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Evaluation of hermetic technologies in the control of insect infestation and mycotoxin contamination in stored maize grains

Jacqueline Namusalisi*[a], Catherine N. Kunyanga[a], Anani Bruce[b], Hugo De Groote[b]

[a]Department of Food Science, Nutrition and Technology, University of Nairobi, P.O. Box 29053-00625 Kangemi, Kenya

[b]International Maize and Wheat Improvement Center (CIMMYT), P.O Box 1041-00621 United Nations Avenue, Nairobi – Kenya

* Corresponding author: namujq.jay@gmail.com

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Abstract

Grain losses due to moulds during on-farm storage increase food insecurity, result in economic losses, negatively affect farmers' livelihoods, and increase exposure to mycotoxins that can harm human and animal health. Hermetic storage technologies provide a reliable solution for maize grain that may also preserve food safety. Several studies report the effectiveness of these technologies against post-harvest insects in Africa but provide limited evidence on effectiveness against mould proliferation and mycotoxin contamination. Hermetic technologies were superior to farmer practice in reducing insect infestations and mycotoxin accumulation. Among hermetic technologies, there were no significant differences ($P>0.05$) in performance between metal silos and hermetic bags for mycotoxin accumulation and insect infestation regardless of the mode of infestation. In non-inoculated grain, fungal populations were varied but included mycotoxin-producing *Aspergillus* and *Fusarium* spp., indicating that the grain was naturally contaminated and acted as a good reservoir for these fungi. Mycotoxin levels increased with higher moisture even in non-inoculated grain. Meanwhile, aflatoxin and fumonisin levels at 4 months were not significantly different from baseline values in dry inoculated grain across all storage technologies ($P>0.05$), indicating that hermetic technologies can prevent mycotoxin contamination in dry grain for at least 4 months of storage. Aflatoxin and fumonisin were significantly higher by 1.69 ppb and 0.25 ppm respectively in non-inoculated grains at high moisture indicating the need to adequately dry grain before storage in hermetic technologies. This trend was observed collectively in all the technologies registering 2.03 ppb and 0.311 ppm respectively. In inoculated grains at high moisture, there was an increase in aflatoxin in both hermetic treatments and the control by 5.7 ppb and 12.14 ppb respectively. Therefore, a trial was conducted to compare hermetic technologies with farmer practice in their effectiveness against both insect infestation and mycotoxin contamination.

Keywords: Insect infestation, mycotoxin contamination, stored maize, hermetic storage, food security

1. Introduction

Maize (*Zea mays* L.) can conveniently be classified as the most important cereal crop owing to its nutritional value and utilization of its by-products. Grain losses due to insect pests during on-farm storage increase food insecurity, result in economic losses, negatively affect farmers' livelihoods, and increase exposure to mycotoxins that can harm human and animal health (Obeng-Ofori, 2008). Among these mycotoxins, the two commonest and highly toxic mycotoxins compound encountered in maize in the tropical and sub-tropical region of the world are aflatoxins and fumonisins (Krska *et al.,* 2008). Aflatoxins are toxic metabolites produced by fungal species during their growth under favorable conditions of temperature and moisture. The major aflatoxin producing species are *Aspergillus flavus* and *Aspergillus parasiticus*. The main cereals affected are maize, sorghum, rice and wheat and other crops like groundnuts and cassava. Aflatoxin-producing fungi have very few nutritional, environmental and reproductive requirements, and that is their strategy to survive and develop (Wu *et al.,* 2011). Fumonisins are mycotoxins produced by the grain moulds *Fusarium verticilliodes* and *Fusarium proliferatum,* which is frequently a universal inhabitant of corn. Fumonisins are categorized as, B1, B2 and B3 and are usually found to be greater than 1

ppm in the corn samples tested. However, the FDA/USDA advises less than 4 ppm in corn meant for human consumption and less than 50 ppm for cattle feed. Fumonisins are not always produced where the fungi have colonized on the kernels, but many factors contribute to the subsequent mycotoxin contamination, including host susceptibility and environmental conditions. All these factors together determine the incidence and severity of mould contamination on the grain. The conditions that favor fumonisin production are not well known; *Fusarium* moulds thrive well in hot followed by cool conditions, in wet conditions during pollination and ear development. The magnitude of the effect of mycotoxin exposure is facilitated by the level and exposure period, as well as health, age and the species of the animal.

Damages caused by insect pests represent a huge setback in the world's effort to achieve food security globally. According to Ileleji *et al*. (2007) and Nukenine, (2010) an estimated 1% to 5% of stored grain in developed countries and 20% to 50% of stored grain in developing countries are lost due to insect damage. Cracked or broken grains provide an entry point for infestation by insects and moulds during storage. Variation in temperature and humidity has been identified to support the metamorphosis of *Prostephanus truncates* (Horn) (Hodges and Meik, 1984). They lay eggs which hatch in about three days at 27 **°C** day temperature and the dust provide the nourishment to the larvae. Larva development to adult stage takes place within 27 days and is facilitated by ideal conditions of 32 °C and 80% relative humidity (Hodges, 1986). Maize weevil, *Sitophilus zeamais* (Motschulsky), is one of the cosmopolitan pests of stored cereals, especially maize (Throne, 1994). It damages stored maize and of cob maize prior to harvest. It may also infest other cereals if the moisture content is moderate or high (Longstaff, 1981). Eggs are laid at temperatures between 15 and 35 °C (with an optimum around 25 °C and at grain moisture contents over 10%). Subsequent infestations in stores result from the transfer of infested grain into store or from the pest flying into storage facilities, probably attracted by the odour of the stored grain. Dry weight loss from *S. zeamais* infestation alone averaged about 5% by weight after six months of storage. The 5% dry weight loss translates into 22% of total grains displaying damage (Holst *et al*., 2000). As a start, it should always be recognized that an intact grain is an essential item for successful storing.

Insect infestation could have significant impact on the mycotoxin contamination of maize. It is worthwhile to know that, the level of insect damage influences the extent of mycotoxins contamination. Insects act as vectors by carrying spores of mycotoxin producing fungi from plant surfaces to the interior of the stalk or kernels or create infection wounds through their feeding habits (Munkvold, 2003). Insects attack in storage could also be devastating because their level of damage influences the extent of mycotoxin production in the store. Hermetic storage technologies provide a reliable solution for maize grain that may also preserve food safety.Several studies report the effectiveness of these technologies against post-harvest insects in Africa but provide limited evidence on effectiveness against mould proliferation and mycotoxin contamination. Hermetic bags have also been known to preserve the quality of grain, appearance and aroma by reducing mould growth (Moussa *et al.,* 2014). Hermetic technology works synergistically to promote conditions of limited oxygen and high carbon dioxide levels produced by aerobic metabolism of insects, micro-organisms and grain respiration, creating a non-toxic, cost effective and environmentally friendly option over the use of chemicals in the control of insects and mycotoxin contamination in stored maize (Williams et al., 2014). Aerobic metabolism uses up oxygen and produce carbon dioxide to levels that are lethal to insects in the grain mass (Yakubu *et al.,* 2011). In the world today, concerns on the environment and food safety have increased and consumers are demanding high quality products that are free from chemical residues, aflatoxin and insect contamination (Weinberg *et al.,* 2008).

Improved storage technologies at both household and national levels which reduce losses by preventing mould growth are important component of food security. Improved storage technologies, based on hermetic sealing in high density polyethylene bag or metal and plastic silos provide affordable and more effective storage alternative for farmers, especially the vulnerable

women, that would markedly contribute to food security (Gitonga *et al.,* 2013; Obeng-Ofori, 2011; Ndegwa *et al.,* 2016; Mutambuki *et al.,* 2012).

This study is, therefore to analyze the synergy effect of hermetic storage to control mould proliferation as well as mycotoxin contamination in safe and environmentally friendly system. The generated data from this study will facilitate sustainable adoption of the hermetic technologies among smallholder farmers in Sub Saharan Africa. This study suggests the ideal storage options for the small holder farmers considering the robustness and cost of the hermetic storage that will have been identified as effective and less expensive. The study also tries to answer the question of how the use of improved storage technology impact the quantity and quality of grain stored and also the length of storage while holding other factors constant at farmers' practice level.

2. Materials and methods

The trial was conducted at CIMMYT/KARLO Kiboko Research Centre (Makuen icounty), 170 km from Nairobi in a semi-arid region in Eastern Kenya. The trial site was selected for being a trouble spot for aflatoxin outbreaks in Kenya. Two factors were used in the design of this study: 1) low (12-13%) or high (14-15%) grain moisture levels; 2) ten storage technologies. The hermetic storage technologies under study were metal and plastic silos, while the hermetic bags were: Super Grain IV-RTM, AGRO-Z with pesticides, AGRO -Z without pesticides, PICS, Elite and ZeroFly. The two controls were two farmer practices, the standard woven polypropylene bags, one with grain treated with insecticide and one without insecticide treatment. The experimental design was a 2 x 10 randomized complete block design (RCBD) with 3 replications. The duration of the experiment was 4 months with non-destructive sampling at baseline and every 120 days afterwards. Each grain sample was divided in two for insect pest testing and mycotoxin analysis.

2.1 Sample collection and preparation

About one kilogram of sample was required for the analysis. Sampling was done from five different points, about 1 inch from the walls of the storage technology using a grain sampling spear. Sampling was done carefully not to puncture the linings of the bags and the spear cleaned with cotton dampened with 75% ethanol before sampling the next storage technology to avoid cross contamination. The sampled grain was transferred into the ziplock plastic bag and sealed carefully to exclude air. Three people were involved in the sampling procedure; one person opens the storage technology, draws samples and transfers to the plastic sample bags held by another person while the other person immediately tightly seals up the bag/silo.

2.2 Materials

The grain used for this study was of H614 and H618 hybrid, purchased from farmers in Nakuru county and Naivasha sub-county. The untreated grain was cleaned by sieving to remove chaff, broken and rotten kernels. At the onset of the experiment, the grain was mixed and conditioned at the appropriate moisture content before transferring in the respective study technologies.

2.3 Grain moisture

The high moisture content (14-15%) was achieved by subjecting the grain to high relative humidity and tests were carried out progressively to determine the required moisture contents. The grain spread on plastic sheet was sprayed with potable water for 1.5 to 2 days. The water was calculated from the formula below:

$$Quantity\ of\ water\ required\ (g) = weight\ of\ grain\ x\ \frac{mcf - mc}{100 - mc}$$

Where *mcf* is the final moisture content; and *mc* the initial moisture content (Kiburi *et al*., 2014).

To achieve the moisture range of 12-13%, the grain will be sun dried in the case their moisture content was above 13%.

Insects assessment

One kilogram of the grain was analyzed for the dead and the alive of insects. This was done to investigate whether the storage technologies are able to prevent entry of insects/encourage insects' activities. The number of live and dead insects, both adult weevils and larger grain borers was counted and recorded. The grains of the subsample were sorted into undamaged, damaged and discolored fractions. The number of kernels and the weight of each fraction were recorded to investigate the extent of damage if any as follows:

$$Discolored\ grain(\%) = \frac{Number\ of\ discolred\ grain}{Total\ number\ of\ grain} \times 100$$

$$weight\ loss(\%) = \frac{[(Wu \times Nd) - (D \times Nu)]}{Wu \times (Nu + Nd)} \times 100$$

Where W_u = Weight of undamaged grain; N_u = Number of undamaged grain; W_d = Weight of damaged grain and N_d = Number of damaged grain

Grain weight loss was determined by count and weight method (Boxall 1986).

Aflatoxin and fumonisin analysis

Aflatoxin and fumonisin levels were determined in each working sample collected at cero and four months after stocking using the VICAM method (VICAM Science Technology, 1998), as describe by (Fandohan *et al.*, 2005). Three samples from each bag were taken.

Statistical analysis

Variances of insect count, (x) was stabilized by log transformation Y=log(x+1) whereas percentage data (P) was arcsine Y=sin−1$\sqrt{P}$), transformed, where Y is the result of transformation. The transformed data was then be subjected to analysis of variance (ANOVA) using Stata SE version 12 (StataCorp LP, Texas, USA). Further due to inherent limitations of ANOVA in describing difference in progression of variables over time, the analysis of covariance (ANCOVA) which combines features of both ANOVA and regression were applied to test effects of treatment and storage duration, and the interaction effects. Means were separated using Bonferroni adjustment at 95% confidence level (Ognakossan *et al.*, 2014).

Results

Aflatoxin and fumonisin

Aflatoxin contamination increased with relative humidity in both hermetic and farmer practice storages at a significance level of P<0.001, it was also observed that aflatoxin contamination increased in all the inoculated storage technologies and very high in the farmer practice (Fig. 1 and table 3). The treatment type had an effect on the level of aflatoxin contamination at the significance level of <0.001 with the mean value of 2.93, 1.31, 2.59, and 1.65 for high humidity, low humidity, inoculated and in not inoculated grains respectively. The level of fumonisin contamination increased in woven storage bags while hermetic storage technologies reduced fumonisin contamination (Table 5 and 6). There was a relationship between moisture levels, mode of inoculation and the fumonisin contamination in the storage technologies with the grand mean of 0.315 and 0.275 respectively, Fig 2. However, there was not a significant difference observed between treatment and the level of fumosin contamination (Table 1). There was no interaction between the aflatoxin and the fumonisin P>0.05 but a strong correlation between the insects and the aflatoxin contamination at P<0.05 and the number of dead insects was linked with the type of storage where hermetic bags had less insects infestation than the farmer practice. At high relative humidity, the aflatoxin, fumonisin and insects was significantly high regardless of the mode of inoculation compared with the dry grains Fig.1.

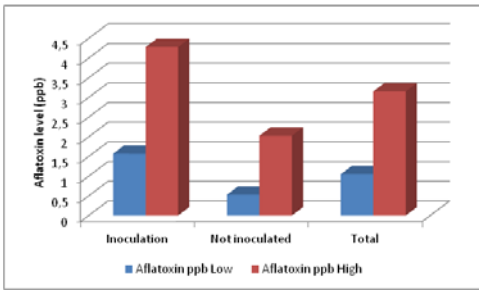

Fig. 1 Mean values of aflatoxin for both hermetic storages and the farmer practice.

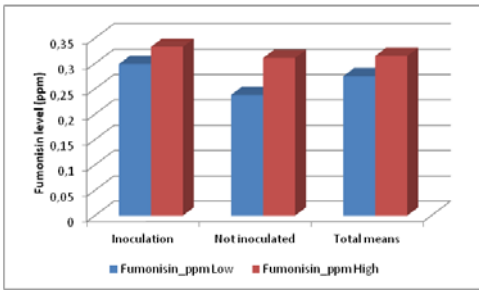

Fig. 2 Mean fumonisin levels in the storage technologies in relation to humidity and mode of inoculation

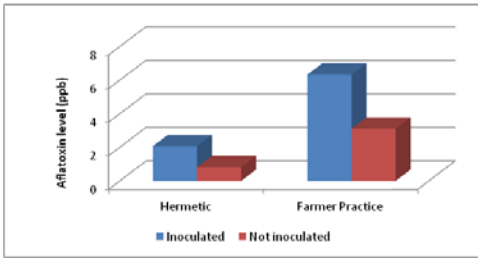

Fig. 3 Effects of technology and inoculation on aflatoxin

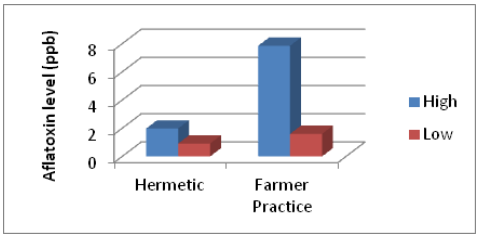

Fig. 4 Effect of technology and RH on aflatoxin

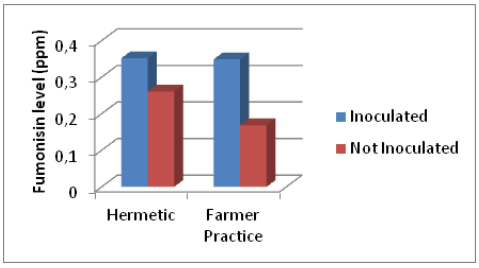

Fig. 5 Effects of technology and inoculation on fumonisin

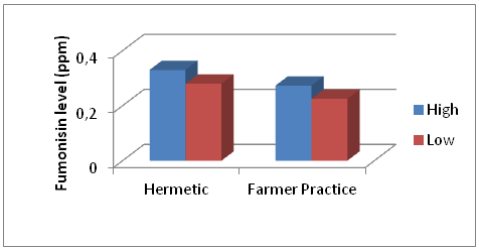

Fig. 6 Effect of technology and RH on fumonisin

Tab. 1 Interaction between aflatoxin/fumonisin and RH, inoculation and treatments

Mycotoxin		P- Value	corrected p-value		Significance
Aflatoxin					
	Relative Humidity	<.001	1.711		Sig.
	Treatment	<.001	0.904		Sig.
	Inoculation	<.001	1.032		Sig.
Fumonisn					
	Relative Humidity	0.555	0.059		n.s
	Treatment	0.092	0.169	Sig.	
	Inoculation	0.413	0.069		n.s

Insect infestation in different sets of treatment

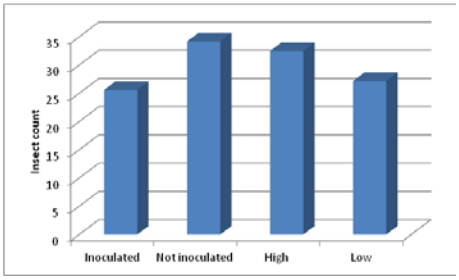

Fig. 7 Insect infestation comparing RH and mode of inoculation.

There was a significant correlation between the total insects infestation and the type of storage technology (treatment), at P= 0.109. Mode of inoculation and RH also did have any effect on the insect infestation in the four months storage period (Fig 7).

Tab. 2 Effects of treatment and insect infestation

Insects				Significance
		P- Value	corrected p-value	
	Inoculation	0.196	0.169	n.s.
	Relative	0.492	0.048	n.s.
	treatment	0.109	0.26	Sig

Discussion

Hermetic storage technologies can be an effective solution to reduce insect infestation and mycotoxin contamination during on-farm storage, thereby reducing potential human and animal exposure to mycotoxins. However, if farmers do not adequately dry grain, even hermetic storage technologies may not be effective in the control of mycotoxin contamination, and contamination will be even greater underconventional storage systems. This observation is in agreement with Cotty (2007), who described water activity as one of the conditions that encourage aflatoxin development high levels of fumonism in woven bags could be attributed to large open spaces that allow for free flow of air hence contamination. Hermetic storage technologies restrict gaseous exchange and act as a barrier hence reduced contamination. There was a correlation between inoculation and insect infestation where insect infestation was higher in the maize that was not inoculated. This is because maize already infested with aflatoxin and fumonism may have reduced in the nutritional components and palatability desired by the insects. This is also agreeable with the findings that mycotoxins development increases with the insects activities in the grain (Munkvold, 2003). This work supports the promotion of both hermetic storage technologies and improved drying practices. Currently the analysis of samples after eight months of storage is ongoing and will also be presented.

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Postharvest treatment research at USDA-ARS: stored product fumigation

Spencer S. Walse*#[1], Matthew Rodriguez[1], John S. Tebbets[1]

[1]USDA-ARS San Joaquin Valley Agricultural Sciences Center, 9611 S. Riverbend Avenue, Parlier, California, USA, 93648-9757

***Corresponding and presenting author: spencer.walse@ars.usda.gov

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Abstract

The overall goal of this USDA-ARS research is to ensure the protection and quality of stored product foodstuffs. The results of this research directly enhance production, distribution, and safety of foodstuffs, promote and retain access of United States-grown crops to domestic and foreign markets, and protect the United States and trading partners from the agricultural, ecological and economic threat posed by quarantine and invasive pests. In general, USDA-ARS research related to the fumigation of stored products focuses on the development of techniques to rapidly disinfest raw products of field pests, control pests in processed products amenable to re-infestation and microbial infection, and reduce reliance on fumigation as a stand-alone measure for postharvest disinfestations and disinfections. Specific research objectives include: comparative evaluation of alternative fumigants to methyl bromide in postharvest applications, development of novel technologies to reduce and eliminate atmospheric emissions from chambers used in postharvest fumigation, and design production strategies that allow for a more strategic postharvest use of methyl bromide and alternative fumigants. Recent research findings will be presented and discussed, including: exposure requirements of phosphine on key stored product pests (as related to resistance management), the establishment of efficacy and experimental criterion for quarantine applications, and the development of models to quantitatively understand the underpinnings of fumigations and related phytosanitary treatments.

Keywords: food security, food safety, quarantine treatments, postharvest methyl bromide

1. Introduction

The use of postharvest phosphine fumigation as a quarantine phytosanitary requirement is increasing coincident with the globalization of agriculture. However, operational and regulatory framework for implementing and certifying efficacious treatments have not bee firmly established. In this work we describe a postharvest fumigation with phosphine to control Warehouse beetle, *Trogoderma variable* (Ballion) (Coleoptera, Dermestidae), a pest of concern to certain countries that import Dried Distillers Grains (DDGs) from USA. A series of laboratory-scale exploratory fumigations with phosphine at 10.0 ± 0.3 ºC ($\bar{x} \pm 2s$) were conducted to evaluate the postharvest control of