Developing integrated strategies to reduce the disease burden of mycotoxin contamination in Ethiopia

Sadik Jemal Awol

PROPOSITIONS

1. Understanding local farming practices is essential to successfully manage mycotoxin contamination.
   (This thesis)
2. The presence of mycotoxins below regulatory limits does not eliminate public health concerns.
   (This thesis).
3. Using AI reduces the capability of non-native speakers to learn writing skills.
4. The rise of multidisciplinary journals has paradoxically made publishing more difficult.
5. Going abroad is the only way to fully understand the importance of inclusiveness.
6. Sharing failure experiences contributes more to learning than sharing success experiences.

Propositions belonging to the thesis, entitled

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Wageningen, 09 January 2026

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This research was conducted under the auspices of the Wageningen School of Social Sciences (WASS)

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Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr C. Kroeze,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Friday 9 January 2026
at 10:30 a.m. in the Omnia Auditorium.

Sadik Jemal Awol
Developing integrated strategies to reduce the disease burden of mycotoxin contamination in Ethiopia, 178 pages.

PhD thesis, Wageningen University, Wageningen, the Netherlands (2026)
With references, with summary in English
DOI http://doi.org/10.18174/681663

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CHAPTER 1

General introduction

1.1 BACKGROUND

Mycotoxins are toxic chemical compounds, produced by certain species of fungi upon and after infection of the crop (Wu *et al*., 2014a). Around the world, the well-known fungal species that produce mycotoxins are *Aspergillus*, *Alternaria*, *Fusarium*, and *Penicillium* mycotoxins (Mohammed *et al*., 2022a; Moretti *et al*., 2019; Wu *et al*., 2014a). One species of fungi can produce different types of mycotoxins, and specific mycotoxins can be produced by different species. However, the most important mycotoxins, based on occurrence and toxicity, are aflatoxins and fumonisins (Braun & Wink, 2018). Mycotoxin exposure occurs in humans and animals primarily via contaminated food and through carry-over into milk and excretion in urine (Yiannikouris & Jouany, 2002). Consequently, a risk-based approach considering all stages from farm to table and focusing on effective prevention and control has been the most accepted food safety risk management strategy (Unnevehr, 2015).

1.1.1 The health burden of aflatoxins and fumonisins in humans

1.1.1.1 Aflatoxins

Aflatoxins are mainly produced by the fungal species *Aspergillus flavus* and *Aspergillus parasiticus* (Chauhan *et al*., 2008; Okechukwu *et al*., 2024; Wu *et al*., 2014a). *Aspergillus flavus* produces mainly aflatoxin B1 (AFB1) and aflatoxin B2 (AFB2) while *Aspergillus parasiticus* produces aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) on top of AFB1 and AFB2 (Okechukwu *et al*., 2024; Wu *et al.,* 2014a)). AFB1 and AFB2 can be hydroxylated to aflatoxin M (AFM1 and AFM2) by humans and animals (Okechukwu *et al*., 2024; Wu *et al*., 2014a). The chemical structure of aflatoxins is shown in Figure 1.1.

Aflatoxins are carcinogenic to humans. The International Agency for Research on Cancer (IARC) has classified naturally occurring aflatoxins (AFB1, AFB2, AFG1, AFG2 and AFM1) (Figure 1.1) as Group1 (Carcinogenic to humans) (Ostry *et al*., 2017). Aflatoxins can cause liver cancer and acute toxicities, reduce protein synthesis, and lower immune responses (Smith *et al*., 2012). AFB1 is the most potent aflatoxin (Sandoval *et al*., 2019; Wu *et al*., 2014a).

Aflatoxins are synergistic with hepatitis B infections. This is because there is an increased risk of hepatocellular carcinoma in populations that are co-exposed (Kucukcakan & Hayrulai-Musliu, 2015). Chen *et al.* (2023) reported synergistic relationship between AFB1 and FB1 within the mitochondrial dysfunction and apoptosis pathways. They reported that the synergistic relationship is most likely triggered by genes in the p53 pathway and the mitochondrial complex. Simultaneous human exposure to hepatitis B virus and aflatoxins is reported to increase the aflatoxin health effects by 30% (Sandoval *et al*., 2019;

Wu *et al*., 2014a). The WHO estimated the burden of disease related to aflatoxins in Africa at 15 Disability Adjusted Life Years (DALY) per 100,000 inhabitants. To compare, aflatoxins are responsible for 0.5 DALYs per 100,000 in Europe (Gibb *et al*., 2010).

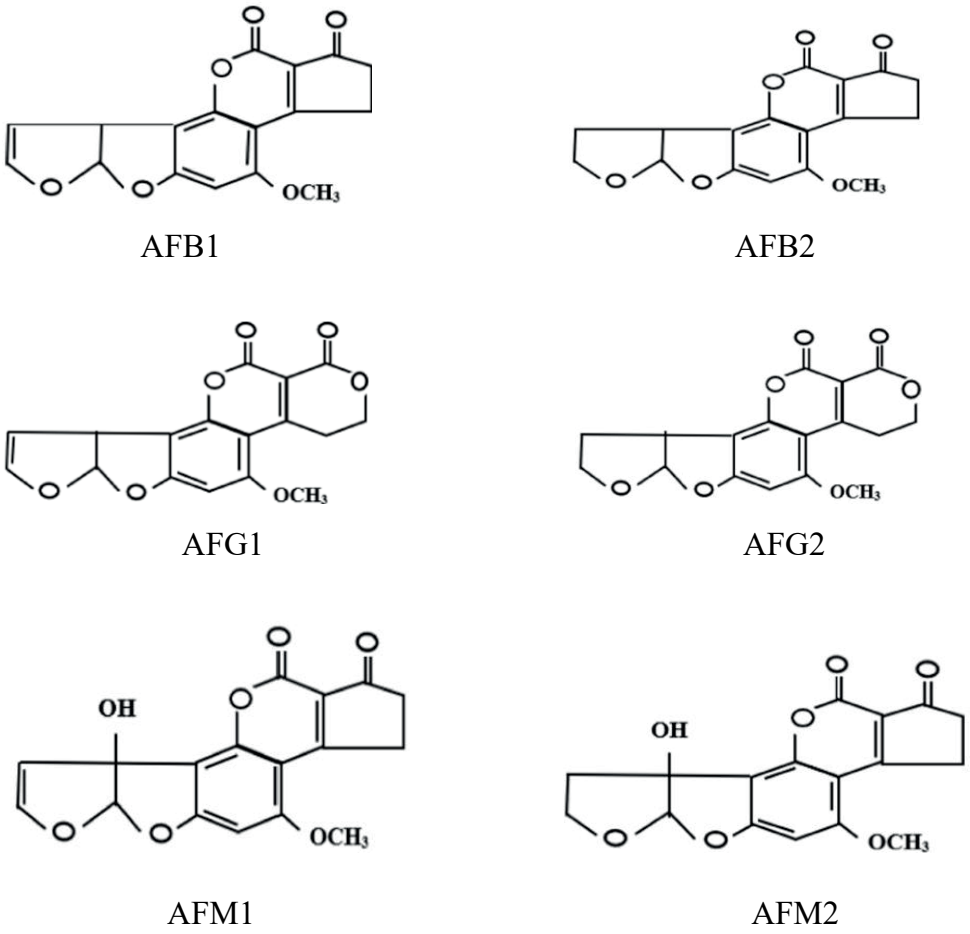


Figure 1.1. Chemical structure of aflatoxins (Okechukwu *et al*., 2024)

Toxicological effects and biomarkers of aflatoxins

After being ingested with food, AFB1 is absorbed into the bloodstream via the small intestine. It then enters the portal circulation and is transported to the liver, the main target organ. Here, cytochrome P450 enzymes (especially CYP1A2 and CYP3A4) convert it into the reactive intermediate AFB_1-8,9-epoxide, which is the key toxic and mutagenic metabolite. This epoxide binds to DNA, forming AFB_1–N^7–guanine adducts, which lead to DNA damage and mutations. A mutation in the TP53 gene (codon 249Ser) in the liver is a hallmark of aflatoxin-induced liver cancer (hepatocellular carcinoma, HCC) (Wild &

Turner, 2002; Gouas *et al*., 2009). The epoxide can also bind to proteins in the blood, forming AFB_1–albumin adducts. The oxidative hydroxylation of AFB_1 by cytochrome P450 enzymes, particularly CYP1A2, in the liver also produces AFM_1.

Various biomarkers are available for studying aflatoxin exposure and risk throughout the aflatoxin metabolic pathway. The AFB_1-Lys adduct in the blood indicates chronic exposure, which is defined as repeated exposure over a long period (usually months to years). TP53 codon 249Ser in plasma and tumour DNA is also used as a chronic aflatoxin exposure biomarker (Gouas *et al*., 2009; Wild & Turner, 2002). Conversely, AFM_1, which is excreted in breast milk and urine, is a biomarker for acute aflatoxin exposure, i.e. recent exposure within days. Additionally, the presence of AFB_1-DNA adducts (AFB_1-N^7-Gua adducts) in the liver and urine indicates acute aflatoxin exposure (for genotoxicity) (Gouas *et al*., 2009; Wild & Turner, 2002).

Margin of exposure for aflatoxin risk metrics

There is no tolerable daily intake (TDI) for aflatoxins, which are both carcinogenic and genotoxic (EFSA, 2020; WHO/FAO, 2014). The margin of exposure (MOE) is one of the methods used to measure the public health risks associated with exposure to aflatoxins. The MOE is the ratio of the benchmark dose lower confidence limit (BMDL10) that causes a 10% increase in liver tumours in animal studies to the estimated daily intake (EDI). An MOE value of 10,000 or higher indicates low public health concern. An MOE value below 10,000, however, suggests potential health concerns and may warrant risk management actions (EFSA, 2020; Sandoval *et al*., 2019; WHO/FAO, 2014).

1.1.1.2. Fumonisins

Fumonisins are common metabolites produced by several species of *Fusarium* such as *Fusarium verticillioides*, *Fusarium proliferatum* and *Fusarium fujikuroi* and some species of *Aspergillus* such as *Aspergillus niger* (Wu *et al*., 2014a). Fumonisins that occur most often are fumonisin B1 (FB1), fumonisin B2 (FB2) and fumonisin FB3 (FB3) (Ren *et al*., 2011).

Fumonisins have been linked to a number of health issues, including esophageal cancer, neural tube defects (Braun & Wink, 2018; Marasas *et al*., 2004), and renal and liver toxicity in humans and animals (Bucci *et al*., 1998). In case of co-exposure of aflatoxins and fumonisins, the human health burden is expected to be increased due to the interaction effects of the two toxins. (Chen *et al*., 2023).

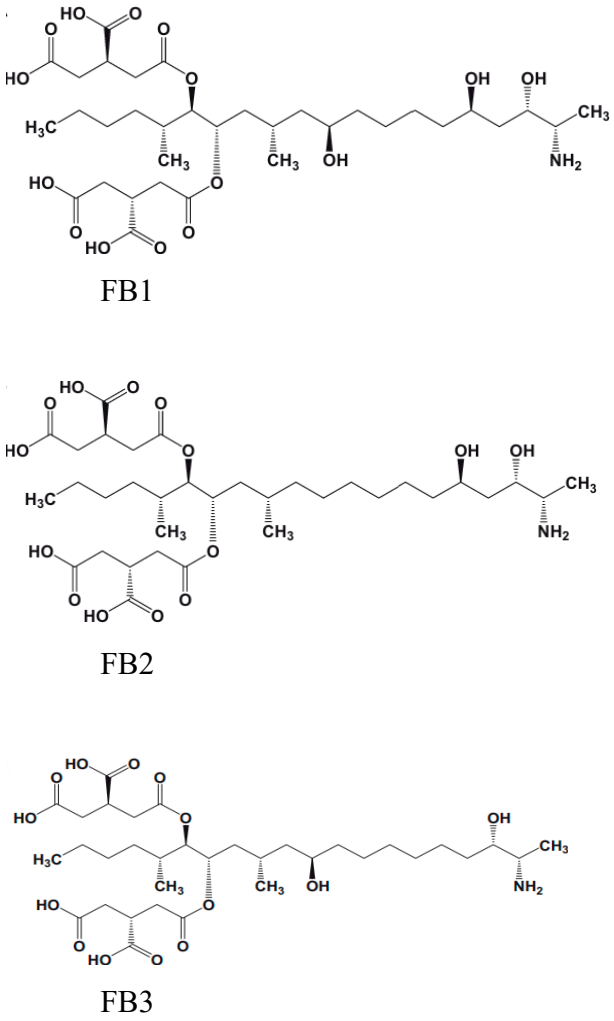


Figure 1.2. Chemical structure of Fumonisins (Ren *et al*., 2011).

Toxicological effects and biomarker for fumonisin

After intake through contaminated foods, fumonisin absorption takes place through the small intestine, mainly via passive diffusion. Fumonisin is mostly confined to the blood plasma, with limited tissue distribution (Voss and Riley, 2013). This makes blood plasma one of the suitable biomarkers (FB1, FB2 and FB3) (Tessema *et al.*, 2020). In the blood, FB1 is metabolized to a limited extent to hydrolysed FB1 while majority of fumonisins are excreted via urine. This makes urinary FB_1, FB2 and FB3 as reliable short-term biomarkers of exposure (Shephard *et al.*, 2007; Boshe *et al.*, 2020; Tessema *et al.*, 2020). On the other hand, a fumonisin is known to inhibit ceramide synthase, disrupting sphingolipid metabolism. This leads to an increase in the sphinganine-to-sphingosine ratio (Sa/So) in

plasma, serving as a biomarker of biological effect rather than direct exposure (Shephard *et al*., 2007; Voss and Riley, 2013). However, due to the low bioavailability of fumonisins, fumonisin biomarkers (FB1, FB2, FB3) from human samples such as urine and blood create analytical challenges. Such investigations should include validation biomarkers. The most used biomarkers are elevated levels of the sphingoid base, sphinganine, or of its ratio with sphingosine (Shephard *et al*., 2007). Analytical details, including LODs and units, for the analysis of fumonisin biomarkers using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) are available in Warth *et al*. (2013). The Provisional Maximum Tolerable Daily Intake (PMTDI) of 2,000 ng/kg bw/day has been established by the FAO & WHO (2017) as a health-based guidance value (HBGV) for FB_1, FB_2 and FB_3 (alone or in combination

Aflatoxin and fumonisin regulation

Besides health-related problems, mycotoxin contamination of agricultural-food commodities affects trade, the economy and the socioeconomic development status of a country may modulate exposure pathways via food systems and regulatory capacity (Atnkut *et al*., 2025; Ortega-Beltran & Bandyopadhyay, 2021; Vipham *et al*., 2020). In Europe, the European Commission (EC) has set regulatory limits for the presence of mycotoxin in foods with the aim to protect human health (Commission Regulation (EC, 2023). This implies that countries, including Africa countries, not meeting these regulatory requirements cannot export their food products to the European Union (EU) market, which may lead to economic losses to these exporting countries.

In Ethiopia, mycotoxin regulatory limits are only available for a small number of mycotoxins and foods (Mamo *et al*., 2020). For wheat grain and teff flour, limits are only available for total aflatoxins, with a limit of 4 ng/g for both. For barley, different limits are available for AFB1 and total aflatoxins: 5 ng/g and 10 ng/g, respectively. The regulatory limits for unprocessed maize and sorghum grains are presented below (Table 1.1). For comparison, the European (EC, 2023) and FAO/WHO (2024) regulatory limits are also presented. Although some African countries have their own regulatory limits for mycotoxins, harmonized mycotoxin regulation in Africa is not yet in place (Chilaka *et al*., 2022).

Table 1.1 Regulatory-framework overview - maximum regulatory limits for aflatoxins and fumonisins in unprocessed maize and sorghum grains (ng/g). The European regulatory limits are based on Commission Regulation (EU) 2023/915 and the FAO/WHO (2024) limits are based on Codex Alimentarius Commission International Food Standards revised in November 2024. The Ethiopian limits are based on Mamo *et al.* (2020).

Mycotoxin	EU limit	FAO/WHO limit	Ethiopian limit
aflatoxins:			
Sorghum			
• AFB1	2	-	5
• Sum of B1, B2, G1 and G2	4	10	10
Maize			
• AFB1	5	-	5
• Sum of B1, B2, G1 and G2	10	15	10
fumonisins:			
Sorghum			
• Sum FUB1, FUB2, FUB3	4000	4000	
Maize			
• Sum FUB1, FUB2, FUB3	4000	4000	

1.1.2. Mycotoxin prevention strategies

Human exposure to mycotoxins is mainly through consumption of contaminated foods (Gibb *et al*., 2015; Ostry *et al*., 2017). To date, effective and practically applicable decontamination processes to reduce mycotoxin contamination in foods are limited (Pandey *et al*., 2023). For instance, complete decontamination of aflatoxins through food processing is extremely challenging because of the high chemical and thermal stability of these mycotoxins (Bhardwaj *et al*., 2023). Consequently, preventing the occurrence of mycotoxins in the crop value chain is the ideal solution. On the other hand, a single intervention to prevent mycotoxin contamination in the crop value chain has very limited effect (Jallow *et al*., 2021). This is because toxigenic fungal species are ubiquitous in nature and contamination can occur at any stage in the value chain (Bhardwaj *et al*., 2023; Jallow *et al*., 2021). Consequently, implementing integrated preventive actions in the crop value chain such as Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs) has been the most important strategy to manage mycotoxin contamination (Jallow *et al*., 2021; Pandey *et al*., 2023). In addition to conventional agricultural practices such as land preparation, sowing, fertilizer application, and harvesting, microbiome-assisted agricultural practices have emerged to improve the sustainability and resilience of food production (Kabir *et al*., 2024). Recently, the use of aflasafe®[1], a novel biological method of preventing aflatoxin contamination, has been reported as effective during the pre-harvest period. Aflasafe uses non-toxigenic strains of native Aspergillus species, excluding aflatoxin-producing species, through competitive mechanisms. This environmentally friendly method is recommended as part of an integrated preventive strategy (Udomkun *et al*., 2017).

The contamination of staple food crops by mycotoxins remains a challenge in low- and middle- income countries. This is primarily due to limited infrastructure and inadequate implementation of Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP), particularly in tropical regions. Consequently, the underdeveloped cultural crop management practices in these countries are favorable for mycotoxin contamination of the produced crops (Jallow *et al*., 2021; Munkvold, 2003). From the total 324.6 million quintals of cereal production in 2020/21 in Ethiopia, about 97% was produced by private peasant holdings (CSA, 2021a) using traditional crop production practices. The current globally available knowledge on mycotoxin management can be adapted to local subsistence farmers' conditions in low- and middle- income countries. Such adapted food safety intervention programmes are most effective if aligned with local value chain settings in the respective countries. This requires a full understanding of local conditions, local farm practices and mycotoxin contamination patterns, followed by designing appropriate and feasible intervention measures (Pitt *et al*., 2013; Udomkun *et al*., 2018; Unnevehr, 2015). Understanding these relationships for cereal value chain enables us to identify the practices that can be promoted further and the practices that need an additional intervention. At the same time, local traditional agricultural practices may provide a rich source of knowledge that, when combined with modern technologies and research, can lead to innovative solutions tailored to specific environments, making local agriculture more sustainable, resilient, and productive (Altieri & Toledo, 2011).

1.1.3. Mycotoxin contamination of staple food crops in Ethiopia

According to the Central Statistics Authority of the Federal Democratic Republic of Ethiopia (CSA) (CSA, 2021a), the first four most produced cereals in Ethiopia for human food consumption, in descending order, are maize, wheat, teff and sorghum, together accounting for about 88% of the total cereal production (324.6 million quintals) in 2020/2021. Despite their importance as staple food crops, the contamination of these cereals with mycotoxins in Ethiopia has frequently been reported, even to concentrations beyond European regulatory limits. Previous research in Ethiopia has focused on the occurrence of mycotoxins in all four crops: in maize (Atnafu *et al*., 2024; Chauhan *et al*., 2016; Getachew *et al*., 2018; Mesfin *et al*., 2022; Mohammed *et al*., 2022b; Mohammed *et al*., 2023; Tsehaye *et al*., 2016), sorghum (Chala *et al*., 2014; Mohammed *et al*., 2022a; Ssepuuya *et al*., 2018; Taye *et al*., 2018), wheat (Getachew *et al*., 2018; Getahun *et al*., 2023), and teff (Ayalew *et al*., 2006; Geremew *et al*., 2018). For instance, Atnafu *et al*. (2024) reported that all the 54 samples of newly harvested maize collected from farmers' fields in southwestern Ethiopia in 2020/21 were contaminated with at least one of the *Fusarium* mycotoxins that are regulated in Europe. In another study, Mohammed *et al*. (2022a) reported the presence of 79 different mycotoxins and related fungal metabolites in stored sorghum samples collected from farmer's households in Eastern Ethiopia (in Doba, Fedis, Gorogutu and Miesso districts) in 2021. The reported toxins included both common (EU regulated) mycotoxins

such as aflatoxins, fumonisins, zearalenone, ochratoxin A, and deoxynivalenol, as well as emerging (unregulated) mycotoxins such as 3-nitropropionic acid, sterigmatocystin, fusaric acid, tenuazonic acid, alternariol, and moniliformin. The occurrence of aflatoxin in Ethiopia was reported by Ayalew *et al*. (2006), and the occurrence of ochratoxin A in teff in Ethiopia was reported by Geremew *et al*. (2018). The AFB1 concentration in teff ranged from 0.0 to 15.6 ng/g. The higher concentrations exceeded the EU's regulatory limit of 4.0 ng/g (European Commission Regulation ((EC, 2023)) and the FAO/WHO (2017) limit of 10 ng/g. The presence of mycotoxins in cereal grains may be related to the climatic conditions in Ethiopia such as temperature and humidity, which are suitable for crop cultivation, but also conducive to fungal infection and mycotoxin production (Tsehaye *et al*., 2016). Tsehay *et al*. (2016) collected climatic data stretching from seeding to harvesting (May to December 2011) and the storage period (January to June 2012) for maize in 20 major maize-growing districts in Ethiopia. They indicated the presence of suitable conditions in different agroecologies in Ethiopia for the growth of *Fusarium* species and fumonisin contamination. Also, local practices for cultivation and storage play a role. Subsistence farmers most often use traditional farming and storage practices of their crops, which may contribute to mycotoxin contamination (Beyene *et al*., 2016; Getachew *et al*., 2018; Mesfin *et al*., 2022). Consequently, fungal species, namely *Aspergillus, Fusarium, Alternaria,* and *Penicillium,* that are known to produce mycotoxins were detected in cereal grains in Ethiopia (Getachew *et al*., 2018; Mohammed *et al*., 2022a). The underdeveloped nature of crop value chain practices in Ethiopia have also contributed to the presence of mycotoxins in the grains (Abamecha, 2021; Beyene *et al*., 2016; Mohammed *et al*., 2022a; Mohammed *et al*., 2022b; Mohammed *et al*., 2023; Taye *et al*., 2016; Taye *et al*., 2018; Taye *et al*., 2022).

1.1.4. Mycotoxin exposure in Ethiopia

Results from mycotoxin exposure studies conducted in Ethiopia, indicated that mycotoxin exposure is a public health problem in the country. Most of the available exposure studies were conducted using measured mycotoxin biomarkers in human blood or the urine. Tesfamariam *et al*. (2022) studied the level of exposure of pregnant women to multiple mycotoxins through serum analysis in a cohort study area in southern Ethiopia. The findings indicated that the women were simultaneously exposed to multiple (5 to 27) mycotoxins, fumonisins being identified in about 99% of the blood of samples. Tessema *et al*. (2021) investigated the exposure of young children aged 6-35 months, selected from an intervention trial on the consumption of quality protein maize in rural Ethiopia, to aflatoxin and fumonisin, by analysing mycotoxin biomarkers in their blood. Their findings indicated that the children were exposed to high amounts of aflatoxins and low amounts of fumonisins. In another study, Boshe *et al*. (2020) investigated the exposure of lactating women in southern Ethiopia to AFB1 by analyzing the concentration of urinary AFM1. Their findings showed the study women were exposed to too high concentra-

tions of AFM1. The median urinary AFM1 level was 0.214 ng/g (range: undetectable to 2.582 ng/g), and AFM1 was present in 53.3% of the research samples. Although there is no established regulatory limit for AFM1 level in the human body fluids, the reported median AFM1 level by far exceeded the FAO/WHO (2009) regulatory limits of 0.5 ng/g in milk. Furthermore, Ayele *et al*. (2022) investigated the exposure of children aged 12-59 months to aflatoxins at a surveillance site in southern Ethiopia. Analysing urinary samples for AFM1, they found that the children had consumed a diet containing high levels of aflatoxins. Another study, conducted in the same study site, investigated the exposure of lactating women to mycotoxins by testing the presence of 16 mycotoxins in their breast milk samples (Mesfin *et al*., 2023). The authors reported the presence of FB2 and FB3 in a portion of the samples, and FB1 and nivalenol in only one sample, and concluded that overall mycotoxin exposure was low. Mohammed *et al*. (2022b) estimated human exposure to fumonisins via a dietary intake study. The results indicated that FB1 exposure from the consumption of stored maize produced in Eastern Ethiopia ranged from 6.1 to 1131.0 ng/kg bw/day, indicating high exposure levels, relative to the tolerable daily intake (TDI) of 2000 ng/kg bw/day set by WHO & FAO (2017).

Although exposure data provides information on the extent of public health risk in relation to the TDI, it does not provide the associated health burden. The population health burden can be quantified by using the DALY metric (Gibb *et al*., 2015). The DALY metric is a widely used public health measure for estimating the overall disease burden (Gibb *et al*, 2010). When applied to aflatoxin exposure, DALY quantifies the combined impact of illness, disability and premature death, primarily from aflatoxin-related diseases such as HCC. Mathematically, DALY is the sum of years lived with disability (YLD) due to illness and years of life lost (YLL) due to premature mortality (Mihalache *et al*, 2024; Wu and Khlangwiset, 2010). The DALY metric provides more important information to policymakers than the mycotoxin contamination and exposure, since it quantifies the number of years lived with disability and the amount of healthy life years lost due to disease. In addition, DALY is useful for evaluating the cost-effectiveness of aflatoxin control strategies, since the benefits of these strategies are improved health outcomes (i.e. reduced DALYs) rather than market outcomes (Wu & Khlangwiset, 2010). To date, DALY estimates for human exposure to mycotoxins via dietary intake in Ethiopia have not been made. The only available DALY estimate is from the global burden of disease study, focusing on the incidence of diseases per category such as liver cancer (ghe2021_daly_bycountry_2021.xlsx).

1.1.5. Literature Gap

Climatic conditions suitable for staple food crop cultivation in Ethiopia are conducive to fungal infection and mycotoxin production (Tsehaye *et al*., 2016). Consequently, the frequent occurrence of mycotoxins in staple cereals in Ethiopia has been reported, even in concentrations above the EC legal limits for food (Atnafu *et al*., 2024; Geremew *et al*.,

2018; Getahun *et al*., 2023; Mohammed *et al*., 2022a). In Ethiopia, regulatory standards are only available for a limited number of mycotoxins and limited number of foods. Aflatoxin limits of 5.0 ng/g for AFB1 and 10.0 ng/g for total aflatoxin are in place for several food grains, including maize and sorghum. However, fumonisin regulations are not in place for cereal grains (Mamo *et al*., 2020). Nevertheless, effective food safety regulation concerning mycotoxins is not in place in local markets in Ethiopia (Global Alliance for Improved Nutrition (GAIN, 2022) leading to mycotoxin exposure of humans via the diet.

To lower the presence of mycotoxins in food and its commodities produced in Ethiopia, insights into the relationships between local agronomic, storage and processing practices of cereal crops prone to mycotoxin contamination is needed. So far, information on mycotoxin related health burden and on relationships between agronomic and storage practices with mycotoxin concentrations in crops are rarely available. Such information is important to guide decision making on priority areas for intervention to reduce mycotoxin contamination.

1.1.6. Aim and objectives of the PhD research

The aim of this PhD research is to develop an integrated approach to reduce the disease burden related to mycotoxin contamination in maize and sorghum in northwest Ethiopia. This part of Ethiopia is among the locations where cereal grains are produced most in the country (CSA, 2021a). Because of the possibility of mycotoxin contamination at any stage of the crop value chain, we investigated the potential of reducing the contamination and related disease burden via a value chain approach, rather than food processing.

The overall objective is broken down into the following four Research Questions (RQ).

- *RQ1*: What is the current knowledge and gaps herein about prevention and control of mycotoxins in staple crops in Ethiopia?
- *RQ2*: Which pre-harvest management practices of staple crop cultivation are related to mycotoxin contamination, and to what extent?
- *RQ3*: Which storage management practices of staple crops are related to mycotoxin contamination, and to what extent?
- *RQ4:* What is the human exposure to and related disease burden of aflatoxins and fumonisins through consumption of staple crops?

Although farming and storage practices in Ethiopia are generally traditional ones, we assumed that there are farming and storage practices that are negatively associated with mycotoxin contamination in the grain, which can be promoted as an intervention option for mycotoxin prevention. On the other hand, we also assumed that there are practices

that are positively associated with mycotoxin contamination. These would be identified in order to plan a potential intervention option.

1.1.7. Outline of Research

The most produced cereal grain in Ethiopia (CSA, 2021a), maize, was considered to address RQ1. A comprehensive literature review was conducted including both preharvest and postharvest maize management practices in relation to mycotoxin contamination to investigate the currently available knowledge and the status of their implementation. The preharvest practices were defined to include cereal production stages from land preparation, including harvest, up to threshing, while the postharvest practices were defined to include long-term storage and processing practices.

The other three RQs (RO2-RO4) were addressed by focusing on sorghum, which is amongst the four most produced cereals in Ethiopia for human food consumption (CSA, 2021a). Although nationally teff is a more widely produced cereal than sorghum (CSA, 2021a), teff has become a more commercial crop in rural communities due to increasing prices (Gebrehiwot & Ndinda, 2024). Teff is traditionally commonly used for the making of *injera*. *Injera* is a staple food in Ethiopia. It is made by mixing teff or other cereal flour with water and a small amount of old fermented dough, called '*ersho*', which acts as a starter culture for fermentation. The mixture is left to ferment for two to three days, which gives *injera* its distinctive, slightly sour taste. The fermented dough is then baked for a few minutes on a flat griddle to produce a soft, spongy bread that resembles a pancake, with a bubbly surface on the top side (Baye *et al*., 2013; Yetneberk *et al*., 2005). However, due to the increasing market price of teff (as a result of commercialization), the use of sorghum has been increasing as a cheaper option to substitute teff for local *injera* making (Fox *et al*, 2020; Suraj *et al*., 2024). Wheat is also produced in greater quantities than sorghum nationally (CSA, 2021a). However, wheat is commercially milled and used for commercial bread making. Here, we assume that mycotoxin contamination in this value chain is under better control than sorghum and maize.

To investigate the relationships between the current preharvest and storage practices with mycotoxin contamination, sorghum samples at harvest and after storage were collected from subsistence sorghum producing farmer households in northwest Ethiopia, and analyzed for the presence of multiple mycotoxins. For the samples collected, related information on the agronomic practices applied to the fields to grow the sorghum and store the respective harvested sorghum was collected by conducting interviews with farmers while collecting the samples and inspection of the practices. Logistic regression was applied to investigate the presence of relationships between the preharvest, and the storage management practices with mycotoxins in the preharvest and stored samples, respectively. With this RQ2 and RQ3 were addressed. The mycotoxin occurrence data

obtained in this research was complemented with sorghum consumption data obtained from the National Food Consumption Survey in Ethiopia to estimate the mycotoxin exposure and associated disease burden, addressing RQ4. With this set of integrated and interdisciplinary research, the overall objective of the PhD thesis was addressed. Information on the extent of mycotoxin contamination of sorghum in northwest Ethiopia was obtained and potential low cost preharvest and postharvest practices that would reduce mycotoxin contamination were identified. Based on the obtained insights of this thesis, recommendations were formulated related to a combination of low cost preharvest and postharvest practices that can effectively reduce the mycotoxin disease burden in Ethiopia.

The conceptual framework for this project is presented below.

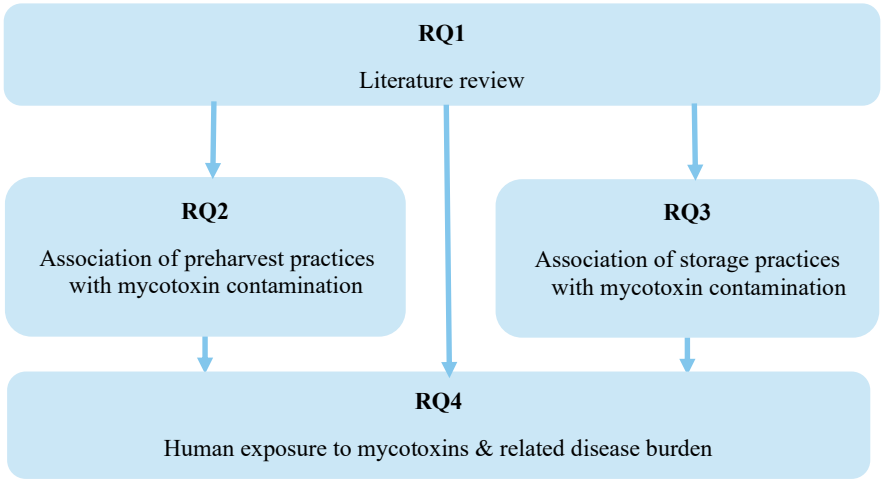


Figure 1.3. The research framework of the PhD project.

1.1.8. Description of the study sites

For RQ2 and RQ3, we used primary data collected from three sorghum-producing areas in northwest Ethiopia, specifically in the Amhara National Regional State. The Amhara National Regional State is the second highest producer of sorghum nationally after the Oromia National Regional State (CSA, 2021a). Three major sorghum-producing administrative zones (Central Gondar, South Wollo and North Shewa) were purposively selected based on CSA (2021) sorghum production data for the 2020/21 period. One high-producing *woreda* was selected from each administrative zone: West Belesa from Central Gondar, Kalu from South Wollo, and Kewet from North Shewa (see Figure 1.4). A *woreda* is an administrative division that functions like a district in Ethiopia. It is one level below a zone and one level above a kebele (a village or ward). We also used primary data

obtained from these *woredas*, namely data on aflatoxin and fumonisin contamination, to address RQ4, complementing it with data from other sources.

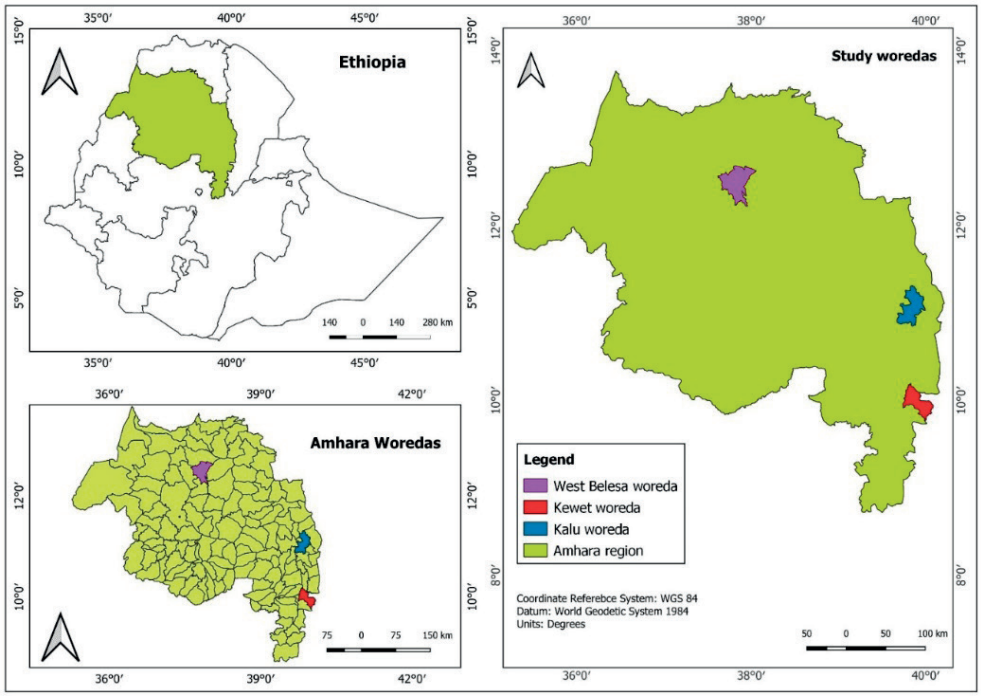


Figure 1.4. Locations of sorghum sample collection *woredas* in Ethiopia

CHAPTER 2

2

Preharvest and postharvest management practices related to mycotoxin contamination in maize in Ethiopia

This Chapter is based on a paper published in an international journal:

Sadik, J. A., Fentahun, N., Brouwer, I. D., Tessema, M., & van der Fels-Klerx, H. J. (2023). Preharvest and postharvest management practices related to mycotoxin contamination in maize in Ethiopia - a review. World Mycotoxin Journal, 16(3):211-226. https://doi.org/10.1163/18750796-20232839

ABSTRACT

Mycotoxins are fungal metabolites that commonly contaminate food crops such as maize. Conducive climatic conditions together with improper crop value chain practices are favorable for mycotoxin contamination. Previous studies in Ethiopia have indicated that mycotoxin contamination in maize is prevalent. For the implementation of proper mycotoxin prevention and control strategies, identifying the current local value chain practices that are related to mycotoxin contamination is needed. This review investigates current preharvest and postharvest management practices of maize cultivation in Ethiopia in relation to mycotoxin contamination and identifies gaps in knowledge and priority areas for future research. Findings indicate that the majority of applied preharvest and postharvest practices of maize in Ethiopia seem to favor mycotoxin contamination. Recent developments in grain drying and storage technologies, which are also potential mycotoxin management strategies, are facing constraints for proper implementation in subsistence farmers' level.

Key words: aflatoxins, fumonisins, maize, staple crop, Africa

2.1. INTRODUCTION

Mycotoxins are toxic chemical compounds produced by certain species of fungi upon and after infection of the crop. Most important mycotoxins known to cause human health burden worldwide are aflatoxins and fumonisins (Jallow *et al*., 2021; Wu *et al*., 2014a). Aflatoxins are mainly produced by the fungal species of *Aspergillus flavus* and *Aspergillus parasiticus* while fumonisins are mainly produced by *Fusarium verticillioides*, *Fusarium proliferatum* and *Aspergillus niger* (Wu *et al*., 2014a). Aflatoxin intake through consumed foods can lead to severe human health effects, including liver cancer, acute toxicity and is also predicted to impair the growth of children leading to stunting (Wu *et al*., 2014a) as well as to lower immune responses of the body and protein synthesis (Smith *et al*., 2012). On the other hand, fumonisin intake causes brain and esophageal cancer, renal and liver toxicity (Bucci *et al*., 1998) and neural tube defects (Wu *et al*., 2014a).

Occurrences of mycotoxins in maize in Ethiopia (*Zea mays L*.), where maize is one of the staple food crops in the country (CSA, 2021a), have been reported, even in concentrations that exceed national or international standards (Alemu *et al*., 2008; Chauhan *et al*., 2016; Getachew *et al*., 2018; Mesfin *et al*., 2022; Tsehaye *et al*., 2016; Tsehaye *et al*., 2017). Chauhan *et al*. (2016) reported that aflatoxin contamination was detected in all the samples collected from 150 maize products bought at local markets in Gedeo zone, South Ethiopia. In all these samples, the quantified aflatoxin concentrations were above EU regulatory limits. In another study, Getachew *et al*. (2018) identified a total of 127 different mycotoxins and derivatives in maize collected from farmers' stores in south and southwestern Ethiopia. To mention one, the FB1 concentration was in the range of 7.0 $*10^3$-1.183$*10^7$ ng/g. These reported high levels of mycotoxin contaminations are presumed to cause severe problems for the maize industry in the country (Getachew *et al*., 2018; Mesfin *et al*., 2022; Tsehaye *et al*., 2017). Several factors may limit effective control of fungal infection and mycotoxin contamination in subsistence maize farming in Ethiopia. First, maize value chains are poorly developed. As a result, subsistence farmers most often use traditional practices, which are prone to fungal infection (Bereka *et al*., 2022; Beyene *et al*., 2016; Getachew *et al*., 2018; Mesfin *et al*., 2022; Tsehaye *et al*., 2017). Secondly, farmers have limited knowledge about mycotoxins and their health impact as well as prevention and control strategies for mycotoxin contamination. This lack of awareness has limited farmers from practicing proper mycotoxin management (Boshe *et al*., 2020; Mohammed *et al*., 2022b). Finally, the lack of proper facilities (such as for improved drying and storage) in rural areas is another limitation (Bereka *et al*., 2022). These factors limit the implementation of effective mycotoxin prevention and control in Ethiopia.

To date, studies in Ethiopia had mainly focused on the occurrence of mycotoxins in the postharvest stage of maize, especially the household and market stage. In these studies,

the relationship with pre- and postharvest maize practices has not been fully explored. Recently, there is interest in studying these practices such as harvesting and drying, and how they are related to mycotoxin contamination (Bereka *et al*., 2022; Mohammed *et al*., 2022b). Despite these good starts, most of the preharvest maize management practices such as land preparation, planting and growing treatment have not been given attention.

To prevent fungal infection and mycotoxin contamination of maize, a full understanding of local practices for both preharvest and postharvest stages together with the state-of-the-art knowledge from literature is crucial (Bereka *et al*., 2022; Beyene *et al*., 2016). Such information will help in identifying gaps for further research, and for planning appropriate intervention strategies. Nevertheless, comprehensive information on the local maize production practices in relation to mycotoxin contamination is limited for Ethiopia. Therefore, this study aimed to investigate the current pre- and postharvest management practices of maize in Ethiopia and evaluate the practices in relation to mycotoxin contamination. For this review, preharvest practice is defined to include land preparation, planting, treatment during growth, harvesting, field drying and shelling. The postharvest practice, on the other hand, is defined to include long-term storage practices and processing at household level.

2.2. MATERIALS AND METHODS

A Prisma 2020 guideline for new systematic reviews was used (Page *et al*., 2021) (Figure 2.1). An extensive literature search was conducted to obtain articles published before November 21, 2022, in the common databases of Web of Science, Pubmed, Scopus and Google Scholar. Only articles published in the English language were considered. Search strings that were used in the search engine included: (maize OR corn OR "zea mays") AND (mycotoxin* OR aflatoxin* OR fumonisin*) AND (harvest OR drying OR storage OR consumption). Additional records on indigenous preharvest and postharvest maize management practice were also searched from google using the phrases- 'land preparation / tillage practice', 'agronomy / agricultural production practice of maize' and 'fungal infection of maize'; each in combination with 'in Ethiopia'. For local practices for which there is not enough information in the literature in relation to mycotoxin contamination, related information from other countries was searched for, using the above search phrases together with 'Africa', 'low- and middle- income countries' OR 'subsistence farming'. All the articles obtained from Google Scholar and Google were considered only if they were confirmed to exist in one of the above databases.

All collected articles were imported to endnote 20 software, after which duplicates were removed. Then articles were screened by exploring the title, key words and the abstract

based on predefined eligibility criteria. These criteria included: research conducted in Ethiopia, describing local preharvest or postharvest practice in relation to fungal infection and/or mycotoxin contamination in maize, and the presence of the article in one of the three major databases (Web of Science, Pubmed, and Scopus). Review articles or unpublished/theses were excluded.

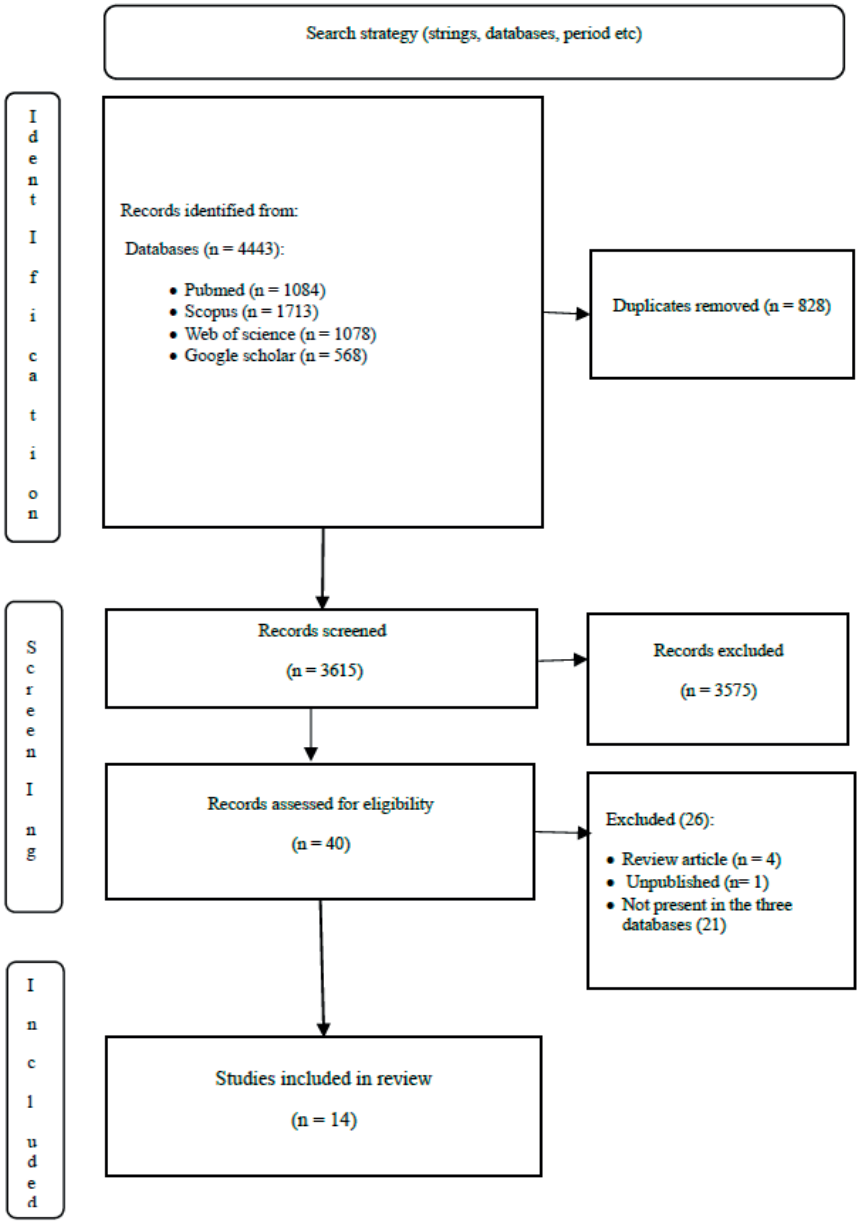


Figure 2.1. A flow diagram of litereature search

2.3. RESULTS AND DISCUSSION

The litereature search led to fourteen relevant and eligible articles, which were then used for this review. The major findings of the reported studies, i.e the association of maize management practices with mycotoxin producing fungi (toxigenic fungi) and/or mycotoxin contamination of maize, were extracted. A summary of the studies that have been included is presented in Table 2.1, showing fungal infection and/or mycotoxin contamination in maize have been reported in samples collected from different parts of the country.

This finding indicates the availability of convenient climatic conditions for the growth of toxigenic fungal species, which together with improper pre- and/or postharvest practices, is a contributing factor for mycotoxin contamination. In Ethiopia, maize is cultivated in different parts of the country that belong to 7-20 agroecological zones each varying in their agroecological indicators namely altitude, temperature, and rainfall (Tsehaye *et al*., 2017; Van Dijk *et al*., 2020). Tsehaye *et al*. (2017) reported the ranges of these indicators for twenty major maize producer areas in Ethiopia. The elevation, temperature, and rainfall ranges were 1206 – 2490m, 16-25^{o}C and 537-1039mm, respectively. Indeed, the requirements for fungal growth related to temperature (10-40^{o}C) and water activity (above 0.7) (Bhat *et al*., 2010) are met in most of the maize producing areas in Ethiopia (Getachew *et al*., 2018; Tsehaye *et al*., 2017). However, the type and extent of mycotoxin contamination in maize could be dependent on the local climatic condition in a particular part of the country. This is because toxigenic fungal species vary in their optimal growth requirements and related mycotoxin production (Dövényi-Nagy *et al*., 2020; Tsehaye *et al*., 2017). A changing climatic condition because of global warming is predicted to increase the risk of mycotoxin contamination in maize in the future. This is partly because, the incidence of extreme climatic conditions such as drought during the growing period weakens the maize immune system which makes the crop more vulnerable to fungal infection and mycotoxin contamination (Chauhan *et al*., 2008). Studies related to predicted levels of mycotoxin contamination in maize considering a forecasted future climate change scenarios in Ethiopia are almost absent in the litereature.

Table 2.1. Eligible articles on the association of maize preharvest (pre) and postharvest (post) practices in relation to mycotoxin contamination in Ethiopia

S. N	part of Ethiopia	Study description	samples collected from (data collected)	relation[1]	stage	Ref.
1	Southwest	association of postharvest practices with aflatoxin awareness	producer farmers" interviewed	fungi	post	Bereka *et al*. (2022)
2	South, Southwest	contamination of maize with *Fusarium* mycotoxins	maize collected from farmers stores	mycot.	post	Mesfin *et al*. (2022)
3	East	association of harvest and postharvest practices with fumonisin contamination	maize collected from farmers stores, markets	mycot.	post	Mohammed *et al*. (2022b)
4	Northwest	performance assessment of improved storage methods for prevention of fungi and mycotoxin	aflatoxin determination	mycot.	post	Worku *et al*. (2022)
5	Southwest	assessment of farmers experience on storage duration and associated pest infestation	interview for the length of storage and degree of insect, rodent and mold infestation	fungi	post-	Abamecha (2021)
6	Southwest	comparison of traditional grain storage structures for postharvest loss reduction and maize safety	above ground and underground storage structures evaluated	fungi	post	Muleta *et al*. (2021)
7	Southwest	potential of weather conditions for fungal growth	relative humidity and temperature before harvest and during on farm storage	fungi	pre	Garbaba *et al*. (2018a)
8	Southwest	maize postharvest practices of actors' (farmer, collectors, and wholesaler) as a potential for toxigenic fungi management	interview with actors and fungi growth during storage	fungi	post	Garbaba *et al*. (2018b)
9	South, Southwest	fungal infection and mycotoxin occurrence	mycotoxin and fungi species determined from farmer stored maize	mycot.	post	Getachew *et al*. (2018)
10	North, South, South, East	infection of maize with *Fusarium* species and contamination with fumonisin	*Fusarium* species identified and fumonisin determined from farmer stored maize	fungi, mycot.	post	Tsehaye *et al*. (2017)

Table 2.1. Eligible articles on the association of maize preharvest (pre) and postharvest (post) practices in relation to mycotoxin contamination in Ethiopia (*continued*)

S. N	part of Ethiopia	Study description	samples collected from (data collected)	relation[1]	stage	Ref.
11	North, northwest, central and south	survey on mothers' awareness and practice about aflatoxin contamination	mothers/caregivers interviewed (for pre and postharvest practices)	fungi	Pre, post	Beyene *et al*. (2016)
12	South	contamination of maize with fungi and aflatoxin	retailers, markets, street maize fruit seller, store house and millers	fungi, mycot.	post	Chauhan *et al*. (2016)
13	North, West, South, Southwest	isolation and genetic characterization of *Fusarium verticillioides*	fungi species identified from Ethiopian maize varieties	fungi	Pre-	Tsehaye *et al*. (2016)
14	South	contamination of maize with mycotoxin	fresh and stored maize grain	mycot.	Pre, post	Alemu *et al*. (2008)

[1] Indicates the reported relationship of maize with fungi or mycotoxin or both

To achieve the objective of this review, the information presented in Table 2.1 was processed to arrive at a summary of the findings, per stage of the maize supply chain, considering pre- and postharvest separately. For each stage of the preharvest and postharvest practices, current local practices are described first, followed by their potential relationship with toxigenic fungal infection and related mycotoxin contamination.

2.3.1. Preharvest practices and mycotoxin contamination

2.3.1.1. Land preparation

In Ethiopia, land is mostly prepared with oxen using an ard or breaking plow (locally called *"maresha"*) and plough to cultivate grains, including maize (Biazin *et al*., 2011; Goe, 1989; Sime *et al*., 2015; Workneh *et al*., 2021). For this purpose, most farmers in the north eastern part of the country own at least one oxen (Derese *et al*., 2017). By using the *"maresha"* and plough method, the topsoil layer can be ploughed to a depth of 0 to 15 cms (Biazin *et al*., 2011; Hussein *et al*., 2019; Sime *et al*., 2015). This depth of tillage belongs to the minimum tillage category of less than 20 cm depth (Arino *et al*., 2009). The number of ploughing times for growing cereal grains is usually one to more than three times. For example, in Southern Nations and Nationalities and Peoples Region, ploughing time is three or less. On the other hand, more than three times ploughing is common in Amhara and Oromia regions (Beyene *et al*., 2016) with the purpose being to improve soil aeration and water infiltration. Manure is added to soil in some areas as part of land preparation to improve soil fertility (Biazin *et al*., 111; Sime *et al*., 2015). According to Desta *et al*. (2021), about 45% of the total rain fed area in Ethiopia is predicted to be acidic (PH < 6.5). Out of this rain fed agricultural area, 12% is extreme to strong acidic (pH < 5.5), 18% is moderate acidic (5.6 < pH < 6.0) and 14.6% is slight acidic (6.0 < pH < 6.5). Recently, treatment of soil acidity using lime has been increasing in the country to improve maize yield (Alemu *et al*., 2022).

Current land preparation practices in Ethiopia may increase mycotoxin contamination. Proper tillage systems contribute to improved aeration of soil and crop water availability. This reduces crop stress during growth and can reduce incidence of fungal infection (Xu *et al*., 2022). There are three common types of tillage systems in the world: plowing with which 30 to 40 cm of the top layer is inverted: minimum tillage, which involves mixing the crop debris with the top 10 to 20 cm of soil; and no tilling, in which seed is directly drilled without tilling soil (Arino *et al*., 2009). Maize grown in plowed soil showed slightly lower fumonisins contamination as compared to minimum tillage, which can be explained by the destroying effect of ploughing on fungi (Arino *et al*., 2009). In another research, Janusauskaite *et al*. (2013) reported about a 12% decrease in fungal population at a minimum tillage level (10 to 20 cm depth) as compared to 0 to 10 cm depth. This decrease could be partly associated with a lower quantity of plant residue at the lower depth.

Despite the unavailability of information on the relationship of *"maresha"* and ploughing method with mycotoxin contamination in Ethiopia, it seems that this method favors mycotoxin contamination. First, when using this method, the topsoil layer can be inverted to about 15 cm depth so only minimum tillage is possible (Biazin *et al*., 2011; Hussein *et al*., 2019; Sime *et al*., 2015). At this depth, important fungal growth simulating factors (nutrient and oxygen) are commonly available, which make fungal proliferation possible (Nesci *et al*., 2006). Second, frequent tillage may increase crop vulnerability to infection. With an increasing number of years of tillage using *"maresha"* and ploughing, soil property deteriorates more. As a result, the soil water infiltration rate decreases and soil moisture loss via evaporation after a rain event increases. Such phenomena may lead to draught stress of the maize plant (Biazin *et al*., 2011), which further makes it prone to fungal infection. Third, the grazing practice in Ethiopia may increase the area coverage of fungal infection. During grazing, animals may distribute the fungal population from its hot spot area to new areas. Nesci *et al*. (2006) studied the distribution of three toxigenic fungal species, *Aspergillus*, *Penicillium* and *Fusarium*, in soil samples collected from conventional tillage, reduced tillage and no tillage practices, with and without grazing in Argentina. The results indicated that *Aspergillus* spp. was the most dominantly isolated species in the samples in all tillage methods, for both with and without grazing. In addition, the incidence rate of this species was increased in no tillage method with grazing practice. Maize sowed after 60 cm manual deep ripping in Ethiopia yielded 6% more maize than the maize sowed at about 15 cm depth (*"maresha"* and plough method) (Hussein *et al*., 2019). For long term impact, the conventional *"maresha"* and plough method shall be improved to enable a deeper tillage, and or tractor access via renting service or sale may be established for subsistence farmers. In the literature, information on the relationship of soil treatment with lime with mycotoxin contamination is rarely available. Despite this gap, it is described that untreated acidic soil causes stress to growing maize (Alemu *et al*., 2022) making the crop vulnerable to fungal infection (Keller *et al*., 2022).

2.3.1.2. Growing period

In Ethiopia, the maize growing period is dependent on the seasonal rainfall (Sime *et al*., 2015). The common growing period is in the main rainy season, which is from May to December (Tsehaye *et al*., 2017). This is mainly because about 99% of the maize is produced by using natural rain (Abate *et al*., 2015). However, in some areas, the growing period may be extended. According to Sime *et al*. (2015), the cereal cropping season in Ziway area, East Shoa Zone of Oromiya Regional State, is from April to October, with the main season being from June to October. In Ethiopia, the use of irrigation water for maize production is limited. According to the (CSA, 2021a) and (CSA, 2021b) reports for the 2020/21 cropping season, only about 37 thousand hectares of land from the total area of 3.4 million hectares used for maize production was irrigated.

The growing period affects mycotoxin contamination given the (indirect) relationship with weather conditions during the maize growing season. Delaying the planting date from mid-March to mid-April was reported to reduce preharvest aflatoxin contamination. This was related to the rain fall pattern which rain mostly before mid-April resulting in reduced contamination, and inadequate rain in mid-March leading to moisture stress favoring contamination (Damianidis *et al*., 2018). According to Arino *et al*. (2009), planting of maize in the dry period increased *Fusarium* species infection and fumonisin contamination as compared to planting in the wet period. Sime *et al*. (2015) reported that farmers in Ziway area, East Shewa Zone of Oromiya Regional State, Ethiopia, practice cropping mid-maturing maize in April while the main rainy season in the area is from June to October. The authors indicated that this practice has been subjected to moisture stress to the growing maize due to cessation of the rain during late May. The incidence of extreme climatic conditions such as drought during the growing period is reported to weaken the crop immune system; making the crop vulnerable to fungal infection and mycotoxin contamination (Chauhan *et al*., 2008). When applicable, the use of early/mid maturing maize varieties or irrigation would be helpful in such situations. Early maturing maize varieties were reported to be contaminated with fumonisin to a lesser extent than late maturing varieties, under local climatic conditions in Zimbabwe (Ndemera *et al*, 2018).

2.3.1.3. Planting

Maize planting practices, namely crop rotation, use of improved seed and seeding density have been exercised to different degrees in Ethiopia. According to Beyene *et al*. (2016), the majority of farmers from major producer regions practice crop rotation, i.e., alternate growing of maize with another cereal or legume in successive cropping seasons. In another study, Assefa *et al* (2021) reported that only about 40% of farmers selected from major maize producer areas in Ethiopia practice crop rotation. For the same cropping season, monocropping has been a common practice (Assefa *et al*., 2021; Sime *et al*., 2015) and intercropping has only been used to a limited extent (Abate *et al*., 2015; Assefa *et al*., 2021). A three-year survey (2015-2017) on agronomic practices of maize in major producer areas in Ethiopia indicated that the mean plant density for 88% of farmers was below the optimum range suggested for yield (44,444 to 62,500 plants per hectare depending on the cultivar); the other 12% of farmers maintained the suggested optimal range (Balemi *et al*., 2019). From the total area of land used for maize production in the 2020/21 cropping seasons (about 3.4 million hectares), the area covered with improved seeds was about 1.5 million hectares (44% of total). For the same year, about 520 thousand quintals of improved seed had been used nationally (CSA, 2021b). On the other hand, Assefa *et al*. (2021) reported that subsistence farmers in major maize producer areas in Oromia and Amhara regions commonly use improved seeds. In another study, Van Dijk *et al*. (2020) reported that only about a quarter of smallholder farmers in the country

use improved seeds, and that the level of use of improved seed (25kg/hectare) is below the requirement for reaching the optimum yield.

The practices of crop rotation and use of improved seed may affect mycotoxin contamination. Maize crop rotation reduces mycotoxin contamination in two ways; it reduces stress during the maize growth period by improving soil fertility (especially when legumes are used) and breaks a pest-disease cycle (Xu *et al*., 2022). According to Venter *et al*. (2016), crop rotation also improves richness and diversity of soil microbial communities. Improvement of the soil microbial community, which can reduce toxigenic fungal infection via competition mechanisms, can reduce mycotoxin contamination (Yin *et al*., 2008). However, fungal spores can stay dormant in the soil to acts as inoculum for contamination during the next cropping season (Reis *et al*., 2010; Xu *et al*., 2022). Yet, as explained above, survival of spores is dependent on the tillage system used for land preparation (Arino *et al*., 2009). Farmers in Ethiopia could have access to different maize varieties (Megerssa *et al*., 2021) including improved varieties such as BH140 (early- to intermediate-maturity breed), BH660 (late maturing breed) and BH661 (draught tolerant breed) (Abate *et al*., 2015). However, information on the relationship of maize varieties available in the country with fungal infection is rarely available at this moment. Despite this gap, the use of improved seeds that are resistant to fungal infection would be helpful (Xu *et al*., 2022). Therefore, evaluation of maize varieties available in the country for their tolerance to toxigenic fungal infection during growth in the field would help in identifying the most tolerant cultivars that are applicable to the specific local agronomic and climatic conditions. The reported (sub) optimal maize plant density may imply that plant density may not be related to mycotoxin contamination. When plant population is not crowded, the competition for nutrients will be limited. In this case, the natural resistance of the crop to fungal infection may not be affected (Xu *et al*., 2022).

2.3.1.4. Maize treatment practices during growth

Current agronomic treatments used to grow maize are below the required level to reach optimum yield. These treatments include fertilizer application, weed removal and pesticide application. Fertilizer is described as the major limiting factor for maize productivity in Ethiopia. The average amount used (119 kg/ha nitrogen) is much lower than the required amount for optimal yield (Van Dijk *et al*., 2020). According to Assefa *et al*. (2021), 90% and 24% of respondents from major maize producer areas in Oromia and Amhara regions use fertilizer and manure, respectively. The same study reported that the use of combinations of fertilizer, improved seed and manure resulted in decreased yield. This situation could be associated to the diluting effect of low quality manure to the fertilizer (Assefa *et al*., 2021). However, it is to be noted that additional manure is possible from animals grazing the field (Van Dijk *et al*., 2020). A survey conducted in major maize producer regions in Ethiopia indicated that the occurrence of weed in maize fields was common,

with a reported weed density from low (0-20 weed m^2) to high (41-100 weed m^2) (Regassa *et al*., 2020). According to Assefa *et al*. (2021), during the main cropping season of 2017 and 2018, the use of herbicide was not common, and pesticides were used to a limited extent among subsistence maize producer farmers present in major producer areas in Ethiopia. The authors described that this practice is partly related to farmers' preferences to use human labor for weeding rather than using herbicides. The limited use of pesticide was associated with the low incident rate of disease/pest in this cropping period.

When optimal growth requirements for maize in Ethiopia are not met, growth stress occurs on the crop which may increase mycotoxin contamination (Keller *et al*., 2022). A low level of nitrogen leads to maize nutrient stress creating favorable conditions for fungal infection (Xu *et al*., 2022). Sub optimal N-fertilizer application to maize was reported to lead to increased contamination of fumonisin contamination in Zimbabwe (Ndemera *et al*, 2018). An increase in weed density during maize growth was indicated to result in a decrease in maize yield in Ethiopia, presumed to be due to a competitive effect of the weed for growth factors (Sime *et al*., 2015). Increase in weed density would also create stress to the growing maize plant making it more vulnerable to fungal infection. Training farmers to control the presence of weed by using manual methods or using recommended pesticides would be helpful. Some mycotoxigenic species may be able to better adapt to stress conditions than others. This feature fundamentally determines ecological dominance in any given environment (Dövényi-Nagy *et al*., 2020).

2.3.1.5. Harvesting

Subsistence farmers in Ethiopia use traditional methods for judging readiness of maize for harvesting. These methods include starting of drying of leaves, colour change from green to yellow, and cob dropping (Garbaba *et al*., 2018b; Mohammed *et al*., 2022b). Other methods such as the crop calendar method, and observation of kernel dryness and shelling property are also used (Garbaba *et al*., 2018b). According to CSA (2021a), the harvesting period for main crops - including maize grown in the main cropping season - is from September to February depending on local weather conditions. Both harvesting at the right maturity and late harvesting practices have been reported. Beyene *et al*. (2016) reported harvesting has been at the right maturity in the major maize producer areas in the country. Similarly, 99% of farmers around Hawasa city in south Ethiopia (Boshe *et al*., 2020) and 93% of maize producer farmers from east Ethiopia (Mohammed *et al*., 2022b) harvest their maize at the right maturity. On the other hand, Bereka *et al*. (2022) reported that all the respondent maize producer farmers in Jimma zone in southwest Ethiopia practice delayed harvesting after maturity, the reason being for further drying. The authors also reported that these farmers wait for sunny days to harvest due to fear of maize produce wetting by accidental rain.

Time of harvesting is described as important agricultural practice to control preharvest mycotoxin contamination. The best time is harvesting at the physiological maturity (Kaaya *et al*., 2005). Thus, harvesting at the right maturity level in Ethiopia should be encouraged. However, in the traditional practice, knowing the actual level of maturity may not be possible since unstandardized and subjective maturity measurements have been used. Thus, more reliable objective methods of testing maturity, determining moisture content (using low-cost portable devices) shall be promoted to determine maturity. With this method, the right time to harvest can be better estimated to effectively prevent mycotoxin contamination at this stage. The practice of delayed harvesting after physiological maturity, which is reported from Jimma zone, should be discouraged. Despite the benefit of drying with delayed harvesting, this practice may lead to fungal infection (Kaaya *et al*., 2005). This is because delaying gives fungal spores surviving in the air more chance for contamination. In addition, rainwater and insects play a role for the dispersion of these spores (Dövényi-Nagy *et al*., 2020). In these perspectives, *Fusarium* species are well known for preharvest infection (Ndemera *et al*, 2018). Further, insect damage at the preharvest period facilitates fungal infection (Jallow *et al*., 2021) which is further associated to risk of mycotoxin contamination.

2.3.1.6. Field drying

Sun drying is the most common drying method in Ethiopia, by using different drying surfaces. For on farm drying, maize drying in a bare ground is quite common (Boshe *et al*., 2020; Garbaba *et al*., 2018b; Mesfin *et al*., 2022). Applying this method, drying is done by spreading the stalks on the ground without detaching the cob or heaping up the stalks (Garbaba *et al*., 2018b). According to Bereka *et al*. (2022), farmers in Jimma zone keep drying their maize on the ground for one to two weeks, and about 30 percent of the respondents practice drying in their home for an extended time. In another study, about 50% and 44% of farmers around Hawassa city are reported for practicing sun drying on bare ground and on top of plastic sheet, respectively. Farmers in this area also use indoor smoke drying to a limited extent (Boshe *et al*., 2020). For this type of drying, maize cobs are suspended above a fire where the smoke and heat produced provide accelerated drying which enables to a good control of insect damage during storage (Kuyu & Bereka, 2020).

The traditional drying methods in Ethiopia seem to favor mycotoxin contamination. Harvesting moisture contents is important factor for mycotoxin contamination. For safe storage, a product should be dried to less than 13% moisture content (Xu *et al*., 2022). Garbaba *et al*. (2018b) reported a moisture content ranging from 16% to 28% for maize samples collected at harvest from Jimma zone of Ethiopia, and the authors described this moisture level makes the grain susceptible to fungi infection. In another research, Getachew *et al*. (2018) reported a moisture content of 9-14.7% for stored maize samples

collected from the south and southwestern Ethiopia. All tested samples in this study were infected with *Aspergillus*, *Fusarium* and *Penicillium* species. In the current practices in Ethiopia, objective measurement of drying levels using moisture content has not been used. As a solution to the problem for persistence farmers in Ethiopia, Beyene *et al*. (2016) suggested to use scratching of the grain by nail and using the sound during teeth breakage. This method could still be ineffective since it is subjective, and difficult to determine the right level of grain dryness. Similar to insufficient drying, over drying of maize may favor fungal infection since it may lead to cracking of kernels, which makes them vulnerable to fungal infection (Garbaba *et al*., 2018b). Field drying by stacking maize for too long period is presumed to favor fungi growth. In this regard, the practice of on farm drying for a long period using bare ground should be discouraged (Garbaba *et al*., 2018b). Leavens *et al*. (2021) demonstrated the possibility of controlling aflatoxin contamination in maize for small holder farmers in Senegal. The researchers implemented a combined method – training of farmers, using tarp for drying surface and using a farmer friendly portable hygrometer to measure moisture content. This approach seems applicable experience worth sharing to subsistence farmers in Ethiopia.

2.3.1.7. Shelling

Grain handling in Ethiopia is by using animals and human labor (Garbaba *et al*., 2018b). Maize shelling, i.e. separating the kernel from the cob, in Ethiopia is described by Tekeste and Degu (2019) and Garbaba *et al*. (2018b). Manual shelling is the common practice. In this method, dry cobs are beaten with stick or rubbed using rough stone. Beating is preferred when the volume of cobs is high (> 1,500 kg) (Tekeste & Degu, 2019). Garbaba *et al*. (2018b) reported that 82% of farmers in Jimma zone use this method for maize shelling. Shelling is also conducted by using finger palm or putting the cobs in a sack and hitting with stick.

Current Ethiopia maize shelling practices may increase mycotoxin contamination. According to Garbaba *et al*. (2018b), a shelling practice where maize cobs are put in a sack which is manually beaten damages the kernels and makes them vulnerable to fungal infection. For subsistence farming situations, separating damaged maize kernels, and using them for immediate consumption while using sound ones for storage seems helpful. In addition, when wetting happens during drying or shelling due to accidental rain, further drying, or using the wet portion for immediate consumption would be helpful, as well as prevention of contact of the maize with soil during shelling. Promoting harvesters and threshers for subsistence farmers could help to reduce fungal infection. These technologies can be made accessible to farmers for renting services through cooperatives. By adopting these technologies complemented with objective moisture measurement tools, maize can be harvested at the right maturity, drying on bare ground can be prevented, contact of maize with soil during threshing can be reduced, and kernel damage

can be reduced. As a result, risk of fungal infection and mycotoxin contamination can be minimized.

2.3.2. Postharvest practices and mycotoxin contamination

2.3.2.1. Off-house storage

Gombisa, a granary, is a commonly used structure for the purpose of both drying and storage of maize. It is common in the southwestern part of Ethiopia (Garbaba *et al*., 2018a). Gombisa is made up of locally available materials, mostly bamboo. The roof is covered with natural or thatch grass. For bulk drying of maize, its wall is made from permeable wood material. Drying of maize occurs exclusively by wind where wind coming through the walls defuses the moisture from the maize surface (Roman *et al*., 2020). Bereka *et al*. (2022) reported that more than 80% of respondents from Jimma area use gombisa for maize storage. According to Megerssa *et al*. (2021), 10 % respondent farmers in North Shewa practice off house hanging of maize cob. In another research, Dirashe people in southern Ethiopia use gotela for maize storage. Gotela is off house storage structure made up of bamboo, wood and clay (Sunano, 2020).

2.3.2.2. Inhouse storage

For inhouse storage, traditional storage structures such as gotera, polyethylene sacks and jute sacks have been used (Beyene *et al*., 2016; Boshe *et al*., 2020; Hengsdijk & de Boer, 2017; Megerssa *et al*., 2021). Gotera is constructed from wood, mud and straw (Boshe *et al*., 2020; Hengsdijk & de Boer, 2017). Structures used less often include metal silos (Hengsdijk & de Boer, 2017), plastic bags (Boshe *et al*., 2020) and clay pots (Megerssa *et al*., 2021). Inhouse storage structures are used for both shelled and unshelled maize grain. However, inhouse maize storage is most often done in a shelled form (Megerssa *et al*., 2021). According to Bereka *et al*. (2022), farmers in Jimma zone prefer to store the unshelled form of maize believing that it prevents insect infestation. Storage structures are commonly disinfected before using them for a new crop, such as by smearing with cow dung or ash (Beyene *et al*., 2016), rubbing the inside wall and the bottom wall with hot pepper or smoking with hot pepper, and using cereal straw at the bottom layer of the structure (Kuyu & Bereka, 2020). Disinfection by smoking with leaves from weira (*Olea Europea subspecies Africana*) and Neem tree (*Azadirachta indica*) and fumigation are also used, but rarely (Beyene *et al*., 2016). According to Boshe *et al*. (2020) about 64 % of respondent farmers around Hawassa city, south Ethiopia disinfect their storage structure before using it for a new harvest. In another research, Garbaba *et al*. (2018b) reported that 99.5% of maize farmers in southern Ethiopia sanitize their store before using it for a new harvest. Several protection methods against insects and fungi are used for grain storage. For instance, the use of pesticides is common for maize (Hengsdijk & de Boer, 2017; Megerssa *et al*., 2021). Mohammed *et al*. (2022b) reported that 87% of their respon-

dent maize producer farmers from eastern and western Hareghe zones use insecticide to protect maize during storage. According to Megerssa *et al*. (2021), celphos, quicphos, deltametrin, malathion dust, deltacal and diazinon obtained from legal or illegal sources are used by farmers in west Showa zone. In another research, Mesfin *et al*. (2022) reported that about two thirds of maize producer farmers in Jimma zone and Sidama Region use dichloro-diphenyl-trichloroethane (DDT) for maize protection. Botanical plants (Kuyu & Bereka, 2020) and elevation of storage structures (Hengsdijk & de Boer, 2017) are also used to protect maize during storage.

Even though they are meant to protect, both the storage structures and treatment methods seem to contribute to fungal infection and mycotoxin contamination in Ethiopia. The common storage structures are influenced by external environmental conditions, such as moisture migration and oxygen permeability. This enables fungal growth and insect infestation (Garbaba *et al*., 2018b; Roman *et al*., 2020). In addition, these structures are prone to internal moisture condensation which also helps fungal growth (Roman *et al*., 2020). High temperature and relative humidity during storage may favor growth of *Aspergillus* species and mycotoxin contamination (Chulze, 2010). Despite different treatment and protection methods have been used to prevent fungal infection, mycotoxin contamination in stored maize has been frequently reported in Ethiopia (Boshe *et al*., 2020; Chauhan *et al*., 2016; Getachew *et al*., 2018; Mesfin *et al*., 2022; Mohammed *et al*., 2022b; Tsehaye *et al*., 2017). Getachew *et al*. (2018) identified several species of fungi, including *Fusarium* and *Aspergillus*, and 127 mycotoxins and derivatives from inhouse stored maize in farmers households from south and southwestern part of Ethiopia. In another study, Mohammed *et al*. (2022b) reported that all maize samples collected from farmers in house stores in eastern Ethiopia were contaminated with fumonisins.

For maize grain stored in jute sacks and polypropylene sacks for 7 months under a laboratory condition, the proportion of maize damaged by weevils increased from 9 to 61% and 5 to 18%, respectively (Kuyu *et al*., 2022). According to Megerssa *et al*. (2021), maize farmers in west Showa reported a grain damage up to 75% in the worst case due to storage pests. Control of insects during maize storage is important since insects damage the grain making it vulnerable to fungal infection or their activities create a moisture accumulation which helps for fungal growth and mycotoxin contamination (Chulze, 2010). If used properly, protection chemicals such as herbicides, fungicides and insecticides during storage can reduce mycotoxin contamination (Ndemera *et al*, 2018). However, the frequent occurrence of mycotoxin contamination in stored maize in Ethiopia has led us to question the effectiveness of treatment chemicals and methods as well as their application practices.

2.3.2.3. Maize processing at household level

Sorting out defected maize kernels is an integral part of processing, which is conducted as the first step. Beyene *et al*. (2016) reported that sorting out damaged kernels has been practiced at household level among the major regions in Ethiopia with variable extent among the regions. According to Boshe *et al*. (2020), about 60 % of respondents around Hawassa city do not sort out fungal contaminated maize kernels. In Ethiopia, maize is processed to produce several type of foods such as *injera*, and porridge (Mohammed *et al*., 2022b) and alcoholic beverages such as *Tella* (Bereka *et al*., 2022). The major processing activities include milling, fermentation, and thermal treatment (baking/boiling/roasting). The detailed processing conditions depend on the specific type of food produced. According to Mesfin *et al*. (2022), about 22% of households in Sidama region practice dehulling, one of the prior activities for milling, while only 2% of households in Jimma zone apply it. The same study indicated that 2% of the households in Sidama and 23% from Jimma zone practice soaking (washing).

Maize processing practices in Ethiopia may reduce mycotoxin contamination. According to Matumba *et al*. (2015), mycotoxins are concentrated in the surface of shrivelled immature, broken and discolored grains. Implementing Good Manufacturing Practices (GMPs) that are applicable to small holder farmers such as sorting, washing and dehulling can reduce mycotoxin contamination (Jallow *et al*., 2021). Matumba *et al*. (2015) reported a mycotoxin reduction of about 96% by using hand sorting. When combined with washing and dehulling, the reduction level was increased to 99%. Information on maize processing practices to foods and beverages such as soaking, roasting, fermenting, and baking in relation to mycotoxin reduction in Ethiopia is hardly available in the literature. Despite the reducing effect of processing, the occurrence of fumonisins in maize flour collected from local markets and households in east Ethiopia was reported (Mohammed *et al*., 2022b).

2.3.3. Developments to prevent mycotoxin contamination in maize in Ethiopia

Selected intervention options which have been studied for postharvest loss reduction and that have direct or indirect impact on reducing mycotoxin contamination in maize are described below. These are recent developments for maize drying and storage.

2.3.3.1. Modified gombisa

In the traditional gombisa, achieving a uniform maize dryness is a problem - relatively faster drying occurs on maize stored in its top. Consequently, this leads to the formation of a microclimate at the center of the stored maize, which is characterized by its own temperature and relative humidity. Surprisingly, the relative humidity in the center of the gombisa can remain above 90% for long time (four weeks) including during day times. This phenomenon was reported to happen when the outside relative humidity was lower

than 40%. Such micro atmosphere could create favorable conditions for fungal growth (Roman *et al*., 2020). Another problem of the traditional gombisa is moisture leakage to the gombisa, especially during rainy season with implications for fungi growth (Garbaba *et al*., 2018b). Once constructed, the same gombisa can be used for 10 years; for about 6 months of storage of a newly harvested crop. Thus, it can be a source of fungal inoculum to a new harvest. To overcome these problems with a subsequent reduction of mycotoxin contamination, the traditional gombisa was modified. A successful pilot scale modification trial was conducted (Roman *et al*., 2020) in which a photovoltaic panel driven fan ventilation system was used to improve the drying process, and to solve the moisture condensation problem (Figure 2.2). This modified gombisa reduced the initial moisture content of maize cobs from 22% to 14% (db) in 11 days at its center while drying took longer times for other locations in the gombisa (Roman *et al*., 2020). In addition, a metal sheet was used underneath the thatch to prevent water leak during raining. However, the performance of this gombisa under actual field conditions in Ethiopia has not been tested yet. In addition, an economic evaluation under subsistence farming situations including the willingness of farmers to adopt the technology has not been performed yet.

Figure 2.2. Modified gombisa (Roman *et al*., 2020)

2.3.3.2. Solar bubble dryer

A novel solar bubble dryer was demonstrated in Ethiopia for on farm maize drying (Figure 2.3). Using this drier, the moisture content of freshly harvested maize grain was reduced from 22-29% to 13% (wet basis) within 24-39 hrs under the experimental location conditions (Asemu *et al*., 2020). Despite its potential in reducing mycotoxin contamination, its practical application is limited. Its availability and relatively high investment cost (cost of

land, the system purchasing, installation and operation) are constraints for farmers. Cost benefit studies and the willingness of farmers to adopt the technology have not been researched.

Figure 2.3. Solar bubble drier (photo with permission from GrainPro®; (Asemu *et al*., 2020)). (Asemu *et al*., 2019)

2.3.3.3. Hermetic storage

Hermetic storage structures which have been recently introduced in Ethiopia are promising techniques for mycotoxin control during storage by subsistence farmers. Purdue Improved Crop Storage (PICs) sack is the most frequently used sack (Mekonen & Wubetie, 2021). Since a sack is the most common storage structure for maize in the country (Hengsdijk & de Boer, 2017), these improved hermetic sacks can easily be adopted. According to Bereka *et al*. (2022), about 8% farmer respondents in their study area use PICs sacks to store maize. PICs sacks are reported to effectively reduce mycotoxin contamination of maize (Leavens *et al*., 2021; Worku *et al*., 2022). As a result, using the PICs sack is one of the recommended GAPs for maize storage (Xu *et al*., 2022). Interestingly, PICs sacks are also proven to reduce maize damage by weevils to below 3.8% during a 7-month storage period in Ethiopia. To compare with, the same study indicated a weevil damage up to 61% of the maize grain stored in a conventional jute sack (Kuyu & Bereka, 2020).

Despite the advantage of reducing mycotoxin contamination, the use of PICs sacks for maize storage in Ethiopia faces some supply chain constraints. Mekonen and Wubetie (2021) found that the initial purchase price of PICs sack was a major determinant for the low level of adoption of the sack for maize storage in Burie area, West Gojam Zone. Consequently, farmers in these areas showed preference for using toxic fumigants as cheaper alternatives. A study by Mohammed *et al*. (2022b) indicated that none of the maize producer respondent farmers from eastern and western Harerghe zones use improved storage structures such as PICs sacks, which the authors associated with lack of farmers' awareness and/or unavailability of the sacks in local markets. According to Leavens *et al*.

(2021), sustainable supply chain of PICs sack has been one of the limitations to effectively reduce mycotoxin contamination of maize for long term food safety benefits in Senegal.

2.4. CONCLUSIONS

Most of the retrieved studies in this review focused on mycotoxin contamination in the postharvest stage of maize production. Despite the limited evidence for the occurrence of mycotoxin contamination in the preharvest stages of maize production in Ethiopia, literature evidence from other countries indicated that the current practices of land preparation, planting, growing, harvesting, drying, and shelling are related to mycotoxin contamination. On the other hand, the occurrence of mycotoxin contamination in the postharvest stage of maize production in Ethiopia is well evident. For this stage of maize production, there are some promising intervention options that are directly (indirectly) related to reducing mycotoxin contamination, particularly during drying and storage. However, these developments are not fully implemented by subsistence maize producer farmers in Ethiopia mainly due to availability, affordability, and/or awareness reasons.

Since most of the current pre-and post-harvest maize management practices in Ethiopia favor mycotoxin contamination, implementing a single intervention strategy is not a feasible option. Therefore, developing integrated intervention options by combining Good Agricultural Practices in the preharvest stage and Good Manufacturing Practices in the postharvest stage that are applicable to subsistence farming conditions are suggested. To complement already available evidence in the litereature to develop integrated intervention options, further research aimed to identify locally available maize varieties for their tolerance to fungal infection and mycotoxin contamination, to improve the *"maresha"* and plough method for deeper ploughing, to optimize growth treatments, to improve the traditional storage structures and treatments, and to modify processing practices are recommended. Predictive modelling of the growth of toxigenic fungal species considering a forecasted local climatic change scenarios in the future, together with a clear understanding of local pre- and postharvest practices, would help in providing early-stage mitigation strategies to prevent mycotoxin contamination in maize in Ethiopia.

CHAPTER 3

3

Association of preharvest practices with multimycotoxin contamination in sorghum (*Sorghum bicolor*) in northwest Ethiopia

This chapter is based on a paper under review in an international journal:

J.A. Sadik, L. Righetti, N. Fentahun, I.D. Brouwer, M. Tessema, M. Abera, H.J. van der Fels-Klerx, 2024. Association of preharvest practices with multimycotoxin contamination in sorghum (*Sorghum bicolor*) in Northwest Ethiopia (Under Review).

ABSTRACT

Mycotoxins are toxic metabolites produced by certain fungal species that can cause animal and human health problems, and can contaminate food crops at any stage of the value chain. Sorghum is one of the important food crops in Ethiopia, where the occurrence of mycotoxins in this grain has been frequently reported. However, information on the relationships between the specific local sorghum production practices and mycotoxin contamination is rarely available. In this study, the occurrence of multiple mycotoxins in newly harvested sorghum samples was determined, as well as potential relationships between preharvest practices for sorghum growing and mycotoxin contamination. In 2022, 120 farmers in northwest Ethiopia were asked about preharvest practices of sorghum fields, and sorghum samples were collected right after harvest. Samples were analysed using UPLC-MS/MS for a total of 33 different mycotoxins. About 75% of the samples were contaminated with at least one specific mycotoxin. The detected mycotoxins belong to one of the four mycotoxin categories, produced by *Aspergillus* spp, *Fusarium* spp, *Penicillium* spp and *Alternaria* spp. The concentrations of regulated mycotoxins were below the EU regulatory limits except for ochratoxin A, which was found in a concentration above the EU regulatory limit for unprocessed cereal grain in four percent of the samples. Several of the investigated preharvest practices showed a significant relationship ($P<0.05$) with either one or more specific mycotoxins and/or with one of the mycotoxin categories. Among these practices, the sowing method and type of fertilizer were the most positively related to multiple mycotoxin contamination. In addition, crop rotation and seed treatment practice before sowing contributed to the low mycotoxin presence. The practices of crop rotation, seed treatment, sowing, and fertilizer application can be further researched to develop a sustainable mycotoxin prevention strategy.

Key words: mycotoxin, agronomic practice, relation, Ethiopia

3.1. INTRODUCTION

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, which are mainly produced by the fungal species of *Aspergillus flavus* and *Aspergillus parasiticus* and fumonisins, which are mainly produced by *Fusarium verticillioides*, *Fusarium proliferatum* and *Aspergillus niger* (Wu *et al*., 2014a). 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*., 2014a).

Sorghum, a staple food grain in Ethiopia (MOA, 2020; Mohammed *et al*., 2022a), has been frequently reported to be contaminated with mycotoxins, being the fourth most produced cereal, next to maize, wheat and teff (*Eragrostis tef*), in 2019/20 cropping season (CSA, 2021a). In this season, about 48 million quintals of sorghum was produced in the country. However, weather conditions in Ethiopia, which were conducive to fungal growth have contributed to the mycotoxin contamination of sorghum (Mohammed *et al*., 2022a; Taye *et al*., 2016; Taye *et al*., 2018). The vulnerability of cultivars in Ethiopia to toxigenic fungal infection has already been shown (Taye *et al*, 2022). Fungal species, namely *Aspergillus, Fusarium, Alternaria*, *Bipolaris, Mucor*, *Penicillium,* and *Rhizoctonia,* that are known to produce mycotoxins were detected in sorghum grain in Ethiopia (Mohammed *et al*., 2022a; Taye *et al*., 2016). As a result of fungal infections, mycotoxin contamination in sorghum has been prevalent. The presence of 79 mycotoxins and related fungal metabolites was reported in sorghum samples collected from farmer's households in Eastern Ethiopia in the year 2021 (Mohammed *et al*., 2022a). The reported toxins included both common mycotoxins such as aflatoxins and fumonisins, and emerging mycotoxins such as moniliformin and tenuazonic acid. In another study, the presence of aflatoxins (AFB1, AFB2, AFG1, AFG2) and sterigmatocystin (a precursor of aflatoxin) with concentrations ranging from 3 to 323 ng/g, and 3 to 1,189 ng/g, respectively, in sorghum samples collected from farmers households and local markets in Ethiopia in 2012/2013 has been reported. Part of these mycotoxin concentrations exceeded national and international regulatory limits, which indicated that mycotoxin presence in sorghum is a public health problem in the country (Ssepuuya *et al*., 2018).

The currently available sorghum preharvest practices, i.e. practices from land preparation up to threshing in Ethiopia, seem to favor mycotoxin contamination (Beyene *et al*., 2016; Mohammed *et al*., 2022a). The inability of farmers to implement recommended practices that prevent mycotoxin contamination is the main contributing factor. This inability may be due to financial constraints to apply optimal inputs during the growing period. Out of the 48 million quintals of sorghum that were produced in Ethiopia in 2020/21, 94% was

produced by private peasant holdings (CSA, 2021a). These farmers use suboptimal inputs for sorghum production (CSA, 2021b), which makes the growing plant vulnerable to fungal infection (Blandino *et al*., 2008b). In addition, farmers often use traditional practices for harvesting, drying, and threshing, which are known to be vulnerable to mycotoxin contamination (Beyene *et al*., 2016; Mohammed *et al*., 2022a; Taye *et al*., 2018). All sorghum samples collected for a particular study from the common threshing surfaces in Eastern Ethiopia, being bare ground, cows' dung smeared ground floor and canvas, were infected with both *Aspergillus* and *Fusarium* species and contaminated with aflatoxins and fumonisins (Taye *et al*., 2018).

Research relating sorghum preharvest practices with mycotoxin contamination in Ethiopia is limited. Available studies focused on the evaluation of sorghum genotypes for fungal infection resistance (Taye *et al*., 2022). and the relationship of field drying and threshing with mycotoxin contamination (Taye *et al*., 2018). Information for other preharvest practices such as land plowing method, weed removal method, and inputs during the growing period such as pesticides in relation to mycotoxin contamination is rarely available. In addition, since Ethiopia is a diverse country in both agroecological conditions and cultural sorghum production practices (Beyene *et al*., 2016; Mohammed *et al*., 2022a; Taye *et al*., 2018), exploring practices could help to generate new or complementary knowledge. Understanding cultural preharvest practices helps to design and implement evidence-based mycotoxin prevention strategies that are suitable for local farming situations (Beyene *et al*., 2016; Ndemera *et al*., 2018; Munkvold 2003). The objective of this research was to investigate current sorghum preharvest practices in northwest Ethiopia and their relationship with mycotoxin contamination. This part of Ethiopia belongs to the leading sorghum producing locations in the country (CSA, 2021a) and, to date, research on the possible relationship of sorghum preharvest practices with mycotoxin contamination in this area is hardly available. According to the Ministry of Agriculture of Ethiopia, (MOA, 2020), sorghum is mostly produced in areas where there is moisture stress during the growing period, being one of the contributing factors to mycotoxin contamination (Medina *et al*., 2015; Ndemera *et al*., 2018; Xu *et al*., 2022). In addition, Amhara National Regional State, located in the northwest Ethiopia, has the highest stunting rate of children in the country (Atalell *et al*., 2023). Mycotoxin exposure is one of the causes of stunting in children (Rasheed *et al*., 2021).

3.2. MATERIALS AND METHODS

3.2.1. 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.

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, 2021a), 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, 2021a) 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 field assessment. Central Gonder administrative zone, which ranked fourth in sorghum production in the Amhara Region (CSA, 2021a) was selected instead. One high sorghum producer *woreda* was selected from each administrative zone, namely West Belesa from Central Gonder, Kalu from South Wollo and Kewet from north Shewa. The geographic locations of the three selected *woredas* are presented in Figure 3.1. The areas considered in the current study belong to moisture stress areas (Ahmed *et al*., 2022; Derese *et al*., 2018; Weledesemayat *et al*., 2016). Weather data collected for the study locations confirmed that all the locations are moisture stress areas. The mean values of rainfall and temperature for the period 2001-2021 are given in Appendix Table 3.1. Weather data is not available for the last few years partly because of the war in northern Ethiopia.

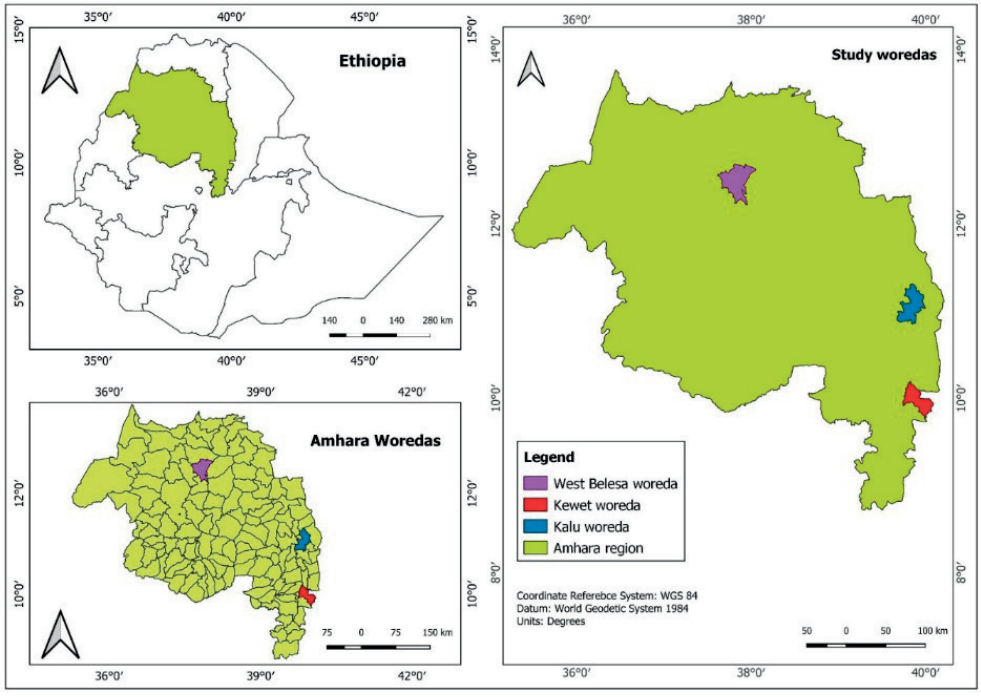


Figure 3.1. Locations of sorghum sample collection *woredas* in Ethiopia

3.2.2. Sample size

A total sample size of 120 households, determined using the G power software, was used; this size would provide about 85% power (with α error probability = 0.05, effect size 0.3) to describe the linear (logistic) relationship of study variables with the presence or absence of mycotoxin contamination in sorghum grain at harvest. The total sample size was equally distributed among the three *woredas* (40 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, respectively. Proportion to population size was applied to distribute the sample size allocated to each *woreda* to its *kebele* administrations (Appendix Table 3.2). 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.

3.2.3. Data collection

Sorghum samples were collected in the last week of December 2022 from the newly harvested sorghum grain produced in the 2022 cropping season for human consumption. The samples were collected right after harvest. In all the locations, sorghum supposed for human consumption was stored in a shelled form. Samples were collected from shelled sorghum temporarily stored in sacks of about 50-100 kg capacity. The quantity of grain produced in the households was in the range of one to thirty quintals. The number of grain storage sacks recommended for sample taking was based on the International Rules for Seed Testing (ISTA) (ISTA, 2016). However, this sampling guideline was not fully implemented, particularly in households that had a relatively higher production volume. This was partly due to poor accessibility of storage sacks for sampling and partly due to 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. 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 sacks 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 1mm particle size according to Ssepuuya *et al*. (2018), sealed in polypropylene plastic films and preserved at -20°C for 8 months. The frozen

samples were individually thawed, thoroughly mixed, about 100 g samples were taken by quartering, packed in a polypropylene sack, and labeled. Then, these 120 samples were transported under cooled conditions to Wageningen Food Safety Research Laboratory, The Netherlands for mycotoxin analysis.

Data about preharvest practices was collected through a face-to-face interview with the main person responsible for farming activities (MPreFA) in each household using a Farming Practices Questionnaire (FPQ) (Appendix Table 3.3. Ethical approval for the interview was obtained from the 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 FPQ was adopted from (Ndemera *et al*, 2018). The questionnaire was initially developed in English language and translated into the local language (*Amharic*) for the interview. The FPQ was prepared in a dichotomous manner where 'yes' (1) represented a farmer who uses/applies the selected practice and 'no' (2) if otherwise. For 'yes' responses, additional closed and open-ended questions were included to gather more detailed information. Additional questions related to sociodemographic characteristics were also included in the FPQ. Awareness about mycotoxins was measured from the response of farmers to three related questions which were prepared in a five-point Likert scale with one additional option for not applicable. During pretesting of the FPQ, it was found that preharvest practices in all the study locations were customized to prevent the presence of ergot alkaloids in sorghum grain. Therefore, mycotoxin awareness questions were administered as related to mycotoxins other than ergot alkaloids. The responses for each question collapsed into dummy variables with one assigned to a farmer who scored 4 or 5 on the six-point Likert scale, representing 'yes' for awareness and zero otherwise. If a farmer's response is 'yes' for the third and at least one of the other two questions, the farmer is assumed to have sufficient awareness about mycotoxins, and insufficient awareness otherwise. A similar method of collapsing mycotoxin awareness responses was also used by (Udomkun *et al*., 2018). The FPQ was pretested by three agricultural extension employees working in the West Belesa *woreda*.

3.2.4. Mycotoxin analysis

Sample preparation and mycotoxin analysis were performed using a fully validated method for multimycotoxin analysis from foods and feed. This method makes use of a calibration curve to determine mycotoxin concentrations. Thirty-three different mycotoxins were analyzed.

3.2.4.1. 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.

3.2.4.2. 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 (10µg/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 minutes. Then, four grams of magnesium-sulphate was added to the individual retrieved samples as per the QuEnChERS method. The samples were subsequently shaken manually for 1 minute, and then centrifuged at 3000 rpm for 10 minutes. 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 the calibration curve solutions (but not yet pushed through), and the filter vials were briefly vortexed and placed in a refrigerator set at a temperature of 4 ℃ for 1 hour. 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.

3.2.4.3. 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 x 2.1 mm) heated at 35 °C was used. The volume of analyte injected was 5µL 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.

3.2.4.4. Validation

To evaluate recovery (extraction efficiency), two blank sorghum samples were spiked by adding mycotoxin standard solutions. The spiked sample was left standing for 10 minutes 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 3.4.

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 3.4. Detailed data on mycotoxin analysis, including the calibration range, linearity (R^2), apparent versus corrected recovery, and the use and identity of the internal standard, will be provided upon request to the first author.

3.2.5. Statistical data analysis

Descriptive statistics were used to investigate the percentages of farmers who applied each of the different preharvest practices, and the percentages of samples that were contaminated with mycotoxins. The presence of covariance between the study variables was tested by using the variance inflation factor (VIF). The 33 mycotoxins were grouped into four mycotoxin categories namely *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria* mycotoxins, as based on Mohammed *et al* (2022a) and Morreti & Suca (2017). Samples were considered contaminated with a specific mycotoxin if at least one of the 33 mycotoxins was detected above LOD. In addition, samples were regarded as contaminated with a particular mycotoxin category if at least one specific mycotoxin belonging to the same category was detected. Univariate logistic regression analysis was used to study the presence of a relationship between study variables with specific mycotoxins and the different categories. A multiple logistic regression analysis was also conducted to further study the relationship between the individual preharvest practices with mycotoxin contamination when all the other variables were kept constant (*ceteris paribus*). Stata software version 16 (StataCorp LLC, College Station, Texas 77845 USA) was used for statistical analysis.

3.3. RESULTS

3.3.1. Demographic and socioeconomic characteristics

Most households (79%) were male headed (Table 3.1) and in 74% of the households the main person responsible for preharvest farming activities (MPreFA) were also men. The MPreFAs age range was 18 to 74 years (mean 47.0 ± 13.4 years). The majority (77%) of the MPreFAs did not attend any formal education. Most of the households (74%) produced sorghum as the main food crop, with the amount of harvested grain being in the range of one to thirty quintals per household (mean 8.9 ± 6.3 Qt). The majority of the households (72%) harvested less than 10 quintals of sorghum in the current season. The total land area used to produce the volume of sorghum harvested per household was less than a hectare, with the majority cultivating less than half a hectare.

Table 3.1. Demographic and socioeconomic characteristics of household participants

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
MPreFA[1]	Husband	74	78	75	70
	Wife	8	10	5	8
	Both	3	5	3	0
	Other[2]	16	8	18	23
Age (yr)	18<-30	20	23	13	25
	31<-50	53	43	53	63
	above 50	28	35	35	13
Farming experience (yr)	<- 15	37	30	38	43
	16<-30	43	45	30	53
	above 30	21	25	33	5
Basic formal education	Yes	23	23	20	28
	No	77	78	80	73
Sorghum was crop produced most.	Yes	74	70	70	85
	No	26	30	30	15
Quantity produced (Qt)	< -10	72	65	95	55
	> 10	28	35	5	45
Total land area (He)	<-0.5	76	83	75	70
	>0.5 <-1.0	24	18	25	30

[1]refers to the main person responsible for preharvest farming activities; [2]refers to either of family sons or other farmers who rented the land to share the sorghum produced. Note that the sum of responses for some of the columns (for several variables) is more than 100 (101) due to the rounding of decimal numbers to the nearest one digit.

3.3.2 Preharvest Practices

Most of the farmers (67%) applied crop rotation, i.e. they produced a product other than sorghum in the previous cropping season on the land they used to produce sorghum in the 2022 cropping season (Table 3.2). Among those who applied crop rotation, most of them (58%) produced cereal, mostly teff, and a considerable proportion of others (30%) produced legumes, chickpea, or mung bean. A limited proportion of the farmers produced vegetables (6%), namely onion, tomato and potato, cereal and legume (teff in a portion of the land and chickpea or mung bean in the remaining portion) (4%) and tobacco (2%) in the previous growing season.

Table 3.2. Sorghum preharvest practices

Variable	Category	Response (%)			
		Total (n=120)	W. Belesa (n=40)	Kalu (n=40)	Kewet (n=40)
Crop rotation[3]	Yes	67	93	63	45
	No	33	8	38	55
Land plowing frequency[3]	2 to 3 times	68	83	73	50
	4 to 5 time	32	18	28	50
Seed treatment method[3]	No treatment	3	0	8	0
	Polishing with fungicide	33	100	0	0
	Washing with water	64	0	93	100
Sowing practice[3]	Broadcasting	58	73	90	90
	Row	43	28	10	10
Type of fertilizer[3]	Synthetic[4]	63	90	10	88
	Organic	38	10	90	13
Pesticide application[3]	Yes	78	80	78	78
	No	22	20	23	23
Weed removal frequency[3]	one to two times	58	83	43	50
	three to four times	42	18	58	50
Incidence of pest infestation	Yes	34	8	28	68
	No	66	93	73	33
Incidence of ergot infection	Yes	17	0	30	20
	No	83	100	70	80
	0-7 days	60	100	58	23
Length of field drying[3]	8-14 days	30	0	30	60
	>14 days	10	0	13	18

[3]refers to the practices where the sum of the results in one or more columns is greater than 100% which is due to rounding of numbers

[4]refers to farmers who used at least one of the synthetic fertilizers (phosphorus (p) or nitrogen (n) fertilizers). From the total, about 75% used both P and N fertilizers, 13% used p only, and 12% used N fertilizer only.

Most of the farmers (97%) treated sorghum seed before sowing, either by polishing (manually mixing) with a fungicide chemical (thiram powder) or washing with water. The seed treatment practice is aimed to remove ergot (*Claviceps Species*) from the seeds, which otherwise the farmers believe leads to the presence of ergot alkaloids during the sorghum growing period particularly after flowering. Polishing seeds with a fungicide (thiram) is practiced only in w. Belesa. All the farmers from Kalu and Kewet treat seeds by washing them with water. Irrespective of the treatment method applied, most of the respondents mentioned that seed is treated for a few to several hours before sowing, or during a night if sowing is planned for the next day. The majority of the respondents (58%) sowed treated seed by broadcasting method (manually spreading seeds over the prepared soil); used synthetic fertilizer (63%); applied pesticide (78%) and removed weed manually two to three times (58%). A considerable number of respondents mentioned the incidence of pest infestation (37%) and fungal infection (17%) during the growth period. Harvesting practice in all households is by cutting the sorghum head with a knife. For drying, the cut head is put on the field where it was produced or on a threshing yard (locally called *awdma*). *Awdma* is a piece of land, usually a circular plot, prepared for the threshing purpose. The duration of field drying is from zero days to a few weeks.

3.3.3. Mycotoxin awareness

All the farmers indicated they have awareness about the growth of fungi in sorghum grain, and most of them (81%) also know fungal infection can occur during the sorghum growth period (Table 3.3). However, most of the farmers (73%) are unaware of the fact that fungi can produce toxic compounds (called mycotoxins). Overall, most farmers (93%) have insufficient awareness about mycotoxins.

Table 3.3. The awareness of farmers about fungal infection and mycotoxins

		response (%)			
Awareness question	Dummy variable[5]	Total (120)	W. Belesa (40)	Kalu (40)	Kewet (40)
Fungi grows in sorghum.	Yes	100	100	100	100
	No	0	0	0	0
Fungi can infect sorghum during growth period.	Yes	81	100	73	70
	No	19	0	28	30
Fungi produce toxic compounds (mycotoxins/aflatoxins)	Yes	27	55	5	20
	No	73	45	95	80
Mycotoxin awareness	Yes	27	55	5	20
	No	73	45	95	80

[5]Variables created by combining the responses of farmers for the awareness questions prepared on six-point Likert scale (Appendix Table 3.3)

3.3.4. Prevalence of mycotoxins

The 33 mycotoxins studied in this research belong to four major categories of mycotoxins - 7 to *Aspergillus* mycotoxins, 20 to *Fusarium* mycotoxins, 4 to *Penicillium* mycotoxins and 2 to *Alternaria* mycotoxins. The occurrence of the mycotoxin categories in decreasing order of prevalence was *Aspergillus* mycotoxins (67%), *Alternaria* mycotoxins (63%), *Fusarium* mycotoxins (58%), and *Penicillium* mycotoxins (23%).

Both *Alternaria* mycotoxins - alternariol and alternariol-methylether were detected in the majority of the samples, with a prevalence of 59% and 60%, respectively. Moniliformin was the most prevalent *Fusarium* mycotoxin, which was detected in about half (48%) of the samples. The prevalence of individual *Penicillium* mycotoxins was comparably lower, with the most prevalent mycophenolic acid being detected in only 21% of the samples. An emerging *Aspergillus* mycotoxin nitropropionic acid was detected in the majority of the samples (56%). The most toxic mycotoxins known, i.e. aflatoxins, were not detected in any of the samples, while the other important mycotoxins – fumonisins were detected in a small proportion of samples (Table 3.4).

About 75% of the collected samples were contaminated with at least one specific mycotoxin. From this total, 67% were contaminated with three or more mycotoxins, indicating the majority of the samples were contaminated with multiple mycotoxins (Figure 3.2).

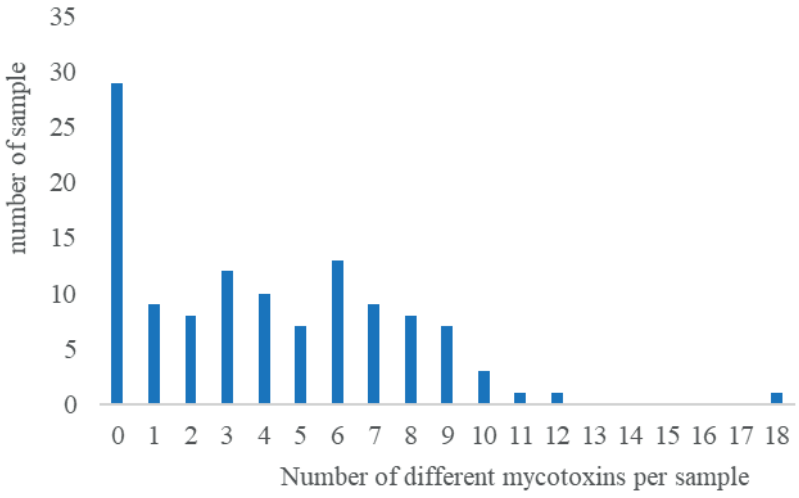


Figure 3.2. The frequency of occurrence of (multi) mycotoxin in sorghum grain

Table 3.4. Prevalence of mycotoxins in sorghum in northwest Ethiopia in 2022

Major category	Specific mycotoxin	Total (120)		W. Belesa (40)		Kalu (40)		Kewet (40)	
		% p (N)	conc. range (ng/g)	% p (N)	conc. range (ng/g)	% p (N)	conc. range (ng/g)	% p (N)	conc. range (ng/g)
Aspergillus mycotoxins	aflatoxin B1	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD
	aflatoxin B2	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD
	aflatoxin G1	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD
	aflatoxin G2	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD
	ochratoxin A	8(9)	< LOD-60.0	10(4)	< LOD-60.04	10(4)	< LOD-15.23	3(1)	< LOD-6.8
	nitropropionic acid	56(67)	< LOD-1111.1	15(6)	< LOD-480.7	88(35)	< LOD-1111.1	65(26)	< LOD-180.4
	sterigmatocystin	28(34)	< LOD-39.1	20(8)	< LOD-4.8	30(12)	< LOD-39.1	35(14)	< LOD-0.7
Fusarium mycotoxins	fumonisin B1	5(6)	< LOD-23.4	0(0)	< LOD	10(4)	< LOD-23.4	5(2)	< LOD-1.4
	fumonisin B2	5(6)	< LOD-18.2	0(0)	< LOD	5(2)	< LOD-18.2	10(4)	< LOD-17.4
	fumonisin B3	5(6)	< LOD-7.0	0(0)	< LOD	5(2)	< LOD-2.6	10(4)	< LOD-7.0
	deoxynivalenol	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD
	nivalenol	1(1)	< LOD-89.5	0(0)	< LOD	5(2)	< LOD-89.5	3(1)	< LOD-42.0
	zearalenone	13(15)	< LOD-591.1	8(3)	< LOD-53.0	15(6)	< LOD-591.1	15(6)	< LOD-19.7
	α-zearalenol	2(2)	< LOD-14.8	0(0)	< LOD	5(2)	< LOD-14.8	0(0)	< LOD
	ß-zearalenol	2(2)	< LOD-15.9	0(0)	< LOD	5(2)	< LOD-15.0	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	28(33)	< LOD-27.6	8(3)	< LOD-9.1	28(7)	< LOD-27.6	58(23)	< LOD-5.9

Table 3.4. Prevalence of mycotoxins in sorghum in northwest Ethiopia in 2022 (*continued*)

Major category	Specific mycotoxin	Total (120)		W. Belesa (40)		Kalu (40)		Kewet (40)	
		% p (N)	conc. range (ng/g)	% p (N)	conc. range (ng/g)	% p (N)	conc. range (ng/g)	% p (N)	conc. range (ng/g)
Fusarium mycotoxins	DON-3-Glucoside	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD
	beauvericin	34(41)	< LOD-21.4	5(2)	< LOD-1.2	28(11)	< LOD-21.4	70(28)	< LOD-6.4
	enniatin A	3(4)	< LOD-1.8	0(0)	< LOD	5(2)	< LOD-0.9	5(2)	< LOD-1.8
	enniatin A1	5(6)	< LOD-6.1	0(0)	< LOD	10(4)	< LOD-0.6	5(2)	< LOD-6.1
	enniatin B	5(6)	< LOD-5.0	0(0)	< LOD	10(4)	< LOD-1.5	5(2)	< LOD-5.0
	enniatin B1	6(7)	< LOD-9.1	0(0)	< LOD	8(3)	< LOD-1.4	10(4)	< LOD-9.1
	moniliformin	48(58)	< LOD-735.3	0(0)	< LOD	48(19)	< LOD-230.4	98(39)	< LOD-735.3
	T-2 Toxin	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD-18.9	0(0)	< LOD
	HT2 toxin	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD
Penicillium mycotoxins	mycophenolic acid	21(25)	< LOD-290.8	10(4)	< LOD-51.9	28(11)	< LOD - 290.8	25(10)	< LOD-9.8
	penicillic acid	4(5)	< LOD-24.7	5(2)	< LOD-24.7	5(2)	< LOD -5.2	3(1)	< LOD-3.6
	roquefortine C	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD	0(0)	< LOD
	citrinin	2(2)	< LOD-3.5	0(0)	< LOD	5(2)	< LOD-3.5	0(0)	< LOD
Alternaria	alternariol	59(71)	< LOD-282.9	8(3)	< LOD-37.7	83(33)	< LOD-282.9	88(35)	< LOD-39.8
	alternariol-methylether	60(72)	< LOD-160.0	10(4)	< LOD-30.8	85(34)	< LOD-160.0	85(34)	< LOD-32.0

% 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 3.4. *DON refers Deoxynivalenol

3.3.5. Relationship of sociodemographic and preharvest variables with mycotoxin contamination

None of the sociodemographic variables showed a significant relationship with one of the four mycotoxin groups or one of the individual mycotoxins ($P>0.05$) (Table 3.5).

Several of the individual preharvest practices showed to be significantly related ($P<0.05$) with mycotoxin contamination. Frequency of land plowing, sowing method, type of fertilizer, weed removal frequency, incidence of pest infestation, incidence of fungi (ergot) infection and duration of field drying were positively related to the contamination of a specific mycotoxin or with each mycotoxin category while crop rotation, seed treatment and mycotoxin awareness were negatively related with mycotoxin contamination. To prevent model overfitting, some variables need to be excluded to conduct multiple logistic regression analysis. The following variables were excluded: those which are not farming practices (location, pest infestation, fungal infection), those which have too little variation (seed treatment), and those practices that are not easy for subsistence farmers to apply, i.e. duration of field drying and weed removal. The decision on the number of field drying days is dependent on the weather conditions, and it is not an easy task to decide the duration of drying for farmers who use traditional sun drying practices. The number of times of weed removal partly depends on the demand for animal feed and partly on the availability of labor, and this is not an easy decision for a farmer provided that grazing land is scarce during the growing season.

The results of the multiple logistic regression analysis are presented in Table 3.6. Sowing method and type of fertilizer were positively ($P<0.05$) related to the occurrence of contamination of both specific mycotoxins and each of the mycotoxin categories. The generated models to describe the relationships were all significant except for the sowing method for the *Penicillium* mycotoxin category. In addition, the Hosmer-Lemeshow test indicated that all the models significantly fit the respective data ($P>0.05$). Compared to sowing by the traditional broadcasting method, sowing by the row planting method increased the occurrence of mycotoxin contamination in the sorghum grain by 0.78 to 1.86, depending on the type of mycotoxin category, *ceteris paribus*. Similarly, compared to synthetic fertilizer application, organic fertilizer applications during sorghum growth increased the occurrence of mycotoxin contamination in the grain by 1.33 to 2.58 depending on the type of mycotoxin category, *ceteris paribus*.

Table 3.5. Univariate analysis - Relationship of sociodemographic and preharvest farming practices with mycotoxin contamination in northwest Ethiopia (for sorghum grown in 2022 cropping season)

Sociodemographic 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
Sociodemographic characteristics										
Gender of head of Household	-0.31(-1.28:0.66)	0.526	-0.17(-1.15:0.80))	0.730	-0.52(-1.45:0.411)	0.274	1.02(-0.27:2.30)	0.122	-0.54(-1.50:0.43)	0.273
MPreFA	-0.27(-1.16:0.62)	0.556	-0.11(-1.01:0.78)	0.801	-0.35(-1.2:0.5)	0.419	0.12(-0.85:1.09)	0.811	-0.31(-1.17:0.56)	0.485
Age (yr)	-0.01(-0.04:0.02)	0.421	-0.01(-0.04:0.02)	0.490	-0.01(-0.04:0.02)	0.657	0.003(-0.03:0.04)	0.842	-0.13(-0.04:0.02)	0.383
Farming experience	-0.01(-0.04:0.02)	0.053	-0.01(-0.04:0.02)	0.549	-0.002(-0.03:0.03)	0.846	-0.001(-0.03:-0.32)	0.955	-0.01(-0.04:0.02)	0.673
Basic formal education	0.07(-0.83:0.97)	0.879	0.37(-0.59:1.33)	0.447	-0.25(-1.10:0.6)	0.560	0.06(-0.92:1.04)	0.906	0.31(-0.59:1.20)	0.505
Sorghum was crop	0.13(-0.73:0.99)	0.768	0.29(-0.58:1.15)	0.516	0.37(-0.45:1.19)	0.379	-0.344(-1.27:0.58)	0.464	0.611(-0.22:1.44)	0.149
Quantity produced	-0.30(-1.13:0.53)	0.475	-0.28(-1.13:0.56)	0.507	0.37(-0.45:1.19)	0.374	0.17(-0.74:1.08)	0.711	-0.39(-1.2:0.42)	0.348
Total land area	1.71(-0.08:3.49)	0.061	1.81(-0.04:3.67)	0.056	-0.11(-0.87:0.66)	0.772	-0.06(-0.98:0.87)	0.905	-0.59(-1.63:0.45)	0.263
Location	1.68(1.03:2.32)	0.000	2.24(1.44:3.05)	0.000	2.93(1.96:3.90)	0.000	0.56(0.03:1.10)	0.040	2.39(1.59:3.19)	0.000
Pre-harvest farming practices										
Crop rotation	-1.69(-2.73: -0.66)	0.001	-2.21 (-0.47: -0.95)	0.001	-1.94(-2.90: -0.96)	0.000	0.14(-0.76:1.04)	0.763	-1.24 (-2.13: -0.35)	0.007
Plowing frequency	0.82(0.24:1.40)	0.006	1.09 (0.44:1.74)	0.001	0.95(.38-1.53)	0.001	-0.02(-0.56:0.53)	0.952	0.8 (0.24:1.36)	0.005
Seed treatment method	-3.16(-4.16: -2.17)	0.000	-3.56(-4.64: -2.49)	0.000	-3.65(-4.83: -2.47)	0.000	-1.36(-2.5: -0.23)	0.019	-4.13(-5.36: -2.90)	0.000

Table 3.5. Univariate analysis - Relationship of sociodemographic and preharvest farming practices with mycotoxin contamination in northwest Ethiopia (for sorghum grown in 2022 cropping season) (*continued*)

Sociodemographic 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
Sowing method	0.47(-0.31:1.25)	0.241	0.61(-0.2:1.4)	0.139	1.06(0.28:1.83)	0.007	0.13(-0.72:0.97)	0.771	0.77 (-0.01:1.54)	0.053
Type of fertilizer	1.68(0.71:2.66)	0.001	1.78(0.75:2.82)	0.001	0.71(-0.06:1.49)	0.071	0.78(-0.07:1.63)	0.072	1.50 (0.62:2.39)	0.001
Pesticide application	-0.15(-1.09:0.79)	0.754	-0.24(-1.20:0.73)	0.626	0.03(-0.85:.91)	0.940	1.08(-0.21:2.36)	0.101	0.52 (-0.84:0.95)	0.909
Weeding frequency	0.86(.17:1.55)	0.015	0.87(0.17:1.58)	0.016	0.77(0.11:1.42)	0.021	0.08(-0.62:0.78)	0.818	1.55 (0.69:2.22)	0.000
Duration of field drying	1.83(0.86:2.80)	0.000	2.6(1.34:3.85)	0.000	2.67(1.62:3.70)	0.000	0.63(-0.21:1.48)	0.142	1.67 (0.78:2.55)	0.000
Incidence of pest infest	0.64(-0.20:1.49)	0.137	0.87(-0.03:1.77)	0.057	2.25(1.22:3.29)	0.000	0.99(0.13:1.84)	0.025	1.74(0.77:2.71)	0.000
Incidence of fungal infest	2.50(0.45:4.55)	0.017	2.37(0.32:4.42)	0.024	2.12(0.60:3.63)	0.006	-0.29(-1.47:0.9)	0.634	2.7(0.65:4.75)	0.01
Mycotoxin awareness	-0.98(-1.81: -0.14)	0.022	-1.16 (-2.00: -0.311)	0.007	-1.17(-2.01: -0.33)	0.006	-0.43(-1.43:0.58)	0.405	-2.15 (-2.09: -0.41)	0.004

Table 3.6. Multiple logistic regression analysis - Relationship of preharvest variables with occurrence of mycotoxin contamination

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
Crop rotation	-0.98(-2.18:0.23)	0.111	-1.36(-2.77: -0.73)	0.063	-1.25(-2.34: -0.16)	0.025	0.34(-0.70:1.38)	0.524	-0.26(-1.36:0.84)	0.642
Plowing frequency	0.59(-0.10:1.28)	0.095	0.92(0.12:1.17)	0.024	0.63(-0.05:1.30)	0.068	-0.06(-0.68:0.57)	0.857	0.64(-0.04:1.33)	0.067
Sowing method	1.31(0.21:2.41)	0.019	1.49(0.32:2.66)	0.013	1.62(0.49:2.75)	0.005	0.78(-0.38:1.94)	0.189	1.86(0.70:3.02)	0.002
Type of fertilizer	2.35(1.11:3.59)	0.000	2.58(1.23:3.92)	0.000	1.41(0.26:2.57)	0.017	1.33(0.17:2.50)	0.025	2.51(1.26:3.75)	0.000
Pesticide application	-0.36(-1.54:0.82)	0.551	-0.63(-1.91:0.66)	0.338	-0.26(-1.37:0.85)	0.645	1.06(-0.29:2.42)	0.125	-0.34(-1.50:0.81)	0.559
Mycotoxin awareness	-0.14(-1.23:0.95)	0.800	-0.31(-1.46:0.84)	0.594	-0.40(-1.48:0.68)	0.466	0.12(-1.03:1.28)	0.833	-0.54(-1.63:0.54)	0.326

Multiple logistic regression model fitness:

* LR chi2(6) = 35.61, Prob > chi2 = 0.0000, Pseudo R2 = 0.2331; Goodness of fit test: Pearson chi2(95) = 33.64, Prob > chi2 = 0.7125;

** LR chi2(6) = 45.38, Prob > chi2 = 0.0000, Pseudo R2 = 0.3061, Goodness of fit test: Pearson chi2(95) = 29.43, Prob > chi2 = 0.8665;

*** LR chi2(6) = 37.49, Prob > chi2 = 0.0000, Pseudo R2 = 0.2300, Goodness of fit test: Pearson chi2(95) = 47.22, Prob > chi2 = 0.1718;

**** LR chi2(6) = 8.99, Prob > chi2 = 0.1743, Pseudo R2 = 0.0677, Goodness of fit test: Pearson chi2(95) = 41.82, Prob > chi2 = 0.3492;

***** LR chi2(6) = 39.03, Prob > chi2 = 0.0000, Pseudo R2 = 0.2458, Goodness of fit test: Pearson chi2(95) = 46.37, Prob > chi2 = 0.1945

3.4. DISCUSSION

The objective of this research was to investigate the relationship between preharvest practices applied by subsistence sorghum producers and mycotoxin contamination in just harvested sorghum. In Ethiopia, regulatory standards are available for a limited number of mycotoxins and for a limited number of foods only. A limit for total aflatoxin in sorghum is available and is 10 ng/g (Mamo *et al*., 2020). All the samples in this study were contaminated with total aflatoxin below this regulatory limit.

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 ng/g, respectively (European Commission Regulation (EC, 2023)). Based on this regulation, most of the just harvested sorghum samples in this study were contaminated with mycotoxins below EU regulatory limits, and only about 4% of the total samples bypassed regulatory limits for ochratoxin A. For emerging mycotoxins such as alternariol, alternariol-methylether, nitropropionic acid and moniliformin, no European regulatory limits have been set yet. The prevalence of the common mycotoxins detected in this study is comparable with a previous report on mycotoxin contamination of stored sorghum grain produced in Eastern Ethiopia (in Doba, Fedis, Goro Gutu and Miesso areas) in the 2021 cropping season (Mohammed *et al*., 2022a). In the current study, a considerable proportion of farmers responded to the incidence of pest infestation in their sorghum plants. However, the majority of the farmers, with a by far higher proportion of the farmers than those who reported the occurrence of pest infestation, applied pesticides (Table 3.3) which could be one of the reasons for the observed low prevalence of the (common) mycotoxins. Pesticide application to control insect infestation is one of the strategies to reduce *Fusarium* spp. and *Aspergillus* spp. infections and related mycotoxin contaminations (D'Mello *et al*., 1998). In this regard, it would be interesting to study if pesticide residues are present in the sorghum grains to a level that can pose risk to human health after sorghum consumption.

The presence of a positive relationship between the majority of the preharvest practices with mycotoxin contamination was expected since these practices are traditional ones which would increase contamination. The results indicated that these practices significantly increased the probability of mycotoxin contamination in sorghum (including both prevalence and/or concentration). Previously we provided the details on how the land plowing with oxen, manual weeding and length of field drying favor mycotoxin contamination in maize in Ethiopia (Sadik *et al*., 2023). The current study indicated similar results that these practices, which are similar for maize and sorghum, also favor mycotoxin contamination in sorghum. In particular, the significant relationships observed for crop rotation, sowing method and type of fertilizer applied could imply that these practices

are most important to consider in mycotoxin preventive intervention studies in the future. Crop rotation has been reported to affect the incidence rate and the composition of mycotoxins in cereal grains (Dong *et al*., 2023). Crop rotation reduces mycotoxin contamination through improving soil fertility, which in turn improves the resistance of the crop to fungal infection (Xie *et al*., 2021). Crop rotation also increases the diversity of soil microbial communities, which reduces available nutrients for toxigenic fungal growth through competitive mechanisms (Yin *et al*., 2008). On the other hand, residues from a previous crop can act as an inoculum for fungal infection in the current crop, increasing mycotoxin contamination (Qiu *et al*., 2016). In our study areas, it is common practice for farmers to collect crop residues after grain harvest for animal feed, which would reduce the volume of substrate available as inoculum for fungal infection and thus reduce mycotoxin contamination. The lower probability of mycotoxin contamination in sorghum when crop rotation was used in this research may mean that the crops planted in the previous season, namely teff, legumes and vegetables, improved soil fertility and/or soil microbial diversity and/or the removal of previous crop residues reduced fungal infection resulting in lower mycotoxin contamination.

The higher probability of mycotoxin contamination obtained when sorghum was sowed in a row than the broadcasting method may be related to seeding density. Increased maize plant density during cultivation was reported to increase mycotoxin contamination, which was considered to be associated with a more favorable microclimatic condition for fungal growth by the higher plant population (Blandino *et al*., 2008a). Despite a scarcity of information in the literature about sorghum plant density related to the different sowing methods in Ethiopia, the small land area (a maximum of 1 hectare) allocated to cultivate sorghum by all the farmers (Table 3.1) may imply the presence of land scarcity, and thus high crop density. Besides to food purpose, sorghum is also grown in Ethiopia for animal feed (Mesfin & Girma, 2022) which means that besides to the grain yield which will be used for human consumption, feed (stalk) yield is also important. Consequently, the farmers probably sow seeds with narrow inter-seed spacing (high plant density) which favors fungal growth and mycotoxin contamination. Unlike maintaining even seed distribution in row planting, in broadcasting, seeds are randomly dispersed in a wide area, and it is difficult for the farmers to keep even inter-seed spacing. As a result, sowing by broadcasting may result in both high- and low-plant densities in different segments of the farm.

The higher probability of mycotoxin contamination when organic fertilizer was applied than synthetic fertilizer application could indicate that the farmers apply an inadequate amount of organic fertilizer than required for optimal growth of sorghum. When suboptimal fertilizer amount is applied, the nutritional status of a growing crop will be altered and as a result becomes vulnerable to mycotoxin contamination (Blandino *et al*., 2008b;

Tubajika *et al*., 1999). Inadequate nitrogen fertilizer application was reported to be related to increased incidence of fungal infection and increased levels of mycotoxin contamination including fumonisins and aflatoxin B1 in maize (Blandino *et al*., 2008b). The farmers could apply inadequate organic fertilizer due to land scarcity. If a farmer owns a small total land area, the number of animals the farmer owns will be limited due to the shortage of land for animal grazing. This would ultimately lead to low manure (organic fertilizer) yield that is insufficient to meet the demand. In this regard, improving organic fertilizer (manure) application in Ethiopia for optimal application during crop cultivation would be important.

Although not included in the multiple regression analysis due to lack of variation in farmers' responses, the seed treatment practice showed a negative and significant relationship with mycotoxin contamination in the univariate analysis. This implies that the probability of mycotoxin contamination is decreased for seeds treated by polishing with a fungicide compared to the seed treated by washing with water. When seed is treated by polishing with a fungicide, the fungicide probably destroys mycotoxin producing fungal species, including those from *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria* genus, from the seeds surface. According to Dabkevičius and SemaŠKienÉ (2002), seed treatment (dressing) with different fungicide chemicals including thiram effectively reduced the incidence of ergot (*Claviceps purpurea* (Fr.) Tul.) in rye in a field trial experiment, making it a feasible option for preharvest ergot control. Despite the current research showing a promising result to be used as a potential mycotoxin prevention option in sorghum, thiram is known to cause a broad spectrum of toxic effects in animals, human and the environment (Liu *et al*., 2022). Therefore, its use in sorghum production should be discouraged. On the other hand, although literature evidence on its effectiveness is lacking, seed treatment by washing with water could be modified to introduce an effective and sustainable seed treatment option that can successfully remove ergot, toxigenic fungal spores, and mycotoxins. For example, a water-soluble and environmentally friendly detergent that is effective in removing all these components from the seed surface can be investigated by studying the nature of attachment (bonding) of these components to the seed surface. Nevertheless, if integrated and holistic preventive options are not implemented, the fact that mycotoxin producing fungal species can exist in soil, air and water (Fumagalli *et al* 2021; Reis *et al*., 2010) would make the seed treatment method meaningless in regard to the prevention of mycotoxin contamination. This could be one of the reasons for the observed concentrations in part of the sorghum samples in west Belesa although all the farmers treated sorghum seed by polishing with fungicide. An important note is that the period in which ergot infection commonly occurs is from sorghum flowering to harvesting (Dabkevičius & SemaŠKienÉ, 2002; Kebede *et al*., 2023). All the farmers indicated they seriously monitor the incidence of ergot during this period, and if they observe the occurrence of ergot or fungal infection, they sort (remove) the in-

fected sorghum plant. Fortunately, this monitoring period corresponds with the period of window of opportunity to prevent fungal infection and related mycotoxin contamination in the preharvest period. This coincidence could be the other reason for the observed low prevalence of common mycotoxins in this study. The presence of more than one variable that is significantly correlated with mycotoxin contamination would imply that a single intervention option would not be sufficient to prevent the occurrence of mycotoxin contamination in sorghum during the preharvest period.

There are some limitations to this study. Potential cofounders were not considered. For example, local weather conditions can affect both agricultural practices and mycotoxin contamination. To mention one, the weather condition during the harvesting period affects the length of the field drying period. The farmers entirely depend on solar energy for field drying. Weather conditions such as temperature and relative humidity also affect mycotoxin contamination since it directly affects the growth of toxigenic fungal species and mycotoxin production. On the other hand, the weather cannot be changed, and we therefore focused on farmers' agronomic practices.

3.5. COST-EFFECTIVENESS OF SUGGESTED INTERVENTIONS

Proper implementation of all the agronomic practices, that showed significant relationships with mycotoxin contamination, i.e. crop rotation, sowing method, type of fertilizer, and seed treatment, could result in a higher impact in reducing the probability of mycotoxin contamination than implementing each of the practices alone. Applying crop rotation, keeping optimal plant density, optimal fertilizer application, and treating seed with fungicide reduces the likelihood of mycotoxin contamination. If the interventions for each of these practices are individually implemented, adaptation of the sowing method seems to be a cost-effective option for subsistence farming situations for a few reasons. First, adapting the nature of the sowing practice probably has the lowest costs over the other practices. In subsistence farming situations in Ethiopia, sowing grains is conducted manually, which means that the associated costs mainly relate to labor. Having either a low or high density of seed planting does not affect these labor costs much, whereas interventions related to the crop rotation, type of fertilizer and seed treatment require additional labor, making them less favorable options in terms of labor costs. In addition, the type of fertilizer application comes with additional costs related to the costs of labor for purchasing and transporting synthetic fertilizer and related costs for the fertilizers itself, and/or related costs for preparing and handling organic fertilizer. Crop rotation comes with costs related to the production of the other crop such as land preparation, seed planting, growth inputs and harvesting. For seed treatment, these costs include the cost of treating the seed and handling the treated seed until sowing and costs for the

fungicides to treat the seeds. Second, the investment required to implement the sowing interventions could be cheaper than the other interventions. All the interventions require the training of farmers. However, training for sowing interventions could cost less investment due to the nature and content of this training. This training is about keeping optimal seed density which would be completed in a few days, and costs less money than the other interventions. Training for crop rotation could include description of crop rotation and its benefits, selection of the other type of crop to plant, management practices during growth, harvesting and handling as well as supply chain perspectives, which require much higher time investment than training for the sowing intervention. Training for types of fertilizer could include manure handling, optimal fertilizer applications, and introducing novel vermicomposting technology as a sustainable alternative. Training on seed treatment intervention could include the impact of using fungicides on soil health and related grain productivity problems as well as issues related to sustainability. Provided that training for optimal fertilizer application and seed treatment interventions require several days to many weeks (including for onsite supervision), both interventions need more investment than the sowing intervention. Finally, based on the coefficients of the logistic regression models (Table 3.5 and 3.6), seed treatment by polishing with fungicide is the most effective intervention to reduce the likelihood of mycotoxin contamination in sorghum. However, regarding sustainability, the sowing method is the most sustainable option among the other practices. Its sustainability partly depends on the specific agricultural practices applied to grow the other crop, including the type of fertilizer used and pesticide application. Seed treatment with fungicide is not a sustainable option since it may affect humans (handling of seeds with fungicides), animals, and the environment. In addition, organic fertilizer application has been associated with emissions that negatively affect the environment. Synthetic fertilizer also affects the environment (e.g. increases soil acidity), and it is also an expensive option for poor farmers. The manual sowing method is environmentally friendly. Overall, if a single intervention option is to be selected, intervention on the sowing method seems the most feasible and sustainable preharvest practice for subsistence farming situations in Ethiopia. Besides to the sowing method, the crop rotation can be a sustainable and feasible option based on its management practices, especially related to the inputs used to grow the crop.

3.6. CONCLUSION

This study provides insights into the presence of multiple mycotoxins in newly harvested sorghum in northwest Ethiopia. The farming practices, namely crop rotation, sowing method (high density), type of fertilizer (organic), and seed treatment were related to multiple mycotoxin contaminations. Therefore, these practices are recommended to be considered in future studies to reduce the occurrence of mycotoxins in sorghum in Ethiopia.

Appendix

Appendix Table 3.1. Weather data for data collection *woredas*

Woreda	Weather data station	Altitude asl (m)	Temp range (T_{min} - T_{max})	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 3.2. 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 3.3. Sociodemographic, farming practices, and mycotoxin awareness 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 person responsible for growing sorghum in the field (MPreFA)	1. Wife 2. Husband 3. both similar responsibility 4. Other, specify, ____
6	Age of MPreFA (in years)	_____
7	Farming experience of MPreFA (in years)	_____
8	Highest level of formal education completed for MPreFA	
9	Mention the list of major food crops you have produced this year (most produced to least produced)	_____
10	How much sorghum did you harvest this season? (express in your own units, quintal, 50 kgs, or other?)	_____
11	Total cultivated land for sorghum growing this year (in hectares or local measurement unit)	_____
	Farming practices	
12	Did you plough the land (tillage) for growing sorghum?	1. Yes 2. No
13 14	If yes, what method did you use for land plowing? Number of land plowing times	1. animal traction (using oxen) 2. Manual (Hand hoe) 3. Both animal traction and hand hoe 4. other, specify _____ 1. once 2. twice 3. three times 4. four times 5. >4 times
15	Did you treat the sorghum seed before sowing?	1. yes 2. no
16	For what purpose did you treat the seed?	_____
17	What chemical did you use for seed treatment?	1. water 2. chemical other than water 3. wood ash 4. other, specify, _____
18	What method did you apply for seed treatment?	1. washing and drying 2. spraying with no drying 3. spraying and drying 4. polishing 5. other, specify _____
19	How many days (weeks) before sowing did you treat seed?	_____
20	Sowing method	1. broadcasting (dispersing) 2. row planting
21	Fertilizer type used	1. organic 2. synthetic 3. both
22	Did you apply synthetic Phosphorus fertilizer?	1. yes 2. No
23	Did you apply synthetic Nitrogen fertilizer?	1. yes 2. No
24	Did you remove weed?	1. yes 2. no
25	If ''yes', what method did you use to remove weed?	1. manual weed removal 2. chemical (herbicide) 3. other, specify _____

Appendix Table 3.3. Sociodemographic, farming practices, and mycotoxin awareness questionnaire (*continued*)

S.No	Questionnaire	Response
26	How many times did you remove weed?	1. every time it appears 2. once 3. twice 4. three times 5. other, specify _____
27	How do you judge readiness of crop for harvesting?	1. eye observation (color change of the plant) 2. kernel hand test (juiciness) 3 kernel teeth biting test 4. another test, specify _____
28	Harvesting of sorghum is	1.by cutting from the bottom of the stalk 2. head cut on the neck 3. by collecting the seed only from the head 4. other, specify ___
29	Sort damaged heads (by bird, mold, etc.) during harvesting?	1. yes 2. no
30	Drying practices for harvested sorghum	1. outdoor 2. indoor 3. both outdoor and indoor
31	Which outdoor drying method do you apply?	1. spreading on bare ground 2. spreading on a mat on the ground 3. hanging on a raised surface 4. other, specify _____
32	How long (in days or weeks) do you dry in outdoor drying situations	_____
33	What method do you use to thresh sorghum (to separate the grain from the head)?	1. manual with hand (fingers) 2. manual beating with wood stick 3. Threshing using animal power 4. using threshing machine 5. other, specify _____
34	Did you face preharvest insect infestation during sorghum cultivation?	1. yes 2. no
35	At what preharvest stage/period did the insect infestation occur this season?	1. early plant growth 2. after growth to optimal plant height and before flowering 3. after flowering and before seed maturation 4. after seed maturity and before harvesting 5. After harvesting and during field drying 6. other, specify _____
36	What intervention did you apply for the insect infestation?	
37	Did you face preharvest fungi infestation during sorghum cultivation this season?	1. yes 2. no
38	At what preharvest period/stage did the fungi infection occur?	1. early plant growth 2. after growth to optimal plant height and before flowering 3. after flowering and before seed maturation 4. after seed maturity and before harvesting 5. After harvesting and during field drying 6. other, specify _____
39	What intervention did you apply for the fungal infection?	
	Mycotoxin Awareness questions	
40	Fungi grow in sorghum grain	1. fully disagree 2. slightly disagree 3. not agree nor disagree 4. slightly agree 5. fully agree 6. I don't know
41	Fungi can infect sorghum during growth in the field	1. fully disagree 2. slightly disagree 3. not agree nor disagree 4. slightly agree 5. fully agree 6. I don't know
42	Fungi produce toxic compounds (mycotoxins (aflatoxins))	1. fully disagree 2. slightly disagree 3. not agree nor disagree 4. slightly agree 5. fully agree 6. I don't know

Appendix Table 3.4. LOD/LOQ values and recovery percentages of specific mycotoxins

S.No.	Name of mycotoxin	LOD (ng/g)	LOQ (ng/g)	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
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	ß-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

CHAPTER 4

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

This chapter is based on a paper published in an international journal:

J.A. Sadik, L. Righetti, N. Fentahun, I.D. Brouwer, M. Tessema, M. Abera, H.J. van der Fels-Klerx, 2025. Storage management practices and mycotoxin contamination of sorghum (*Sorghum bicolor*) in northwest Ethiopia, Journal of Stored Products Research, 111: 10253. https://doi.org/10.1016/j.jspr.2024.102535

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 (ng/g) for total aflatoxins, 5.31, 12.50, 14.94, 15.77, 32.94, 56.81, 58.07 and 112.59 (ng/g) for ochratoxin A, and 123.48, 238.43 and 431.78 (ng/g) 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.

Key words: Mycotoxin, Sorghum, Storage Practice, management, Ethiopia

4.1. INTRODUCTION

Sorghum (*Sorghum bicolor*) is an important staple food grain in Ethiopia (MOA, 2020; Mohammed *et al*., 2022a). 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, 2021a).

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 *et al*., 2022a; 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*., 2014a). 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*., 2014a). In the year 2021, Mohammed *et al*. (2022a) 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 & 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*., 2022a; Taye *et al*., 2016; Taye *et al*., 2018; Taye *et al*., 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*., 2022a; 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 favour 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 & 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 PICs (Purdue Improved Crop Storage) sack, which is a hermetic sack 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 & Wubetie, 2021). Instead, indigenous storage structures are commonly used to store grains (Garbaba *et al*., 2018a; 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 low- and middle- income 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 & 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, 2021a). To date, research on the possible relationship of sorghum storage practices with mycotoxin contamination in this area is hardly available.

4.2. MATERIALS AND METHODS

4.2.1. 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, 2021a), 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, 2021a) 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 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 Figure 4.1. Weather data has been collected from the National Meteorology Institute of Ethiopia, Addis Ababa for the years 2001-2021 (and presented in Appendix Table 4.1). Weather data is not available for the last few years partly because of the war in north Ethiopia.

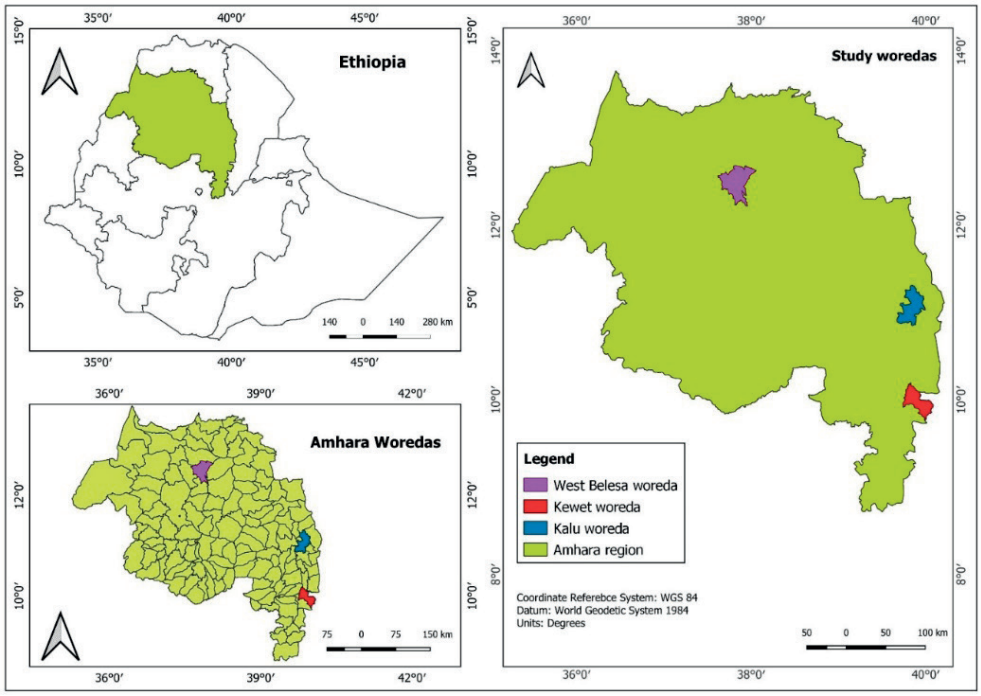


Figure 4.1. Locations of sorghum sample collection *woredas* in Ethiopia

4.2.2. 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 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 4.2). 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.

4.2.3. Data collection

4.2.3.1. 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 polypro-

pylene (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 1-2kg based on the European Commission Regulation (EC, 2006). The aggregate samples were thoroughly mixed manually on the spot, and packed in labeled fabric sacks 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 1mm 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 sack, and labeled. Then these 120 samples were transported under cooled conditions to Wageningen Food Safety Research Laboratory, The Netherlands for mycotoxin analysis.

4.2.3.2. 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 4.3). Ethical approval for the interview was obtained from the 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 & 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.

4.2.3.3**.** 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 4.4.

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.

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 (10µg/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 minutes. Then, four grams of magnesium-sulphate was added to the individual retrieved samples as per the QuEChERS method. The samples were subsequently shaken manually for 1 minute, and then centrifuged at 3000 rpm for 10 minutes. 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 ℃ for 1 hour. 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.

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 x 2.1 mm) heated at 35 °C was used. The volume of analyte injected was 5µL 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.

Validation

To evaluate recovery (extraction efficiency), two blank sorghum samples were spiked with the 33 mycotoxins listed in Appendix Table 4.4. The spiked sample was left standing for 10 minutes 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) (EC, 2006). The percent recovery values for each mycotoxin are summarized in Supplementary material.

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 4.4.

4.2.4. 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* mycotoxins as based on (Mohammed *et al*., 2022a; Moretti & 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.

4.3. RESULTS

4.3.1. 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 to 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 4.1).

Table 4.1. 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**	Wife	63	73	55	63
	Other**	37	28	45	38
Age (yr)*	18<-30	14	15	15	13
	31<-50	65	58	58	80
	above 50	21	28	28	8
*Basic formal education**	Yes	19	18	15	25
	No	81	83	85	75

*Refers to the Main Persons Responsible for sorghum Storage management. ** 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.

4.3.2. Sorghum storage management practices

Both introduced and indigenous storage structures were used to store sorghum grain (Table 4.2). In comparison, a slightly lower proportion of farmers (43%) than those who used the introduced storage structures, 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 4.2). 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 Figure 4.2.

Table 4.2. 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 sack	49	100	15	88
	- PICs sack	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

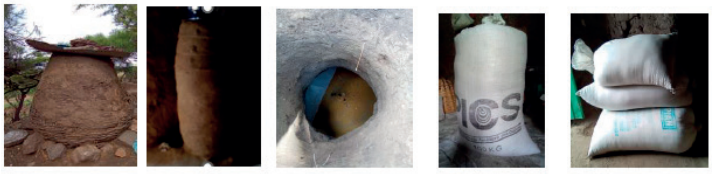


Fig 4.2. Sorghum storage structures

4.3.3. Prevalence of mycotoxin contamination

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

The presence of mycotoxins is summarized in Table 4.3. 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 4.3).

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 (Figure 4.3).

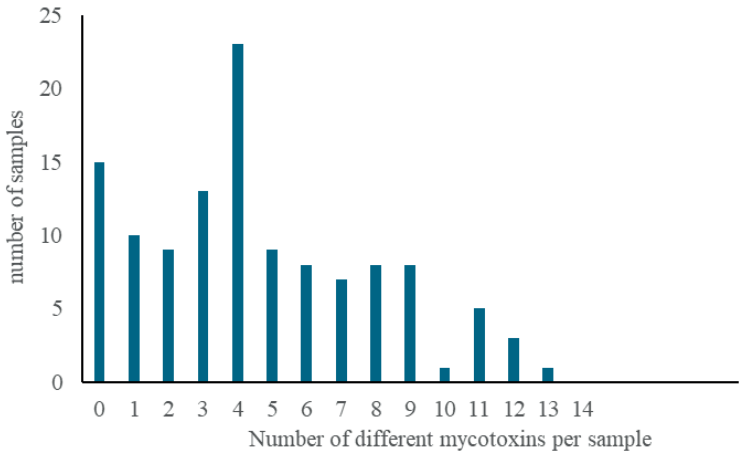


Figure 4.3. Occurrence of multiple mycotoxins in in stored sorghum in northwest Ethiopia, 2023

Table 4.3. 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 (ng/g)	% p (N)	conc. Range (ng/g)	% p (N)	conc. Range (ng/g)	% p (N)	conc. Range (ng/g)
Aspergillus mycotoxins	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 mycotoxins	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
	ß-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

Table 4.3. Prevalence of mycotoxin contamination in stored sorghum in northwest Ethiopia, 2023 (*continued*)

Major category	Specific mycotoxin	Total (120)		w. Belesa (40)		Kalu (40)		Kewet (40)	
		% p (N)	conc. Range (ng/g)	% p (N)	conc. Range (ng/g)	% p (N)	conc. Range (ng/g)	% p (N)	conc. Range (ng/g)
Fusarium mycotoxins	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 mycotoxins	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 mycotoxins	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 4.4). *DON refers to Deoxynivalenol

4.3.4. 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 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 4.5). 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.

Table 4.4. 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

Table 4.5. Multiple logistic regression - 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	0.39(-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:

* 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;

** 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;

*** 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;

**** 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;

***** 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

4.4. 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 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 ng/g (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 ng/g.

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 ng/g, 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 (ng/g) for total aflatoxins, 5.31, 12.50, 14.94, 15.77, 32.94, 56.81, 58.07 and 112.59 (ng/g) for ochratoxin A, and 123.48, 238.43 and 431.78 (ng/g) 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*., 2022a). 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*., 2022a) 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, (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 to 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 4.1). 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*., 2018a; 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 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 4.4). 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 (Milania & Malekib, 2014). 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 4.2), 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 *woreda*s (Table 4.3). 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 minutes 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 4.2). 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 to 53 years of storage experience (Table 4.3) 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 select-

ing damaged crops intended for human consumption (Cervini *et al*, 2023). Provided that 63% of the MPRS in this study were women (Table 4.1), 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 4.4). 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, 2018; Wu 2014a), 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 when other variables are kept constant. 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 4.4). 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 (Table 4.4 and 3.5). 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, 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.

4.5. COST-EFFECTIVENESS OF 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 *et al* (2017) 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.

4.6. 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.

Appendix

Appendix Table 4.1. Weather data for data collection *woredas*

Woreda	Weather data station	Altitude asl (m)	Temp range (T_{min} - T_{max}) (°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 4.2. 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 4.3. 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 4.4. LOD/LOQ values and recovery percentages of specific mycotoxins

S.No.	Name of mycotoxin	LOD (ng/g)	LOQ (ng/g)	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
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

Appendix Table 4.4. LOD/LOQ values and recovery percentages of specific mycotoxins (*continued*)

S.No.	Name of mycotoxin	LOD (ng/g)	LOQ (ng/g)	Percent recovery (±SD)
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	ß-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

CHAPTER 5

5

Exposure and disease burden of fumonisins and aflatoxins from sorghum consumption in Ethiopia

This chapter is based on a paper published in an international journal:

J.A. Sadik, N. Fentahun, I.D. Brouwer, M. Tessema, and H.J. van der Fels-Klerx, 2025. Exposure and disease burden of fumonisins and aflatoxins from sorghum consumption in Ethiopia. Journal of Regulatory Toxicology and Pharmacology, 164:105966.

ABSTRACT

Studies on mycotoxin exposure from sorghum consumption and related public health risk estimation are rarely available in Ethiopia. The aim of this research was to assess fumonisin and aflatoxin exposure of adults through sorghum consumption in the Amhara National Regional State (ANRS) and at national level in Ethiopia and to estimate related health risks. Data on fumonisin and aflatoxin concentrations in sorghum samples were collected from a survey and literature. Estimated fumonisin exposure in the ANRS and at national level were below the FAO/WHO limit of 2000 ng/kg bw day to be considered a health concern. The estimated aflatoxin exposure levels in the ANRS and at national level fall below the Margin of Exposure value of 10000, indicating potential health concern. The incidence of hepatocellular carcinoma due to aflatoxin exposure in the ANRS ranges from 0.0003 to 0.017 while at national level, it ranges from 0.181 to 8.47 (per100.000 persons/year). The related disability-adjusted life years estimates for the ANRS and at national level ranged from 0.0003 to 0.019 and 0.204 to 11.230, respectively. Aflatoxin exposures were driven more by sorghum intake than aflatoxin contamination. Dietary intervention could further reduce the health risk estimates.

Key words: Mycotoxin, Exposure, Hepatocellular carcinoma, DALY, Ethiopia

5.1. INTRODUCTION

Mycotoxins are metabolites produced by certain species of fungi. The most well-known mycotoxins which occur in food are aflatoxins, fumonisins, ochratoxins, patulin, deoxynivalenol, and zearalenone (Pandey *et al*, 2023). Aflatoxins and fumonisins are the major mycotoxins known to cause a public health burden worldwide (Wu *et al*., 2014a). Aflatoxins can lead to liver injury after acute exposure and cancer due to chronic exposure. Aflatoxins also reduce weight gain in children, leading to stunting after chronic exposure (Braun & Wink, 2018). The major aflatoxins are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) (Okechukwu *et al*., 2024). From these four, AFB1 is the most potent mycotoxin, and its health concern is of greatest concern (Sandoval *et al*., 2019). AFB1 is hydroxylated to aflatoxin M1 (AFM1) (Okechukwu *et al*., 2024; Wu *et al*., 2014a) which is seen in cow's milk. Simultaneous exposure to hepatitis B virus in addition to aflatoxin exposure increases the risk of hepatocellular carcinoma (Sandoval *et al*., 2019; Wu *et al*., 2014a). Fumonisin intake is associated with brain and esophageal cancer, renal and liver toxicity (Bucci *et al*., 1998) , and neural tube defects (Marasas *et al*., 2004; Wu *et al*., 2014a). The major types of fumonisins are fumonisin B1 (FB1), fumonisin B2 (FB2), and fumonisin 3 (FB3) (Ren *et al*., 2011). Simultaneous exposure to aflatoxins and fumonisins would exacerbate the mycotoxin health burden on humans (Nikie`ma *et al*., 2004). One of the most used methods to estimate the burden of dietary exposure to food safety hazards is disability-adjusted life years (DALY) where one DALY represents the one year of healthy life lost (Gibb *et al*., 2015). WHO estimated the burden of disease related to aflatoxins in Africa at 15 DALY per 100,000 persons. To compare, aflatoxins are responsible for 0.5 DALYs per 100,000 in Europe (Gibb *et al*., 2015).

Sorghum (*Sorghum bicolor* L. *Moench*) is one of the staple food grains in Ethiopia (Atnkut *et al*., 2025; Mohammed *et al*., 2022a). In the year 2020/21, sorghum was the fourth most produced cereal in the country, next to maize, wheat and teff. In that year, about 4.8 million tons of sorghum grain was produced (Central Statistics Authority of Ethiopia (CSA, 2021a). In areas of Ethiopia experiencing moisture stress, including those considered in this research, sorghum is the most widely produced and consumed crop (Ministry of Agriculture (MOA, 2020)). According to FAOSTAT Food Balances (2022), about 3.8 million tons of sorghum was used for human consumption in 2022 nationally, supplying about 209 kcal/capita/day. Sorghum is mainly used to produce *injera* (a flat pancake-like product) for human consumption. Next to teff, sorghum is the most suitable cereal to produce a good quality *injera* (Baye *et al*., 2013; Fox *et al*., 2020; Mohammed *et al*., 2022a; Yetneberk *et al*., 2005). It is also used to produce complementary foods for young children (Beyene *et al*., 2016) and to produce local alcoholic beverages such as *tella* and *areki* (Atnkut *et al*., 2025; Derese *et al*., 2018). The use of sorghum to produce *injera* has been increasing in recent years due to the rising trend of the market price of teff (Fox *et al*., 2020).

Despite its importance as a staple food in Ethiopia, sorghum can be contaminated with mycotoxins, even to concentrations beyond regulatory limits (Chala *et al*., 2014; Mohammed *et al*., 2022; Ssepuuya *et al*., 2018; Taye *et al*., 2018). For instance, Ssepuuya *et al*. (2018) reported the presence of aflatoxins B1, B2, G1, G2 concentrations ranging from 3 to 323 ng/g in sorghum samples collected from farmers households and local markets in Ethiopia in 2012/2013. Mycotoxin concentrations in part of the samples exceeded national and international regulatory limits, indicating that mycotoxin presence in sorghum can pose a public health problem in the country. In another research, Mohammed *et al*. (2022a) reported 79 mycotoxins in sorghum samples collected from farmers' households in eastern Ethiopia. The list included both aflatoxins and fumonisins. The presence of conducive climatic conditions in Ethiopia for fungal growth together with the underdeveloped nature of sorghum value chain practices favorable for fungal infection (Mohammed *et al*., 2022a; Taye *et al*., 2016; Taye *et al*., 2018) as well as the low level of awareness of farmers about mycotoxins and their control (Beyene *et al*., 2016) have been mentioned as a reason for the mycotoxin contamination.

An appreciable number of mycotoxin exposure studies have been conducted in Ethiopia. These studies have been conducted by using biomarker studies for mycotoxins in blood/plasma, urine and breast milk (Ayele *et al*., 2022; Boshe *et al*., 2020; Mesfin *et al*., 2023; Mulisa *et al*., 2024; Tessema *et al*., 2021). Although these studies did not indicate whether the results were due to acute or chronic exposure, the findings of most of these studies indicated that mycotoxin exposure is a public health problem in Ethiopia and suggested the implementation of appropriate interventions to reduce the associated problems. However, biomarker-based studies are known to provide more reliable information for mycotoxin exposure than dietary intake-based studies, the dietary intake-based studies can provide better directions to intervention to reduce the problem. In addition, the limitation to the above studies is that the quantitative public health burden related to mycotoxin exposure, such as hepatocellular carcinoma (HCC) and DALY was not estimated. A quantitative estimate of the public health burden is more important information to public health policymakers than exposure information (Gibb *et al*., 2015). In addition, understanding the risk factors for mycotoxin exposure and related health burden helps to identify priority areas for intervention.

This study aimed to assess the aflatoxin and fumonisin exposure of adults through sorghum consumption, and to assess the related public health burden. The risk assessment was conducted for northwest Ethiopia and at national level.

5.2. MATERIALS AND METHODS

5.2.1. Mycotoxin data collection

Data from a previous study on mycotoxin concentration in 240 sorghum samples grown in northwest Ethiopia in the 2022 cropping season, collected at harvest and after six months of storage, was used (Details of sampling provided in Chapter 3 & 4).

5.2.2. Management of left censored data

Fumonisin and aflatoxin concentrations that are lower than their LOD values were managed according to the EFSA (2010) recommendations with slight modifications. Based on EFSA (2010), when the proportion of samples with results less than the LOD values is above 80 % of the total samples (left censoring data), estimation of the statistical mean, median, and standard deviation is considered not practicable. That is because for data where more than 80% of the data is left censored, the mean does not ensure the reliability and comparability of the results. It also does not provide a realistic description of food contamination and exposure assessment, thus not providing sound information on which to base decisions (GEMS/Food-EURO, 1995). For such cases, a simple estimate of the mean can be conducted by producing two estimates - one is by substitution of all the non-detectable results by a value of zero (lower bound) and the other is by substitution of all the non-detectable results by the numerical value of the LOD (upper bound). In our case, we used the average values of both lower bound and upper bound values for risk assessment because the LOD values for the fumonisins (1.5 ng/g) and aflatoxins (0.06 ng/g) were low.

5.2.3. Sorghum processing practices for regular food preparation

Data about sorghum processing practices was collected through a face-to-face interview with the person responsible for food preparation in 120 households. Interview data were collected from the end of March to mid-April 2023. The basic sorghum *injera* processing questionnaire was adopted from Yetneberk *et al*. (2005) and Baye *et al*. (2013) (Supplementary Figure 5.1). The questionnaire was initially developed in English language and was translated into the local language (*Amharic*). Ethical approval for the interview was obtained from the Bahir Dar University Institutional Review Board (Protocol number 12/IRB/23) before respondent data collection. Written Informed Consent was received before the interview.

5.2.5 Mycotoxin exposure

All the respondents mentioned regularly using sorghum grain to prepare *injera* for their family consumption (Supplementary Table 5.1). Therefore, sorghum *injera* (hereunder referred to as '*injera*') was used to estimate the mycotoxin exposure and the related public health risk.

Sorghum processing can lead to mycotoxin reduction of the foods, with variability in reduction depending on the processing conditions (Adebo *et al*., 2019; Fandohan *et al*., 2005; Matumba *et al*., 2015; Udovicki *et al*., 2019). Since all the respondents in the present study mentioned milling sorghum and *teff* grains without any prior decortication (without dehulling), we assumed the whole milling of these grains does not have an impact on mycotoxin reduction. In *West Belesa woreda*, all the respondents mentioned blending *teff* with sorghum for making *injera*. *Woreda* is an administrative division that functions like a district in Ethiopia. It is one level below a zone and one level above a kebele (village or ward). Previous research by Geremew *et al*. (2018) and Ayalew *et al*. (2006) reported the occurrence of mycotoxins in *teff* grain/flour samples. Therefore, we assumed the two grains are similarly vulnerable to mycotoxin contamination, and as a result, blending *teff* to sorghum has no impact in reducing mycotoxin concentration due to dilution effects. Further, the temperature and time of baking to obtain optimal quality *teff injera* range from 230 to 260^{o}C for 2 to 3 minutes (Bikila *et al*., 2024). Because of the very short *injera* baking time, we assumed that baking injera does not reduce the concentration of mycotoxins. The local *injera* processing practices, complemented with litereature data for the extent of mycotoxin reduction during sorghum processing, were used to calculate the mycotoxin concentration in the *injera* (*FC*), by adopting the method that was used for mycotoxin reduction in maize products (Udovicki *et al*., 2019):

$$FC = IC \times (100\ \% \times RF1 \times RF2 \times RF3) \quad \text{Eq.1}$$

Where: *IC* refers to the initial mycotoxin concentration in the sorghum grain. *RF1* refers to the mycotoxin remaining after reduction due to the combined effect of manual sorting and windowing, *RF2* refers to the mycotoxin remaining after reduction due to dilution effect of water addition, and *RF3* refers to the mycotoxin remaining after reduction due to 2-3 days natural fermentation. The average reduction factors obtained from literature are given in Supplementary Table 5.2.

Monte Carlo simulation was used to estimate the mycotoxin exposure so as to consider the natural variation in mycotoxin occurrence, sorghum intake and body weight (Liang *et al*., 2021). Total aflatoxins (sum of AFB1, AFB2, AFG1 and AFG2) and total fumonisins (sum of FB1, FB2 and FB3) were individually used in the simulation applying 100,000 iterations using Eq.2 according to (Udovicki *et al*., 2019).

Exposure to aflatoxin and fumonisins was determined for adults by calculating the Estimated Daily Intake (EDI) of the mycotoxins due to *injera* intake (Eq.2) based on (Udovicki *et al*., 2019).

$$EDI\ (ng\ kg^{-1}bw\ day\ ) = \frac{average\ injera\ consumption\ (g/day) \times mycotoxin\ \ concentration\ (ng/g)}{body\ weight\ (kg)}$$

Eq 2

The best fit distribution models for the aflatoxin and fumonisin occurrence data were selected based on the Model-then-Add approach (MTA) using the @Risk software (Andrade *et al*., 2020). Accordingly, the Pareto statistical distribution was used for both aflatoxins and fumonisins (Supplementary Figure 5.2). This distribution was selected based on the Akaike Information Criteria, which is calculated from the log-likelihood function and takes into account the number of parameters of the fitted distribution using the @Risk software. The sorghum intake data for the Amhara National Regional State (ANRS) and at the national level in Ethiopia were obtained from the National Food and Nutrition Strategy survey (Ethiopian Public Health Institute ((EPHI, 2025)) (Supplementary Table 5.3). This survey was conducted at a national level from 2021 to 2024, and the ANRS data was collected between 2021 and 2022. We used sorghum intake data collected from women of reproductive age (14–59 years old) using a 24-hour recall method. A total of 944 and 10,006 women of this age were surveyed for the ANRS and nationally, respectively. Details of the survey, including the sample size, the sampling method and the quantitative dietary assessment, are available in the Ethiopian Public Health Institute (2025) report. We assumed that the sorghum intake of adult women and men was the same. We assumed all the sorghum intake is in the form of *injera*. The intake data showed a large variation between mean and median values, both in the ANRS and at national level, thus data were not normally distributed. In addition, from the large standard deviation values, we understand that the sorghum intake level is widely varying among the consumers. Therefore, we applied a statistical distribution to account for variability in consumption. A triangle distribution was used for sorghum intake by using the minimum intake (the 2.5 percentile intake), the most likely intake (the median), and the maximum (97.5 percentile intake) values. Further, the mean intake quantities for both the ANRS and the National level were much higher than the respective median intake levels, implying that the raw intake data were skewed to the right. Therefore, the 2.5 and the 97.5 percentile intake quantities were calculated from a log transformed mean and standard deviation of the intake data. The 2.5 % and 97.5 % intake values obtained for the ANRS were 71 g and 1039 g, respectively; while for the National level, the values were 77 g and 1014 g, respectively (Supplementary Figure 5.3).

For body weight, the mean and median values were similar for the ANRS and for the National level, indicating data for both the ANRS, and at the National level were normally distributed (Supplementary Table 5.3; Supplementary Figure 5.4). In addition, because the food consumption survey was conducted at national level, we assumed the sample mean and standard deviation of body weight for the national level data would represent the population mean (μ) and variance (σ2), respectively.

For the mycotoxin exposure assessment at national level, we used mycotoxin occurrence data from literature. From a literature search, six articles were obtained on the occurrence of mycotoxins in sorghum in Ethiopia (Supplementary Table 5.4). However, the fumonisin and aflatoxin occurrence data reported by Ssepuuya *et al*. (2018) was used in this study. This study was selected because the samples in this study were collected from a survey conducted at national level, while the other studies were conducted in a specific part of Ethiopia. In addition, the other articles missed at least one of the specific aflatoxins or fumonisins required in the current study, which made it difficult to determine the total aflatoxins and total fumonisins. Further, Ssepuuya *et al*. (2018) collected the samples from a newly harvested and stored grain in farmers households as well as from the market, making the results more realistic since the different stages of the sorghum value chain were considered. For the other studies, samples were collected only from the farmers' households or from controlled experiments.

5.2.6. Sensitivity analysis

Sensitivity analysis was conducted graphically using a tornado plot to investigate how the percent changes in the input variables (aflatoxin concentration, sorghum intake and body weight) influence exposure (EDI) based on the @Risk software instructions ((Risk and decision analysis platform for Microsoft Excel, version 8.8.1, Palisade Company LLC, NY, USA) according to Herojeet *et al*. (2023).

5.2.7. Risk characterization

The public health risk for fumonisin exposure was evaluated by comparing the estimated EDI value with the provisional maximum tolerable daily intakes (TDI) value of 2000 ng/kg bw day (WHO & FAO, 2017). For aflatoxins, which are both carcinogenic and genotoxic, there is no Tolerable Daily Intake (TDI) (EFSA, 2020). The risk of exposure to aflatoxins was estimated by determining the Margin of Exposure (MOE), the risk of incidence of hepatocellular carcinoma (HCC), and the disability-adjusted life years (DALY) (Mihalache *et al*., 2024).

5.2.7.1. Risk of characterization for aflatoxin

Margin of exposure (MOE)

MOE is a dividend of the BMDL10 (benchmark dose lower limit), a value of aflatoxin derived from animal studies (400 ng/kg bw/day) by the EDI. MOE value below 10000 is an indicator for the presence of public health concern (FAO/WHO, 2014).

Liver cancer risk estimation

The average cancer potency of AFB1 (Pcancer) was estimated considering the proportion of individuals having hepatitis B surface antigen positive (HBsAg+) and hepatitis B

surface antigen negative (HBsAg-) from the total population (WHO & FAO, 2017) (Eq 3). The prevalence of HBsAg+ in rural Ethiopia (9.7 %) and the pooled prevalence of HBsAg+ at national level (9.4 %) reported for ages above 15 years in Ethiopia (Ethiopian Ministry of Health (MOH, 2021) were used for the determination of *Pcancer* in Amhara regional national state and at national level, respectively.

$$Pcancer = 0.01 \times HBsAg^{-} + \ 0.3 \times HBsAg^{+} \quad \text{Eq.3}$$

The risk of incidence of hepatocellular carcinoma (HCC) (case number/100,000 persons/year) was simulated using Eq 4 (Udovicki *et al*., 2019):

$$Risk\ of\ HCC = Pcancer \times EDI \quad \text{Eq.4}$$

According to Sandoval *et al*. (2019), estimation of extra cancer cases (HCC) for a lifetime exposure to aflatoxin, and its expression per million population, is a more common way of expressing HCC results in toxicology research. To estimate the risk of lifetime exposure to aflatoxins, the HCC result obtained from equation 4 was multiplied by the average life expectancy years for Ethiopians, which is 67.8 years (WHO, 2023, https://data.who.int/countries/231), and by a factor of 10 (Sandoval *et al*., 2019). For lifetime risk estimation, we assumed that individuals' EDI remains constant throughout their lifetime.

Estimation of DALY of aflatoxins

DALY (Disability-adjusted life years) due to aflatoxin exposure was estimated by using the HCC result obtained from Eq.5 and additional data obtained from the Global Cancer Observatory (GCO)(231-ethiopia-fact-sheet.pdf). From the GCO database, the total number of new liver cancer cases for both sexes and all ages in Ethiopia in 2022 were 2,798 and the total number deaths due to liver cancer were 2,683. This would mean that the death rate from the incidence of HCC cases was 95.9 % (2.683/2.798=0.956). The DALY per capita at national level due to the incidence of HCC from aflatoxin exposure was obtained by using the equation adopted from Mihalache *et al*. (2024). Although morbidity is also important to be considered for the DALY calculation (Gibb *et al*., 2015), morbidity data related to aflatoxin exposure in Ethiopia, such as numbers of illness cases, the duration of illness is not available to date.

$$DALY = 0.959 * incidence\ of\ HCC \quad \text{(Eq.5)}$$

5.2.7. Statistical data analysis

The data distribution models and the Monte Carlo simulations were conducted by using the @ Risk software (Risk and decision analysis platform for Microsoft Excel, version 8.8.1, Palisade Company LLC, NY, USA). The random values of the input variables (aflatoxin/fu-

monisin concentrations, sorghum intake and body weight) obtained from the statistical distribution models of the respective data were used for the simulations.

5.3. RESULTS

5.3.1 Occurrence of Aflatoxins and fumonisins

Fumonisins were detected above LOD in 32 samples (13.3 % of the total) while aflatoxins were detected above LOD in 5 samples (2.1 % of total) (Table 5.1). Shapiro-Wilk test result indicated that neither the aflatoxin nor the fumonisin data were normally distributed (P values = 0.000). In addition, the skewness test indicated that both aflatoxin and fumonisin data were highly skewed to the left (P=0.000).

Table 5.1. The occurrence of fumonisin and aflatoxin in sorghum grain in northwest Ethiopia in 2022 cropping season

Mycotoxin	LOD (ng/g)	LOQ (ng/g)	Proportion of samples > LOD (%)	Conc range (ng/g)	Reference
Aflatoxin	0.06	0.15	2.1	LOD-29.19 (mean = 0.37±2.29)	This study
Fumonisin	1.5	3.0	13.3	LOD-81.40 (mean=4.19±8.20)	This study
Aflatoxin	Not given	Not given	Not given	93.32*	(Ssepuuya *et al*., 2018)
Fumonisin	Not given	Not given	Not given	178.9*	(Ssepuuya *et al*., 2018)

*Mean values calculated from the occurrence data of the individual aflatoxins/fumonisins. We assumed a worst-case scenario where the reported mean values for the individual mycotoxins occur in the same samples (thus the mean results are additive). These values are used for risk assessment at national level.

5.3.2 Fumonisin and aflatoxin exposure

The simulated results for fumonisin and aflatoxin exposure are presented in Figure 5.1. The fumonisin exposure ranges from 0.10 to 14.78 ng/kg bw day in the ANRS, and from 7.47 to 299.16 ng/kg bw day at the national level. These exposure levels are much lower than the maximum exposure limit of 2000 ng/kg bw day set by (WHO & FAO, 2017) indicating that the associated public health risk is negligible. The aflatoxin exposure ranges from 0.007 to 0.267 in the ANRS, and from 5.08 to 300.00 at national level (Figure 1). The different percentiles of aflatoxin exposures and related public health risk estimates are shown in Supplementary Table 5.5.

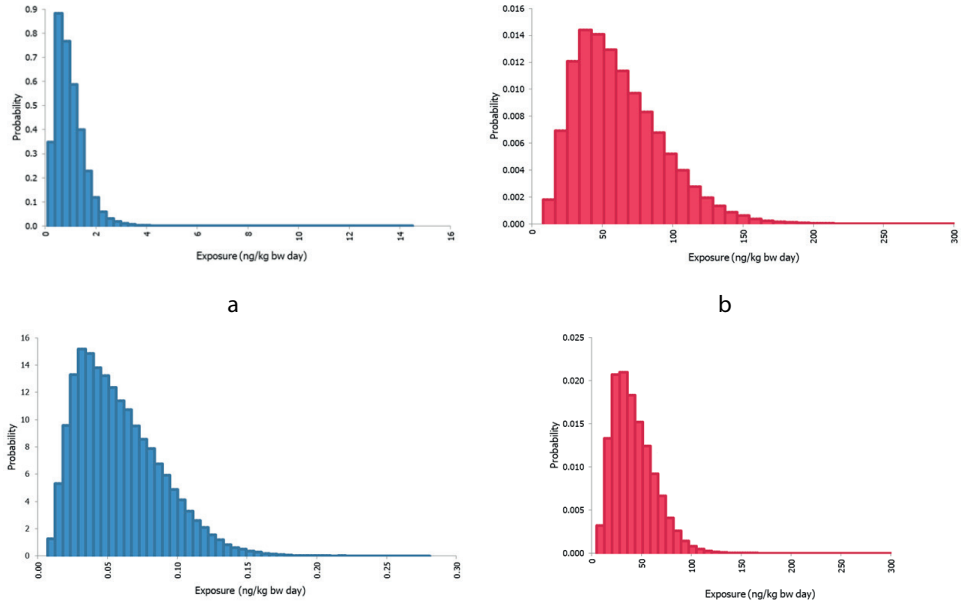


Figure 5.1. Fumonisin exposure in the ANRS (a) and at National level (b); and aflatoxin exposure in the ANRS (c) and at national level (d)

The simulated MOE results obtained for the aflatoxin exposure are presented in Figure 5.2. Most of the estimated results in the ANRS, and all the results at national level were below the MOE value of 10000, a maximum value that is used as a cut-off point for the presence of public health concern for aflatoxin based on EFSA (2020).

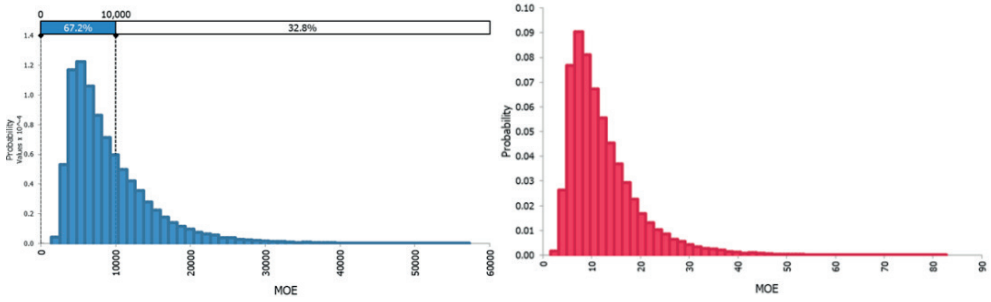


Figure 5.2. MOE in the ANRS (e) and at national level (f)

The estimated incidence of HCC due to aflatoxin exposure in the ANRS ranges from 0.0003 to 0.017 while at national level, the HCC ranges from 0.181 to 8.47 (per100,000 persons/year) (Figure 5.3). For a lifetime aflatoxin exposure, the incidence of new HCC cases per capita would be 0.2 to 11.5 in the ANRS, and 122.7 to 5742.66 at national level (per million persons), respectively. The related DALY for lifetime aflatoxin exposure in the ANRS and at national level ranged from 0.2 to 11.05, and 117.69 to 5489.98 (per million), respectively. For the total of 125 million population in Ethiopia in 2022 (https://data.who.int/countries/231), the related total DALY in the year 2022 was estimated to be 216.96 to 10,121.64.

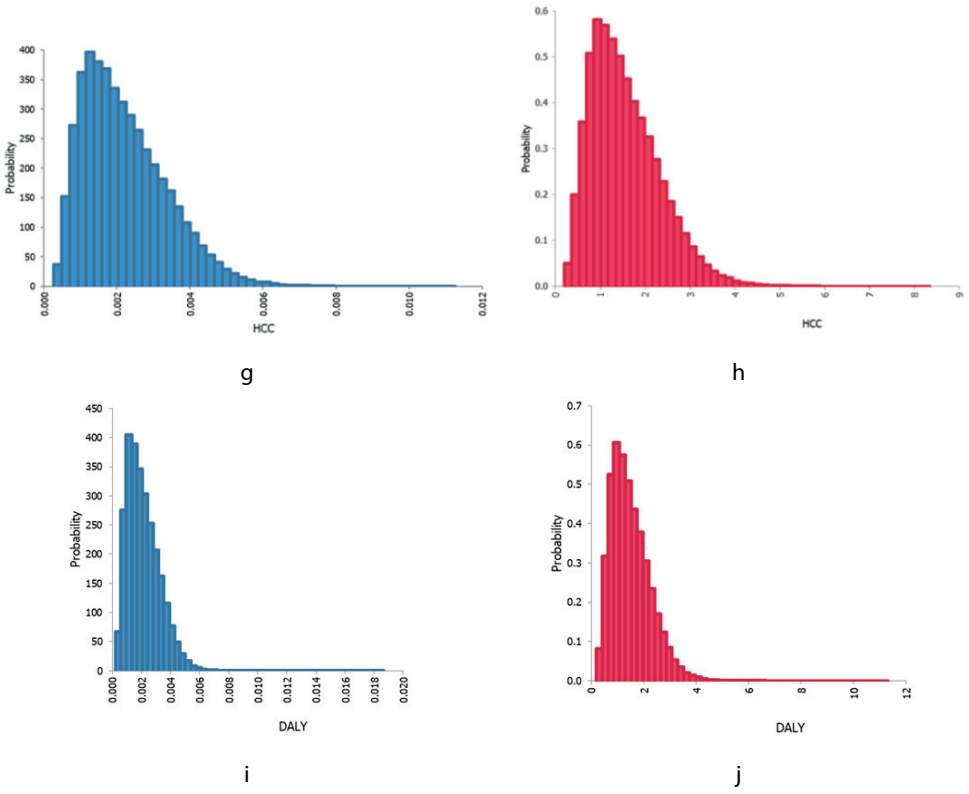
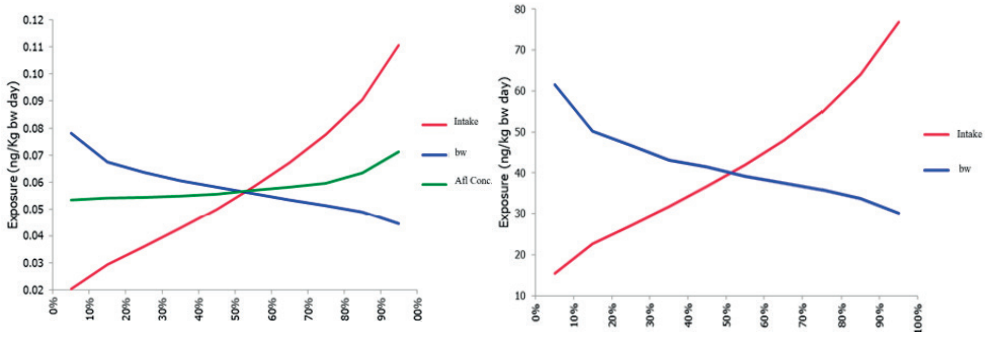


Fig.5.3. HCC in the ANRS (g) and National level (h); and DALY in the ANRS (i) and at national level (j)

5.3.3. The contribution of study variables to aflatoxin exposure

The pattern of aflatoxin exposure changes as the aflatoxin concentration in the grain, daily sorghum intake and assumed average body weight change are shown in the tornado plot (Figure 5.4). The change in exposure showed a steeper line (indicating a slightly linear relationship) for change in sorghum consumption than for aflatoxin concentration

and body weight, indicating sorghum intake having a greater impact on aflatoxin exposure than the other variables.



k l

Figure 5.4. The impact of aflatoxin concentration, sorghum intake and assumed average body weight on aflatoxin exposure in the ANRS (k) and at national level (l). In Figure 4l, the aflatoxin concentration is not shown because we used a single parameter for aflatoxin concentration (mean) for exposure estimation at national level

5.4. DISCUSSION

The objective of this research was to assess the aflatoxin and fumonisin exposure and related public health risks from sorghum consumption in the ANRS and at national level in Ethiopia.

Compared to the FAO/WHO (2024) limits of 10 ng/g for aflatoxin and 4000 ng/g for fumonisin, only two of the samples (0.8%) bypassed the regulatory limit for aflatoxins, while none of the samples bypassed the limit for fumonisin. The concentrations of the two samples, which exceeded the limit for aflatoxin, were 18.34 and 29.25 (ng/g). Apart from the fact that there is no TDI set for human aflatoxin exposure due to its genotoxic and carcinogenic nature (EFSA, 2020), the exposure in the ANRS can be taken as low for subsistence farmers situations who apply traditional practices to grow and store sorghum. However, the low levels of fumonisin and aflatoxin concentrations in the ANRS seem to be achieved from farm agronomic practices on the use of pesticides and insecticides, during the growth and storage period of sorghum (Sadik *et al*., 2025) which probably pose additional public health risks beyond those posed by the mycotoxins themselves. Therefore, it would be interesting to study the extent of pesticide residues in sorghum and their related public health risks as well.

The aflatoxin and fumonisin exposure levels obtained in our study are lower than a previous similar study by Ssepuuya *et al*. (2018). Ssepuuya *et al*. (2018) deterministically estimated the fumonisin and aflatoxin exposure from sorghum consumption at national level in Ethiopia for a sorghum intake of 69 g/d and for a 60 kg body weight. Like our finding, the estimated fumonisin exposure (50 to 700 ng/kg bw/day) was lower than the EFSA (2018) limit of 1000 ng/kg bw day. On the other hand, the estimated aflatoxin exposure (5-100 ng/kg bw day) indicated associated health concerns. Our exposure estimates are much lower than Ssepuuya *et al*. (2018) partly because we applied processing reduction factors to estimate the EDI, thus the concentrations in food products were different. The processing of sorghum *injera* (Supplementary Figure 5.2) involves several steps including manual sorting, windowing, adding water (dilution) and fermentation. A literature review of similar products showed that a combination of manual sorting and windowing, water addition, and spontaneous fermentation for 2-3 days, reduces mycotoxin contamination by 60-69%, 61% and 68%, respectively (Supplementary Table 5.2). These practices should be encouraged to reduce mycotoxin exposure. Investigating the actual reduction levels of mycotoxins in sorghum *injera* processing in Ethiopia would provide more realistic data on the extent to which processing reduces mycotoxins. Despite processing factors reducing mycotoxins, the probability of aflatoxin exposure to be a priority for risk management (the MOE value to be less than 10000), was about 96 % and 100 % in the ANRS and at national level, respectively (Figure 5.2).

Because the mean value for sorghum intake in the ANRS (450 g) estimated from the statistical distribution (Supplementary Figure 5.3) was higher than the actual mean value of 344 g, our simulation result seems to be overestimating the actual aflatoxin exposure. We therefore also deterministically calculated the aflatoxin exposure values for the actual mean and median intake levels in the ANRS. The results obtained were 0.124 ng/kg bw/day and 3230, for exposure and MOE, respectively (Supplementary Table 5.6). The values show the presence of health concern as the simulated results.

The median aflatoxin exposure at national level in our study (38.14 ng/kg bw day) is in the range of aflatoxin exposure from sorghum consumption in Mali (2-133 ng/kg bw day), but much lower than the exposure level in Niger (706-2221 ng/kg bw day) (Falade *et al*., 2022), and higher than in Uganda (19 ng/kg bw day) (Wokorach *et al*., 2021). This difference could be due to the possible variations in climatic conditions, sorghum agricultural production and storage practices, intake as well as variations in the body weight of adults among these countries. The associated HCC risk predicted for Ethiopia is also lower than in Mali and Niger. Besides to variations in aflatoxin exposure, the lower prevalence of Hepatitis B virus infection in Ethiopia (9.4 % a) (MOH, 2021) than in Mali (20 %) and in Niger (16 %) (Breakwell *et al*., 2017; Falade *et al*., 2022) could have contributed for the lower risk of HCC in Ethiopia. Simultaneous human exposure to hepatitis B virus and aflatoxin

is reported to increase the oxidative stress in the population contributing to higher HCC risk (Liu *et al*., 2008). Although Hepatitis C infection is also positively related to HCC risk, with the risk getting worse when hepatitis C and hepatitis B infections co-exist (Okeke *et al*., 2020), a quantitative relationship between aflatoxin exposure and hepatitis C infection has not been established (Liu & Wu, 2010). In Ethiopia, a total of 69,2000 Hepatitis C infection cases were reported for all ages in 2022 (WHO, 2024).

Although the concentrations of aflatoxin in about 99.2 % of samples were below the (FAO/WHO, 2024) regulatory limits of 10 ng/g for aflatoxin in unprocessed cereal grains, and process factors were applied for possible reduction of aflatoxins during *injera* processing, the estimated MOE results showed priority for risk management. The estimated median MOE value estimated for the median intake and median body weight in ANRS without considering the reduction factors for aflatoxin concentration (mean=0.1321 ng/g) was 656, which is much lower than the simulated results estimated considering the process factors as given in Figure 5.2. Similar results where aflatoxin concentrations in foods that were below the EU regulatory limits leading to MOE values below 10000 were also reported in Europe (Udovicki *et al*., 2019) and in Mexico (Sandoval *et al*., 2019). Indeed, contamination levels in foods below the maximum regulatory limits may not always protect consumers health (Nacim *et al*., 2017). The simulated DALY results in the ANRS and at national level (Figure 5.3) seem to be overestimating the aflatoxin disease burden. That is because for the DALY calculation (Eq. 5), we used the total number of DALY cases for all age groups since the Global Cancer Observatory (GCO) report (231-ethiopia-fact-sheet.pdf) doesn't provide the data for each age category. Thus, we assumed all liver cancer cases and related DALYs occur on people older than 14 years of age (adults). Although the DALY data available in the Global Disease Burden report (ghe2021_daly_bycountry_2021.xlsx) provides DALY data by different age groups, we didn't use these data because WHO mentioned that the death registration data for Ethiopia are unusable due to quality issues. However, it is important to note that our DALY calculation only considered mortality and not morbidity (EQ 5). This means that our DALY estimates would underestimate the actual disease burden. Nevertheless, the total DALY due to aflatoxin exposure in Africa ranges from 50-400/one million persons/year (Gibb *et al*., 2015).

For the 97.5% of sorghum *injera* consumption in the ANRS, the estimated DALY was about 0.05/million persons (Supplementary Table 5.5). Besides sorghum, other food crops grown in our study sites, such as *teff* and chickpea, are also susceptible to mycotoxin contamination (Alemayehu *et al*., 2020; Geremew *et al*., 2018). Consequently, the total exposure to fumonisin and aflatoxin, and the resulting disease burden, could be higher than estimated from sorghum consumption alone. Therefore, future studies need to consider the aflatoxin and fumonisin exposure from all food sources. To further reduce the aflatoxin exposure in the ANRS, interventions on sorghum intake are more interest-

ing than on aflatoxin occurrence (Figure 5.4). Provided that aflatoxin exposure is virtually through food intake (Gibb *et al*., 2015), diversification of food intake, i.e. reducing the daily sorghum intake by partially replacing the sorghum that is used for *injera* preparation with other alternative crops that are less susceptible to mycotoxin contamination, and/or replacing part of the *injera* meal with other safer and healthier alternative foods would be helpful. However, this option is not easy to achieve since finding an alternative grain locally that is less vulnerable to mycotoxin contamination seems difficult. Since sorghum is a staple food, reducing its consumption may be difficult or inappropriate for the local population. A more effective strategy would be to develop and implement a code of practice to mitigate aflatoxin contamination in the local food supply chain, rather than reducing sorghum consumption.

Compared to the estimated aflatoxin exposure and risk in the ANRS, the estimated aflatoxin exposure and risk at the national level were much higher (Supplementary Table 5.5; Figures 5.1-5.3). These variations are due to higher input values used for the national-level calculations, namely aflatoxin concentration, sorghum intake, and the proportion of hepatitis B surface antigen-positive individuals, than at the ANRS level (Eq2, Eq4, Eq5). An increase in body weight would lead to decreased exposure and risk estimates (Eq2); however, the body weight values used at the national level and in the ANRS were similar, as evidenced by the mean body weight of 53 kg nationally and 52 kg in the ANRS. This indicates that body weight contributes very little to the variation in exposure and risk estimates. Despite sorghum intake contributed to aflatoxin exposure more than aflatoxin concentration (Figure 5.4), sorghum intake levels are similar both in the ANRS and at national level (Supplementary Table 5.3). This would imply that the higher aflatoxin concentration in sorghum used to estimate the aflatoxin exposure at national level (93.31 ng/g), which is far higher (about 250%) than the mean aflatoxin concentration in the ANRS (0.37±2.29 ng/g) (Table 5.1) contributed to the high aflatoxin exposure and disease burden at national level. It is important to note that for the mycotoxin concentration at national level, we assumed a worst-case scenario in which the reported mean values for the individual aflatoxins (AFB1, AFB2, AFG1 and AFG2) were present in the same samples. The concentrations were then added together to calculate the total aflatoxin concentration (Table 5.1). This could lead to an overestimation of aflatoxin exposure and the resulting disease burden at a national level. For lifetime risk estimation, we assumed that individuals' EDI remains constant throughout their lifetime. Previous biomarker-based studies in Ethiopia indicated unsafe levels of aflatoxin exposure (Ayele *et al*., 2022; Boshe *et al*., 2020; Tessema *et al*., 2021). Besides intervention on sorghum intake, intervention on reducing aflatoxin contamination is also important.

The intervention would be applied through training consumers to create awareness about safer and healthier alternatives to sorghum *injera*. Intervention on hepatitis B virus

infection would also be important. According to WHO and FAO (2017), the probability of incidence of HCC is 30 times lower in persons that don't have hepatitis B infection story (HBsAg- surface antigen) than in persons with hepatitis infection (HBsAg+ surface antigen).

Although sorghum is the most widely consumed food in our study sites (Supplementary Table 5.1), other grains, such as maize, wheat, and teff, are also main staples in other parts of the country, depending on agroecological conditions that allow for the growth of these grains, cultural food consumption practices, etc. Therefore, aflatoxin exposure results at the national level may over- or underestimate actual exposure levels, depending on the typical staple food crop in each area. For example, teff is the main staple food crop in the highlands of northwest Ethiopia, and sorghum is consumed to a limited extent. Therefore, the aflatoxin exposure assessment results presented in this study may not accurately reflect the actual risk in this region of Ethiopia, as teff, rather than sorghum, may be the primary source of aflatoxin exposure. In addition, although the results of a lifetime aflatoxin exposure assessment provided insight into the extent of aflatoxin risk with lifetime exposure, this estimation may not be realistic. This is because we assumed, for the lifetime exposure assessment, that individuals could be exposed to the same level of aflatoxin throughout their life. However, this assumption is not true since the variables used to estimate risk may change over time. For example, aflatoxin concentration levels may change due to changes in agricultural practices or climate, while sorghum intake levels may change due to public dietary shifts. Therefore, future research should consider a lifespan of 5 or 10 years, during which time changes are presumed minimal.

5.6. CONCLUSIONS

The results in this research provided insights on the fumonisin and aflatoxin exposure from sorghum consumption in the ANRS and at national level in Ethiopia. The related fumonisin exposure does not seem to pose a public health concern in either the ANRS or at national level. On the other hand, aflatoxin exposure is a priority for risk management. Despite aflatoxin exposure showed the presence of public health concern, the corresponding DALY estimates were low in the ANRS. Health concerns have been related mainly to sorghum intake. Therefore, dietary intervention at consumers' level is recommended to reduce aflatoxin exposure. In addition, it would be helpful to implement integrated preventive strategies along the sorghum value chain, covering agricultural production, storage, and handling.

Appendix

Supplementary Table 5.1. Sorghum processing and consumption practices

Variable	Category	Response (%)			
		Total (n=120)	W. Belesa (n=40)	Kalu (n=40)	Kewet (n=40)
Sorghum was the food crop produced most	Yes	74	70	70	85
	No	26	30	30	15
Source of sorghum for food	Own produce	53	65	20	73
	Own produce + market	48	35	80	28
Regular sorghum-based food	*Injera*	100	100	100	100
	Other	0	0	0	0
Washing sorghum grain	Yes	65	0	95	100
	No	35	100	5	0
Dehulling	Yes	0	0	0	0
	No	100	100	100	100
Blending other grain/flour	Yes	77	100	85	45
	No	23	0	15	55
Milling (whole milling)	Yes	100	100	100	100
	No	0	0	0	0
Fermentation	Yes	100	100	100	100
	No	0	0	0	0
Baking	Yes	100	100	100	100
	No	0	0	0	0

Supplementary Table 5.2. Aflatoxin and fumonisin reduction factors during sorghum processing

Process	Mycotoxin reduced	% (mean) reduction	Reference	Average reduction used in this study
RF1	- Aflatoxin - Fumonisin	- 59.36 % - 68.75 %	(Fandohan *et al*., 2005)	- 60 % for aflatoxin - 69 % for fumonisin
RF2	- Aflatoxin - fumonisin	*61 %	(Yetneberk *et al*., 2005)	61 % for
RF3	Fumonisin	57-79 %	(Adebo *et al*., 2019)	68 %** for

*The aflatoxin/fumonisin reduction due to water dilution was determined from the total volume of water added to sorghum flour to prepare a standard sorghum *injera* based on Yetneberk *et al* (2004) assuming the aflatoxin/fumonisin concentration in water is negligible. **The rate of aflatoxin reduction is assumed to be the same as that of fumonisin. Note – *injera* undergoes natural fermentation for 2 to 3 days (Baye *et al* 2013)

Supplementary Table 5.3. Per capita intake of sorghum (in grams) among those who consumed sorghum and sorghum products

*Intake (g)	Mean	SD	median	P25	P75
Amhara National Regional State (ANRS)	344	265	240	134	536
Ethiopia (National)	347	255	284	142	536
*Body wt					
	Mean	SD	Median		P75
Amhara National Regional State	52	8	51	46	56
Ethiopia	53	10	51	47	57

*Survey data was collected from 2019-2023 for women (age: 14-59 years). Data collection took several years due to security situations in Ethiopia

Supplementary Table 5.4. Litereature review data on the occurrence of aflatoxins and fumonisins in sorghum in Ethiopia

S. No	Reference	Studied aflatoxins and fumonisins	Sampling location	Sampling Year	Number of samples	Mycotoxin analysis method
1	Taye. W *et al* 2018	AFB1; Total fumonisin	East Ethiopia (Haramaya University Farm)	2012 and 2013	controlled storage expt	ELISA kit
2	Mohammed *et al* 2022	AFB1, AFB2, AFG1, AFG2, FUB1, FUB2	East Ethiopia (farmers stores -Doba, Fedis, Goro Gutu. and Miesso)	2021	80	LC-MS/MS
3	Chala *et al* 2014	AFB1, AFB2, AFG1, AFG2; FUB1, FB2, FB3	South, East and Northwest Ethiopia (Farmers stores)	not given	70	LC-MS/MS
4	Ayalew *et al* 2006	AFB1; FUMB1. FUMB2. FUMB3	North and East Ethiopia (farmers stores)	1999	82	HPLC (AFT) and ELISA (FUM)
5	Taye *et al* 2016	AFB1	East Ethiopia (farmers stores - Babile)	2013	90	ELISA
6	Ssepuuya *et al* 2018	AFB1, AFB2. AFG1, G2, FMB1, FB2, FB3	value chain actors	2012-2013	381	LC-MS/MS

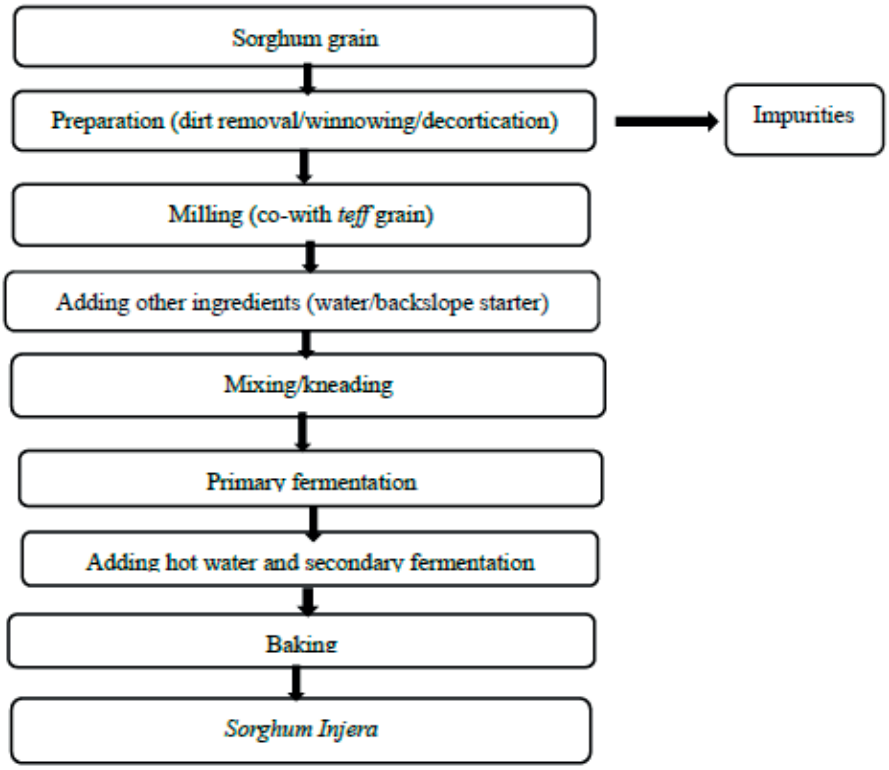
Supplementary Table 5.5. Aflatoxin exposure and related risk from sorghum consumption for adults in Ethiopia (2023)

Percentiles	Exposure (ng/kg bw day)		MOE		HCC/100.000/year		DALY/100.000/year	
	ANRS	National	ANRS	National	ANRS	National	ANRS	National
P25	0.035	26.07	5209.99	7.373	0.0013	0.969	0.001	0.932
P50	0.053	38.14	7564.19	10.465	0.002	1.424	0.002	1.363
P75	0.077	54.20	11518.59	15.369	0.003	2.025	0.003	1.934
P97.5	0.127	89.88	20322.56	32.314	0.005	3.3355	0.005	3.225

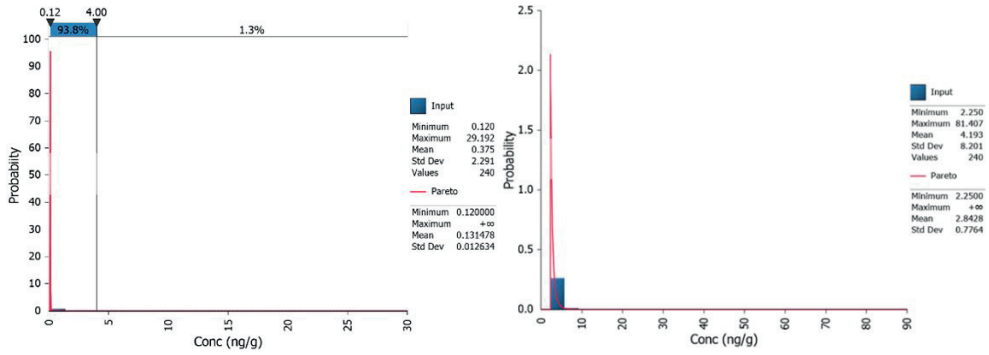
Supplementary Table 5.6. Deterministic determination of aflatoxin exposure and MOE in ANRS considering actual sorghum intake levels

Intake level	mean	Median	P25	P75
Intake weight (g)	344 g	240	134	536
Exposure (ng/g kg bw)	0.124	0.087	0.048	0.193
MOE	3229.974	4622.012	8299.616	2074.904

Supplementary Figure 5.1. Sorghum *injera* processing flow diagram (Baye *et al*. 2014)

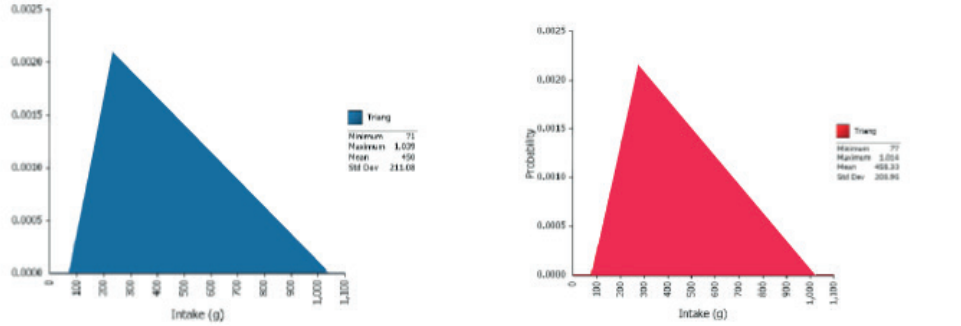


Supplementary Figure 5.2. Statistical data distribution for the occurrence of aflatoxins (a) and fumonisins (b) in sorghum in 2022 in northwest Ethiopia



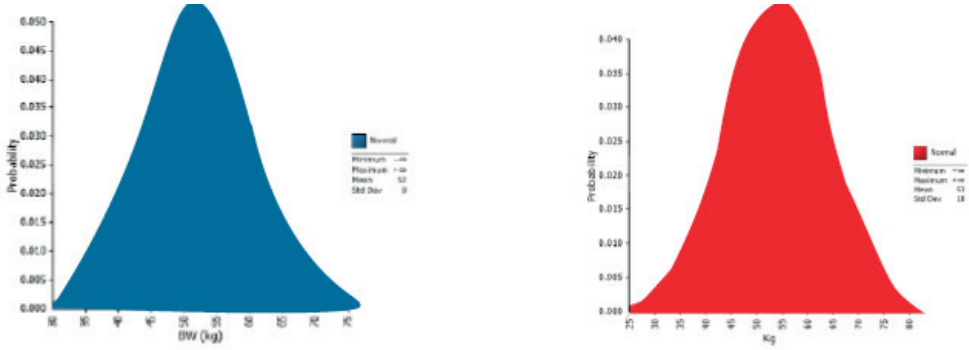
(a) (b)

Supplementary Figure 5.3. Statistical data distribution for sorghum intake data in the ANRS (c) and at National level (d) (Triangle distribution)



c d

Supplementary Figure 5.4. Statistical data distribution for body weight data in the ANRS (a) and national level (b) (Normal distribution)



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CHAPTER 6

General discussion

6.1. OVERVIEW OF THE CHAPTER

To develop and implement feasible measures to prevent and control mycotoxin contamination of staple crops, a full understanding of the local value chain practices, from production to consumption, is important. The overall aim of this study was to develop an integrated approach to reduce the disease burden related to mycotoxin contamination in maize and sorghum in northwest Ethiopia, focusing on effective and low-cost management practices along the crop value chain.

Fungal infection can occur at any stage of the crop value chain, favoring mycotoxin contamination (Dövényi-Nagy *et al*., 2020). In this research, we studied the association between the currently available cereal value chain practices with mycotoxin contamination, in particular for pre-and post-harvest management practices of maize production (Chapter 2), and preharvest (Chapter 3) and postharvest (Chapter 4) management practices of sorghum production in Ethiopia. In Chapters 3 and 4, mycotoxin contamination in sorghum was determined by chemical analysis of the presence of 33 individual mycotoxins that belonged to four different groups of mycotoxins namely *Aspergillus*, *Alternaria*, *Fusarium*, and *Penicillium* mycotoxins. We also estimated human exposure to aflatoxins and fumonisins due to sorghum consumption, and the related disease burden, at national level and in the Amhara National Regional State (ANRS) specifically, the important sorghum producing area in Ethiopia (Chapter 5).

The results of Chapters 2 to 5 are synthesized and discussed in comparison to previous literature (6.1), reflections on the methods and data used in the current research are provided (6.2), and policy implications and future research directions are highlighted (6.3). Finally, overall conclusions obtained from the different research activities are listed.

6.2. SYNTHESIS OF RESULTS

6.2.1. Occurrence of mycotoxins in sorghum

We collected sorghum samples right after harvest (referred to as preharvest samples, Chapter 3), and after storage for six months in the same farmers households (referred to as storage samples, Chapter 4), in Ethiopia in the year 2022-2023. Preharvest sorghum was found to be contaminated with multiple mycotoxins, and mycotoxin type and concentration slightly increased during the 6 months storage period. Only about 4%, 0%, and 0% of the preharvest samples and 7%, 3% and 3% of the stored samples were contaminated with ochratoxin A, aflatoxin and zearalenone, respectively, in concentrations above the EU regulatory limits (Chapter 2 & Chapter 3). Although there is a scarcity of published national level data on the presence of mycotoxins in preharvest sorghum in Ethiopia,

concentrations of both regulated and emerging mycotoxins measured in our samples are in general comparable with results of Mohammed *et al*. (2022a) who reported the presence of multiple regulated and emerging mycotoxins in stored sorghum grain produced in Eastern Ethiopia (in Doba, Fedis, Goro Gutu and Miesso areas) in the 2021 cropping season (Chapter 3 and 4). The slight differences in prevalence and concentration of individual mycotoxins as compared to the study by Mohammed *et al*. (2022a) could be partly due to variabilities in climatic conditions in different agro-ecological zones and years as the geographical distribution and mycotoxin production of fungi depend on season and the climatic conditions present (Adelusi *et al*., 2023; Casu *et al*., 2024; Zingales *et al*., 2022).

In our study, concentrations of the FAO/WHO (2024) and the European (EC (2023)) regulated mycotoxins in the pre- and post-harvest sorghum samples were generally low, with only some of the specific mycotoxins in several samples exceeding the regulatory limits (Chapter 3-5), suggesting that a minor intervention is required to meet international food safety regulations. However, these low concentrations could still pose serious public health concerns for local consumers such as related to exposure to aflatoxins as seen in Chapter 5. Emerging mycotoxins, such as 3-nitropropionic acid, sterigmatocystin, alternariol, alternariol methylether, mycophenolic acid and moniliformin, occurred at higher prevalence and concentrations in both the pre-harvest and stored sorghum samples than the EU regulated mycotoxins (Chapter 3 & 4). These emerging mycotoxins are known to cause lower toxicity to humans and animals than aflatoxins and fumonisins. Although they are less toxic individually, the combined toxicity of exposure to multiple emerging mycotoxins, or their interaction with regulated mycotoxins, is presumed to significantly affect human and animal health (Kolawole *et al.*, 2024).

6.2.2. Preharvest practices influencing mycotoxin contamination

The contamination of about 75% of the preharvest samples with at least one specific mycotoxin would indicate that most of the preharvest management practices are positively related to mycotoxin contamination. Although logistic regression analysis does not allow expressing the total variability explained by the used variables, the results of the Hosmer-Lemeshow test showed that all developed logistic models significantly fit the data regarding the relationship between preharvest variables and mycotoxin contamination ($P > 0.05$) (Chapter 3). The beta coefficients of the regression models for the preharvest practices were low (the maximum being 4.13 for seed treatment). This implies that for one specific agronomical practice, the probability of mycotoxin contamination would change only slightly if the alternative was used. This would also mean that, rather than implementing a single preharvest practice, an integrated combination of practices needs to be implemented to minimize mycotoxin contamination in preharvest samples. Seed treatment which is aimed at preventing ergot infection seemed to contribute to the low mycotoxin contamination of the sorghum samples, indicating that this practice

can contribute to the prevention of both regulated and emerging mycotoxins. However, the fungicide used for seed treatment, thiram powder, is known to be toxic to animals, humans and the environment (Liu *et al*., 2022). Proper regulation is necessary for correct and limited application of seed treatment, or its avoidance is advisable.

The preharvest practices in sorghum could have contributed to the presence of a variety of fungal species that resulted into the occurrence of multiple mycotoxins in the grain. When the growth requirements such as fertilizer application, seedling density, weed density, pesticide application, are below the required level to meet optimal growth requirements, the crop becomes vulnerable to mycotoxin contamination (Dovenyi-Nagy *et al*., 2020; Sadik *et al*., 2023). Although 81% of the respondents responsible for pre-harvest activities indicated to be aware that fungi can infect sorghum during its growth period, only 27% indicated to know that these fungi can produce mycotoxins (Chapter 3). Furthermore, smallholder farmers may lack the resources necessary to properly manage and treat their crops, even if they are aware of fungal infections and mycotoxin contamination. Recently, Atnafu *et al*. (2024) reported the presence of multiple regulated and emerging mycotoxins that belong to the *Fusarium* and *Penicillium* mycotoxin categories in maize samples collected from farmers' growing fields in Southwest Ethiopia in the 2020/2021 cropping season. Our literature review in Chapter 2 also revealed that the majority of preharvest practices for maize in Ethiopia favor mycotoxin contamination.

The application of organic fertilizer during sorghum growth is related to greater mycotoxin contamination than the application of synthetic fertilizer (Chapter 3). Vermicomposting could be adopted as an alternative to organic fertilizer, since farmers' use of inadequate organic fertilizer (animal manure) could be due to capacity issues relating to insufficient cattle for manure production. Vermicomposting is the process of converting organic waste into nutrient-rich compost using earthworms and microorganisms. The resulting compost is a nutrient-rich biological fertilizer containing a variety of microorganisms thought to significantly increase the growth and yield of various field crops. Vermicomposting has attracted a lot of attention, for example as a sustainable biofertilizer and as a means of enhancing crop resistance to disease (Mohite *et al*., 2024). Vermicompost and its derivatives, such as vermiwash, along with associated decomposer bacteria, combat fungal pathogens. The antifungal efficacy of vermicompost may be due to the presence of bioactive compounds in earthworm castings, mucus, skin secretions, and metabolites secreted by decomposer bacteria, which inhibit the growth of various fungal pathogens, including *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, and *Fusarium graminearum* (Gudeta *et al*., 2022).

6.2.3. Storage practices influencing mycotoxin contamination

Unlike the storage stage, the sociodemographic variables were not found to be significantly associated with mycotoxin contamination during the preharvest stage (Chapter 3 & 4). It is important to note that in the majority of households (74%), men are primarily responsible for preharvest farming management activities (MPRP) (Chapter 3), while in the majority of households (63%), women are primarily responsible for storage management activities (MPRS) (Chapter 4). The difference in the influence of sociodemographic variables on mycotoxin contamination may be related to differences in experience (Chapters 3 and 4). The fact that 77% and 81%, respectively, of those responsible for preharvest and storage management did not attend any formal education programs (Chapters 3 and 4) implies that experience is the main sociodemographic variable in their learning about sorghum storage management. However, knowledge of farming practices gained through experience may not be sufficient to implement the correct preventive measures for mycotoxin contamination in the preharvest stage because farmers do not know about mycotoxins. This is due to the need to apply a combination of integrated practices, which smallholder farmers probably cannot apply due to resource constraints, such as a lack of fertilizer, information on weather forecasts for deciding the harvesting period, and pesticides.

On the other hand, knowledge gained through experience can significantly reduce mycotoxin contamination with minor changes to storage management. For instance, 59% of farmers applied insecticides during sorghum storage, and insecticide application was negatively correlated with mycotoxin contamination (Chapter 4). Controlling insects during grain storage is important because insects can damage grain, making the crop vulnerable to fungal infection. Insects can also create moisture accumulation, which can lead to fungal growth and mycotoxin contamination (Chulze, 2010).

Compared to the preharvest stage, the prevalence of multimycotoxin contamination in the sorghum increased during the storage period for six months; from 75 to 88% for any mycotoxin, 67 to 72% for *Aspergillus* mycotoxins, 63 to 67% for *Alternaria*, 58 to 61% for *Fusarium*, and 23 to 31% for *Penicillium* mycotoxins (Chapter 3 & Chapter 4). The increase can be considered as low, indicating the majority of farmers' adherence to good practices has resulted in only a slight increase during storage.

One of the risk factors for mycotoxin contamination, the climatic condition, cannot be controlled during the preharvest period, while it can be controlled during storage (Dövényi-Nagy *et al*., 2020), depending on the storage facilities. It is known that increase in temperature during grain storage increases fungal growth and mycotoxin contamination (Mannaa & Kim, 2017). In this respect, the storage ecosystem (e.g. temperature and relative humidity) in both the indigenous and introduced structures were such that they

limited the growth of fungal species resulting in low mycotoxin contaminations. The lower increase in mycotoxin prevalence in sorghum when stored in indigenous structures as compared to the introduced structures could be due to the better ecosystem in indigenous storage structures, particularly the lower temperature (Chapter 4). The indigenous outdoor storage structures - *sherfa* and pit, and the indoor structure - *gota*, are presumed to create a lower temperature during storage than the ambient temperature. This is because soil (and mud), used to build these structures, are poor conductors of heat (Ochsner, 2019), which means that heat transfer from the external environment or a living room to the grain stored in these structures is low. The temperature of sorghum stored in the introduced structures (sacks), which are always placed indoors, may be higher than the external temperature due to heating effects from food preparation facilities in a living room.

Recently, Ponce *et al*. (2025) discovered that the simultaneous presence of the insect *Sitophilus oryzae* L. and the fungus *Aspergillus flavus* during wheat storage optimizes the abiotic conditions for improved insect fitness. The relative humidity was 7-8% higher in the presence of both species, with insect feeding and movement (plus fungal metabolism) appearing to drive this microclimatic change. Gallan *et al*. (2023) also indicated that such interactions influence the colonisation and transmission of storage fungi through the insect gut. Our results showed that indigenous storage structures are a promising, low-cost option for reducing the prevalence of mycotoxin contamination, outperforming introduced structures. Further improvements to indigenous storage structures require consideration of the dynamics and interactions within the storage ecosystem, including air composition, temperature, relative humidity, insect infestation, fungal growth and mycotoxin contamination.

Storing grain in polypropylene sacks increases the probability of mycotoxin contamination due to the sacks' vulnerability to insect attack and moisture permeability. These factors alter the grain storage ecosystem by increasing the temperature and moisture levels, both of which favor mycotoxin contamination (Kuyu *et al*., 2022; Turner *et al*., 2005). The supply of improved grain storage structures (PICs sacks) for storing grain safely in Ethiopia was identified as problematic due to the sacks being unavailable in local markets, their purchase price, and a lack of awareness about their existence (Mekonen & Wubetie, 2021; Mohammed *et al*., 2022b) (Chapter 2). On the other hand, our results showed that the mycotoxin prevalence in sorghum stored in these sacks was higher than in sorghum stored in local storage structures (Chapter 5). This is probably due to improper use of PICs sacks, and hence, introducing PICs sacks in Ethiopia should be accompanied by proper farmer training on how to use the sacks (Chapter 4).

In our study, we classified mycotoxins based on the major fungal genera that produce them (*Aspergillus*, *Fusarium*, *Alternaria* and *Penicillium* species). Since a single fungal species can produce different types of mycotoxins and specific mycotoxins can be produced by different species (Braun & Wink, 2018), it would be interesting to include the functional and morphological roles of fungi alongside mycotoxin occurrence in future research. Including the ecological functions and morphological diversity of fungi could provide more robust evidence for targeted control strategies, such as pre- and post-harvest interventions.

6.2.4. Mycotoxin exposure and disease burden

Results of Chapter 5 revealed that the measured fumonisin concentrations in all sorghum samples (Chapters 3 and 4) were below the FAO/WHO and European regulatory limits of 4,000 ng/g. This implied a negligible public health risk in the Amhara National Regional State and at the national level.

On the other hand, although only 1.25 % of the sorghum samples in the current research bypassed the European regulatory limit of 4.0 ng/g, and only 0.8% of the samples bypassed the Ethiopian and the FAO/WHO limit of 10.0 ng/g for aflatoxin, the estimated Margin of Exposure (MOE) values of less than 10,000 implied a public health concern at both the ANRS and the national level (Chapter 5), stressing the need for interventions to reduce human exposure to aflatoxin. However, the related health burden estimates, namely the Hepatocellular carcinoma (HCC) and the Disability Adjusted Life Years (DALY), are reasonably low. The low health risk is mainly a result of the relative low prevalence of Hepatitis B virus infection (HBsAg+ surface antigen) in Ethiopia (Chapter 5). For the same aflatoxin exposure, the probability of incidence of HCC is 30 times lower in persons that do not have hepatitis B infection story (HBsAg- surface antigen) compared to persons with hepatitis infection (HBsAg+ surface antigen) (WHO & FAO, 2017). Since the incidence of HCC is directly related to the EDI of aflatoxin, and the MOE is inversely related to EDI (Udovicki *et al*., 2019), the reduction of hepatitis B infection could lead to a decrease in HCC, but increase in MOE. Our DALY calculation considered only mortality, not morbidity, due to the unavailability of data to calculate morbidity from aflatoxin exposure. For instance, the Global Cancer Observatory database (231-ethiopia-fact-sheet.pdf), from which we obtained data on the incidence of new liver cases and related deaths for our DALY estimation (Chapter 5), does not provide data on morbidity. Consequently, the actual DALY estimates may be slightly higher than our estimates, as morbidity adds to DALYs.

We estimated the disease burden of aflatoxin exposure from sorghum consumption for adults. However, besides the disease burden related to HCC, for which we estimated our DALY, aflatoxin exposure is also associated to stunting in children. Stunting is a condi-

tion that reflects chronic undernutrition during the most critical periods of growth and development in early life. It affects physical growth, cognitive development, immune function, and is associated to long-term health impacts (Knipstein1 *et al.*, 2015; Soliman *et al.*, 2021). Tessema (2020) indicated that children aged 6-35 months, selected from rural Ethiopia in 2015–16, were exposed to high levels of aflatoxins through maize consumption. Therefore, it would be interesting to study the extent to which children are stunted as a result of exposure to aflatoxins.

Despite this, we also detected multiple mycotoxin contaminations, including other regulated and emerging mycotoxins. This would imply that there could be additional health burden presumed to occur due to a combined effect of (co-exposure to) multiple mycotoxins (Atnafu *et al*., 2024; Chen *et al*., 2023). Depending on the chemistry of mycotoxins, exposure to multiple mycotoxins can produce synergic, additive, or antagonist toxicity (Karsauliya *et al*., 2022). Unfortunately, there is no method available in the literature to estimate the health burden due to multiple exposure to and the interaction effects of different mycotoxins. In addition, any mycotoxin exposure in human also indirectly aggravates other human diseases by increasing the growth of infectious pathogenic bacteria, damaging the intestinal barrier to pathogens, increasing the host's susceptibility to infectious diseases, and suppressing the immune system, resulting in reduced resistance to infectious diseases (Sun *et al*., 2023).

Besides to sorghum, the most widely consumed crop in our study areas, teff, chickpea, mung bean and tomato produced and consumed in our study sites, are also vulnerable to mycotoxin contamination (Alemayehu *et al*., 2020; Atasever *et al*., 2025; Sori *et al*., 2025). This implies that the estimated health risk in our study can be considered as conservative. Ayalew *et al*. (2006) reported the presence of aflatoxin B1 in teff grain in Ethiopia. The concentrations ranged from 0.0 to 15.6 ng/g. The higher concentrations exceeded the FAO/WHO and EU regulatory limit of 4.0 ng/g for total aflatoxins. In another research, Alemayehu *et al*. (2020) examined the levels of aflatoxin and fumonisin in fresh and stored chickpea samples in different types of sacks in a laboratory setting in Ethiopia. Their results showed that the concentrations of both fumonisins and aflatoxins were below the FAO/WHO (2017) limits. As discussed earlier, the consumption of these other food crops is lower than that of sorghum. Therefore, they can be considered to contribute less to aflatoxin and fumonisin exposure than sorghum. Nevertheless, it is important to note that even low concentrations can increase mycotoxin exposure when combined with other foods, thereby increasing the related risk. However, other data on the occurrence of mycotoxins in these crops produced at our specific study sites is not available in the literature. Maize and wheat are also vulnerable to aflatoxin and fumonisin contamination in Ethiopia (Mamo *et al*., 2020), but they are rarely produced in our study areas. However,

these products can be purchased at the market and consumed, potentially resulting in additional exposure to aflatoxins (fumonisins) and related DALYs.

The relatively low levels of aflatoxins and fumonisins found in sorghum may partly be due to the use of pesticides. Pesticides were commonly applied during both the growth and storage of sorghum (chapters 3 and 4). Control of insect infestation by using pesticides is one of the strategies to reduce the presence of fungal infection and mycotoxins in food crops (D'Mello *et al*., 1998). Although there is a Pesticide Registration and Control Proclamation in Ethiopia which mainly focuses on control across the supply chain actors (Federal Negarit Gazeta (FNG), (FNG, 2010), pesticides have been mis-used in the country, such as the use of illegal and unregistered pesticides, application of higher doses than required, and improper handling. For example, Negede *et al*. (2023) reported that maize growers in the East Wollega zone of Ethiopia widely use a highly toxic fumigant rather than PICs sacks to prevent grain loss due to insect infestation during storage. Although the proper use of pesticides may reduce mycotoxins contamination, their use may also pose a human health risk on its own. Besides to food consumption, humans can be exposed to pesticides through skin, water and inhalation, which would lead to user exposure and related health risk (Hernandez *et al*., 2013). The health risk could even be further increased by co-exposure to mycotoxins and pesticides. For instance, simultaneous exposure to the mycotoxin patulin and the pesticide chlorpyrifos has been reported to enhance toxicity compared to exposure to the two chemicals individually (Fu *et al*., 2022). Training of the local stakeholders in Ethiopia who are involved in pesticide research, regulation, production, use, and marketing has been recommended to reduce the possible risks associated to pesticide application, and at the same time to sustainably improve agricultural productivity (Teshome *et al*., 2023).

6.3. REFLECTIONS ON METHODS AND DATA

6.3.1. Reflection on methods

For practical reasons, preharvest sorghum samples for mycotoxin analysis were collected right after harvest from the batches transported from the field to the farmers' households, rather than at actual harvest, before the sorghum was put into permanent storage structures. The farmers mentioned that they usually put their harvested crops into permanent storage structures a few weeks after harvesting. This is to allow the grain to cool, and to give the farmers time to clean and prepare the storage structures and apply insecticides. The storage structures, *sherfa*, *gota* and *pit*, are usually cleaned and painted with mud and left to dry in the sun before new harvests are stored in these structures. The small delay in sample collection, which occurred because ethical approval of the study was not received in time, may be considered a limitation. New fungal infection may

occur during grain handling, such as during transportation, or the already present fungi may proliferate, which could result in higher levels of mycotoxin contaminations (both in type and concentration). Sorghum can become infected with toxigenic fungi from the soil when it is threshed on the ground (Taye *et al*., 2018). For future research, samples can be taken from the dried sorghum cob while it is still in the field, just before harvesting and threshing, to obtain insights for preharvest mycotoxin contamination. A similar approach was used to collect pre-harvest maize samples from smallholder farmers' farms in southwestern Ethiopia (Atnafu *et al*., 2024). Such an approach, however, does require an incredible higher time and investment effort.

Our initial plan to collect data from 120 households every season for two to three seasons could not be implemented due to security issues related to the outbreak of war in north Ethiopia. As a result, we used data collected from 120 households in one year only. This small sample size limited the number of variables that could be included in the multiple logistic regression. Therefore, we only included variables that showed significant relationships ($P < 0.05$) with mycotoxin contamination in the univariate regression analysis. In addition, we also excluded a few more variables, such as location and field drying duration, since implementing these alternative options for subsistence farmers is not currently achievable (Chapter 3 & 4). Not using all the variables in the multiple logistic regression might be considered a study limitation. This is partly because variables that individually showed insignificant relationships with mycotoxin contamination may show significant relationships when used in combination with other variables, and/or may affect the significance of other individually insignificant variables due to interaction or confounding effects.

Since this study does not assess climatic factors, it is biologically plausible that variations in temperature, rainfall and humidity could influence sorghum preharvest and storage practices, as well as fungal growth and mycotoxin contamination (Magan *et al*., 2011; Paterson & Lima, 2010). Weather conditions during the preharvest period, namely temperature, rainfall and relative humidity, impact preharvest practices such as the incidence of insect infestation and weeds, and their management. They also affect the duration of field drying. Furthermore, weather conditions affect fungal growth and mycotoxin contamination. Future studies should incorporate weather data to clarify these interactions and provide a more comprehensive understanding of the relationship between pre-harvest and storage practices and mycotoxin contamination.

Furthermore, we only had data from one season. As farmers in our study areas only produce sorghum once a year, data from one season would represent one year. While farmer data on preharvest and storage practices could possibly be similar across different years, the data on mycotoxin contamination in sorghum may vary across years. This variation is

related to differences in weather patterns between one year and the other, and possible also indirectly to the variations in the length of the growing seasons, flowering and harvesting dates due to variations in weather conditions (Van der Fels-Klerx *et al*., 2016). This implies that future research should consider data collection in more than one year. In addition, we collected mycotoxin contamination data in six months stored sorghum. Future research should also consider a storage period of around one year, given that farmers would usually store their sorghum until the next harvest is ready to be consumed. The level of mycotoxin contamination usually increases with the duration of grain storage (Taye *et al*., 2018; Wawrzyniak *et al*., 2018).

The delay in receiving ethical approval for the research caused the data collection for preharvest and storage management practices through interviews to be delayed. Since documentation of agricultural management activities by smallholder farmers in Ethiopia is poor, farmers rely on remembering specific events, such as church ceremonies and holidays, to recall exact harvest dates, how many times they plowed land, how many times they removed weeds, and so on. For future studies related to agricultural practices, we recommend that data are collected during or directly after the period each farming activity is carried out. As this practice requires a considerable investment of time and resources from researchers, it would probably be more feasible to train local agricultural development agents to collect and document farm management data. Documented data on the frequency of land plowing, the quantity of inputs used (e.g., grain, fertilizer, and pesticides), the frequency of weed removal, the dates of harvesting and threshing, and the quantity of produce helps obtain the necessary data for mycotoxin and other related research at a lower cost than if researchers collected the data themselves.

Although we focused on the most produced and consumed crops in northwest Ethiopia, being sorghum, in the exposure assessment, other foods consumed may have contributed as well to mycotoxin exposure. Since the total internal dose of mycotoxins comes from all food sources and routes, it would be helpful to validate our results using a human biomonitoring approach. This approach, which measures mycotoxin levels in serum, plasma and urine, provides a better indication of the true internal exposure of mycotoxins (Arce-Lopez *et al*., 2020; Namorado *et al*., 2024). For such a study, in addition to sorghum, other food sources that potentially contribute to internal exposure would also be considered. However, a biomonitoring study faces certain challenges, such as the inclusion of other mycotoxins in human biomonitoring programmes and the need to increase knowledge of mycotoxin metabolism and toxicokinetics (including emerging mycotoxins). Additionally, the absence of biomarkers for modified mycotoxins and the cost of analyzing multiple mycotoxins present challenges (Arce-Lopez *et al*., 2020).

For cost and effectiveness of the suggested management interventions, we used theoretical justifications to compare the costs of the practices that are related to the lower probability of mycotoxin contamination. In this way, a rough indication of the most preferred (considering cost of application and effectiveness) practice could be obtained. In the future, cost and effectiveness studies shall include primary research data from farmers, and the willingness of farmers to switch to the alternative farming practices suggested in this research (Chapter 3 and 4). Moreover, a more comprehensive investigation of the economic impact of the mycotoxin problem and integrated mycotoxin prevention strategies using a One Health Approach would be interesting for the future. The One Health approach incorporates the health of humans, animals and plants, as well as their shared environment (Gomes *et al*., 2023). In addition to the burden on human health, information about animal health burdens, financial costs, and environmental impact helps with integrated decision-making.

6.3.2. Reflection on data

To account for the samples with concentrations below LOD, we applied the substitution method to manage left censored data, based on the EFSA (2010) recommendation with slight modifications. Instead of using the lower and upper bound values separately based on EFSA (2010), we used the average of both lower and upper bound values because the LOD values for the fumonisins (1.5 ng/g) and aflatoxins (0.06 ng/g) were low (Chapter 5). Although a combination of censored and uncensored data can be used to fit the probability function of the distribution data with assumptions, such assumptions for data distributions could have limitations in calculating the maximum-likelihood estimator. The limitation arises from a potential bias resulting from the failure to consider different distributions for the mycotoxin data for undetected (mycotoxin concentrations below the LOD) and detected (mycotoxin concentrations above the LOD) data separately, in case they have different distributions (Tekindal *et al*., 2017). In our research, only 2.1% of the aflatoxin data showed concentrations above the LOD value of 0.06 ng/g, and only 3 of the total samples (1.25 %) bypassed the EU regulatory limit for aflatoxins (4 ng/g) (European Commission Regulation (EC, 2023)), indicating aflatoxin contamination was low. Therefore, we assumed the pareto distribution for both detected and undetected data combined based on the Model-then-Add approach (MTA) using the @Risk software according to Andrade *et al*. (2020). When conducting an exposure assessment of mycotoxins using a large set of data, values below the LOD can be better managed by applying non-parametric probabilistic methods (Tressou *et al*., 2004) which can be considered for future research.

For aflatoxin and fumonisin occurrence data at national level, we used data reported in one research article (Ssepuuya *et al*., 2018) only, since only this study showed practical relevance, as described in Chapter 5. However, this can be taken as one limitation since

these data, collected in December 2012 and December 2013, are more than one decade old. Although the use of this data for exposure assessments have provided some insight into the extent of the problem, the results may not fully reflect the current situation. The levels of mycotoxin contamination observed in Ssepuuya *et al*. (2018) far exceeded the contamination levels obtained in our own study. For example, the concentration ranges (and means) of AFB1, AFB2, AFG1 and AFG2 in this study were 8.9-126 (42.7); 2.7-24 (6.7); 8.1-157 (32.8); and 8.1-157 (32.8) (ng/g), respectively. This would suggest that the disease burden estimates at the national level are overestimated (Chapter 5). However, the use of recent sorghum intake and body weight data for exposure assessment can be considered a strength of the research. These data, which were collected via a survey of a large sample size at both the ANRS and national levels, would provide a sound basis for exposure assessment (Chapter 5).

The weather data obtained from the National Meteorological Institute of Ethiopia for our study sites for the period from 2000 to 2021 (Chapter 3 & 4) showed a slight increase in temperature over time. According to Dusseaua *et al*. (2025) the temperature in Ethiopia has risen by an average of 1.1°C a decade later compared to the temperature in 2000. According to Casu *et al*. (2024), a rise in temperature due to global warming affects fungal physiology towards the production of mycotoxins with greater diversity or concentration. However, our results showed the opposite since our mycotoxin contamination results was lower as observe by Ssepuuya *et al* (2018).

Despite the increase in temperature, which is likely to increase mycotoxin contamination in our research compared to the previous study by Ssepuuya *et al* (2018), the lower mycotoxin contamination in our study could be due to changes in agricultural and storage practices. According to FAO (2020), the activities of Agricultural Transformation Agency (currently the Agricultural Transformation Institute) in Ethiopia through its contribution to smallholder farmers in providing access to agricultural inputs such as fertilizer, extension services such as training of farmers, and agricultural technology has greatly impacted the agricultural food production improving yield. Optimal inputs during the crop-growing stage that improve yield also reduce the crop's vulnerability to mycotoxin contamination (Dovenyi-Nagy *et al*., 2020). In addition, soil management practices to reduce soil acidity such as lime application (Alemu *et al*., 2022; Desta *et al*., 2021) could also have helped to reduce the level of mycotoxin contamination. Reducing crops stress during growth reduces vulnerability of crops to mycotoxin contamination (Keller *et al*., 2022). However, more recent data on weather conditions and soil management practices should be better considered in future research. As described in Chapters 3 and 4, the presence of multiple mycotoxins in sorghum, some of which were detected above regulatory limits, suggests that current pre-harvest and storage practices are insufficient to prevent contamination levels from exceeding these limits.

The unavailability of appropriate and recent weather data for our study sites in the National Meteorology Institute of Ethiopia is also a limitation to this study since we are unable to provide statistical correlations between weather data from the pre-harvest and storage periods and mycotoxin contamination. We could not collect such data ourselves due to limitations in our research budget to procure important data collection tools to measure temperature, relative humidity and rainfall. The use of weather data for the same season as sorghum sample collection, together with preharvest and storage practices data, would provide an opportunity to understand a three-way association of weather conditions, preharvest and storage practices and mycotoxin contamination, which would provide a deeper insight into the risk factors contributing to mycotoxin contamination (Stutt *et al*., 2023). Ideally, this would be done in combination with multiple years of data collection related to mycotoxin contamination in the samples. Such information helps to develop predictive models which will further support the development of an early warning system to prevent the occurrence of mycotoxins, thus providing an opportunity to control mycotoxin contamination. Mycotoxin predictive modelling is a computational or statistical model that estimates the likelihood or level of mycotoxin contamination in crops. It considers factors such as weather conditions, agricultural practices, crop type and fungal ecology. (Cui *et al*., 2022; Dövényi-Nagy *et al*., 2020; Fu *et al*., 2024; Van der Fels-Klerx *et al*., 2016). With effective communication, such information would help farmers to get proper advice on how and when to modify their local preharvest and storage management practices, such as the time when to harvest sorghum and whether to further dry the harvested crop before storage, with the aim to prevent further mycotoxin formation.

We did not consider sorghum variety as a study variable in our research. This is because, with the exception of one of the study sites (Belesa *woreda*), where a limited number of farmers cultivate the improved Melkam variety of sorghum, all the farmers at the other research sites grow local varieties. Therefore, we did not consider the variety of sorghum (improved or local) in our regression analysis, as this might have caused the model to become biased towards predicting the mycotoxin status of the local variety. Additionally, we noted variations in color and local names among the different varieties in each research site. This makes it difficult to group the varieties into two color-based categories for the logistic regression. However, variety requires attention in future research. Taye *et al*. (2022) reported variations in the vulnerability of ten sorghum varieties in Ethiopia to two common fungal species that produce mycotoxins namely *Aspergillus flavus* and *Fusarium verticillioides*, in a field experiment in eastern Ethiopia. Results showed that the Long Muyera variety was the most vulnerable to both fungal species and was contaminated with aflatoxin B1 and total fumonisin beyond regulatory limits. Melkam variety, which is grown in our studied sites, was among the ten varieties that were studied. The findings indicated that this variety is susceptible to infection by the fungus *Fusarium*

verticillioides. Research work on the relationships between sorghum color and mycotoxin contamination in Ethiopia is currently unavailable.

6.4. IMPLICATIONS OF OUR FINDINGS FOR POLICY, BUSINESS, AND FUTURE RESEARCH

Based on the synthesis of our study results, we summarized the findings for policy and business implications below.

6.4.1. Implications for policy

The preharvest interventions suggested in Chapter 2 & 3 for maize and sorghum require transformation of traditional preharvest practices in Ethiopia to improved alternatives while some of them should be kept as is. The traditional method of land tillage with oxen in Ethiopia, which is presumed to favor mycotoxin contamination due to minimal plowing capacity (Chapter 2 & Chapter 3), shall be improved to a deeper plowing capacity and/or it can be replaced by other technologies such as using a tractor that has a deeper plowing capacity. Despite deep plowing negatively affecting soil texture for soils with high silt content (labile soil structure), it is an effective method of improving crop yield in areas experiencing drought stress (Schneider *et al*, 2017). Deep inverted soil (30-40 cm) is reported to result in lower mycotoxin contamination than minimally inverted soil (10-20 cm deep) (Arino *et al*., 2009). In addition, deep plowing has also been reported to increase water storage capacity of soil and improve grain yield (Shi *et al*., 2024). Since sorghum grows in semi-arid areas in Ethiopia (Ministry of Agriculture ((MOA, 2020)), deep plowing would reduce mycotoxin contamination, and at the same time improve yield. Workneh *et al*. (2021) reported that wheat growing smallholder farmers in the Debre Eliyas district, Ethiopia, showed a preference to using a tractor for land plowing than using oxen. Tractors, however, require a high investment of local farmers, and it should be investigated if farmer cooperations could be helpful. In addition, the current practices of fertilizer application (Chapter 2 & Chapter 3) and types of storage structure (Chapter 4) are positively related to mycotoxin contamination in sorghum. This implies that there is a need to improve the supply chain of agricultural inputs, such as fertilizers (subsidies to synthetic fertilizers, training on optimal application of organic fertilizers) to meet optimal growth requirements for sorghum, and PICs sacks (subsidies, improved supply chains) to safer grain storage (Chapter 3 & Chapter 4). Further, in Ethiopia, effective food safety regulation is not in place in local markets (Global Alliance for Improved Nutrition (GAIN, 2022) and regulatory standards are available for a limited number of mycotoxins and limited number of foods only (Mamo *et al*., 2020). Besides reducing mycotoxin exposure for local consumers, the presence of effective regulation can help the country to fully use

regional trade agreements in the future. Aflatoxin control is one of the most important food safety challenges in Africa, and development of regulation has been an important agenda point for the establishment of the African Continental Free Trade Area (AfCFTA) (African Union (AU, 2018)). Proper implementation of the developed national strategic plan for the prevention and control of viral hepatitis in Ethiopia, 2021 - 2025, which aims to eradicate the virus by 2030 (MOH, 2021)) would help in reducing the risk to HCC and related DALY. Interestingly, Aychew *et al*. (2023) reported that households living in the city of Bahir Dar, Ethiopia are willing to pay for hepatitis B vaccination when available for a reasonable price.

Animals can be exposed to mycotoxins such as AFB1 through their feed. Consequently, humans can be exposed to AFM1 through dairy products. Gizachew *et al*. (2016) tested milk samples collected in the Addis Ababa milk shed between September 2014 and February 2015 for AFM1. AFM1 was found in all samples, with contamination levels ranging from 28 to 4,980 ng/g. Overall, only nine (8.2%) of the 110 samples contained 0.5 ng/g or less of AFM1. This suggests that the majority of the samples were contaminated with AFM1 at concentrations higher than the regulatory limit of 0.5 ng/g for milk, as set out by the FAO/WHO (2009). Therefore, it is important to establish regulatory guidelines for mycotoxin contamination in animal feed to reduce human exposure to mycotoxins through animal-based products. Currently, such guidance is not available in Ethiopia.

Since aflatoxin concentrations measured in most of the preharvest and stored sorghum samples of our study were below the regulatory limits, which still would raise public health concern based on the MOE value (MOE < 100,000 (Chapter 5), dietary interventions seem to be more important than preharvest and storage interventions. Dietary interventions can be divided into two categories: processing interventions and diet diversification interventions. A literature review of similar products showed that a combination of manual sorting and windowing, water addition, and spontaneous fermentation for 2–3 days, reduces mycotoxin contamination in sorghum by 60–69%, 61% and 68%, respectively (Chapter 5). These practices should be encouraged and promoted at the consumer level because they can reduce the mycotoxin contamination of sorghum produced at one's own farm or bought from the market. In Chapter 5, almost all the households in Kalu and Kewet *woredas* mentioned washing sorghum before milling it for *injera* preparation. According to Matumba *et al*. (2015), when combined with hand sorting, washing maize grain can remove more than 90% of mycotoxins, including aflatoxins and fumonisins. This practice can be promoted as an intervention option. Since fungi can be present in water (Reis *et al*., 2010), adding disinfectants or properly boiling the water would destroy the fungi and prevent it from becoming a source of infection. The presence of direct sunlight for most of the year for sun drying of grain after washing with water in sorghum producing areas (Chapter 3 & Chapter 4) provides an opportunity for public acceptance of wash-

ing with water as a low-cost intervention option for mycotoxin mitigation. Ultimately, this can reduce mycotoxin exposure. In addition, dietary diversity, important for overall diet quality supporting healthiness of diets (FAO/WHO 2024), has also been already mentioned as one of the possible intervention options to reduce aflatoxin exposure (Wu *et al*., 2014b). Applying a social norms-based intervention for training consumers for dietary diversification helps to achieve societal behavioral change (Talegawkar *et al*., 2021). However, increased knowledge alone does not lead to behavioral change. It should be complemented by interventions to increase the availability, accessibility, affordability and desirability of alternatives locally (Brouwer *et al*., 2021). This option may not be easy to achieve. Finding an alternative grain locally that is less vulnerable to mycotoxin contamination seems challenging (Chapter 5).

6.4.2. Business recommendations

The intervention options mentioned above can be used to create income generation business opportunities as well as achieving positive food safety outcomes. Promoting agricultural entrepreneurship, for example on farm drying methods, improving traditional storage structures, together with training farmers on how to use PICs sacks, are some of the income generating business possibilities. Leavens *et al*. (2021) demonstrated that using a plastic tarp as a drying surface for maize, instead of directly on bare soil, in combination with other mitigation measures in the value chain, is a low cost and effective means of reducing aflatoxin contamination for smallholder farmers in Senegal. Similar interventions could be adopted for drying sorghum cobs in Ethiopia to reduce mycotoxin contamination. Farmers usually sun dry harvested sorghum cob on the ground in the field. Although we did not include the results of sorghum threshing practice in this research because of lack of diversity of practices used among our respondents, the grain will possibly be infected with mycotoxin producing fungi during threshing through the soil contact. All farmers thresh sorghum by putting the sun-dried sorghum cob on top of a bare ground that is painted with cow's dung. Contact with soil during harvesting or threshing of food crops increases fungal infection to grains (Oyesigye *et al*., 2024). Therefore, the development or adoption of sorghum threshing technology could be another opportunity for entrepreneurs. Entrepreneurs can also establish income generating business that improves the supply chain of agricultural and storage inputs including legal agrochemicals such as insecticides and pesticides, production and distribution of organic fertilizer, developing and adopting of low cost and portable grain moisture testers as well as on PICs sacks. For subsistence farmers, improving the indigenous storage structure, *sherfa*, to prevent moisture and air permeability could be a low-cost way of preventing mycotoxin contamination during storage. This could also be considered an income generation activity for agricultural entrepreneurs.

This study was conducted in three locations in the Amhara National Regional State, one of the regions (provinces) of Ethiopia (Chapter 1). However, the findings can also be applied to other regions of the country. Although there could be variations in the weather conditions and agroecological conditions (Tsehaye *et al*., 2016) as well as cultural sorghum production practices across different regions in Ethiopia, the majority of the preharvest and postharvest practices in the country are traditional ones, and similar in different regions (Beyene *et al*., 2016; Mohammed *et al*., 2022a; Taye *et al*., 2018). However, there may also be cultural diversities in the types, applications and skills of these practices across different locations, which should be considered when implementing the recommendations of this research.

6.4.3. Implications for future research

A summary of future research areas is provided below:

- Further research is required, particularly during the pre-harvest stage of crop production, given the limited knowledge of mycotoxin contamination in Ethiopia at this stage.
- Relationship between pre- and post-harvest practices and mycotoxin contamination in multiple years.
- Improvement of indigenous storage structures (*sherfa*, *gota* and *pits)*, to ensure safer grain storage.
- Study pre-harvest and storage management practices for sorghum in other parts of Ethiopia and for other staple food crops, such as wheat, barley and teff.
- Investigate the influence of soil management practices aimed at reducing soil acidity on mycotoxin contamination in the crop.
- Study the effect of *injera* processing practices on the reduction of mycotoxins.
- Study safer alternatives to replace part or all of the sorghum used to make *injera*, or to reduce the amount of sorghum *injera* consumed.
- Investigate the exposure assessment and disease burden related to the consumption of other staple crops by using a combination of dietary intake-based studies and biomonitoring approaches.
- Evaluate the resistance of crop varieties to fungal infection and mycotoxin contamination.
- Study on local ergot preventive practices as potential alternatives to reduce mycotoxin contamination.
- Study the human exposure to, and related disease burden of, the consumption of multiple mycotoxins and their interaction effects.
- Study the cost-effectiveness and environmental sustainability of mycotoxin-reducing pre-harvest and post-harvest practices.

- Study the influence of weather and agroecological conditions, including future predicted weather scenarios, and developing predictive modelling to create early warning systems.
- Study the human exposure and environmental impact of agrochemicals (pesticides, insecticides and herbicides).
- Establishing platforms for documenting agricultural production data for smallholder farmers. This makes data collection and future research easier.
- Study behavioral aspects, such as the willingness of farmers to adopt improved practices and their willingness to pay for them.

6.5. Status of thesis objective

Using both primary and secondary data, we applied relevant statistical methodologies and interpreted statistical outputs to achieve our overall aim of developing an integrated approach to reducing the disease burden related to mycotoxin contamination in maize and sorghum in Ethiopia.

Through a literature review, we adequately addressed RQ1, reporting on the current knowledge regarding the prevention and control of mycotoxins in maize in Ethiopia and highlighting knowledge gaps in pre- and post-harvest practices. We found limited evidence of mycotoxin contamination in maize at the pre-harvest stage, whereas sufficient evidence exists for contamination at the post-harvest stage. Several interventions that can directly or indirectly reduce mycotoxin contamination, such as solar bubble drying and PICs sacks for grain storage, have been implemented at the postharvest stage to some extent.

Using primary data, we were also able to address RQ2 and RQ3 sufficiently. We reported on the extent of mycotoxin contamination in pre-harvested and stored sorghum, caused by 33 different mycotoxins, including both regulated and unregulated ones. For RQ2, we generated new knowledge on the extent of mycotoxin contamination in sorghum and identified practices related to mycotoxin contamination for which data is limited in Ethiopia. For RQ3, we expanded on existing knowledge by generating new insights on the extent of mycotoxin contamination in stored sorghum and identifying practices related to mycotoxin contamination.

We also adequately addressed RQ4. We estimated human exposure and related disease burden from sorghum consumption in the Amhara National Regional State and at national level using the aflatoxin and fumonisin data obtained from RQ2 and RQ3, complemented with additional primary and secondary data. Implementing the recommended

local practices from each of the RQs, along with appropriate intervention options for practices requiring intervention, would reduce the occurrence of mycotoxins and ultimately reduce the disease burden, thus achieving the overall objective.

6.6. CONCLUSIONS

The main conclusions drawn from the results of the PhD research are the following:

- The available literature on mycotoxin contamination of maize in Ethiopia mainly focuses on the post-harvest stage, particularly storage. There is limited knowledge regarding the occurrence of mycotoxin contamination during the pre-harvest stage, and the impact of local processing methods on mycotoxin contamination. Similarly, there is limited research on dietary based exposure assessment.
- Ethiopian farmers have limited awareness about mycotoxins. Mycotoxin issue is not a perceived a concern during the preharvest management and storage management of grains.
- Concentrations of 33 different mycotoxins in both preharvest and stored sorghum samples collected in 2022 in Ethiopia were generally low.
- Most households applied traditional sorghum preharvest and storage management practices. Despite this, concentrations of regulated mycotoxins in most of the sorghum samples were below FAO/WHO and European regulatory limits.
- Both the preharvest and storage management practices are associated with multimycotoxin contamination. The prevalence of mycotoxin contamination is slightly higher in stored samples than preharvest samples.
- For pre-harvest mycotoxin contamination, only practice variables play a significant role, whereas both sociodemographic and practice variables play a role in stored sorghum contamination.
- Human exposure to aflatoxin and fumonisin from sorghum consumption is low in northwestern Ethiopia and in the entire country. However, the low exposure levels seem to be achieved partly by introducing other potential risks to human and the environment such as the use of chemical pesticides.
- Fumonisin exposure showed low public health concern. Based on the MOE value (MOE < 10000), aflatoxin exposure showed priority for risk management.
- A better understanding of local practices, such as sorghum seed treatment with washing with water, can potentially be a source of innovation for developing a sustainable local mycotoxin mitigation strategy.
- To reduce mycotoxin exposure and disease burden, integrated strategies combining good pre-harvest, storage management and dietary intervention practices are needed, rather than a single intervention option.

7. REFERENCES

Abamecha, N. (2021). A survey of farmers' perceptions on maize and sorghum storage duration and level of pest infestations in the case of two selected districts of Jimma zone, Ethiopia. *Hindawi International Journal of Food Science*, 5583387. https://doi.org/10.1155/2021/5583387

Abate, T., Shiferaw, B., Menkir, A., Wegary, D., Kebede, Y., Tesfaye, K., Kassie, M., Bogale, B., Tadesse, B., & Keno, T. (2015). Factors that transformed maize productivity in Ethiopia. *Food Security*, *7*, 965–981. https://doi.org/10.1007/s12571-015-0488-z

Adebo, O. A., Kayitesi, E., & Njobeh, P. B. (2019). Reduction of mycotoxins during fermentation of whole grain sorghum to whole grain *Ting* (a Southern African Food). *Toxins (Basel)*, *11*(3). https://doi.org/10.3390/toxins11030180

Adelusi, O. A., Gbashi, S., Adebo, J. A., Aasa, A. O., Oladeji, O. M., Kah, G., Adebo, O. A., Changwa, R., & Njobeh, P. B. (2023). Seasonal and geographical impact on the mycotoxigenicity of *Aspergillus* and *Fusarium* species isolated from smallholder dairy cattle feeds and feedstuffs in free state and limpopo provinces of South Africa. *Toxins (Basel)*, *15*(2). https://doi.org/10.3390/toxins15020128

Ahmed, Y., Tesfye, E., & Ahmed Yasin, M. (2022). Farmers' willingness to pay for rehabilitation of degraded natural resources under watershed development: The case of Belesa districts, Amhara region of Ethiopia. *Cogent Economics & Finance*, *10*(1). https://doi.org/10.1080/23322039.2022.2041261

Alemayehu, S., Abay, F., Ayimut, K. M., Assefa, D., Chala, A., Mahroof, R., Harvey, J., & Subramanyam, B. (2020). Evaluating different hermetic storage technologies to arrest mold growth, prevent mycotoxin accumulation and preserve germination quality of stored chickpea in Ethiopia. *Journal of Stored Products Research*, *85*. https://doi.org/10.1016/j.jspr.2019.101526

Alemu, E., Selassie, Y. G., & Yitaferu, B. (2022). Effect of lime on selected soil chemical properties, maize (*Zea mays L.*) yield and determination of rate and method of its application in Northwestern Ethiopia. *Heliyon*, *8*(1), e08657.

Alemu, T., Birhanu, G., Azerefgne, F., & Skinnes, H. (2008). Evidence for mycotoxin contamination of maize in Southern Ethiopia: The need for further multidisciplinary research. *Cereal Research Communications*, *36*(6), 337–411.

Altieri, M. A., & Toledo, V. M. (2011). The agroecological revolution in Latin America: rescuing nature, ensuring food sovereignty and empowering peasants. *Journal of Peasant Studies*, *38*(38), 587–612. https://doi.org/10.1080/03066150.2011.582947

Andrade, P. D., Dias, J. V., Souza, D. M., Brito, A. P., van Donkersgoed, G., Pizzutti, I. R., & Caldas, E. D. (2020). Mycotoxins in cereals and cereal-based products: Incidence and probabilistic dietary risk assessment for the Brazilian population. *Food and Chemical Toxicology*, *143*, 111572. https://doi.org/10.1016/j.fct.2020.111572

Arce-Lopez, B., Lizarraga, E., Vettorazzi, A., & Gonzalez-Penas, E. (2020). Human biomonitoring of mycotoxins in blood, plasma and serum in recent years: A review. *Toxins (Basel)*, *12*(3). https://doi.org/10.3390/toxins12030147

Arino, A., Herrera Juan, T., Estopanan, G., Carraminana, J. J., Rota, C., & Herrera, A. (2009). Influence of agricultural practices on the contamination of maize by fumonisin mycotoxins. *Journal of Food Protection*, *72*(4), 898–902.

Asemu, A. M., Habtu, N. G., Delele, M. A., Subramanyam, B., & Alavi, S. (2019). Drying characteristics of maize grain in solar bubble dryer. *Journal of Food Process Engineering*, *43*(2). https://doi.org/10.1111/jfpe.13312

Asemu, A. M., Habtu, N. G., Subramanyam, B., Delele, M. A., Kalsa, K. K., & Alavi, S. (2020). Effects of grain drying methods on postharvest insect infestation and physicochemical characteristics of maize grain. *Journal of Food Process Engineering*, *43*(7), Article e13423. https://doi.org/10.1111/jfpe.13423

Assefa, B. T., Chamberlin, J., van Ittersum, M. K., & Reidsma, P. (2021). Usage and impacts of technologies and management practices in Ethiopian smallholder maize production. *Agriculture*, *11*, 938. https://doi.org/10.3390/agriculture11100938

Atalell, K. A., Techane, M. A., Terefe, B., & Tamir, T. T. (2023). Mapping stunted children in Ethiopia using two decades of data between 2000 and 2019. A geospatial analysis through the Bayesian approach. *Journal of Health Population and Nutrition*, *42*(1), 113. https://doi.org/10.1186/s41043-023-00412-3

Atnafu, B., Garbaba, C. A., Lemessa, F., Migheli, Q., Sulyok, M., & Chala, A. (2024). Multiple mycotoxins associated with maize (*Zea mays* L.) grains harvested from subsistence farmers' fields in southwestern Ethiopia. *Mycotoxin Research*. https://doi.org/10.1007/s12550-024-00536-3

Atnkut, B., Nigussie, A., Berza, B., Mikru, A., Zhao, B., & Astatkie, T. (2025). Mycotoxins contaminations in Ethiopian food: Impacts, challenges, and mitigation strategies. *Food Control*, *171*. https://doi.org/10.1016/j.foodcont.2024.111066

AU. (2018). *First Progress Report of the Chairperson of the Commission on Food Safety, Meeting of the Parliament Representatives' Committee*. Addis Ababa

Ayalew, A., Fehrmann, H., Lepschy, J., Beck, R., & Abate, D. (2006). Natural occurrence of mycotoxins in staple cereals from Ethiopia. *Mycopathologia*, *162*(1), 57–63. https://doi.org/10.1007/s11046-006-0027-8

Aychew, A., Minyihun, A., Tsehay, C. T., Amare, T., & Aschalew, A. Y. (2023). Willingness to pay for hepatitis B virus vaccine and associated factors among households in Bahir Dar City, northwest Ethiopia: using contingent valuation method. *Frontiers Public Health*, *11*, 1058026. https://doi.org/10.3389/fpubh.2023.1058026

Atasever, A., M., Özlü, H., & Atasever, M. (2025). Occurrence and dietary risk assessment of AFB1 in tomato and pepper pastes in Türkiye. *Italian Journal of Food Science*, *37*(1), 410–420. https://doi.org/10.15586/ijfs.v37i1.2678

Ayele, M., Haile, D., Alonso, S., Sime, H., Abera, A., Balcha, K. H., Roba, K. T., Guma, G. T., & Endris, B. S. (2022). Aflatoxin exposure among children of age 12–59 Months in Butajira District, South-Central Ethiopia: a community based cross-sectional study. *BMC Pediatrics*, *22*, 326. https://doi.org/10.1186/s12887-022-03389-w

Balemi, T., Kebede, M., Abera, T., Hailu, G., Gurmu, G., & Getaneh, F. (2019). Some maize agronomic practices in Ethiopia: A review of research experiences and lessons from agronomic panel survey in Oromia and Amhara regions. *African Journal of Agricultural Research in Plant Disease*, *14*(33), 1749–1763. https://doi.org/10.5897/AJAR2019.14338

Baye, K., Mouquet-Rivier, C., Icard-Vernière, C., Picq, C., & Guyot, J. P. (2013). Changes in mineral absorption inhibitors consequent to fermentation of Ethiopian injera: implications for predicted iron bioavailability and bioaccessibility. *International Journal of Food Science & Technology*, *49*(1), 174–180. https://doi.org/10.1111/ijfs.12295

Bereka, T. Y., Kuyu, C. G., Tolera, K. D., & Addis, E. M. (2022). Current postharvest practices and aflatoxin contamination awareness amongst maize producers in Jimma Zone, Southwest of Ethiopia. *World Mycotoxin Journal*, *15*(1), 35–43.

Beyene, A. A., Woldegiorgis, A. Z., Adish, A. A., De Saeger, S., & Tolossa, A. L. (2016). Assessment of mothers' knowledge and practice towards aflatoxin contamination in complementary foods

in Ethiopia: from pre-harvest to household. *World Mycotoxin Journal*, *9*(4), 535–544. https://doi.org/10.3920/wmj2016.2088

Beyene, A. A., Woldegiorgis, A. Z., Adish, A. A., Saeger, S. D., & Tolossa, A. L. (2016). Assessment of mothers' knowledge and practice towards aflatoxin contamination in complementary foods in Ethiopia: from pre-harvest to household. *World Mycotoxin Journal*, *9*(4), 535–544. https://doi.org/10.3920/WMJ2016.2088

Bhardwaj, K., Meneely, J. P., Haughey, S. A., Dean, M., Wall, P., Zhang, G., Baker, B., & Elliott, C. T. (2023). Risk assessments for the dietary intake aflatoxins in food: A systematic review (2016–2022). *Food Control*, *149*, 109687. https://doi.org/10.1016/j.foodcont.2023.109687

Bhat, R., Rai, R. V., & Karim, A. A. (2010). Mycotoxins in food and feed: present status and future concerns. *Comprehensive Reviews in Food Science and Food Safety*, *9*, 57–81.

Biazin, B., Stroosnijder, L., Temesgen, M., AbdulKedir, A., & Sterk, G. (111). The effect of long-term Maresha ploughing on soil physical properties in the Central Rift Valley of Ethiopia. *Soil & Tillage Research*, *111*, 115–122. https://doi.org/10.1016/j.still.2010.09.006

Bikila, G. M., Tola, Y. B., & Kuyu, C. G. (2024). Standardization of teff (*Eragrostis* teff) injera making process conditions for better physicochemical and sensory quality. *Food Science and Nutrition*, *12*(5), 3417-3432. https://doi.org/10.1002/fsn3.4006

Blandino, M., Reyneri, A., & Vanara, F. (2008a). Effect of plant density on toxigenic fungal infection and mycotoxin contamination of maize kernels. *Field Crops Research*, *106*(3), 234–241. https://doi.org/10.1016/j.fcr.2007.12.004

Blandino, M., Reyneri, A., & Vanara, F. (2008b). Influence of nitrogen fertilization on mycotoxin contamination of maize kernels. *Crop Protection*, *27*(2), 222–230. https://doi.org/10.1016/j.cropro.2007.05.008

Boshe, B., Gebremedhin, S., Alemayehu, F., Eshete, M., Taye, M., & Stoecker, B. J. (2020). Aflatoxin exposure among lactating women in southern Ethiopia. *Food Science and Nutrition.*, *8*, 6738–6745. https://doi.org/10.1002/fsn3.1968

Braun, M. S., & Wink, M. (2018). Exposure, occurrence, and chemistry of fumonisins and their cryptic derivatives. *Comprehensive Reviews in Food Science and Food Safety*, *17*(3), 769–791. https://doi.org/10.1111/1541-4337.12334

Breakwell, L., Tevi-Benissan, C., Childs, L., Mihigo, R., & Tohme, R. (2017). The status of hepatitis B control in the African region. *Pan African Medical Journal*, *27*, 17. https://doi.org/10.11604/pamj.supp.2017.27.3.11981

Brouwer, I. D., van Liere, M. J., de Brauw, A., Dominguez-Salas, P., Herforth, A., Kennedy, G., Lachat, C., Omosa, E. B., Talsma, E. F., Vandevijvere, S., Fanzo, J., & Ruel, M. (2021). Reverse thinking: taking a healthy diet perspective towards food systems transformations. *Food Security*, *13*(6), 1497–1523. https://doi.org/10.1007/s12571-021-01204-5

Bucci, T. J., Howard, P. C., Tolleson, W. H., Laborde, J. B., & Hansen, D. K. (1998). Renal effects of fumonisin mycotoxins in animals. *Toxicologic Pathology*, *26*(1), 160–164.

Casu, A., Camardo Leggieri, M., Toscano, P., & Battilani, P. (2024). Changing climate, shifting mycotoxins: A comprehensive review of climate change impact on mycotoxin contamination. *Compr Rev Food Sci Food Saf*, *23*(2), e13323. https://doi.org/10.1111/1541-4337.13323

Cervini, C., Abegaz, B., Mohammed, A., Elias, R., Medina, A., Gebre, K., & Verheecke-Vaessen, C. (2023). Assessment of agricultural practices by Ethiopian women farmers: existence of gender disparities in access to mycotoxins training. *World Mycotoxin Journal*, *16*(3), 227–238. https://doi.org/10.3920/wmj2022.2827

Chala, A., Taye, W., Ayalew, A., Krska, R., Sulyok, M., & Logrieco, A. (2014). Multimycotoxin analysis of sorghum (*Sorghum bicolor* L. Moench) and finger millet (*Eleusine coracana L.* Garten) from Ethiopia. *Food Control*, *45*, 29e35. https://doi.org/10.1016/j.foodcont.2014.04.018

Chauhan, N. M., Washe, A. P., & Minota, T. (2016). Fungal infection and aflatoxin contamination in maize collected from Gedeo zone, Ethiopia. *Springerplus*, *5*(1), 753. https://doi.org/10.1186/s40064-016-2485-x

Chauhan, Y., Wrighta, G. C., & Rachaputi, N. C. (2008). Modelling climatic risks of aflatoxin contamination in maize. *Australian Journal of Experimental Agriculture*, *48*, 358–366. https://doi.org/10.1071/EA06101

Chen, X., Abdallah, M. F., Grootaert, C., Van Nieuwerburgh, F., & Rajkovic, A. (2023). New insights into the combined toxicity of aflatoxin B1 and fumonisin B1 in HepG2 cells using Seahorse respirometry analysis and RNA transcriptome sequencing. *Environonment International*, *175*, 107945. https://doi.org/10.1016/j.envint.2023.107945

Chilaka, C. A., Obidiegwu, J. E., Chilaka, A. C., Atanda, O. O., Mally, M., 2022. Mycotoxin Regulatory Status in Africa: A Decade of Weak Institutional Efforts. *Toxins*, 14, 442. https://doi.org/10.3390/ toxins1407044

Chulze, S. N. (2010). Strategies to reduce mycotoxin levels in maize during storage: a review. *Food Additives and Contaminants: Part A Chemistry, analysis, control, exposure & risk assessment*, *27*(5), 651–657. https://doi.org/10.1080/19440040903573032

CSA. (2021a). *The Federal Democratic Republic of Ethiopia Central Statistical Agency, Agricultural sample survey, 2020 / 2021 (2013 E.C.)(September – January 2020/2021), volume VII, Report on crop and livestock product utilization (private peasant holdings, meher season)*.

CSA. (2021b). *The Federal Democratic Republic of Ethiopia Central Statistical Agency, Agricultural sample survey, 2020/21(2013 E.C.) 2020/21(2013 E.C.) (September – December, 2020), volume III, Report on farm management practices (private peasant holdings, meher season)*

Cui, H., Wang, S., Yang, X., Zhang, W., Chen, M., Wu, Y., Li, S., Li, L., Cai, D., Guo, B., Ye, J., & Wang, S. (2022). Predictive models for assessing the risk of *Fusarium pseudograminearum* mycotoxin contamination in post-harvest wheat with multi-parameter integrated sensors. *Food Chemistry X*, *16*, 100472. https://doi.org/10.1016/j.fochx.2022.100472

D'Mello, J. P. F., Macdonald, A. M. C., Postel, D., Dijksma, W. T. P., Dujardin, A., & Placinta, C. M. (1998). Pesticide use and mycotoxin production in *Fusarium* and *Aspergillus* phytopathogens. *European Journal of Plant Pathology*, *104*, 741–751.

Dabkevičius, Z., & SemaŠKienÉ, R. (2002). Control of ergot (*Claviceps purpurea* (Fr.) Tul.) ascoscarpus formation under the impact of chemical, biological seed dressing. *Plant Protection Science*, *38*(SI 2 - 6th Conf EFPP), 681–683. https://doi.org/10.17221/10589-pps

Damianidis, D., Ortiz, B. V., Bowen, K. L., Windham, G. L., Hoogenboom, G., Hagan, A., Knappenberger, T., Abbas, H. K., Scully, B. T., & Mourtzinis, S. (2018). Minimum temperature, rainfall, and agronomic management impacts on corn grain aflatoxin contamination. *Agronomy Journal*, *110*(5), 1–12. https://doi.org/10.2134/agronj2017.11.0628

Dejene, M., Yuen, J., & Sigvald, R. (2004). The impact of storage methods on storage environment and sorghum grain quality. *Seed Science and Technology*, *32*(2), 511–529. https://doi.org/10.15258/sst.2004.32.2.21

Derese, S. A., Shimelis, H., Laing, M., & Mengistu, F. (2018). The impact of drought on sorghum production, and farmer's varietal and trait preferences, in the north eastern Ethiopia: implications for breeding. *ACTA Agriculture Scandinavica, Section B - Soil & Plant Science*, *68*(5), 424–436. https://doi.org/10.1080/09064710.2017.1418018

Desta, G., Kassawmar, T., Tadesse, M., & Zeleke, G. (2021). Extent and distribution of surface soil acidity in the rainfed areas of Ethiopia. *Land Degradation and Devevopment*, *32*, 5348–5359. https://doi.org/10.1002/ldr.4113

Dong, F., Chen, X., Lei, X., Wu, D., Zhang, Y., Lee, Y. W., Mokoena, M. P., Olaniran, A. O., Li, Y., Shen, G., Liu, X., Xu, J. H., & Shi, J. R. (2023). Effect of Crop Rotation on *Fusarium* Mycotoxins and *Fusarium* Species in Cereals in Sichuan Province (China). *Plant Disease*, *107*(4), 1060–1066. https://doi.org/10.1094/PDIS-01-22-0024-RE

Dovenyi-Nagy, T., Racz, C., Molnar, K., Bako, K., Szlama, Z., Jozwiak, A., Farkas, Z., Pocsi, I., & Dobos, A. C. (2020). Pre-harvest modelling and mitigation of aflatoxins in maize in a changing climatic environment-a review. *Toxins*, *12*(12), 768. https://doi.org/10.3390/toxins12120768

Dusseaua, D., Lute, A., Ezra, A., Cummings, J., Condia, A., Fennelly, A., Gassert, K., Carlos, Dobler-Morales, Fedor, P., McGlinchey, D., & Schwalm, C. (2025). Climate risk assessment in Ethiopia. Policy brief, Woodwell climate research center

EC. (2006). Laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs, Commission Regulation (EC) No 401/2006 of 23 February 2006. *Official Journal of the European Union*, *L 70/12*(9.3.2006).

EC. (2023). Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006 (Text with EEA relevance). *Official Journal of the European Union*, *5.5.2023*(L 119/103).

EFSA. (2020). Risk assessment of aflatoxins in food. *EFSA Journal*, *18*(3), 6040. https://doi.org/10.2903/j.efsa.2020.604010.2903/j

EFSA. (2010). Management of left-censored data in dietary exposure assessment of chemical substances. Scientific report of European Food Safety Authority, *EFSA Journal*, 8(3), 1557.

EPHI. (2025). *Ethiopia Food and Nutrition Strategy Baseline Survey 2021–2022*. Addis Ababa

Fandohan, P., Zoumenou, D., Hounhouigan, D. J., Marasas, W. F. O., Wingfield, M. J., Hell, K., 2005. Fate of aflatoxins and fumonisins during the processing of maize into food products in Benin. *International Journal of Food Microbiology*, 98, 249–259. doi:10.1016/j.ijfoodmicro.2004.07.007

FAO/WHO, 2024. General standard for contaminants and toxins in foods and feed. Revised in forty-seventh session of the codex alimentarius commission in November 2024, CXS 193-1995.

FAOSTAT 2022. Food Balances (2010-). FAOSTAT

FAO. (2020). *Ten years of the Ethiopian Agricultural Transformation Agency. An FAO evaluation of the Agency's impact on agricultural growth and poverty reduction brief* Addis Ababa

FAO/WHO. (2017). Evaluation of certain contaminants in food. In *Eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA/83/SC). WHO Technical Report Series 1002*. Geneva, Switzerland.

FAO/WHO, 2014. Joint FAO and WHO food standards programme codex committee on contaminants. *In food, Eighth Session, Discussion paper on aflatoxin levels in cereals*, CX/CF 14/8/15.

FAO/WHO, 2009. Codex general standard for contaminants and toxins in food and feed (CODEX STAN 193-1995), Amended 2009

FNG. (2010). *Pesticide Registration and Control Proclamation, Federal Negarit Gazeta of the Federal Democratic Republic of Ethiopia*, Addis Ababa.

Fox, G., Nugusu, Y., Nida, H., Tedessa, T., McLean, G., & Jordan, D. (2020). Evaluation of variation in Ethiopian sorghum injera quality with new imaging techniques. *Cereal Chemistry*, *97*, 362–372. https://doi.org/10.1002/cche.10252

Fu, J., Yue, X., Zhang, Q., & Li, P. (2024). Early warning technologies for mycotoxins in grains and oilseeds: A review. *Trends in Food Science & Technology*, 148. https://doi.org/10.1016/j.tifs.2024.104479

Fu, Y., Yin, S., Zhao, C., Fan, L., & Hu, H. (2022). Combined toxicity of food-borne mycotoxins and heavy metals or pesticides. *Toxicon*, *217*, 148–154. https://doi.org/10.1016/j.toxicon.2022.08.012

Fumagalli, F., Ottoboni, M., Pinotti, L., & Cheli, F. (2021). Integrated Mycotoxin Management System in the Feed Supply Chain: Innovative Approaches. In *Toxins* (13):572. https://doi.org/10.3390/toxins13080572

GAIN. (2022). Consumer and vendor perspectives on and practices related to food safety in Ethiopia: A Review. A USAID EatSafe Project Report.

Gallan, D. Z., Henrique, M. O., Silva-Filho, . Silva-Filh., (2023). The Phytopathogen *Fusarium verticillioides* Modifies the Intestinal Morphology of the Sugarcane Borer. *Pathogenes*,12, 443. https://doi.org/10.3390/pathogens12030443

Garbaba, C. A., Denboba, L. G., Mendesil, E., Ocho, F. L., & Hensel, O. (2018b). Actors' post-harvest maize handling practices and allied mycoflora epidemiology in southwestern Ethiopia: Potential for mycotoxin-producing fungi management. *Journal of Applied Botany and Food Quality*, *91*, 237–248.

Garbaba, C. A., Diriba, S., Ocho, F. L., & Hensel, O. (2018a). Potential for mycotoxin-producing fungal growth in various agro-ecological settings and maize storage systems in southwestern Ethiopia. *Journal of Stored Products Research*, *76*, 22–29. https://doi.org/10.1016/j.jspr.2017.12.001

Gebrehiwot, N. T., & Ndinda, C. (2024). The role of teff crop in Ethiopian life: consumption and contribution to rural livelihoods. *CABI Agriculture and Bioscience*. https://doi.org/10.1186/s43170-024-00297-0

Geremew, T., Haesaert, G., Abate, D., & Audenaert, K. (2018). An HPLC-FLD method to measure ochratoxin A in teff (*Eragrostis tef*) and wheat (*Triticum* spp.) destined for the local Ethiopian market. *World Mycotoxin Journal*, *11*(3), 359–368. https://doi.org/10.3920/wmj2017.2175

Getachew, A., Chala, A., Hofgaard, I. S., Brurberg, M. B., Sulyok, M., & Tronsmo, A. M. (2018). Multi-mycotoxin and fungal analysis of maize grains from south and southwestern Ethiopia. *Food Addititives and Contaminants: Part B Surveilliance*, *11*(1), 64–74. https://doi.org/10.1080/19393210.2017.1408698

Getahun, M., Fininsa, C., Mohammed, A., & Bekeko, Z. (2023). Integrated management of wheat (*Triticum aestivum* L.) *Fusarium* head blight and deoxynivalenol contamination through host resistance and fungicide application in Ethiopia. *Journal of Crop Science and Biotechnology*, *27*(2), 195–209. https://doi.org/10.1007/s12892-023-00223-2

Gibb, Devleesschauwer, B., Bolger, P. M., Wu, F., Ezendam, J., Cliff, J., Zeilmaker, M., Verger, P., Pitt, J., Baines, J., Adegoke, G., Afshari, R., Liu, Y., Bokkers, B., van Loveren, H., Mengelers, M., Brandon, E., Havelaar, A. H., & Bellinger, D. (2015). World Health Organization estimates of the global and regional disease burden of four foodborne chemical toxins, 2010: a data synthesis. *F1000Reseaqrch*, *4*, 1393. https://doi.org/10.12688/f1000research.7340.1

Gizachew, D., Szonyi, B., Tegegne, A., Hanson, J., Grace, D., 2016. Aflatoxin contamination of milk and dairy feeds in the Greater Addis Ababa milk shed, Ethiopia. *Food Control* 59:773e779.

Goe, M. R. (1989). The Ethiopian maresha: clarifying design and development. *Northeast African Studies*, *11* (3), 71–112.

Gomes, B., Dias, M., Cervantes, R., Pena, P., Santos, J., Vasconcelos Pinto, M., & Viegas, C. (2023). One Health Approach to Tackle Microbial Contamination on Poultries-A Systematic Review. *Toxins, 11(4), 374*. https://doi.org/10.3390/toxics11040374

Gouas, D., Shi, H., Hainaut, P., 2009. The aflatoxin-induced TP53 mutation at codon 249 (R249S): Biomarker of exposure, early detection and target for therapy. *Cancer Letters,* 286:29-37

Gudeta, K., Bhagat, A., Julka, J. M., Sinha, R., Kumar, A., Kumari, S., Ameen, F., Bhat, S. A., Verma, B., Amarowicz, R., & Sharma, M., (2022). Vermicompost and Its Derivatives against Phytopatho-

genic Fungi in the Soil: A Review: *Horticulturae*, 8:311. https://doi.org/10.3390/horticulturae8040311

Hengsdijk, H., & de Boer, W. J. (2017). Post-harvest management and post-harvest losses of cereals in Ethiopia. *Food Security*, *9*(5), 945–958. https://doi.org/10.1007/s12571-017-0714-y

Hernandez, A. F., Parron, T., Tsatsakis, A. M., Requena, M., Alarcon, R., & Lopez-Guarnido, O. (2013). Toxic effects of pesticide mixtures at a molecular level: their relevance to human health. *Toxicology*, *307*, 136–145. https://doi.org/10.1016/j.tox.2012.06.009

Herojeet, R., Dewangan, R. K., Naik, P. K., & Verma, J. R. (2023). Probabilistic modelling is superior to deterministic approaches in the human health risk assessment: an example from a tribal stretch in central India. *Scientific Reports*, *13*(1), 19351. https://doi.org/10.1038/s41598-023-45622-1

Hussein, M. A., Muche, H., Schmitter, P., Nakawuka, P., Tilahun, S. A., Langan, S., Barron, J., & Steenhuis, T. S. (2019). Deep tillage improves degraded soils in the (Sub) humid Ethiopian highlands. *Land*, *8*, 159. https://doi.org/10.3390/land8110159

ISTA, (2016). International seed testing association, vol. 2016. Chapter 2, i-2-40(46. http://doi.org/10.15258/istarules.2016.02

Jallow, A., Xie, H., Tang, X., Qi, Z., & Li, P. (2021). Worldwide aflatoxin contamination of agricultural products and foods: From occurrence to control. *Comprehensive Reviews in Food Science and Food Safety*, *20*(3), 2332–2381. https://doi.org/10.1111/1541-4337.12734

Janusauskaite, D., Kadžienė, G., & Auškalnienė, O. (2013). The Effect of Tillage System on Soil Microbiota in Relation to Soil Structure. *Polish Journal of Environental Studies*, *22*(5), 1387–1391.

Jian, F., Jayas, D. S., & White, N. D. G. (2009). Temperature fluctuations and moisture migration in wheat stored for 15 months in a metal silo in Canada. *Journal of Stored Products Research*, *45*(2), 82–90. https://doi.org/10.1016/j.jspr.2008.09.004

Kaaya, A. N., Warren, H. L., Kyamanywa, S., & Kyamuhangire, W. (2005). The effect of delayed harvest on moisture content, insect damage, moulds and aflatoxin contamination of maize in Mayuge district of Uganda. *Journal of the Science of Food and Agriculture*, *85*(15), 2595–2599. https://doi.org/10.1002/jsfa.2313

Kabir, A. H., Baki, M. Z. I., Ahmed, B., & Mostofa, M. G. (2024). Current, faltering, and future strategies for advancing microbiome-assisted sustainable agriculture and environmental resilience. *New Crops*, *1*. https://doi.org/10.1016/j.ncrops.2024.100013

Karsauliya, K., Yahavi, C., Pandey, A., Bhateria, M., Sonker, A. K., Pandey, H., Sharma, M., & Singh, S. P. (2022). Co-occurrence of mycotoxins: A review on bioanalytical methods for simultaneous analysis in human biological samples, mixture toxicity and risk assessment strategies. *Toxicon*, *218*, 25–39. https://doi.org/10.1016/j.toxicon.2022.08.016

Kebede, D., Dramadri, I. O., Rubaihayo, P., Odong, T., & Edema, R. (2023). Resistance of Sorghum Genotypes to Ergot (*Claviceps Species*). *Agriculture*, *13,* 1100. https://doi.org/10.3390/agriculture13051100

Keller, B., Russo, T., Rembold, F., Chauhan, Y., Battilani, P., Wenndt, A., & Connett, M. (2022). The potential for aflatoxin predictive risk modelling in sub-Saharan Africa: a review. *World Mycotoxin Journal*, *15*(2), 101–118.

Knipstein, B., Huang, J., Barr, E., Sossenheimer, P., Dietzen, D., Egner, P. A., Groopman, J. D., and Rudnick, D., 2015. Dietary aflatoxin-induced stunting in a novel rat model: evidence for toxin-induced liver injury and hepatic growth hormone resistance. *Pediatric Research*, 78:2

Kolawole, O., Siri-Anusornsak, W., Petchkongkaew, A., Elliott, C., 2024. A systematic review of global occurrence of emerging mycotoxins in crops and animal feeds, and their toxicity in livestock. *Emerging Contaminants*, 10, 100305.

Kucukcakan, B. & Hayrulai-Musliu, Z., 2015. Challenging role of dietary aflatoxin B1 exposure and hepatitis B infection on risk of hepatocellular carcinoma. *Open Access Macedonian Journal of Medical Sciences*. 3(2):363-369

Kuyu, C. G., & Bereka, T. Y. (2020). Review on contribution of indigenous food preparation and preservation techniques to attainment of food security in Ethiopian. *Food Science and Nutrition*, *8*, 3–15. https://doi.org/10.1002/fsn3.1274

Kuyu, C. G., Tola, Y. B., Mohammed, A., Mengesh, A., & Mpagalile, J. J. (2022). Evaluation of different grain storage technologies against storage insect pests over an extended storage time. *Journal of Stored Products Research*, *96*, 101945. https://doi.org/10.1016/j.jspr.2022.101945

Lahouar, A., Marin, S., Crespo-Sempere, A., Said, S., & Sanchis, V. (2016). Effects of temperature, water activity and incubation time on fungal growth and aflatoxin B1 production by toxinogenic *Aspergillus flavus* isolates on sorghum seeds. *Argentinean Journal of Microbiology*, *48*(1), 78–85. https://doi.org/10.1016/j.ram.2015.10.001

Leavens, L., Bauchet, J., & Ricker-Gilbert, J. (2021). After the project is over: Measuring longer-term impacts of a food safety intervention in Senegal. *World Development*, *141*, 105414. https://doi.org/10.1016/j.worlddev.2021.105414

Liang, J., Ning, M., Guan, S., Fang, L., Chen, X., Dong, Z., & Fan, L. (2021). Risk assessment of multiple mycotoxin exposure for consumers of chestnuts in Shandong Province markets in China. *Food Additives & Contaminants: Part A*, *38*(12), 2137–2150. https://doi.org/10.1080/19440049.2021.1970240

Liu, K., Li, Y., Iqbal, M., Tang, Z., & Zhang, H. (2022). Thiram exposure in environment: A critical review on cytotoxicity. *Chemosphere*, *295*, 133928. https://doi.org/10.1016/j.chemosphere.2022.133928

Liu, Y., & Wu, F. (2010). Global burden of aflatoxin-induced hepatocellular carcinoma: A risk Assessment. *Environmental Health Perspectives*, *118*(6), 818–824. https://doi.org/10.1289/ehp.0901388

Liu, Z. M., Li, L. Q., Peng, M. H., Liu, T. W., Qin, Z., Guo, Y., Xiao, K. Y., Ye, X. P., Mo, X. S., Qin, X., Li, S., Yan, L. N., Shen, H. M., Wang, L., Wang, Q., Wang, K. B., Liang, R. X., Wei, Z. L., Ong, C. N.,…Peng, T. (2008). Hepatitis B virus infection contributes to oxidative stress in a population exposed to aflatoxin B1 and high-risk for hepatocellular carcinoma. *Cancer Letters*, *263*(2), 212–222. https://doi.org/10.1016/j.canlet.2008.01.006

Magan, N., Medina, A., & Aldred, D., (2011). Possible climate-change effects on mycotoxincontamination of food crops pre- and postharvest. *Plant Pathology,* 60:150-163. Doi: 10.1111/j.1365-3059.2010.02412.x

Mamo, F. T., Abate, B. A., Tesfaye, K., Nie, C., Wang, G., & Liu, Y. (2020). Mycotoxins in Ethiopia: A review on prevalence, economic and health impacts. *Toxins*, *12*(10). https://doi.org/10.3390/toxins12100648

Mannaa, M., & Kim, K. D. (2017). Influence of temperature and water activity on deleterious fungi and mycotoxin production during grain storage. *Mycobiology*, *45*(4), 240–254. https://doi.org/10.5941/myco.2017.45.4.240

Manu, N., Opit, G. P., Osekre, E. A., Arthur, F. H., Mbata, G., Armstrong, P., Danso, J. K., McNeill, S. G., & Campbell, J. F. (2019). Moisture content, insect pest infestation and mycotoxin levels of maize in markets in the northern region of Ghana. *Journal of Stored Products Research*, *80*, 10–20. https://doi.org/10.1016/j.jspr.2018.10.007

Marasas, W. F., Riley, R. T., Hendricks, K. A., Stevens, V. L., Sadler, T. W., Gelineau-van Waes, J., Missmer, S. A., Cabrera, J., Torres, O., Gelderblom, W. C., Allegood, J., Martinez, C., Maddox, J., Miller, J. D., Starr, L., Sullards, M. C., Roman, A. V., Voss, K. A., Wang, E., & Merrill, A. H., Jr. (2004). Fumonisins

disrupt sphingolipid metabolism, folate transport, and neural tube development in embryo culture and in vivo: a potential risk factor for human neural tube defects among populations consuming fumonisin-contaminated maize. *Journal of Nutrition*, *134*(4), 711–716. https://doi.org/10.1093/jn/134.4.711

Matumba, L., Namaumbo, S., Ngoma, T., Meleke, N., De Boevre, M., Logrieco, A. F., & De Saeger, S. (2021). Five keys to prevention and control of mycotoxins in grains: A proposal. *Global Food Security*, *30*. https://doi.org/10.1016/j.gfs.2021.100562

Matumba, L., Poucke, C. V., Ediage, E. N., Jacobs, B., & Saeger, S. D. (2015). Effectiveness of hand sorting, flotation/washing, dehulling and combinations thereof on the decontamination of mycotoxin-contaminated white maize. *Food Additives & Contaminants: Part A*, *32*(6), 960–969. https://doi.org/10.1080/19440049.2015.1029535

Medina, Á., Rodríguez, A., Sultan, Y., & Magan, N. (2015). Climate change factors and *Aspergillus flavus*: effects on gene expression, growth and aflatoxin production. *World Mycotoxin Journal*, *8*(2), 171–179. https://doi.org/10.3920/wmj2014.1726

Megerssa, M., Negeri, M., Getu, E., Demissie, G., & Selvaraj, S. (2021). Farmers' perceptions, existing knowledge and current control methods of major stored maize grain insect pests in West Showa, Ethiopia. *Archives of Phytopathology and Plant Protection*, *54*(19–20), 1778–1796. https://doi.org/10.1080/03235408.2021.1940648

Mekonen, T. K., & Wubetie, B. Y. (2021). Determinants of the use of hermetic storage bags for maize storage among smallholder farmers in northwest Ethiopia. *Advances in Agriculture*, ID 6644039. https://doi.org/doi.org/10.1155/2021/6644039

Mesfin, A., Lachat, C., Gebreyesus, S. H., Roro, M., Tesfamariam, K., Belachew, T., Boevre, M. D., & Saeger, S. D. (2023). Mycotoxins exposure of lactating women and its relationship with dietary and pre/post-harvest practices in rural Ethiopia. *Toxins*, *15*, 285. https://doi.org/10.3390/toxins15040285

Mesfin, A., Tesfamariam, K., Belachew, T., De Saeger, S., Lachat, C., & De Boevre, M. (2022). Multi-mycotoxin profiling in maize reveals prevalence of *Fusarium* mycotoxins in South and West Ethiopia. *World Mycotoxin Journal*, *15*(1), 73–83. https://doi.org/10.3920/wmj2020.2645

Mesfin, A. H., & Girma, F. (2022). Understanding sorghum farming system and its implication for future research strategies in humid agro ecologies in Western Ethiopia. *Journal of Agriculture and Food Researc*, *10*, 100456. https://doi.org/10.1016/j.jafr.2022.100456

Mihalache, A. O., Torrijos, R., & Dall'Asta, C. (2024). Occurrence of mycotoxins in meat alternatives: Dietary exposure, potential health risks, and burden of disease. *Environment Intternational*, *185*, 108537. https://doi.org/10.1016/j.envint.2024.108537

Milania, J., & Malekib, G. (2014). Review - Effects of processing on mycotoxin stability in cereals. *Journal of the science of food and agriculture*, *94*, 2372–2375. https://doi.org/10.1002/jsfa.6600

MOA. (2020). *Crop Variety Register*. Addis Ababa: Plant Variety Release, Protection and Seed Quality Control Directorate, Ministry of Agriculture, Federal Democratic Republic of Ethiopia

MOH. (2021). *National strategic plan for prevention and control of viral hepatiti in Ethiopia, 2021-2025*. Addis Ababa, Ethiopia

Mohammed, A., Bekeko, Z., Yusufe, M., Sulyok, M., & Krska, R. (2022a). Fungal species and multi-mycotoxin associated with post-harvest sorghum (*Sorghum bicolor* (L.) Moench) grain in Eastern Ethiopia. *Toxins*, *14*, 473. https://doi.org/10.3390/toxins14070473

Mohammed, A., Seid, A., Terefe, H., Cervini, C., & Verheecke-Vaessen, C. (2022b). Harvest and post-harvest handling practices associated with fumonisin B1 contamination in maize (*Zea mays* L.): dietary exposure and risk characterization in eastern Ethiopia. *Mycotoxin Research*, *38*(4), 275–287. https://doi.org/10.1007/s12550-022-00468-w

Mohammed, A., Seyoum, C., Yousuf, J., Mweetwa, A., Odera, J. A., Okello, D. K., Bekeko, Z., Tadessa, T., & Sulyok, M. (2023). Multi-mycotoxins analysis in post-harvest maize (Zea mays L.) grain from major producing areas of Ethiopia. *World Mycotoxin Journal*, *16*(3), 261–272. https://doi.org/10.3920/wmj2022.2829

Mohite, D. D., Chavan, S. S., Jadhav, V. S., Kanase, T., Kadam, M. A., Singh, A. S., (2024). Vermicomposting: a holistic approach for sustainable crop production, nutrient-rich bio fertilizer, and environmental restoration. *Discover Sustainability,* (2024) 5:60 | https://doi.org/10.1007/s43621-024-00245-y

Moretti, A., Pascale, M., & Logrieco, A. F. (2019). Mycotoxin risks under a climate change scenario in Europe. *Trends in Food Science & Technology*, *84*, 38–40. https://doi.org/10.1016/j.tifs.2018.03.008

Moretti, A., & Susca, A. (2017). *Mycotoxigenic Fungi Methods and Protocols* (Vol. 1542). Springer.

Muleta, O. D., Tola, Y. B., & Hofacker, W. C. (2021). Assessment of storage losses and comparison of underground and aboveground storage for better stability and quality of maize (*zea mays* l.) and sorghum (S*orghum bicolor* l.) grains in selected districts of jimma zone, Ethiopia. *Journal of Stored Products Research*, *93*, 101847.

Munkvold, G. P. (2003). Cultural and genetic approaches to managing mycotoxins in maize. *Annual Reviews in Phytopathology*, *41*, 99–116. https://doi.org/10.1146/annurev.phyto.41.052002.095510

Nacim, N. M., Hammoudi, A., Radjef, M. S., Hamza, O., & Perito, M. A. (2017). How do food safety regulations influence market price? A theoretical analysis. *British Food Journal*, *119*(8), 1687–1704. https://doi.org/10.1108/bfj-12-2016-0594

Nakano, Y., Tsusaka, T. W., Aida, T., & Pede, V. O. (2018). Is farmer-to-farmer extension effective? The impact of training on technology adoption and rice farming productivity in Tanzania. *World Development*, *105*, 336–351. https://doi.org/10.1016/j.worlddev.2017.12.013

Namorado, S., Martins, C., Ogura, J., Assuncao, R., Vasco, E., Appenzeller, B., T, I. H., Janasik, B., Kolossa-Gehring, M., Van Nieuwenhuyse, A., Olafsdottir, K., Rambaud, L., Riou, M., Silva, S., Wasowicz, W., Weber, T., Esteban-Lopez, M., Castano, A., Gilles, L.,…Alvito, P. (2024). Exposure assessment of the European adult population to deoxynivalenol - Results from the HBM4EU Aligned Studies. *Food Research International*, *198*, 115281. https://doi.org/10.1016/j.foodres.2024.115281

Ndemera, M., Landschoot, S., Boevre, M. D., Nyanga, L. K., & Saeger, S. D. (2018). Effect of agronomic practices and weather conditions on mycotoxins in maize: a case study of subsistence farming households in Zimbabwe. *World Mycotoxin Journal,*, *11*(3), 421–436. https://doi.org/10.3920/WMJ2017.2227

Negede, B. M., De Groote, H., Minten, B., & Voors, M. (2023). Does access to improved grain storage technology increase farmers' welfare? Experimental evidence from maize farming in Ethiopia. *Journal of Agricultural Economics*. https://doi.org/10.1111/1477-9552.12546

Neme, K., & Mohammed, A. (2017). Mycotoxin occurrence in grains and the role of postharvest management as a mitigation strategies. A review. *Food Control*, *78*, 412e425. https://doi.org/10.1016/j.foodcont.2017.03.012

Nesci, A., Barros, G., Castillo, C., & Etcheverry, M. (2006). Soil fungal population in preharvest maize ecosystem in different tillage practices in Argentina. *Soil & Tillage Research*, *91*, 143–149. https://doi.org/10.1016/j.still.2005.11.014

Ochsner, T. (2019). *Rain or Shine, An Introduction to Soil Physical Processes* (Vol. 3). Oklahoma State University Libraries Stillwater.

Okechukwu, V. O., Adelusi, O. A., Kappo, A. P., Njobeh, P. B., & Mamo, M. A. (2024). Aflatoxins: Occurrence, biosynthesis, mechanism of action and effects, conventional/emerging detection techniques. *Food Chemistry*, *436*, 137775. https://doi.org/10.1016/j.foodchem.2023.137775

Omodara, M. A., McNeill, S. G., & Montross, M. D. (2021). Water vapor permeability of bag materials used for corn storage. *Agricultural Engineering International: CIGR Journal*, *23*(4), 329–340.

Ortega-Beltran, A., & Bandyopadhyay, R. (2021). Contributions of integrated aflatoxin management strategies to achieve the sustainable development goals in various African countries. *Global Food Security*, *30*. https://doi.org/10.1016/j.gfs.2021.100559

Ostry, V., Malir, F., Toman, J., & Grosse, Y. (2017). Mycotoxins as human carcinogens-the IARC Monographs classification. *Mycotoxin Res*, *33*, 65–73. https://doi.org/10.1007/s12550-016-0265-7

Oyesigye, E., Cervini, C., Mahuku, G., & Medina, A. (2024). Potential impact of current agricultural practices on mycotoxin occurrence and mycotoxin knowledge along the cassava value chain in Uganda. *Journal of Food Protection*, *87*(9), 100340. https://doi.org/10.1016/j.jfp.2024.100340

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., & al., e. (2021). PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews *BMJ*, *372*, 160. https://doi.org/10.1136/bmj.n160

Pandey, A. K., Samota, M. K., Kumar, A., Silva, A. S., & Dubey, N. K. (202). Fungal mycotoxins in food commodities: present status and future concerns [Review]. *Frontiers in Sustainable Food Systems*, *7*, Article 1162595. https://doi.org/10.3389/fsufs.2023.1162595

Paterson, r. r. m., & Lima, n., (2010). How will climate change affect mycotoxins in food? *Food Research International*, 43:1902-1914. doi:10.1016/j.foodres.2009.07.010

Pitt, J. I., Taniwaki, M. H., & Cole, M. B. (2013). Mycotoxin production in major crops as influenced by growing, harvesting, storage and processing, with emphasis on the achievement of Food Safety Objectives. *Food Control*, *32*, 205e215. https://doi.org/10.1016/j.foodcont.2012.11.023

Ponce, M. A., Kim, T. N., Morrison, W. R., (2025). Multiple infestation of a grain mass by *Sitophilus oryzae* L. (Coleoptera: Curculionidae) and the fungus, *Aspergillus flavus,* optimizes abiotic conditions for improved insect fitness. *Environmental Entomology*, 54(4): 802-809. https://doi.org/10.1093/ee/nvaf067

Qiu, J., Dong, F., Yu, M., Xu, J., & Shi, J. (2016). Effect of preceding crop on *Fusarium* species and mycotoxin contamination of wheat grains. *Journal of the science of food and agriculture*, *96*(13), 4536–4541. https://doi.org/10.1002/jsfa.7670

Rasheed, H., Xu, Y., Kimanya, M. E., Pan, X., Li, Z., Zou, X., Shirima, C. P., Holmes, M., Routledge, M. N., & Gong, Y. Y. (2021). Estimating the health burden of aflatoxin attributable stunting among children in low income countries of Africa. *Scientific Reports*, *11*(1), 1619. https://doi.org/10.1038/s41598-020-80356-4

Regassa, B., Abraham, A., Fininsa, C., Wegary, D., & WoldeHawariat, Y. (2020). Distribution of maize lethal necrosis epidemics and its association with cropping systems and cultural practices in Ethiopia. *Crop Protection*, *134*, 105151. https://doi.org/10.1016/j.cropro.2020.105151

Reis, T. A. D., Zorzete, P., Pozzi, C. R., Nascimento da Silva, V., Ortega, E., & Correa, B. (2010). Mycoflora and fumonisin contamination in Brazilian sorghum from sowing to harvest. *Journal of the Science of Food and Agriculture*, *90*(9), 1445–1451. https://doi.org/10.1002/jsfa.3962

Ren, Y., Zhang, Y., Lai, S., Han, Z., & Wu, Y. (2011). Simultaneous determination of fumonisins B1, B2 and B3 contaminants in maize by ultra high-performance liquid chromatography tandem mass spectrometry. *Analytica Chimica Acta*, *692*(1-2), 138–145. https://doi.org/10.1016/j.aca.2011.03.005

Roman, F., Garbaba, C. A., Schellert, C., & Hensel, O. (2020a). A modification of a traditional Ethiopian maize store for solar powered ambient drying to reduce post-harvest losses. *Scientific Reports*, *10*(1), 11588. https://doi.org/10.1038/s41598-020-68485-2

Sadik, J. A., Fentahun, N., Brouwer, I. D., Tessema, M., Abera M., & van der Fels-Klerx, H. J. (2025). Storage management practices and mycotoxin contamination of sorghum (*Sorghum bicolor*) in northwest Ethiopia. *Journal of Stored Products Research*, 111:102535.

Sadik, J. A., Fentahun, N., Brouwer, I. D., Tessema, M., & van der Fels-Klerx, H. J. (2023). Preharvest and postharvest management practices related to mycotoxin contamination in maize in Ethiopia – a review. *World Mycotoxin Journal*, *16*(3), 211–226. https://doi.org/10.1163/18750796-20232839

Sandoval, I. G., Sebastiaan Wesseling, & Rietjens, I. M. C. M. (2019). Aflatoxin B1 in nixtamalized maize in Mexico; Occurrence and accompanying risk assessment. *Toxicology Reports*, *6*, 1135–1142. https://doi.org/10.1016/j.toxrep.2019.10.008

Schneider, F., Don, A., Hennings, I., Schmittmann, O., Seidel, S. J., 2017. The effect of deep tillage on crop yield – What do we really know? - Review. *Soil & Tillage Research*, 174, 193–204.

Shephard, G. S., Westhuizen, L. V. D., & Sewram, V., 2007. Biomarkers of exposure to fumonisin mycotoxins: A review. *Food Additives and Contaminants*, 24(10): 1196-1201.

Shi, X., Li, C., Li, P., Zong, Y., Zhang, D., Gao, Z., Hao, X., Wang, J., & Lam, S. K. (2024). Deep plowing increases soil water storage and wheat yield in a semiarid region of Loess Plateau in China: A simulation study. *Field Crops Research*, *308*. https://doi.org/10.1016/j.fcr.2024.109299

Sime, G., Aune, J. B., & Mohammed, H. (2015). Agronomic and economic response of tillage and water conservation management in maize, central rift valley in Ethiopia. *Soil and Tillage Research*, *148*, 20–30. https://doi.org/10.1016/j.still.2014.12.001

Smith, L. E., Stoltzfus, R. J., & Prendergast, A. (2012). Food chain mycotoxin exposure, gut health, and impaired growth: a conceptual framework. *Advances in Nutrition*, *3*(4), 526–531. https://doi.org/10.3945/an.112.002188

Soliman, A., Sanctis, V. D., Alaaraj, N., Ahmed, S., Alyafei, F., Hamed, N., Soliman, N., 2021. Early and long-term consequences of nutritional stunting: From childhood to adulthood. *Acta Biomedica*; 92:1: e2021168.

Sori, A., Bishaw, Z., Fikre, A., Dejene, M., & Kaske, K. (2025). Assessment of on-farm saved chickpea (*Cicer arietinum* L.) seed quality in central Ethiopia. *Legume Science*, *7*(1). https://doi.org/10.1002/leg3.70020

Ssepuuya, G., Ediage, C. V. P. E. N., Tritscher, C. M. A., Verger, P., Kenny, M., Bessy, C., & Saeger, S. D. (2018). Mycotoxin contamination of sorghum and its contribution to human dietary exposure in four sub-Saharan countries. *Food Additives and Contaminants Part A*, *35*(7), 1384–1393. https://doi.org/10.1080/19440049.2018.1461253

Stutt, R., Castle, M. D., Markwell, P., Baker, R., & Gilligan, C. A. (2023). An integrated model for pre- and post-harvest aflatoxin contamination in maize. *NPJ Science of Food*, *7*(1), 60. https://doi.org/10.1038/s41538-023-00238-7

Sun, Y., Song, Y., Long, M., & Yang, S. (2023). Immunotoxicity of three environmental mycotoxins and their risks of increasing pathogen infections. *Toxins*, *15*(3). https://doi.org/10.3390/toxins15030187

Sunano, Y. (2020). Traditional storage pit, polota, for storing sorghum as a long-term survival strategy in Dirashe special Worenda, Southern Ethiopia. *Agroecology and Sustainable Food Systems*, *44* (4), 536–559. https://doi.org/10.1080/21683565.2019.1631933

Suraj, M., Abewaa, M., Mengistu, A., Bultosa, G., & Bussa, N. (2024). Influence of fermentation conditions, and the blends of sorghum and carrot pulp supplementation on the nutritional and

sensory quality of tef injera. *Scientific Reports*, *14*(1), 12819. https://doi.org/10.1038/s41598-024-62420-5

Talegawkar, S. A., Jin, Y., Sedlander, E., Ganjoo, R., Behera, S., DiPietro, L., & Rimal, R. (2021). A social norms-based intervention improves dietary diversity among women in rural India: The reduction in anemia through Normative Innovations (RANI) Project. *Nutrients*, *13*(8). https://doi.org/10.3390/nu13082822

Taye, W., Ayalew, A., Challa, A., & Dejene, M. (2016). Aflatoxin B1 and total fumonisin contamination and their producing fungi in fresh and stored sorghum grain in East Hararghe, Ethiopia. *Food Additives & Contaminants: Part B Surveillance*, 1939–3229. https://doi.org/10.1080/19393210.2016.1184190

Taye, W., Ayalew, A., Dejene, M., & Chala, A. (2018). Fungal invasion and mycotoxin contamination of stored sorghum grain as influenced by threshing methods. *International Journal of Pest Management*, *64*(1), 66–76. https://doi.org/10.1080/09670874.2017.1327681

Taye, W., Dejene, M., Ayalew, A., & Chala, A. (2022). Evaluation of sorghum genotypes for their reaction to major grain molds and mycotoxin-producing fungi in two climates of Ethiopia. *Israel Journal of Plant Sciences*, *69*(1-2), 87–99. https://doi.org/10.1163/22238980-bja10050

Tekeste, S., & Degu, Y. M. (2019). Performance evaluation of motorized maize sheller. Advances of science and technology, 7th EAI international conference (ICAST 2019) Proceedings, Bahir Dar, Ethiopia, August 2–4,.

Tekindal, M. A., Erdogan, B. D., & Yavuz, Y. (2017). Evaluating left-censored data through substitution, parametric, semi-parametric, and nonparametric methods: A simulation study. *Interdisciplinary Sciences*, *9*(2), 153–172. https://doi.org/10.1007/s12539-015-0132-9

Tesfamariam, K., Argaw, A., Hanley-Cook, G. T., Gebreyesus, S. H., Kolsteren, P., Belachew, T., Velde, M. V. d., Saeger, S. D., Boevre, M. D., & Lachat, C. (2022). Multiple mycotoxin exposure during pregnancy and risks of adverse birth outcomes: a prospective cohort study in rural Ethiopia. *Environment International*, *160*, 107052. https://doi.org/10.1016/j.envint.2021.107052

Teshome, A., Terefe, G., Getaw, H., & Koomen, I. (2023). *Pesticide Lifecycle in Ethiopia: challenges, opportunities and leverage points* (Resilient Agriculture for Inclusive and Sustainable Ethiopian Food Systems (RAISE FS), Issue.

Tessema, M., Groote, H. D., Brouwer, I. D., Boevre, M. D., Corominas, A. V., Stoecker, B. J., Feskens, E. J., Belachew, T., Karakitsou, A., & Gunaratna, N. S. (2021). Exposure to aflatoxins and fumonisins and linear growth of children in rural Ethiopia: a longitudinal study. *Public Health Nutrition*. https://doi.org/10.1017/S1368980021000422

Tressou, J., Leblanc, J., Feinberg, M., & Bertail, P. (2004). Statistical methodology to evaluate food exposure to a contaminant and influence of sanitary limits: application to Ochratoxin A. *Regulatory Toxicology and Pharmacology*, *40*(3), 252–263. https://doi.org/10.1016/j.yrtph.2004.07.005

Tsehaye, H., Brurberg, M. B., Sundheim, L., Assefa, D., Tronsmo, A., & Tronsmo, A. M. (2017). Natural occurrence of *Fusarium* species and fumonisin on maize grains in Ethiopia. *European Journal of Plant Pathology*, *147*(1), 141–155.

Tsehaye, H., Elameen, A., Tronsmo, A. M., Sundheim, L., Tronsmo, A., Assefa, D., & Brurberg, M. B. (2016). Genetic variation among *Fusarium verticillioides* isolates associated with Ethiopian maize kernels as revealed by AFLP analysis. *European Journal of Plant Pathology*, *146*(4), 807–816.

Tubajika, K. M., Mascagni, H. J., Damann, K. E., & Russin, J. S. (1999). Nitrogen fertilizer influence on aflatoxin contamination of corn in Louisiana. *Journal of Agriculture & Food Chemistry*, *47*, 5257–5260.

Tubbs, T., Baributsa, D., & Woloshuk, C. (2016). Impact of opening hermetic storage bags on grain quality, fungal growth and aflatoxin accumulation. *Journal of Stored Products Research*, *69*, 276–281. https://doi.org/10.1016/j.jspr.2016.10.003

Turner, P. C., Sylla, A., YGong, Y., Diallo, M. S., Sutcliffe, A. E., Hall, A. J., & Wild, C. P. (2005). Reduction in exposure to carcinogenic aflatoxins by postharvest intervention measures in west Africa: a community-based intervention study. *Lancet* (365), 1950–1956.

Udomkun, P., Wiredu, A. N., Nagle, M., Muller, J., Vanlauwe, B., & Bandyopadhyay, R. (2017). Innovative technologies to manage aflatoxins in foods and feeds and the profitability of application - A review. *Food Control*, *76*, 127–138. https://doi.org/10.1016/j.foodcont.2017.01.008

Udomkun, P., Wossen, T., Nabahungu, N. L., Mutegi, C., Vanlauwe, B., & Bandyopadhyay, R. (2018). Incidence and farmers' knowledge of aflatoxin contamination and control in Eastern Democratic Republic of Congo. *Food Science and Nutrition* (6), 1607–1620. https://doi.org/10.1002/fsn3.735

Udovicki, B., Djekic, I., Kljusuric, J. G., Papageorgiou, M., Skendi, A., Djugum, J., & Rajkovic, A. (2019). Exposure assessment and risk characterization of aflatoxins intake through consumption of maize products in the adult populations of Serbia, Croatia and Greece. *Food Additives & Contaminants: Part A*, *36*(6), 940–951. https://doi.org/10.1080/19440049.2019.1600748

Unnevehr, L. (2015). Food safety in developing countries: Moving beyond exports. *Global Food Security*, *4*, 24–29. https://doi.org/10.1016/j.gfs.2014.12.001

Van den Berg, H., & Jiggins, J. (2007). Investing in Farmers—The impacts of farmer field schools in relation to integrated pest management. *World Development*, *35*(4), 663–686. https://doi.org/10.1016/j.worlddev.2006.05.004

Van der Fels-Klerx, H. J., Liu, C., & Battilani, P. (2016). Modelling climate change impacts on mycotoxin contamination. *World Mycotoxin Journal*, *9*(5), 717–726. https://doi.org/10.3920/wmj2016.2066

Van Dijk, M., Morley, T., Van Loond, M., Reidsmad, P., Tesfayee, K., & Van Ittersum, M. K. (2020). Reducing the maize yield gap in Ethiopia: Decomposition and policy simulation. *Agricultural Systems*, *183*, 102828. https://doi.org/10.1016/j.agsy.2020.102828

Venter, Z. S., Jacobs, K., & Hawkins, H.-J. (2016). The impact of crop rotation on soil microbial diversity: A meta-analysis. *Pedobiologia*, *59*(4), 215–223. https://doi.org/10.1016/j.pedobi.2016.04.001

Vipham, J. L., Amenu, K., Alonso, S., Ndahetuye, J.-B., Zereyesus, Y., Nishimwe, K., Bowers, E., Maier, D., Sah, K., Havelaar, A., & Grace, D. (2020). No food security without food safety: Lessons from livestock related research. *Global Food Security*, *26*. https://doi.org/10.1016/j.gfs.2020.100382

Walker, S., Jaime, R., Kagot, V., & Probst, C. (2018). Comparative effects of hermetic and traditional storage devices on maize grain: Mycotoxin development, insect infestation and grain quality. *Journal of Stored Products Research*, *77*, 34–44. https://doi.org/10.1016/j.jspr.2018.02.002

Warth, B., Sulyok, M., & Krska, R., 2013. LC-MS/MS-based multibiomarker approaches for the assessment of human exposure to mycotoxins. *Analytical and Bioanalytical Chemistry*, 405:5687-5695.

Wawrzyniak, J., Waśkiewicz, A., & Ryniecki, A. (2018). Evaluation of critical points of mould growth and mycotoxin production in the stored barley ecosystem with a hazardous initial microbiological state of grain. *Journal of Stored Products Research*, *77*, 166–176. https://doi.org/10.1016/j.jspr.2018.04.008

Weledesemayat, G. T., Gezmu, T. B., Woldegiorgis, A. Z., & Gemede, H. F. (2016). Study on *Aspergillus* species and aflatoxin levels in sorghum (*Sorghum bicolor* L.) stored for different period and storage system in Kewet districts, northern Shewa, Ethiopia. *Journal of Food Science and Nutrition*, *010*(010). https://doi.org/10.24966/FSN-1076/100010

Wild, C. P and Turner, P. C., 2002. The toxicology of aflatoxins as a basis for public health decisions. *Mutagenesis,*17 (6):471–481.

Williams, S. B., Baributsa, D., & Woloshuk, C. (2014). Assessing purdue improved crop storage (PICs) bags to mitigate fungal growth and aflatoxin contamination. *Journal of Stored Products Research*, *59*, 190e196. https://doi.org/10.1016/j.jspr.2014.08.003

Wokorach, G., Landschoot, S., Anena, J., Audenaert, K., Echodu, R., & Haesaert, G. (2021). Mycotoxin profile of staple grains in northern Uganda: Understanding the level of human exposure and potential risks. *Food Control, 122,* 107813. https://doi.org/10.1016/j.foodcont.2020.107813

Workneh, W. A., Ujiie, K., & Matsushita, S. (2021). Farmers' agricultural tractor preferences in Ethiopia: a choice experiment approach. *Discover Sustainability*, *2*(1). https://doi.org/10.1007/s43621-021-00021-2

Worku, A. F., Kalsa, K. K., Abera, M., Tenagashaw, M. W., & Habtu, N. G. (2022). Evaluation of various maize storage techniques on total aflatoxins prevalence and nutrient preservation. *Journal of Stored Products Research*, *95*, 101913.

Wu, F., and Khlangwiset, P. (2010). Health economic impacts and cost-effectiveness of aflatoxin-reduction strategies in Africa:case studies in biocontrol and post-harvest interventions. *Food Additives and Contaminants*, 27(4):496–509

Wu, F., Groopman, J. D., & Pestka, J. (2014a). Public Health Impacts of Foodborne Mycotoxins. *Annual Reviews in Food Science and Technology* (5), 351–372. https://doi.org/10.1146/annurev-food-030713-092431

Wu, F., Mitchell, N. J., Male, D., & Kensler, T. W. (2014b). Reduced foodborne toxin Exposure is a benefit of improving dietary diversity. *Toxicological Sciences*, *141*(2), 329–334. https://doi.org/10.1093/toxsci/kfu137

Xie, L., Wu, Y., Wang, Y., Jiang, Y., Yang, B., Duan, X., & Li, T. (2021). Fumonisin B1 induced aggressiveness and infection mechanism of *Fusarium proliferatum* on banana fruit. *Environmental Pollution, 288*, 117793. https://doi.org/10.1016/j.envpol.2021.117793

Xu, F., Baker, R. C., Whitaker, T. B., Luo, H., Zhao, Y., Stevenson, A., Boesch, C. J., & Zhang, G. (2022). Review of good agricultural practices for smallholder maize farmers to minimise aflatoxin contamination: Review. *World Mycotoxin Journal*, *15*(2), 171–186. https://doi.org/10.3920/wmj2021.2685

Yetneberk, S., Rooney, L. W., & Taylor, J. R. N. (2005). Improving the quality of sorghum injera by decortication and compositing with tef. *Journal of the Science of Food and Agriculture*, *85*(8), 1252–1258. https://doi.org/10.1002/jsfa.2103

Yiannikouris, a., & Jouany, j., 2002. Mycotoxins in feeds and their fate in animals: a review. *Animal Research*, 51:81–99.

Yin, Y. N., Yan, L. Y., Jiang, J. H., & Ma, Z. H. (2008). Biological control of aflatoxin contamination of crops. *Journal of Zhejiang University of Science B*, *9*(10), 787–792. https://doi.org/10.1631/jzus.B0860003

Zingales, V., Taroncher, M., Martino, P. A., Ruiz, M. J., & Caloni, F. (2022). Climate Change and Effects on Molds and Mycotoxins. *Toxins*, *14*(7). https://doi.org/10.3390/toxins14070445

SUMMARY

To prevent fungal infection and the occurrence of mycotoxins in food crops, implementing Good Agricultural and Storage practices is important. However, smallholder farmers in Ethiopia do not have sufficient resources to implement recommended practices and/or do not have sufficient awareness about mycotoxins, their health impacts and their preventive and control measures. Consequently, mycotoxin contamination in staple food crops produced in Ethiopia has been frequently reported. At the same time, information on the health related to mycotoxin contamination, and the relationships between preharvest and storage practices with mycotoxin contamination in food crops in Ethiopia is fragmented. Generating evidence on the extent of the health burden of mycotoxins in food crops helps to develop evidence-based measures and policies in the country. Implementation of effective mycotoxin policies not only reduces local human health burden but also provides opportunities for international food trade.

The aim of this PhD research was to investigate the currently available local pre- and post-harvest practices in relation to mycotoxin contamination in maize and sorghum and to assess human health burden to mycotoxin exposure via sorghum consumption in Northwest Ethiopia, with the underlying goal of developing knowledge and an integrated methodology to reduce the related disease burden. A summary of the different research activities conducted to achieve the overall research objective and the findings from each activity are described in Chapters 2-6.

In **Chapter 2**, we performed a comprehensive review of scientific literature on both the preharvest and postharvest management practices of maize in relation to mycotoxin contamination in Ethiopia, to identify the gaps in knowledge and priority areas for future research. A systematic literature review was performed using the Prisma 2020 guideline for literature search and organization. Most of the investigated preharvest practices showed to be related to maize mycotoxin contamination. Land preparation with oxen provides a shallow plowing, growth inputs such as fertilizer and pesticide application are sub optimal, harvesting period is judged by subjective and traditional methods, field drying is done with sun drying and its duration is long, and the shelling practices damage kernels; all these practices are positively related with mycotoxin contamination. In addition, postharvest practices such as of-house and in-house storage structures are related to maize mycotoxin contamination. Both indoor (sack, *gota*, *gotera*) and outdoor (*gombisa*) maize storage structures are affected by environmental factors leading to moisture leakage, temperature increase, and insect infestation in the stored grain favoring mycotoxin contamination. Recent developments in postharvest loss reduction, which are also promising intervention options for mycotoxin contamination, such as using a so-

lar bubble dryer for field drying and PICs sacks for long term storage, are not sufficiently adopted by smallholder farmers mainly due to supply chain constraints.

In **Chapter 3**, we investigated the occurrence of mycotoxins in preharvest sorghum samples and potential relationships between preharvest management practices with mycotoxin contamination in sorghum in Ethiopia. For this study, we collected freshly harvested sorghum samples from 120 smallholder households in northwest Ethiopia during the 2011/2022 cropping season. The samples were analysed using UPLC-MS/MS for a total of 33 different mycotoxins. We also collected data on preharvest practices by interviewing the main persons responsible for the preharvest activities in each of the households. We assumed a sample was contaminated with a specific mycotoxin if at least one of the 33 mycotoxins was detected above its respective Limit of Detection (LOD). Logistic regression was applied to statistically test the presence of significant relationships between preharvest practices and mycotoxin contamination. Results showed that 75% of the samples were contaminated with at least one specific mycotoxin. The concentrations of regulated mycotoxins were below the EU regulatory limits except for ochratoxin A, which was found in a concentration above the EU regulatory limit for unprocessed cereal grain in four percent of the samples. In total 23 out of the 33 different tested mycotoxins were found to be present in the sorghum samples. The detected mycotoxins belong to one of the four mycotoxin categories, produced by *Aspergillus* spp, *Fusarium* spp, *Penicillium* spp and *Alternaria* spp. Several of the preharvest practices showed to be significantly ($P<0.05$) related with mycotoxin contamination, including the sowing method, type of fertilizer applied, crop rotation and seed treatment.

In **Chapter 4**, the occurrence of mycotoxins in stored sorghum samples and the relationships between storage management practices with mycotoxin contamination were investigated. For this study, we collected sorghum samples stored for six months from the 120 smallholder households mentioned above. Data on storage management practices were collected by interviewing the main person responsible for grain storage in each of the households. The methods of mycotoxin analysis and statistical analysis were similar to the methods described in Chapter 3. Results showed that about 88% of the samples was contaminated with at least one specific mycotoxin (out of the 33 different mycotoxins analysed). From the total, 3%, 7%, and 3% of the samples were contaminated with aflatoxins, ochratoxin A, and zearalenone, respectively, in concentrations above their respective EU regulatory limits. We found the presence of statistically significant relationships between the majority of the storage management practices with mycotoxin contamination, namely the experience of the main person responsible for storage management, the educational status of this person, the type of storage structure, the placement of the storage structure, and the insecticide application.

In **Chapter 5**, we assessed the exposure of adults to aflatoxins and fumonisins through sorghum consumption in in northwest Ethiopia (the Amhara National Regional State (ANRS)), and at national level in Ethiopia, and estimated the associated disease burden. We used the mycotoxin analysis data from the 240 sorghum samples (120 preharvest samples, 120 stored sorghum samples) of Chapter 3 and 4 for the exposure assessment in the ANRS, and we used litereature data for the exposure assessment at national level. Sorghum *injera*, the most regularly consumed sorghum-based food in the study locations, was used for the exposure assessment. Sorghum (*injera*) consumption data were obtained from the National Food and Nutrition Strategy (FNSS) survey 2022/2023 in Ethiopia. To assess fumonisin exposure, the Estimated Daily Intake (EDI) was estimated and compared with the FAO/WHO provisional maximum tolerable daily intakes (PMTDI) value of 2000 ng/kg bw day. To assess the aflatoxin exposure, the Margin of exposure (MOE), liver cancer risk (hepatocellular carcinoma (HCC)), and Disability-adjusted life years (DALY) values were estimated. In the exposure assessment, Monte Carlo simulation was applied to include potential variation and uncertainty in mycotoxin occurrence, sorghum intake and body weight. Results indicated that the predicted fumonisin exposure levels in the ANRS (0.101 to 14.78 ng/kg bw day) and at national level (7.47 to 299.16 ng/kg bw day) were far below the FAO/WHO (2017) PMTDI limit of 2000 ng/kg bw day to be a health concern. Most of the aflatoxin exposure levels in the ANRS and all the exposure levels at national level fall below the Margin of Exposure (MOE) value of 10,000, indicating a potential public health concern. However, the public health risk estimates for aflatoxin exposure (HCC and DALY) were reasonably low in both the ANRS and at national level.

In **Chapter 6,** we synthesized and discussed the results obtained from Chapters 2 to 5 to get a comprehensive overview for achieving the overall objective, addressed the limitations of our research methods and discussed our findings in relation to scientific literature. We discussed the occurrence of mycotoxins in sorghum, the preharvest and storage practices contributing to mycotoxin contamination, and the human exposure to aflatoxins and fumonisins through sorghum consumption. In this Chapter, we also summarized our findings for policy and business implications, and provided reflections on our methods and data. Our research faced certain limitations, including the use of primary data collected for only one season at our research sites and the use of mycotoxin contamination levels from a single article for the aflatoxin risk assessment at a national level. Additionally, we assumed that aflatoxin exposure remains constant throughout one's lifetime. Despite the limitations, we addressed the overall aim of the research by using both primary and secondary data. In this Chapter, we also outlined the implications of our findings for future research. We recommend studying the cost-effectiveness of the proposed practices, as well as investigating farmers' willingness to adopt and pay for recommended methods. Further research into the mycotoxin contamination of maize, sorghum and other food crops, especially during the pre-harvest period, was also recom-

mended. This should involve considering variables that were not considered in our study, such as soil management practices and weather conditions over several years.

The main conclusions drawn from this research are the following:

- The available literature on mycotoxin contamination of maize in Ethiopia mainly focuses on the post-harvest stage, particularly storage. There is limited knowledge regarding the occurrence of mycotoxin contamination during the pre-harvest stage, and the impact of local processing methods on mycotoxin contamination. Similarly, there is limited research on dietary based exposure assessment.
- Ethiopian farmers have limited awareness about mycotoxins. Mycotoxin issue is not a perceived a concern during the preharvest management and storage management of grains.
- Concentrations of 33 different mycotoxins in both preharvest and stored sorghum samples collected in 2022 in Ethiopia were generally low.
- Most households applied traditional sorghum preharvest and storage management practices. Despite this, concentrations of regulated mycotoxins in most of the sorghum samples were below FAO/WHO and European regulatory limits.
- Both the preharvest and storage management practices are associated with multimycotoxin contamination. The prevalence of mycotoxin contamination is slightly higher in stored samples than preharvest samples.
- For pre-harvest mycotoxin contamination, only practice variables play a significant role, whereas both sociodemographic and practice variables play a role in stored sorghum contamination.
- Human exposure to aflatoxin and fumonisin from sorghum consumption is low in northwestern Ethiopia and in the entire country. However, the low exposure levels seem to be achieved partly by introducing other potential risks to human and the environment such as the use of chemical pesticides.
- Fumonisin exposure showed low public health concern. Based on the MOE value (MOE < 10000), aflatoxin exposure showed priority for risk management.
- A better understanding of local practices, such as sorghum seed treatment with washing with water, can potentially be a source of innovation for developing a sustainable local mycotoxin mitigation strategy.
- To reduce mycotoxin exposure and disease burden, integrated strategies combining good pre-harvest, storage management and dietary intervention practices are needed, rather than a single intervention option.

SAMENVATTING

Om schimmelinfecties en het optreden van mycotoxinen in voedselgewassen te voorkomen, is het belangrijk om goede landbouw- en opslagpraktijken toe te passen. Kleine boeren in Ethiopië beschikken echter niet over voldoende middelen om de aanbevolen praktijken toe te passen en/of zijn onvoldoende op de hoogte van mycotoxinen, de gevolgen daarvan voor de gezondheid en de maatregelen om deze te voorkomen en te bestrijden. Als gevolg daarvan wordt er regelmatig melding gemaakt van mycotoxineverontreiniging in voedselgewassen. Tegelijkertijd is de informatie over de gezondheids- en economische lasten in verband met mycotoxinebesmetting en de verbanden tussen praktijken vóór de oogst en opslag en mycotoxinebesmetting in voedselgewassen in Ethiopië fragmentarisch. Het verzamelen van bewijsmateriaal over de omvang van de gezondheids lasten van mycotoxinen in voedingsgewassen helpt bij het ontwikkelen van op wetenschap gebaseerde maatregelen en beleidsmaatregelen in het land. De uitvoering van een effectief mycotoxinebeleid vermindert niet alleen de lokale lasten voor de menselijke gezondheid, maar biedt ook kansen voor de internationale handel in voedingsmiddelen.

Het doel van dit doctoraatsonderzoek was om de momenteel beschikbare lokale agrarische praktijken vóór en na de oogst te onderzoeken in verband met mycotoxineverontreiniging in maïs en sorghum en om de gevolgen voor de menselijke gezondheid van blootstelling aan mycotoxines via de consumptie van sorghum in te schatten, teneinde kennis en een geïntegreerde methodologie te ontwikkelen om de ziektelast in Noordwest-Ethiopië te verminderen. Een samenvatting van de verschillende onderzoeksactiviteiten die zijn uitgevoerd om de algemene onderzoeksdoelstelling te bereiken en de bevindingen van elke activiteit worden beschreven in de hoofdstukken 2-6.

In **hoofdstuk 2** hebben we een uitgebreid overzicht gemaakt van de wetenschappelijke literatuur over zowel de agrarische praktijken voor de productie van maïs vóór als na de oogst in verband met mycotoxineverontreiniging in Ethiopië, en de hiaten in de kennis en de prioritaire gebieden voor toekomstig onderzoek in kaart gebracht. Er is een systematisch literatuuroverzicht gemaakt aan de hand van de Prisma 2020-richtlijn voor literatuuronderzoek en -organisatie. De meeste onderzochte praktijken vóór de oogst bleken verband te houden met mycotoxineverontreiniging van maïs. De methode van landbewerking met ossen zorgt voor een ondiepe ploeg, de groei-inputs zoals meststoffen en pesticiden zijn suboptimaal, de oogstperiode wordt beoordeeld aan de hand van subjectieve en traditionele methoden, het drogen op het veld gebeurt door middel van zonnedrogen en duurt lang, en de praktijken voor het pellen beschadigen de korrels. Al deze praktijken hebben een positief verband met mycotoxinebesmetting. Bovendien houden praktijken na de oogst, zoals opslagstructuren buiten en binnen, verband met

de besmetting van maïs met mycotoxinen. Zowel binnen- (zakken, gota, gotera) als buitenopslagstructuren (gombisa) voor maïs worden beïnvloed door omgevingsfactoren die leiden tot vochtlekkage, temperatuurstijging en insectenplagen in het opgeslagen graan, wat de besmetting met mycotoxinen bevordert. Recente ontwikkelingen op het gebied van het terugdringen van verliezen na de oogst, die ook veelbelovende interventiemogelijkheden bieden voor mycotoxinebesmetting, zoals het gebruik van zonnebubbel-drogers voor het drogen op het veld en PICs-zakken voor langdurige opslag, worden door kleine boeren onvoldoende toegepast, voornamelijk vanwege beperkingen in de toeleveringsketen.

In **hoofdstuk 3** hebben we het optreden van mycotoxinen in sorghummonsters vóór de oogst onderzocht, evenals mogelijke verbanden tussen agrarische praktijken tijdens de teelt en mycotoxinebesmetting in sorghum in Ethiopië. Voor dit onderzoek hebben we tijdens het teeltseizoen 2011/2022 vers geoogste sorghummonsters verzameld bij 120 kleine boerenhuishoudens in het noordwesten van Ethiopië. De monsters zijn geanalyseerd met behulp van UPLC-MS/MS op in totaal 33 verschillende mycotoxinen. We hebben ook gegevens verzameld over praktijken vóór de oogst door de belangrijkste personen die verantwoordelijk zijn voor de agrarische praktijken tijdens de teelt in elk van de huishoudens te interviewen. We gingen ervan uit dat een monster besmet was met een mycotoxines als ten minste één van de 33 mycotoxines werd gedetecteerd boven de respectieve detectielimiet (LOD). Logistische regressie is toegepast om statistisch te testen of er een significant verband bestond tussen praktijken vóór de oogst en mycotoxinebesmetting. Uit de resultaten bleek dat 75% van de monsters met ten minste één specifieke mycotoxine was besmet. De concentraties van in de EU gereguleerde mycotoxinen lagen onder de wettelijke EU-normen, met uitzondering van ochratoxine A, dat in vier procent van de monsters in een concentratie boven de EU-norm voor onbewerkte graankorrels is aangetroffen. In totaal zijn 23 van de 33 verschillende geteste mycotoxinen in de sorghummonsters aangetroffen. De gedetecteerde mycotoxinen behoren tot een van de vier mycotoxinecategorieën, geproduceerd door *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. en *Alternaria* spp. Bepaalde teelt praktijken vóór de oogst bleken significant ($P<0,05$) verband te houden met mycotoxinebesmetting, waaronder de zaaimethode, het type meststof dat werd gebruikt, de vruchtwisseling en de zaadbehandeling.

In **hoofdstuk 4** is het optreden van mycotoxinen in opgeslagen sorghummonsters en het verband tussen opslagpraktijken en mycotoxinebesmetting onderzocht. Voor dit onderzoek hebben we sorghummonsters verzameld die zes maanden waren opgeslagen bij de 120 bovengenoemde kleine boerenhuishoudens. Gegevens over opslagbeheerpraktijken zijn verzameld door de belangrijkste persoon die verantwoordelijk is voor graanopslag in elk van de huishoudens te interviewen. De methoden voor mycotoxineanalyse en statistische analyse waren vergelijkbaar met de methoden die in hoofdstuk 3 zijn beschreven.

Uit de resultaten bleek dat ongeveer 88% van de monsters besmet was met ten minste één specifieke mycotoxine (van de 33 verschillende geanalyseerde mycotoxines). Van het totaal was respectievelijk 3%, 7% en 3% van de monsters verontreinigd met aflatoxinen, ochratoxine A en zearalenon in concentraties die hoger waren dan de respectieve wettelijke EU-normen. We hebben een statistisch significant verband gevonden tussen de meeste opslagbeheerpraktijken en mycotoxinebesmetting, namelijk de ervaring van de hoofdverantwoordelijke voor het opslagbeheer, het opleidingsniveau van deze persoon, het type opslagstructuur, de plaatsing van de opslagstructuur, en het gebruik van insecticiden.

In **hoofdstuk 5** hebben we de blootstelling van volwassenen aan aflatoxinen en fumonisinen door de consumptie van sorghum in de nationale regionale staat Amhara (ANRS) en op nationaal niveau in Ethiopië beoordeeld en de daarmee samenhangende ziektelast ingeschat. We hebben de mycotoxineanalysegegevens van de 240 sorghummonsters (120 monsters vóór de oogst, 120 opgeslagen sorghummonsters) uit hoofdstukken 3 en 4 gebruikt voor de blootstellingsschatting in de ANRS, en we hebben literatuurgegevens gebruikt voor de blootstellingsschatting op nationaal niveau. Sorghum *Injera*, het meest geconsumeerde voedingsmiddel op basis van sorghum in de onderzoeksgebieden, is gebruikt in de blootstellingsbeoordeling. De gegevens over de consumptie van sorghum (*Injera*) zijn afkomstig uit de enquête van de Nationale Voedsel- en Voedingsstrategie (FNSS) 2022/2023 in Ethiopië. Om de blootstelling aan fumonisine te beoordelen, is de geschatte dagelijkse inname (EDI) geschat en vergeleken met de voorlopige maximaal toelaatbare dagelijkse inname (PMTDI) vastgesteld door FAO/WHO van 2000 ng/kg lichaamsgewicht per dag. Om de blootstelling aan aflatoxine te beoordelen, zijn de blootstellingsmarge (MOE), het risico op leverkanker (hepatocellulair carcinoom (HCC)) en de waarden voor disability-adjusted life years (DALY) geschat. Bij de blootstellingsbeoordeling is Monte Carlo-simulatie toegepast om rekening te houden met mogelijke variaties en onzekerheden in het optreden van mycotoxinen, de inname van sorghum en het lichaamsgewicht. Uit de resultaten bleek dat de voorspelde blootstellingsniveaus aan fumonisine in de ANRS (0,101 tot 14,78 ng/kg lichaamsgewicht per dag) en op nationaal niveau (7,47 tot 299,16 ng/kg lichaamsgewicht per dag) ver onder de PMTDI-grens van 2000 ng/kg lichaamsgewicht per dag van de FAO/WHO (2017) lagen, die als een gevaar voor de gezondheid wordt beschouwd. De meeste blootstellingsniveaus aan aflatoxine in de ANRS en alle blootstellingsniveaus op nationaal niveau liggen onder de blootstellingsmarge (MOE) van 10.000, wat wijst op een potentieel gevaar voor de volksgezondheid. De schattingen van het risico voor de volksgezondheid als gevolg van blootstelling aan aflatoxine (HCC en DALY) waren echter redelijk laag, zowel in de ANRS als op nationaal niveau.

In **hoofdstuk 6** hebben we de resultaten uit de hoofdstukken 2 tot en met 5 samengevat en besproken om een uitgebreid overzicht te krijgen voor het bereiken van de algemene doelstelling, hebben we de beperkingen van onze onderzoeksmethoden besproken en hebben we onze bevindingen in relatie tot de wetenschappelijke literatuur besproken. We hebben het voorkomen van mycotoxinen in sorghum besproken, evenals de praktijken vóór de oogst en tijdens de opslag die bijdragen aan mycotoxinebesmetting, en de blootstelling van mensen aan aflatoxinen en fumonisinen door de consumptie van sorghum. In dit hoofdstuk hebben we ook onze bevindingen samengevat voor beleids- en zakelijke implicaties, en hebben we reflecties gegeven op onze methoden en gegevens. Ons onderzoek kende bepaalde beperkingen, waaronder het gebruik van primaire gegevens die slechts voor één seizoen op onze onderzoekslocaties waren verzameld en het gebruik van mycotoxinebesmettingsniveaus uit één enkel artikel voor de risicobeoordeling van aflatoxine op nationaal niveau. Bovendien gingen we ervan uit dat de blootstelling aan aflatoxine gedurende het hele leven constant blijft. Ondanks deze beperkingen hebben we het algemene doel van het onderzoek bereikt door zowel primaire als secundaire gegevens te gebruiken. In dit hoofdstuk hebben we ook de implicaties van onze bevindingen voor toekomstig onderzoek uiteengezet. We bevelen aan om de kosteneffectiviteit van de voorgestelde praktijken te bestuderen en te onderzoeken in hoeverre boeren bereid zijn om de aanbevolen methoden toe te passen en ervoor te betalen. Verder onderzoek naar de mycotoxinebesmetting van maïs, sorghum en andere voedingsgewassen, met name tijdens de periode vóór de oogst, werd ook aanbevolen. Hierbij moet rekening worden gehouden met variabelen die in ons onderzoek niet in aanmerking zijn genomen, zoals bodembeheerpraktijken en weersomstandigheden over meerdere jaren.

De belangrijkste conclusies uit dit onderzoek zijn de volgende:

- De beschikbare literatuur over mycotoxineverontreiniging van maïs in Ethiopië richt zich voornamelijk op de fase na de oogst, met name opslag. Er is beperkte kennis over het voorkomen van mycotoxineverontreiniging tijdens de fase vóór de oogst en over de invloed van lokale verwerkingsmethoden op mycotoxineverontreiniging. Ook is er beperkt onderzoek gedaan naar de blootstelling via de voeding.
- Ethiopische boeren hebben weinig kennis over mycotoxinen. Mycotoxinen worden niet als een probleem gezien tijdens het beheer vóór de oogst en de opslag van granen.
- De concentraties van 33 verschillende mycotoxinen in zowel vóór de oogst als opgeslagen sorghummonsters die in 2022 in Ethiopië zijn verzameld, waren over het algemeen laag.
- De meeste huishoudens pasten traditionele praktijken toe voor het beheer van sorghum vóór de oogst en tijdens de opslag. Desondanks lagen de concentraties van

gereguleerde mycotoxinen in de meeste sorghummonsters onder de FAO/WHO- en Europese regelgevingslimieten.

- Zowel de praktijken voor het beheer vóór de oogst als tijdens de opslag worden in verband gebracht met besmetting met meerdere mycotoxinen. De prevalentie van mycotoxinebesmetting is iets hoger in opgeslagen monsters dan in monsters vóór de oogst.
- Voor mycotoxinebesmetting vóór de oogst spelen alleen praktijkvariabelen een belangrijke rol, terwijl zowel sociodemografische als praktijkvariabelen een rol spelen bij besmetting van opgeslagen sorghum.
- De blootstelling van mensen aan aflatoxine en fumonisine door de consumptie van sorghum is laag in het noordwesten van Ethiopië en in het hele land. De lage blootstellingsniveaus lijken echter gedeeltelijk te worden bereikt door de introductie van andere potentiële risico's voor mens en milieu, zoals het gebruik van chemische bestrijdingsmiddelen.
- Blootstelling aan fumonisine bleek weinig zorgwekkend voor de volksgezondheid. Op basis van de MOE-waarde (MOE < 10000) bleek blootstelling aan aflatoxine prioriteit te hebben voor risicobeheer.
- Een beter begrip van lokale praktijken, zoals het behandelen van sorghumzaad door het met water te wassen, kan mogelijk een bron van innovatie zijn voor het ontwikkelen van een duurzame lokale strategie voor het verminderen van mycotoxinen.
- Om de blootstelling aan mycotoxinen en de ziektelast te verminderen, zijn geïntegreerde strategieën nodig die goede praktijken op het gebied van vooroogst, opslagbeheer en voedingsinterventies combineren, in plaats van één enkele interventieoptie.

Acknowledgement

Although I carried out most of the activities for this research as required, I would also like to thank my supervisors, colleagues, friends and family for their help in successfully completing my PhD.

Dear Prof. Ine, I have no words that can fully express my feelings to your support that exceed my expectations. I am so lucky that you are both my promoter and daily supervisor, which gave me an opportunity to discuss directly with you, not only academic but also personal issues with full understanding, which has helped me to go smoothly in my research as planned. You always provided me with positive and timely responses to my questions, whether I asked the questions in person or via email. Your positive attitude motivated me to work hard. As my local university is located in an area of Ethiopia that is still at risk of war (2023-2025), it would have been very difficult to complete my research work in time if you hadn't helped me move to Wageningen at the beginning of my third year, a year earlier than the initially planned. Security issues such as travel restrictions and internet interruptions in Ethiopia would have made my personal life and research a challenging one. With no hesitation to support, you always look for opportunities to help my research go well and my stay be extended in the Netherlands. For instance, together with Inge, you initiated and facilitated a funding application to Wageningen Graduate School for extending my stay in the Netherlands, which has contributed to complete my PhD in time. I greatly appreciate your recruitment of one MSc student for the mycotoxin analysis in our sorghum samples under our supervision. This helped me a lot in shortening the time required for the PhD. I also greatly appreciate your step-by-step guidance with a Mega Project Proposal writing, which we submitted to the Wageningen Global Sustainability Programme. Given your busy schedule as a scientist and your additional administrative duties, I never expected you be willing to allocate time to supervise the write up of the new grant proposal to fund my extended stay in the Netherlands. Although our proposal was unsuccessful, it helped me practice how to write a Grant proposal in several aspects such as how to form a research team, how to develop a research hypothesis and how to develop research questions. I greatly acknowledge your contributions to my successful completion of the PhD study.

Dear Prof. Inge, Thank you for your support as a supervisor and for your motivation at different times. Your questions during our meetings helped me to look at the research project from a more practical perspective. Your invitations to attend several diner parties (to your full professor ship Dinner Party in Wageningen, your Christmas-2024 dinner invitation in Amersfoort, your cake invitation which I received after completing my first stay in the Netherlands in 2022), didn't just provide me with happy short moments; but also gave me more responsibilities for my research. In addition, your co-financing of my

extended stay in the Netherlands very positively contributed to my work. The process of acquiring data on sorghum consumption from the Ethiopian Public Health Institute was made easier thanks to your assistance. This greatly helped me to use more practical data than using literature based secondary data for my research. It was also nice to meet you in Addis Ababa and enjoy traditional Ethiopian coffee and dinner together. Thank you so much for all your contributions.

Dear Prof. Netsanet: Thank you for your acceptance of my request for you to be my local promoter during the PhD application, and for your motivation and encouragement to apply. Your willingness for a face-to-face meeting during the field data collection, even in difficult security situations in Bahir Dar city, is greatly appreciated. Your suggestion to include Ethical Approval for the farmers interview was a critical one. I realized how important ethical approval was when the journals to which we submitted our manuscripts for publication requested it.

Dear Dr. Masresha: Thank you for your facilitation to obtain Sorghum Consumption data from your Institute, and your help during the export of sorghum samples to the Netherlands for mycotoxin analysis. Thanks also for your invitation to co-write a proposal on adolescent nutrition aimed to get partial funding for my field work in Ethiopia. Although not selected for the grant, our proposal was one of the best, and I learned several new lessons while developing the proposal.

Dear Prof. Merkuz: I am thankful for your close supervision during the development of field data collection tool (questionnaire), support for getting part of the local research funding from Bahir Dar University for part of my field work in Ethiopia, and motivations during the stressful field data collection period in Ethiopia.

Dear Dr. Laura: Thank you so much for your motivation and your facilitation of access to mycotoxin analysis laboratory in the Wageningen Food Safety Research. Your encouragement during our face-to-face meetings to continue further research on emerging mycotoxins motivated me to aspire about my next research. Thank you also for your facilitation of alkaloid analysis of the sorghum samples at the Wageningen Food Safety Research, which was a piece of exploratory research that we conducted to determine the importance of conducting further research on the topic.

Dear Dr. Beshir: The then postdoctoral researcher from BEC, Thank you for recommending me to Ine as a potential candidate for the PhD competition. Otherwise, I would never have had the opportunity to work on this research project.

Dear BEC staff, colleagues and secretariat: I am naturally a shy person to openly express my feelings. However, through the interactions I had with you such as during the PhD meetings, the coffee breaks, and the different social events, I learnt openness to ideas and even to improve my English communication skills. Your congratulations messages for my achievements such as when papers are published in a journal, when research abstracts were accepted for a conference, when my stay in the Netherlands was extended, provided me a positive energy for my research. Thank you **Alfons** for your positive feedback and facilitation of my application to the Wageningen Graduate Schools to extend my stay in the Netherlands. Thank you **Julia Hoehler** for your feedback on the proposal we have made to compete for the Wageningen Global Sustainability Programme, and your encouragement to apply. Thank you **Monique** for your help with purchasing the @Risk software online. Thank you **Miranda**, **Yan**, **Coco**, **Jan-Philip and Nina** for reminding me of the coffee breaks. Thank you **Nina** also for your support in communicating with the DigiD office about my re-registration after I lost my mobile phone, and for your acceptance to join you for traveling from Wageningen to Salzburg (Austria) for the World Mycotoxin Conference by car. I enjoyed the journey, and it provided me an opportunity to explore the geography of Germany briefly. I would also like to express my deep acknowledgement to my Pizza team (**Lukas**, **Mohsin**, **Teguh and Oscar)**, I enjoyed the time we ate new york pizza together every Tuesdays. Thank you, my current and former officemates, (**Triana, Julia Virol**, **Coco**, **Iris**, **Berliana**, **Luca**, **Shambachew**, **Jan-Philip,** and **Guy**), it was a pleasure to work alongside you. Thank you for sharing with me your experiences, for answering my questions, your nice talks and nice smiles, these all helped me love our office for work. Thank you to all the other staff and colleagues for the positive interactions we have had throughout our daily lives (**Henk**, **Jaap**, **Mariska**, **Frederic**, **Tobias**, **Max**, **Bart**, Floor **Biemans, Guy**, **Murilo**, **Loekie**, **Emmanuel**, **Youssef**, **Chiara**, **Harold**, **Yimin**, **Riccardo**, **Reyza**, **Jonathan**, **Megi, Marie-Fleur, Xiaomei, Mahdi**, **Ikhsa**n, J**asper, Ardilasunu, Cyntia, Evie,** and **Okta**). Thank you, the former and current secretariat, at BEC (**Anne**, **Christa**, **Jeannette**, **Minke**, **Rena**) for your support during my travel preparation from Ethiopia to the Netherlands, your facilitation of my resident permit extension and for our nice conversations.

Dear paranymphs Lukas and Mesfin: I would like to express my heartfelt thanks for your support during my PhD defense. Having you by my side on such a meaningful and nerve-wracking day meant a great deal to me. Thank you also for your help with preparing for the ceremony and organizing related activities. Your presence, encouragement and friendship were a great source of strength and comfort to me.

Dear **Mom**: Thank you for your positive attitude towards all situations, good or bad, which you have passed on to me. I will never forget your advice 'do always good thing for all people especially without their knowledge, good deeds pay off later'. This is one of my

personal sources of inspiration, which has helped me to develop a more positive attitude towards my relationships with other people, including during this PhD. Thank you, Dad, for all the sacrifices you made to improve my life. I have found your advice during our conversations to be extremely helpful. It has given me more energy and motivation to complete my PhD study.

Dear wife Mahbuba: Thank you for the excellent care you provided for our children in my absence. Managing the children alongside your other responsibilities was a huge undertaking, but you did it exceptionally well. I would also like to express my gratitude for your understanding when I am unable to provide full support during my study abroad due to my study commitments. Thank you, my daughter Meryem and my son Hamza, for being patient and understanding my situation while I was living abroad. I couldn't treat you the way you deserved. I apologize profusely for any upset you experienced during my absence. During my absence, you did not have any particularly negative experiences, which I am sure is thanks to your mother.

For those of you whose names are not mentioned above, please know that my appreciation also extends to you. Thank you for the help you provided in the form of ideas, advice and support, or other, all of which contributed to my successful completion of the PhD.

Sadik Jemal Awol
Wageningen School of Social Sciences (WASS)
Completed Training and Supervision Plan

Wageningen School of Social Sciences

Name of the learning activity	Department/Institute	Year	ECTS*
A. Project related competences **A1. Managing a research project**			
WASS PhD Introduction	WASS	2022	1
Scientific writing	Wageningen in'to Languages	2022	1.8
Writing PhD research proposal	BEC, WUR	2022	6
BEC PhD Meetings	WUR	2022-2025	1.5
'Developing cost-effective strategies to reduce the disease burden of mycotoxins in Ethiopia'	WASS PhD Day	2022	0.5
'Association of preharvest practices with multimycotoxin contamination in sorghum (Sorghum bicolor) in northwest Ethiopia'	International Conference on Mycotoxins Forum, Salzburg, Austria	2025	1
'Storage management practices and mycotoxin contamination of sorghum (Sorghum bicolor) in northwest Ethiopia'	ANH Academy 2025, Dar es Salaam, Tanzania	2025	1
'Preharvest and postharvest management practices related to mycotoxin contamination in maize in Ethiopia'	National Conference of the Society of Postharvest Management of Ethiopia, Addis Ababa	2023	1
Reviewing paper	World Mycotoxin Journal	2025	1
A2. Integrating research in the corresponding discipline			
Food safety economics, BEC-21306	WUR	2022	6
Risk analysis and risk management in agriculture: Updates on modelling and applications	WASS	2022	3
Research Methodology: from research question to research proposal	WASS	2022	4
B. General research related competences **B1. Placing research in a broader scientific context**			
Exposure Assessment in Nutrition research	VLAG	2022	1
Food Systems for Healthier and Sustainable Diets	WCDI	2024	4
Introduction to R	VLAG	2022	0.7
Applied Statistics	VLAG	2022	1
Ethics for Social Sciences Research	WGS	2022	0.5
Scientific Integrity	WGS	2022	0.6
Adobe Illustrator: Scientific Artwork and Infographics	WUR Library	2025	1.2
B2. Placing research in a societal context			
Result Dissemination training workshop	Aflatoxin sensitization training to stakeholders, Injibara, Ethiopia	2023	1

Completed Training and Supervision Plan (*continued*)

Name of the learning activity	Department/Institute	Year	ECTS*
C. Career-related competences/personal development **C1. Employing transferable skills in different domains/careers**			
PhD Competence Assessment	WGS	2022	0.3
Supervision to MSc thesis	WFSR	2024	0.5
Total			**38.8**

*One credit according to ECTS is on average equivalent to 28 hours of study load

About the Author

Sadik Jemal Awol was born and grew up in a rural village in the now Central Ethiopia Regional State, Silte zone, Mirab Azernet Berbere *woreda*, Mugo area (village - Azobad). **Sadik** followed his bachelor education in Food Science and Postharvest Technology (2002-2006), and master's education in Food Science and Technology (2008-2010), both at Haramaya University, Ethiopia. Starting from his graduation in BSc degree, **Sadik** has been employed by Bahir Dar University, and has worked in different academic positions as assistant lecturer and lecturer. After working for the University for about 10 years, **Sadik** followed a second master education in Applied Food Safety at Wageningen University (2018-2020). During this MSc study, he got opportunities to work for his Thesis and Internships in areas where he had limited knowledge, which helped him understand the recent areas of food safety research. For his MSc, he worked on his thesis with the title - *Influence of antibiotics on the gut microbial metabolism of daidzein in rats*, and on his internship with the title - *Distribution of clonal complexes of Listeria monocytogenes along the different food matrix and the relationships between the presence of virulence genes and particular food matrix*. After MSc graduation at Wageningen University, **Sadik** went back to his home university as a lecturer in the Food Engineering department.

About one year after his graduation, while Sadik was looking for opportunities to pursue his studies to PhD level, he received an incidental email from Prof.dr.ir. HJ (Ine) van der Fels-Klerx asking if he was interested in competing to apply for a Sandwich PhD application to Wageningen University with the topic of Reducing the human disease burden to mycotoxins in Ethiopia. **Sadik** showed interest, successfully passed the PhD candidate screening processes, applied for the Scholarship, and was granted the requested fund the same year. In his sandwich PhD research, **Sadik** worked on the relationships between farming and storage management practices of maize and sorghum in Ethiopia with mycotoxin contamination and estimated the human exposure to aflatoxins and fumonisins from sorghum consumption. **Sadik** developed an integrated strategy to reduce the disease burden resulting from the consumption of mycotoxin-contaminated sorghum in northwest Ethiopia. These strategies combined better practices in the pre- and postharvest stages of the sorghum value chain. With contributions from his supervisors and colleagues, **Sadik** completed the PhD study based on the initially planned period (February 2022 to February 2026).

Sadik presented the results of his PhD research at the annual conference of the Society of the Ethiopian Postharvest Management in Addis Ababa, the WASS PhD Day 2024 in Wageningen University, the World Mycotoxin Forum Conference in Salzburg (Austria), and the 10th Annual Agriculture, Nutrition and Health (ANH) academy week in Tanzania (online). **Sadik** also published the research results in reputable scientific journals. For any questions related to this thesis, please contact me at Jemal.sadik@gmail.com

List of Publications:

1. J.A. **Sadik**, N. Fentahun, I.D. Brouwer, M. Tessema and H.J. van der Fels-Klerx, 2023. Preharvest and postharvest management practices related to mycotoxin contamination in maize in Ethiopia – a review. *World Mycotoxin Journal*, 16 (3): 211-226.
2. J.A. **Sadik**, L. Righetti, N. Fentahun, I.D. Brouwer, M. Tessema, M. Abera, H.J. van der Fels-Klerx, 2025. Storage management practices and mycotoxin contamination of sorghum (Sorghum bicolor) in northwest Ethiopia. *Journal of Stored Products Research*, 111:102535.
3. J.A. **Sadik**, L. Righetti, N. Fentahun, I.D. Brouwer, M. Tessema, M. Abera, H.J. van der Fels-Klerx, 2025. Association of preharvest practices with multimycotoxin contamination in sorghum (*Sorghum bicolor*) in northwest Ethiopia, Under review.
4. J.A. **Sadik**, N. Fentahun, I.D. Brouwer, M. Tessema, H.J. van der Fels-Klerx, 2025. Exposure and disease burden of fumonisins and aflatoxins from sorghum consumption in Ethiopia. *Journal of Regulatory Toxicology and Pharmacology*, 164:105966.

Acknowledgements of financial support

The research described in this thesis was financially supported by Wageningen University, Wageningen Food Safety Research, and Bahir Dar University.

Financial support from Wageningen University for printing this thesis is greatly acknowledged.

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