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Aspergillus SPECIES AND AFLATOXIN CONTAMINATION IN PEPPER (*Capsicum annuum l.*) IN WEST GOJJAM, ETHIOPIA

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ABSTRACT

Pepper (*Capsicum annuum* L.) is an important spice and source of income for smallholder producers in Ethiopia. Since the larger proportion is for the market, it takes a significant share of the national income from export commodity. However, often the product was rejected by some of the European Union markets due to the maximum aflatoxin level accumulations beyond their acceptable limits. So, the present study was carried out to highlight the importance of *Aspergillus* species invasion in pepper, and levels of aflatoxin contamination at maturity in the field (pod form), farmers' storage and local market (powder form) in West Gojjam, Ethiopia. A total of 135 pepper samples were collected from three districts of West Gojjam (Burie, Jabitehnan, and Fnoteselam), Ethiopia for fungal and aflatoxin analysis. The producers used a pre-validated structured questionnaire to obtain information on pepper production practices. *Aspergillus* species isolates were recovered using potato dextrose agar (PDA) medium and counting was through dilution method (cfu g^{-1}). The pepper pods were ground to a fine powder for aflatoxin analysis using Enzyme-Linked Immunosorbent Assay (ELISA). The prevalence of infected samples revealed that, pre-harvest samples (51%) were less infected by *Aspergillus* species, compared to local markets (65 %) and storage (79 %). *Aspergillus flavus* species were recovered in pre-harvest samples, whilst *A. niger* were found in local market samples. Aflatoxin contamination at pre-harvest, storage, and local market were 10, 47, and 42 % with levels which ranged from non-detected to 10.6, 0.3 - 17.1 and 3.1 - 19.2 ppb, respectively. The mean aflatoxin concentration detected from storage samples (10.6 ppb) and local market (12.6 ppb) were found to be above the tolerable limits set by EU (5 ppb) in pepper products. From the findings in this study, proper drying, physical separation of molded pods and use of clean storage structures should be implemented along the production chain in order to reduce aflatoxin contamination in pepper in Ethiopia.

Key words: Aflatoxin, *Aspergillus* species, farmer's storage, local market, pre-harvest

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INTRODUCTION

Pepper (*Capsicum annum* L.) is an exotic crop for Ethiopia and believed to be introduced by the Portuguese in the 17th century [1] and currently considered as the national spice. It is widely cultivated in different regions of Ethiopia specifically, Amhara, Oromiya, and Southern Nation Nationalities and Peoples Regional State. The estimated national production of dry red and green pepper were 264,722.5 and 63,240.5 tons with an average productivity of 1.7 and 6.2 tons ha^{-1}, respectively [2]. However, the national average yield found low (1.7 and 6.2 ton ha^{-1}) compared to the global pepper production of dry red and green peppers estimated at 3.9 and 34.5 million tons, harvested from 1.8 and 1.9 million hectares for both dry red and green peppers, respectively [3].

Pepper is an important agricultural crop because of its economic importance, nutritional and medicinal value of its fruit. It is an important source of nutrients like vitamins A and C content; high iron, potassium, and magnesium provided in the human diet [4] and it can be consumed fresh or dried. The range of food products that contain pepper or its chemical constituent is broad and includes ethnic foods, meat, salad dressings, mayonnaise, dairy products, and candies, packed foods, snack foods, salsa, and hot sauces. The crop is also an important source of spice extraction due to the presence of various oleoresin for dying of food items [5]. Ethiopia is among the few developing countries that have been producing paprika and *Capsicum* oleoresins for the export market [5]. Apart from its food importance, pepper is one of the most important spices that serve as the cash crop, and source of income for smallholder producers in many parts of rural Ethiopia. The worth of pepper production income contributed to growers and countries is increasing over time.

Despite its importance, pepper is vulnerable to mycotoxin contamination along the production chains by *Aspergillus* species which exhibit immense ecological and metabolic differences [6]. These fungi are capable of growing on a great variety of food commodities and animal feed materials when the conditions of temperature, relative humidity, and product moisture are favorable for development [7]. Pepper crop has been reported as aflatoxin-contaminated [8], a major group of mycotoxins, produced by species of *Aspergillus,* primarily *A. flavus* and *A. parasiticus*. Contamination of pepper with aflatoxins may start at field (pre-harvest) conditions or during drying, storage or processing stages (post-harvest). Due to its health and economic impacts, different countries have their own limits of tolerances, for example, European Commission Regulation has 4 ppb limits for total aflatoxin in cereals and peanuts, and 10 to 15 ppb for spices [9]. However, Ethiopia has no tolerable limits for all agricultural crops. Though, maximum contamination of aflatoxin has caused massive economic losses with export and import markets and diseases such as impaired immune system, cancer and stunted growth in infants have been reported [10].

The occurrence of aflatoxin in pepper and its product was limited; however, Besrat and Gebre [11] first reported the mean aflatoxin level of 32 and 102 ppb in red pepper powder and its paste in Ethiopia. According to Capital Ethiopia, A Crown Publishing Company [12,] a pack of Ethiopian hot red pepper worth economic value of USD 10 million was rejected from the European Union markets, due to the presence of maximum aflatoxin

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concentration. In the last five years, from 2016 an increasing amount of Ethiopian pepper has had unsafe amounts of aflatoxin, close to 78 ppb, while the tolerance limit is 5 ppb in pepper products [12]. Such market rejection could lead to great economic losses of the growers and the country too. Aflatoxin can contaminate pepper during processing due to poor harvesting practices, improper drying, handling, packaging, storing and inadequate transport facilities. The current study was initiated to generate important data for various stakeholders such as Agriculture and Extension officers, and Ministry of Health.

West Gojjam of Amhara regional state of Ethiopia is an area known for pepper production, consumption, and domestic markets. However, there was a limited study of aflatoxin contamination of pepper in the area. Therefore, assessment of aflatoxin contamination in pepper and contaminating fungi are needed for the collection of coherent data at the country level. The aims of this study were to determine the major *Aspergillus* species contaminating pepper and also quantify the level*s* of aflatoxin in pepper along the value chains in the study areas.

MATERIALS AND METHODS

Survey and pepper sample collection

A total of 135 pepper samples were collected from fields (pre-harvest), farmer's storage and local market (powder) from November 2017 to April 2018, from three potential pepper producing districts (Burie, Fnoteselam, and Jabitehnan) of West Gojjam zone, Amhara regional state, Ethiopia (Table 1). Systematic random sampling was used to collect samples from these selected districts. From each district 45 samples, weighing 500 g at pre-harvest (n = 15), storage (n = 15) and local market (n = 15) were collected. Pre-harvest samples were collected in cross-sectional ways or X fashion from farmers' fields. Three months later from February to April 2018 equal amounts were collected from farmers' storage, likewise, local market samples were collected in powder form from a different retailer. Multiple sub-samples were collected, blended and representative of each lot considered. All samples were properly labeled with the name of the location and collection date and brought to Plant Pathology Laboratory, Haramaya University and stored at 4 ^{o}C for analyses.

During sample collections relevant pre and post-harvest information on the management practice of the pepper, such as: ways of planting, time of harvest (at optimum, delay harvesting), storage practices, and planting varieties were collected using a standard questionnaire (Table 2). A total of 45 (3 districts X 15 farmers = 45) pepper growing farmers have participated in the study. These farmers were randomly selected, while the villages were selected purposely depending on the pepper producing potential.

Fungal isolation

Pepper pod samples were ground to a fine powder using sterilized laboratory mill (Kanchan Multipurpose Kitchen, India). About 500 g was taken from each sample and divided into two parts for mycology and aflatoxin analysis (each 250 g). Fungal isolation from each sample was through dilution plating method and pour plate technique on potato dextrose agar (PDA) medium. The numbers of fungal colonies of samples were

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expressed as the logarithm of colony-forming units (cfu) per gram of sample as indicated [13], and prevalence of each *Aspergillus* species was carried out.

$$\text{Log}_{10}\ (cfu\ g-1) = \frac{number of colonies of a\ fungal\ species}{amount\ plated\ *\ dillution\ factor}$$

$$\text{Prevalence (\%)} = \frac{number\ of\ isolates\ of a fungal\ species}{total\ nubers\ of fungal\ speices}\ x100$$

***Aspergillus* species identification**

Aspergillus species colonies inoculated in PDA were counted and purified on freshly prepared CDA (Czapex Dox Agar) and incubated for 5-7 days at 25 °C. Plates were examined daily, with repeated sub-culturing into another freshly prepared CDA medium in order to get pure cultures using sterilized loops. Isolates were stained with lactophenol cotton blue and observed under the light microscope (40x). Fungal colonies that grew rapidly and produced colors of white, yellow, yellow-brown, brown to black or shades of green, mostly consisting of a dense felt of erect conidiophores were broadly classified as *Aspergillus* species. Isolates with dark green colonies and rough conidia were considered as *A. parasiticus*, while isolates that produced light green and smooth conidia were classified as *A. flavus* [14]. The major distinction separating *A. niger* from the other species of *Aspergillus* is the production of carbon black or very dark brown spores from biseriate phialides. Further, isolates were distinguished based on the exudates, pigments, and size of sclerotia. Pure culture of each *Aspergillus* isolates was identified using the laboratory manual (14).

Aflatoxin extraction and analysis

From each sample of pepper, 50 g was ground and mixed using laboratory mill (Kanchan Multipurpose Kitchen, India) to form fine powder 1 mm size. Then, 20 g of powder sub-sample was suspended to 250 mL conical flask containing 20:80 mL of distilled water and methanol. Each flask was shaken at 500 rpm for 3 minutes on a rotator flask shaker (Shaker 8 ErlenmeyerStuarts^{TM}SF1, UK). Extracts were allowed to settle, the top layer of the extract filtered through a Whatman No.1 filter paper, and the filtrate was collected to the clean falcon tubes of 50mL and used for total aflatoxin analysis. The extract dilution of 1 mL of the filtered extract was put into a 14 mL falcon tube and diluted with an aliquot of the extract 1:10 with re-constituted washing buffer.

Total aflatoxin concentration in the sample was determined using enzyme-linked immunoassay (ELISA) kit (RIDASCREEN® Aflatoxin Total Art.No.:R4701, Darmstadt, Germany), a competitive enzyme immunoassay for the quantitative analysis of total aflatoxin, according to the manufacturer's recommendation. Then, 50 µL of enzyme conjugate was added in each premixing wells, followed by addition of 50 µL of each standard/sample into the correspondent premixing wells. Then it was mixed three times with micropipette and immediately 100 µL was transferred from each premixing well into corresponding anti-aflatoxins antibody-coated micro-well and incubated for 30 minutes at room temperature and the liquid was poured out from the wells. This was followed by completely filling all the wells with working washing buffer and the liquid was poured out from the wells (repeated 3 times in a row). Then, 100 µL of the substrate

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(chromogen) was added into each well, mixed gently by shaking the plate manually and incubated for 15 minutes at room temperature. Finally, 100 µL of stop solution was added to each well and mixed by shaking the plate manually for 10 seconds and absorbance was measured at 450 nm within 30 minutes after the addition of stopping solution.

Data analysis

Data obtained from the questionnaire were analyzed as proportional values. Laboratory data from each district and sampled stages, were entered into calculated Microsoft Excel sheets and analyzed using SPSS v.20 for prevalence, and further crosscheck using one-way analysis of variance (ANOVA). The differences in group mean of the ranked scores for fungal and aflatoxin contamination in each district sampled was tested for significance ($\rho < 0.05$). Total aflatoxin concentration was calculated as ppb.

RESULTS AND DISCUSSION

Farmers' management practices of pepper

The demographic characteristics of growers in terms of sex, age and education level were considered in the questionnaire. Among the total farmers interviewed, 84.4% (n= 38) were male and the remaining 15.6 % (n= 7) female (Table 2). The age intervals of farmers ranged from 26 to 62 years with a mean of 40 years. Majority of the respondents had an age from 20 to 40 years old (n= 26, 57.8 %), followed by less frequent from 40 to 60 years old (n= 18, 40.0 %). According to the Ethiopian age group classification, these groups are considered a productive group. The lowest age group (n= 1, 2.2 %) had an age of beyond 60 years old. The educational background of farmer also considered as variable and about 24.5 % were uneducated, while 31.1 % found able to read and write due to they joined basic education system and religious school. However, 24.4 % were those who attended primary school, while 20.0 % were joined secondary school and above.

In case of pepper production constraint factors, all of the interviewed farmers (100 %), heard about mold associated with pepper products. However, limitations of improved seed varieties made them to use local varieties for the planting of peppers. Farmers used, pepper seeds for planting either from their own harvests or through sharing among partners and neighbor. Such planting materials are often mixtures of different varieties with impurities and likely harboring pathogens. Planting of poor pepper seed and local varieties can be attributed to poor yields and prevalence of fungal contamination [15]. The current study affirmed that, the pepper varieties planted in the surveyed areas are local and calling for the adaptation of improved varieties against *Aspergillus* infection and aflatoxin accumulation in West Gojjam.

In the present study, 77.8 % of respondents used transplant (raising seedlings in a nursery) and 22.2 % direct planting system. In the surveyed districts, pepper harvesting starts 4 to 5 months after transplanting and manually picking. Maturity is determined by the color changes (green to light red) when the fruit physically attained maturity [16]. However, a proportion of immature fruits are also picked up during the process and quick plucking action, fruits are often picked in a way that pedicle remains onto the plants. Such fruits as a result of opening up of their viscera are more liable to fungal

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contamination that may lead to aflatoxin production. The collection of immature and mature pods with physical damage aggravates for mold development and considered as the chief source of aflatoxin contamination [17].

Pepper drying is also an important practice, in the production chain. About 91.1 % of participants dry their pepper directly on the ground using the sun, while 8.9 % used the top of their houses. Indeed, over drying of pepper directly on the sun for long period on top of houses would not advisable as it decreases the pepper pungency. Some of the researchers revealed that pepper with a high concentration of capsaicin (the pungent component of peppers) had lower concentrations of aflatoxin [18]. Poor storage conditions could also influence mold development and aflatoxin contamination in the stored products. In the districts surveyed during the sample collection, it was observed that the common storage materials of pepper were regular sack, in which most of the participants (82.2 %) stored pepper yield in an old sack and 17.8 % used new sack, while 69.6 % store with other product, which could likely affect the quality of the products. Some study in India revealed that, during storage conditions, aflatoxin contamination presumably due to inappropriate handling with insufficiently dried pepper products [19].

Fungal population in pepper samples

Aspergillus species contamination of pepper samples were expressed as colony-forming unit of fungi and converted to the log of cfu g^{-1}. The maximum contamination occurred in the farmers' storage (1.45 $\log_{10}$ cfu g^{-1}), followed by pre-harvest (1.23 $\log_{10}$ $cfug^{-1}$), and local market (1.20 $\log_{10}$ cfu g^{-1}) samples. Researchers also reported that fungal contamination can occur throughout the production chain, from the harvesting, drying and storage phases to product transportation and marketing [20]. Peppers are susceptible to fungal contamination during drying and storage condition [21], supported our finding.

Fungal invasion across the districts at each sampled stage was evaluated. At the pre-harvest stage, mean fungal invasion of 1.24±0.21, 1.20±0.22, 1.26±0.16 $\log_{10}$ $cfug^{-1}$ from Burie, Fnoteselam, and Jabitehnan, respectively were recorded, with non-significant differences across the districts. Pepper pods infected by insects at field stage could influence prevalence's of fungal infection due to it creates routine fungal entrances. The insect wounds on the pistil of the flowers might serve as the germinating beds for mold spores and growth of aflatoxigenic fungi like *Aspergillus* species, thus, resulting in aflatoxin accumulation [22]. Moreover, the insect presences or incidences were not recorded during the sample collection in the current study.

Mean fungal population in storage samples were 1.45±0.03, 1.44±0.04, 1.46±0.01 $\log_{10}$ $cfug^{-1}$ from Burie, Fnoteselam and Jabitehnan, respectively, and indicated greater infection compared to pre-harvest samples. It might have been due to, the farmers management practice such as; drying the pepper through spreading on the direct ground or roadside, enhances mold development. It is observed that sometimes farmers sprinkled water on pepper during packaging to reduce the strong spicy smell. This little moisture on the fruit enhanced mold development during storage [23]. In the study areas, it was observed that farmers store the damaged and healthy pods together, which needs proper screening to reduce further mold development.

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The mean fungal population in the local market samples was 1.14±0.14, 1.22±0.17 and 1.22±0.11 $\log_{10}$ $cfug^{-1}$ from Burie, Fnoteselam, and Jabitehnan, respectively. Even though the samples were collected from the local markets, in which some were supposed to be sold directly for consumers, the fungal invasion was observed. Such fungal contamination in the products ready for consumption may threaten human health. Though, sorting and market contamination could be reduced through buying the healthy pods, physical separation, as well as applications of safe anti-microbial properties of essential oils, has synergistic effects against fungal growth. Perhaps, physical sorting is the effective measure in the reduction of mold, as high as 40 to 80 % [24], such practices could be practiced among the pepper grower.

Prevalence of *Aspergillus* species in pepper samples

The mean prevalence of *Aspergillus* species in pepper samples across surveyed districts and sampled stages varied with locations (Table 3). Three common *Aspergillus* species associated with pepper in West Gojjam were identified as, *A. flavus*, *A. parasiticus,* and *A. niger.* In the pre- and post-harvest samples, *A. flavus* 26.1 and 30.6 %, recovered than *A. parasiticus* (25.5 %, each), while *A. niger* isolated in less frequency (7.0 and 23.4 %), respectively. *A. flavus* is the predominant species in pepper samples in several cases [25]. It might be due to their ability to survive in a wide range of environmental factors (temperature and humidity) compared to other species. *A. flavus* is a saprophytic soil fungus that infects and contaminates pre and post-harvest crops [26]. Studies have demonstrated peppers are susceptible to aflatoxin producing *A. flavus* during drying and storage conditions [27].

In the samples collected from the local market (powder form), *A. niger* (32.1 %), followed by *A. flavus* (17.5 %) and *A. parasiticus* (15.6 %) were recovered. Researchers revealed that, *Aspergillus* species such as; *A. fumigatus*, *A. flavus*, and *A. niger* isolated in red pepper powder samples [28], in which *A. fumigatus* did not isolated in the present study with the highest presence of *A. niger* in local market samples. *A. flavus* and *A. parasiticus* were isolated in low frequency than pre-harvest and storage. Moreover, among *Aspergillus* species, *A. flavus* had the lowest occurrence in garlic, ginger, and pepper powder in another study [29]. Across the surveyed districts, pepper powder sold in the local market might have been prepared from low-quality yield and sold unpacked likely exposed to *Aspergillus* species contamination.

Total aflatoxin in pepper

The levels of total aflatoxin from non-detected to 10.6, 0.3 to13.9, and 3.6 to 19.2 ppb, in pre-harvest, farmers' storage, and local market samples, respectively were detected (Table 4), with the means of 2.2, 10.7 and 11.7 ppb. Aflatoxin level was high in the local market and farmers' storage samples, than pre-harvest, which was corroborated with findings reported in Africa [30], stated aflatoxin production continues with increasing at post-harvest, storage conditions and processing. Likewise, the mean prevalence of fungal infection were also higher in storage (26.5 %) and local market (21.7 %) samples, compared to pre-harvest (17.1 %), might have been responsible for aflatoxin contamination. However, another researcher indicated, there was no relationship between the number of *Aspergillus* species isolated from peppers and the level of concentration of aflatoxin [31], contradicting with the current finding.

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The proportion of positive samples for aflatoxin from each sampled stage pre-harvest, storage, and local market were 10.3, 47.0 and 42.4 %, respectively, in which storage samples found highly contaminated, followed by the local market. However, in the earlier study, powder pepper samples positive for aflatoxin contamination was 13.3 %, which collected from government-owned food stores, retail shops and open market of Addis Ababa, Ethiopia [32]. In this study pre-harvest (10.3 %) and storage (47.0 %) samples were in pod forms, likewise in Iran, the incidence of aflatoxin-contaminated samples (70.0 %) was found in pod form peppers [33]. Similarly, various incidences of aflatoxin contaminations of powdered (46.0 %) and crushed (52.8 %) pepper samples were reported from Pakistan, with concentration beyond the maximum limits (20 ppb) assigned by the USDA [34]. However, the total aflatoxin detected in 12 spices (13.6 %) at the level of 0.08 to 4.7 ppb [35], found less than our results. Contamination of pepper with aflatoxin could occur due to poor post-harvest handling and non-sanitary conditions. Poor handling at post-harvest storage and transportation increases aflatoxin contamination [19]. Peppers are susceptible to aflatoxin contamination in the field during production and at storage conditions when atmospheric temperature and humidity favor mold development and subsequent aflatoxin production [21].

The proportion of samples positive for aflatoxin contamination across sampled areas in both pre-harvest and storage condition were as follows: Burie (4.3 and 37.6%), Finoteselam (2.4 and 42.9 %) and Jabitehnan (25.2 and 60.0 %), respectively. Aflatoxin contamination is influenced by various co-factors. During sample collection, Jabitehnan district had a maximum average temperature (23 °C) and rainfall (1250 mm) with low altitude (1900 m.a.s.l.) compared to Burie and Finoteselam, might have been contributed for maximum aflatoxin contamination. Virtually in these three districts, storage samples had a greater proportion of contamination compared to pre-harvest samples. Other factors that can facilitate aflatoxin contamination are mechanical damage, insect and bird damage, drought, stress, and excessive rainfall. Inadequate drying practices, direct on the floor, on top of the house with extended drying period and stored in poor conditions commonly practiced by the growers in the study areas, could contribute for mold development and aflatoxin production [36]. Post-harvest growth of fungi in a commodity also determined by length of time in storage, meant that the possibility environmental conditions could lead to a proliferation of aflatoxigenic molds and subsequent aflatoxin production was greater [30].

The proportion of samples positive for aflatoxin from the local market (powder form), Burie (58.0 %), Finoteselam (54.0 %) and Jabitehnan (14.6 %) across sampled areas. Burie found more contaminated than those two districts. In Ethiopia, red pepper powder is processed in the traditional method. Contamination of aflatoxin may occur through inadequate processing, or infected pods in the field or sometimes made from low-graded and poor- quality pods. Perhaps, pepper powder sold in wholesale local markets with poor and loose packaging or open-air likely contaminated with airborne aflatoxigenic molds. Likewise, a report from India revealed that, aflatoxin contamination of pepper was shown to be high, particularly, for low-graded pepper and pepper powder sold in local markets [37].

Maximum accepted levels of aflatoxins in foods and products for human consumption range from 0.5 ppb in milk to 20 ppb for processed foods. The European Commission (EC) established the most rigorous legislation for mycotoxin in food and in feed, including regulations for aflatoxins in pepper with maximum tolerable limits set at 10 ppb for total aflatoxin and 5 ppb for aflatoxin B_1 [38], while it is 20 ppb in the United State [39]. In the current study, 24.4, 96.0 and 98.0 % of pepper samples obtained from pre-harvest, storage and local markets, respectively had total aflatoxin exceeded EU recommended maximum limit 4 ppb and might have been unfit for human consumptions. In this regard, this much percentages of pepper samples were ineligible neither for consumers nor for traded in European markets and worthless and would be rejected. United Arab Emirates (UAE) also established acceptance limit of total aflatoxin (<10 ppb), in this study, about 4.4 % of pre-harvest, 71.0 % of storage and 87.0 % of local market samples had levels beyond acceptance limit of UAE. However, the aflatoxin levels of all pepper samples (n= 126, 100 %) collected from pre-harvest, storage, and local market did not exceed the legal limit (20 ppb) prescribed by the USA, Food and Drug Administration (FDA). In Ethiopia there is no acceptable limits of aflatoxin in all agricultural food products and the country has to set for further regulatory measures.

CONCLUSION

Pepper is one of the most important crops cultivated as a cash crop and for spices in West Gojjam, Ethiopia. However, the crop is contaminated with *Aspergillus* species and aflatoxin along the production chain. *A. flavus* was primarily recovered in pre-harvest and storage, while *A. niger* was abundantly isolated from local market samples. The levels of aflatoxin detected in the current study exceeded the tolerable limits set by EU and UAE.

The present study has shown that urgent intervention strategies are needed such as awareness creation, farmers' training on good agronomic and cultural practices at pre-harvest, as well as proper handling such as appropriate drying and good storage conditions, with accurate aeration. Care should be taken to reduce aflatoxigenic mold development and aflatoxin contamination to manage *Aspergillus* infection and aflatoxin contamination in pepper in Ethiopia.

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Table 1: Geographical description of those three surveyed districts, West Gojjam, Ethiopia

Districts	Latitude	Longitude	Altitude (m.a.s.l)	Annual average temperature (^{0}C)	Annual average rainfall (mm)
Burie	10°42′N	37°4′E	2091	19.0	1200
Fnoteselam	10°42′N	37°16′E	1917	16.7	1250
Jabitehnan	10°41'N	37°10'E	1900	23.0	1250

Sources: Agricultural office of the West Gojjam

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Table 2: Information on farmers interviewed and pepper management practices in West Gojjam, Ethiopia

Variables	Responses	N%
Age	20-40	26 (57.8)
	40-60	18 (40.0)
	>60	1(2.2)
Sex	Male	38(84.4)
	Female	7(15.6)
Education	Illiterate	11 (24.4)
	Read and write	14(31.1)
	Primary(1-8)	11(24.4)
	Secondary and above(>9)	9(20)
Heard about mold	Yes	45(100.0)
	No	0(0.0)
Way of planting	Transplant	26(77.8)
	Direct	19(22.2)
Crop Rotation	Yes	26(57.8)
	No	19(42.2)
Harvest the crop as soon as maturity	Yes	33(35.6)
	No	12(64.4)
After harvesting did you dry	Yes	45(100)
	No	0(0.0)
Storage location	In the house	36(80.0)
	Courtyard	9(20.0)
Clean the storehouse before storage	Yes	32 (71.1)
	No	13 (28.9)
Which storage problem is the most important	Mould	20(44.4)
	Rodent	14(31.1)
	Insect	11(24.4)
What did you do to solve this problem	No treatment	27(60.0)
	Rodenticide	12(26.7)
	Storage insecticide	6(13.3)
Do you Store other products with pepper	Yes	32(69.6)
	No	13(30.4)
Storage time	6 month-1year	32 (71.1)
	1-2year	13(28.9)
Drying place	On ground	41(91.1)
	Top house	4(8.9)
Storage material	New sack	8(17.8)
	Old sack	37(82.2)

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Table 3: Prevalence of *Aspergillus* species isolated in pepper samples in the surveyed areas, West Gojjam, Ethiopia

***Aspergillus* species**		**Districts (%)**			
		Burie	**Fnoteselam**	**Jabitehnan**	**Mean**
Pre-harvest	*A. flavus*	27.8	29.8	20.6	26.1
	A. parasiticus	20.9	15.5	17.6	18.1
	A. niger	6. 5	8.8	6.0	7.0
	Mean	18.4	18.0	14.7	17.1
Storage	*A.flavus*	33.6	27.2	31.2	30.6
	A. parasiticus	25.5	20.3	25.5	25.5
	A. niger	22.1	20.5	27.5	23.4
	Mean	27.1	22.7	28.1	26.5
Local market	*A.flavus*	15.2	17.1	19.8	17.5
	A. parasiticus	14.2	15.7	16.9	15.6
	A. niger	33.7	30.5	32.0	32.1
	Mean	21.0	21.1	22.9	21.7

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Table 4: Total aflatoxin concentration (ppb) in pepper, pre-harvest and storage (n = 45 samples, each) and local market (n = 36) samples, from those three districts, West Gojjam, Ethiopia

District	Preharvest		Storage		Local market	
	Range	Mean	Range	Mean	Range	Mean
Burie	nd-6.6	1.0	0.3 - 13.9	8.6	3.6-16.3	7.3
Fnoteselam	nd-4.7	0.6	0.3 - 12.2	11.2	11.5-19.2	13.2
Jabitehnan	0.2 -10.6	5.1	10.6 - 13.9	12.3	11.2-14.5	14.2
Total mean		2.2		10.7		11.7

*nd- non-detectable level

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