

## **EFFECTIVENESS OF THE TRIPLE-LAYER HERMETIC BIODEGRADABLE BAGS FOR BIO-RATIONAL MANAGEMENT OF AFLATOXINS IN STORED MAIZE**

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### **ABSTRACT**

Aflatoxin contamination of maize remains a serious challenge in developing countries in the tropics where it is pervasive due to favourable environmental conditions and high consumption of maize. An assessment of the triple-layer hermetic bag and a conventional bag (polypropylene bag) effectiveness to control the aflatoxin content in insect-free and insect-infested stored maize was studied over a six month period at the farm level. Moisture content, temperature, relative humidity, oxygen depletion, carbondioxide elevation and grain damage were analysed during storage. Seasonal changes resulted in significant fluctuation in grain moisture content, temperature and relative humidity in different storage bag technologies and storage environment. Analysis of variance also showed significantly differences ( $p < 0.05$ ) in the degree of insect damage and the corresponding levels of aflatoxins in hermetic and polypropylene bags. The initial mean percentage damage and aflatoxin content of 1.24% and 38.2  $\mu\text{g/kg}$  respectively, in either storage bag technology increased significantly following seasonal changes and insect metabolic activities. *Prostephanus truncatus*-infested maize in the polypropylene bags recorded the highest damage (97.9%) with a corresponding aflatoxin level of 227.7  $\mu\text{g/kg}$  after six (6) months storage. An aflatoxins level ( $>70 \mu\text{g/kg}$ ) was recorded in the triple-layer hermetic bags despite the bags excellent prevention of insect activities ( $<5\%$ ) over the same period of storage. Triple-layer hermetic bags have limited capacity to abate further accumulation of aflatoxins in previously aflatoxin-contaminated grains in the tropics.

**Keywords:** aflatoxin, maize, Triple-layer hermetic bags, polypropylene bags, moisture content, temperature and relative humidity

## 1.0 INTRODUCTION

Africa contributes only 7% of the global maize production (FARA, 2009), ironically, significant proportion of its population prefers maize to other cereals such as sorghum, rice, and millet. Maize consumption is high in sub-Sahara Africa ranging from 85 kg/year per person in Eastern and Southern Africa to 105 kg/year per person in West Africa (FAO, 2005). In recent years considerable effort to improve maize production through plant breeding and improved management options have made remarkable progress in increasing yield. However, limited attentions to efficient postharvest system to restrict grain destruction agents such as insects and microorganisms have created the situation whereby many individuals still remain food insecure. Preventing postharvest losses is a serious challenge for large number of resourced-poor farmers who produce the bulk of maize in Africa. Small-holder farmers generally rely on direct sunlight to dry maize grains, which apart from being labour intensive and time consuming, hardly dry maize to safe moisture level of  $\leq 14$  (Weinberg *et al.*, 2008). Inadequate drying and storage predispose maize to mycotoxin accumulation in warm, humid environment available in the tropics (Bankole *et al.*, 2006).

Mycotoxins are structurally diverse group of mostly small molecular weight compounds produce mainly by the metabolism of some filamentous fungi in the presence of suitable temperature, moisture and relative humidity (Zain, 2011). Prominent mycotoxins of greatest public and agro-economic importance are; aflatoxins, fumonisins, ochratoxins, ergot alkaloid and, zearalenone. Aflatoxins contamination of maize, peanuts, and oilseeds can be considered in terms of diet exposure the most significant worldwide (Benford *et al.*, 2010). The contamination of maize by Aflatoxins is deemed unavoidable (Ozay *et al.*, 2007). Whiles severe acute exposure to aflatoxins may result in death, chronic exposure result immune suppression, malnutrition and liver cancer which is the third most common cause of death from cancer in Africa (Williamset *al.*, 2004)

The profound level of ignorance among large portion of the Africa population regarding aflatoxins couple with the dangers and cost of using synthetic antifungal chemicals has necessitated the need for simple, inexpensive and environmentally friendly technology to manage aflatoxins. Across sub-Sahara Africa, triple-layer hermetic (Purdue Improved Crop Storage) bags have been disseminated and adopted by millions of farmers due to its exceptional ability to control insect pests in cowpea, maize, and peanut (Baoua *et al.*, 2014; Anankware *et al.*, 2013; Mutungi *et al.*, 2014). These hermetic triple-layer bags consist of two separate high-density polyethylene (HDPE) bags with 80 micron wall thickness, one fitted inside the other. The third bag surrounding completely the two inner bags is made of woven polypropylene.

The technology works by creating an airtight seal in which oxygen levels are drastically reduced in relatively short period through the metabolic activities of insects, fungi and grains (Murdoch

*et al.*, 2012). A study by Williamset *al.* (2014) concluded that the TLHB was effective in preventing aflatoxin contamination under a control environment. The current study was undertaken to establish the effectiveness of triple- layer hermetic bag (TLHB) against aflatoxins in stored maize under field conditions. Also, as part of the study aflatoxins levels of insect-infested maize stored in hermetic and polypropylene bag were assessed after six (6) months storage.

## **2.0 MATERIALS AND METHODS**

### **2.1 Treatment and experimental design**

An improved maize variety (Obatanpa) cultivated on farmer's field in Techiman Brong Ahafo was brought to the Entomology Laboratory of Department of Crop Science, School of Agriculture, College of Basic and Applied Sciences (CBAS), University of Ghana (UG), Legon, for the study.

A factorial treatment combination of two (2) insects (*Sitophilus zeamais* and *Prostephanus truncatus*) and two (2) bagging technologies (triple-layer hermetic bag and polypropylene interwoven bag) were used for the experiment in a completely randomized design.

A five kilogram triple-layer bag (150 micron thick and measure 34 cm× 62 cm in width and length, respectively) supplied by Bioplastic Company Limited Accra was used for the study. Polypropylene bag of the same dimension as the hermetic bag was also bought from the open market to serve as the control for the study.

### **2.2 Culturing of insects**

An unsexed adults *Sitophilus zeamais* and *Prostephanus truncatus* obtained from the Entomology Laboratory of Department of Crop Science, School of Agriculture, College of Basic and Applied Sciences (CBAS), University of Ghana (UG), Legon. Insects were cultured to obtain insect population of known age at culture conditions of  $28 \pm 2$  °C, 65 % Relative humidity and 12:12 LD photo regime (Bonu-Ire, 2001). About 500 unsexed adult *S. zeamais* and 500 unsexed adults *P. truncatus* were introduced into separate glass jar containing 1 kg of sterilized maize. The grains were sterilised in a refrigerator for 24 hours and in an oven at 40°C for six hours (Bonu-Ire, 2001). After 15 days, all the insects were removed and the maize grains kept in the same condition for 2 months. After this period, the adult insects that emerged from the culture were used for the experiments.

### **2.3 Experimental procedure**

Impurities and broken kernels were removed by screening the maize. Prior to filling the triple-layer hermetic bags, they were tested for hermetic seal by filling each of the two inner high-density polyethylene (HDPE) envelopes with air to ensure freedom from tear and leakage. Each of the 5 kg triple-layer hermetic bags (TLHB) and the polypropylene bags (PPB) was filled with 2.5 kg of maize and grouped into three sets.

In the first sets, 50 unsexed adults of *S. zeamais* from the laboratory culture were introduced into the maize with a camel brush, to simulate storage of pre-storage infested maize. Each bag was gently pressed to evacuate all air present and tied quickly with cotton rope.

In the other sets of 5 kg TLHB and PPB bags, 50 unsexed adults of *Prostephanus truncatus* was introduced into each of the maize samples, following the same procedure as stated above.

The third set of bags contained a mixture of the *S. zeamais* and *P. truncatus* in the same proportion i.e. 50 adult insects from each. These set of bags were also tied and stored.

The last sets of TLHB and PPB bags were filled with 2.5 kg maize sterilized at 60°C for 3 hours to kill all insects present. The bags were gently pressed to remove all air present and tied immediately with cotton rope.

Each treatment was replicated three (3) times and stored in an improved crib at the University of Ghana campus farm, Accra for six months.

In all, there were 144 experimental units. Destructive and replacement sampling was done at one month interval (i.e. 24 bags per month) for six months.

Moisture content was recorded monthly for six months whiles temperature and relative humidity were measured daily. Percentage insect damage and Aflatoxins levels were assessed before, 1, 3 and 6 months after storage.

### **2.4 Determination of Temperature and relative humidity**

A thermo hydrometer data logger (EL-USB-2, LASCAR electronics,  $\pm 0.5$  °C,  $\pm 3\%$ ) was used to measure the temperature and relative humidity in the hermetic bags, polypropylene bag and surrounding environment for the duration of the study. The thermo hydrometer logger was programmed to continuously measure the temperature and relative humidity every thirty minutes. Data logger was inserted into the triple-layer hermetic bag and the conventional bag before they were tied to measure the internal temperature and relative humidity. Similarly, the external

environmental conditions of the storage structure were also measured by placing the data logger on the platform of the storage structure.

## **2.5 Determination of oxygen (O<sub>2</sub>) and carbon dioxide (CO<sub>2</sub>)**

Prior to the opening of the triple-layer hermetic bags, oxygen and carbon dioxide levels were measured with the aid of GrainPro oxygen analyser (SCY-2A, MAOAN, O<sub>2</sub>  $\leq \pm 1.0$  and CO<sub>2</sub>  $\leq \pm 3.0$ ), butterfly needle and epoxy glue. To take measurements the inner HDPE lining was punctured with the analyser needle at the top, middle and bottom. All punctures on the bag walls were sealed with epoxy glue. Subsequent measurement was performed from the same spot by simply opening and closing the lid of the butterfly needle. This was done immediately after the set up and repeated daily.

## **2.6 Sampling for aflatoxin determination in maize**

Maize transported to the experimental site was sun dried to moisture content of between 12%-13% and mixed thoroughly in a container to obtain a uniformly mixed sample. Four composite samples, each weighing 2 kg was taken from the mixed sample, by sub-sampling from the different parts of the container for the initial aflatoxins analysis. Similarly, a uniform mixture of 1 kg was taken from 2.5 kg of maize from the different storage bag technologies in the cause of storage for aflatoxin analysis.

## **2.7 Determination of aflatoxin content in stored maize**

The aflatoxin levels were determined at the Mycotoxins and Histamine Laboratory of the Ghana Standard Authority, Accra, according to ISO 16050: 2003 test methods. One (1) kilogram sub-sample maize was ground with laboratory mill (IKA-Werke, IKA, and MF10B) and blender and mixed thoroughly before sampling into sample containers. Two grams (2 g) of sodium chloride and 100ml of extraction solvent were added to 20 g of milled maize. The solution was shaken and homogenised for 3 minutes. The extract was filtered using a paper filter and 20ml of the filtrate was diluted with 60ml of PBS, mixed well and filtered again using glass microfiber filter. Purification was carried out by passing the extract through immunoaffinity columns (IAC) containing antibodies specific for aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. The aflatoxins were eluted with 1.0ml of methanol and quantified by reverse-phase High Performance Liquid Chromatography (HPLC) with spectrofluorometric detector (RF-20A, Shimadzu Corporation, Japan). Aflatoxins were derivatised in a post-column reaction chamber (Kobra cell) by adding potassium bromide of 0.119 g to 1L of the mobile phase followed by fluorescence detection. Aflatoxins are identified by comparing the retention time of the peak detected in the chromatogram of the test solution with the retention time of the peaks of the standard for aflatoxins.

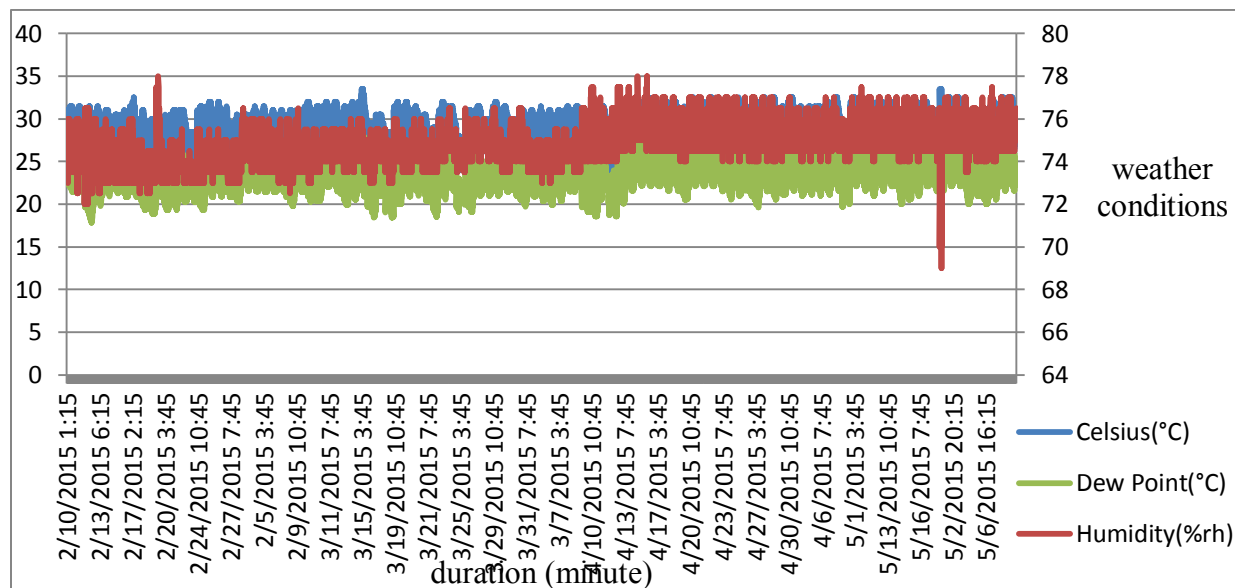
## 2.8 Analysis of Data

The data for the different treatments were entered in Excel. ANOVA followed by LSD was used to separate the means when there were significance differences.

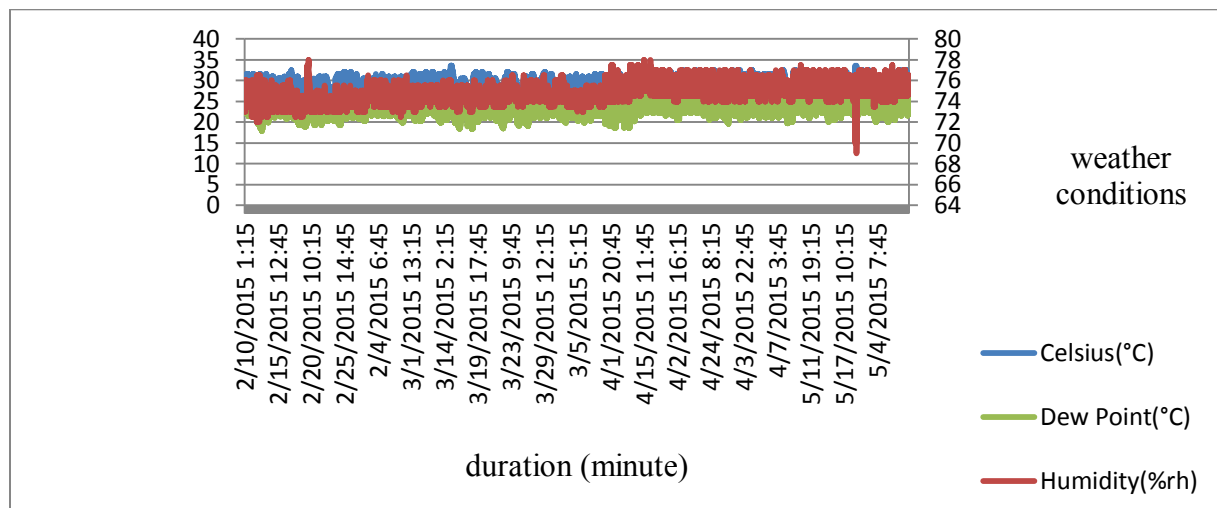
## 3.0 RESULTS

### 3.1 Changes in temperature, relative humidity and dew point within the storage structure, hermetic bag and polypropylene bag.

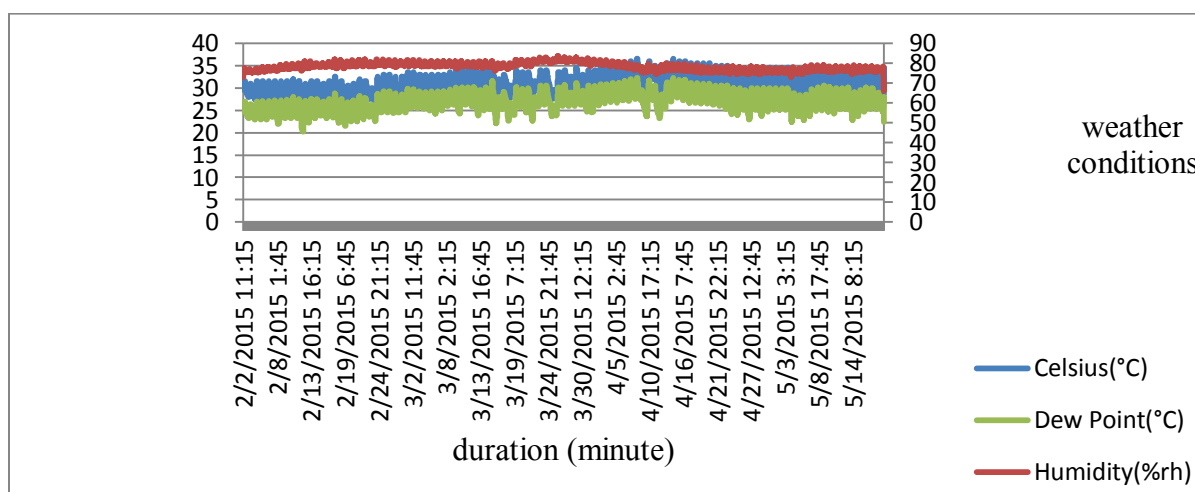
Temperature, relative humidity and dew point fluctuated both within the storage structure and the different storage bag technology during the storage period. A maximum and minimum temperature of 36.5°C and 22°C were recorded within the crib whiles a maximum and minimum relative humidity and dew point of 98.5 % to 50% and 30.5°C and 19.7°C were respectively recorded as shown in Figure 1. Similarly, internal temperature, relative humidity and dew point within the triple-layer hermetic bag and the polypropylene bag fluctuated as the season changes (Figure 2). The minimum and maximum relative humidity recorded in the conventional bag during the period of storage were 68.5% and 79.5% respectively whilst the highest dew point recorded in the same period was 32.5°C as shown in Figure 3.



**Figure 1: Daily Temperature, dew point and relative humidity in the maize storage structure**



**Figure 2: Internal daily temperature, dew point and relative humidity in the triple-layer hermetic bag**

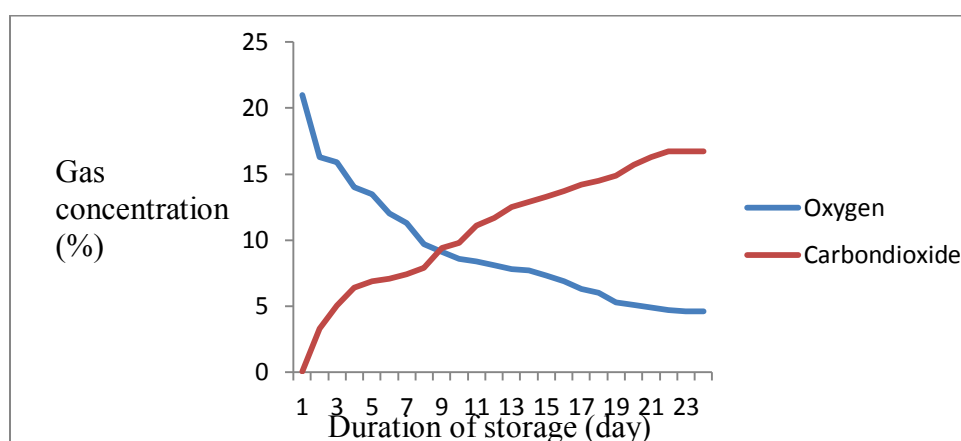


**Figure 3: Internal daily Temperature, dew point and relative humidity in the polypropylene bag**

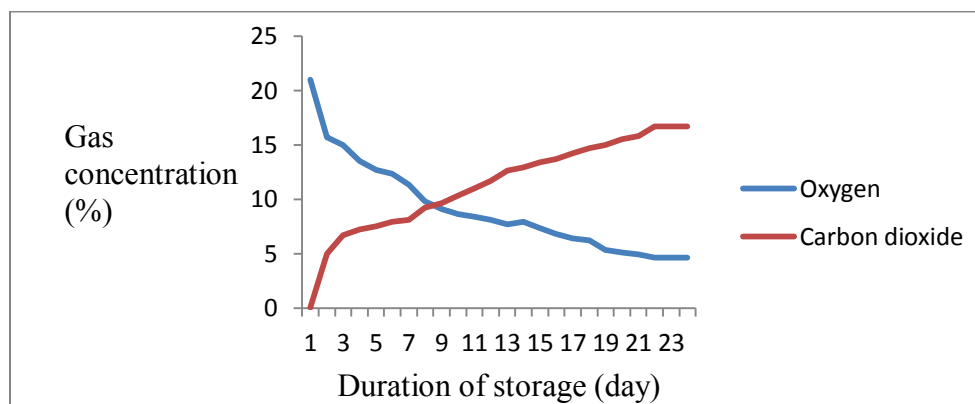
### 3.2 Changes in oxygen and carbon dioxide concentration the triple-layer hermetic bag

Figures 4 and 5 show the changes in the atmospheric gas content in insect-free and insect-infested maize in the triple-layer hermetic bag. During the first couple of days (day 1 to day 4)

after sealing of the hermetic bags the initial of oxygen of 21% in the insect-infested maize dropped greatly to 13.5%. The corresponding carbon dioxide concentration (7.2%) was recorded after four days of storage. Similar trend was observed in the insect-free maize with oxygen dropping from 21% to 13.5% after 5 days of storage. However the levels of oxygen depletion and carbon dioxide elevation remained relatively constant after 20 days of storage in the hermetic bags, regardless of the present or absent of insect on the maize. The lowest concentration of oxygen and highest carbon dioxide of 4.6% and 16.7% was recorded respectively after 23 days maize storage in the triple-layer hermetic bags.



**Figure 4: Changes in atmospheric gas concentration in insect-free maize in triple-layer hermetic bag**

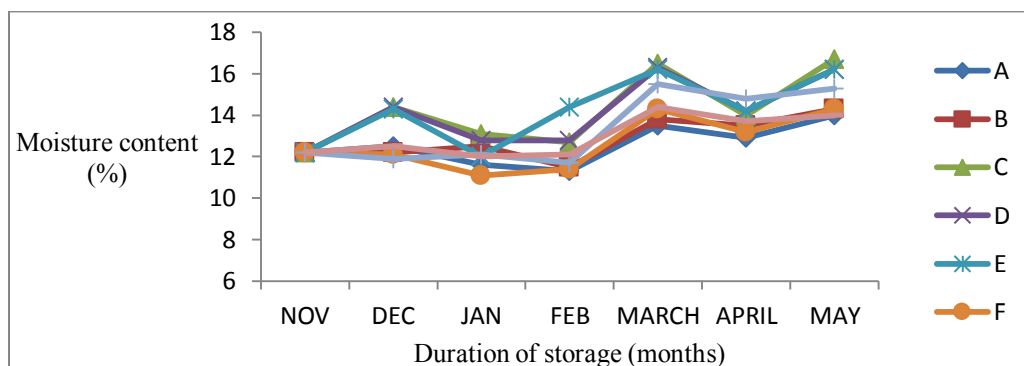


**Figure 5: Changes in atmospheric gas concentration in insect-infested maize in triple-layer hermetic bag**



### 3.3 Changes in moisture content of maize grain in different storage bag technologies.

The initial mean moisture content (12.2%) of maize generally remained relatively stable in the hermetic bag after one (1) month, but dropped slightly below the initial mean moisture level after two (2) months of storage to 11.1% in insect-free maize in hermetic bag. On the contrary, the moisture content in the polypropylene interwoven bags increased from the initial 12.2% to 14.4% after one month and reduced to near the initial moisture level after 2 months of storage. Likewise, the moisture content of maize in the hermetic bags, the moisture level of insect-free maize in the conventional storage bag reduced after one month to 11.9%, after which it remained relatively stable until the third month of storage. Overall, there was a surge in grain moisture from the third month (March) of storage with analysis of variance showing significant difference ( $p < 0.05$ ) in moisture contents of maize stored in either storage bag technologies as shown in Figure 6. The highest moisture content of 16.7% was recorded in maize infested with *S. zeamais* in polypropylene bag at the sixth month with 14.3% recorded in insect-free maize in the triple-layer hermetic bag.



**Figure 6: Changes in moisture content of maize stored in hermetic and polypropylene bags**

A= *S. zeamais* in hermetic bag, B= *P. truncatus* in hermetic bag, C= *S. zeamais* in polypropylene bag, D= *P. truncatus* in polypropylene bag, E= *S. zeamais* and *P. truncatus* in hermetic bag, F= insect-free maize in hermetic bag, G= insect-free maize in polypropylene bag, H= *S. zeamais* and *P. truncatus* in polypropylene bag

### 3.4 LEVELS OF INSECT DAMAGE AND AFLATOXIN CONTAMINATION

#### 3.4.1 Percentage damage of stored maize due to insect infestation

Percentage damage by insects and the resultant levels of aflatoxins produced are presented in Tables 1-4. Analysis of variance results showed significant differences ( $p < 0.05$ ) in the percentage damage between insect infested grains in polypropylene bag and hermetic bag. While percentage damage in both insect-infested and insect-free maize in hermetic bags remains

fairly constant over time, the level of damage of insect-infested maize in polypropylene bags continued to increase over time at an exponential rate. The mean level of damage of 1.3% at the inception of storage increased significantly to 97.9% in the *P. truncatus* infested maize grains in the polypropylene bag after the six (6) months of storage. There was however no significant difference ( $p < 0.05$ ) in the levels of damage in insect-infested maize in polypropylene bags over the 6 months storage period.

### **3.4.2 Changes in aflatoxin concentration in stored maize in triple-layer hermetic bag and polypropylene interwoven bag.**

The aflatoxin level of 38.2  $\mu\text{g/kg}$  detected in the maize sample prior to storage increased over the course of storage to 321  $\mu\text{g/kg}$ .

The level of aflatoxin G<sub>2</sub> (AFG<sub>2</sub>) was 0.0  $\mu\text{g/kg}$  for the first one month in both insect-infested maize and insect-free maize stored in the different storage bag technologies. Apart from *P. truncatus* infested maize in polypropylene bag whose level of AFG<sub>2</sub> changed (2.4  $\mu\text{g/kg}$ ) during the third month of storage, all other treatments showed no AFG<sub>2</sub>. Also, regardless of the kind of treatment, the last month of storage produced AFG<sub>2</sub> that was significantly different ( $p < 0.05$ ).

Aflatoxin G<sub>1</sub> level at the beginning of storage was 0.0  $\mu\text{g/kg}$  which remained constant until month 6 when the highest level of 22.20  $\mu\text{g/kg}$  was recorded in maize grain devastated by the cumulative activities of *S. zeamais* and *P. truncatus* in polypropylene bag.

There was no significant difference ( $p < 0.05$ ) in the level of AFB<sub>2</sub> in the insect infested grains and the insect-free maize stored in hermetic bag at the third month of storage. On the contrary, insect-damaged grains and insect-free maize in the different storage bags at the same month showed significant differences ( $p < 0.05$ ).

The level of aflatoxin B<sub>1</sub> was the highest (34.0  $\mu\text{g/kg}$ ) prior to maize storage and continues to increase over the course of storage to 293.1  $\mu\text{g/kg}$  as the percentage damage by insect increased. There was an exponential increment of AFB<sub>1</sub> in insect-damaged grains in polypropylene interwoven bags, with *S. zeamais* infested-grains recording the highest level of AFB<sub>1</sub>. The lowest level of AFB<sub>1</sub> (64.5  $\mu\text{g/kg}$ ) was recorded in *S. zeamais* infested grains in hermetic bag during the sixth months. Analysis of variance showed no significant difference ( $p < 0.05$ ) in the level of aflatoxin B<sub>1</sub> in *P. truncatus*-infested maize, combined activities of the two insects and insect-free maize by month six in hermetic bags.

**Table 1: Effect of insect damage on aflatoxins levels in maize at time of storage**

Storage treatment	Initial level of damage (%)	Aflatoxins (µg/kg)				
		G2	G1	B2	B1	TOTAL
Weevil (HB)	1.3	0.0	0.0	4.2	34.0	38.2
LGB(HB)	1.3	0.0	0.0	4.2	34.0	38.2
Weevil+LGB (HB)	1.3	0.0	0.0	4.2	34.0	38.2
IFM (HB)	1.3	0.0	0.0	4.2	34.0	38.2
Weevil (PB)	1.3	0.0	0.0	4.2	34.0	38.2
LGB (PB)	1.3	0.0	0.0	4.2	34.0	38.2
Weevil+LGB (PB)	1.3	0.0	0.0	4.2	34.0	38.2
IFM (PB)	1.3	0.0	0.0	4.2	34.0	38.2

Weevil (HB)= *S. zeamais* in hermetic bag, LGB (HB)= *P. truncatus* in hermetic bag, Weevil+LGB = *S. zeamais* and *P. truncatus* in hermetic bag, IFM (HB)= insect-free maize in hermetic bag, Weevil (PB)= *S. zeamais* in polypropylene bag, LGB (PB)= *P. truncatus* in polypropylene bag, Weevil+LGB (PB)= *S. zeamais* and *P. truncatus* in polypropylene bag, IFM (PB)= insect-free maize in polypropylene bag,

**Table 2: Effect of insect damage on aflatoxins levels in maize after one month of storage**

Storage treatment	Damage level (%) month 1	Aflatoxins (µg/kg)				
		G2	G1	B2	B1	TOTAL
Weevil(HB)	5.9 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	5.4 <sup>bc</sup>	62.0 <sup>b</sup>	67.4 <sup>b</sup>
LGB (HB)	4.0 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	5.9 <sup>b</sup>	57.3 <sup>bc</sup>	63.2 <sup>b</sup>
Weevil+LGB (HB)	2.8 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	5.6 <sup>b</sup>	62.6 <sup>b</sup>	68.2 <sup>b</sup>
IFM (HB)	0.0	0.00 <sup>a</sup>	0.00 <sup>a</sup>	3.9 <sup>d</sup>	48.2 <sup>d</sup>	52.1 <sup>d</sup>
Weevil (PB)	59.7 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	5.0 <sup>c</sup>	52.6 <sup>cd</sup>	57.6 <sup>c</sup>
LGB (PB)	66.6 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	10.4 <sup>a</sup>	93.8 <sup>a</sup>	104.2 <sup>a</sup>
Weevil+LGB (PB)	63.3 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	4.8 <sup>cd</sup>	36.6 <sup>e</sup>	41.4 <sup>e</sup>
IFM (PB)	0.00	0.00 <sup>a</sup>	0.00 <sup>a</sup>	5.0 <sup>c</sup>	39.0 <sup>e</sup>	44.0 <sup>e</sup>

<sup>a-c</sup> Values in the same column not sharing a common superscript are significantly different at LSD (p<0.05)

Weevil (HB)= *S. zeamais* in hermetic bag, LGB (HB)= *P. truncatus* in hermetic bag, Weevil+LGB = *S. zeamais* and *P. truncatus* in hermetic bag, IFM (HB)= insect-free maize in hermetic bag, Weevil (PB)= *S. zeamais* in polypropylene bag, LGB (PB)= *P. truncatus* in polypropylene bag, Weevil+LGB (PB)= *S. zeamais* and *P. truncatus* in polypropylene bag, IFM (PB)= insect-free maize in polypropylene bag,

**Table 3: Effect of insect damage on aflatoxins levels in maize after three months of storage**

Storage treatment	Damage level (%) month 3	Aflatoxins (µg/kg)				
		G2	G1	B2	B1	TOTAL
Weevil (HB)	4.3 <sup>b</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>	7.3 <sup>c</sup>	60.1 <sup>d</sup>	67.4 <sup>d</sup>
LGB (HB)	5.7 <sup>b</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>	5.1 <sup>c</sup>	56.6 <sup>d</sup>	61.7 <sup>d</sup>
Weevil+LGB (HB)	4.7 <sup>b</sup>	0.0 <sup>b</sup>	0.7 <sup>a</sup>	6.7 <sup>c</sup>	63.6 <sup>d</sup>	71.9 <sup>d</sup>
IFM (HB)	0.0	0.0 <sup>b</sup>	0.0 <sup>b</sup>	6.7 <sup>c</sup>	54.0 <sup>d</sup>	60.7 <sup>d</sup>
Weevil (PB)	92.2 <sup>a</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>	17.9 <sup>a</sup>	268.4 <sup>a</sup>	286.3 <sup>a</sup>
LGB (PB)	94.2 <sup>a</sup>	2.4 <sup>a</sup>	0.0 <sup>b</sup>	11.7 <sup>b</sup>	133.5 <sup>b</sup>	147.6 <sup>b</sup>
Weevil+LGB (PB)	94.2 <sup>a</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>	10.0 <sup>b</sup>	112.1 <sup>c</sup>	122.1 <sup>c</sup>
IFM (PB)	0.0	0.0 <sup>b</sup>	0.0 <sup>b</sup>	10.2 <sup>b</sup>	50.6 <sup>d</sup>	60.8 <sup>d</sup>

<sup>a-c</sup> Values in the same column not sharing a common superscript are significantly different at LSD (p<0.05)

Weevil (HB)= *S. zeamais* in hermetic bag, LGB (HB)= *P. truncatus* in hermetic bag, Weevil+LGB = *S. zeamais* and *P. truncatus* in hermetic bag, IFM (HB)= insect-free maize in hermetic bag, Weevil (PB)= *S. zeamais* in polypropylene bag, LGB (PB)= *P. truncatus* in polypropylene bag, Weevil+LGB (PB)= *S. zeamais* and *P. truncatus* in polypropylene bag, IFM (PB)= insect-free maize in polypropylene bag,

**Table 4: Effect of insect damage on aflatoxins levels in maize after six months of storage**

Storage treatment	Damage level month 6 (%)	Aflatoxins (µg/kg)				
		G2	G1	B2	B1	TOTAL
Weevil(HB)	2.3 <sup>b</sup>	0.0 <sup>d</sup>	1.2 <sup>c</sup>	8.0 <sup>e</sup>	64.5 <sup>f</sup>	73.7 <sup>e</sup>
LGB (HB)	4.8 <sup>b</sup>	0.0 <sup>d</sup>	0.0 <sup>d</sup>	7.9 <sup>e</sup>	75.7 <sup>ef</sup>	83.6 <sup>e</sup>
Weevil+LGB (HB)	2.4 <sup>b</sup>	1.4 <sup>c</sup>	0.0 <sup>d</sup>	8.5 <sup>de</sup>	81.1 <sup>e</sup>	89.6 <sup>e</sup>
IFM (HB)	0.0	0.0 <sup>d</sup>	0.0 <sup>d</sup>	10.4 <sup>cd</sup>	75.6 <sup>ef</sup>	86.0 <sup>e</sup>
Weevil (PB)	96.8 <sup>a</sup>	4.6 <sup>a</sup>	0.0 <sup>d</sup>	24.1 <sup>a</sup>	293.1 <sup>a</sup>	321.8 <sup>a</sup>
LGB (PB)	97.9 <sup>a</sup>	3.4 <sup>b</sup>	5.1 <sup>b</sup>	18.9 <sup>b</sup>	200.3 <sup>b</sup>	227.7 <sup>c</sup>
Weevil+LGB (PB)	97.8 <sup>a</sup>	1.9 <sup>c</sup>	22.2 <sup>a</sup>	20.0 <sup>b</sup>	264.7 <sup>b</sup>	308.8 <sup>b</sup>
IFM (PB)	0.00	2.8 <sup>b</sup>	0.0 <sup>d</sup>	11.5 <sup>c</sup>	124.1 <sup>d</sup>	138.4 <sup>d</sup>

<sup>a-f</sup> Values in the same column not sharing a common superscript are significantly different at LSD (p<0.05)

Weevil (HB)= *S. zeamais* in hermetic bag, LGB (HB)= *P. truncatus* in hermetic bag, Weevil+LGB = *S. zeamais* and *P. truncatus* in hermetic bag, IFM (HB)= insect-free maize in hermetic bag, Weevil (PB)= *S. zeamais* in polypropylene bag, LGB (PB)= *P. truncatus* in polypropylene bag, Weevil+LGB (PB)= *S. zeamais* and *P. truncatus* in polypropylene bag, IFM (PB)= insect-free maize in polypropylene bag,

## **4.0 DISCUSSION**

### **4.1 Changes in moisture content of maize grains in storage**

Many subsistent farmers in Africa depend on traditional and improved storage structures to prevent their farm produce from deterioration and ensure household food security. The experiment revealed fluctuation in moisture content of grains stored at farm level in the crib during the period of storage. The increase in moisture content in the triple-layer hermetic bags could be attributed to the extreme temperature fluctuation experienced during the first-three month after storage (November-February). The frequent temperature fluctuations that characterized this period (harmattan) led to the formation of condensed water at the sides of the hermetic bags that trickled down to the bottom of the bags. This concurs with Obeng-Ofori and Boateng (2008) who reported that condensed water on the walls and bottom of metal silos are absorbed by grains to increase its moisture content.

The staggering increased of moisture content from 12.2% to 16.7% after six (6) months of storage is due to poor barrier protection of the polypropylene interwoven bags against water, oxygen and sun created optimum conditions to elevate insect metabolic activities. The increased metabolic activities of insects in polypropylene interwoven bags were accompanied with heat and moisture that was absorbed by the grains leading to the rise in moisture content, hot spot and subsequent caking of the grains (Sinha and Sinha, 1992; Obeng-Ofori and Boateng, 2008). The better barrier provided by the triple-layer hermetic bag against oxygen, moisture and insects might have accounted for the relatively lower moisture of maize in the hermetic bags compare with polypropylene interwoven bags. Similar incidence was reported by Williamset *al.* (2014) when maize stored in the triple-layer hermetic bag maintained its moisture content close to the initial moisture content of the maize.

### **4.2 Changes in Temperature, dew point and relative humidity of the storage environment**

The relative higher variability in temperature, dew point and relative humidity within the crib were due to poor protection offered by crib against these climatic factors. This was consistent with the report by Olakojo and Akinlosotu (2004) that despite the more ventilation capacity of the crib, it does not provide full protection to stored product against harsh environmental conditions. The significant difference in the abiotic factors in the triple-layer hermetic bag and the polypropylene interwoven bag might also be due to the multiple covering of the hermetic bag which minimizes the amount of heat radiation from the sun on the hermetic bag (Jonfia-Essien *et al.*, 2010).

#### **4.3 Effect of insect damage and aflatoxin contamination of maize stored in the triple-layer hermetic bag and polypropylene interwoven bag**

Oxygen depletion and carbon dioxide elevation was noted by Moreno-Martinez *et al.* (2000) as function of element of the storage system, including the population density of insect, moisture content of grain, fungal inoculum, quality of grains and the degree of gas tightness.

The unrestricted amount of oxygen in the conventional bag provided an ideal condition for the metabolic activities of insects leading to the exponential increment of aflatoxins during the period of storage. The high metabolic activities of insects increased temperature, relative humidity and moisture content of grains, creating fertile grounds for *Aspergillus species* proliferation and aflatoxins contamination (Sinha and Sinha, 1991; Beti *et al.*, 1995).

Even though the level of insect damage of grains in the triple-layer hermetic bags remained relatively stable during the storage period, the level of aflatoxin accumulation after storage was relatively higher than the average initial amount. This may be due the surge in the grains moisture content as a result of temperature fluctuation during storage that led condensation and absorption of condensed water by the grain. Barney *et al.*, (1995) and Rees (2004) reported that fungal growth in stored grain in the tropical countries is mainly associated with increases in grain moisture contents, and fluctuation in temperatures resulting in unsafe storage of high-moisture grain and moisture migration and condensation. Similar conclusion was also drawn by Ojeda *et al.* (2009) that *Aspergillus flavus* grew on oxo-biodegradable polyethylene film as used in the triple-layer bagging technology, while moisture content increased to produce aflatoxins.

The general rise in the level of aflatoxins in both bag technologies may have been significantly influenced by the change in season (harmattan and rain) and length of storage. Aflatoxin levels in insect-free maize in the polypropylene remained relatively stable for the first-three month due to the marginal reduction of the moisture content of grains as a result of the dry, windy and hazy experienced during the harmattan. Following the onset of rains in March, the previously dried grains in the hermetic and polypropylene bags absorbed moisture from the humid air to create suitable conditions for aflatoxin contamination. This was confirmed by Choudary and Sinha (1993) in India when they observed that aflatoxin accumulation was highest in maize stored for 52 weeks during the monsoon, a season with high relative humidity. The same authors reported of a decline of 33% in the level of aflatoxin B<sub>1</sub> in the winter when relative humidity was low. Seasonal and diurnal temperature difference between stored grains and surrounding environment can result in moisture translocation or migration among quantities of bulk grains or in condensation of moisture on the grain (FAO, 1979).

The crib storage structure failed to protect the different storage bag technology from rainstorm from March-May leading to significant accumulation of aflatoxins. After reporting that aflatoxin

accumulation increase with storage time, Hell *et al.* (2000) concluded that, the level of aflatoxins contamination after harvest or during storage is dependent on the type of storage structure. Several other researchers also observed that aflatoxins concentration was related to storage structure (Ansah, 2012; Ahmad, 1993 and Prasad *et al.*, 1987), storage time (Lillehoj and Zuber, 1988) and storage insect pests (Sinha and Sinha, 1992).

Studies by Ognakosson *et al.* (2013) noted that oxygen level could be modified by the respiration and metabolic activities of insects, fungi and the grain itself to a level of 1%-2% and below. Such an inimical level of oxygen was reported by Murdoch *et al.* (2003) from the use of TLHB of thickness of 80 microns to control the cowpea beetle *Callosobruchus maculatus*. Despite the extremely low permeability of oxygen through the walls of HDPE (150 microns) bag used, the somewhat inflexible nature of the bags allows the ingress of oxygen through the entrance of the bags. Similar observation was reported by Anankware *et al.* (2013) when oxygen level of below < 5% was achieved to control bruchids in TLHB. But unlike insects, many storage fungi are capable of growing in low pressure of oxygen (O<sub>2</sub>) and reduction of oxygen is often not sufficient to prevent moulding (Hocking, 1989). The failure of the triple- layer hermetic bag to decreasing oxygen to <0.14% and elevating carbon dioxide to >50% resulted in incomplete inhibition of growth and subsequent aflatoxin contamination by aflatoxigenic fungi (Magan and Lacey, 1984).

## **5. CONCLUSION**

The high accumulation of aflatoxins in insect-infested maize stored in interwoven polypropylene bag shows that, insect metabolic and damaging activities play a pivotal role in aflatoxins production in stored maize. Triple-layer hermetic bags have the capacity to deter effectively insects and hence prevent insect-induced aflatoxins contamination. The low oxygen/high carbon dioxide conditions generated in TLHB cannot completely prevent further accumulation of aflatoxins in previously dried maize at safe moisture content ( $\leq 14$ ) in the tropics but can effectively minimize the rate aflatoxins accumulation. The widely used crib storage structure should be restricted to storing maize from aflatoxins in the dry season due to its high ventilation capacity and not the rain season due to its poor protection against rainstorm and high relative humidity.

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