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Trends and critical points of *Aspergillus* contamination along Ethiopian chili postharvest value chain

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Abstract

Background Chili is the most commonly grown spice in Ethiopia and is a high-value crop for household consumption and sale both at domestic and export markets. However, an unsafe level of fungal toxins is becoming a problem leading to challenges in exporting. This study assessed trends, possible points of *Aspergillus* contamination, and contamination risk factors along the Ethiopian chili postharvest value chain (PVC).

Methods Chili handling practices, value chain actors, and their respective roles were investigated along the PVC through an exploratory type of research, a participant unstructured observation. A total of 214 individual sample units composed of multiple subsamples consisting of aseptically picked matured red pods (PiPP), dried red pods (DPP), crushed chili (CP), unpacked (UpPPo), and packed chili powder (PaPPo) were randomly collected along the PVC from different major chili growing localities of Ethiopia during 2017/2018 main cropping season. Individual sample units were further homogenized into a fine powder and composited. *Aspergillus* was analyzed using *Aspergillus flavus* and *parasiticus* agar medium. To monitor *Aspergillus* contamination, trend analysis was done using the mean of count data and biological inference was made in association with stages of operations and postharvest handling practices.

Results *Aspergillus* was detected in 44% of PiPP, all (100%) of DPP, CP, UpPPo, and PaPPo. Counts were in the range of 5.00×10^3 to 2.10×10^5 CFU g^{-1} up along the PVC with fold changes of 19.6, 30, 42, and 38-fold in DPP, CP, UpPPo, and PaPPo, respectively. *Nigri* (99%), *Flavi* (85%), and *Circumdati* (56%) were the most detected sections with relative densities of 50, 29, and 14%, respectively. Postharvest handling practices such as harvesting, sun-drying, and transporting were generally found poor and unhygienic.

Conclusions Counts of *Aspergillus* showed gradually increasing trends up along the PVC. The poor and unhygienic handling practices probably contributed to the contamination. Harvesting and direct open sun-drying were likely initial and critical points of contamination while wetting and tight stacking likely contributed to aggravated growth and proliferation of aspergilli leading to further consecutive buildup. Intervention at these stages would make a significant difference.

Keywords *Aspergillus* contamination, *Capsicum* spp., Postharvest handling, Postharvest value chain

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Introduction

In Ethiopia, chili is the most commonly grown spice and accounts for over 80% of total spice production (ENTAG 2020). It is a crop of high value for household consumption and sale both at domestic and export markets contributing to the national economy as a source of foreign exchange earnings (Strategy 2010). In 2013 and 2014, out of the spice export total earnings of US $26 million, chili accounted for a value of US $6.1 million (23%) (Herms 2015). However, unsafe levels of fungal toxins (mycotoxins) become a problem leading to challenges in exporting chili. Various developed countries have established stringent maximum levels for certain mycotoxins like aflatoxins to regulate them in agri-foods trade as dietary mycotoxins exposures are associated with many chronic health risks such as cancer (Asai et al. 2012; Ikoma et al. 2015; IFPRI 2013), immune suppression (EAC 2018), growth retardation (child stunting) (Smith et al. 2015), genotoxicity (Kumar et al. 2017; Liew and Mohd-Redzwan 2018). For example, the European Union has established maximum limits (μg/kg) of 5.00 and 10.00 for aflatoxin B1 and aflatoxin total, respectively in chili powder intended for human consumption (EU 2010). Such stringent standards make it difficult for countries with weak food safety systems like Ethiopia to export their potential raw and/or processed foods (FAO/WHO 2005). As a result of such stringent limits, in May 2017, Ethiopian chili powder worth 10 million USD has been returned to Ethiopia at an additional cost from European markets when it was found to have aflatoxins and ochratoxins above the limits (Muluken 2017). If this problem is not addressed sustainably, it is likely to lead to the loss of chili export market opportunities directly affecting the economic benefit the country should get through the export of chili. Preventing and/or reducing *Aspergillus* contamination along the postharvest value chain (PVC) of chili can improve the mycotoxicological safety of chili enhancing export market opportunities as well as the income of smallholder farmers in particular and the national economy at large. To prevent and/or reduce *Aspergillus* contamination along the PVC of chili, comprehensive information about factors leading to contamination along the PVC is necessary. In this regard, the few previous studies of Ethiopian chili mainly concentrated on levels of fungal toxins and fungal loads (Fufa and Urga 1996; Fufa and Urga 2001; Bedada et al. 2018; Tsehaynesh et al. 2021). The issues of possible points of fungal contamination and contributing risk factors along the PVC were not well addressed. The present study was conducted to investigate *Aspergillus* contamination, possible points of contamination, and contributing risk factors along the Ethiopian chili postharvest value chain.

Materials and methods

Investigating chili postharvest handling practices with respect to *Aspergillus* contamination

Chili handling practices, value chain actors, and their respective activities/roles were investigated from harvest to chili powder through an exploratory type of research (Sahu 2013). Informal discussions/conversations were made, and information/data were collected by a participant unstructured observation (Sahu 2013) depending on the willingness of the participant. Discussions/conversations were conducted using the appropriate local language and also local translators were used when needed.

Chili sample collection

During sample collection, due to the non-availability of full information, inaccessibility, security, and cost issues, judgment/purposive (non-probability) sampling (Sahu 2013) was followed for the selection of study localities. Once the study localities were selected, depending on road accessibility, a total of 214 samples composed of aseptically picked matured red pods (PiPP) (directly picked from the plants by the researchers) (n = 9, about 8000 g each), dried red pods (DPP) (pods already harvested and dried by farmers, collected mostly from market) (n = 105, about 1500 g each), crushed chili (CP) (the DPP pounded with other additional spices, collected from small/medium scale and household processors) (n = 20, about 500 g each), unpacked chili powder (UpPPo) (powder kept in open bowls, jute bags and sacks for marketing, collected from market) (n = 44, about 300 g each), and packed chili powder (PaPPo) (powder packed in polyethylene plastic bags for marketing, collected from market and retail shops) (n = 36, about 125–500 g each) were collected from markets, retail shops and farm fields around Bako, Tibe, Jare, Nono, Mareko, Alaba, Gojam, Metekel, Assossa, Nekemte, Ambo, Addis Ababa and the surroundings during 2017/2018 main cropping season. The PiPP samples were collected following stops at about five to ten kilometers of driving intervals and following the 'W" walking fashion at each field. The DPP and UpPPo samples were collected with a systematic selection of markets as appropriate, and following a 'zig-zag' walking fashion at each market. The CP and PaPPo samples were collected as encountered as these were found infrequently. Within the different categories of samples, individual sample units were composed of multiple subsamples. Finally, each package of PaPPo and all categories of samples were brought to the laboratory in a sterile double-lined paper bag, labeled with all necessary information. The PiPP samples were dried in the laboratory soon after arrival with maximum care to minimize possible contamination. The

DPP samples were further sun-dried following common practices of small/medium scale and household processors, and each of the PiPP and DPP samples was destemmed and pounded into a fine powder using laboratory mortar and pestle sterilized after every sample. The CP samples were also further sun-dried and pounded into fine powder in the same way. All categories of samples were stored in dry places on laboratory shelves until laboratory analysis.

Analysis of *Aspergillus*

The PiPP samples were analyzed after 4 months of storage in the laboratory while the DPP, CP, UpPPo, and PaPPo were analyzed within a month. Except for the PiPP, some of the individual sample units of all respective samples (DDP, CP, UpPPo, and PaPPo) were composited as needed (e.g., based on sampling area/locality/market), after thoroughly mixing the individual sample units, each contributing equally (100 g). Then, *Aspergillus* was analyzed as follows. One gram of each powder sample was suspended in 9 ml of 0.1% sterile peptone water and serially diluted. Aliquots of 0.1 ml were spread plated onto *Aspergillus flavus* and *parasiticus* agar (AFPA, yeast extract, 20 g; bacteriological peptone, 10 g; iron (III) citrate, 0.5 g; Chloramphenicol, 0.1 g; Agar, 15 g; in a liter of distilled water) (Pitt et al. 1983; Abbas et al. 2004). To facilitate counting, rose Bengal (15 µg/ml) was added to the culture medium for retarding the fungal colony diameter of fast-growing fungi (King et al. 1979; Abbas et al. 2004). To suppress bacterial growth, the medium was aseptically supplemented with chloramphenicol (100 µg /ml). Duplicate plates were incubated at 28 °C for 5 to 7 days with continuous follow-up for the emergence of colonies. After incubation, colonies within the range of 10–150 (Pitt and Hocking 2009) were counted and reported as colony-forming units (CFU) per gram of sample tested. Isolates were further confirmed based on an aspergillum-like spore-bearing erect hyphal branch (Rodrigues et al. 2007) and counted as *Aspergillus*, and micromorphologically further characterized into subgenus and sections. Detection frequency (DF) and relative density (RD) were calculated.

$$\begin{aligned} &Aspergillus\ DF\ (\%) \\ &= \frac{Number\ of\ samples\ infected\ with\ aspergillus}{Total\ number\ of\ samples\ analyzed} \\ &\times 100 \end{aligned}$$

$$Aspergillus\ section\ DF\ (\%) = \frac{Count\ of\ a\ section}{Total\ positive\ samples} \times 100$$

$$Aspergillus\ section\ RD\ (\%) = \frac{Count\ of\ a\ section}{total\ of\ sections} \times 100$$

Data analysis

Aspergillus contamination was monitored along the PVC as the product sequentially moved through certain stages of operations: harvesting, sun-drying, crushing, and pounding into chili powder. Mean of *Aspergillus* counts data from each sequential stage were used to determine trends (a pattern, or changes in a pattern), and possible points of contamination. Count fold change up along the PVC was generally computed using counts in PiPP samples as a base for comparison, and by comparing counts of each stage with counts of the next stage(s). Based on the trend analysis (changes or patterns in the data), the biological inference was made in association with stages of operations and postharvest handling practices.

Results

Postharvest handling practices with respect to *Aspergillus* contamination

During this study, starting at harvesting, direct open sun-drying on soil platform, sorting, grading, storing, transporting, marketing, and processing were found as the most common postharvest handling practices of chili in all study localities. Farmers, collectors, wholesalers, retailers, processors, and consumers were the main actors along the PVC.

Harvesting

Harvesting was done at the red maturity stage by hand picking, mostly two to three times per plot of plantation as all pods did not reach the optimum red maturity stage at the same time. Farmers also collected pods already dropped on the ground, insect damaged/wounded, diseased/stressed ones together with the main harvested pods to increase the bulk. During harvesting, farmers used varieties of harvesting containers such as bamboo baskets with rough structure, plastic containers, jute bags, sacks, and also mosquito nets that were mostly found visually dirty, and tightly stacked the pods into containers. They did the harvesting at any time of the day at their convenience even during wet/dewy conditions. Though not quantified, a larger number of mechanically injured/damaged and stem detached pods (caused by unsafe picking, harvesting containers with rough structures such as bamboo baskets, and tight stacking) were found.

Direct open sun-drying

Drying of harvested red pods was done commonly by spreading the pods on a soil platform in a layer exposing them to direct open sun-drying (Fig. 1) with frequent turning of the pods until assumed dryness is achieved. The sun-drying took about 2 to 3 weeks depending on

Fig. 1 Farmers direct open sun-drying practice of harvested chili pods on soil platforms in the Bako area

sunlight intensity, length, diameter, and thickness of the pods which also varies depending on the chili variety.

Sorting, grading, and storage

The harvested and dried chili pods were sorted and graded (when presumed important) at least into two grade levels (though no established standard for grading) depending on size, color/bleaching, and damage level to add an 'incremental value' in terms of price or eye appeal and market acceptability. The most damaged/broken, bleached pods such as the ones collected from the ground were graded as poor grade. Storage was done either before or after sorting and grading using any suitable and available materials such as plastic bags, jute bags, sacks, mosquito nets, and also on the storehouse floor (mostly by collectors, wholesaler particularly to store a larger bulk). Farmers did not keep/store their dried chili pods for long.

Marketing

The dried chili pods and/or powder moved through complex market channels/networks of interactions of most actors that came into the chili market. Farmers were the base of the value chain supplying the dried chili pods to the nearest markets. Collectors collected and bulked the dried chili pods and sold them at any appropriate market level especially to consumers, small/medium scale processors, and wholesalers as well. Wholesalers bought large quantities of dried chili pods and also sold them at any appropriate next-market level, especially to medium and factory-level processors. Retailers bought dried chili pods (also chili powder) in small limited quantities and sold them mostly to domestic consumers. Processors (only small/medium and household processors were contacted during this study) bought dried chili pods from any market level seems appropriate to them and processed it into powder and supplied mostly to the domestic market. Consumers, as end-users of chili, purchased already processed chili powder or did the processing on their own after buying the dried chili pods.

During this study, wetting of dried chili pods by sprinkling water, 1 or 2 days ahead of transporting/displaying to market was commonly practiced by collectors, wholesalers, and retailers. However, wetting was not commonly practiced by farmers, though some farmers (46%) practiced it. The DDP samples further sun-dried to acceptable dryness (as judged by consumers) lost a mean of 15% (9–21% range) extra moisture. Actors claim that wetting was practiced to minimize smashing of the pods during stacking, packing, and transporting, though consumers claim that it was practiced to gain false extra weight. Water from any available source was used for the wetting without considering its sanitary status. In the market, pieces of plastics, clothes, and rugs were used to display the dried pods with the larger portion of the bulk even falling on bare soil. Displaying/heaping dried pods on the roadside (Fig. 2a) where they can easily be covered by blowing dust was also commonly practiced, particularly by collectors.

Processing

During this study, only small/medium and household processors were contacted. Chili powder preparation starts with dried pods as the main ingredient. The dried pods purchased from the market were further sun-dried to the needed dryness level for ease of destemming

Fig. 2 Chili pods bulked on the roadside (**a**); and were tightly stacked into jute bags for transportation to the next market level (**b**) in the Mareko area

Table 1 Counts and detection frequency of *Aspergillus* from Ethiopian chili postharvest value chain

Sample types (original no.)	Composited/samples analyzed (no.)	*Aspergillus* count (CFU g^{-1}) on AFPA		
		Positive samples (Freq. %)	Count[a]	Range[b]
PiPP (9)	(9)	4 (44)	5.00×10^3	$<1\times10^4$–1.50×10^4
DPP (105)	(42)	42 (100)	9.80×10^4	8.50×10^4–1.10×10^5
CP (20)	(13)	13 (100)	1.50×10^5	1.40×10^5–1.60×10^5
UpPPo (44)	(23)	23 (100)	2.10×10^5	2.00×10^5–2.20×10^5
PaPPo (36)	(18)	18 (100)	1.90×10^5	1.80×10^5–2.00×10^5
214	105	100 (95)		

CFU colony forming units, *PiPP* picked matured red pods, *DPP* dried red pods, *CP* crushed chili, *UpPPo* unpacked chili powder, *PaPPo* packed chili powder

[a] Values are the mean of means of duplicate countable plates of each sample from 10^{-3} dilution with 0.1 ml amount plated

[b] Values are mean of duplicate countable plates of each sample from 10^{-3} dilution with 0.1 ml amount plated

and pounding. Once the pods were further dried and destemmed, pounding was done mostly with traditional mortar and pestle resulting pounded chili. The pounded chili was again further sun-dried and then re-pounded with some additional fresh green and/or dried spices such as garlic (*Allium sativum*), ginger (*Zingiber officinale*), cardamom (*Aframomum corrorima*), basil (*Ocimum basilicum*), rosemary (*Salvia rosmarinus*), Rue (*Ruta graveolens*), black caraway (*Nigella sativa*) and cumin (*Cuminum cyminum*) giving the re-pounded mass. The re-pounded mass physically appeared wetted and clumped (particularly when fresh spices were used), and then further sun-dried and finally became crushed chili. Sun-drying of the re-pounded mass took 2 to 3 days depending on the intensity of the sunlight. The well-dried crushed chili is then slightly warmed on a hot iron or clay plate (optional), salted (optional), and finally milled or pounded into fine powder. Once powdered, kept as unpacked (in open bowls, jute bags, and sacks) or packed (in polyethylene plastic bag) powder, particularly for marketing purposes by small/medium scale processors. At the household level, mostly kept as an unpacked powder using containers like tin and plastic cans. According to household processors, it is the additional spices that give the required flavor and aroma to powder as a spice. Types and quantities of the additional spices used were different from household to household depending on the accustomed flavor and aroma of the individual household. During chili powder preparation, every sun-drying step was done by spreading the item to be sun-dried on a sheet of plastic, cloth, rug, etc. as appropriate. The whole steps of chili powder preparation took about a week depending on the amount being processed, sunlight intensity, and available labor. Along the entire PVC (harvest to powder), direct bare-hand contact was common and frequent, and generally, the postharvest handling practices were poor and unhygienic.

Analysis of Aspergillus

Aspergillus was detected in 44% of PiPP, all (100%) of DPP, CP, UpPPo, and PaPPo samples with counts ranging from 5.00×10^3 to 2.10×10^5 CFU g^{-1} along the PVC

Table 2 *Aspergillus* fold changes among sample types along the Ethiopian chili postharvest value chain

	Sample types and *Aspergillus* count (CFU g^{-1})				
	PiPP (5.00×10^3)	**DPP (9.80×10^4)**	**CP (1.50×10^5)**	**UpPPo (2.10×10^5)**	**PaPPo (1.90×10^5)**
PiPP		19.6	30	42	38
DPP			1.53	2.14	1.94
CP				1.4	1.04
UpPPo					0.90
PaPPo					

Values > 1 show increasing fold change; values = 1 show no counting change (0% change); values between 0 and 1 show decreasing fold change; values = 0 show no count detected (100% decrease)

CFU colony forming units, *PiPP* picked matured red pods, *DPP* dried red pods, *CP* crushed chili, *UpPPo* unpacked chili powder, *PaPPo* packed chili powder

(Table 1). The highest and lowest average counts were recorded in UpPPo and PiPP samples, respectively. The highest fold change (42-fold) was recorded in UpPPo (Table 2). Counts of *Aspergillus* showed distinctive trends of variations among sample types, though did not show uniform increasing or decreasing trends across study localities and storage time.

Based on micromorphological characterization, isolates were identified and categorized into two subgenera (*Circumdati* and *Fumigati)* and four sections (*Flavi*, *Nigri*, *Circumdati*, and *Fumigati*). Figure 3 presents representative colony colors of sections of *Aspergillus* detected during this study. Section *Nigri* was the most frequently detected (99%) with the highest relative density (50%) followed by section *Flavi* both in detection frequency (85%) and relative density (29%). All sections were detected in all sample types except sections *Circumdati* and *Fumigati* were absent in PiPP samples. Tables 3 and 4 show the detection frequency and relative density of sections of *Aspergillus* detected during this study.

A total of 85 representative isolates of section *Flavi* were morphologically further characterized into species. Seventy-six percent (65/85) of them were presumably identified whereas 24% (20/85) of them were not because of doubtful identification. Out of the identified isolates, *Aspergillus flavus* was found predominant at 58% (38/65) followed by *A. parasiticus at* 42% (27/65).

Discussion

The results of this study showed gradually increasing trends of *Aspergillus* count up along the PVC, with the maximum count in UpPPo exceeding the lowest count in PiPP (aseptically picked and processed in the laboratory as a base for comparison) by 5.31 log CFU g^{-1}. Count in PiPP, most likely originated from incidental contamination from airborne fungi, soil contact with pods during tilling/agronomic practices, and lodging of the plants. Similar to this study, fungal contamination of fresh chili in the field has been reported (Adebanjo and Shopeju 1993). Count in DPP increased by 4.97 log CFU g^{-1} as compared to the base count in PiPP, and this could likely be attributed to contaminations from pods collected from the ground and dirty containers during harvesting, direct contact of pods with soil during sun-drying, displaying/heaping of dried pods on the roadside and storing on bare soil/house floor, of course in addition to the add up from contamination at the preceding stage (PiPP). Similarly, previous reports also indicated open sun-drying on soil platforms as a source of fungal infection (Adebanjo and Shopeju 1993; Erdogan 2004; Iqbal et al. 2011).

The raised count in the DPP could also be attributed to further growth and proliferation of the inocula that happened due to increased moisture (15%) of the dried pods as a result of wetting practices. In line with this work, moisture re-introduction during the storage/transportation of Ethiopian spices/herbs and pulses was indicated as one of the main factors favoring mold spore germination, growth, and proliferation (Admasu and Fentahun 2018).

The count in CP increased by 5.16 log CFU g^{-1} as compared to the base count in PiPP. This increase likely originated from the dried and/or fresh green spices used during chili powder preparation in addition to the add-up from the preceding stages (PiPP and DPP). Spices are well known to harbor fungal flora (Matthews and Jack 2011). The maximum increased count (5.31 log CFU g^{-1}) recorded in UpPPo as compared to PiPP could likely be attributed to the cumulative buildup of inocula from the preceding stages (PiPP, DPP, and CP).

In addition, contamination sources like airborne spores, direct bare-hand contact, and equipment were also possible sources contributing to counts at all stages. Airborne spores are known to be a possible source of contamination (Adams and Moss 2008). Mechanically damaged, injured, and point of stem detachment on pods inflicted during harvesting and at various stages of the postharvest likely facilitated contamination by creating

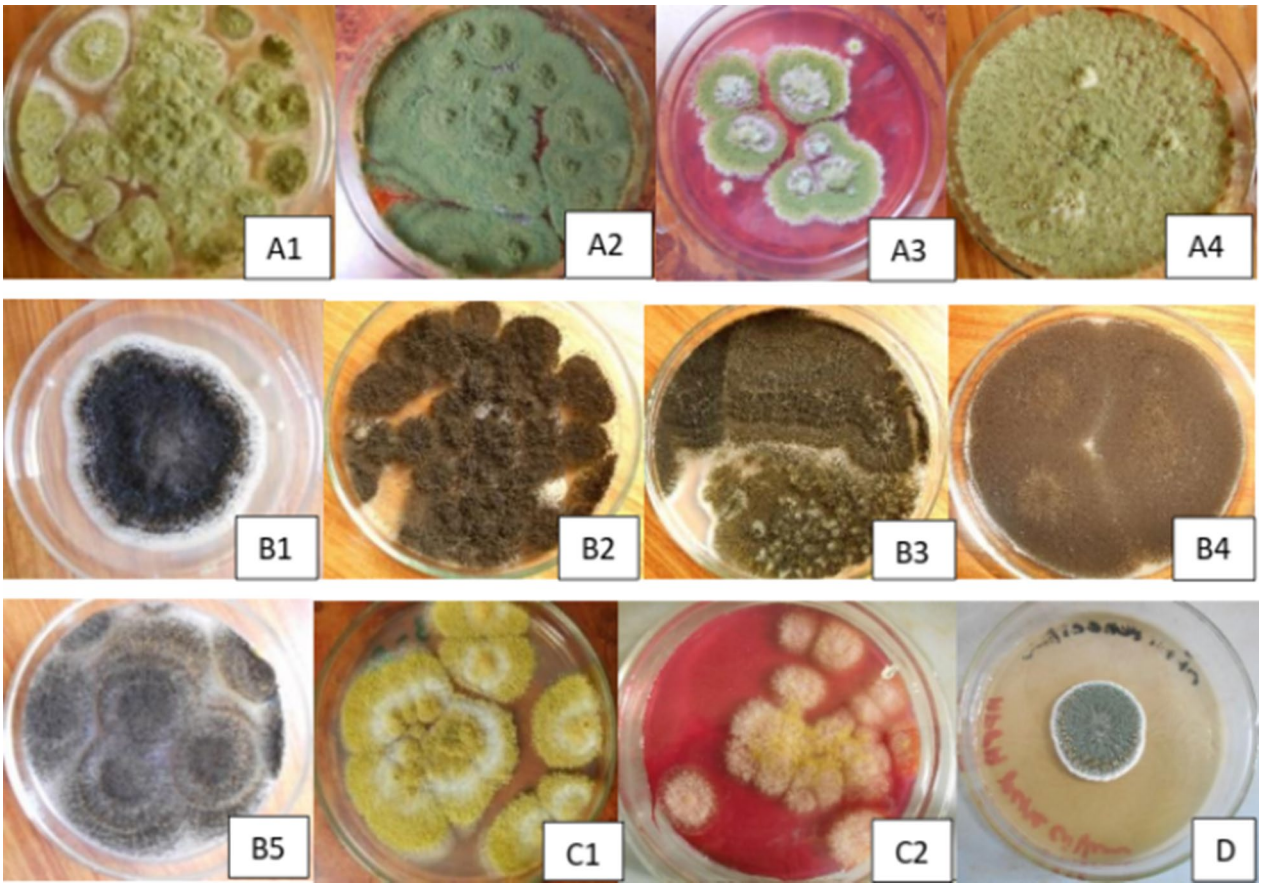

Fig. 3 Representative colony colors of sections of *Aspergillus*. *Flavi* (**A1**–**A4**), yellow-green, parrot green, deep green colony color (Rodrigues et al. 2007, 2009; Houbraken et al. 2014; Moore et al. 2015; Zulkifli and Zakaria 2017); *Nigri* (**B1**–**B5**), dark-brown to black colony color (Silva et al. 2011; Houbraken et al. 2014); *Circumdati* (**C1**–**C2**), yellow to yellowish-brown/ochre colony color (Visagie et al. 2014), and *Fumigati* (**D**) on PDA, velutinous blue-green colony color (Samson et al. 2007; Zulkifli and Zakaria 2017). **A3** and **C2** are with rose Bengal added

Table 3 Detection frequency of sections of *Aspergillus* from Ethiopian chili postharvest value chain

Subgenus	Section	Sample type (n = total positive samples)											
		Picked chili pods (n = 4)		Dried chili pods (n = 42)		Crushed chili (n = 13)		Unpacked chili powder (n = 23)		Packed chili powder (n = 18)		Total (n = 100)	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Circumdati	*Flavi*	1	25	30	71	13	100	23	100	18	100	85	85
Circumdati	*Nigri*	3	75	42	100	13	100	23	100	18	100	99	99
Circumdati	*Circumdati*	0	0	9	21	9	69	22	96	16	89	56	56
Fumigati	*Fumigati*	0	0	4	10	7	54	12	52	13	72	36	36

possible points of entries/contamination and favoring fast growth and proliferation of inocula accounting for raised counts (fold changes) at all stages of the PVC. Conducive conditions such as moisturization of dried pods, tight stacking, free aeration along the PVC, continuous growth, and proliferation of inocula at each stage likely contributed to the increased counts (fold changes) at that particular and subsequent stage of the PVC.

Though increased trends of counts were recorded along the PVC, counts in PaPPo showed a slight decline, by 4.3 log CFU g^{-1} as compared to the UpPPo. This decline can likely be attributed to reduced further contamination

Table 4 Relative density of sections of *Aspergillus* from Ethiopian chili postharvest value chain

Subgenus	Section	Sample type (n = total positive samples)																
		Picked chili pods (n = 4)			Dried chili pods (n = 42)			Crushed chili pods (n = 13)			Unpacked chili powder (n = 23)			Packed chili powder (n = 18)			Total (n = 100)	
		No.	%[a]	%[b]	No.	%[a]	%[b]	No.	%[a]	%[b]	No.	%[a]	%[b]	No.	%[a]	%[b]	No.	%[c]
Circumdati	*Flavi*	1	25	1	33	30	19	27	33	16	58	28	34	51	28	30	170	29
Circumdati	*Nigri*	3	75	1	65	58	22	36	43	12	97	48	33	91	49	31	292	50
Circumdati	*Circumdati*	0	0	0	9	8	11	13	16	15	37	18	44	25	14	30	84	14
Fumigati	*Fumigati*	0	0	0	4	4	10	7	8	17	12	6	29	18	10	44	41	7
Total of a sample type		4	100		111	100		83	100		204	100		185	100		587	100

[a] Relative density of isolate(s) of each section in relation to the total of each sample type

[b] Relative density of isolate(s) of each section of each sample type in relation to a total of each section

[c] Relative density of total isolates of each section in relation to a total of all sections or all sample types

(minimized entry) and reduced growth and proliferation of inocula possibly due to limited aeration as a result of the packaging. This is consistent with the findings of Iqbal et al. (2011) who reported significantly lower counts of total mold and *Aspergillus* counts from chilies packed in polyethylene bags as compared to chilies stored in jute bags.

The generally increased trends of counts (fold-changes) up along the PVC evidenced role of the poor and unhygienic handling practices as possible risk factors (in addition to other extrinsic and intrinsic factors) for the continuous *Aspergillus* contamination, growth, proliferation, buildup, and survival of conidia/sclerotia up along the PVC. The relatively minimal counts in PiPP and variations of counts (fold-changes) among sample types, and non-uniform increasing or decreasing trends across study localities and storage time also demonstrated the importance of the poor and unhygienic handling practices over localities and storage time alone. Previous work on Ethiopian chili (Tsehaynesh et al. 2021) also reported non-significant differences in fungal counts across localities and counts which are far below the counts of our study. This dissimilarity of counts could be due to differences in handling practices in addition to other extrinsic and intrinsic factors. Existing food safety microbiology facts also show favorable environmental conditions as the most determining factors that support microbial contamination of agri-foods at any stage of food production (Choudhary and Kumari 2010).

During this study, the postharvest practices were generally identified as practices that can cause cross-contamination, can expose the chili to further contamination, and can create conducive conditions for the growth and proliferation of inocula and possible points of entry/contamination. Some of the practices such as collecting dropped, insect damaged/wounded, and diseased/stressed pods to increase the bulk, and wetting of dried pods to gain false extra weight were considered as 'economically motivated adulteration' (a food fraud).

The detection of sections of *Aspergillus* such as *Nigri* and *Circumdati* known to consist of species capable of producing ochratoxin A (Samson et al. 2004; Visagie et al. 2014), and *Flavi*, having species known to produce aflatoxins (Kumar et al. 2017) warns possibility of mycotoxin contamination and high potential health risks. Similar to this work, the presence of *Aspergillus flavus, A. parasiticus*, and *A. niger* in chili samples collected from some districts of Ethiopia has been reported (Tsehaynesh et al. 2021). Recently, aflatoxin G1 (43.61 μg kg^{-1}) and B1 (22.18 μg kg^{-1}) contaminations from Ethiopian chili powder, and cancer risk due to consumption of aflatoxin-contaminated chili were reported (Tariku et al. 2020). Studies in Chile, Bolivia, and Peru also suggested the possibility of the development of gallbladder cancer (GBC) with a high-level consumption of aflatoxin and ochratoxin A (OTA) contaminated chilies (Ikoma et al. 2015; Asai et al. 2012). During this study, a mycotoxigenicity test for each *Aspergillus* subgroup/species using biochemical/analytical methods was not done due to the costly nature of the methods, and as the study was concerned more with trends, possible points of aspergilli contamination and contributing risk factors. In future studies, considering the association of the presence of *Aspergillus* subgroups/species, and mycotoxins and factors favoring mycotoxin production would give more supportive information. Besides, the study was mainly based on morphological characterization for the identification of *Aspergillus*, and as a result, due to morphological complexities of genus *Aspergillus* in general and *Aspergillus* section *Flavi* in particular, a number of isolates that even appeared morphologically doubtful were not included in this report. Though the study is concerned more with trends, possible

points of aspergilli contamination, and contributing risk factors, in future studies, the use of advanced tools such as molecular analysis, particularly if the taxonomic categories make a difference in the results of the study would give more detailed taxonomic information. During this investigation, in addition to mycotoxin-producing aspergilli, the detection of section *fumigati*, the main etiologic agent of invasive aspergillosis (Bart-Delabesse et al. 2001) also indicated the possibility of additional health burden.

Conclusions and recommendations

The chili samples analyzed from different stages of the PVC contained *Aspergillus* with increasing counts (fold-changes) up along the chain. Most of the handling practices appeared to be poor and unhygienic and probably contributed to the contamination. Harvesting and direct open sun-drying were likely initial and critical points of *Aspergillus* contamination while wetting and tight stacking likely contributed to aggravated growth and proliferation of aspergilli leading to further consecutive buildup. This study revealed trends, possible points of aspergilli contamination, and contributing contamination risk factors indicating possible points of intervention to protect against aspergilli contamination of Ethiopian chili. Intervention at these stages would make a significant difference.

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Author contributions
THT developed research proposal, conducted field and laboratory diagnosis, data analysis and wrote the article, AT participated in field diagnosis, research proposal development and reviewing, MA assisted in proposal development and reviewing.

Declarations

Competing interests
The author declares that there are no commercial or financial competing interests.

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References

Abbas HK, Shier WT, Horn BW, Weaver MA. Cultural methods for aflatoxin detection. J Toxicol. 2004;23(2):295–315.
Adams MR, Moss MO. Food microbiology. 3rd ed. Cambridge: RSC Publishing; 2008.
Adebanjo A, Shopeju E. Sources of mycoflora associated with some sundried vegetables in storage. Int Biodeterior Biodegrad. 1993;31:255–63.
Admasu and Fentahun. Aflatoxin related challenges and mitigation strategies of Ethiopia in spices, herbs and pulses' domestic and international markets. 2018. https://www.researchgate.net/publication/337211699. Accessed 20 July 2022.
Asai T, Tsuchiya Y, Okano K, Piscoya A, Nishi CY, et al. Aflatoxin contamination of red chili pepper from Bolivia and Peru, countries with high gallbladder cancer incidence rates. Asian Pac J Cancer Prev. 2012;13(10):5167–70. https://doi.org/10.7314/APJCP.2012.13.10.5167.
Bart-Delabesse E, Sarfati J, Leeuwen WV, Belkum AV, Bretagne S, Latge JP. Comparison of Restriction Fragment Length Polymorphism, Microsatellite Length Polymorphism, and Random Amplification of Polymorphic DNA Analyses for Fingerprinting Aspergillus fumigatus Isolates. J Clin Microbiol. 2001;39(7):2683–6.
Bedada TL, Derra FA, Gebre SG, Sima WG, Edicho RM, Maheder RF, Negassi TY, Asefa YB. Microbial evaluation of spices in Ethiopia. Open Microbiol J. 2018;12:422–9.
Choudhary AK, Kumari P. Management of mycotoxin contamination in preharvest and postharvest crops: present status and future prospects. J Phytol. 2010;2(7):37–52.
EAC (East African Community). Harmful effects of aflatoxin and its impact on human health. EAC policy brief on aflatoxin prevention and control. Policy brief no. 1; 2018.
ENTAG (Ethiopia–Netherlands trade for Agricultural Growth). Business opportunity report. Spices sector in Ethiopia. 2020. https://www.advanceconsulting.nl/assets/Uploads/spicesbor-ethiopia.pdf. Accessed 23 Aug 2022.
Erdogan A. The aflatoxin contamination of some chilichilitypes sold in Turkey. Chemosphere. 2004;56:321–5.
EU (European Union). Commission regulation (EU) no 165/2010. Amending regulation (EC) no 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. Off J Eur Union L 50/8. 2010.
FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization). FAO/WHO regional conference on food safety for Africa, Harare, Zimbabwe, 3–6 Oct 2005. National food system in Ethiopia, a situation analysis (prepared by Ethiopia). 2005.
Fufa H, Urga K. Screening of aflatoxins in Shiro and ground red pepper in Addis Ababa (Abstract). Ethiop Med J. 1996;34:243–9.
Fufa H, Urga K. Survey of aflatoxin contamination in Ethiopia. Ethiop J Health Sci. 2001;11(1):17–25.
Herms S. Business opportunities report spices #6 in the series written for the Ethiopian Netherlands business event 5–6 Nov 2015, Rijswijk, The Netherlands.
Houbraken J, de Vries RP, Samson RS. Modern taxonomy of biotechnologically important *Aspergillus* and *Penicillium* species. In: Sariaslani S, Gadd GM, editors. Advances in applied microbiology. Amsterdam: Elsevier; 2014. p. 199–249.
IFPRI (International food policy research institute). Aflatoxins finding solutions for improved food safety. Washington: IFPRI; 2013.
Ikoma T, Tsuchiya Y, Asai T, Okano K, Edoh K, et al. Ochratoxin contamination of red chili peppers from Chile, Bolivia and Peru showing high incidences of gallbladder cancer. Asian Pac J Cancer Prev. 2015;16(14):5897–991. https://doi.org/10.7314/APJCP.2015.16.14.5987.
Iqbal Q, Amjad M, Asi MR, Arino A. Assessment of hot peppers for aflatoxin and mold proliferation during storage. J Food Prot. 2011;74(5):830–5.
King AD Jr., Hocking AD, Pitt J. I.1979. Dichloran-rose bengal medium for enumeration and isolation of molds from foods. Appl Environ Microbiol 37:959–64.
Kumar P, Mahato PDK, Kamle M, Mohanta TK, Kang SG. Aflatoxins: a global concern for food safety, human health and their management. Front Microbiol. 2017;7: 2170. https://doi.org/10.3389/fmicb.2016.02170.
Liew WPP, Mohd-Redzwan S. Mycotoxin: its impact on gut health and microbiota. Front Cell Infect Microbiol. 2018;8: 60. https://doi.org/10.3389/fcimb.2018.00060.
Matthews M, Jack M. Spices and herbs for home and market. Rome: Food and Agriculture Organization of the United Nations (FAO); 2011.
Moore GG, Mack BM, Beltz SB. Genomic sequence of the aflatoxigenic filamentous fungus *Aspergillus nomius*. BMC Genom. 2015;16:551. https://doi.org/10.1186/s12864-015-1719-6.
Muluken Yewondwossen. EU rejects Ethiopian red pepper for unsafe levels of toxins. 2017. https://health-ethiopianism.blogspot.com/2017/05/eu-rejects-ethiopian-red-pepper-for.html. Accessed 18 Dec 2019.

Pitt JI, Hocking AD. Fungi and food spoilage. 3rd ed. New York: Springer; 2009. p. 519.
Pitt JI, Hocking AD, Glen D. An improved medium for the detection of *Aspergillus flavus* and *A. parasiticus*. J Appl Bacreriol. 1983;54:109–14.
Rodrigues P, Soares C, Kozakiewicz Z, Paterson RRM, Lima N. Identification and characterization of *Aspergillus flavus* and aflatoxins. Commun Curr Res Educ Top Trends Appl Microbiol. 2007;2007:527–34.
Rodrigues P, Vena˜ncio A, Kozakiewicz Z, Lima N. A polyphasic approach to theidentification of aflatoxigenic and non-aflatoxigenic strains of Aspergillus Section Flavi isolated fromPortuguese almonds. Int J Food Microbiol. 2009;129:187–193.
Sahu PK. Research methodology: a guide for researchers in agricultural science, social science and other related fields. West Bengal: Springer; 2013.
Samson RA, Houbraken JAMP, Kuijpers AFA, Mick Frank J, Frisvad JC. New ochratoxin a or sclerotium producing species in *Aspergillus* section *Nigri*. Stud Mycol. 2004;50:45–61.
Samson RA, Hong S, Peterson SW, Frisvad J, Varga J. Polyphasic taxonomy of *Aspergillus* section *Fumigati* and its teleomorph *Neosartorya*. Stud Mycol. 2007;59:147–203.
Silva DM, Batista LR, Rezende EF, Fungaro MHP, Sartori D, Alves E. Identification of fungi of the genus *Aspergillus* section Nigri using polyphasic taxonomy. Braz J Microbiol. 2011;42:761–73.
Smith LE, Prendergast AJ, Turner PC, Mbuya MN, Mutasa K, Kembo G, Stoltzfus RJ. The potential role of mycotoxins as a contributor to stunting in the SHINE trial. Clin Infect Dis. 2015;61(7):S733–7.
Strategy. All ACP agricultural commodities programme. Spice sub-sector strategy for ETHIOPIA. Submitted to the Government of Ethiopia by the spice sector strategy coordinating committee, February 2010; 2010.
Tolera TH, Tesfaye A, Alemu M. Aflatoxin contamination of Ethiopian hot red pepper and risk characterization: dietary exposure assessment and estimated aflatoxin-induced hepatocellular carcinoma. Ethiop J Agric Sci. 2020;30(4):63–81.
Tsehaynesh T, Abdi M, Hassen S, Taye W. *Aspergillus* species and aflatoxin contamination in pepper (*Capsicum annuum* L.) in West Gojjam, Ethiopia. Afr J Food Agric Nutr. 2021;21(1):17178–94.
Visagie CM, Varga J, Houbraken J, Meijer M, Kocsube S, Yilmaz N, Fotedar R, Seifert KA, Frisvad JC, Samson RA. Ochratoxin production and taxonomy of the yellow aspergilli (*Aspergillus* section *Circumdati*). Stud Mycol. 2014;78:1–61.
Zulkifli NA, Zakaria L. Morphological and molecular diversity of *Aspergillus* from corn grain used as livestock feed. HAYATI J Biosci. 2017;24:26–34.

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