Evaluation of Commercial Maize Cultivars for their Resistance to Aflatoxigenic Fungi under Storage Conditions in Ethiopia

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Abstract: A seed system can be defined as all activities related to seed production, seed storage, seed management, seed dissemination and seed use. The aim of the study was to evaluate commercial maize cultivars for their resistance to aflatoxigenic fungi under storage conditions in Ethiopia. The collected samples were analyzed using two-way analysis of variance (ANOVA, SAS version: 9.4) and the mean differences were separated by t test (LSD). There was significantly different ($p<0.05$) of storage fungi incidence on the commercial maize cultivars and the highest incidence 30.00 and 30.33% of A. flavus and A. niger was recorded in BH661 and BH546, respectively. There were significantly different ($p<0.05$) variations of germination percentage of the maize cultivars over the locations. The highest germination 97.33% was obtained from Jibat cultivar whereas the lowest 72.67%% was recorded in BH546. In all the five commercial maize cultivars analyzed, aflatoxin types were not detected and quantified because it was below the quantification limits of 0.35μg/kg and the development of secondary metabolite was low due to less development of the factors that aggravated it in the seed's storage. This study showed storage periods have low effect on the development of mycotoxins/secondary metabolite on the seeds of commercial maize cultivars since the taken samples were stored for more than one to three years but no aflatoxins types were detected. Therefore, from this study it was concluded that seed storage has less impact for the aflatoxin types development but has a high impact on the seed germinations.

Keywords: Chromatogram, maize cultivars, mycotoxin, seed quality, storage fungi, germination

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1. Introduction

Maize is one of such crops cultivated extensively in warm regions and consumed worldwide by both humans and livestock (Ranum *et al*., 2014). Maize is prone to infection by several fungal species, which can slow the growth and reduce the yield of the plant (Muthomi *et al*., 2012). Mycotoxin contamination of maize can take place at different stages of production including in the field during cultivation, during processing, storage or transportation (Coulibaly *et al.*, 2008). In Africa, certain aflatoxin productions are associated with hot, dry agroecological zones with latitudinal shifts in climate influencing the fungal community structure (Cotty and Jaime-Garcia, 2007). Interactions between fungi and their plant hosts or insects govern the infestation of fungi in the field whereas the availability of nutrients, temperature, moisture, and biotic factors such as insect infestation, govern the invasion of fungi after harvesting (Miller, 1994). The susceptibility of maize plants towards plant pathogenic fungi in the field has been extensively studied. Sources of resistance to *A. flavus* and *Fusarium spp*., particularly *F. verticillioides* have been identified and have been incorporated into public and private

breeding programs (Munkvold, 2003). There were a number of factors that aggravated the incidence of mycotoxigenic fungi and mycotoxins during storage. Some these factors are extrinsic and intrinsic, some as physical, chemical and biological factors though others categorized them as ecological, environmental and storage factors (Zain, 2011). Mycotoxins occur more frequently in areas with a hot and humid climate, favourable for the growth of moulds. The most prevalent fungi that can produce mycotoxins belong to the genera *Alternaria, Aspergillus, Fusarium*, and *Penicillium (*Tsitsigiannis *et al*., 2012). Seed health that is the absence of any pathogens causing seed deterioration or plant diseases after germination, can only be controlled through the source of seed, i.e., the seed system (Bishaw *et al.*, 2013). A seed system can be defined as all activities related to seed production, seed storage, seed management, seed dissemination, and seed use.

A sustainable seed system can ensure that high-quality seeds of a wide range of varieties and crops are produced and fully available in time and affordable to farmers and other stakeholders (Louwaars, 2007). Many organizations such as IITA are continuously working on resistance breeding programs in Africa (Hell *et al*., 2005). To devise effective strategies to control fungal infection and minimize mycotoxin production in host plants, a better knowledge of genetic variability and population structure at the intra-specific level and ability to detect cryptic populations or lineages which might arise that possess significant features in terms of toxin profile or host preferences is necessary (Mule *et al*., 2005). However, the ability of maize kernels to withstand the infestation of fungi during storage has not yet been reported even though infestation levels of fungi in stored maize throughout Africa is high (Bankole *et al*., 2006). In Ethiopia, there was limited studies on the resistance of commercial maize cultivars for mycotoxigenic fungi and mycotoxin during storage. Therefore, the objective this study was to evaluate commercial maize cultivars for their ability to resist against Aflatoxigenic fungi associated with maize during storage.

2. Materials and Methods

Study areas and Sample collection

The samples were collected from three agricultural research centers, Bako, Melkasa and Ambo. The laboratory experiment was arranged in CRD with three replications (Ambo, Melkasa and Bako). The treatments are: five commercial maize varieties MH140, BH546, M6Q, BH661 and Jibat. 250 to 500 grams of samples were taken from each commercial maize varieties and locations.

Agar plate method: Samples of commercial maize grains with and without surface disinfection were used and 10 grains of each treatment were aseptically placed on potato dextrose agar (PDA) by the method of agar plate according to the procedures used by Binyam and Girma (2016). The laboratory analysis was carried out in the Ambo Plant Protection Research Center mycology laboratory department. Firstly, from each sample, 360 maize grains; in 3 replications of 120 seeds were selected. Initially, freshly harvested seed of BH661 was used and periodically the stored maize grains were used and thoroughly washed with distilled water at each period. From surface disinfected and non-disinfected samples, 10 grains/Petri-dish/plate (9 cm diameter plates) containing potato dextrose agar (PDA) were aseptically placed. The plate that contains fungus was incubated at 26°C for 7 days and after 7 days of incubation, the identification of fungi isolates was done based on: septate, growth rate, color, and morphology of mycelia, conidia and sporulation structures. Then, the isolated fungi were subculture after three days of incubation for purification of the isolate. Finally, incidence of isolation fungi (%) and frequency of isolation of fungi (%) were calculated as follows: Incidence of fungi: Incidence of fungi infections on each sample was calculated by using the following formula:

$$\text{Incidence } (\%) = \frac{\text{Number of cultured grains}}{\text{Total number of grains used}} \times 100$$

3. Detection and Quantification of Mycotoxins Using HPLC Method

50.14gm of disodium phosphate (DSP) was dissolved with 700ml of distilled water in a 1000ml flask. 42.50gm of Sodium phosphate monobasic was dissolved in 350ml of distilled water. The two dissolved solutions were mixed to adjust it to 7.4 PH. 200ml of buffer were filled into 1000ml of graduating cylinder. Take 230ml of buffer from the prepared and add 20ml polytene 2020. 20gm of samples were weighed and 2gm of NaCl was added into conical flask and Shaked by using mechanical shaker, and then filtered by vacuum pump. The two layers were separated and the bottom layer was used for the analysis. Take 7ml of samples and 43ml of buffer (Figure 3). Elute 50ml of solution in ingenuity affinity column (Afla CLEAN) and wash by distill water. Then add 2ml methanol to degrade the proteins and wait for 5 minutes and elute. Finally use the preserve glass and take into vial and inject and the analysis undergone. 200 gm of samples were weighed and placed in labeled paper bags before they were sent to the Bless Agri food laboratories services PLC (ISO/IEC 17025:2017 Accredited) which was established by the joint venture of Ethiopia and French investors. The total Aflatoxin content analysis in the samples were performed using HPLC protocols consisting of two chromatographic pumps, sampling system, and fluorescence detector (HPLC-FLD).

Figure 1. Sample preparation methods. A = The two layers top and bottom, B = Mechanical Shaker

Data Analysis

The data was analyzed using two-way analysis of variance (ANOVA) and the mean of fungi incidence were separated by t test (LSD).

4. Results and Discussions

Isolation and identification Fungi from the commercial maize cultivars

There was significantly different ($p<0.05$) among the fungi species grown on the commercial maize varieties and locations (Table 1). A total of fourteen (14) commercial maize samples were collected, from which nine from Melkasa, five from Bako and one from Ambo. Among these, five commercial maize varieties, MH-140, BH-546, M-6Q, BH-661 and Jibat were used for mycotoxins analysis because these commercial cultivars were recently used by farmers. The result indicated that six fungi species, *Aspergillus flavus, Aspergillus niger, Aspergillus nomius, Fusarium moniliforme, Penicillium spp. and Rhizopus stolonifer* were identified through agar plate method. The incidence of *A. flavus, A. niger, A. nomius, F. moniliforme, penicillium and R. stolonifera* was recorded in low 18.33, 15.12, 5.33, 9.67, 2.00 and 3.10 in Jibat variety of Ambo agricultural research center than the commercial maize

varieties sampled from the two agricultural research centers. This is due to three reasons: 1) freshly harvested during the sample taken for analysis. 2) The environmental condition of Ambo agricultural research center lies on Woina Dega where there are few variations in storage temperature and relative humidity of the areas. 3) Commercial maize varieties of MQ6, BH546, MH140 and BH661 were stored for more than one year and additionally MQ6 has a soft endosperm than the other because it is quality protein maize. *These commercial maize cultivars were stored for more than one to three years but no aflatoxins types were detected in all the cultivars.* Likewise, Aliyu and Kutama (2007) identified six fungal species, *Aspergillus Rhizopus, Penicillium Curvularia, Fusarium and Mucor*. Ihejirika *et al*. (2005) reported that *A. niger* was occurred with the highest incidence 60% followed by *A. versicolor* 25% whereas, *A. fumigatus* occurred with the lowest incidence of 15%. The fungal development was highly obtained as the storage period increased because of the metabolic activity of the produce, inappropriate storage conditions and moisture increment due to microbial activities.

Evaluation of germination percentage of commercial maize cultivars

Table 1 indicated that there were significant differences ($p<0.05$) between cultivars on germination percentage. The highest germination 97.33% was obtained from Jibat cultivar whereas the lowest 72.67%% was recorded in BH546 which was collected from Ambo and Bako, respectively. This is due to Jibat cultivar being freshly harvested and also Ambo was located at woyinadega than Bako which have kola conditions that shorten the viability of the seeds due to biochemical analysis in the seeds. However, Louwaars (1994) reported that loss of viability was due to poor seed storage conditions which caused up to 10% loss in seed quality mainly in the tropics. Bosci and Kovacs (1990) reports germination of seed may vary with the temperature management. This is because temperature is a modifying factor in germination since it can influence available soil water and nutrient supply necessary for maize growth and development (Bosci and Kovacs, 1990; Keeling and Greaves, 1990).

Table 1. Mean percentage incidence of fungi species identified from the commercial maize

Commercial maize varieties	***A. flavus* (%)**	***A. niger* (%)**	***A. nomius* (%)**	***F. moniliform* (%)**	***Penicillium* (%)**	***R. stolonifera* (%)**	***Ger %***
Jibat	18.33b	15.12b	5.33c	9.67b	2.00b	3.10b	97.33a
MQ6	25.62ab	17.67b	27.20a	24.94a	16.11a	7.85a	88.00b
BH546	30. 00a	24.63a	16.33b	24.67a	4.67b	3.33b	72.67d
MH140	24.43ab	29.99a	8.77c	22.98a	11.66a	7.33a	80.00c
BH661	29.62a	30.33a	16.67b	24.33a	14.67a	6.63a	94.00ab
LSD (%)	10.98	5.95	6.85	9.60	1.2	2.44	6.24
CV (%)	22.79	13.44	24.51	23.93	4.47	23.94	3.84

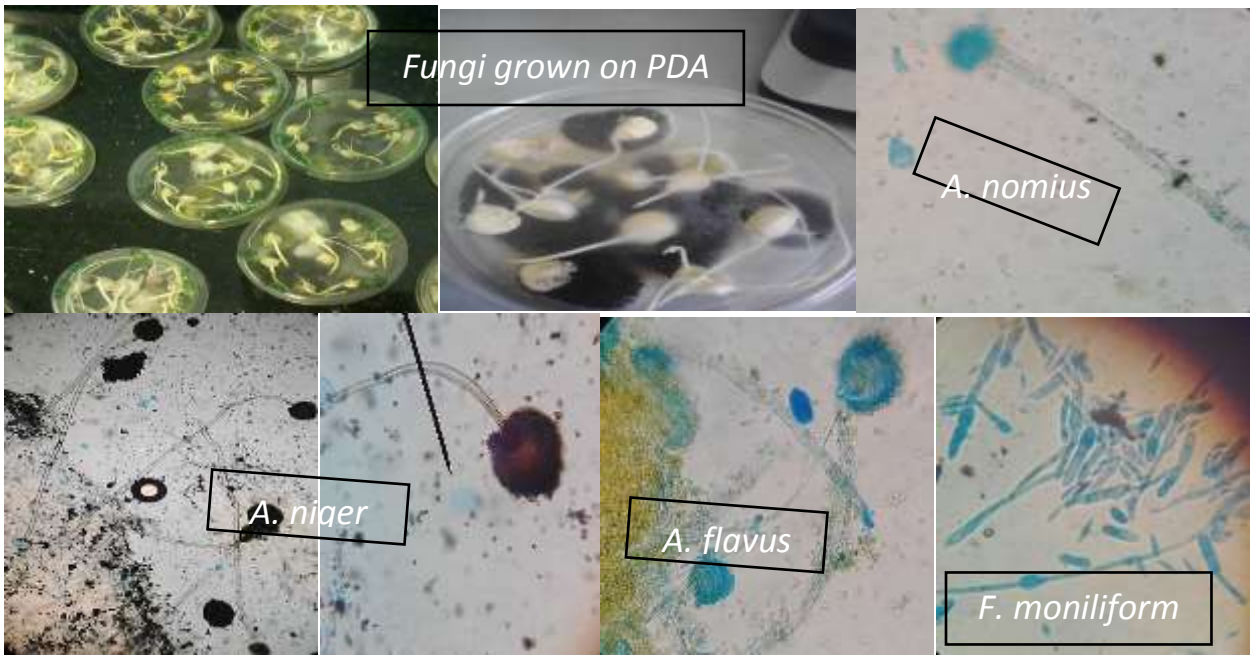


Figure 2 Fungal species identified on the agar plate media

Client Address
AMBO AGRICLTURE RESEARCH CENTER
Tel: (+251) 962 40 89 85
ADDIS ABABA
ETHIOPIA

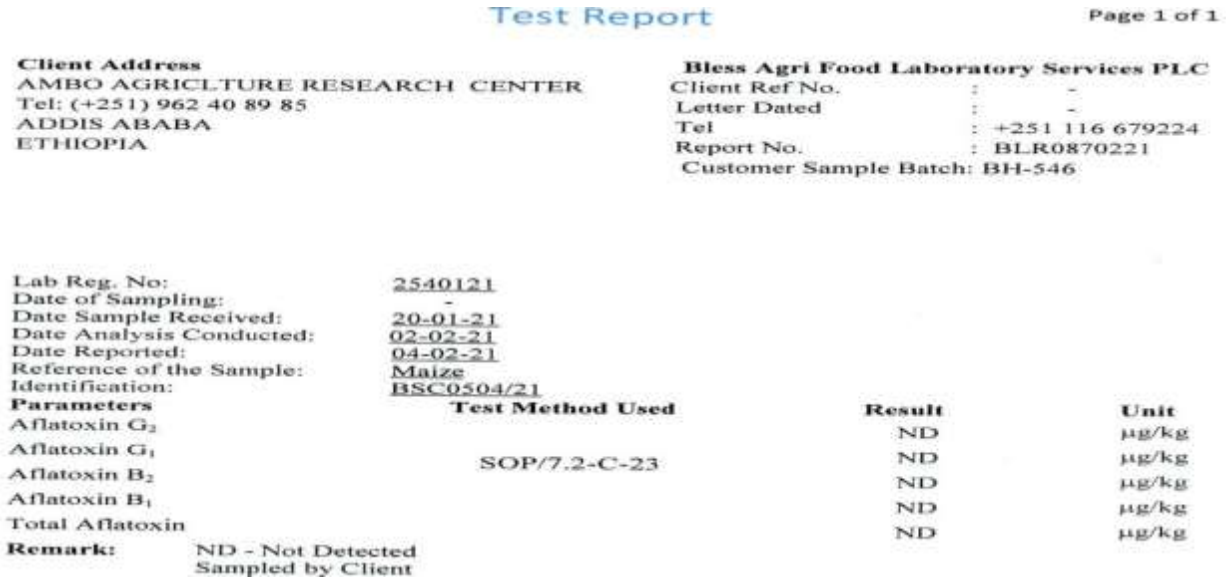
Bless Agri Food Laboratory Services PLC
Client Ref No. : -
Letter Dated : -
Tel : +251 116 679224
Report No. : BLR0880221
Customer Sample Batch: MH-140

Lab Reg. No:	2550121
Date of Sampling:	-
Date Sample Received:	20-01-21
Date Analysis Conducted:	02-02-21
Date Reported:	04-02-21
Reference of the Sample:	Maize
Identification:	BSC0505/21

Parameters	Test Method Used	Result	Unit
Aflatoxin G_2	SOP/7.2-C-23	ND	µg/kg
Aflatoxin G_1		ND	µg/kg
Aflatoxin B_2		ND	µg/kg
Aflatoxin B_1		ND	µg/kg
Total Aflatoxin		ND	µg/kg

Remark: ND - Not Detected
Sampled by Client

Figure 3. Commercial maize variety of MH-140 laboratory analysis using HPLC



Test Report

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Client Address
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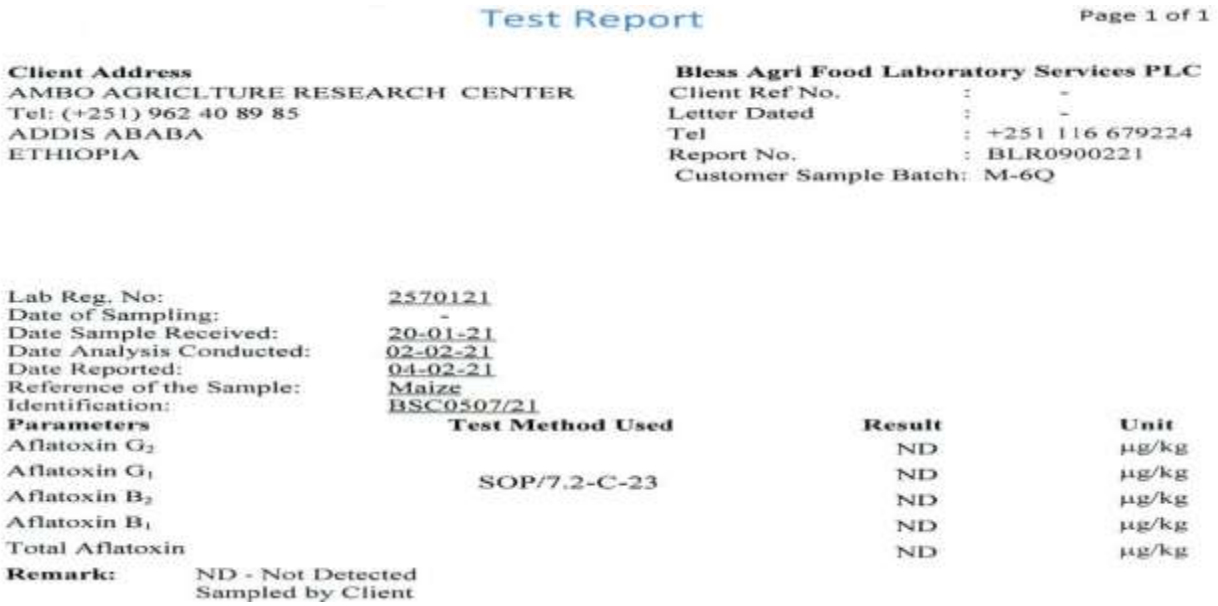
Bless Agri Food Laboratory Services PLC
Client Ref No. : -
Letter Dated : -
Tel : +251 116 679224
Report No. : BLR0870221
Customer Sample Batch: BH-546

Lab Reg. No: 2540121
Date of Sampling: -
Date Sample Received: 20-01-21
Date Analysis Conducted: 02-02-21
Date Reported: 04-02-21
Reference of the Sample: Maize
Identification: BSC0504/21

Parameters	Test Method Used	Result	Unit
Aflatoxin G_2	SOP/7.2-C-23	ND	µg/kg
Aflatoxin G_1		ND	µg/kg
Aflatoxin B_2		ND	µg/kg
Aflatoxin B_1		ND	µg/kg
Total Aflatoxin		ND	µg/kg

Remark: ND - Not Detected
Sampled by Client

Figure 4. Commercial maize variety of BH-546 laboratory analysis using HPLC



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Bless Agri Food Laboratory Services PLC
Client Ref No. : -
Letter Dated : -
Tel : +251 116 679224
Report No. : BLR0900221
Customer Sample Batch: M-6Q

Lab Reg. No: 2570121
Date of Sampling: -
Date Sample Received: 20-01-21
Date Analysis Conducted: 02-02-21
Date Reported: 04-02-21
Reference of the Sample: Maize
Identification: BSC0507/21

Parameters	Test Method Used	Result	Unit
Aflatoxin G_2	SOP/7.2-C-23	ND	µg/kg
Aflatoxin G_1		ND	µg/kg
Aflatoxin B_2		ND	µg/kg
Aflatoxin B_1		ND	µg/kg
Total Aflatoxin		ND	µg/kg

Remark: ND - Not Detected
Sampled by Client

Figure 5. Commercial maize variety of M-6Q laboratory analysis using HPLC

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Client Ref No. : -
Letter Dated : -
Tel : +251 116 679224
Report No. : BLR0910221
Customer Sample Batch: BH-661

Lab Reg. No: 2580121
Date of Sampling: -
Date Sample Received: 20-01-21
Date Analysis Conducted: 02-02-21
Date Reported: 04-02-21
Reference of the Sample: Maize
Identification: BSC0508/21

Parameters	Test Method Used	Result	Unit
Aflatoxin G_2	SOP/7.2-C-23	ND	µg/kg
Aflatoxin G_1		ND	µg/kg
Aflatoxin B_2		ND	µg/kg
Aflatoxin B_1		ND	µg/kg
Total Aflatoxin		ND	µg/kg

Remark: ND - Not Detected
Sampled by Client

Figure 6. Commercial maize variety of BH-661 laboratory analysis using HPLC

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Client Address
AMBO AGRICLTURE RESEARCH CENTER
Tel: (+251) 962 40 89 85
ADDIS ABABA
ETHIOPIA

Bless Agri Food Laboratory Services PLC
Client Ref No. : -
Letter Dated : -
Tel : +251 116 679224
Report No. : BLR0890221
Customer Sample Batch: AMJ

Lab Reg. No: 2560121
Date of Sampling: -
Date Sample Received: 20-01-21
Date Analysis Conducted: 02-02-21
Date Reported: 04-02-21
Reference of the Sample: Maize
Identification: BSC0506/21

Parameters	Test Method Used	Result	Unit
Aflatoxin G_2	SOP/7.2-C-23	ND	µg/kg
Aflatoxin G_1		ND	µg/kg
Aflatoxin B_2		ND	µg/kg
Aflatoxin B_1		ND	µg/kg
Total Aflatoxin		ND	µg/kg

Remark: ND - Not Detected
Sampled by Client

Figure 7. Commercial maize variety of AMJ-Jibat laboratory analysis using HPLC

Aflatoxin detected from the commercial maize cultivars using HPLC

A total of fourteen (14) commercial maize varieties nine from Melkasa, five from Bako and one from Ambo were collected. Five commercial maize varieties, MH-140, BH-546, M-6Q, BH-661, and Jibat were used for laboratory analysis. The aflatoxin types were not detected in all the five commercial maize cultivars because they were below the quantification limits 0.35µg/kg. This might be because maize endosperm was hard to degrade with few storage periods and secondary metabolite was developed slowly. Since mycotoxins development was aggravated by moisture content, temperature and relative

humidity of the storage and environment. The figures below represent aflatoxin was not detected in all the analyzed commercial maize varieties (Figure 3 - 7).

5. Conclusions and Recommendations

Maize is prone to infection by several fungal species, which can slow the growth and reduce the yield of the plant. Six fungi species, *Aspergillus flavus, Aspergillus niger, Aspergillus nomius, Fusarium moniliforme, Penicillium spp. and Rhizopus stolonifer* were identified through agar plate method. The incidence of Aspergillus flavus and Aspergillus niger was high in the commercial maize cultivars of BH661 and BH546 and low in the Jibat cultivar. This is due to Jibat cultivar being freshly harvested and the environmental condition of Ambo lies on Woina Deg where there are few variations in storage temperature and relative humidity of the areas. There were significant differences ($p<0.05$) among cultivars on germination percentage. The highest germination percentage was obtained from Jibat cultivar whereas the lowest was recorded in BH546, respectively. The aflatoxin types were not detected in all the five commercial maize cultivars because they were below the quantification limits 0.35μg/kg. This might be because maize endosperm was hard to degrade with few storage periods and secondary metabolite was developed slowly. Aflatoxin's development was aggravated by the quantity of the stored produce, storage condition, moisture content, temperature and relative humidity of the storage and the environment. The storage periods have a minimum effect for the detection and quantification of aflatoxin types on the analyzed seeds of commercial maize cultivars since the samples were taken from one to three years of stored seeds. Seed storage has less impact for the aflatoxin types development but has a high impact on the seed germination percentage.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests regarding to the materials.

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