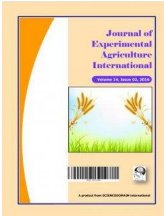

Journal of Experimental Agriculture International

29(3): 1-8, 2019; Article no.JEAI.45958
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Effect of Packaging Materials on Insect Mortality and Aflatoxin Contamination in Stored Maize under Different Conditions

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2019/45958
Editor(s):
(1) Dr. Hugo Daniel Solana, Professor, Department of Biological Science, National University of Central Buenos Aires, Argentina.
Reviewers:
(1) Isela Quintero Zapata, Universidad Autónoma de Nuevo León, Mexico.
(2) S. Sathish, Mother Terasa College of Agriculture (Affiliated to Tamil Nadu Agricultural University), India.
(3) Bonaventure January, Sokoine University of Agriculture, Tanzania.
Complete Peer review History: http://www.sciencedomain.org/review-history/28085

Original Research Article

Received 02 October 2018
Accepted 18 December 2018
Published 03 January 2019

ABSTRACT

Postharvest losses in stored maize is alarmingly huge due to destructive storage pests. The main objective of this study was, therefore, to evaluate the effectiveness of different packaging materials for the protection of stored maize against infestation by maize weevils under different storage conditions. A 2×2×2 factorial experiment in a Completely Randomized Design (CRD) with three replications was used. *Obaatanpa* maize variety was packaged in triple-layer hermetic and standard woven polypropylene bags. Half of each treatment samples were then artificially infested with 20 live *Prostephanus truncatus* (Horn) and the other half with 20 live *Sitophilus zeamais* (Motschulsky) before being stored under ambient (28.5°C, 73.53%RH) and simulated hot (37.7°C, 48.64%RH) environment for 6 months. From the results, the triple layer hermetic bags could significantly ($p \leq 0.01$) lower population growth and greatly increase mortality of *P. truncatus* and *S. zeamais* during storage than woven polypropylene. Under both ambient and simulated hot storage

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environment, aflatoxin count was significantly (*p≤0.01*) worse in woven polypropylene packaging (16.39%) than in the triple layer hermetic bags (3%). The *P. truncatus* was found to be more destructive (26.26%) than *S. zeamais* (14.03%). It can be concluded that Triple layer packaging bags could be used for maize storage in both ambient and hot storage environment, protecting it against insect infestation, aflatoxin infestation and maintaining the quality of maize without the need for insecticide use.

Keywords: Simulated; mycotoxins; contamination; tensile; degradation and hermetic.

1. INTRODUCTION

(Maize (*Zea mays* L.) is referred to as the cereal of the future because of its nutritional value and utilization of its by-products [1]. According to FAO [2], the global attention is continuously focused on maize because it is one of the most important dietary staple foods in the world. In sub-Saharan Africa, maize is one of the most important grain staples and a source of caloric intake, protein, iron, minerals and vitamin B, accounting for nearly 20% of plant-based food supply [3]. It is used for the preparation of several kinds of dishes which are consumed on a daily basis in Africa. Additionally, the fresh maize is eaten boiled, baked or roasted and provides needed food to fill the hunger gap in situations where adequately prepared dishes may not be available. In Ghana it is the most important cereal and a staple food for over 90% of the population [4]. Maize yield registered by the Ministry of Food and Agriculture in 2015 was 1,692,000 Mt. The current value of maize production in Ghana is approximately US$ 530 million annually [5].

Over 20 different insect pests attack stored maize. These include the maize weevil, *Sitophilus zeamais* (Mot) (Coleoptera: Curculionidae) and the Larger Grain Borer (LGB), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). Insect pests damage to stored grains results in major economic losses to farmers throughout the world [6]. Several millions of rural farmers produce maize, but unfortunately do not have access to improved postharvest technology to safely keep their produce until such a time when the value peaks before they are sold. Another challenge in maize storage is the build-up of aflatoxin. According to Miller [7], aflatoxin poses a major health concern in consumption of cereal grains, especially in maize. It is produced by three main species of fungi, *Aspergillus flavus, A. parasiticus, and A. nomius*. Aflatoxins contamination is facilitated by long- term storage under unhygienic and unventilated conditions [8].

Hermetic storage consists of a sealed storage system with a modified atmosphere. As a result of respiration, Oxygen (O_2) level is lowered with high accumulation of Carbon Dioxide (CO_2) causing asphyxiation leading to death of the insects. According to Montross et al. [9], propagation and development of insects depend on several factors, including moisture content and temperature of the grain, the level of damage and foreign-material of the grain, and atmosphere around the grain. Hayma [10] found that favorable conditions for most grain storage insects development is between 25°C to 30°C, and relative humidity between 70% and 80%. A research conducted by Yakubu et al. [11] showed that insect infestation problems can be controlled under hermetic storage conditions at moisture content and temperature ranges of 6% to 16% and 10°C to 27°C respectively. In order to surmount emerging challenges confronting maize storage, there is the need to explore further new and emerging solutions. The main objective of the study were: to determine the mortality of *S. zeamais* and *P. truncatus* in the different packaging materials under different storage conditions; and aflatoxin levels in the stored maize under the different treatments for a six-month storage period; and the tensile strength of the packaging materials used for the study.

2. MATERIALS AND METHODS

2.1 Experimental Site

The study was conducted in the laboratory of the Department of Horticulture of the College of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology in the Ashanti Region of Ghana from October 2017 to March 2018.

2.2 Source of Maize Variety and Moisture Content

Three 50 kg bags of maize (*Obataanpa*) harvested in the 2017 major farming season was obtained from the Crops Research Institute, Kumasi. The initial moisture content of the maize was determined using Hydromette G8 Grain Moisture Meter. The maize sample was dried to a moisture content of 12% to 14% which is the recommended range for long-term storage of maize by farmers. The maize was cleaned and all debris removed. The initial base moisture content averaging 13% was established using moisture meter.

2.3 Characteristics of the Storage Materials Used

Two sets of storage materials were used for this experiment. Thirty (30) triple-layer hermetic bags were obtained from the Forum for Agricultural Research in Africa, (FARA) through a local representative, Bioplastics. The triple-layer hermetic bag consists of two plastic bags made of polyethylene and a woven polypropylene to give additional support and protection. The bags measured 100 μm in thickness and were resized into 144 pieces of 15 cm × 15 cm in length and width and sealed using electric manual sealer after filling content with maize and insects.

Thirty (30) woven polypropylene bags (popularly known as ‘fertilizer bags’ in Ghana) were obtained from the Kumasi Central Market. Each of these was resized and sewn into 144 pieces of 15 cm × 15 cm in length and width after filling with maize sample and insects.

2.4 Culturing of Experimental Insects

Culture of experimental weevils was done at two places. The Entomology Unit of the Department of Crop and Soil Sciences, KNUST and Crop Sciences Department of University of Ghana in Accra to raise 3,000 *Sitophilus zeamais* and 3,000 *Prostephanus truncatus* unsexed adults respectively. The insect multiplication lasted for a period of six weeks after which they were put into clean jars suitable for transportation. Insect infested maize was used as source of insects and were allowed to multiply over the period. The insects were separated from the maize by sieving using a set of USA standard sieve series (Nos. 10 to 35 mm).

2.5 Experimental Design

A 2×2×2 factorial experiment with 2 packaging materials, two insect types and 2 storage conditions arranged in Completely Randomized Design (CRD) with three replications.

2.6 Sample Preparation

Three 50 kg bags of maize harvested in 2017 major farming season was obtained from the Crops Research Institute, Kumasi. The initial moisture content of the maize was determined to be 12% to 14% using a moisture meter. The moisture content chosen was the recommended range for long-term storage of maize. Thirty (30) triple-layer hermetic bags were resized and sealed using Heat Sealing Impulse Manual Sealer to obtain 144 pieces of 15 cm×15 cm mini packaging materials. Thirty (30) woven polypropylene bags were resized and sewn into 144 pieces of 15 cm×15 cm. Five hundred (500) g of *Obaatanpa* maize grains were weighed using Digital Precision Lab Scale and poured into each of the 288 resized storage materials. Twenty (20) unsexed adult *S. zeamais* live insects were introduced to each of the 72 packs of TLH and each of the 72 packs of woven polypropylene with maize grains and half of each set stored in ambient and simulated hot storage environment. Twenty (20) unsexed adult *P. truncatus* live insects were introduced to each of 72 packs of TLH and each of 72 packs of woven polypropylene with grains and half of each set stored in ambient and simulated hot storage environment.

2.7 Inoculation of Insects

Each of the 72 pieces of hermetic bags and 72 pieces of the polypropylene bags containing 500 g of maize sample was inoculated with 20 unsexed live *S. zeamais* using camel hair brush. Each of the remaining 72 pieces of hermetic bags and 72 pieces of the polypropylene bags containing 500 g of maize was also inoculated with 20 unsexed live *P. truncatus* insects using camel hair brush. The inoculated mini-sacks were then sealed. The hermetic bags were sealed with Heat Sealing Impulse Manual Sealer and the polypropylene coverings were sewn. The standard polypropylene bags were all sewn. The experiment was set up in two different temperature storage conditions to assess the performance of the selected storage packages against insect infestations. The first storage environment was ambient (laboratory of the Department of Horticulture, expected to mimic storage conditions for maize storage in southern part of Ghana) with temperature range of 21°C to 28°C. A second storage environment similar to conditions in northern Ghana with temperatures ranging from 21°C to 45°C was set up in one of the Department of Horticulture’s greenhouse sheds, KNUST. A temperature/humidity data loggers (EL-USB-2, LASCAR Electronics, USA) was placed in each of the storage environment to log daily temperature (°C) and relative humidity readings (%).

2.8 Data Collection and Parameters Monitored

2.8.1 Determination of temperature and relative humidity

A temperature/humidity data loggers (EL-USB-2, LASCAR Electronics, USA) was used to measure the temperature and relative humidity in the storage environment where the hermetic bags and the polypropylene bags were stored for the entire duration of the study. The data logger was programmed to continuously measure the temperature and relative humidity every thirty minutes for the total duration of the experiment. Data logger was placed on the platform where the packaged materials of triple-layer hermetic bags and the polypropylene bags were stored in the ambient and the simulated hot environment to measure the surrounding temperature and relative humidity.

2.8.2 Determination of insect mortality and multiplication

At each sampling occasion, the contents of each experimental package was opened and sieved using a set of USA standard sieve series (Nos. 10 to 35). Adult insects and the dead were collected separately. The count of insects sieved out (live and dead together) was recorded.

2.8.3 Determination of aflatoxin levels in stored maize

Aflatoxin levels of the stored maize was determined at two stages of the duration of experiment. The first was at the start of the experiment and the last at the end of the sixth month. Packaged samples of both storage materials were collected for analysis at the Mycotoxin Laboratory of Food Science Department of KNUST according to ISO 16050: 2003 test methods. Thirty (30 g) sub-sample maize was ground with laboratory mill and blender and mixed thoroughly before sampling into sample containers. Two grams (2 g) of sodium chloride and 100 ml of extraction solvent were added to 20 g of milled maize. The solution was shaken and homogenized for 3 minutes. The extract was filtered using a paper filter and 20 ml of the filtrate was diluted with 60 ml of PBS, mixed well and filtered again using glass microfiber filter. Purification was carried out by passing the extract through Immuno-Affinity Columns (IAC) containing antibodies specific for aflatoxin B1, B2, G1 and G2. The aflatoxins were eluted with 1.0 ml of methanol and quantified by reverse-phase High Performance Liquid Chromatography (HPLC) with spectrofluorometric detector. 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 were 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.

2.8.4 Determination of tensile strength of packaging materials

According to Anankware et al. (2013), each triple-layer hermetic bag consists of two plastic bags (made of polyethylene) put inside a third bag made of woven polypropylene to give additional protection and strength. These bags are 100 µm thick and measure 34×62 cm in width and length, respectively. The experiment determined the tensile strength of the bags as a measure of deterioration during storage. This assessment was done at the start, midway and end of the period. The test was done at the Materials Unit of the Faculty of Engineering, University of Ghana in Accra.

2.9 Data Analysis

Data obtained was subjected to analysis of variance (ANOVA) using Statistix Software Version 10 and means were separated using Tukeys HSD for laboratory data at 1% ($P < 0.01$).

3. RESULTS

3.1 Temperature and Relative Humidity of Storage Environment

Table 1 illustrates the temperature and relative humidity values for both the ambient and simulated hot environment for the maize storage from October, 2017 to March, 2018.

In the ambient environment, the highest average temperature was recorded in March, 2018 and the least was recorded in January, 2018. The highest relative humidity was recorded in November, 2017 and the least was March, 2018. In the simulated hot environment, the highest average temperature was recorded in February, 2018 and the least was recorded in October, 2017. The highest relative humidity was recorded in November, 2017 and the least was March, 2018.

3.2 Insect Mortality as Influenced by Different Packaging Materials and Storage Environment

There were significant ($p \leq 0.01$) weevil type x packaging material x storage environment interaction on insect mortality (Table 2). Significantly highest insect mortality was recorded by maize grains stored in triple-layer packaging material under ambient and simulated hot environment with *Prostephanus truncatus* and *Sitophilus zeamais* whiles the least was recorded by maize grains stored with *Prostephanus truncatus* in single-lined packaging material. Among the weevil types, there were no differences in the means for insect mortality. Across the storage environment, the highest insect mortality was recorded by grains stored under ambient environment and the least was recorded by grains stored under simulated hot environment.

As regards insect mortality (%) there were significant differences among the packaging materials for insect mortality (Table 2). The highest insect mortality was recorded by grains stored in triple–layer packaging material and the least was recorded by those stored in woven polypropylene bags.

3.3 Aflatoxin Count (%) in Stored Maize as Influenced by Packaging Materials

There were significant differences ($p \leq 0.01$) between the packaging materials for Aflatoxin count. Highest Aflatoxin count was recorded by maize grains stored in Woven polypropylene packaging material and the least was recorded by the triple-layer hermetic bags (Table 3).

Table 1. Temperature and relative humidity of storage environment

Month/Year	Ambient storage environment		Simulated hot storage environment	
	Average temperature (℃)	Ave. relative humidity (%RH)	Average temperature (℃)	Ave. relative humidity (%RH)
Oct-17	29.14	75.12	34.75	61.23
Nov-17	28.34	76.14	35.25	56.35
Dec-17	27.51	78.03	36.25	50.36
Jan-18	26.61	71.82	38.26	41.34
Feb-18	29.51	70.03	40.62	41.85
Mar-18	30.14	70.01	41.09	40.72
Mean	28.54	73.53	37.70	48.64

Table 2. Insect mortality as influenced by different packaging materials and storage environment

Weevil (%)	Package (%)	Storage environment		Mean
		Ambient (%)	Hot Environ (%)	
Prostephanus truncatus	Woven polypropylene	36.00^{bc*}	21.00^{d}	28.50
	Triple-layer	89.00^{a}	91.00^{a}	90.00
Mean		**62.50^{a}**	**56.00^{b}**	59.00^{a}
Sitophilus zeamais	Woven polypropylene	34.00^{bc}	27.00^{cd}	30.50
	Triple-layer	91.00^{a}	91.00^{a}	91.00
Mean		**62.5^{a}**	**59.00^{b}**	60.75^{a}
Grand Mean		63.00^{a}	57.00^{b}	
Package Mean	Woven polypropylene	35.00^{a}	24.00^{a}	**29.50^{a}**
	Triple-layer	90.00^{b}	91.00^{b}	**90.50^{b}**

CV=4.17
HSD (0.01) Weevil=3.00 Package mat=3.00 storage environment= 3.00 Weevil x Package mat. X storage environment=8.00

**Means followed by the same alphabets are notsignificantly different ($p \leq 0.01$) from each other*

Table 3. Aflatoxin count (%) as influenced by different weevil types, packaging materials and storage environment

Weevil (%)	Package (%)	Storage environment		Mean
		Ambient (%)	Hot Environ (%)	
Prostephanus truncatus	Woven polypropylene	21.40[a*]	18.03[a]	19.72
	Triple-layer	0.00[a]	6.00[a]	5.00
Mean		**10.7[a]**	**12.02[a]**	**9.37[a]**
Sitophilus zeamais	Woven polypropylene	6.80[a]	19.32[a]	13.06
	Triple-layer	0.00[a]	8.00[a]	4.00
Mean		**3.4[a]**	**13.66[a]**	**6.51[a]**
Grand Mean		7.05[a]	9.37[a]	
Package Mean	Woven polypropylene	14.1[b]	18.68[a]	**16.39[a]**
	Triple-layer	0.00[a]	7.00[b]	**4.50[b]**

CV=2.18
HSD (0.01) Weevil=2.00; Package mat.= 2.00, storage environment=2.00;
Weevil x Package mat. X storage environment= 5.00

** Means followed by the same alphabets are not significantly different (p≤0.01) from each other.*

There were no significant (p≤0.01) weevil type x packaging material x storage environment interaction on Aflatoxin count (Table 3).

3.4 Tensile Strength of Packaging Materials before and after Six Months Storage of Maize

Table 4 shows the tensile strength of the packaging materials used under different storage environment. At 0 month of storage, highest tensile strength was recorded in triple-layer hermetic bag used in both simulated hot and ambient conditions and the least was single layer with ambient and simulated hot environment.

At third month of storage, highest tensile strength was recorded in triple-layer hermetic bag used in simulated hot and the least was single layer with ambient and simulated hot environment.

At the sixth month of storage, highest tensile strength was recorded in triple-layer hermetic bags used in simulated hot and the least was single layer with simulated hot environment.

4. DISCUSSION

4.1 Insect Mortality (%) as Influenced by Different Packaging Materials and Storage Environment

Results showed that highest insect mortality was recorded by maize grains stored in triple-layer hermetic bags under ambient and simulated hot environment with *Prostephanus truncatus* and *Sitophilus zeamais* whiles the least was recorded by maize grains stored with *Prostephanus truncatus* in Woven polypropylene packaging material. This is because the insects which were in the triple-layer hermetic bags could not fly away due to the barrier the bag created as compared to the Woven polypropylene packaging material. Insects in the Woven polypropylene material could easily escape by flying through the openings in the package material. Moreover, the openings of the woven polypropylene material permitted the exchange of oxygen and moisture which might have given life to those insects to survive than those in the triple-layer hermetic bags which had no oxygen for the insects to respire, survive and reproduce. Higher insect mortality was recorded by insects in the simulated hot environment than those in the ambient environment due to the high temperatures in the simulated hot environment. Baoua et al. [12], reported that the low oxygen levels under hermetic conditions result in the suppression of feeding, growth, development and population expansion of insects. In addition, Njoroge et al. [13], stated that before the insects succumb, they respond to hypoxia by metabolic down-regulation which reduces overall insect movement. Furthermore Hashem et al. [14] reported similar findings that the lethal action of low oxygen and oxygen-free atmospheres on postharvest insect pests during storage has been studied over many years.

4.2 Aflatoxin Count (%) in Stored Maize

Results showed that highest Aflatoxin count was recorded by maize grains stored in Woven polypropylene packaging material and the least was recorded by the triple-layer hermetic bags. This could be that triple-layer hermetic bags provided resistance or barrier for the exchange of

Table 4. Tensile strength of packaging materials during six months storage of maize

Packaging material/Storage environment	Period of storage		
	0 month (kpa)	Three months (kpa)	Six months (kpa)
Triple layer, Ambient	28	29	30
Triple layer, Simulated hot	28	30	37
Woven polypropylene, Ambient	26	26	27
Woven polypropylene, Simulated hot	26	26	20

moisture between the grains and the ambient environment. Maize grains are hygroscopic and therefore have the tendency to absorb moisture from the environment during storage when temperature and relative humidity were high. Aflatoxins are fungal mycotoxins that normally proliferate when dried products have higher moisture and temperature. Castellari et al. [15] indicated that the proliferation of these fungi are stimulated by higher grains moisture content, higher temperature during storage, long storage period, intensive infection by fungi before storage and by higher activity of insects and mites. Therefore, it is important to identify the species of fungi in stored maize grains with special emphasis on mycotoxigenic species, which pose a potential risk to human and animal health. Moreover, Amézqueta et al. [16] reported that temperature and moisture content of maize are two crucial parameters that affect significantly the quality of grains, biochemical reactions, dry matter losses, allowable storage times and overall storage management of the grains. Furthermore, Fuseini [17] explained that fluctuations in temperature and relative humidity in tropical countries accelerate rapid multiplication and proliferation of molds and insects which undermine grain quality. In the current study, results clearly confirm these findings.

4.3 Tensile Strength of Packaging Materials as Influenced by the Storage Environment

The relatively high temperatures in the simulated hot storage environment (average of 37.7°C for the storage period with corresponding relative humidity of 48.64%RH) were seen to increase the ductility and tensile strength of the hermetic bag as compared to the original state. This was because the longer the period the hermetic bag was kept in that environment, the higher its tensile strength. On the other hand, the relatively lower but stable temperatures in the ambient environment (average of 28.54°C for the storage period with corresponding relative humidity of 73.53%) tend to maintain the ductility hence, a uniform tensile strength realized.

In respect of the woven polypropylene bags, the relatively high temperature condition in the simulated hot storage environment affected the stress and strain properties of the strips of the woven polypropylene bags making it brittle hence weakening it towards the end of the storage period. This was evident in the course of storage as some of the bags had completely deteriorated at the end of the storage period hence exposing their contents. The ductility and the tensile strength of the woven polypropylene bags stored under ambient conditions was stable but got weakened towards the end of storage period. This could be attributed to the stable temperature conditions prevailing in the storage environment.

5. CONCLUSION

The triple-layer hermetic bag can be said to have effective control against *P. truncatus* and *S. zeamais.* The results showed that highest insect mortality was recorded by maize grains stored in triple-layer hermetic bags under ambient and simulated hot environment with *P. truncatus* and *S. zeamais* whiles the least was recorded by maize grains stored with *P. truncatus* in Woven polypropylene packaging material. Aflatoxins counts of maize was found to be low in the triple-layer hermetic bags in both ambient and simulated hot storage conditions. Meanwhile, aflatoxins count was found to be high in maize packaged in the woven polypropylene packaging material under both storage conditions. Degradation of the tensile strength of the packaging materials over the six month storage period was highest in the woven polypropylene packaging material than the triple-layer hermetic bag. From these findings, it can be deduced that triple-layer hermetic bag is very effective for the protection of stored maize against infestation by maize weevils (*Sitophilus zeamais*) and larger grain borer (*Prostephanus truncatus*) irrespective of the storage conditions (ambient and simulated hot environment) they are kept.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/28085