Ethiop. J. Agric. Sci. 30(4) 63-81 (2020)

Aflatoxin Contamination of Ethiopian Hot Red Pepper and Risk Characterization: Dietary Exposure Assessment and Estimated Aflatoxin-Induced Hepatocellular Carcinoma

Tariku Hunduma Tolera[1,2*], Antenen Tesfaye[2] and Melaku Alemu[3]

[1] *Ethiopian Institute of Agricultural Research, Ambo ARC, Ambo, Ethiopia*

[2] *Institute of Biotechnology, Addis Ababa University, Addis Ababa, Ethiopia*

[3] *Ethiopian Agricultural Research Council Secretariat, Addis Ababa, Ethiopia*

[*] *Corresponding author: tarikuh2012@gmail.com*

አህፅሮት

በምግብ ሰንሰለት ውስጥ በምግብ ወለድ ሻጋታዎች የሚመነጩ የተለያዩ መርዛማ ኬሚካሎች በሰው ጤና፣ በምግብ ንግድና በኢኮኖሚ ላይ ከፍተኛ ጫና ያስከትላሉ። ይህ ጥናት በበርበሬ ድህረ-ምርት የምግብ ሰንሰለት ሂደት ላይ በሻጋታዎች ሊመነጩ የሚችሉ አፍላቶክሲን የሚባሉ መርዛማ ኬሚካሎች ብክለትን፣ የሰው ተጋላጭነትንና ሊከሰት የሚችሉ ተዛማች የጤና ጠንቆችን ገምግሟል። በአጠቃላይ በ25 ድብልቅ የላቦራቶሪ ናሙናዎች ላይ ምርመራ ተደርጎ በ48 ከመቶ ናሙናዎች ውስጥ አፍላቶክሲን ተገኝቷል። አፍላቶክሲን ጅ1 (aflatoxin G1) የሚባለው የአፍላቶክሲን ዓይነት በከፍተኛ ድግግሞሽና መጠን የተገኘ ሲሆን አፍላቶክሲን ቢ1 (aflatoxin B1) የተባለው ደግሞ በተከታይነት ተመዝግቧል። በዚህ ጥናት ወቅት ከፍተኛ የአፍላቶክሲን መጠን (ማግ/ኪግ) (43.61 አፍላቶክሲን ጅ1 እና 22.18 አፍላቶክሲን ቢ1) ከታሸጉ የበርበሬ ዱቄት ናሙናዎች ሲመዘገብ በተከታይነት ካልታሸጉ የበርበሬ ዱቄት ናሙናዎች (30.53 አፍላቶክሲን ጅ1 እና 13.50 አፍላቶክሲን ቢ1) ተመዝግቧል። አፍላቶክሲን ከተገኙባቸው ናሙናዎች ውስጥ 42 በመቶ በአፍላቶክሲን ቢ1 ይዘታቸው ከአውሮፓ ህብረት የደህንነት ደረጃ (5 ማግ/ኪግ) በላይ ሆነው ተገኝተዋል። አፍላቶክሲን በድግግሞሽ፣ በዓይነትና በመጠን በምግብ ሰንሰለቱ ሂደት ከታች ወደ ላይ የመጨመር እዝማሚያ አሳይቷል። በዚህ ጥናት አንድ ሰው በአማካይ በቀን 1.04 ናግ አፍላቶክሲን በ1/ኪግ የሰውነት ክብደት የሚወስድ ሆኖ የተገኘ ሲሆን በዚህ መጠን 0.0188, 0.0098 እና 0.0286 የጉበት ካንሰር ክስተት/ዓመት/100,000 ሕዝብ በሄፓታይትስ ቢ ፖዝቲቭ፣ ኤጋቲቭ እና ጎልማሳ ማህበረሰብ እንደ አዳጃፉ ቅደም ተከተል ሊከሰት እንደሚችል ተገምቷል። ይህ የካንሰር ክስተት አንዳንድ ድርጅቶች ባስቀመጡት የካንሰር ክስተት ደረጃ መጠን (1 በ100,000 ካንሰር) ሲታይ እምብዛም አሳሳቢ አይደለም። ነገር ግን ይህ ጥናት በአንድ የምግብ ዓይነት ብቻ ላይ የተሰራ ከመሆኑም በላይ የአፍላቶክሲን ስርጭት ከዓመት ወደ ዓመት እና ከቦታ ወደ ቦታ የሚለያይ ስለሆነ የዚህ ጥናት የካንሰር ክስተት ውጤት እንደ ደህንነት መተማመኛ ሊወሰድ አይገባም። በተጨማሪ የተጋላጭነት ጥናቱ በብዙ የምድብ ዓይነት ላይ ቢሰራ ሊከሰት የሚችለው የጤና ጠንቅ ከዚህ ሊብስ ይችላል። እንደ ማጠቃለያ የአፍላቶክሲን በድግግሞሽ፣ በዓይነትና በመጠን በምግብ ሰንሰለቱ ሂደት እየመጨረ የመሄድ እዝማሚያ አፍላቶክሲኑና ተዛማች የጤና ጠንቁ ምግቡ ለምግብነት እስከሚቀርብበት ድረስ ሊኖርና ሊከሰት እንደሚችል ማሳያ ነው። ስለዚህ አፍላቶክሲን አንድ ጊዜ በምግብ ውስጥ ከመነጨ በኋላ ሙሉ በሙሉ ለማስወገድ አዳጋች በመሆኑ ቅድመ የመከላከል ዘዴን በምግብ ሰንሰለት ላይ አትኩሮ መስራት ሊከሰት የሚችለውን ጉዳት ሊቀንስ ይችላል።

መግለጫ: ማግ/ኪግ = ማይክሮ ግራም/ኪሎ ግራም፤ ናግ/ኪግ = ናኖ ግራም/ኪሎ ግራም፤ 1 ኪሎ ግራም 1000 ግራም ነው፤ 1 ማግ 1/1000000 ግራም ነው፤ 1 ናግ 1/1000000000 ግራም ነው።

Abstract

Aflatoxins are toxic fungal secondary metabolites, and their presence in the food chain can cause adverse health effects, impair trade and pose a significant economic burden. This study analyzed aflatoxin contamination along a hot pepper postharvest value chain, estimated its dietary exposure and its associated potential health risk to consumers. A total of 25 composite samples were analyzed for aflatoxins using immunoaffinity column cleanup and HPLC. Aflatoxins were detected in 48 % of the tested samples. Aflatoxin G1 was recorded at highest frequencies and contamination levels followed by AFB1. Uppermost contaminations (µg/kg) were recorded from packed pepper powder (43.61 AFG1 and 22.18 AFB1) followed by unpacked pepper powder (30.53 AFG1 and 13.50 AFB1). Five (42 %) of the positive samples exceeded the EU regulatory limits for AFB1 (> 5 µg/kg). Aflatoxin detection frequencies, aflatoxin types and contamination levels generally increased up along the chain. The mean daily intake dose was found as 1.04 ng AFB1/kg bw/day and the cancer risk was estimated to be 0.0188, 0.0098 and 0.0286 cancer cases/year/100,000 population of hepatitis B surface antigen positive, negative and adult subpopulation, respectively. This cancer risk level can be considered "essentially negligible" as compared to 1 x 10^{-5} cancer risk level established by some agencies. However, as this study was dependent on a single food commodity, and aflatoxin contamination level varies from year to year and location to locations, the risk level of this study should not be taken as assurance for safe risk level. In addition, if aggregate dietary exposure is considered, possible health risk would be high. In conclusion, the increased trends of detection frequencies, aflatoxin types and contamination levels up along the value chain signified the possible occurrence of the toxin and their associated health risk as the food commodity approaches consumption. Because complete elimination of aflatoxin is almost unachievable once contamination has happened, preventative management efforts should target the value chain.

Keywords: Aflatoxins, liver cancer risk, dietary exposure, pepper, postharvest value chain

Introduction

In agricultural food commodities production systems, food products can become microbiologically contaminated at any point along the entire continuum from production to consumption (Thakur and Kniel, 2018). Mycotoxins, the toxic fungal secondary metabolites, contaminate a wide variety of agricultural food commodities along the continuum when environmental conditions are favorable (Choudhary and Kumari, 2010). *Capsicum* fruit (*Capsicum* spp.), commonly known as "red pepper", "pepper", "hot red pepper", "tabasco", "paprika", and "cayenne" (Costa *et al.,* 2019) is the most important world spice crop (Matthews and Jack, 2011) and the second largest consumed spice throughout the world, after black pepper (Costa *et al.,* 2019). It usually gets contaminated by mycotoxins (Costa *et al.,* 2019; Ikoma *et al.,* 2015). In Ethiopia, pepper is the most dominantly

grown spice and is a high value crop for household consumption and for sale both at domestic and export markets playing an important role in the national economy (Strategy, 2010). At household level, pepper is an indispensable ingredient in Ethiopian daily cuisine. It is widely used as paste or sauce and also to modify the color, flavor and aroma of almost every cuisine. Despite the role of pepper in household consumption, households' income and national economy, pepper production system has been facing food safety challenges from contamination of mycotoxins. Of all known mycotoxins, aflatoxins are highly toxic fungal secondary metabolites mainly produced by toxigenic species (strains) of *Aspergillus. flavus* and *A. parasiticus (*Zhang *et al.,* 2014*)*. Presence of aflatoxins in the food chain can cause adverse health effects to humans and livestock, impair trade and also pose a significant economic burden. The recent rejection of hot peppers from Ethiopia by the European market that worth over 10 million USD for unsafe levels of aflatoxins and ochratoxins is a good example of trade impact (ENTAG, 2018a; ENTAG, 2018b; Muluken, 2017).

In addition to its market consequence, aflatoxins demonstrate health impact. Particularly, aflatoxin B1 (AFB1) demonstrates carcinogenic, teratogenic, hepatotoxic, mutagenic and immunosuppressive effects on human and animals (Iram *et al.,* 2016; CAST, 2003). The International Agency for Research on Cancer (IARC) classified AFB1 as group 1 carcinogens ("carcinogenic to humans") (IARC, 2009). It has been suggested that developing countries are chronically exposed to largely uncontrolled amounts of aflatoxins (Yu, 2012) with estimated mean aflatoxin dietary exposures exceeding 100 ng/kg bw/day in some sub-Saharan African countries (WHO, 2018) in which the HBsAg-positive prevalence rate was generally assumed to be 25 % (Benkerroum, 2020). From decades of epidemiological research, it has been well established that aflatoxin dietary exposures cause hepatocellular carcinoma (HCC) in humans (IARC, 2012) which is the third leading cause of cancer deaths worldwide, with roughly 550,000–600,000 new HCC cases globally each year (Liu and Wu, 2010). Of these new cases, about 25,200–155,000 may be attributable to aflatoxin exposure, mostly occurring in sub-Saharan Africa, Southeast Asia and China where populations suff er from both high hepatitis B virus (HBV) prevalence and largely uncontrolled aflatoxin exposure in food (Liu and Wu, 2010). Aflatoxin exposure in food is a significant risk factor for HCC (Liu and Wu, 2010). It is estimated that 26,000 Africans living south of the Sahara die annually of liver cancer associated with aflatoxin exposure (IFPRI, 2013). Study in Chile, Bolivia and Peru suggested the possibility of the development of gallbladder cancer (GBC) with a high-level consumption of aflatoxin and ochratoxin A (OTA) contaminated red chili peppers (Ikoma *et al.,* 2015; Asai *et al.,* 2012).

Aflatoxin B1 has consistently been found to be genotoxic carcinogens (EFSA, 2005). In rodents, the principal tumors occurred in the liver, primarily hepatocellular carcinoma (HCC), though tumors were also found in aflatoxin-treated animals at other sites including the lung, kidney, and colon (Benford *et al.*, 2010). Genotoxic carcinogens (non-threshold contaminants) are considered to pose a non-zero health risk at any level of exposure with risks expected to increase with increasing exposure. For this type of genotoxic carcinogen, it is generally assumed that there is no threshold dose below which no tumor formation would occur, i.e. there is no dose without a potential effect; only a zero level of exposure will result in no risk (EFSA, 2005). As theoretically assumed, even a single molecular event could evoke changes in genomic DNA leading to mutations, selective cellular proliferation, and cancer (IARC, 2012). However, there are arguments that low dose exposures to direct acting genotoxins may be tolerated by cells through homeostatic mechanisms such as DNA repair, and DNA-reactive genotoxic carcinogens may have practical threshold for their action (Fukushima *et al.*, 2012; Jenkins *et al.*, 2010).

In Ethiopia, hot red pepper is consumed almost on a daily basis and aflatoxins are assumed to be stable during food processing (Sakuma *et al.*, 2013: Macdonald and Castle, 1996). Despite the impact of aflatoxins in Ethiopian hot red pepper, its heat stability during thermal food processing and the daily usage of hot red pepper, information on aflatoxin contamination along hot red pepper postharvest value chain, dietary exposure to aflatoxins and associated potential health risk to consumers are not available. Therefore, this study was conducted to analyze aflatoxin contamination along the chain, estimate dietary exposure to AFB1 and associated potential health risk to Ethiopian adult subpopulation due to consumption of contaminated hot red pepper.

Materials and Methods

Sample collection and aflatoxin analysis

Hot red pepper samples were collected along the postharvest value chain from different pepper growing agro-ecologies, open markets, supermarkets and retail shops in Bako, Tibe, Jare, Silk-Amba, Mareko, Alaba, Gojam, Metekel, Assossa, Nekemte, Ambo, Addis Ababa and the surroundings, during main season of 2017-2018. A total of 214 samples were collected of which 25 composite samples composed of aseptically picked and dried pepper pods (PiPP) (n=3), pepper pods harvested and dried by farmers (DPP) (n=6), crushed pepper, the DPPs pounded with other additional spices (n=4), unpacked pepper powder kept in open bowls, jute bags and sacks after milled (UpPPo) (n=6) and pepper powder packed in polyethylene plastic bags after milled (PaPPo) (n=6) were analyzed for the presence of the aflatoxins (AFB1, AFB2, AFG1 and AFG2).

The aflatoxin contamination level was determined using a high-performance liquid chromatography (HPLC) system (e.g. Agilent Corporation, USA model 120) consisting of an autosampler with injector, pump, column oven, fluorescence detector and computer with chromatography software. The chromatographic separation was performed on a stainless-steel C-18 reversed phase HPLC column. The limit of detection and the limit of quantification were 0.15 and 0.5 μg/kg, respectively. All solvents used for aflatoxin analysis were of HPLC grade. The analysis was done at Bless Agri Food Laboratory services Plc. The laboratory has been certified for chemical analyses of foods under the International Organization for Standardization (ISO) 17025 guidelines and the analysis was conducted according to the method of AOAC Official Method 2005.08 and LCTech Sample Preparation and Analysis Manual.

Health risk characterization

Dietary exposure and health risk due to consumption of aflatoxin contaminated hot red pepper were assessed for AFB1 as it is well known genotoxic carcinogens using the equations presented in the following sections.

Dietary exposure assessment to aflatoxin B1

For lifetime human exposure, dietary exposure to AFB1 due to consumption of contaminated hot red pepper was estimated based on ingestion lifetime average daily dose (LADD), also called chronic daily intake (CDI) (Pawełczyk, 2013; US EPA, 2005) assessment approach using Equation (1).

$$\text{Ingestion LADD} = \frac{C \; x \; IR \; x \; EF \; x \; ED}{BW \; x \; AT} \qquad (1)$$

Where: LADD = Lifetime average daily dose (ng AFB1/kg bw/day); C = concentration (mean) of AFB1 in food (ng/g); IR = daily average ingestion (intake) rate (g/day); EF = Exposure frequency (days/year); ED = Exposure duration (year); BW = Body weight (kg); AT = Averaging time (ED x 365 days/year) (For carcinogenic substances ED = lifetime years, and then AT = (lifetime years x 365 days/year).

Population health risk estimation

Population health risk due to the consumption of aflatoxin contaminated hot red pepper was assessed by estimating cases of hepatocellular carcinoma (HCC) for AFB1 as most epidemiological studies show a correlation between exposure to AFB1 and an increased incidence of liver cancer (Joint FAO/WHO, 1999). Health risk estimation was done using carcinogenic potency approach developed by Joint FAO/WHO committee on food additives (Joint FAO/WHO, 1999). An average cancer potency of AFB1 for aflatoxin-induced HCC was calculated by summing

product of their respective carcinogenic potency/weighted potency (slope factor) and proportion of hepatitis B surface antigen positive ($HBsAg^+$) and negative ($HBsAg^-$) individuals using Equation (2) (Joint FAO/WHO, 1999).

$$Average\ P_{cancer} = (\mathrm{PHBsAg}^+ \ x\ \mathrm{FHBsAg}^+) + (\mathrm{PHBsAg}^- \ x\ \mathrm{FHBsAg}^-) \quad (2)$$

Where: P_{cancer} = carcinogenic potency of AFB1 (cancer cases/year/100,000 individuals/ng of AFB1 ingested/kg bw/day); PHBsAg+ = carcinogenic potency (slope factor) of AFB1 in hepatitis B surface antigen positive (HBsAg+) individuals; PHBsAg- = carcinogenic potency (slope factor) of AFB1 in hepatitis B surface antigen negative (HBsAg-) individuals; FHBsAg+ = population fraction (prevalence rate) of HBsAg+; FHBsAg- = population fraction (prevalence rate) of HBsAg-

According to Joint FAO/WHO (1999), carcinogenic potency (slope factor) of AFB1 in hepatitis B surface antigen positive (HBsAg+) and negative (HBsAg-) individuals were estimated to be 0.3 (range 0.05-0.5) and 0.01 (range 0.002-0.03) cancer cases/year/100,000 individuals/ng AFB1/kg bw/day, respectively. For this particular study, the estimated prevalence of 6.03 % for HBsAg+ population fraction of Ethiopia was used as reported by (Schweitzer *et al.*, 2015). Population fraction for HBsAg- groups was extrapolated to be 93.97 %. Based on this prevalence rate, cancer potency of AFB1 for $HBsAg^+$, $HBsAg^-$ and Ethiopian population (average potency) was calculated as follows:

$$P_{cancer}\ for\ \mathrm{HBsAg}^+\ \text{individuals} = (0.3\ x\ 0.0603) = 0.0181$$
$$cancer\ cases/year\ /\ 100{,}000\ \ individuals/ng\ of\ AFB1\ ingested/kg\ bw/day$$

$$P_{cancer}\ for\ \mathrm{HBsAg}^-\ \text{individuals} = (0.01\ x\ 0.9397)\ = 0.0094$$
$$cancer\ cases/year\ /\ 100{,}000\ \ individuals/ng\ of\ AFB1\ ingested/kg\ bw/day$$

$$\text{Average } P_{cancer} = (0.3\ x\ 0.0603) + (0.01\ x\ 0.9397)\ = 0.0275$$
$$cancer\ cases/year\ /\ 100{,}000\ \ individuals/ng\ of\ AFB1\ ingested/kg\ bw/day$$

Because carcinogen risk assessment models have generally been based on the premise that risk is proportional to cumulative lifetime dose (US EPA, 2005) (i.e. cancer is thought to be a function of long-term rather than short-term exposure), population cancer risk for AFB1 was estimated as an excess lifetime cancer risk (ELCR) (WHO, 2010). Due to the synergistic hepatocarcinogenic effects of AFB1 and hepatitis B virus infection (HBV), ELCR was estimated from multiplying the estimated ingestion LADD with the carcinogenic potency of AFB1 using Equation (3) (Joint FAO/WHO, 1999).

$$Population\ cancer\ \ risk\ (ELCR) = LADD\ x\ \ P_{cancer} \quad (3)$$

Where: ELCR = excess lifetime cancer risk expressed as numbers of cancer cases/year/100,000 population; LADD = lifetime average daily dose of the carcinogen; P_{cancer} = carcinogenic potency of AFB1 (cancer cases/year/100,000 individuals/ng of AFB1 ingested/kg bw/day.

Results and Discussion

Aflatoxins contamination levels

Detection frequencies and co-occurrence of the aflatoxins are presented in Table 1. Aflatoxins were detected in 12 (48 %) of the composite samples tested. The highest detection frequencies were recorded in unpacked pepper powder 5 (83 %) followed by crushed pepper 3 (75 %), packed pepper powder 3 (50 %) and dried pepper pods 1 (17 %). None of the picked pepper pod samples were contaminated (< LOD). Aflatoxin B1, B2, G1 and G2 were detected in 11 (92 %), 1 (8 %), 12 (100 %) and 1 (8 %) of the positive samples, respectively. Aflatoxin B1 and AFG1 and total aflatoxins (AFB1, AFB2, AFG1, AFG2) were co-occurred in 10 (83 %) and 1(8 %) of the positive samples, respectively. Of the positive samples, one (8 %) sample contained only one aflatoxin type (AFG1).

Aflatoxin contamination levels are summarized in Table 2. The highest contamination level was recorded from the packed pepper powder followed by the unpacked pepper powder, while the highest detection frequencies and types of aflatoxins were recorded in the unpacked pepper powder. Aflatoxin G1 was the highest contamination level recorded followed by AFB1. Of positive samples, a total of five (42 %) (four unpacked pepper powder and one packed pepper powder) samples exceeded the EU regulatory limits for AFB1 (>5 µg/kg) (EU, 2006).

Table 1. Detection frequencies and co-occurrence of aflatoxins in Ethiopian hot red pepper samples

Original Samples (No.)	Composite samples (No.)	Total positive sample(s) out of composite samples [%]	Detection frequency of aflatoxins					Co-occurrence of aflatoxins			
								No. of aflatoxin(s) per positive sample(s)			
			AFB1	AFB2	AFG1	AFG2	TAFT*	1	2	3	4
			Positive sample(s) out of composite samples (%)	Positive sample(s) out of composite samples (%)	Positive sample(s) out of composite samples (%)	Positive sample(s) out of composite samples (%)	Positive sample(s) out of composite samples (%)	positive sample(s) [% out of total positive samples] (type of aflatoxin)	positive sample(s) [% out of total positive samples] (type of aflatoxins)	positive sample(s) [%]out of total positive samples (type of aflatoxin)	positive sample(s) [% out of total positive samples] (type of aflatoxins)
PiPP (9)	(3)	0 [0]	0(0)	0(0)	0(0)	0(0)	0(0)	0 [0]	0 [0]	0 [0]	0 [0]
DPP (105)	(6)	1 [17]	0(0)	0(0)	1(17)	0(0)	0(0)	1 [100] (G1)	0 [0]	0 [0]	0 [0]
CP (20)	(4)	3 [75]	3(75)	0(0)	3(75)	0(0)	0(0)	0 [0]	3 [100] (B1, G1)	0 [0]	0 [0]
UpPPo (44)	(6)	5 [83]	5(83)	1(17)	5(83)	1(17)	1(17)	0 [0]	4 [80] (B1, G1)	0 [0]	1 [20] (B1, B2, G1, G2)
PaPPo (36)	(6)	3 [50]	3(50)	0(0)	3(50)	0(0)	0(0)	0 [0]	3 [100] (B1, G1)	0 [0]	0 [0]
Percentage of positive sample(s) to composite samples tested, 25)		12[48]	11(44)	1(4)	12(48)	1(4)	1(4)	1 [4] (G1)	10[40] (B1, G1)	0 [0]	1[4] (B1, B2, G1, G2)
Percentage of positive sample(s) to total positive samples, 12)		12[100]	11(92)	1(8)	12(100)	1(8)	1(8)	1[8] (G1)	10[83] (B1, G1)	0 [0]	1[8] (B1, B2, G1, G2)

PiPP-picked pepper pods; DPP- dried pepper pods; CP-crushed pepper; UpPPo- unpacked pepper powder; PaPPo-packed pepper powder; TAFT- total aflatoxins

* Total aflatoxins are indicated only when the four aflatoxins (AFB1, AFB2, AFG1 and AFG2) were co-occurred in a sample type

Previous studies on Ethiopian hot red pepper reported AFB1 contamination levels ranging from 250-525 µg/kg (Habtamu and Kelbessa, 1996), and AFB1 and AFG1 average contamination ranging from 26-75 and 32-120 µg/kg, respectively (Habtamu and Kelbessa, 2001). These previous studies reported much higher results than our present findings. These differences could be due to differences in postharvest handling and processing practices over years and among stakeholders in addition to other factors. The 'not detected' result from picked pepper pod samples might be due to better drying and storage conditions done in the laboratory during this work. Reddy *et al.* (2001) indicated that the level of aflatoxin contamination increased when chilies were prepared and kept in conditions which encouraged fungal growth. Fofana-Diomande *et al* (2019) also associated low level mycotoxin contamination of spices to better drying and storage conditions.

The increased detection frequency and types of aflatoxins up along the value chain through the unpacked pepper powder may be attributed to increased chance of progressive contaminations by different strains of aflatoxin-producing fungi and favorable conditions such as aeration. The slight decline in frequency of detection and the standstill in types of aflatoxins detected up through packed pepper powder could be due to restricted contamination as a result of the packaging. But the highest contamination level recorded from the packed pepper powder could be from the type of aflatoxin-producing fungal strains that contaminated the matrix at some stage along the chain and aggravated due to growth restriction as a result of the packaging, as these secondary metabolites are produced when growth is temporarily restricted (Soso *et al.,* 2014; Magan and Aldred, 2007).

Table 2. Contamination levels of aflatoxins in Ethiopian hot red pepper samples collected along postharvest value chain

			Range and average of aflatoxins (µg/kg)									
			AFB1		AFB2		AFG1		AFG2		Total aflatoxins [d]	
Original samples (No.)	Composite sample (No.)	Positive samples [%]	Range (µg/kg)	Mean (µg/kg)	Range. (µg/kg)	Mean (µg/kg)	Range (µg/kg)	Mean (µg/kg)	Range (µg/kg)	Mean (µg/kg)	Range (µg/kg)	Mean (µg/kg)
PiPP (9)	(3) [a]	0 [0]	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	**	**
DPP (105)	(6) [b]	1 [17]	< LOD	< LOD	< LOD	< LOD	< LOD-4.93	0.88	< LOD	< LOD	**	**
CP (20)	(4)	3 [75]	< LOD-4.17	1.90	< LOD	< LOD	0.99-4.20	1.80	< LOD	< LOD	**	**
UpPPo (44)	(6)	5 [83]	< LOD-13.50	5.68	< LOD-0.99	0.23	< LOD-30.53	11.95	< LOD-1.21	0.26	0.99-30.53	7.71
PaPPo (36)	(6)	3 [50]	< LOD-22.18	4.87	< LOD	< LOD	< LOD-43.61	9.08	< LOD	< LOD	**	**
UpPPo + PaPPo (80)[c]	(12)	8 (67)	< LOD-22.18	5.27	< LOD-0.99	0.15	< LOD-43.61	10.52	< LOD-1.21	0.17	1.21-30.53	3.85

LOD = limit of detection

[a] 100 % of the samples were < LOD

[b] > 80 % of the samples were < LOD

[c] Pepper powder as prepared for human consumption

[d] Total aflatoxins are indicated only when the four aflatoxins (AFB1, AFB2, AFG1 and AFG2) were co-occurred in a sample type

** The four aflatoxins (AFB1, AFB2, AFG1 and AFG2) were not co-occurred

PiPP-picked pepper pods, DPP- dried pepper pods, CP-crushed pepper, UpPPo- unpacked pepper powder, PaPPo-packed pepper powder

Set and Erkmen (2014) reported lower occurrence of aflatoxins in packed ground red chili pepper than in unpacked, and generally related the situation to controlled processing conditions (such as drying in dryer, supplying good manufacturing practice) and packaging without air. Ozturkoglu-Budak (2017) also suggested careful handling from harvesting to retailing of the packaged product in order to assure quality of red dried chili pepper. It has to be noted that in our study, pepper powder samples were packed from dried pepper pods treated with the usual traditional method and packed with no special packaging conditions. In agreement with the works of Set and Erkmen (2014) and Ozturkoglu-Budak (2017), our study results also signified that packaging may reduce further contamination if sanitary conditions are well kept in the preceding steps along the value chain.

In aflatoxin biosynthesis, the pathway branches into two main pathways that leads to formation of AFB1/AFG1, and AFB2/AFG2 (Soso *et al.*, 2014). The nearly similar detection frequencies and co-occurrences of AFB1 and AFG1 probably indicated that the intrinsic and extrinsic conditions along the postharvest value chain (during this study) might have supported production of both AFB1 and AFG1. These aflatoxins (B1 and G1) were detected at high frequencies and levels both during this and the previous studies ((Habtamu and Kelbessa, 2001) and require especial attention as they are well known for their carcinogenicity/toxicity than AFB2 and AFG2 (Iram *et al.*, 2016). The results of this study clearly indicated that there is a trend of increase in the aflatoxin detection frequencies, types of aflatoxins detected and the levels of contamination up along the value chain from harvest to processed pepper powder. This signifies the probability of their occurrence in foods at the time of consumption and the chance of exposure to them and their health risks.

Estimated lifetime average daily dose

Ingestion lifetime average daily dose (LADD) of AFB1 contamination level detected during this study was estimated for Ethiopian adult subpopulation. For this particular study, average hot red pepper consumed by Ethiopian adults (15 g/person/day) (MARC, 2004 as cited in Mekdes *et al.*, 2017), mean contamination level of AFB1 (5.27 µg/kg) in hot red pepper powder recorded in the present study (Table 2), 60 kg standard average body weight of an adult as proposed by FAO/WHO (2009), the 2019 Ethiopian demographic data (63,324,482 adults of $\geq$ 15 years old) and 66.34 years life expectancy (macrotrends website) were used. With exposure frequency of 365 days/year, exposure duration of 52.34 years and averaging time of 24,214.1 days, the lifetime average daily dose of the adult subpopulation group was estimated at level of 1.04 ng AFB1/kg bw/day as calculated using Equation (1). The LADD is typically an estimate of the daily intake of a carcinogenic agent throughout the entire life of an individual (US EPA, 2005). Exposure level of 1 ng/kg bw/day in industrialized countries has been

considered low exposure as compared to exposure level of 100 ng/kg bw/day in developing African and Asian countries (Benkerroum, 2020). Though there is no threshold level (i.e. no acceptable daily intake) for AFB1 as it is genotoxic carcinogen, just for the purpose of comparison, exposure level recorded from this study can be considered low as compared to what has been reported (100 ng/kg bw/day) in developing African and Asian countries. Shephard (2008), highlighted the fact that even meeting a maximum tolerable limit (MTL) does not of itself guarantee food safety as low levels of contamination which might of themselves fall within legislated limits can have serious health implications due to excessive consumption of foods meeting MTLs. As complete elimination of aflatoxin is almost unachievable (Shephard, 2008) (once contamination has happened), the approach of "As Low as Reasonably Achievable" (ALARA) is usually adopted to reduce the exposure to aflatoxins (EFSA, 2005).

Estimated population health risk

From the estimated cancer potencies of AFB1 in the Ethiopian population and estimated LADD (1.04 ng /kg bw/day), the number of population cancer risk (ELCR) attributable to exposure to AFB1 (aflatoxin-induced HCC cases) due to consumption of contaminated hot red pepper was calculated as follows using Equation (3).

$$Popolation\ risk\ (ELCR)\ for\ \mathrm{HBsAg^{+}}\ \text{individuals} = (1.04 \times 0.0181) = 0.0188$$
$$cancer\ cases/year\ /\ 100{,}000\ \ individuals/ng\ of\ AFB1\ ingested/kg\ bw/day$$

$$Popolation\ risk\ (ELCR)\ for\ \mathrm{HBsAg^{-}}\ \text{individuals} = (1.04 \times 0.0094) = 0.0098$$
$$cancer\ cases/year\ /\ 100{,}000\ \ individuals/ng\ of\ AFB1\ ingested/kg\ bw/day$$

$$Popolation\ risk\ (ELCR)\ for\ adult\ subpopulation\ = (1.04 \times 0.0275) = 0.0268$$
$$cancer\ cases/year\ /\ 100{,}000\ \ individuals/ng\ of\ AFB1\ ingested/kg\ bw/day$$

Risk level of the estimated population risk

For genotoxic carcinogens, as aforementioned, there is no safe dose above zero (i.e. any level of exposure above zero may pose some probability of risk). For example, it is assumed that there is no threshold of exposure to AFB1 below which cancer would never occur, because AFB1 has a reactive metabolite that interacts directly with DNA (IARC, 2012). However, for genotoxic carcinogens, different regulatory agencies and authorities have established different guidelines regarding cancer risk levels that are deemed as acceptable, tolerable, or negligible, though there is no overall international scientific consensus among different agencies/authorities on the selected cancer risk level as an 'acceptable' cancer risk (Safe Work Australia, 2018). Most regulatory agencies in the food industries and drinking water generally set acceptable or regulatory limits between 1×10^{-5} and 1

x 10^{-6} (Safe Work Australia, 2018). For example, according to the WHO guideline for drinking-water quality (WHO, 2011), an excess lifetime cancer risk of 1 x 10^{-5} or less is considered to be of low risk for health concern. And also, according to Health Canada (2004), cancer risks will be considered to be "essentially negligible" where the estimated value is $\leq$ 1 x 10^{-5} (1-in-100,000). Taking such established cancer level (e.g. 1 x 10^{-5}) into account, the excess lifetime cancer risk from this study (0.0188, 0.0098 and 0.0286 cancer cases/year/100,000 individuals in HBsAg+, HBsAg- and whole adult subpopulation group, respectively) can be considered "essentially negligible" at the current contamination and exposure level for this particular agro-food commodity. However, as aflatoxin contamination level varies from year to year and location to locations and food intake rates also varies, the risk level of this study should not be taken as assurance for safe risk level. Table 3 shows a hypothetical scenario of how population cancer risk varies depending on ranges of intake rates and AFB1 contamination levels for this particular agro-food commodity. The underlined values indicate the current study's cancer cases for the recorded mean AFB1 contamination level and the average daily intake rate. The shaded values represent region of risk in excess of one in a million of population in case acceptable or regulatory limit of 1 x 10^{-6} is considered. For instance, if the mean AFB1 contamination level increases to 20 ng/g and the intake rate to 25 g/person/day, cancer cases increase by about six-folds. In addition, if aggregate dietary exposure from different agro-food commodities susceptible to aflatoxins are considered, possible health risk from consumption of such diversified diet would be high. Estimated national cancer prevalence rate also affects the estimation of aflatoxin-induced HCC cases as it is well established that the risk of HCC attributable to aflatoxins is up to 30-fold higher in populations chronically infected with HBV than in uninfected populations.

Table 3. Population cancer risk (cancer cases/year/100,000 individuals/ng of AFB1 ingested/ kg bw/day) as a function of ranges of hot red pepper intake rates and AFB1 contamination levels (including mean contamination level and mean intake rate of the current study)

AFB1 (ng/g)	HBsAg+ individuals intake (g/person*/day)					HBsAg- individuals intake (g/person*/day)					Adult subpopulation intake (g/person*/day)				
	5	10	15	20	25	5	10	15	20	25	5	10	15	20	25
0.50	0.0006	0.0012	0.0018	0.0024	0.0030	0.0003	0.0006	0.0009	0.0012	0.0015	0.0009	0.0018	0.0027	0.0036	0.0045
1.00	0.0012	0.0024	0.0036	0.0048	0.0060	0.0006	0.0012	0.0019	0.0025	0.0031	0.0018	0.0036	0.0054	0.0072	0.0090
5.00	0.0060	0.0119	0.0179	0.0238	0.0298	0.0031	0.0062	0.0093	0.0124	0.0155	0.0090	0.0181	0.0271	0.0362	0.0452
5.27	0.0063	0.0125	0.0188	0.0251	0.0314	0.0033	0.0065	0.0098	0.0130	0.0163	0.0095	0.0191	0.0286	0.0381	0.0476
10.00	0.0119	0.0238	0.0357	0.0476	0.0595	0.0062	0.0124	0.0185	0.0247	0.0309	0.0181	0.0362	0.0542	0.0723	0.0904
20.00	0.0238	0.0476	0.0714	0.0952	0.1190	0.0124	0.0247	0.0371	0.0494	0.0618	0.0362	0.0723	0.1085	0.1446	0.1808
30.00	0.0357	0.0714	0.1071	0.1428	0.1785	0.0185	0.0371	0.0556	0.0742	0.0927	0.0542	0.1085	0.1627	0.2170	0.2712
50.00	0.0595	0.1190	0.1785	0.2380	0.2975	0.0309	0.0618	0.0927	0.1236	0.1545	0.0904	0.1808	0.2712	0.3616	0.4520
100.00	0.1190	0.2380	0.3570	0.4760	0.5950	0.0618	0.1236	0.1854	0.2472	0.3090	0.1808	0.3616	0.5424	0.7232	0.9040

HBsAg+ = hepatitis B surface antigen positive
HBsAg- = hepatitis B surface antigen negative
Adult population = population of ≥ 15 years old
* 60 kg body weight

Note:

- Calculation was done based on cancer potency approach as used above
- The above estimated national carcinogenic potency of AFB1 was used for the respective groups
- The underlined values indicate the current study's cancer cases
- Shaded values represent region of risk in excess of one in a million of population

For developing countries including sub-Saharan Africa, $HBsAg^+$ prevalence rate was generally assumed to be 25 %. During this study, the estimated Ethiopian national carcinogenic potency of AFB1 was lower than potency of developing countries. In our study, a prevalence rate far lower than the assumed value for the developing countries was used. Had this assumed HBsAg-positive prevalence rate been considered, the Ethiopian national carcinogenic potency of AFB1 would also rise. In Ethiopia, national, regional and localized area HCC prevalence rate is not available, though the disease burden is expected to be significant (Ferehiwot and Asfaw, 2020**)**. A study conducted on patients with a diagnosis of HCC reported hepatitis B and C viruses as causative agents in 48% of the cases, and also claimed alcohol intake and unidentified risk factors to have contributed for another half of the causes (Hailemichael *et al*., 2015). The risk factors claimed as 'unidentified' could be dietary sources as shown in our study. It has been indicated that aflatoxin may play a causative role in 4.6–28.2 % of all global HCC cases (Liu and Wu, 2010). In Ethiopia, further HCC prevalence rate studies at national, regional and localized area are needed associating dietary habit of patients as exposure to AFB1 (consumption of aflatoxin contaminated foods) and liver cancer cases correlate positively.

In conclusion, the generally increased trends of detection frequencies, aflatoxin types and contamination levels up along the value chain exhibited possible occurrence of the toxin and the associated health risks as the agro-food commodity approaches consumption. Therefore, for the reason that complete elimination of aflatoxin is almost unachievable once contamination has happened, preventative management efforts should target the value chain, particularly at postharvest handling and processing stages. Appropriate application of the Codex-Code of hygienic practice for spices and dried aromatic herbs (CAC, 1995) and the Codex-Code of hygienic practice for fresh fruits and vegetables (CAC, 2003) could help to minimize contamination by aflatoxigenic fungi and subsequent production of aflatoxins and hence possible health risks. Generally, because these toxins have repercussions in terms of public health, trade and economy, ratifying good practices and legislation for the production system of Ethiopian hot red pepper may also help in this regard.

Acknowledgment

The authors are grateful to the Ethiopian Institute of Agricultural Research (EIAR), and EIAR-AGP II project for financing this work.

References

Asai T, Tsuchiya Y, Okano K, Piscoya A, Nishi CY, Ikoma T, Oyama T, Ikegami K and Yamamoto M. 2012. Aflatoxin contamination of red chili pepper from Bolivia and Peru, countries with high gallbladder cancer incidence rates. *Asian Pacifc J Cancer Prev.,* 13 (10): 5167-5170, doi*:10.7314/APJCP.2012.13.10.5167*

Benford D, Leblanc JC, and Setzer RW. 2010. Application of the margin of exposure (MoE) approach to substances in food that are genotoxic and carcinogenic Example: Aflatoxin B1 (AFB1). *Food and Chemical Toxicology*, 48: S34–S41

Benkerroum N. 2020. Aflatoxins: Producing-Molds, Structure, Health Issues and Incidence in Southeast Asian and Sub-Saharan African Countries. *Int. J. Environ. Res. Public Health*, 17:1215, doi:10.3390/ijerph17041215

Choudhary AK, and Kumari P. 2010. Management of mycotoxin contamination in pre-harvest and post-harvest crops: present status and future prospects. *Journal of Phytology*, 2(7): 37-52

Codex Alimentarius Commission (CAC). 1995. Code of Hygienic Practice for Spices and Dried Aromatic Plants. *CAC/RCP 42-1995*.

Codex Alimentarius Commission (CAC). 2003. Code of hygienic practice for fresh fruits and vegetables. CXC 53-2003.

Costa J, Rodríguez R, Garcia-Cela E, Medina A, Magan N, Lima N, Battilani P, and Santos C. 2019. Overview of fungi and mycotoxin contamination in *Capsicum* pepper and in its derivatives. *Toxins*, 11(27): 1-16

Council for Agricultural Science and Technology (CAST). 2003. Mycotoxins: Risks in plant, animal, and human systems. Task Force Report No. 139 January 2003.

European Food Safety Authority (EFSA). 2005. Opinion of the scientific committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. *The EFSA Journal*, 282: 1-31

Ethiopia–Netherlands trade for agricultural growth (ENTAG). 2018a. Newsletter Issue 1, April 2018.

Ethiopia–Netherlands trade for agricultural growth (ENTAG). 2018b. Reopening the European spice market for Ethiopian exporters, March 2018.

European Union (EU). 2006. Setting maximum levels for certain contaminants in foodstuffs. commission regulation (EC) No 1881/2006 of 19 December 2006. *Official Journal of the European Union*, 364: 5–24

Ferehiwot, G. and Asfaw A. 2020. Patterns of Hepatocellular Carcinoma on Computed Tomography at Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia. *Ethiop J Health Sci.,* 30(2): 209-214, doi: 10.4314/ejhs.v30i2.2

FAO/WHO. 2009. Principles and methods for the risk assessment of chemicals in food. Dietary exposure assessment of chemicals in food (Chapter 6). Environmental Health Criteria 240

Fofana-Diomande A, Kouakou KJM, Aboul KN, Traore KS and Dembele A. 2019. Study of the contamination of some spices from Côte d'Ivoire by mycotoxins (AFB1 and OTA). *Journal of Chemical, Biological and Physical Sciences,* June 2019, doi: 10.24214/jcbps.B.9.3.38999.

Fukushima S, Wei M, Kakehashi A, and Wanibuchi H. 2012. Threshold for genotoxic carcinogens: The central concern in carcinogenic risk assessment. *Genes and Environment*, 34 (4):153-156

Habtamu F and Kelbessa U. 2001. Survey of aflatoxin contamination in Ethiopia. *Ethiop. J Health Sci*, 11(1):17-25

Habtamu F and Kelbessa U. 1996, Screening of aflatoxins in Shiro and ground red pepper in Addis Ababa. *Ethiopean Medical Journal*, 34**:**243-249

Hailemichael DM, Suraj S, Abate S, Feld J and Endale L. 2015. Major risk factors, clinical and laboratory characteristics of patients with hepatocellular carcinoma; a retrospective study at Tikur Anbassa Hospital, Addis Ababa university, Addis Ababa, Ethiopia. *Ethiop Med J*, 53 (3): 127-132

Health Canada. 2004. Federal contaminated site risk assessment in Canada. Part I: Guidance on human health preliminary quantitative risk assessment (PQRA). September 2004.

Ikoma T, Tsuchiya Y, Asai T., Okano K, Endoh K, Yamamoto M and Nakamura K. 2015. Ochratoxin contamination of red chili peppers from Chile, Bolivia and Peru showing high incidences of gallbladder cancer. *Asian Pac. J. Cancer Prev*., *16(*14): 5897–5991, doi:10.7314/APJCP.2015.16.14.5987

International Agency for Research on Cancer (IARC). 2012. Improving public health though mycotoxin control. IARC scientific publication No. 158. Lyon, France.

International Agency for Research on Cancer (IARC). 2009. Aflatoxins. Summary of evaluations: Vol.100F

International food policy research institute (IFPRI). 2013. Aflatoxins finding solutions for improved food safety. Focus 20.

Iram W, Anjum T, Iqbal M, Ghaffar A, and Abbas M. 2016. Structural elucidation and toxicity assessment of degraded products of aflatoxin B1 and B2 by aqueous extracts of *Trachyspermum ammi*. *Frontiers in microbiology*, doi:10.3389/fmicb.2016.00346

Jenkins GJS, Zair Z, Johnson GE, and Doak SH. 2010. Genotoxic thresholds, DNA repair, and susceptibility in human populations. *Toxicology*, 278: 305–310

Joint FAO/WHO. 1999. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO technical report series No. 884. WHO. Geneva, 1999.

Liu Y, and Wu F. 2010. Global burden of aflatoxin-induced hepatocellular carcinoma: A risk assessment. *Environmental health perspectives*, 118(6):818-824

Macdonald S, and Castle L. 1996. A UK retail survey of aflatoxins in herbs and spices and their fate during cooking. *Food Additives and Contaminants*, 13(1):121-128

Magan N, and Aldred D. 2007. Why do fungi produce mycotoxins? P.121-133. *In:* Dijksterhuis, J. and Samson, R. A. (eds). Food Mycology: A multifaceted approach to fungi and food. Mycology, Vol. 25. CRC press, New York

Matthews M, and Jack M. 2011. Spices and herbs for home and market. FAO diversification booklet number 20.

Mekdes D, Teshale W and Getachew M. 2017. Value chain analysis of red pepper: The case of Abeshge district, Guragie Zone, South Ethiopia. *Int J Environ Sci Nat Res* 2(3): 001-08, doi: 10.19080/IJESNR.2017.02.555590

Muluken Y. 2017. EU rejects Ethiopian red pepper for unsafe levels of toxins. ttps://health-ethiopianism.blogspot.com/2017/05/eu-rejects-ethiopian-red-pepper-for.html (accessed on 18 December, 2019)

Ozturkoglu-Budak S. 2017. A model for implementation of HACCP system for prevention and control of mycotoxins during the production of red dried chili pepper. *Food Sci. Technol*, Campinas, 37(Suppl. 1): 24-29, Dec. 2017.

Pawełczyk A. 2013. Assessment of health risk associated with persistent organic pollutants in water. *Environ Monit Assess*., 185: 497–508, doi:10.1007/s10661-012-2570-8

Reddy SV, Mayi DK, Reddy MU, Thirumala-Devi K, and Reddy DVR. 2001. Aflatoxins B1 in different grades of chillies (*Capsicum annum* L.) in India as determined by indirect competitive ELISA. *Food Additives and Contaminants,* 18 (6): 553-558, doi: 10.1080/02652030119491

Safe Work Australia. 2018. WES review 2018. Non-threshold based genotoxic carcinogens. Accessory document to recommending health-based workplace exposure standards and notations. Australian workplace exposure standards and advisory notations.

Sakuma H, Watanabe Y, Furusawa H, Yoshinari T, Akashi H, Kawakami H, Saito S, and Sugita-Konishi Y. 2013. Estimated dietary exposure to mycotoxins after taking into account the cooking of staple foods in Japan. *Toxins,* 5:1032-1042, doi:10.3390/ toxins5051032

Schweitzer A, Horn J, Mikolajczyk RT, Krause G, and Ott JJ. 2015. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. *Lancet,* 386:1546–55**,** doi:10.1016/S0140-6736(15) 61412-X

Set E, and Erkmen O. 2014. Occurrence of aflatoxins in ground red chili pepper and Pistachio Nut. *International Journal of Food Properties*, 17:2322–2331, doi: 10.1080/10942912.2013.800985

Shephard GS. 2008. Risk assessment of aflatoxins in food in Africa. *Food Additives and Contaminants,* 25 (10): 1246–1256

Soso VM, Skrinjar MM, and Blagojev NT. 2014. Identification of aflatoxigenic fungi using polymerase chain reaction-based assy. *APTEFF*, 45:1-283, doi: 10.2298/APT1445259S

Strategy. 2010. Spice sub-sector strategy for Ethiopia. Submitted to the Government of Ethiopia by the Spice Sector Strategy Coordinating Committee.

Thakur, S. and K.F. Kniel. 2018. Preharvest food safety. ASM press, Washington DC

United States Environmental Protection Agency (U.S. EPA). 2005. Guidelines for carcinogen risk assessment. EPA/630/P-03/001B. Risk assessment forum. U.S. EPA, Washington, DC, March, 2005.

WHO. 2018. Food safety digest. REF. No.: WHO/NHM/FOS/RAM/18.1

WHO. 2011. Guidelines for drinking-water quality. 4th edition.

WHO. 2010. WHO Human health risk assessment toolkit: Chemical Hazards. IPCS harmonization project document; no.8.

Yu J. 2012. Current understanding on aflatoxin biosynthesis and future perspective in reducing aflatoxin contamination. *Review*. *Toxins,* 4:1024-1057, doi:10.3390/toxins4111024

Zhang X, Kuca K, Dohnal V, Dohnalova L, Wu Q and Wu C. 2014. Military potential of biological toxins. Review. *Journal of applied medicine,* 12: 63-77.