6.22	2.47	0.11 - 2.23	0.71
on cow dung painted ground	nd- 6.13	1.4	nd - 0.56	0.23	-	-
on canvas	nd - 0.28	0.28	0.05	0.05	-	-
	Total fumonisin (μgkg^{-1})					
on bare ground	nd,1085.1	1085.1	nd -1102.5	789.8	nd -2002.8	1065.4
on cow dung painted ground	nd - 1269.3	715.26	nd - 986.4	499.4	-	-
on canvas	nd – 586	404.4	nd	nd	-	-

nd= not detected

Table 4: Level of aflatoxin B_1 and total fumonisin in sorghum grain stored in underground pit in different ways in three districts of East Hararghe, Ethiopia

Sorghum grain stored in underground pit	Aflatoxin B_1 (μgkg^{-1})					
	Babile		Kersa		Haramaya	
	Range	Mean	Range	Mean	Range	Mean
with plastic bag	0.3 - 1.2	0.9	0.3	0.3	0.8 - 1.2	1
in bare pit	1.7 - 16.3	7.2	1.0 - 33.1	11.3	0.9 - 11.8	4.6
with plastic bag in cemented wall pit	-	-	-	-	0.3	0.3
without plastic bag in cemented wall	-	-	-	-	0.5	0.5
in plastic lined wall pit	0.6	0.6	0.7 - 1.4	0.9	0.5 - 1.2	0.9
with mosquito net in bare pit	-	-	2.0 , 3.0	2.5	-	-
	Total fumonisin (μgkg^{-1})					
with plastic bag	nd - 1535	982	nd	nd	nd - 1868	1241
in bare pit	nd - 2041	1401	nd - 1136	1136	624 - 1933	1075
with plastic bag in cemented wall pit	-	-	-	-	1547	1547
without plastic bag in cemented wall	-	-	-	-	1229	1229
in plastic lined wall pit	1184	1184	nd - 1415	766	1031, 1476	1254
with mosquito net in bare pit	1885	1885	nd, 841	421	-	-

nd= not detected

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