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Aflatoxin Levels in Wheat Flour in Afar Region, Northeastern Ethiopia

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ABSTRACT

Aflatoxins are common contaminants in Agricultural commodities including wheat flour which pose a considerable risk to human health and have significant economic implications. The objective of this study was to determine the level of aflatoxin concentrations in wheat flour samples at vendors and wholesalers from Logia-Samara, Dubti and Assaita town, Northeastern Ethiopia. A total of 60 flour samples were collected and analyzed for aflatoxin contamination using an ELISA test. Of the total 60 samples, aflatoxin was detected in 51, ranging from 1 ppb to 25 ppb. Aflatoxin concentrations were above 15 ppb in 65% of the positive samples collected from wholesalers in Assaita town. Moisture content and aflatoxin levels of flour samples were positively correlated ($r = 0.917$) and significant ($p \leq 0.05$). This study identified heavy aflatoxin contamination in wheat flour beyond the maximum tolerable level by the CODEX (15 ppb). Therefore, the negative impacts of aflatoxin on health, and aflatoxin contamination should be considered in future programs. We suggest improvements be made to post-harvest management (proper drying, sorting, and storage) to reduce the level of aflatoxin contamination of wheat flour at vendors and wholesalers.

Keywords: Afar region, Aflatoxin, ELISA, Ethiopia, Wheat flour.

INTRODUCTION

Wheat (Triticum aestivum) is an important food crop worldwide a staple food in Africa (Manjula et al., 2009), and all over the world. Wheat and wheat products are the main food for four billion people and constitute a major part of the daily human and animal diet (Food and Agricultural Organization, 2016). Wheat is utilized mainly as flour (whole grain or refined) to produce a large variety of leavened and flatbreads and for the manufacture of a wide variety of other baked products contributing 339 kcal and 13.70 g to energy and proteins intake, respectively (Emily & Sherry, 2010). Wheat is milled into flour, which is used for human food in a variety of recipes. In Ethiopia, wheat flour is used for making bread, porridge, cake, cookies, pastries, etc. Porridge is eaten by all age groups including babies as young as 6 months. (Emily & Sherry, 2010).

During the wheat production chain, that is harvest, handling, and storage, the grain is inevitably contaminated with various contaminants including moulds (Hejri et al., 2013). Ethiopia has a climate that is conducive to fungal growth. Mould growth leads to the production of mycotoxins, which are defined as secondary metabolites of toxigenic moulds (Hejri et al., 2013).

Nowadays, over 400 mycotoxins are known, however, the most investigated include aflatoxins, trichothecenes, fuminosins, zearaleunone, and ochratoxins (Ediage et al., 2011). Aflatoxins are produced by Aspergillus flavus, A. parasiticus and A. nominus (Magan et al., 2011). They contaminate a wide range of agricultural food commodities including beans, sorghum, groundnuts, millet, peas, cassava, rice, maize, and wheat, with the latter being the most significantly contaminated by aflatoxins (Kaaya & Warren, 2005; Magan et al., 2011). Aflatoxins B1 (AFB1) and B2 (AFB2), produced typically by the virulent Aspergillus flavus, are the most serious mycotoxins affecting wheat (Cheli et al., 2013).

Aflatoxins are poisonous. They can cause serious harm to health including death when food or feed containing them is eaten by humans and animals. Aflatoxins are acutely and chronically toxic,

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immunosuppressive, mutagenic, teratogenic, and carcinogenic compounds targeting mainly the liver for toxicity and carcinogenicity (Bennett & Klich, 2003). Although acute aflatoxin poisoning is rare, 25% of these cases are fatal (Williams et al., 2011). Chronic exposure rates are high especially in developing counties (Williams et al., 2011).

In Africa, the consumption of aflatoxin-contaminated food may be linked to recent increased liver cancer cases and even fatalities. Indeed, in 2004, in rural Kenya, an Eastern African country, the consumption of aflatoxin-contaminated maize resulted in 317 cases of aflatoxicosis, with the death of 125 persons (Lewis et al., 2005). Aflatoxins are also believed to be positively correlated to malnutrition disorders like stunting, underweight and decreased level of hemoglobin in children (Bankole & Adebanjo, 2003). Thus, along with other factors such as parasites and bacterial infections, lack of food to eat and less education on a balanced diet, human exposure to aflatoxins and eventually other mycotoxins would worsen the malnutrition problem in African countries including Ethiopia, especially in children (Bankole & Adebanjo, 2003).

As may be true for other mycotoxins, aflatoxins do not have an adverse impact only on the health of consumers, but also on the economy of Ethiopia and other African countries. This is in terms of large losses of contaminated food commodities and the cost involved in agricultural inputs and the destruction of the contaminated consignments and/or lots (Williams 2011). Also, importantly, noncompliance with set maximum tolerable limits prevents small-scale farmers, processors, and marketers from accessing highly remunerating markets and tarnishes the image of the exporting countries.

According to Williams (2011), 40% of commodities in local African markets exceed permissible levels of aflatoxins in foods. Ephrem et al. (2015) found that the prevalence of total aflatoxin concentration in 87% of the positive samples was above 10 ppb and 13% were less than 10 ppb among traders in the Fedis district, indicating heavy contamination of groundnut by aflatoxin beyond the maximum tolerable level by the CODEX Alimentarius Commission (10 ppb) in Eastern Ethiopia.

In Ethiopia, mycotoxins produced by several fungal species are common in barley, sorghum, wheat, maize and teff (Amare et al., 2006). Amare (2010) identified 15 species of fungi from 17 maize grain samples collected from Dire Dawa, Adama, and Ambo.

He also detected aflatoxins, flumonisins, deoxynivalenol and nivalenol with varying concentrations. A need, therefore, exists for a research series on aflatoxins and other mycotoxin contamination of local and imported food products. Wheat and maize flour are used for food for all age groups of Ethiopians, especially in the Afar region among all socio-economical classes. Ephrem, et al. (2014) found that 90% of the actors, i.e., 98.7% of farmers, 96.7% of traders and 70% of consumers were unaware of aflatoxin contamination of groundnut seeds and its consequence in Eastern Ethiopia. In addition, data on aflatoxin contamination of wheat flour is not available or has not been sought so far and hence studies are warranted. Therefore, there was a need to extend the database both spatially and temporally to get conclusive evidence on the level of contamination of wheat flour. The present study was, therefore, launched to detect and quantify aflatoxin levels in wheat flour in northeastern Ethiopia.

MATERIALS AND METHODS

Description of the study area:

The study was conducted at Logia-Samara, Dubti and Assaita towns, in the Afar Region, Northeastern Ethiopia. Dubti town is situated at about 600 km northeast of Addis Ababa. Geographically, it is located between 11°39’-11°48' N latitudes and 041°6˙-041°o12˙ E longitudes with an altitude that ranges from 339 to 381 m above sea level. Based on traditional classification methods using altitude, temperature, and rainfall, the Dubti area is classified under tropical desert (Bereha) i.e., arid climate categories. Based on meteorological data obtained from Dubti station, the average annual 28-years (1986-2014) rainfall is 222 mm, which is characterized by a bimodal rainfall pattern, and the major season is “karma”, the long rainy season (mid-June to mid-September), while the short rainy season that extends from March to April is “sugum”. The rainfall is erratic in nature and is not sufficient for rainfed agriculture. Hence, crop production is practiced based on irrigation. The annual mean minimum and maximum temperatures are 22.6 °Cand 48.8 °C, respectively, with mean annual evapo-transpiration of 2854 mm. Assaita town is situated at about 630 km northeast of Addis Ababa, at a latitude and longitude of 11°34′N and 41°26′E, an elevation of 300 meters (980 ft), and is 50 km (31 mi) south by unpaved road from the Awash-Asseb highway. In Assaita, the average annual temperature is 28.7 °C. About 144 mm of rain (precipitation) falls annually. At an average temperature of 33.4 °C, June is the hottest month of the year. The lowest average temperatures in the year occur in January, when it is around 24.5 °C. Logia town is situated at about 570 km northeast of Addis Ababa, the country's capital city. The average annual temperature at Logia is 28.2 °C. Rainfall (precipitation) here averages 203 mm annually. The warmest month of the year is June, with an average temperature of 32.5 °C. January is the coldest month, with temperatures averaging 24.3 °C.

Sampling:

Wheat flour samples were collected from the three towns purposively (using the purposive sampling method). Accordingly, samples were randomly drawn from wholesalers' storage and vendors as described below. Samples were 1 kg each, comprising five sub-samples drawn from different parts of the lot (composite samples). The samples were placed in amber paper bags. Samples were properly labeled and relevant information on the locality, Global Positioning System (GPS) coordinates, altitude, date of sampling, type and duration of storage, etc. were recorded. Samples were transported on the same day to Samara University and maintained at about 4 °C until laboratory analyses were done.

Sampling from wholesalers and vendors:

A total of 60 flour samples were collected from Logia-Semera, Dubti and Assaita town wholesalers' stores and vendors, that is ten samples from wholesalers and ten samples from vendors from each town. Samples were transported on the same day to Samara University and maintained at about 4 °C until laboratory analyses.

Determination of moisture content:

Moisture content (%) of flour was determined using the oven-drying method. Five grams of the sample were placed on an aluminum dish, which was placed in a dry air oven. The samples were dried at 105 °C for 3 hours and the net weight of the dried sample was determined. Each sample was replicated twice, and the moisture content was calculated as follows:

$$\textbf{Moisture Content}\ (\%) = \frac{\textbf{M0} - \textbf{M1}}{\textbf{M0}}\ \textbf{x 100}$$

Where: M0 - initial weight; M1 - final weight, in grams of wet and dried test portion, 184 respectively

Aflatoxin analysis using ELISA test:

Flour samples obtained from stores of wholesalers and vendors of the three towns (Logia- Samara, Dubti and Assaita) were used for the determination of total aflatoxin concentration in the flour. Collected flour samples were further air dried and brought to uniform moisture content (7%) immediately after collection and serologically assayed for total aflatoxins (AFT) contamination within four weeks of collection using the Indirect Enzyme Linked Immunosorbent Assay (ELISA). From each 1 kg sample, 100 g of flour was weighed and blended. Then 100 ml of 70% methanol (v/v) containing 0.5% KCl, was added to 20 g of the blended flour sample and blended further. The mixture was then transferred to a 250 ml conical flask and shaken at 300 rpm for 30 min (Gallen Kamp Orbital Shaker). The mixture was then sieved with a 1.00 mm sieve size into a 250 g bottle and diluted 1:10 in phosphate buffer saline with Tween 20 (1 ml filtrate in 9 ml buffer). Microtiter plates sensitized with aflatoxin B1 BSA conjugate were incubated at 37 °C for 2 h followed by a wash with PBS Tween. In all the steps, 150 ul/well of appropriate wash solution was used. Then the plates were washed with PBS Tween followed by the addition of blocking solution (0.2% Bovine serum albumin) before 30-45 min incubation at 37 °C and washing. The extracts of the samples, and/or AFB1 standard solution of 100 ul/well, were incubated with 50 ul/ well of polyclonal antibody solution in the plate for 60 min. Polyclonal antibodies were cross absorbed with 0.2% BSA for 30 min at 37 °C prior to addition to the plates. Then diluted anti-rabbit Igls labeled with Alkaline Phosphatase was added to each well and the plates were incubated at 37 °C for 60 min. After washing, p-Nitro phenyl phosphate prepared in 10% di-ethanolamine was added and the plates were read at 405 nm in the Multiskan Plus (Laboratory system) ELISA reader. The principle of ELISA lies in immobilizing the antigen onto a solid surface capturing antigen by specific antibodies and probing with specific immunoglobulin carrying an enzyme label. The enzyme retained in case positive reaction is detected by adding a suitable substrate. The enzyme converts the substrate to a product, which can easily be recognized by its color (Waliyar, et al., 2009).

Data analysis:

Data were analyzed by descriptive statistics using SPSS version 16 for Windows (SPSS. Inc., Chicago, Illinois, USA). The mean contents of aflatoxins were transformed into a log (x+1) to normalize data prior to analysis. Regression and correlation analyses of moisture contents with aflatoxin levels were done using Minitab version 17 for windows. Results of aflatoxin levels were compared against the standards set by Codex Alimentarius Commission (CODEX, 2004).

RESULTS

A total of 60 flour samples were collected from vendors and wholesalers at Logia-Samara, Dubti, and Assaita towns for total aflatoxin concentration analysis and moisture content determination. From the total 60 flour samples, of which 51 samples were positive for aflatoxin. Aflatoxin concentration in the positive samples ranged from 1 ppb to 21 ppb, the latter indicating heavy contamination of flour by aflatoxin beyond the maximum tolerable level by the World Health Organization (WHO) (15 ppb) and CODEX Alimentarius Commission (15 ppb).

Prevalence of total aflatoxin at vendors and wholesalers in Logia-Semera town:

CODEX categorizes samples with over 15 ppb as unfit for human consumption, and all the present study's results were compared using CODEX standard limits (CODEX, 2004). Percentage of flour samples from vendors and wholesalers with aflatoxin levels above and below 15 ppb at Logia-Semera town are shown in (Fig.1). Ten samples were tested for total aflatoxin concentration from vendors; only 4 samples were negative while the remaining 6 samples tested positive for aflatoxins. Total aflatoxin concentration from 55% of the positive samples was above 15 ppb and from the other 45% was less than 15 ppb at vendors in Logia-Samara town. From wholesalers, a total of 10 flour samples were tested for total aflatoxin concentration and only 2 samples tested negative while the rest,8 samples, were positive. Total aflatoxin concentration in 70% of the positive samples was above 15 ppb and in the other 30% was less than 15 ppb at wholesalers in Logia-Samara town. When we compared aflatoxin at vendors and wholesalers, there was less flour contamination by aflatoxin at vendors. Regression analysis showed that aflatoxin levels with moisture contents were positively correlated ($r = 0.922$) and significant ($p \leq 0.05$) in flour at vendors. Aflatoxin levels were correlated to the moisture contents of flour following the equation of total aflatoxin at vendors LS-T = 3.145 + 3.818 Moisture Content at vendors LS-T, with R2 = 0.91(Fig. 2).

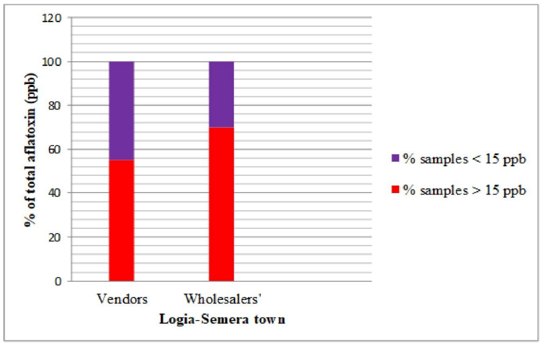

Fig. 1: Percent of flour samples from vendors and wholesalers with aflatoxin levels above 494 and below 15 ppb in Logia-Samara town.

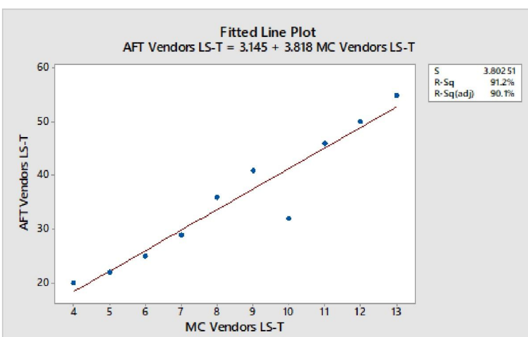

Fig. 2: Regression analysis of moisture contents of flour with total aflatoxin levels at vendors in Logia-Samara.

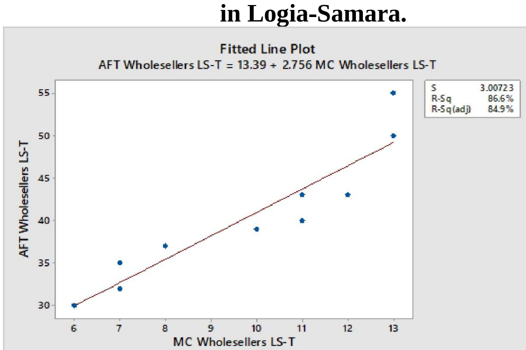

Fig. 3: Regression analysis of moisture contents of flour with total aflatoxin levels at wholesalers in Logia-Samara town.

Regression analysis showed aflatoxin levels with moisture contents were also positively correlated ($r = 0.931$) and significant ($p \leq 0.05$) in flour at wholesalers. Aflatoxin levels were correlated to the moisture contents of flour following the equation of Total Aflatoxin at wholesalers' LS-T = 13.39 + 2.756 Moisture Content at wholesalers' LS-T, with R2 258 = 0.86 (Fig. 3).

Prevalence of total aflatoxin at vendors and wholesalers in Dubti town:

A total of 20 flour samples were collected from vendors and wholesalers; that is 10 samples were collected from vendors and 10 samples from wholesalers in Dubti town. As a standard, CODEX categorizes samples with over 15 ppb as unfit for human consumption, and all the present study's results were compared using CODEX standard limits (CODEX, 2004). Percent of flour samples from vendors and wholesalers with aflatoxin levels above and below 15 ppb at Dubti town is shown (Fig. 4). Ten samples were tested for total aflatoxin concentration from vendors, of which only 4 samples were negative while the remaining 6 samples tested positive for aflatoxins. Total aflatoxin concentration from 50% of the positive samples was above 15 ppb and from the other 50% less than 15 ppb at vendors in Dubti town. From wholesalers, a total of 10 flour samples were tested for total aflatoxin concentration, of which only 3 samples tested negative while the rest of the samples (7) were positive. Total aflatoxin concentration from 60% of the positive samples was above 15 ppb and from the other 40% less than 15 ppb at wholesalers in Dubti town. When we compared aflatoxin at vendors and wholesalers, there was less flour contamination by aflatoxin at vendors. Our observations showed that increases in contamination were due to poor storage, aflatoxigenic fungi infestation, pest damage, inappropriate cultural practices, and lack of knowledge of proper drying methods.

Regression analysis showed that aflatoxin levels with moisture contents were positively correlated ($r = 0.890$) and significant ($p \leq 0.05$) in flour at vendors. Aflatoxin levels were correlated to moisture contents of flour following the equation of total aflatoxin at vendors D-T = 26.75 + 1.408 Moisture Content at vendors D-T, with R2 = 0.79 means that 79% of the variability of AFT at vendors around the mean was explained by moisture contents of flour (Fig. 5). This is because as moisture content of the flour increases, aflatoxigenic fungi development is promoted, thereby increasing aflatoxin contamination. The moisture content of the flour affects both the metabolism and physiological function of aflatoxigenic fungi.

Through regression analysis, it was found that aflatoxin levels with moisture contents were positively correlated ($r = 0.901$) and significant ($p \leq 0.05$) in flour at wholesalers. Aflatoxin levels were correlated to moisture contents of flour following the equation of Total Aflatoxin at wholesalers D-T = 22.81 + 1.912 Moisture Content at wholesalers D-T, with R2 = 0.85, meaning that 85% of the variability of AFT at wholesalers around the mean was explained by the moisture content of flour (Fig. 6). This is because as moisture content of the flour increases, it promotes aflatoxigenic fungi development, thereby increasing aflatoxin contamination. The moisture content of the flour affects both the metabolism and physiological function of aflatoxigenic fungi.

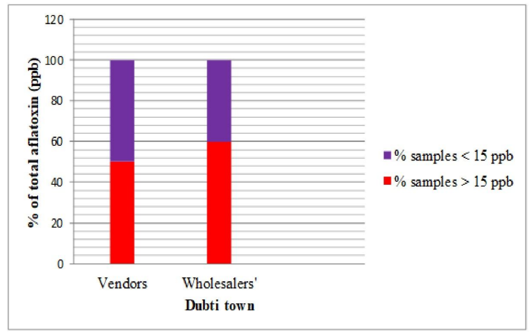

Fig. 4. Percent of flour samples from vendors and wholesalers with aflatoxin levels above and below 15 ppb in Dubti town.

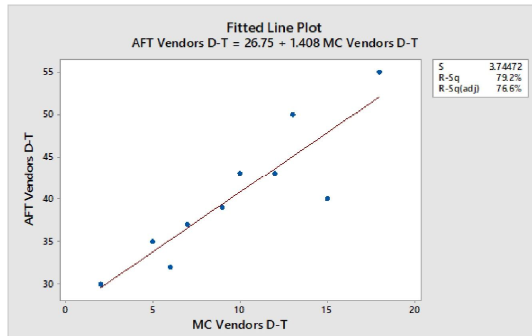

Fig. 5. Regression analysis of moisture contents of flour with total aflatoxin levels at vendors at Dubti town.

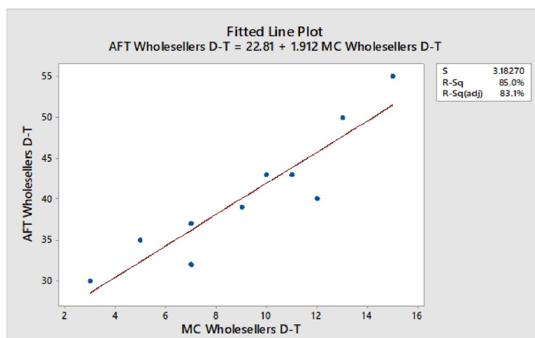

Fig. 6: Regression analysis of moisture contents of flour with total aflatoxin levels at wholesalers in Dubti town.

Prevalence of total aflatoxin at vendors and wholesalers in Assaita town:

A total of 20 flour samples were collected from vendors and wholesalers, including 10 samples from vendors and 10 samples from wholesalers in Assaita town. As a standard, CODEX categorizes samples with over 15 ppb as unfit for human consumption; all the present study's results were compared using CODEX standard limits (CODEX, 2004). Percent of flour samples from vendors and

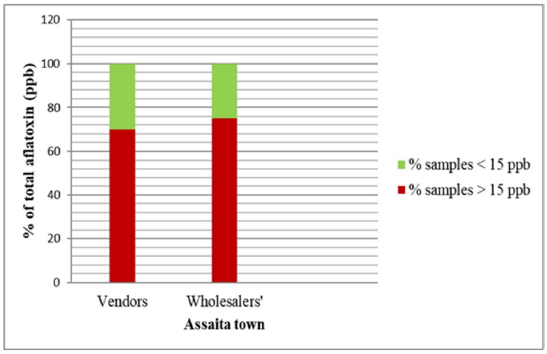

Fig. 7: Percent of flour samples from vendors and wholesalers with aflatoxin levels above and below 15 ppb in Assaita town.

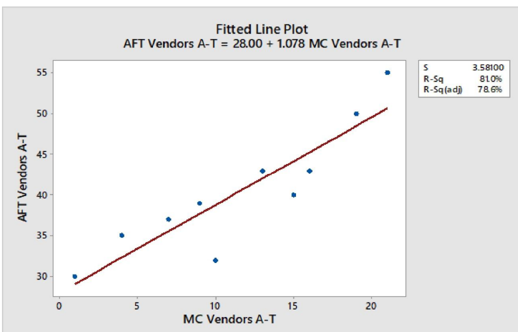

Fig. 8. Regression analysis of moisture contents of flour with total aflatoxin levels at vendors in Assaita town.

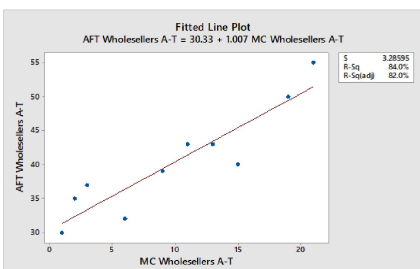

Fig. 9: Regression analysis of moisture contents of flour with total aflatoxin levels at wholesalers in Assaita town.

wholesalers with aflatoxin levels above and below 15 ppb at Assaita town is shown in (Fig. 7). Of the ten samples tested for total aflatoxin concentration from vendors, only 3 samples were negative while the remaining 7 samples tested positive for aflatoxins. Total aflatoxin concentration from 70% of the positive samples was above 15 ppb and from the remaining 30% was less than 15 ppb at vendors in Assaita town. From wholesalers, of a total of 10 flour samples tested for total aflatoxin concentration, only 1 sample was negative while the other 9 samples were positive. The percent prevalence of total aflatoxin concentration from 75% of the samples that tested positive was above 15 ppb and from the other 25% of positive samples was less than 15 ppb at wholesalers in Assaita town. When we compared aflatoxin at vendors and wholesalers, there was less flour contamination by aflatoxin at vendors. Our observations showed that these increases were due to poor storage, aflatoxigenic fungi infestation, pest damage, inappropriate cultural practices, and a lack of knowledge of proper drying methods.

Through regression analysis, it was found that aflatoxin levels and moisture content were positively correlated ($r = 0.900$) and significant ($p \leq 0.05$) in flour at vendors. Aflatoxin levels were correlated to moisture contents of flour following the equation of total Aflatoxin at vendors A-T = 28.00 + 1.078 Moisture Content at vendors A-T, with $R2 = 0.81$, meaning that 81% of the variability of AFT around the mean at vendors was explained by moisture contents of flour (Fig. 8). This is because as moisture content of the flour increases, it promotes aflatoxigenic fungi development, thereby increasing aflatoxin contamination. The moisture content of the flour affects both the metabolism and physiological function of aflatoxigenic fungi.

Through regression analysis, it was found that aflatoxin levels and moisture contents were positively correlated ($r = 0.917$) and significant ($p \leq 0.05$) in flour at wholesalers. Aflatoxin levels were correlated to moisture contents of flour following the equation of Total Aflatoxin at wholesalers' A-T = 30.33 + 1.007 Moisture Content at wholesalers' A-T, with $R2 = 0.84$, meaning that 84% of the variability of AFT around the mean at wholesalers was explained by moisture contents of flour (Fig.9). This is because as moisture content of the flour increases, it promotes aflatoxigenic fungi development, thereby increasing aflatoxin contamination. The moisture content of the flour affects both the metabolism and physiological function of aflatoxigenic fungi.

DISCUSSION

Results from across the three towns surveyed suggest the presence of high aflatoxin contamination of flour samples. In general, when actors were compared, the highest prevalence of total aflatoxin contamination was recorded at wholesalers in Assaita town, and the least was recorded at vendors in Dubti town. The high temperature and periodic drought prevalent in a study town could explain the higher levels of aflatoxin contamination in that climate. In addition, unfavorable drying and storage practices may aggravate the problem. Moreover, environmental conditions, especially temperature and relative humidity and/or moisture prevailing in the study towns, may be responsible for this established trend.

Regression analysis showed that aflatoxin levels and moisture content were positively correlated and significant in flour samples at vendors and wholesalers. The research results on flour moisture showed that the moisture content of samples ranged between 3% and 11%; the lowest was obtained from flour samples collected from vendors and the highest was from wholesalers in Assaita town. According to Codex Alimentarius Commission, the maximum allowable moisture content in flour is 10%; it is known that above this maximum range, moisture can support mold growth during storage and can lead to aflatoxin contamination (CODEX, 2004).

Rahmianna, et al. (2015) reported that kernel moisture content is crucial in the incidence of aflatoxin contamination, where the range of % to 28% moisture content is the critical level that is suitable for aflatoxin production. Another publication reported 15% to 30% soil moisture content and soil temperature higher than 28 °C as crucial for aflatoxin production. Below 15% and above 30% of moisture content were the safe levels. According to Wagacha, et al., (2013), moisture contents were significantly ($p \leq 0.05$) and positively correlated ($r = 0.76$) to the aflatoxin contamination level of the flour samples, a finding that agrees with the present study. This correlation explained that fungal growth and aflatoxin contamination were the consequence of an interaction among the fungus, the host, and the environment, where the appropriate combination of these factors determine the infestation and colonization of the fungus, and the amount of aflatoxin produced.

In a 2007 study where wheat flour samples were analyzed by using ELISA, the mean level of aflatoxin B1 was 0.93 ng/g and 6.5% of samples had higher levels than the maximum limits set by the worldwide regulations for aflatoxin B1 (Azizi, et al., 2007). In another study, 2.54% of samples were contaminated with aflatoxin, and aflatoxin B1, and G1 were detected in 2.54% and 3.39% of samples, respectively. Total aflatoxin and aflatoxin B1 levels in samples ranged between 1.3 ng/g – 7.1 ng/g and 1.36 ng/g – 1.78 ng/g, respectively (Hedayati & Mohammadpour, 2005). Taheri et al. (2019) researched aflatoxin contamination of wheat flour in Iran and found that the mean total aflatoxin levels of samples were 0.82 ng/g and 1.99 ng/g in summer and winter, respectively. Aflatoxin B1 levels were detected at 3.1%, and 7.4% over permissible limits by worldwide regulations in samples collected in summer and winter, respectively. Aflatoxins in winter were higher than in summer. The highest frequency of aflatoxin contamination in winter was B2 (98%) and in summer G1 (51%). The relationship between humidity and rate of aflatoxin B1 and total aflatoxin was significant in winter. Results of multivariate regression showed the strongest relationship between humidity and aflatoxin level. In Ethiopia, preparation of some food materials such as red pepper powder and its paste showed some aflatoxin contamination (mean 32 μg/Kg for powder; 1 paste sample had 102.2 μg/Kg aflatoxin B1, respectively) while samples of groundnuts and peanut butter had aflatoxin B1 at mean values of 34.7 μg/Kg and 105 μg/Kg-1, respectively (Abraham & Petros, 1981). In the European Union, regulations limit the number of total aflatoxins to 4 ppb whereas guidelines in a few developing countries and the US limit total aflatoxins to no more than 20 ppb in foodstuffs intended for human consumption (Food and Agricultural Organization, 2011). Following international standards for levels of aflatoxin, the flour samples analyzed were grouped into three categories: samples containing 0-4 ppb, samples with 4-20 ppb, and samples with > 20 ppb. Compared to this standard, most of the flour samples from northeastern Ethiopia had aflatoxin at a level much higher than any of these three classes.

Wheat flour is one of the most important main foods in the Afar region, and aflatoxins are common contaminants in flour across the study areas. Out of the total 60 samples, aflatoxin was detected in 51 samples, ranging from 1 ppb to 21 ppb. Aflatoxin concentrations were above 15 ppb in 75% of the positive samples collected from wholesalers in Assaita town. Moisture contents and aflatoxin level of flour samples were positively correlated ($r = 0.917$) and significant ($p \leq 0.05$). This study identified heavy aflatoxin contamination of wheat flour beyond the maximum tolerable level by the CODEX (15 ppb). Results of the current study suggest heavy contamination of flour by aflatoxin in northeastern Ethiopia. Investigation of flour contamination by aflatoxigenic fungi and associated aflatoxins should continue across the country to come up with a complete picture of flour contamination both temporally and spatially. Such studies will serve as an important basis to understand the full extent of the problem and to work on appropriate control measures. The current result suggests that there is an urgent need to control aflatoxin contamination. Therefore, research about the negative impacts of aflatoxin on health and aflatoxin contamination should be considered in future programs. A decrease in aflatoxin contamination may be made practical by reducing the duration of wheat flour storage and controlling humidity. It is also necessary to develop concerted campaigns to create awareness among traders about aflatoxin contamination.

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Data Availability Statement:

Data are available from the corresponding author on reasonable request.

Declarations:

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

The authors have provided their consent for publication.

Competing interests:

The authors declare that they have no competing interests.

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