



## ENZYME ACTIVITY OF FUNGI ASSOCIATED WITH STORED MAIZE GRAINS OBTAINED FROM SOME SELECTED FARMS NEAR BENIN CITY, NIGERIA

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### ABSTRACT

Global concern about food safety has led to increased interest in the study of food-spoilage fungi. Contaminated food affects human and animal health when such food items are consumed. This study was carried out to determine the fungi associated with stored corn in a bid to establishing their possible role in deterioration of corn. Using standard procedures, these fungi were isolated: *Aspergillus*, *Trichoderma*, *Penicillium*, and *Rhizopus* species. Results revealed the production of enzymes (protease, amylase, and lipase) by the fungi. The activity of these enzymes could play a role in the spoilage of corn by the fungi. In addition, the isolated fungi are known to be associated with postharvest yield losses in stored produce as well as producing toxins. Therefore, the data presented will help in choosing postharvest practices that will reduce contamination by these fungi. Protease activity values of the isolates ranged from  $1.426 \pm 0.042 - 1.748 \pm 0.023 \mu\text{ml}^{-1}$  (in *Aspergillus* sp.),  $1.599 \pm 0.018 - 1.990 \pm 0.019 \mu\text{ml}^{-1}$  (in *Rhizopus* sp.),  $1.364 \pm 0.018 - 1.679 \pm 0.012 \mu\text{ml}^{-1}$  (in *Trichoderma* sp.) and  $(1.544 \pm 0.017 - 1.714 \pm 0.013 \mu\text{ml}^{-1})$  (in *Penicillium* sp.). Amylase activity was highest in *Rhizopus* ( $1.625 \pm 0.054 - 1.790 \pm 0.013 \mu\text{ml}^{-1}$ ), followed by *Penicillium* sp and lowest in *Aspergillus* sp. Lipase activity was highest in *Penicillium* and lowest in *Aspergillus*. The detection and identification of fungi are crucial to developing appropriate management strategies for stored corn. Postharvest losses due to these fungi will lead to reduced income for the farmers.

**Keywords:** Characterization, Enzyme Activity, Isolation, Postharvest, Toxins, Stored Maize Grains

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## INTRODUCTION

The world is concerned with food safety and this has enhanced interest in fungi and subsequently, food spoilage (Chilaka *et al.*, 2012). Fungal spoilage of corn reduces the nutritional value and palatability of foods, thereby increasing its allergic potential and may result in mycotoxin contamination. Another issue is that some fungal species present in corn have been linked to mystic infection of cattle, particularly *Aspergillus fumigatus* (Griffin, 2010). Maize is one of the oldest human-cultivated crops, dating back to around the 15th century (Phoku *et al.*, 2012).

Fungi can grow on grains in the field as well as in storage. Contamination of maize grains with fungi is regarded as one of the most serious safety problems in the tropical countries and throughout the world (Chilaka *et al.*, 2012). Toxigenic fungi invading maize are divided into two distinct groups, field fungi and storage fungi. Field fungi invade maize and produce toxins before harvest or before the grains are threshed, and can develop under high relative humidity of over 80 %, with moisture content of 22 % to 33 % and a wide range of temperature ( $10 \pm 35$  °C). These fungi usually die out in storage, but some can live under storage conditions, cause significant damage, reducing the yield and quality, especially in warm humid climates (Dutton *et al.*, 2013). Conversely, storage fungi invade grain primarily during storage and require moisture content in equilibrium with relative humidity of 70 % to 90 %. In both circumstances, fungi originated from the field. Storage moulds replace field moulds that invade/ contaminate the maize before harvest. A number of fungal species are associated with stored grains; *Fusarium*, *Penicillium*, *Rhizopus*, *Aspergillus* and *Tilletia* species. (Egbuta *et al.*, 2015). Infection of maize grain by storage fungi results in discoloration, dry matter loss, chemical and nutritional changes and overall reduction of maize grain quality. It has been reported by Mohale *et al.* (2013) that storage fungi contribute to loss of more than 50 % of maize grain in tropical countries, and ranks second after insects as the major cause of deterioration and loss of maize.

According to Mulunda *et al.* (2013), when storage moulds invade maize grain they cause rot, kernel discoloration, loss of viability, mycotoxin contamination, and subsequent seedling blights. It was revealed that broken or mechanically damaged maize promotes development of storage moulds because fungi more easily enter the grain via the wounds (Ncube *et al.*, 2011; Phoku *et al.*, 2012; Saleemi *et al.*, 2012).

Therefore, the study sought to isolate and identify the fungi associated with stored corn, and determine the enzyme activity of the species of fungi with a view to evaluating their impact on nutritional quality of the corn.

## MATERIALS AND METHODS

### COLLECTION AND SERIAL DILUTION OF SAMPLES

Corn samples were collected from eight (8) farmers in Evboneka, Ukpoke, Iyowa and Ugbogiobo communities in Ovia North East Local Government Area (LGA), near Benin City, Edo State, the communities being among the major farming areas in the LGA. Samples were kept in clean polythene bags for laboratory analyses. Each corn sample was pounded in a mortar with pestle. Then 5g of the pounded corn was transferred to a test tube and 5ml of water was added to form the stock. The mixture was sterilized at 121°C for 15psi, allowed to cool and incubated for 7days. Sterile McCartney bottles were divided and labeled  $10^1$  to  $10^5$  with each of the bottles (2 - 5) containing 9ml sterile distilled water. With a sterile pipette, 1ml each was transferred from the stock bottle into the first bottle and from the first bottle to the next until a dilution of  $10^5$  was obtained.

## ISOLATION AND IDENTIFICATION OF FUNGI ASSOCIATED WITH STORED CORN

Using pour-plate method, 1ml of each serially diluted sample was transferred with a syringe into the corresponding labeled Petri dish. Then 9ml of PDA containing 10g/200ml chloramphenicol was dispensed into the Petri dish and plate was swirled gently for proper mixing of content. The plates were in duplicates and were incubated at room temperature ( $28 \pm 2^\circ\text{C}$ ) for 72hrs. Fungi associated with the maize samples were identified by using their cultural characteristics (Samson *et al.*, 2002) and under a microscope fitted with a camera (Motic B1 Digital camera) using the cover-slip method in which a little quantity of each culture was transferred onto the base of a cover slip buried in PDA (Samson *et al.*, 2002).

## DETERMINATION OF FUNGAL LOAD

The microbial load of the samples was determined by visibly counting the colony forming units (CFU) after 72hrs of incubation. The morphological characterization of the isolates was also determined. The microbial load/ml was then determined by the formula shown below (Griffin, 2010).

$$\text{Count/ml} = \frac{\text{No of colonies on plate} \times 1}{\text{Amount plated} \quad \text{dilution factor}} \quad \text{Eqn. 1}$$

## Determination of percentage occurrence

This was done using the formula stated below (Griffin, 2010).

$$\% \text{ occurrence} = \frac{\text{No of a particular isolate on plate} \times 100}{\text{Total number of isolates on plate} \quad 1} \quad \text{Eqn. 2}$$

## SUB-CULTURING OF FUNGI AND MAINTENANCE OF STOCK CULTURES

Subcultures were made by carefully taking a growing portion of the old culture with a sterilized needle into a fresh PDA plate. The fresh culture plates were in duplicates and were incubated at room temperature for 72 hours. The fungal cultures were aseptically transferred into PDA slopes in McCartney bottles and stored at  $5^\circ\text{C}$  in a Gallenkamp incubator (Model 1H150).

## DETERMINATION OF ACTIVITY OF EXTRACELLULAR ENZYMES PRODUCED BY THE ISOLATED FUNGI

The biochemical characterization of the isolated fungi was done by measuring the extracellular enzymes produced by the fungi in Czapeck dox broth. The broth was prepared by suspending 35g of the medium in one liter of distilled water. The suspension was properly mixed using a magnetic stirrer with frequent agitation until complete dissolution. The suspension was dispensed into appropriate containers and autoclaved at  $121^\circ\text{C}$  for 15 minutes.

Mycelial disc of 48-hour old culture of each fungus formed by using a cork borer of 5mm in diameter, was introduced separately into 50 ml of Czapeck dox broth. The broth was incubated at room temperature for 7 days for the fungus to grow. After the period of incubation, the mycelial mat was harvested by centrifuging the broth at 3000rpm for 15min. The supernatant served as a source of inoculum for the extracellular enzyme activity determination. The assayed enzymes were protease, amylase, and lipase, following the methods described below.

#### **QUANTITATIVE ESTIMATION OF $\alpha$ - AMYLASE**

This was done according to the method described by Yoo *et al.* (1987) using starch powder as the enzyme substrate. Starch solution was prepared by weighing 0.15 g of starch powder into 300 ml of phosphate buffer. The solution was boiled for 1 min. Iodine solution was prepared by weighing 0.15g of iodine crystals and 0.3g of potassium iodide into 300 ml of distilled water. Then 1ml of inoculum (obtained as explained above) was transferred into a McCartney bottle and 1ml of starch solution added. The reaction was stopped by adding 0.5 ml of 0.1 N HCl and then incubated for 30 minutes after which 3drops of iodine solution were added; the blue colour developed was read at 620 nm.

#### **QUANTITATIVE ESTIMATION OF PROTEASE**

Casein was prepared by dissolving 1g of casein (Merck quality) in a conical flask and 3 drops of sodium hydroxide solution and 80ml buffer solution were added. The mixture was stirred and heated to boil for 30min. The solution was allowed to cool and phosphate buffer solution was added to get a total volume of 100ml. This was followed by preparation of 5% trichloroacetic acid (TCA) solution (16.4g TCA in 250ml of distilled water). The assay according to Kempka *et al.* (2008) was followed.

One millilitre of test sample was incubated at 25°C for 2 minutes, 1ml of casein solution was added and mixed well. The solution was left at room temperature for 10mins, after which 2ml of trichloroacetic acid was added and mixed thoroughly. The solution was incubated at 40°C for 10min, then filtered through a membrane filter and 5ml of sodium carbonate solution was added to 1ml of the filtrate followed by addition of 1ml of Folin's reagent. Incubation was done at 40°C for 20mins, after which absorbance was read at 680nm. The following calculation was used to determine protease activity. One unit of protease was equivalent to the amount of enzyme required to release 1 $\mu$ g of tyrosine/ml/min under standard assay conditions.

$$\text{Protease activity (U/ml)} = \frac{\text{Absorbance} \times \text{dilution factor} \times \text{total volume}}{\text{Reaction time}} \quad \text{Eqn. 3}$$

#### **QUANTITATIVE ESTIMATION OF LIPASE**

Lipase activity was determined titrimetrically on the basis of olive oil hydrolysis by the modified method of Kempka *et al.* (2008). One millilitre of culture supernatant was added to assay substrate, containing 5ml of 10% (v/v) homogenized olive oil in 10% (w/v) gum acacia, 1.0 ml of 0.6% CaCl<sub>2</sub> solution and 5 ml of phosphate buffer (pH 7). The enzyme- substrate mixture was incubated on a rotary shaker at 150 rpm and 30°C for one hour. Then 5ml of alcohol: acetone (1:1) mixture was added to the reaction mixture. Liberated fatty acids were titrated with 0.1N NaOH

using phenolphthalein as an indicator. The end point was light pink in colour. One unit of lipase was defined as the amount of enzyme which released one micro mole fatty acid per minute under specified assay conditions as shown below. Extracellular lipase activity was recorded as units per ml (U ml<sup>-1</sup>) while intracellular activity was calculated in units per gram (U g<sup>-1</sup>).

$$\text{Lipase activity} = \frac{\Delta V \times N \times 1000}{V_{\text{sample}} \times 60} \quad \text{Eqn. 4}$$

$$\Delta V = V_2 - V_1$$

V1= Volume of NaOH used against control flask

V2= Volume of NaOH used against experimental flask

N= Normality of NaOH

V<sub>(Sample)</sub> = Volume of enzyme extract

## RESULTS

Fungi isolated from stored white corn collected from selected farms in Ovia North East LGA are presented in Table 1. They include *Aspergillus*, *Trichoderma*, *Penicillium* and *Rhizopus* species. *Aspergillus* species had the highest percentage occurrence of 40.3% followed by *Penicillium* (29.57) while *Rhizopus* had the least (7.53). The highest colony forming units (CFU) value was found in *Aspergillus* and the least, in *Rhizopus*. The fungi isolated from yellow corn are shown in Table 2.

**Table 1:** Fungi isolated from stored white corn collected from selected farms in Ovia North East LGA

Maize Sample	CFU/ml	Fungal Isolate	% Occurrence
ASP (F1-8)	75×10 <sup>5</sup>	<i>Aspergillus</i> species	40.30
TRI (F1-6)	42×10 <sup>5</sup>	<i>Trichoderma</i> species	22.58
PEN (F1-8)	55×10 <sup>5</sup>	<i>Penicillium</i> species	29.57
RHI (F1-2)	14×10 <sup>5</sup>	<i>Rhizopus</i> species	7.53

**Table 2:** Fungi isolated from stored yellow maize collected from selected farms in Ovia North East LGA

Maize Sample	CFU/ml	Fungal Isolate	% Occurrence
ASP (F1-8)	105×10 <sup>5</sup>	<i>Aspergillus</i> species	36.46
TRI (F1-7)	70×10 <sup>5</sup>	<i>Trichoderma</i> species	24.31
PEN(F1-8)	85×10 <sup>5</sup>	<i>Penicillium</i> species	29.51
RHI (F1-6)	28×10 <sup>5</sup>	<i>Rhizopus</i> species	9.72

The enzyme activity of fungal isolates obtained from white and yellow corn for Day One is presented in Table 3. Protease activity of *Rhizopus* ranged from 0.729±0.017 - 1.333±0.011 µml<sup>-1</sup>, which was the highest. This was followed by *Penicillium* (0.379±0.014 - 0.836±0.018 µml<sup>-1</sup>), *Aspergillus* (0.480±0.090 - 0.662±0.010 µml<sup>-1</sup>) and *Trichoderma* (0.371±0.016 - 0.607±0.019 µml<sup>-1</sup>). In the case of amylase, the activity was in the order: *Rhizopus* > *Aspergillus* > *Trichoderma* > *Penicillium*. The values for lipase activity were highest in *Aspergillus* (0.250±0.017 - 0.500±0.011 µml<sup>-1</sup>) followed by *Penicillium* (0.050±0.016 - 0.300±0.012) while *Rhizopus* (0.050±0.016 - 0.200±0.018 µml<sup>-1</sup>) and *Trichoderma* (0.050±0.019 - 0.200±0.015 µml<sup>-1</sup>) had similar activity

**Table 3:** Enzyme activity of fungal isolates from white and yellow corn on Day One

Isolate	Protease	Amylase	Lipase
<i>Aspergillus</i> (Y)	0.662±0.010	0.539±0.012	0.500±0.011
<i>Aspergillus</i> (YC)	0.512±0.030	0.389±0.014	0.350±0.013
<i>Aspergillus</i> (W)	0.630±0.060	0.639±0.016	0.400±0.015
<i>Aspergillus</i> (WC)	0.480±0.090	0.489±0.018	0.250±0.017
<i>Rhizopus</i> (Y)	1.333±0.011	0.505±0.013	0.200±0.019
<i>Rhizopus</i> (YC)	1.183±0.013	0.355±0.016	0.050±0.012
<i>Rhizopus</i> (W)	0.879±0.015	0.746±0.019	0.100±0.014
<i>Rhizopus</i> (WC)	0.729±0.017	0.596±0.011	0.050±0.016
<i>Trichoderma</i> (Y)	0.607±0.019	0.558±0.012	0.200±0.018
<i>Trichoderma</i> (YC)	0.457±0.012	0.408±0.015	0.050±0.010
<i>Trichoderma</i> (W)	0.521±0.014	0.655±0.016	0.200±0.015
<i>Trichoderma</i> (WC)	0.371±0.016	0.505±0.019	0.050±0.019
<i>Penicillium</i> (Y)	0.836±0.018	0.351±0.011	0.300±0.012
<i>Penicillium</i> (YC)	0.686±0.010	0.201±0.014	0.150±0.011
<i>Penicillium</i> (W)	0.529±0.019	0.434±0.015	0.200±0.014
<i>Penicillium</i> (WC)	0.379±0.014	0.284±0.011	0.050±0.016

Values are mean ± SEM; n=3

**Key:** Y=yellow corn, YC= control /yellow corn, W= white corn, WC=control/white corn

The enzyme activity of fungal isolates obtained from white and yellow corn for Day Two is shown in Table 4. Protease activity was in the order: *Rhizopus* > *Trichoderma* > *Aspergillus* and *Penicillium* (which had similar values of

0.624±0.017 - 0.897±0.011 µml<sup>-1</sup> and 0.738±0.011 - 0.896±0.015 µml<sup>-1</sup>, respectively). Amylase activity was highest in *Penicillium* (0.774±0.019 - 1.501±0.016 µml<sup>-1</sup>) and lowest in *Trichoderma* (0.663±0.017 - 1.101±0.012 µml<sup>-1</sup>) while lipase activity was highest in *Penicillium* (0.250±0.016 - 0.500±0.010 µml<sup>-1</sup>) and lowest in *Aspergillus* (0.150±0.011 - 0.350±0.013).

**Table 4:** Enzyme activity of fungal isolates from white and yellow on Day Two

Isolate	Protease	Amylase	Lipase
<i>Aspergillus</i> (Y)	0.897±0.011	1.102±0.010	0.300±0.013
<i>Aspergillus</i> (YC)	0.729±0.013	0.952±0.080	0.150±0.011
<i>Aspergillus</i> (W)	0.774±0.015	1.179±0.016	0.500±0.016
<i>Aspergillus</i> (WC)	0.624±0.017	1.029±0.015	0.350±0.013
<i>Rhizopus</i> (Y)	1.485±0.019	1.325±0.014	0.200±0.017
<i>Rhizopus</i> (YC)	1.335±0.012	1.175±0.012	0.050±0.012
<i>Rhizopus</i> (W)	1.079±0.014	1.372±0.017	0.350±0.015
<i>Rhizopus</i> (WC)	0.929±0.016	1.222±0.019	0.400±0.013
<i>Trichoderma</i> (Y)	0.693±0.018	1.101±0.012	0.250±0.011
<i>Trichoderma</i> (YC)	0.543±0.010	0.860±0.013	0.250±0.016
<i>Trichoderma</i> (W)	1.312±0.011	0.813±0.016	0.400±0.013
<i>Trichoderma</i> (WC)	1.162±0.014	0.663±0.017	0.250±0.012
<i>Penicillium</i> (Y)	0.896±0.015	0.924±0.011	0.400±0.013
<i>Penicillium</i> (YC)	0.746±0.013	0.774±0.019	0.250±0.016
<i>Penicillium</i> (W)	0.888±0.012	1.501±0.016	0.500±0.010
<i>Penicillium</i> (WC)	0.738±0.011	1.351±0.015	0.350±0.011

Values are mean ± SEM; n=3

**Key:-**Y=yellow corn, YC= control /yellow corn, W= white corn, WC=control/white corn.

The enzyme activity of fungal isolates obtained from white and yellow corn after three days is presented in Table 5. The highest value of protease activity was recorded in *Rhizopus* (1.599±0.018 – 1.990±0.019 µml<sup>-1</sup>) while the least was found in *Trichoderma* (1.364±0.018 – 1.679±0.012 µml<sup>-1</sup>). *Aspergillus* (1.426±0.042 – 1.748±0.023 µml<sup>-1</sup>) and *Penicillium* (1.544±0.017 – 1.714±0.013 µml<sup>-1</sup>) had similar activity values. For lipase, activity values ranged from 0.350±0.016 – 0.700±0.012 µml<sup>-1</sup> (*Aspergillus*), 0.550±0.013 – 0.700±0.018 (*Trichoderma*), 0.550±0.015 – 0.800±0.010 (*Rhizopus*) to 0.650±0.011 – 0.800±0.012 (*Penicillium*).

**Table 5:** Enzyme activity of fungal isolates from white and yellow corn on day three

Isolate	Protease ( $\mu\text{ml}^{-1}$ )	Amylase ( $\mu\text{ml}^{-1}$ )	Lipase ( $\mu\text{ml}^{-1}$ )
<i>Aspergillus</i> (Y)	1.748 $\pm$ 0.023	1.252 $\pm$ 0.044	0.700 $\pm$ 0.012
<i>Aspergillus</i> (YC)	1.598 $\pm$ 0.048	1.102 $\pm$ 0.036	0.550 $\pm$ 0.013
<i>Aspergillus</i> (W)	1.576 $\pm$ 0.051	1.426 $\pm$ 0.049	0.500 $\pm$ 0.014
<i>Aspergillus</i> (WC)	1.426 $\pm$ 0.042	1.276 $\pm$ 0.052	0.350 $\pm$ 0.016
<i>Rhizopus</i> (Y)	1.990 $\pm$ 0.019	1.775 $\pm$ 0.061	0.700 $\pm$ 0.018
<i>Rhizopus</i> (YC)	1.850 $\pm$ 0.015	1.625 $\pm$ 0.054	0.550 $\pm$ 0.013
<i>Rhizopus</i> (W)	1.749 $\pm$ 0.016	1.790 $\pm$ 0.013	0.700 $\pm$ 0.015
<i>Rhizopus</i> (WC)	1.599 $\pm$ 0.018	1.640 $\pm$ 0.018	0.550 $\pm$ 0.017
<i>Trichoderma</i> (Y)	1.679 $\pm$ 0.012	1.666 $\pm$ 0.013	0.700 $\pm$ 0.019
<i>Trichoderma</i> (YC)	1.529 $\pm$ 0.019	1.516 $\pm$ 0.019	0.550 $\pm$ 0.015
<i>Trichoderma</i> (W)	1.514 $\pm$ 0.014	1.685 $\pm$ 0.013	0.800 $\pm$ 0.010
<i>Trichoderma</i> (WC)	1.364 $\pm$ 0.018	1.535 $\pm$ 0.085	0.650 $\pm$ 0.015
<i>Penicillium</i> (Y)	1.714 $\pm$ 0.013	1.535 $\pm$ 0.030	0.800 $\pm$ 0.011
<i>Penicillium</i> (YC)	1.564 $\pm$ 0.011	1.385 $\pm$ 0.025	0.650 $\pm$ 0.016
<i>Penicillium</i> (W)	1.694 $\pm$ 0.013	1.729 $\pm$ 0.029	0.800 $\pm$ 0.012
<i>Penicillium</i> (WC)	1.544 $\pm$ 0.017	1.579 $\pm$ 0.010	0.650 $\pm$ 0.011

Values are mean  $\pm$  SEM; n=3.

**Key:** Y=yellow corn, YC= control /yellow corn, W= white corn, WC=control/white corn.

## DISCUSSION

Some of the isolated fungi, especially *Aspergillus*, are known to contain species that produce toxins which may have adverse effects in plants, animals, and humans, as well as causing spoilage of food and feed (Wu *et al.*, 2012). These fungi cause food spoilage during preharvest and postharvest stages of production, and release mycotoxins (Pavlova *et al.*, 2011).

The results obtained from this study showed that various field and storage fungi are associated with stored maize. In addition, the analysis also showed contamination, predominantly by the four major toxigenic fungal genera. The CFU values were in the following range; yellow corn (28 - 105 CFU/ml) and white corn ( $14 \times 10^5$  -  $75 \times 10^5$  CFU/ml). The findings are in line with the study of Mahapatra and Banerjee (2012) who reported that the major fungal genera commonly encountered in maize-producing regions are *Aspergillus* and *Penicillium* spp. with count of  $95 \times 10^4$  -  $361 \times 10^4$  CFU. In line with the result of this study, Feng *et al.* (2010) reported that in Ethiopia various grain fungi including *Fusarium*, *Penicillium*, *Aspergillus* and *Nigropora* spp. have been detected in maize samples collected from Hawassa, Areka, Billito, Shallo and Arsi-Negele. The populations of all the fungi were higher in samples collected from farmers' stores than in the samples collected from research and seed multiplication stores. In the present study *Aspergillus* and *Fusarium* were frequently isolated from maize seeds and *Penicillium* was the second most frequent fungal genus. Charity *et al.* (2010) also identified four *Fusarium* species associated with maize grain in Nigeria. The major genera commonly encountered in maize grain in tropical regions are *Fusarium*, *Aspergillus* and *Penicillium* (Chulze, 2010).

Ortiz *et al.* (2010) reported that the development of these fungi can be affected by moisture content of the product, temperature, storage time and degree of fungal contamination prior to storage. Insect and mite activity facilitate fungi dissemination (Griffin, 2010). During storage, several kinds of fungi can remain associated with corn

seeds either causing their deterioration or simply remaining viable to infect germinating seedling. The fungal genera typically found in stored grains are *Aspergillus*, *Penicillium*, *Fusarium* and some xerophytic species, several of them with capabilities of producing toxins (Adetunji *et al.*, 2014). *Aspergillus*, *Fusarium*, *Penicillium* and *Cladosporium* are the predominant fungal genera associated with grains in storage (Chilaka *et al.*, 2012). *Aspergillus. flavus* can infect maize pre- and post-harvest and an increase in aflatoxin content can occur if the phases of drying and storage are poorly managed (Dutton *et al.*, 2013). Several phytopathogenic species of *Fusarium* are found to be associated with maize including *F. verticillioides*, *F. proliferatum*, *F. graminearum* and *F. anthophilum* (Mohale *et al.*, 2013). Egbuta *et al.* (2015) reported that fungi from five genera (*Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria* and *Calviceps*) are responsible for the production of the great majority of the mycotoxins that are of agricultural relevance (Mulunda *et al.*, 2013). The losses caused by seed fungi may occur during seed development, storage or germination. Damage may result from loss of seed viability or from seedling infection following germination. Mulunda *et al.* (2013) also reported that *Fusarium*, *Aspergillus* and *Penicillium* were the most common genera isolated from maize samples collected around Shashemene and Alemaya. In general, the percentage occurrence of the different genera isolated showed the predominance of *Aspergillus* (38.46 – 40.30 %), *Penicillium* (22.58 – 24.31 %), *Trichoderma* (29.51 – 29.57 %) and *Rhizopus* (20.51%). This is in line with the study conducted by Phoku *et al.* (2012) which revealed the predominance of *Penicillium* species to be 57.2 % in South African maize cultivars. Saleemi *et al.* (2012) reported that *Penicillium* spp. (34.5 %) are the most abundant genus after *Fusarium* spp. (65.5 %) in corn from Spain. Oda (2012) also reported *Aspergillus* spp. (20.1 %) at lower occurrence when compared to *Penicillium* species (40.9 %) and *Fusarium* spp. (39.0 %) in maize.

Results obtained for enzyme activity revealed the enzymes had different activity in different fungi. The results are also in line with those of Feijoo-Siota and Villa (2010) who reported that the enzyme activities of *Aspergillus* range from 0.986 - 1.411, while *Penicillium* was 0.601 – 1.691. Yilmaz *et al.* (2016) stated that protease is an enzyme that catalyzes proteolysis, the breakdown of proteins into smaller polypeptides or single amino acids. They do this by cleaving the peptide bonds within proteins by hydrolysis, a reaction where water breaks bonds. Skoreński and Sieńczyk (2013) also reported that amylase is an enzyme that catalyses the hydrolysis of starch into sugars. They further stated that the presence of amylase can serve as an additional method of selecting for successful integration of a reporter construct in addition to antibiotic resistance. In addition Rejzek *et al.* (2011) reported that among a large number of non-pathogenic microorganisms capable of producing useful enzymes, filamentous fungi are particularly interesting due to their easy cultivation, and high production of extracellular enzymes of large industrial potential (Kundu *et al.*, 2011). These enzymes are applied in the industry for detergents, starch, drinks, food, textile, animal feed, baking, pulp and paper, leather, chemical and for biomedical products.

## CONCLUSION

This study has shown various fungi that affect stored corn, and how different enzymes are produced by the isolated fungi. *Aspergillus* species were the most predominant storage fungi followed by *Penicillium* species. These fungi are known for producing secondary metabolites (mycotoxins) which are carcinogenic to both humans and animals. Since these isolated fungi are very important in causing postharvest yield losses and production of mycotoxins, seed selection

is useful in reducing the fungal load on seeds. Accurate detection and removal of infected seeds as well as identification of appropriate storage conditions that do not encourage fungal growth would help in reducing corn seed spoilage and postharvest losses.

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