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Storage management practices and mycotoxin contamination of sorghum (*Sorghum bicolor*) in northwest Ethiopia

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ABSTRACT

Mycotoxins are toxic metabolites produced by certain fungal species that affect animal and human health. Data on the relationships between specific traditional storage management practices of sorghum and mycotoxin contamination are rarely available in Ethiopia. The aim of this study was to investigate current sorghum storage management practices in major sorghum producer locations in Northwest Ethiopia and their relationships with mycotoxin contamination. Sorghum storage management practices of 120 farmers were surveyed, the occurrence of multiple mycotoxins in samples from their stored sorghum was determined, and potential relationships between the traditional storage management practices and mycotoxin contamination were analyzed. Samples were analyzed using UPLC-MS/MS for 33 different mycotoxins. About 88% of the samples were contaminated with at least one mycotoxin. The detected mycotoxins belong to one of the four mycotoxin categories, produced by *Aspergillus* spp, *Fusarium* spp, *Penicillium* spp, and *Alternaria* spp. From the total, 3%, 7%, and 3% of the samples were contaminated with aflatoxins, ochratoxin A, and zearalenone, respectively, above the EU regulatory limits. The measured concentrations that bypassed EU regulatory limits were 9.14, 18.34 and 29.13 (μg/kg) for total aflatoxins, 5.31, 12.50, 14.94, 15.77, 32.94, 56.81, 58.07 and 112.59 (μg/kg) for Ochratoxin A, and 123.48, 238.43 and 431.78 (μg/kg) for Zearalenone, respectively. Logistic regression showed relationships between the traditional storage management practices with mycotoxin contamination. The age and the experience of the Main Person Responsible for Storage management (MPRS), the placement of the storage structure, and the insecticide application showed negative relationships with multi-mycotoxin contamination. On the other hand, the educational status of the MPRS and the type of storage structure showed positive relationships with mycotoxin contamination. Therefore, it is recommended that farmers receive training in proper sorghum storage management to further reduce the mycotoxin contamination in the grain.

1. Introduction

Sorghum (*Sorghum bicolor*) is an important staple food grain in Ethiopia (MOA, 2020; Mohammed et al., 2022). In the 2019/20 cropping season, it was the fourth most produced cereal, next to maize, wheat, and teff. In this season, about 48 million quintals of sorghum was produced in the country (CSA, 2020/21).

Despite its importance as a staple food, the contamination of sorghum with mycotoxins in Ethiopia has been frequently reported, even to concentrations beyond regulatory limits (Chala et al., 2014; Mohammed

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et al., 2022; Ssepuuya et al., 2018; Taye et al., 2018). Mycotoxins are toxic secondary metabolites produced by certain fungal species that can cause animal and human health problems. Two of the most known mycotoxins to cause human health problems are aflatoxins and fumonisins. Aflatoxins are mainly produced by the fungal species of *Aspergillus flavus* and *Aspergillus parasiticus*, and fumonisins are mainly produced by the fungal species of *Fusarium verticillioides*, *Fusarium proliferatum* and *Aspergillus niger* (Wu et al., 2014). Aflatoxins can cause liver cancer and acute toxicities, reduce protein synthesis, and lower immune responses (Smith et al., 2012). Fumonisins can cause neural tube defects and esophageal cancer (Wu et al., 2014). In the year 2021, Mohammed et al. (2022) reported the presence of 79 different mycotoxins and related fungal metabolites in stored sorghum samples collected from farmer's households in Eastern Ethiopia. The reported toxins included both regulated mycotoxins such as aflatoxins, fumonisins, zearalenone, ochratoxin A, and deoxynivalenol; and emerging mycotoxins such as 3-nitropropionic acid, sterigmatocystin, fusaric acid, tenuazonic acid, alternariol, and moniliformin. In another study, Ssepuuya et al. (2018) reported the presence of both common mycotoxins including the above-mentioned ones and emerging mycotoxins such as sterigmatocystin, alternariol, and altenuene in sorghum samples collected from newly harvested grain and stored grain in farmers' households and local markets in Ethiopia in 2012/2013. Parts of the concentrations of the regulated mycotoxins in both studies were above regulatory limits. Further, Weledesemayat et al. (2016) reported that all the sorghum samples collected from farmers' households in the Kewet district in the North Shewa zone were contaminated with aflatoxins, all above regulatory limits. Besides to health related problems, mycotoxin contamination also affects trade and the economy since contaminated products above regulatory limits is not allowed in many countries (Ortega-Beltran and Bandyopadhyay, 2021; Vipham et al., 2020).

Weather conditions in Ethiopia, which are conducive to fungal growth, have contributed to the mycotoxin contamination of sorghum together with the underdeveloped nature of sorghum value chain practices that are favorable for fungal infection (Abamecha, 2021; Mohammed et al., 2022; Taye et al., 2016, 2018, 2022). The presence of toxigenic fungal species in soil, water, and air makes infection possible at any stage of the value chain (Reis et al., 2010). When convenient weather conditions are available, the fungal species proliferate and produce mycotoxins. Consequently, fungal species, namely *Aspergillus*, *Fusarium*, *Alternaria*, *Bipolaris*, *Mucor*, *Penicillium*, and *Rhizoctonia*, that are known to produce mycotoxins, were detected in sorghum grain samples in Ethiopia (Mohammed et al., 2022; Taye et al., 2016). Besides to the weather condition, the low level of awareness of farmers about mycotoxins and their control has been mentioned as a contributing factor for the mycotoxin contamination (Beyene et al., 2016; Cervini et al., 2023).

Implementing appropriate agricultural practices is claimed as the most important strategy for managing mycotoxin contamination. This is because effective and practically applicable decontamination processes to reduce mycotoxin contamination are limited to date (Pandey et al., 2023). The fact that fungal infection is affected by climatic conditions that human beings cannot control, makes prevention of mycotoxin contamination during the preharvest period, the period from land preparation to harvesting, challenging (Dövényi-Nagy et al., 2020). On the other hand, fungal infection of grain can also occur during the storage period; yet, the growth of fungi and their toxin production can be controlled by applying proper storage management practices, since the ecological conditions inside the storage structures that favor fungal growth can be controlled (Roman et al., 2020; Walker et al., 2018; Wawrzyniak et al., 2018). Mycotoxin contamination during grain storage is affected by the grain storage ecosystem including ecological conditions such as temperature and relative humidity (Mannaa and Kim, 2017; Wawrzyniak et al., 2018); air composition (Williams et al., 2014) as well as moisture and air barrier properties of the structure (Jian et al., 2009; Omodara et al., 2021; Williams et al., 2014). Reducing the growth of toxigenic fungal species during storage is one of the key strategies proposed to prevent mycotoxin contamination in grains (Matumba et al., 2021). Reduction of mycotoxin contamination has been achieved for instance by storing grain in PIC (Purdue Improved Crop Storage) sack, which is a hermetic bag that has a three-layer system that acts as a barrier for preventing oxygen entry and release of carbon dioxide (Williams et al., 2014). When storing grain in this sack, the gas composition created inside the sack by biological respiration within the sack makes the storage environment unsuitable for insect development and fungal growth (Tubbs et al., 2016). The use of this introduced PICs sacks in Ethiopia is limited due to supply chain constraints (Mekonen et al., 2021). Instead, indigenous storage structures are commonly used to store grains (Garbaba et al., 2018; Sadik et al., 2023; Taye et al., 2016).

Despite previous studies on the occurrence of mycotoxin contamination in stored sorghum grain in Ethiopia, studies on the relationships between the specific traditional storage structures used by subsistence farmers with mycotoxin contamination is limited. Taye et al. (2016) reported the presence of positive relationship between sorghum stored in pits in Eastern Ethiopia with the contamination of *Aspergillus* and *Fusarium* species, and aflatoxin B. Indigenous and introduced grain storage structures used by farmers in developing countries vary in their storage ecosystems leading to variability in mycotoxin contamination (Sadik et al., 2023; Walker et al., 2018). Proper storage management of grains considering the storage ecosystems helps to develop a feasible intervention option to control mycotoxin contamination (Neme and Mohammed, 2017).

This study aimed to investigate the traditional sorghum storage management practices in major sorghum producer locations in Northwest Ethiopia and their relationship with mycotoxin contamination. This part of Ethiopia belongs to the leading sorghum producing locations in the country (CSA, 2020/21). To date, research on the possible relationship of sorghum storage practices with mycotoxin contamination in this area is hardly available.

2. Materials and methods

2.1. Selection of study sites

The research was conducted in three purposively selected districts (locally called *woredas*), ensuring sufficient diversity in agroecological conditions and agronomic practices. A multistage sampling technique was applied to select the *woredas*. First, Amhara National Region State, located in Northwest Ethiopia, which is the second (next to Oromia National Regional State) highest producer of sorghum nationally (CSA, 2020/21), was purposively selected. Second, three leading sorghum producer administrative *zones* (West Gondar, South Wollo and North Shewa administrative *zones*) were purposively selected based on CSA (2020/21) sorghum production data. West Gondar administrative *zone* was excluded as sampling location due to security reasons related to the ongoing war in North Ethiopia during the field assessment (2020–2022). Central Gondar administrative zone, which ranked fourth in sorghum production in the Amhara Region CSA (2020/21) was selected instead. One high sorghum producer *woreda* was selected from each administrative zone, namely West Belesa from Central Gondar, Kalu from South Wollo and Kewet from North Shewa. The geographic locations of the three selected *woredas* are presented in Fig. 1. Weather data has been collected from the National Meteorology Institute of Ethiopia, Addis Ababa for the years 2001–2021 (and presented in Appendix Table 1). Weather data is not available for the last few years partly because of the war in northern Ethiopia.

2.2. Sample size

A total sample size of 120 households was used for the study. The total sample size was equally distributed among the three *woredas* (40

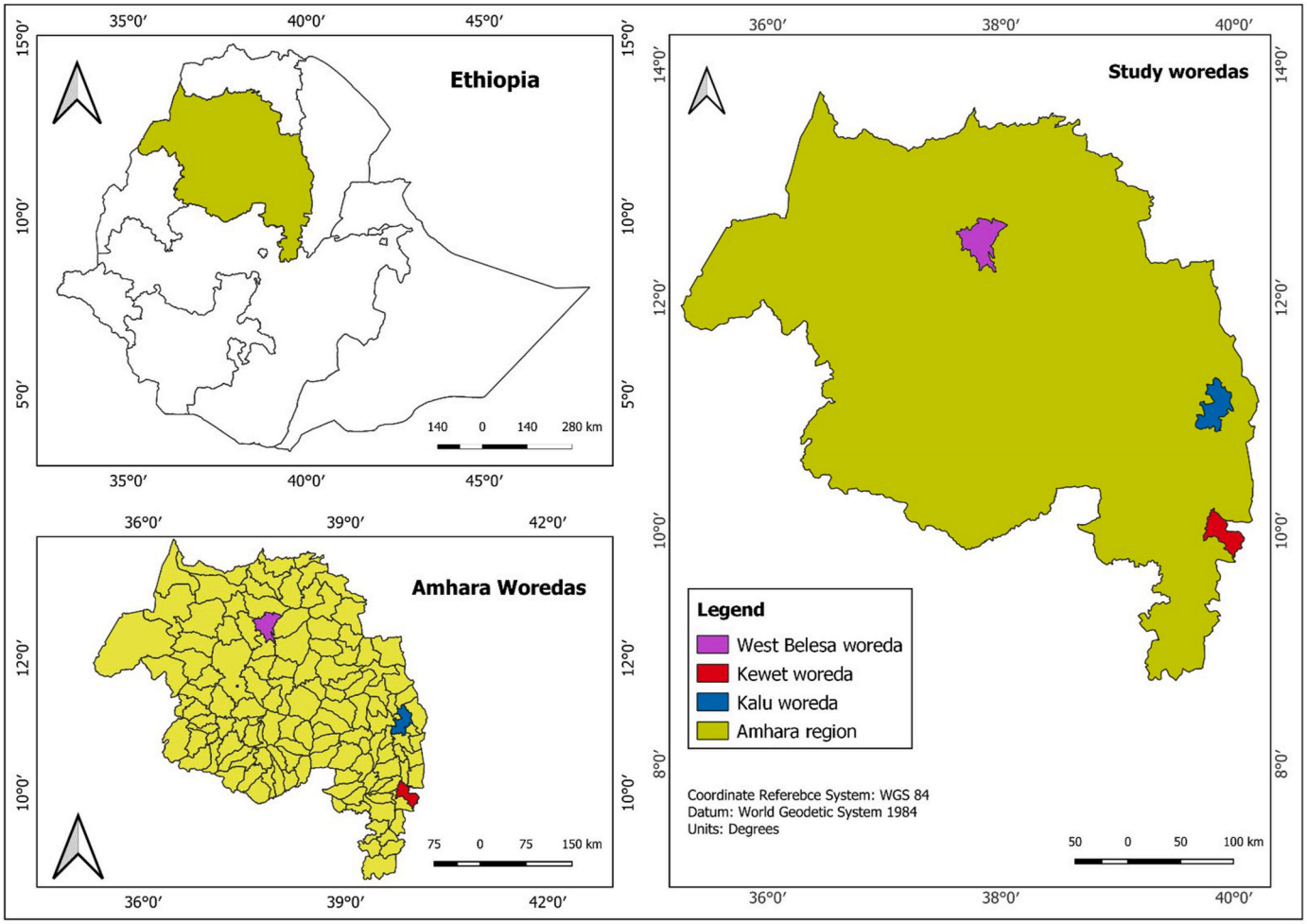

Fig. 1. Locations of sorghum sample collection *woredas* in Ethiopia.

households each). From each *woreda*, the leading sorghum producer rural villages (locally called *kebele* administrations) were selected purposely by the Crop Research Directorate officers from the respective *woredas* Agriculture Offices. The selected *kebele* administrations were Abay Tara, Diquana, Ebrarag and Qaley from West Belesa; Miawa, Woraba, Agamssa and Chorasa from Kalu, and Terie, Yelen and Birbira from Kewet *woredas*, respectively. Proportion to population size was applied to distribute the sample size allocated to each *woreda* to its *kebele* administrations (Appendix Table 2). The required numbers of farmer households from each *kebele* administration were randomly selected from the list of registered farmer households available in the respective *kebele* administration.

2.3. Data collection

2.3.1. Sorghum sample collection

Samples were collected in the last week of June 2023 from sorghum grain stored for six months. In all the locations, sorghum supposed for human consumption was stored in a shelled form. Sorghum was stored in introduced storage structures namely polypropylene (pp) and PICs sacks, and in indigenous storage structures namely *gota, sherfa* and underground pit. *Gota* is a traditional structure that is made up of mud mixed with straw, and it is cylindrical in shape. *Sherfa* (also called *gotera* in other places in Ethiopia) is a basket work that is usually thatched with conical roofing (Ayalew et al., 2006). For sack storage, the number of grain storage sacks recommended for sample taking was based on the International Rules for Seed Testing, ISTA (2016). However, this sampling guideline could not be fully implemented, particularly in households that had a relatively higher production volume. This was due to poor accessibility of storage sacks for sampling and the willingness of farmers to provide samples only from a small number of sacks due to fear of disordering of an organized set of sacks. Consequently, samples were taken from two to six sacks per household. Three to five incremental samples were taken from every sack, from selected points from the top, middle, and bottom by scooping with hand according to ISTA (2016) procedure. For the indigenous storage structures, incremental samples were collected from various points of the structures using a grain sampling trier (probe). The aggregate weight of samples collected from each household was 1–2 kg based on the European Commission Regulation (EC, 2006). The aggregate samples were thoroughly mixed manually on the spot, and packed in labeled fabric bags that had a fine mesh according to Ssepuuya et al. (2018). All the samples were then packed together in a cartoon box and transported to the Food Process Laboratory, Bahir Dar University, within three days after collection. Immediately after reception in the laboratory, the samples were milled using a coffee grinder machine (Zaiba®, Model No: ZA-728, China), sieved to less than 1 mm particle size according to Ssepuuya et al. (2018), sealed in polypropylene plastic films and preserved at −20 °C for about two months. The frozen samples were individually thawed, and thoroughly mixed, about 100 g samples were taken by quartering, packed in a polypropylene bag, and labeled. Then these 120 samples were transported under cooled conditions to Wageningen Food Safety Research Laboratory, The Netherlands for mycotoxin analysis.

2.3.2. Sorghum storage management practices

Data about storage practices was collected through a face-to-face

interview with the Main Persons Responsible for Storage management (MPRS) (Appendix Table 3). Ethical approval for the interview was obtained from Bahir Dar University Institutional Review Board (Protocol number 12/IRB/23), before respondent data collection. Interview data was collected from the end of March to mid-April 2023, and Written Informed Consent was received before the interview. The basic storage practices questionnaire was adopted from previous research works (Ayalew et al., 2006; Baye et al., 2013; Dejene et al., 2004; Hengsdijk and de Boer, 2017; Yetneberk et al., 2005). The questionnaire was initially developed in English language and translated into the local language (*Amharic*) for the interview.

2.4. Mycotoxin analysis

Thirty-three different mycotoxins were analyzed using a validated LC-MS/MS method described below. Both regulated (European Commission Regulation (EC, 2023)) and emerging mycotoxins were considered, as listed in Appendix Table 4 (Sadik et al., 2024; another manuscript under press in another Journal).

2.4.1. Chemicals

Analytical standards of mycotoxins were purchased individually from Sigma-Aldrich, CoringSystem DiagnostiX and BioAustralis. Mixtures containing different standards were prepared in 50% acetonitrile (acidified 0.1% acetic acid). HPLC-grade methanol and acetonitrile were purchased from Biosolve (Valkenswaard, the Netherlands). Formic acid, ammonium formiate, acetic acid, ultrapure water (Milli-Q Gradient A10) and ^{13}C-Caffein were from Sigma-Aldrich (Darmstadt, Germany). Magnesium sulphate dried from VWR was also used.

2.4.2. Sample preparation

Sorghum flour samples (2.5 ± 0.05g each) were individually weighed in 50 ml Greiner Tubes and 25 µl of ^{13}C-caffeine internal standard (10 µg/ml) was added to each sample. Bi-distilled water (7.5 ml) was added to each sample with a subsequent mixing by using a vortex mixer. Next, 10 ml of acetonitrile with 1% acetic acid was added and mixed using an overhead shaker, for 30 min. Then, 4 g of magnesium-sulphate was added to the individual retrieved samples as per the QuEChERS method. The samples were subsequently shaken manually for 1 min, and then centrifuged at 3000 rpm for 10 min. A 250 µl of the resulting supernatant was transferred to 0.5 ml filter vials, followed by addition of 250 µl acetonitrile (50%). The filter caps were subsequently placed on top of these vials and on the vials containing solutions for the calibration curves (but not yet pushed through), and the filter vials were briefly vortexed and placed in a refrigerator set at a temperature of 4 °C for 1 h. The retrieved vials were briefly vortexed again before the filter caps were pushed through with the aid of a vial closure tool. The resulting extract was used for the UPLC-MS/MS analysis.

2.4.3. UPLC-MS/MS analysis

The multimycotoxin analysis was conducted by using an Exion LC (Applied Biosystem) system coupled with QTRAP 6500 MS/MS Mass spectrometer (Applied Biosystem). For chromatographic separation, a reverse phase C18 column (Acquity HSS T3 1.8 µm 100 × 2.1 mm) heated at 35 °C was used. The volume of analyte injected was 5 µl and the flow rate of elution was 0.4 ml/min. Gradient elution was performed by using 1 mM ammonium formate in water (mobile phase A), and 1 mM ammonium formate in methanol/water (95/5, v/v) (mobile phase B), both acidified with 1 % formic acid. Initial conditions were set at 100% mobile phase A, then mobile phase B was increased to 50% in 3 min and to 100 % in 5 min; after 2 min of isocratic step at 100% B, the system was re-equilibrated to initial conditions for 4.5 min. The total run time was 15 min.

The analyses were performed using both positive and negative electrospray ionization (ESI) modes. The operating conditions for the analysis were the following: ion spray voltage, +4000 V (ESI pos) and -4000V (ESI neg); curtain gas, 35 (arbitrary units); GS1 and GS2, 50 psi; probe temperature (TEM), 400 °C. Nitrogen served as the nebulizer and collision gas. The MS was operated in multiple reaction monitoring (MRM) mode with the resolution set to unit resolution for Q1 and Q3.

Matrix-matched calibration curves were used for target analyte quantification. Sorghum blank matrices were previously checked using the above-described method. For positive samples, peak area values of specific mycotoxins corresponding to its retention time were obtained from the UPLC-MS/MS array with the Analyst software program and checked using Multiquant 3.0.3. program.

2.4.4. Validation

To evaluate recovery (extraction efficiency), two blank sorghum samples were spiked with the 33 mycotoxins listed in Appendix Table 4. The spiked sample was left standing for 10 min to allow the spiked solution to be absorbed by the sorghum flour. The efficiency of mycotoxin extraction was evaluated based on the percent recovery of spiked blank samples and in accordance with the EU regulation (EC) No 401/2006). The percent recovery values for each mycotoxin are summarized in Appendix Table 4.

The limit of Detection (LOD) and the limit of Quantification (LOQ) were determined from the chromatogram signal by visual inspection. LODs were estimated from signal-to-noise (S/N = 3) in chromatograms obtained from the diluted calibration curves. LOQs were estimated by S/N = 10. The LOD and LOQ values of all the mycotoxins are presented in Appendix Table 4.

2.5. Statistical data analysis

Descriptive statistics were used to investigate the percentages of farmers who applied each of the different storage management practices and to describe the percentages of samples that were contaminated with mycotoxins. The 33 mycotoxins were grouped into four mycotoxin categories namely Aspergillus, Fusarium, Penicillium, and Alternaria toxins as based on (Mohammed et al., 2022; Moretti and Susca, 2017). Samples were regarded as contaminated with a particular mycotoxin category if at least one of the specific mycotoxins belonging to the same category was detected above its limit of detection (LOD). Univariate logistic regression analysis was used to investigate the relationship between individual study variables with specific mycotoxins and the different mycotoxin categories. Multiple logistic regression analysis was also applied to further study the relationships between the individual storage management practices with mycotoxin contamination when all the other variables were kept constant. Both common (regulated) and emerging (unregulated) mycotoxins were treated as equally important in logistic modeling. Stata software version 16 (StataCorp LLC, College Station, Texas 77845 USA) was used for statistical analysis.

3. Results

3.1. Sociodemographic characteristics

In most of the households (79%), husbands, all men, were the heads of the households. In the majority of the households (63%), wives, all women, were the Main Persons Responsible for Storage management (MPRS) of sorghum. The age range of the MPRS was 22–74 years (mean 43.12 ± 11.40 years). Apart from minor differences in the MPRS experiences between the total and each *woreda*, similarities were observed between the responses obtained in each *woreda* and the total (Table 1).

3.2. Sorghum storage management practices

Both introduced and indigenous storage structures were used to store sorghum grain (Table 2). In comparison, a slightly lower proportion of farmers (43%) than those who used the introduced storage structures,

Table 1
Sociodemographic characteristics of sorghum producer farmers in Northwest Ethiopia (2023).

Variable	Category	Response (%)			
		Total (n = 120)	W. Belesa (n = 40)	Kalu (n = 40)	Kewet (n = 40)
Gender of Head of Household	Male	79	88	80	70
	Female	21	13	20	30
MPRS[a]	Wife	63	73	55	63
	Other[b]	37	28	45	38
Age[a] *(yr)*	18<−30	14	15	15	13
	31<−50	65	58	58	80
	above 50	21	28	28	8
Basic formal education[a]	Yes	19	18	15	25
	No	81	83	85	75

[a] Refers to the Main Persons Responsible for sorghum Storage management.
[b] Refers to households where the MPRS is the husband, both wife and husband with equal responsibility, or other family member (son). Among the MPRS categorized under Other* category, 45% were husbands, 2% were sons, and 52% were households where both wives and husbands had similar responsibility for the sorghum storage management. The sum of responses for some of the columns for several variables is greater than 100 (101) due to the rounding of decimal numbers to the nearest one digit.

Table 2
Sorghum storage management practices of sorghum producer farmers in Northwest Ethiopia (2023).

Variable	Category	Response (%)			
		Total (n = 120)	W. Belesa (n = 40)	Kalu (n = 40)	Kewet (n = 40)
Storage experience (yr)*	<- 15	27	25	33	23
	16<−30	48	45	33	68
	>30	25	30	35	10
Type of storage structure	Indigenous	43	90	3	35
	Introduced	58	10	98	65
Name of storage structure	Sack	58	13	98	65
	- PP bag	49	100	15	88
	- PICs bag	51	0	85	12
	Gota	10	30	0	0
	Sherfa	19	58	0	0
	Pit	13	0	3	35
Placement of storage structure	Indoor	68	43	98	63
	Outdoor	33	58	3	38
Insecticide application	Yes	59	80	13	85
	No	41	20	88	15

used the indigenous storage structures namely *gota*, *sherfa* and underground pit. *Gota* is a traditional structure that is made up of mud mixed with straw, and it is cylindrical in shape. *Sherfa* (also called *gotera* in other places in Ethiopia) is a basket work that is usually thatched with conical roofing (Ayalew et al., 2006). *Gota* is a fixed structure that is built inside a room (indoors). On the other hand, *sherfa* is a moveable structure, which can be placed indoors or outdoors. A remarkable difference was observed in the types of storage structures used by farmers in different *woredas* (Table 2). Most farmers in West Belesa use the indigenous structures, particularly *gota* and *sherfa*, while the majority in the other two *woredas* use the introduced storage structures. The pictures for the grain storage structures are given in Fig. 2.

3.3. Prevalence of mycotoxin contamination

The 33 mycotoxins found in the sorghum samples belong to four major categories of mycotoxins – 7 to Aspergillus, 20 to Fusarium, 4 to Penicillium and 2 to Alternaria. The occurrence of the mycotoxin categories in decreasing order of prevalence was Aspergillus (72%), Alternaria (67%), Fusarium (61%), and Penicillium (31%).

The presence of mycotoxins is summarized in Table 3. Comparing the individual mycotoxins – both Alternaria mycotoxins, i.e. alternariol-methylether and alternariol, were the most detected, with a prevalence of 64 and 60%, respectively. Two other mycotoxins that belong to the Aspergillus category, nitropropionic acid and sterigmatocystin, were also detected in more than half of the samples, with a prevalence of 56 and 54%, respectively. Among the mycotoxins in the Fusarium category, beauvericin and moniliformin were the most prevalent, detected in 39 and 40% of the samples, respectively. From the penicillium category, mycophenolic acid was the most prevalent toxin which was detected in 28% of the samples. In comparison, the common mycotoxins occurred in a lower prevalence than the emerging mycotoxins. Among the common mycotoxins, ochratoxin A was the most prevalent, which was detected in 13% of the samples. The prevalence of aflatoxins and fumonisins was relatively low, yet the prevalence of fumonisins was slightly higher than that of aflatoxins (Table 3).

About 88% of the collected samples were contaminated with at least one specific mycotoxin. From the total samples, 72% of samples were contaminated with three or more mycotoxins, indicating the majority of the samples were contaminated with multiple mycotoxins (Fig. 3).

3.4. Variables associated with mycotoxin contamination

The age of the MPRS, storage experience of the MPRS, storage placement and insecticide application showed negative relationships with mycotoxin contamination. This means the probability of mycotoxin contamination in sorghum decreases with increase in years of either the age or the storage experience of the MPRS. In addition, compared to sorghum stored in indoor structures, the probability of the occurrence of mycotoxin in sorghum decreases when sorghum was stored in outdoor structures. Further, compared to sorghum grain stored with no insecticide application, the probability of mycotoxin contamination in the grain decreases when insecticide was applied. On the other hand, both the educational status of the MPRS and the type of storage structure showed positive relationships with mycotoxin contamination. This means that compared to sorghum storage management by MPRS who have received no formal education, storage management by MPRS who have received a basic formal education increased the probability of mycotoxin contamination. In addition, compared to sorghum stored in indigenous storage structures, the probability of mycotoxin contamination increased when sorghum was stored in introduced storage

Fig. 2. Sorghum storage structures.

Table 3
Prevalence of mycotoxin contamination in stored sorghum in Northwest Ethiopia (2023).

Major category	Specific mycotoxin	Total (120)		W. Belesa (40)		Kalu (40)		Kewet (40)	
		% p* (N)	conc. range (μg/Kg)	% p (N)	conc. range (μg/Kg)	% p (N)	conc. range (μg/Kg)	% p (N)	conc. range (μg/Kg)
Aspergillus	Aflatoxin B1	4(5)	< LOD-27.00	5(2)	< LOD-10,63	8(3)	< LOD-27,00	0(0)	< LOD
	Aflatoxin B2	3(4)	< LOD-2.13	5(2)	< LOD-1,67	5(2)	< LOD-2.13	0(0)	< LOD
	Aflatoxin G1	3(3)	< LOD-5.36	5(2)	< LOD-5.36	3(1)	< LOD-1,28	0(0)	< LOD
	Aflatoxin G2	2(2)	< LOD-0.67	5(2)	< LOD-0.67	0(0)	< LOD	0(0)	< LOD
	Ochratoxin A	13(15)	<LOD-112.59	10(4)	<LOD-112.59	15(6)	< LOD-58.07	13(5)	< LOD-14.94
	Nitropropionic acid	56(67)	<LOD-1407.28	20(8)	<LOD-555.83	83(33)	< LOD-1407.28	65(26)	< LOD-308.68
	Sterigmatocystin	54(65)	<LOD-81.05	53(21)	<LOD-81.05	45(18)	< LOD-8.33	65(26)	< LOD-55.54
Fusarium	Fumonisin B1	14(17)	< LOD-45.4	0(0)	< LOD	10(4)	< LOD-45.4	33(13)	< LOD-35,66
	Fumonisin B2	8(10)	< LOD-12.01	5(2)	< LOD-6.23	0(0)	< LOD	20(8)	< LOD-12.01
	Fumonisin B3	4(5)	< LOD-8.01	0(0)	< LOD	3(1)	< LOD-1.81	10(4)	< LOD-8.01
	Deoxynivalenol	2(2)	<LOD-57.19	0(0)	< LOD	0(0)	< LOD	5(2)	<LOD-57.19
	Nivalenol	4(5)	LOD < −85,61	0(0)	< LOD	0(0)	< LOD	13(5)	LOD < −85,61
	Zearalenone	18(21)	<LOD-431.78	13(5)	<LOD-11.92	25(10)	<LOD-431.78	15(6)	<LOD-7.86
	α-Zearalenol	1(1)	<LOD-2.6	0(0)	< LOD	3(1)	<LOD-2.6	0(0)	< LOD
	β-Zearalenol	1(1)	<LOD-6.20	0(0)	< LOD	3(1)	<LOD-6.20	0(0)	< LOD
	3-Acetyl-DON	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD
	15AcetylDON	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD
	Diacetoxyscirpenol	23(28)	<LOD-6.42	5(2)	<LOD-2.57	20(8)	<LOD-4.28	45(18)	<LOD-6.42
	DON-3-Glucoside	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD
	Beauvericin	39(47)	<LOD-59.09	10(4)	<LOD-1.76	40(16)	<LOD-59.09	68(27)	<LOD-32.96
	Enniatin A	3(4)	<LOD-18.3	0(0)	< LOD	8(3)	<LOD-18.3	3(1)	<LOD-0.47
	Enniatin A1	3(4)	<LOD-9.34	0(0)	< LOD	8(3)	<LOD-9.34	3(1)	<LOD-2.88
	Enniatin B	4(5)	<LOD-12.391	3(1)	<LOD-0.67	5(2)	<LOD-4.99	5(2)	<LOD-12.391
	Enniatin B1	3(3)	< LOD-8.08	0(0)	< LOD	3(1)	<LOD-6.99	5(2)	< LOD-8.08
	Moniliformin	40(49)	< LOD	0(0)	< LOD	35(14)	< LOD-241.09	88(35)	< LOD-1009.04
	T-2 Toxin	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD
	HT2 toxin	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD
Penicillium	Mycophenolic acid	28(34)	< LOD	28(11)	< LOD-55.37	35(14)	< LOD-125.93	23(9)	< LOD-42.83
	Penicillic acid	3(4)	< LOD-13.22	3(1)	< LOD-13.22	5(2)	< LOD-5.51	3(1)	< LOD-3.27
	Roquefortine C	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD
	Citrinin	7(8)	< LOD-286.97	10(4)	< LOD-286.97	0(0)	< LOD	10(4)	< LOD-32.99
Alternaria	Alternariol	60(72)	< LOD-39.51	5(2)	< LOD-39.51	85(34)	< LOD-35.98	90(36)	< LOD-31.20
	Alternariol-methylether	64(77)	< LOD	20(8)	< LOD-27.62	83(33)	< LOD-36.07	90(36)	< LOD-44.59

% P and N refer to the percentage and the number of contaminated samples with mycotoxins, respectively. LOD values for individual mycotoxins are given in Appendix (Table 4).

Table 4
Univariate analysis - Relationship of sociodemographic characteristics and storage management practices with multimycotoxin contamination in stored sorghum in northwest Ethiopia, 2023.

Variable	Any mycotoxin		Aspergillus mycotoxin		Fusarium mycotoxin		Penicillium mycotoxin		Alternaria mycotoxin	
	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value
Gender of MPRS	0.53(-0.68:1.74)	0.394	0.08(-0.75:0.91)	0.844	0.34(-0.43:1.11)	0.387	0.73(-0.06:1.53)	0.071	0.27(-0.53:1.07)	0.504
Age	−0.05(-0.10: 0.003)	0.039	−0.05(-0.09: 0.02)	0.005	−0.003 (-0.04:0.03)	0.849	−0.02 (-0.06:0.02)	0.265	−0.05(-0.08: 0.01)	0.01
Storage experience	−0.06(-0.11: 0.01)	0.012	−0.05(-0.08: 0.01)	0.007	−0.01 (-0.04:0.02)	0.632	−0.02 (-0.05:0.01)	0.265	−0.04(-0.08: 0.01)	0.012
Basic Education	1.31(-0.77:3.39)	0.217	0.75(-0.41:1.92)	0.203	0.47(-0.5:1.45)	0.343	0.46(-0.49:1.41)	0.34	1.41(0.13:2.69)	0.031
Type of storage structure	2.44(0.90:3.98)	0.002	0.59(-0.21:1.39)	0.148	1.64(0.84:2.43)	0.000	−0.04 (-0.83:0.74)	0.912	2.13(1.26:3.00)	0.000
Storage placement	−1.42(-2.54: 0.3)	0.013	−0.29 (-1.13:0.56)	0.507	−0.57 (-1.35:0.23)	0.157	0.29(-0.54:1.11)	0.496	−1.14(-1.95: 0.32)	0.006
Insecticide application	−1.67(-3.2: 0.12)	0.034	0.02(-0.79:0.83)	0.962	0.12(-0.63:0.86)	0.758	−0.14 (-0.93:0.64)	0.72	−0.87(-1.69: 0.05)	0.038

structure.

Multiple logistic regression was applied to test for differences between the alternative options of each of the study variables when all the other variables were kept constant. The age of the MPRS showed a significant covariance with the storage experience of the MPRS (Pearson correlation = 0.9369) indicating the presence of multicollinearity. Therefore, the storage experience of the MPRS was selected to be included in the multiple logistic regression. The Hosmer and Lemeshow goodness of fit test results showed that all the generated models significantly described the data (Table 5). The storage experience of the MPRS showed negative and significant relationships with mycotoxin contamination when other variables remain the same while the type of storage structure showed positive and significant relationships.

4. Discussion

The objective of this research was to investigate the relationship between sorghum grain storage management practices applied by subsistence sorghum producing farmers in Northwest Ethiopia with multi mycotoxin contamination in the stored grain. In Ethiopia, regulatory

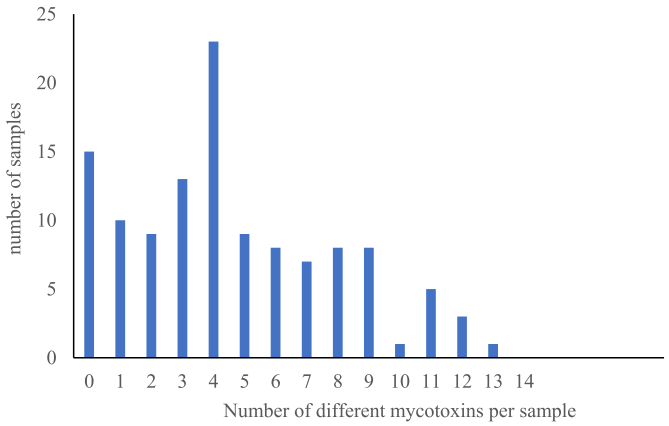

Fig. 3. Occurrence of multiple mycotoxins in stored sorghum in Northwest Ethiopia (2023).

standards are available for a limited number of mycotoxins and for a limited number of foods only. A legal limit for total aflatoxin in sorghum is available and is 10 μg/kg (Mamo et al., 2020). Only two percent of the samples in the current study bypassed this regulatory limit for total aflatoxin the concentrations that bypassed the limit being 18.34 and 29.13 μg/kg.

Following EU regulations, the maximum limit for the presence of aflatoxins (AFB1+AFB2+AFG1+AFG2), fumonisins (FB1+FB2), Ochratoxin A, Deoxynivalenol and Zearalenone in unprocessed cereals is 4, 4000, 5, 1250 and 100 μg/kg, respectively (European Commission Regulation (EC, 2023)). Based on this regulation, most of the stored sorghum samples in this study were contaminated with mycotoxins below the respective regulatory limits. About 3%, 7%, and 3% of the samples were contaminated with aflatoxins, ochratoxin A, and Zearalenone, respectively, above the EU regulatory limits. The measured concentrations that bypassed the EU regulatory limits were 9.14, 18.34 and 29.13 (μg/kg) for total aflatoxins, 5.31, 12.50, 14.94, 15.77, 32.94, 56.81, 58.07 and 112.59 (μg/kg) for Ochratoxin A, and 123.48, 238.43 and 431.78 (μg/kg) for Zearalenone, respectively. For emerging mycotoxins such as Alternariol, Alternariol-methylether, Nitropropionic acid, and Moniliformin, no European regulatory limits have been set yet. Both the prevalence and concentrations of common mycotoxins - namely aflatoxins, fumonisins, deoxynivalenol, ochratoxin A, nivalenol, and zearalenone - were similar, with a slight differences, to a previous study that reported the occurrence of multiple mycotoxins in stored sorghum samples collected from farmers households in Eastern Ethiopia (in Doba, Fedis, Goro Gutu and Miesso areas) in the 2021 cropping season (Mohammed et al., 2022). The slight differences could be due to the possible variations in agroecological conditions and cultural storage management practices in the sample collection sites. Despite this, like the findings of this study, the previous research by (Mohammed et al., 2022) also reported that the prevalence and concentration of emerging mycotoxins were far higher than the prevalence of the common mycotoxins in the sorghum samples.

The presence of multiple mycotoxins that belong to the four different mycotoxin categories could imply the presence of several species of fungi infecting the samples. On the other hand, the low concentrations of mycotoxins (especially the common mycotoxins) measured in the samples would indicate that the growth of infecting fungal species was limited during storage. Several factors could have contributed to the low fungal growth and related low mycotoxin contaminations. First, the ecological conditions in the sample collection areas could cause the growth of fungi to be low during the grain storage period. Conducting a controlled experiment on barely storage ecological conditions related to toxigenic fungal growth and related mycotoxin production, Wawrzyniak et al. (2018) reported that the growth of *Aspergillus ochraceus* and *Penicillium verrucosum* were intense when the storage relative air humidity was above 0.90 and when the storage temperature was between 24 and 30 °C. On the contrary, the same study indicated that both the growth of these fungal species and their produced concentrations of mycotoxins were lower when the relative humidity was below 0.8, and when the storage temperature was between 12 and 24 (°C). The sample collection areas in the current research belong to moisture-stress areas, which are characterized by low rainfall and warm temperatures (Appendix Table 1). Despite its negative impact on sorghum during the growing period, low rainfall could have a positive impact on sorghum storage regarding mycotoxin contamination. The low rainfall would mean the risk of water leakage to sorghum stored in indigenous outdoor storage structures namely *sherfa* and pit would be low. Moisture leakage, especially during rainy seasons, is one of the challenges of outdoor grain storage structures in Ethiopia, which could create a convenient moisture level in the grain with implications to support fungal growth and mycotoxin contamination (Garbaba et al., 2018; Roman et al., 2020). The presence of a relatively high ambient temperature in the study areas would also provide subsistence farmers who entirely depend on sun drying of harvested grain the opportunity to adequately dry the harvested grain in the field (before storage). A moisture content of grain

Table 5
Multiple logistic regression analysis - Relationship of sociodemographic characteristics and storage management practices with multimycotoxin contamination in stored sorghum in northwest Ethiopia, 2023.

Variable	Any mycotoxin[a]		Aspergillus mycotoxin[b]		Fusarium mycotoxin[c]		Penicillium mycotoxin[d]		Alternaria mycotoxin[c,b]	
	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value
Gender of MPRS	0.39(-0.97:1.74)	0.578	0.13(-0.86:0.88)	0.975	0.28(-0.57:1.13)	0.516	0.72(-0.09:1.52)	0.081	0.14(-0.82:1.10)	0.773
Storage experience	−0.07(-0.12: 0.01)	0.021	−0.05(-0.09: 0.11)	0.012	−0.01 (-0.05:0.03)	0.685	−0.01 (-0.05:0.02)	0.473	−0.05(-0.09: 0.003)	0.037
Basic Education	−0.05 (-2.46:2.36)	0.968	−0.06 (-1.38:1.26)	0.930	0.07(-1.13:1.27)	0.914	0.25(-0.87:1.38)	0.661	0.73(-0.79:2.25)	0.346
Type of storage structure	2.23(0.55:3.91)	0.009	0.74(-0.20:1.68)	0.122	2.08(1.07:3.09)	0.000	−0.13 (-1.02:0.76)	0.778	2.16(1.13:3.20)	0.000
Storage placement	−0.89 (-2.22:0.43)	0.185	−0.20 (-1.16:0.76)	0.681	−0.46 (-1.41:0.49)	0.345	0.38(-0.53:1.29)	0.415	−0.75 (-1.75:0.25)	0.140
Insecticide application	−0.75 (-2.50:1.00)	0.401	0.38(-0.60:1.35)	0.448	1.21(0.15:2.28)	0.025	−0.33 (-1.24:0.59)	0.484	0.01(-1.05:1.07)	0.988

Multiple logistic regression model fitness information.
[e]LR chi2(6) = 39.32, Prob > chi2 = 0.0000, Pseudo R2 = 0.2574, Goodness of fit test: Pearson chi2(95) = 79.52, Prob > chi2 = 0.8731.
[a] LR chi2(6) = 25.68, Prob > chi2 = 0.0003, Pseudo R2 = 0.2840; Goodness of fit test: Pearson chi2(95) = 68.08, Prob > chi2 = 0.9833.
[b] LR chi2(6) = 10.91, Prob > chi2 = 0.0913, Pseudo R2 = 0.0762, Goodness of fit test: Pearson chi2(95) = 104.33, Prob > chi2 = 0.2408.
[c] LR chi2(6) = 24.40, Prob > chi2 = 0.0004, Pseudo R2 = 0.1518, Goodness of fit test: Pearson chi2(95) = 96.30, Prob > chi2 = 0.4434.
[d] LR chi2(6) = 5.49, Prob > chi2 = 0.4826, Pseudo R2 = 0.0370, Goodness of fit test: Pearson chi2(95) = 103.41, Prob > chi2 = 0.2608.

below 13% has been reported to limit mycotoxin contamination and insect infestation during grain storage (Manu et al., 2019; Walker et al., 2018). The temperature of sorghum stored in the introduced structures, which are always placed indoors, may be higher than the ambient temperature due to heating effects from food preparation facilities in a living room. This could be the reason for the increase in the probability of mycotoxin contamination of sorghum when stored in indoor structures compared to the extent of contamination obtained in outdoor storage structures (Table 4). On the other hand, the indigenous storage structures - *sherfa* and pit, and the indoor structure - *gota*, are presumed to create a cooler temperature during storage than the ambient temperature. This is because soil (and mud) is a poor conductor of heat (Ochsner, 2019), which means that heat transfer from the external environment or a living room to the grain stored in the structures made with mud is low. Therefore, sorghum stored in the indigenous structures may have a cooler ecosystem than sorghum stored in the introduced structures, which may be one of the reasons for the observed lower probability of mycotoxin contamination in sorghum stored in indigenous storage structures compared to sorghum stored in the introduced structures. Indeed, water availability and temperature are the major ecological conditions that affect fungal growth and mycotoxin contamination in cereals (Milani, 2013). One of the limitations in this study was that the storage ecosystem parameters such as temperature and relative humidity inside the storage structures as well as the moisture content of the stored grain were not investigated. These parameters are believed to vary among the individual farmers and would contribute to the variations in the extent of mycotoxin contamination. It is important to include these parameters in future studies.

Unexpectedly, sorghum stored in the PICs sacks didn't result in a lower level of mycotoxin contamination than sorghum stored in indigenous structures. In Kalu *woreda*, where 98% of the households stored sorghum in sacks, of which 85% of them in PICs sacks (Table 2), the recorded prevalence of Nitropropionic acid, Alternariol, and Alternariol-methylether detected in the samples collected from this *woreda* were 83%, 85%, and 83% respectively, all with higher prevalence than in the other *woredas* (Table 3). The presence of a high prevalence and concentrations of mycotoxins in the samples stored in PICs sacks may imply that the farmers packed the sacks improperly. Tubbs et al. (2016) demonstrated that opening properly packed PICs sacks containing maize grain every week for 30 min increased the fungal growth and aflatoxin contamination. Since the households in our study areas are subsistence farmers, they probably take portions of the stored sorghum at different time intervals, allowing an influx of air and moisture into the grain during withdrawal, which might have caused fungal growth and related mycotoxin contamination. In this regard, providing training to the farmers on the proper use of the PICs sacks would be important. On the other hand, in West Belesa *woreda*, where 90% of the farmers used the indigenous storage structures namely *sherfa* and *gota*, the prevalence and concentration of mycotoxins were lower compared to the other two *woredas*. This could be partly because insecticides are used during storage in the indigenous structures while not used in PICs sacks. About 80% of farmers in West Belesa used insecticides (Table 2). Despite this, the indigenous structures, *gota* and *sherfa*, which are also hermetic if properly managed, can be improved to better prevent mycotoxin contamination and insect infestation. Particularly, the *sherfa,* due to its movability advantages to place indoors or outdoors based on local circumstances (weather, theft, etc), can be a low-cost option for storing sorghum safely for subsistence farmers. The change in air composition during the storage of sorghum was not included in our study. However, it would be interesting to investigate the change in air composition during sorghum storage and the related change in mycotoxin contamination, which would provide a better insight for the potential use of *gota* and *sherfa* as a mycotoxin preventive storage structures.

The negative relationships between both the age and the experience of the MPRS and multimycotoxin would mean an increase in the years of age and experiences of farmers decreases the probability of mycotoxin contamination in the stored grain. Although most of the farmers (81%) did not attend any formal education, their 3–53 years of storage experience (Table 3) is presumed to provide them with an awareness of the suitable conditions during sorghum storage that favor fungal growth. A survey conducted in the Oromia and Amhara regions in Ethiopia indicated that women are responsible for selecting damaged crops intended for human consumption (Cervini et al., 2023). Provided that 63% of the MPRS in this study were women (Table 1), sorting damaged grains would be a practical learning experience for them to learn the causes of the grain damage. Sorting out damaged seeds is one of the Good Practices to reduce mycotoxin contamination (Matumba et al., 2015). Consequently, the farmers could have implemented preventive measures such as proper cleaning of the storage structures before putting in a new harvest and use of insecticides to control insect infestation, which could be some of the reasons for the low prevalence of mycotoxin contaminations detected in the samples.

The negative relationship of insecticide application with mycotoxin contamination obtained from the univariate analysis was expected (Table 4). That is because the presence of insects in storage facilities and sacks increases the humidity in the structures due to metabolic activities and the spreading of fungal spores (Turner et al., 2005), i.e. if insect infestation is controlled by using insecticides, the increase in humidity will be low and fungal growth and mycotoxin contamination would be limited. However, the positive and significant relationship of insecticide application with fusarium mycotoxin contamination obtained in the multiple logistic regression was not expected. This could be related to the phytopathogenic relationship of *Fusarium* species with insects as described by Gallan et al. (2023). According to Gallan et al. (2023), the species *Fusarium verticillioides,* which is one of the major producers of fusarium mycotoxins (Braun and Wink, 2018; Wu et al., 2014), showed a symbiotic relationship with sugarcane borer *Diatraea saccharalis*. The researchers reported that the colonization of this fungal species increased the thickness of the midgut of the *Diatraea saccharalis* by 3.3 times compared to the control. A similar relationship between fusarium species and storage insects in sorghum might have caused the positive relationship of insecticide application and fusarium mycotoxins obtained in this research. If there is an insect infestation, some of the fungal spores may have colonized the insect's gut, reducing the number of spores that can produce mycotoxins in the grain. Killing the insects with insecticides would mean that all the fungal spores could infect the grain and produce mycotoxins. This situation could have increased the likelihood of mycotoxin contamination in sorghum stored without insecticide application compared to the other stored with insecticide application. However, such relationships may depend on the type of fungal species, as some of the mycotoxin categories showed negative relationships with insecticide application. An unexpected result was also obtained for the basic education. Basic education showed a positive relationship with mycotoxin contamination (Table 4). That means, for a farmer who attended basic education, the probability of mycotoxin contamination in sorghum was higher than for the farmer with no formal education. This might be due to the fact that mycotoxin awareness is not part of the academics in lower-level education. About 81% of the participants haven't attended any formal education, and even among the 19% of the participants who attended basic formal education, the majority of them attended only primary level education. It may be inferred that the farmers who attended the basic formal education, are actually not aware of mycotoxins through their education. The preventive measures they apply to prevent mycotoxin contamination would be entirely due to their learnings from their previous experiences, as storage experience showed negative relationships with mycotoxin contamination.

It is important to mention that without considering statistical significance, the relationships between several of the traditional sorghum storage management practices with multimycotoxin contamination were bi-directional, meaning both positive and negative relationships were obtained for the mycotoxins belonging to the different categories

(Tables 4 and 5). This implies that a specific management practice may reduce mycotoxins from one category but increase the presence of mycotoxins from another category. This could be due to differences in the ecosystem of the different storage management practices, particularly the storage structures, which may lead to differences in the types of fungal species present (Cao et al., 2022). In addition, the different sorghum varieties commonly grown in the study locations could differ in their vulnerability to fungal infection and mycotoxin contamination. However, sorghum variety was not considered in our study and would be interesting to consider it in future studies.

5. Cost-effectiveness of a training intervention

Training farmers to improve the traditional storage management practices of sorghum could be a feasible intervention option to reduce mycotoxin contamination for some reasons. First, creating awareness about sorghum storage ecosystems in relation to mycotoxin contamination, especially temperature control, could help farmers place the storage structures in cooler areas among available storage spaces, which need a limited investment. Whether indoor or outdoor storage structures are used, placement of the structure in areas having lower temperatures reduces the rate of fungal infection and mycotoxin contamination (Lahouar et al., 2016; Wawrzyniak et al., 2018). In the current practice, part of the farmers prepare shade for their structures with metal roofing sheets, or they place the structures under a tree shade, or they place the structure beside to the wall of a living room, and others use no shade. Since all the research areas belong to dry climates with relatively high temperatures, proper management of shades is important, especially for outdoor storage structures. The metal sheet is a good conductor of heat, thus its contribution to reducing the solar heat seems low; the tree may not provide complete shade from the sun which means that the ambient temperature could be lowered only to a limited extent; and placing the structure adjacent to a living room may prevent rain, but may not control temperature since smoke coming from the living room could have a heating effect on the structure which increases the temperature. The use of proper shades helps to prevent moisture leakage to the storage structure during rainy times, which reduces the possibility of damage to the structure by rain and ultimately prevents moisture leakage to the grain. Second, the availability of established Farmer Training Centers in Ethiopia could help in providing the training investment to be cost-effective. The presence of Farmer Training centers is an opportunity to organize the training at a low cost since communication with farmers costs low investment in money and time. Training of farmers about better storage Management Practices selected from the currently used local practices such as storage placement, temperature control, and moisture control as well as the construction of improved indigenous storage structures would be important. Van den Berg and Jiggins (2007) reported that training farmers to implement Integrated Pest Management practices in Field Schools is a cost-effective investment. The incorporation of farmers who have applied one or more of the exemplary storage management practices in the training is believed to improve the learning outcomes of farmers via an experience-sharing scheme. Nakano et al. (2018) demonstrated the cost-effectiveness of farmer-to-farmer extension programs in agricultural technology adoption in Tanzania. Our results showed that the increase in the storage experience of farmers is related to the decrease in the probabilities of mycotoxin contamination. The knowledge that needs to be acquired through experience, which requires years of practice, can be cost-effectively achieved with properly managed short-term training.

6. Conclusions

Results obtained in this study showed that - in general - the prevalence of regulated mycotoxins was low, with only 3%, 7%, and 3% of the samples being contaminated with aflatoxins, ochratoxin A, and zearalenone, respectively, above the EU regulatory limits. However, the majority of the total samples (about 72%) were contaminated with multiple (three or more) mycotoxins. The probability of mycotoxin contamination in sorghum samples stored in indigenous storage structures was lower than those samples stored in the introduced structures. This would imply that with further improvement, indigenous storage structures themselves are promising intervention options to manage mycotoxin contamination. In addition, when the other variables were kept constant, the increase in the storage experience of the MPRS showed a decrease in the probability of mycotoxin contamination. This would imply that training to farmers about proper management of storage structures would be an important intervention to reduce the probability of occurrence of mycotoxins in sorghum.

CRediT authorship contribution statement

J.A. Sadik: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **L. Righetti:** Writing – review & editing, Validation, Supervision, Resources, Methodology. **N. Fentahun:** Writing – review & editing, Supervision, Methodology. **I.D. Brouwer:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **M. Tessema:** Writing – review & editing, Supervision, Methodology. **M. Abera:** Writing – review & editing, Supervision, Methodology. **H.J. van der Fels-Klerx:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix

Appendix Table 1
Weather data for data collection *woredas*.

Woreda	Weather data station	Altitude asl (m)	Temp range (T_{min} - T_{max}) (^{o}C)	Rainfall (mm)***	Latitude	Longitude	**Weather data (period in years)
W. Belesa	Arbaya	1831	13.4–31.9	2.9	12.28	37.49	2003–2018
Kalu	Harbu	1507	11.9–30.7	2.9	10.90	39.79	2001–2021
Kewet	Shewa Robit	1277	14.6–30.6	2.4	10.01	39.89	2006–2021

The weather data was obtained from the National Meteorology Institute of Ethiopia, Addis Ababa. *A mean value of data was computed for the range of years mentioned. i.e. using all the data available in the Institute for each location.

Appendix Table 2
Sample size determination.

S. No.	*Woreda*	Selected kebeles	Size of registered households	Ratio of households in each kebele	Sample size (household) distribution (*40)	Number of households
1	West Belesa	Abay Tara	928	0.25	9.98	10
2	West Belesa	Diquana	1050	0.28	11.29	11
3	West Belesa	Ebrarag	898	0.24	9.66	10
4	West Belesa	Qaley	844	0.23	9.08	9
	Sum		3720			
1	Kalu	Woraba	925	0.29	11.53	12
2	Kalu	Miawa	986	0.31	12.29	12
3	Kalu	Agamssa	640	0.20	7.98	8
4	Kalu	Chorasa	657	0.20	8.19	8
	Sum		3208			
1	Kewet	Terie	1740	0.49	19.52	20
2	Kewet	Yelen	1097	0.31	12.31	12
3	Kewet	Birbira	729	0.20	8.18	8
	Sum		3566			

Appendix Table 3
Sociodemographic and storage management practices questionnaire.

S.No	Questionnaire	Response
	Sociodemographic characteristics	
1	Name of *woreda*	_____
2	Name of *kebele*	_____
3	Household ID number	_____
4	Head of household	1. Husband 2. Wife 3. Other, specify _____
5	The main Persons Responsible for Storage management (MPRS) of sorghum	1. Wife 2. Husband 3. Both have similar responsibilities 4. Other, specify, ___
6	Age of MPRS (in years)	_____
7	Sorghum storage experience (in years)	_____
8	Highest level of formal education completed for MPRS	_____
	Storage management practices	
9	Name of sorghum grain storage structure	1. PP sack 2. PICs sack 3. *gota* 4. *sherfa* 5. underground pit 6. Other, specify ___
10	The currently used sorghum storage structure is	1. Indigenous 2. Introduced 3. other, specify ___
11	Placement of the storage structure	1. Indoor 2. Outdoor
12	Insecticide application	1.Yes 2. No

Appendix Table 4
LOD/LOQ values and recovery percentages of specific mycotoxins.

S.No.	Name of mycotoxin	LOD (μg/Kg)	LOQ (μg/Kg)	Percent recovery (±SD)
1	15AcetylDON	12	24	96 ± 13
2	3-Acetyl-DON	12	40	87 ± 14
3	Aflatoxin B1	0.06	0.15	93 ± 15
4	Aflatoxin B2	0.06	0.15	94 ± 15
5	Aflatoxin G1	0.06	0.15	95 ± 11
6	Aflatoxin G2	0.06	1.25	95 ± 11
7	Alternariol	0.3	1.2	92 ± 11
8	Alternariol-methylether	0.3	1.2	97 ± 12
9	Beauvericin	0.06	0.15	93 ± 13

(*continued on next page*)

Appendix Table 4 (*continued*)

S.No.	Name of mycotoxin	LOD (μg/Kg)	LOQ (μg/Kg)	Percent recovery (±SD)
10	Deoxynivalenol	12	60	90 ± 11
11	DON-3-Glucoside	60	125	83 ± 14
12	Diacetoxyscirpenol	0.75	1.5	86 ± 17
13	Enniatin A	0.3	0.75	95 ± 12
14	Enniatin A1	0.3	0.75	95 ± 9
15	Enniatin B	0.3	0.75	96 ± 12
16	Enniatin B1	0.3	0.75	97 ± 10
17	Fumonisin B1	1.5	3	86 ± 16
18	Fumonisin B2	1.5	3	89 ± 15
19	Fumonisin B3	1.5	3	89 ± 16
20	Moniliformin	1.5	3.75	50 ± 15
21	Mycophenolic acid	1.5	3	96 ± 15
22	Nitropropionic acid	7.5	15	79 ± 12
23	Nivalenol	30	125	82 ± 13
24	Ochratoxin A	0.3	0.6	90 ± 13
25	Penicillic acid	3	6	90 ± 17
26	Roquefortine C	0.06	0.15	91 ± 12
27	Sterigmatocystin	0.015	0.06	96 ± 14
28	ZON	1.5	3	97 ± 12
29	α-Zearalenol	1.5	3	95 ± 15
30	β-Zearalenol	1.5	3	100 ± 14
31	T-2 Toxin	3	6	84 ± 16
32	HT2 toxin	3	6	105 ± 12
33	Citrinin	0.06	0.3	34 ± 6

Data availability

Data will be made available on request.

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