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Harvest and Post-harvest Handling practices associated with Fumonisin B₁ Contamination in Maize (*Zea mays* L.): Dietary exposure and risk characterization in Eastern Ethiopia

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Abstract

Maize is the main staple food crop in the Eastern part of Ethiopia. However, maize loss is a major issue due to fungal contamination especially at the post-harvest stage owing to inadequate handling practices. This study aimed to assess post-harvest handling and awareness against fungal development and Fumonisin B_1 (FB_1) in maize, and to calculate risk exposures of FB_1. A total of 197 maize samples (grain and flour) were collected from five districts (Haramaya, Kersa, Meta, Oda Bultum, and Tullo). FB_1 was detected using LC-MS/MS qTRAP. Exposure assessment was done based on the maize consumption rate per day in Ethiopia for different age groups (infants, children and adults). Risk characterization depends on the Margin of Exposure (MoE) combined with the lower confidence limit of the benchmark dose level (BMDL). About 81% of farmers were not physically separating undamaged maize ears with damaged from either birds or fungi. Moreover, 100% were not using improved storage material. In storage samples, FB_1 was detected as high as 1058 μg/kg ± 234 in Kersa district, while the minimum 22.60 μg/kg ± 5.27 in Meta. In flour samples, the maximum FB_1 (327 μg/kg) was detected from Oda Bultum district. The maximum exposure of infants was estimated at Kersa (1131 µg/kg bw/day), followed by Oda Bultum (1073 µg/kg bw/day) and Haramaya (854 µg/kg bw/day). Overall, FB_1 exposures ranged from 6.09 - 1131 µg/kg bw/day, which is 3 to 500 µg/kg bw/day higher than the maximum tolerable daily intake of 2 µg/kg bw/day recommended by the World Health Organization. The MoE, ranged from 0.15 - 176, with infants being at higher risk than adults. The study highlights the urgent need to enhance growers' awareness and knowledge of good post-harvest practices to reduce mycotoxin contamination in maize. Further biomarkers analysis must be pursued to determine the risk exposure assessment for different age groups in these areas with a priority for Kersa district.

Keywords Awareness assessment · Exposure assessment · fumonisin contamination · maize · post-harvest handling · Ethiopia

Introduction

Maize (*Zea mays* L.) is one of the main staple crops used worldwide and an estimated amount of 1.15 billion tons were harvested globally in 2019 FAOSTAT (2020). In Ethiopia, maize is widely growing and covers 90% of arable land as an important food crop, in which 95% of the total production covered by smallholder farmers USDA (2020). It grows across an agro-ecology which have an altitude of 1000 to 1800 m above sea level and receives a reliable average annual rainfall from 1000 to 1500 mm/year suitable for maize production (Abera et al. 2013).

The wide adaptability of the crop and the potential to produce more calories and food per area of land cultivated than all major cereals grown in Ethiopia were important factors in considering maize as part of the national food security strategy, including its inclusion under the government-led intensive agricultural extension program (Abate et al. 2015). There is evidence that the increased production and productivity of maize is also having a significant positive impact on poverty reduction (Dercon et al. 2009). For instance, maize alone constitutes more than 60% of the caloric intake of a typical household in terms of consumption. It is also the most affordable grain for rural communities and poor urban consumers USDA (2020). In the 2020 cropping season, maize production is projected to be at 8.6 million metric tons of harvest from 2.34 million hectares in Ethiopia USDA (2020). Moreover, the area coverage and productivity have been increasing since the early 1990s, with an average yield reaching more than 3.5 t/ha, 2.2-fold more compared to the East African average (1.6 t/ha) productivity. Ethiopia has the ambitious target to increase the national average yield up to 7.0 t/ha under well-managed farm conditions, improved storage conditions, improved extension, and marketing system USDA (2020).

Maize is susceptible to contamination by mycotoxigenic fungi, especially those belonging to the genera of *Aspergillus, Fusarium* and *Penicillium.* These genera produce mycotoxins, which could accumulate throughout the food chain including pre-harvest, at harvest, drying, transportation, storage and processing stages (Liu et al. 2016; Biemond et al. 2021). *Aspergillus* and *Fusarium* species invade maize at different stages. *Fusarium* species mainly cause a variety of diseases in maize at pre-harvest stage, including seedling disease, stalk rots, and ear rots (Marín et al. 1998). Among *Fusarium* species, *F. graminearum, F. proliferatum,* and *F. verticillioides* are the major species contaminating maize along the production chains, reported from China (Sun et al. 2014). Moreover, *F. verticillioides* is one of the most important species associated with maize throughout the world and produces high levels of fumonisins. Contamination with these mycotoxins has a significant impact, compromising the safety of maize products (De La Campa et al. 2005).

Naturally, maize and maize-based products are contaminated by fumonisins and associated metabolites (Dall'Asta and Battilani, 2016; Cendoya et al. 2018). FB_1, FB_2, and FB_3 are the most frequently naturally occurring toxins (Rheeder et al. 2002; Sewram et al. 2005). And, FB_1 is classified as Group 2B (possibly carcinogenic to humans) by the International Agency for Research on Cancer (IARC 2002).

Fumonisins have been reported as an abundant mycotoxin in agricultural products of sub-Saharan African countries (Bankole et al. 2006). FB_1 contamination levels ranged from 3600 - 11600 μg/kg in Kenya (Kedera et al. 1999), 70.46 to 213 μg/kg of FB_1 in Tanzania (Nyang et al. 2016), and 700 - 2400 μg/kg of total fumonisin in Ethiopia (Ayalew 2010). Moreover, a study by Tsehaye et al. (2017) documented total fumonisin levels of 25 - 4500 μg/kg in maize collected from 20 major maize growing areas in Ethiopia. About 7% of the samples exceeded the maximum limit set by the European Union in maize flour which is 1000 μg/kg EC (2007). Likewise, Getachew et al. (2017) reported about 70.0% of maize samples were positive with FB_1 that ranged from 7 - 11830 μg/kg. Recently, Getahun et al. (2019) reported a total fumonisin concentration ranged from 105 - 5460 μg/kg in freshly harvested and three months storage of maize samples from eastern Ethiopia. So far, eastern Ethiopia has never been deeply assessed for fumonisin exposure and associated health risk assessment related to with consumption of contaminated maize and maize-based products.

Risk assessments by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) have assigned a group provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg body weight per day for FB_1, FB_2 and FB_3 alone or in combination (Bolger et al. 2001; Bulder et al. 2012; JECFA 2016). Recently, EFSA published a scientific opinion on the appropriateness to set a group health-based guidance value for fumonisins and their modified forms. Tolerable daily intake (TDI) was established at 1 μg FB_1/kg bw/day based on the benchmark dose lower confidence limit (BMDL10) of 0.1. mg/day derived on the induction of megalocytic hepatocytes in mice and an uncertainty factor (UF) of 100 was considered for intra and interspecies variability. Based on structural similarity and limited data available indicating similar mode of action (MoA) and similar toxicity, FB_2, FB_3, and FB_4 were included in the TDI group with FB_1 EFSA (2018).

Unfortunately, Ethiopia do not have enforced maximum levels in food commodities leading to potential exposure by the community. The East African Community through the East African Bureau of Standards set regulatory limits for total fumonisins in maize at 2000 μg/kg. Assessments have found high fumonisin exposures in adults and children in Kenya, Tanzania and Uganda (Kimanya 2015). Moreover, about 11% of children exposure to fumonisin was also reported in southern Ethiopia (Tessema et al. 2021).

The lack of maximum legislative levels coupled with improper post-harvest practices and poor storage conditions are some of the factors leading to mycotoxin contamination in maize products. Post-harvest strategies to reduce mycotoxins contamination need to be implemented to maintain proper storage conditions such as temperature and relative humidity, insect, and fungal control (Munkvold 2003). Hence, potential losses and hazards due to mycotoxins can be reduced by adopting improved storage materials, and effective strategies to promote farmers awareness, and to campaign health risks associated with mycotoxins (Strosnider et al. 2006).

Thus, this study aimed to (1) assess maize growers post-harvest handling practices and awareness towards fungal development, (2) determine FB_1 concentration in post-harvest maize products and (3) assess FB_1 dietary exposure and to characterize its risk in Ethiopian maize products. Our findings would generate new empirical data about contamination levels of FB_1 in maize products and Ethiopian people exposure risk to this mycotoxin. Also, it would provide information on the awareness of local people about mycotoxin issue and on current post-harvest handling practices in the maize production chain. Such findings will be necessary to address specific mitigation strategies.

Materials and Methods

Maize Sample Collection

Maize samples were collected in 2018/2019 cropping season from five districts of eastern (Haramaya, Kersa, Meta) and western (Oda Bultum and Tulo) Hararghe, eastern Ethiopia (Fig.1) and selected kebeles (i.e., farmer associations or the lowest administrative units). Additional information such as the altitude (m.a.s.l) and the average amount of rainfall received during harvesting time (mm) was provided by the Agricultural and Natural Resources offices of each respective district (Table S1). Samples were collected from different sampling sources, namely farmers' storages, local markets and maize flour ready for consumption from local households. A total of 99 storage samples (each 500 g) were collected 3-4 months post-harvest, from January to February 2019. During sampling, households were randomly selected and the amounts of maize grain packsacks in the storage house were examined, and the purpose of the study was explained and then requested to obtained the representative sample from their maize grain. Sampling spear was used to take small portion of samples from different points of packs or lots and combined, thoroughly to make an aggregated samples and then divided into four parts equally. Then one part, weighted 500 grams was taken and considered as household sample, placed in sample bag, recorded the sample numbers and date, per district. From May to June 2019, 51 maize samples (each 500 g) and 47 maize flour samples (each 100 g) ready for consumptions were collected from local markets and from local households, respectively.

Survey to assess harvest and post-harvest handling practices and mycotoxin awareness among Ethiopian maize growers

A total of 75 maize growing households from the five districts were interviewed through one-to-one and face-to-face interactions (Table S2). Pre-developed questionnaire was used to collect data on socio-demography (age group and gender), harvest and post-harvest farming practices (including storage) and mycotoxin knowledge. The recorded data from each district were compared with the proportions of contaminated samples and levels of fumonisin detected in maize samples.

Multi-mycotoxin analysis by LC/MS-MS

FB_1 extraction and analysis were conducted at Applied Mycology Group, Cranfield University, UK according to Malachová et al. (2014). Briefly, 50 g of maize grain was homogenously ground, and a representative 5 g flour sample was suspended in 20 ml of extraction solvent (acetonitrile/ water/ acetic acid, 79:20:1 v/v/v). After 90 min shaking using Stuart Orbital Shaker (SSL1) a GFL 3017 (GFL, Burgwedel, Germany) and centrifuged using Thermoscientific for 2 min at 3000 x g (radius 15 cm), extracts were transferred into vials and 350 µL aliquots were diluted with the same volume of dilution solvent (acetonitrile/water/acetic acid, 20:79:1, v/v/v). Five µL of the diluted extract was injected into the sampling port of the LC-MS/MS Sciex 6500 qTRAP system.

Mobile phase was also performed as follows: Phases A and B contained 5 mM ammonium acetate and were composed of methanol/water/acetic acid at 10:89:1 (v/v/v; eluent A) and at 97:2:1 (v/v/v; eluent B), respectively. After an initial time of 2 min at 100% A, the proportion of B was increased linearly to 50% within 3 min. Further linear increase of B to 100% within 9 min was followed by a hold-time of 4 min at 100% B and 2.5 min column re-equilibration at 100% A. The oven temperature was 60 °C, with speed limit of 5700 psi, with an eluent flow rate of 0.6 mL/min.

Quantification was performed by external calibration using serial dilutions of the following standard stock-solutions: 3-acetyldeoxynivalenol, deoxynivalenol, nivalenol, zearalenone, Alternariol methylether, alternariol, cyclopiazonic acid, HT-2, T-2, aflatoxins B_1, B_2, G_1 and G_2, M_2, FB_1, FB_2, diacetoxyscirpenol, neosolaniol, beauvericin, 15-monoacetoxyscirpenol and ochratoxin A. Additional information are provided (Table S3).

Assessment of Consumers Dietary Exposure Risk to Fumonisins in Maize

The maize consumer exposure risk assessment of fumonisin was based on information such as, types of food products (porridges, injera, bread, beverages, and snacks) made from maize, processing, handling, and consumption patterns of different age groups per day across the studied districts. The recorded data were associated with the levels of FB_1 detected in maize samples per district. The average maize consumption in Ethiopia was estimated from the FAO food balance sheets (FAO 2015). Maize is the second in quantity of consumption with an average daily consumption per head of 115 g/person/day in Ethiopia. There was no officially published data for all age groups, and hence, the same data were applied. The average body weight (bw) and the consumers age groups; infants, children, and adults were used according to Liu and Wu (2010) and Rodriguez-Carrasco et al. (2013), when 10, 25 and 64 kg and 0–4, 5–18, and 19–65 years, for infants, children, and adults, respectively. The levels of exposure of FB_1 in maize grains and the products for all age groups were estimated, and FB_1 dietary exposure assessment was calculated according to Adetuniji et al. (2014) as follows:

$$\text{Dietary Exposure } (\mu\frac{\text{g}}{\text{kg}}) \text{ (bw/day)}$$

$$= \frac{\text{Fumonisin concentration } \left(\mu\frac{\text{g}}{\text{kg}}\right) \text{x Amount consumed } \left(\frac{\text{g}}{\text{day}}\right)}{\text{Body weight (kg)of each group}}$$

Fumonisin risk characterization was done from Provisional Maximum Tolerable Daily Intake (PMTDI) of 2 µg/kg bw/day allocated based on a No-observed-adverse-effect level (NOAEL) of 0.2 mg FB_1/kg bw/day based on the joint FAO and WHO expert committee on food additives JECFA (2016).

Risk characterization for fumonisin in maize for consumers

Risk characterization of fumonisin in maize for consumer comprised different age group (infants, children and adults) were computed. For this purpose, the Margin of Exposure (MoE) combined with the lower confidence limit of the benchmark dose level (BMDL) of each group was calculated. Likewise, the $BMDL_{10}$ value of 165 µg/kg bw/day for fumonisin (EFSA 2007; Yogendrarajah et al. 2014; JECFA 2016) was assumed as a Point of Departure (PoD) to calculate MoE for FB_1 levels in maize samples. Moreover, additional procedure is adopted from the information published in Modupeade et al. (2017) which modifications were applied. The MoE was calculated according to Chacha et al. (2018) as follows for all age group:

$$\mathrm{MoE} = \frac{\mathrm{BMDL}_{10}}{\text{Exposure of each group}}$$

In this case the larger the MoE, the smaller the risk, and a value lower than 10000 ($10000>X$) indicated a human health concern, while any of MoE values greater than 10000 ($10000<X$) is considered of low health concern to the public according to the European Food Safety Authority EFSA (2005). This can be determined as 165 µg/kg bw/day of fumonisin /10000, which gives 0.0165 represented a risk of public health concern of fumonisin in maize. So that, MoE of FB_1 values above 0.0165 µg/kg bw/day considered as high public health concern according to Modupeade et al. (2017).

Data Analysis

Survey data on farmers' awareness and post-harvest practices were analyzed using the IBM® SPSS® statistical version-20. Questionaries were used as variables to assess the levels of proportions important for the mycotoxin development in maize products. Statistical analyses were carried out using SAS software version 9.2 for window 9 and mean separation was done using Least Significant Difference (LSD) post hoc tests.

FB_1 concentration was calculated as µg/kg in maize samples. Consumers exposure assessment and risk characterizations of FB_1 in maize products from all districts were done as per the equations mentioned above. For a two-tailed 95% confidence interval (CI), means were used to calculate the lower and upper bounds of the confidence intervals for each group.

Results and Discussion

Harvest and Post-harvest Handling practices and mycotoxin awareness among maize growers

Maize growers' harvest, post-harvest handling practices and mycotoxin awareness were assessed during the survey and results are reported in table S2. Of the total respondents (n = 75, 15 per district), about 53% were male, while the remaining (47%) were female. Among the age groups assessed (18–30 years, 31–45 years and >46 years), the majority (49%) of the respondents belonged to 31–45 years old. The average age of respondents was 35 years with a minimum and maximum age of 18 and 62 years old, respectively, indicating that most of the respondents were adults of a productive group.

Regarding the harvesting practices, about 93% of the growers claimed that they harvested maize at its optimum maturity time. Usually, growers determine the maturity period when the maize ear becomes dry and bend down, kernels are completely dry and show black layer at the base removed from the cobs and it is hard while chopped by teeth. Indeed, it's challenging for the growers to quantify the moisture contents for proper time harvesting. Likewise, Liu et al. (2016) revealed a common practice among growers that they estimate maize moisture content for harvesting by puncturing kernels with their thumbnail or biting kernels with their teeth. From our results, 81% of farmers did not purposely screen and physically separate damaged maize cobs after harvest. It was revealed that, grading of maize cobs damaged either by birds or with visible fungal contamination was not done neither in the field nor during harvest or storage~~.~~

Maize crops can be primarily infected by toxigenic fungi at field, and subsequent mycotoxin production, including fumonisin, may occur before harvest time (Fandohan et al. 2003). Thus, sorting practice of maize at harvest has been found to significantly decrease fumonisin contamination under experimental and field conditions (Kimanya et al. 2008; Matumba et al. 2015). It was reported that physical separation and density-based sorting could reduce about 66%, of total fumonisin contamination compared to unsorted maize (Ngure et al.2020). Based on our findings, 90% and 100% of the growers involved in this study did not know about fungal development and mycotoxin contamination, respectively, under storage conditions. Therefore, limited control measures against fungal spoilage and mycotoxin contamination were taken. Indeed, none of the respondents used improved storage bags for maize grains and 87% of them controlled storage pests by applying only insecticide. Insect pests under storage conditions lead to huge post-harvest losses in developing countries (Sawicka 2019), sometimes caused more than 30% of the maize grain loss (Kiaya 2014). Overall, the use of improved storage materials (e.g. Purdue Improved Crop Storage (PICS) bags, ZeroFly®), rapid and effective drying conditions to reduce moisture contents (e.g. passive solar dryer), adequate storage conditions (e.g. controlled T and a_w, no water exposure, no rainfall percolating), simple food processing (e.g. nixtamalization) significantly influence fungal growth and mycotoxin contamination.

Fumonisin B_1 Contamination in Maize Grain and Flour

The study was proposed to detect and quantify multi-mycotoxin in maize samples harvested in 2018/2019 cropping seasons in the eastern Ethiopia, however, only FB_1 were found beyond the levels of quantifications and considered to be discussed and presented hereafter. Other mycotoxins may likely occur in different harvesting or storage seasons, and it will be urged to investigate in further study.

FB_1 was detected in all grain samples collected in the current study from East and West Hararghe zones. Of the total maize samples (n = 197) collected, about 62.9 % (n = 124) had detectable levels of FB_1 from 22.60 to 1058 μg/kg in storage samples. All storage samples had the maximum concentrations of FB_1 in all districts (Table 1).

Among the storage samples, the maximum incidences of positive samples (75%) with detectable levels of FB_1 were obtained from Haramaya and Oda Bultum districts, while the lowest (9%) was from Meta district. In their studies conducted on maize and total fumonisin contamination in Ethiopia, Ayalew (2010) reported 23.5% of the maize samples were contaminated with fumonisin, following that Tsehaye et al. (2017) explored about 77 % of samples were positive for fumonisin contamination. Later, in the study conducted by Getachew et al. (2018) conveyed similar proportions (70%) were contaminated with FB_1. Those findings agree with the current study affirmed that Ethiopian maize at post-harvest stages is abundantly contaminated with fumonisin. Streit et al. (2013) reported that 72% of samples (n= 17300) from different parts of the world collected over 8 years contained mycotoxins. Kovalsky et al. (2016) reported that mycotoxin contamination in feeds could be up to 79% or even above in about 2000 samples from 52 countries. All market samples (100 %) obtained from Kersa, Oda Bultum and Tulo districts were contaminated with FB_1, indicating that, fumonisin can contaminate maize grain at market stage, whenever there is conducive environment for toxigenic mold developments. Both Oda Bultum and Tulo districts, are based in West Hararghe, while Kersa, located in East Hararghe zone. Geographical variations, such as altitude and annual rainfall recorded from each district didn't affect the FB_1 contamination, except samples collected from Meta district, had fewer positive samples obtained from storage and non-detectable in market and flour samples.

In the current study, the maximum concentration of FB_1 as high as 1058 µg/kg was detected in storage samples from Kersa district, found to have a higher level of fumonisin beyond the EU maximum limit (1000 µg/kg) set for other cereals like maize for direct human consumption EC (2007). However, in similar study, Tsehaye et al. (2017) detected fumonisin level up to 4500 µg/kg in maize, of which about 7% exceeded the maximum tolerable limit set by EU in maize flour, likewise Getachew et al. (2018) revealed FB_1 concentration ranged from 7 - 11830 µg/kg in maize grain. Differences in FB_1 content in maize samples, could depend on many factors such as type of variety, different agro-ecology of the cultivated areas, and post-harvest handling practices (e.g. moisture content, insect infestations, storage conditions).

In another study, 85% of maize samples collected from several agro-ecologies of Tanzania were positive for fumonisin with levels ranged from 49 to 18273 µg/kg (Kamala et al. 2016), and up to 10140 µg/kg in South African maize samples (Shephard et al. 2007a). On the contrary, the maximum concentration (1058 µg/kg) of FB_1 detected in the present study was significantly lower than those reports in other countries. Those maximum concentration of fumonisin levels in other countries could be affected by moisture contents during storage and duration, and other promoting factors like insect infestations, storage temperatures, and storage materials, while relatively less FB_1 was detected in Ethiopian maize, considered in the current study. Maize genetic variation and growers handling contribute to mycotoxin contamination might have been different from location to location.

Oda Bultum district had the maximum concentration of 338 of FB_1 for market samples and 327 µg/kg of FB_1 for maize flour samples. Nonetheless, grain and flour sample collected from Meta district had neither positive nor detectable levels of fumonisin though it doesn't guarantee that maize grains at market levels around Meta district collected during 2018/2019 are free from fumonisin, due to the limited number of samples collected and tested for this study. Perhaps, it would have been adequately handled as compared to samples collected from other districts. Ayalew (2010) in similar market samples, reported a total fumonisin in two samples from Dire Dawa at the concentrations of 700 and 2400 µg/ kg, greater than a values of the current study, and similar levels of 300 µg/kg in one sample each from Adama and Ambo. Kimanya et al. (2010) reported that the frequency of fumonisin contamination in ready-to-cook maize flour consumed by infants in the division of Tarakea, Tanzania, in which 68.6% of samples were contaminated with fumonisin that ranged from 21 - 3201 µg/kg. Overall, maize flour samples intended for direct household consumptions had contamination incidences ranged from 10 to 100 % with mean FB_1 level as high as 91.0 µg/kg and a maximum concentration of 327 µg/kg in a single sample. In this case, the maximum concentration detected in the present study found below the reports from Tanzania (Kimanya et al. 2010).

The extent of fumonisin contamination in home-based maize product for human consumption in Tanzania was reported to be up to 11048 µg/kg (Kimanya et al. 2008) and up to 2.28 µg/kg in maize based complementary children's foods (Kimanya et al. 2014), which is found below the highest levels detected in the current study. Overall, fumonisin levels in flour samples were relatively lower than levels detected in grain samples, which could as a result of cleaning for further milling process likely reduce the contaminated grains in the maize flour. Apparently, there was a finding reported a high concentration of FB_1 (1903 µg/kg) in the processed maize food products called *ogi* (fermented maize gruel) in Nigeria (Chilaka et al. 2016). Such finding is a calling up for further investigation of mycotoxin or fumonisin in the maize-based processed food products, which was not including in the current study and urged in any further study.

Apart from sampled types, the mean levels of FB_1 detected in all maize-based samples (grain and flour) were compared across the districts (Fig. 2). The maximum mean (99.4 µg/kg) detected in samples from Kersa district followed by Oda Bultum (96.8 µg/kg), while minimum (0.9 µg/kg) concentration detected from Meta district.

Maize Food Product Consumption Pattern in Eastern Ethiopia

In the present study, the types of food products made from maize and consumption pattern per day for different age groups were assessed. In Ethiopia, different staple foods are prepared from maize including *injera*, bread, porridge, *nufro* (boiled maize grain with bean), shorba, among others. Beverages made from maize in Ethiopia include *borde, tella,* and *checka* (Ekpaa et al. 2018). Globally, maize consumed in various forms, such as green maize roasted or boiled, steamed products, porridges, beverages, bread, and snacks (Ranum et al. 2014). Similarly, in the studied areas, porridge and *Injera* (flat bread) were the main food types (100%) made from maize. Of course, *Injera* is a common traditional Ethiopian food types made from maize, sorghum or *teff* flours either in blended or sole forms, and consumed with different types of sauces or stew (*wot*). Indeed, it's reported that, different maize-based foods are available in Africa with various processing methods, food products and forms of consumption (Mensah et al. 2013). At a global level, maize can be also processed into a variety of industrial products, including starch, sweeteners, oil, glue industrial alcohol and fuel ethanol. However, in Ethiopia maize is mainly used for food purposes. In the eastern parts of Ethiopia, maize-based beverages are not as common as other parts of the country.

In the studied areas, all respondents used to access maize from their own harvest and purchased when only grain shortage occurs at household levels. According to the 2019 cropping season, about 90% of maize produced goes for domestic food consumptions, both as green and dry cereals, which may close to an estimate of 8.5 million metric tons of harvested maize yield in Ethiopia USDA (2020). This contributes to about 20% of the total calorie intake of Ethiopia and remains the primary food source for the poor community.

A study on the effectiveness of quality protein maize in improving the nutritional status of children in the highlands of Ethiopia revealed that, majority of the children were dependent on maize-based foods, while 31% of families reported feeding their children foods other than maize (Girma et al. 2010). It was also confirmed that, children's complementary diets consisted of cereal grains in which over 95% of them were maize based foods (Girma et al. 2010). However, in our study different age groups (Infants, children and adults) were targeted for maize-based food consumptions at household level, revealed that all age groups reported to consume maize-based food. The consumption rates of infants and adults did not significantly different ($P > 0.05$) among districts, while significant variation observed for children from Tulo district compared to Haramaya and Kersa (Table 2). Nevertheless, as this study was focusing only on the consumption rates per day, the volume of consumed maize products per day needs to be investigated further.

Exposure Risk Assessment of Fumonisin B_1 in Maize

The levels of FB_1 detected in maize samples from the respective districts were correlated combined with the consumption amounts of maize-based food products to determine exposure risk assessment for those targeted different age groups (infants, children and adults). In various regions of the world including Ethiopia, maize intake has been estimated for various populations who consume maize either as a major or a minor part of their diets (Shiferaw et al. 2011). Maize is the second important food commodity in quantity of consumption with an average daily consumption per head of 115 g/day FAO (2015), and the first important in terms of its contribution to daily caloric intakes (20%) among food crops USDA (2020). Due to lack of up-to-date data on the consumption rate of maize per day for infants and children in Ethiopia, 115g/day was taken over as an average consumption rate per day for all consumers and used in the current study. However, Ethiopian consumption rate is found below the rates reported from Tanzania (356 g/day) and South Africa (397 g/day) (Shephard et al. 2007a). That is to mean, maize consumption rate in Ethiopia is 68% less than those countries, which could be associated with the availability of maize food products as well as diversification of food habits at household levels.

The overall FB_1 exposure was ranged from 6.09 - 1131 µg/kg bw/day, which is 3 to 500 folds higher than the maximum tolerable daily intake of 2 µg/kg bw/day JECFA (2016). FB_1 exposure assessment for infants group revealed that the maximum exposure was recorded from Kersa (1131 µg/kg bw/day), followed by Oda Bultum (1073 µg/kg bw/day), Haramaya (853 µg/kg bw/day) and

Tulo (465 µg/kg bw/day) districts, while the minimum exposure was assessed from Meta (6.09 µg/kg bw/day) district. Similarly, in Tanzania, about 12% of infants exceeded the provisional maximum tolerable daily intake of 2 µg/kg bw/day of fumonisin (Kimanya et al. 2010). In rural populations from Tanzania, exposure was reported to range from 0.78 - 142 µg/kg bw/day (Kimanya et al. 2008), which is much higher than the tolerable daily intake JECFA (2016). This can be attributed to differences in fumonisin contamination of maize, with Ethiopian and Tanzanian maize being highly contaminated by fumonisin as documented by several studies. Such exposure assessment results are corresponding with the mean values of fumonisin detected in the districts considered in the present study (Table 3). The study also indicated that the inspected districts with maximum exposure values found vulnerable for fumonisin contamination in maize food products.

Much lower fumonisin exposure was reported to be at 0.063 µg/kg bw/day in Brazilian populations (Bordin et al. 2014) than the standard set. In Brazil populations, fumonisin exposure was less than 2 µg/kg bw/day of the PMTDI. Comparably, exposure assessment made in eastern Ethiopia in the present study was 500 times higher than the exposure reported in Brazil. The lowest exposure of 0.94 µg/kg bw/day was recorded among the adults age group, which was the only level below the PMTDI (2 µg/kg bw/day) noted from Meta district, due to less amount of fumonisin detected in maize samples compared to other districts. The finding revealed that, health risk exposure in the maize-based food product was high in the studied districts as compared to the reports from Brazil. The dietary exposure based on maize food product was estimated indirectly through combining levels of fumonisin detected in maize samples and average consumption rate data with body weight as it has been done in other studies (Shephard et al. 2007a; Burger et al. 2010; Lombard et al. 2012; Bordin et al. 2014). Likewise, dietary exposure through direct approaches were employed

(Shephard et al. 2007b; Ediage et al. 2012; Ezekiel et al. 2014; Heyndrickx et al. 2015), which this study could not manage to do so due to data gap.

The present study proved that infants represent the most vulnerable age groups for food-based contaminants than children and adults. These findings are in line with the reports of Modupeade et al. (2017) indicted, infants > children > adults in terms of levels of exposure of fumonisin in stored maize consumption. There are also other supportive findings which implied that infants and children are particularly vulnerable to mycotoxin exposure, mostly because of a lower detoxification capacity, rapid growth and high intake of food and water per kg body weight (Lombard 2014). Despite these results, a comprehensive study is suggested for Ethiopia to confirm the severe exposure of those highly vulnerable age groups for various mycotoxins using direct parameters like biomarkers and biological based samples.

Moreover, the maximum children exposure assessment of FB_1 was recorded from Kersa (452 µg/kg bw/day) and Oda Bultum (429 µg/kg bw/day) compared to other districts, associated with the maximum concentration of FB_1 detected in maize samples of respective district (Table 3). The results obtained in the present study affirmed that the entire population depends on maize-based food products are at risk of fumonisin exposure being infants and children are at the highest risks. Consequently, adverse effects and biomarker risk analysis becomes important in the targeted areas for further study. In scenarios of greater exposure through consumption of highly contaminated maize-based food products, exposure associated with other food sources such as groundnut and sorghum crops had been reported with maximum mycotoxin contamination (Chala et al. 2014; Wondimeneh et al. 2018).

Risk Characterization of Fumonisin B_1 in Maize

The mean levels of fumonisin detected in maize samples were used for risk cauterization in the current study, according to FAO/WHO (2006), for different age groups of consumers (Infants, children and adults) across East and West Hararghe zones. The provisional maximum tolerable daily intake (PMTDI) of fumonisin, set by the Joint FAO/WHO expert committee on food additives ~~(JECFA)~~ is 2 µg/kg bw/day JECFA (2016). The Margin of Exposure (MoE) determined from the mean daily exposure of fumonisin values, and BenchMark Dose Lower limit ($BMDL_{10}$) 165 µg/kg bw/day were also done according to the previous reports (EFSA 2005, 2007; Shephard 2008; Yogendrarajah et al. 2014; JECFA 2016) (Table 4).

The results with the larger the MoE, is the smaller the risk. As already stated above, a value lower than 10000 is indicating a human health concern EFSA (2005). In this case, all values are below 10000 and indicated public health concerns across those districts. The MoEs ranged from 0.15 – 176 where the age group of infants is at greatest risk in Oda Bultum whereas the group of adults turned out to be at least risk in the Meta district. Such studies are limited in the considered areas as well as in the whole country. There is an urgent need of intervention strategies and calling up for any mycotoxin mitigation approaches in maize and other food products. Similarly, Chacha et al. (2018) conducted risk characterization of aflatoxin and fumonisin contamination in Tanzanian maize and reported that, the findings were below 10000 and considered as public health concern in the study area and other region with similar agro-ecological zones. According to the authors, reported MoE values (2.84 -50.9) were comparable to those reported from different African countries (0.10-850) (Shephard 2008; Yogendrarajah et al. 2014). The MoE calculated in the present study are also within the reported range of the literature.

Exposure risk characterization of FB_1 based on the 95% CI (95 percentile, confidence intervals) depends on the lower bound (LB) and upper bound (UB) scenarios in line with the FAO/WHO suggestions for each targeted group. Then, the 95% CI values had a minimum exposure for adults ranged from LB, 72 - UB, 147 µg/kg bw/day, as well as the maximum LB, 468 to UB, 953 µg/kg bw/day for the infants group (Table 5). According to the joint FAO/WHO expert committee on food additives, the tolerable daily intake of FB_1 in maize predominant source of exposure with 95% CI is 33.3 µg/kg bw/day JECFA (2011). However, the minimum (LB, 72 µg/kg bw/day) and maximum (UB, 953µg/kg bw/day) exposure values revealed in the present study were surpassed by 2- and 28.6 times recommended tolerable daily exposure of FAO/WHO JECFA (2016).

Comparing our results with other studies conducted in different countries, in Hungary the mean daily intake for all maize-product consumers based on the LB and UB scenarios was 0.045 to 0.120 µg/kg bw/day (Andrea et al. 2019). Regarding children (aged 0–18 years), the mean intake was 0.056 to 0.167 µg/kg bw/day, and the high intake (95%CI) was 0.244 - 0.537 µg/kg bw/day (Andrea et al. 2019). An exposure levels between 0.03 and 0.81 µg/kg bw/day in the LB scenario and between 0.15 and 1.19 µg/kg bw/day in the UB scenario in adults group (Andrea et al. 2019). On the basis of French contamination data of 2013, the mean exposure levels in children groups ranged between 0.17 and 1.52 µg/kg bw/day in the LB scenario and between 0.47 and 2.11 µg/kg bw/day in the UB scenario EFSA (2014). However, the present study had a value significantly greater than those reports from Europe.

Maize is an important staple food crop in SSA including Ethiopia. However, fungal infection and mycotoxin contamination affect its production and become the major food safety concerns. In the present study, a survey was conducted to assess the farmer's awareness of the post-harvest handling practices of maize and furthermore, FB_1 contamination detected in maize samples and exposure risk assessment investigated. Survey data showed inadequate post-harvest handling of maize by growers becomes the main factor for fumonisin contamination along the value chains. Overall, good post-harvest handling practices of maize from harvesting, drying to processing should be adopted by growers to reduce the levels FB_1 in maize as well as to halt the health risk exposure for the consumers. Farmers can be an important target group to be trained on the handling practices of maize. The use of improved storage materials is limited in the studied areas due to lack of access or awareness. So, training focuses on the proper harvesting, appropriate drying to remove the moisture contents, adequate threshing, physical separations of healthy and damaged cobs and grain, transportation and storing in the improved storage materials like hermetic bags and ZeroFly®, should be practiced by the growers.

Due to the public health impacts, the maximum tolerable levels and daily acceptable of either FB_1 or total fumonisin in various agricultural products for different age group of consumers are commended in Ethiopia, to reduce health risk exposures of mycotoxins. Presently, the exposure assessment and risk cancerizations were done using the maize-based food product consumption for infants, children and adults. Infants are more vulnerable for FB_1, so, risk communication and creating awareness in the affected communities are also critical, through farmers training, workshops, and newsletter using local languages, in collaboration with the required stakeholders like village level agricultural and health extensions. To the best of our knowledge this is the first of FB_1 exposure assessment in eastern part of Ethiopia and as country level too in associated with maize food products. Therefore, such study is imperative to reduce the dietary exposure of fumonisin specifically and mycotoxin in general in the study area and country wide in large. Fumonisin mitigation strategy in maize targeting the whole value chains should be implemented.

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Declarations

Conflict of interest the authors declare no competing interests.

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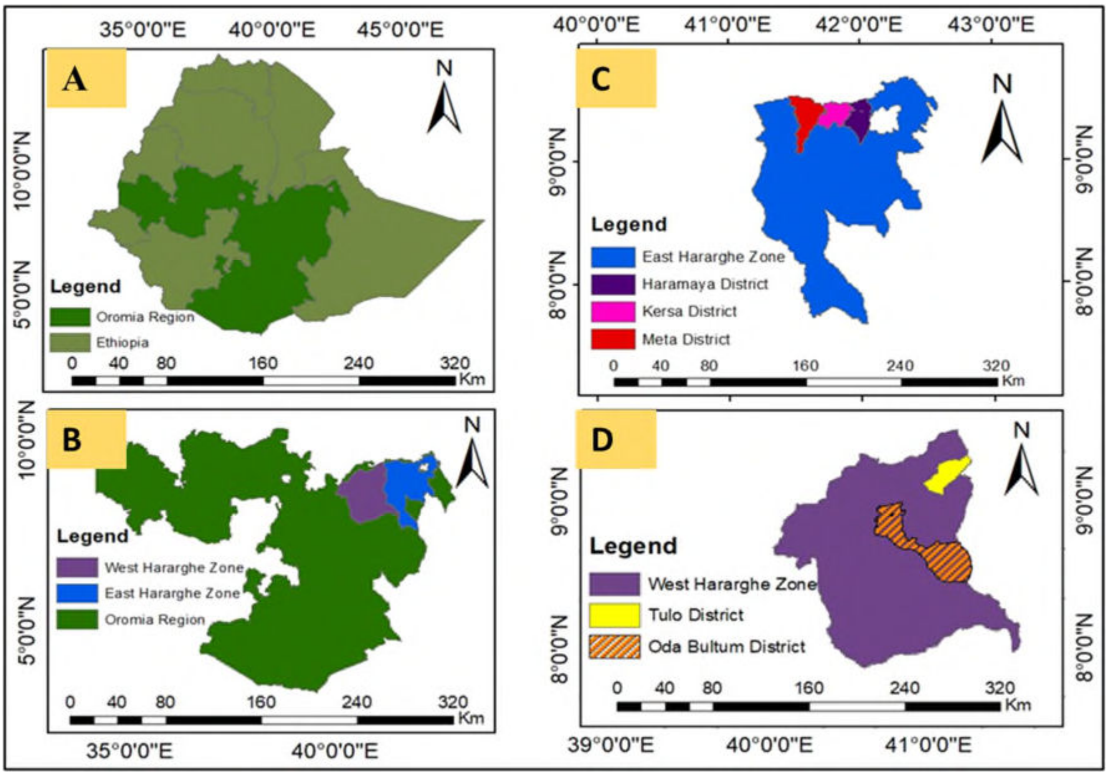

Fig. 1 Schematic representation of the surveyed areas; A) Ethiopia, B) Oromiya region, C) East Hararghe zone, and D) West Hararghe zone.

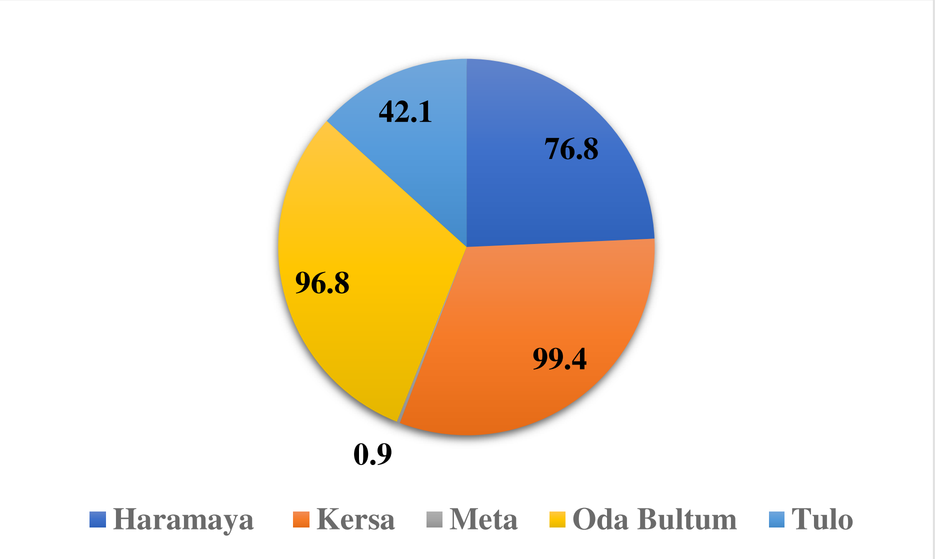

Fig. 2. The levels of FB_1 (µg/kg) means for districts comparisons.

Table 1 Levels of FB_1 (µg/kg) in maize samples (n = 197) collected from eastern Ethiopia during the 2018/2019 cropping season

District	Sample source	Positive sample (%)	Maximum concentration (µg/kg)	Mean concentration (µg/kg)	Median concentration (µg/kg)	Standard deviation (µg/kg)
Haramaya (n = 43)	Storage	75	530	85	28.4	127
	Market	15	266	77	79	76
	Flour	10	243	61	43.7	71
Kersa (n = 37)	Storage	65	1058	100	15.5	234
	Market	100	265	110	89	88
	Flour	14	179	86	84	52.5
Meta (n = 40)	Storage	9	22.6	1.6	<LOQ	5.3
	Market	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Flour	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Oda Bultum (n = 40)	Storage	75	856	107	15.4	254
	Market	100	338	82	55.3	97
	Flour	100	327	91	73	91
Tulo (n = 37)	Storage	61	453	47.9	11.8	106
	Market	100	110	39.8	23.7	36.2
	Flour	60	229	33.6	<LOQ	72

*<LOQ: refers to the levels of FB_1 concentration less than the limit of quantifications.

Table 2 Means of consumption rate (µg/kg) of maize-based food products for different age groups [infants (0–4 yr/10 kg), children (5–18 yr/25 kg) and adults (19–65 yr/64 kg)], per day across the surveyed areas, during 2018/2019 (n = 75)

District	Infants	Children	Adults
Haramaya	2.33^{a}	3.13^{a}	2.47^{ab}
Kersa	2.66^{a}	3.13^{a}	3.06^{a}
Metta	2.60^{a}	2.93^{ab}	2.40^{b}
Oda Bultum	2.93^{a}	2.93^{ab}	2.40^{b}
Tulo	2.53^{a}	2.53^{b}	2.47^{ab}
LSD (0.05)	0.65	0.58	0.60
CV (%)	34.50	27.35	32.21

*Means in a column followed by the same letter are not significantly different according to LSD at $P > 0.05$ probability level. LSD = Least significant difference and CV =Coefficient of variation.

Table 3 Consumer's exposure assessment of FB_1 based on maize samples collected across eastern and western Hararghe Zones, eastern Ethiopia, during 2018/2019 cropping season

District	Age groups	Mean FB_1 (µg/kg)	Consumption rate (g) per day	Average body weight (kg)	Exposure (µg/kg bw/day)
Haramaya	Infants	74.16	115	10	853
	Children	74.16	115	25	341
	Adults	74.16	115	65	131
Kersa	Infants	98.32	115	10	1131
	Children	98.32	115	25	452
	Adults	98.32	115	65	179
Meta	Infants	0.53	115	10	6.09
	Children	0.53	115	25	2.44
	Adults	0.53	115	65	0.94
Oda Bultum	Infants	93.29	115	10	1073
	Children	93.29	115	25	429
	Adults	93.29	115	65	165
Tulo	Infants	40.45	115	10	465
	Children	40.45	115	25	186
	Adults	40.45	115	65	72

FB_1: refers to mean FB_1 concentration detected in maize samples.

Table 4 Risk characterization of FB_1 for different age groups from consumptions of maize based on MoE in eastern Ethiopia

District	Age groups	Exposure (µg/kg bw/day)	MoE
Haramaya	Infants	853	0.19
	Children	341	0.48
	Adults	131	1.26
Kersa	Infants	1131	0.15
	Children	452	0.36
	Adults	179	0.92
Meta	Infants	6.09	27.07
	Children	2.44	67.7
	Adults	0.94	176
Oda Bultum	Infants	1073	0.15
	Children	429	0.38
	Adults	165	0.99
Tulo	Infants	465	0.35
	Children	186	0.88
	Adults	72	2.31

Table 5 Risk characterizations of FB_1 from maize consumptions rates of different age groups, infants (0-4 yr/ 10 kg), children (5-18 yr/ 25 kg) and adults (19-65 yr/ 64 kg)

Age groups	Mean and SD	Median	Maximum	95% CI	
				LB	UB
Infants	711±438	882	1265	468	953
Children	284±175	353	506	187	381
Adults	109±67	136	195	72	147

CI: Confidence Intervals; LB: Lower bound; UB: Upper bound.

Supplementary Table 1 Surveyed and maize samples collected districts from eastern Ethiopia in 2018/2019 cropping season

Zones	**Districts**	**Altitude (m.a.s.l.)**	**Average annual rainfall (mm)**	**Selected kebeles**
East Hararghe	Haramaya	2230	950	Adele Kuro Jalala Tinike Tuji Gabissa
	Kersa	2032	1150	Golawatchu Harodimtu Kufanzik Yabeta Lencha
	Meta	1990	975	Chelenko Lola Gamachu Duse Goro Biyo Ifa Biftu
West Hararghe	Oda Bultum	2200	1050	Makanissa Odabasso Odaroba Odabiyo
	Tulo	2240	820	Bate Debesso Lubudekeb Tarkanfata

Sources: Agricultural and Natural Resources offices of the respective districts. m.a.s.l (meter above sea levels).

Supplementary Table 2 Responses of maize growers (%) regarding post-harvest handling practices across the studied areas (n = 15 per each district)

Variable/probe/questions used for data collection	Variable class	Districts assessed during the survey and number of respondents					%
		Haramaya	Kersa	Meta	Oda-Bultum	Tulo	
Gender	Male	9	9	7	8	7	53.33
	Female	6	6	8	7	8	46.67
Age group	18–30 y	4	5	7	6	4	34.67
	31–45 y	5	9	6	8	9	49.33
	>46 y	6	1	2	1	2	16.00
Do you harvest maize at the right maturity time?	Yes	13	15	15	14	13	93.3
	No	2	0	0	1	2	6.67
Do you physically screen molded or bird affected maize heads upon harvesting and storage?	Yes	3	0	4	5	2	18.67
	No	12	15	11	10	13	81.33
Do you maintain/dry moisture contents before storing the maize grains?	Yes	13	15	15	15	12	93.33
	No	2	0	0	0	3	6.67
Do you know toxigenic mold development in maize grains?	Yes	4	0	3	0	0	9.33
	No	11	15	12	15	15	90.67
Do you know that mycotoxin can contaminate maize grains?	Yes	0	0	0	0	0	0.00
	No	15	15	15	15	15	100
Do you use improved storage (hermetic/pics) bags for maize grains?	Yes	0	0	0	0	0	0.00
	No	15	15	15	15	15	100
Do you use new bags every year or recycle the previously used bags?	Yes	0	2	2	0	0	5.33
	No	15	13	13	15	15	94.67
For how long do you store maize grains?	1–3 months	7	7	8	4	5	41.33
	3–6 months	5	5	5	5	6	34.67
	>6 months	3	3	2	6	4	24.00
Do you face any storage pest of maize grains?	Insect	15	15	15	15	15	100
	Fungus	0	0	0	0	0	0.00
	Rodent	0	0	0	0	0	0.00
Do you apply any control measures against storage pests?	Insecticide	15	12	13	10	15	86.67
	Others	0	3	2	5	0	13.33

Supplementary Table 3 LC-MS/MS parameters used in this study.

Mycotoxin	Retention time (min)	m/z Q1	DP (V)	m/z Q3	CE (V)	CXP (V)	LOD	LOQ
T-2	6.73	484.3	57/27	215.2/185.1	29/33	17/11	0.44	1.47
HT-2	6.17	447.4	131/50	345.1/323.2	15/29	20/16	0.40	1.35
3-Acetyldeoxynivalenol	4.36	397.3	-70	59.2/307.1	-38/-20	-8/-7	3.48	11.61
15-Acetyldeoxynivalenol	4.38	397.1	91	137.2/321.2	17/13	8/18	0.41	1.36
Zearalenone	6.92	317.1	-110	175.0/121.1	-34/-42	-13/-8	0.1	0.34
Nivalenol	1.01	371.1	-75	59.1/281.1	-42/-22	-14/-19	10.71	46.68
Deoxynivalenol	1.78	355.1	-70	59.2/265.2	-40/-22	-13/-10	14.9	49.65
Fusarenon X	3.30	413.2	-70/-25	59.1/352.8	-44/-14	-9/-19	0.73	2.42
Alternariol Methyl Ether	7.18	271.023	-60	255.7/227.8	-32/-38	-55/-11	0.65	2.18
Alternariol	5.67	257.0	-100	213.0/215.0	-34/-36	-11	0.27	0.91
Fumonisin B1	6.40	722.5	121	352.3/334.4	55/57	12/4	133.54	445.14
Fumonisin B2	7.88	706.5	126	336.4/318.4	59/51	8/2	51.18	170.6
Diacetoxyscirpenol	5.51	384.2	71	307.0/246.9	15/21	28/14	0.91	3.02
Neosolaniol	3.99	400.2	76	215.0/185.0	25/29	12/14	1.45	4.85
Beauvericin	11.03	801.5	116/191	244.2/384.4	47/73	12/10	0.59	1.95
15-Acetoxyscirpenol	5.00	342.2	71	265.1/307.2	13	26/8	2.11	7.02

LOD: Limit of detection in µg.kg^{-1}; LOQ: Limit of quantification µg/ kg