

**PERFORMANCE OF POST-HARVEST STORAGE BAGS AND
DIATOMACEOUS EARTH FOR THE PROTECTION OF STORED MAIZE
AGAINST INSECT PEST INFESTATIONS**

BY

Shekinat Kehinde ASIWAJU-BELLO

Matric No.: 172792

B.Tech. Biology (Storage Technology) (Akure), M.Sc. Zoology (Ibadan)

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CERTIFICATION

I certify that this work was carried out by Mrs S.K. Asiwaju-Bello in the Department of Zoology, University of Ibadan.

.....

Supervisor
K.O.K. Popoola
B.Sc., M.Sc., Ph.D (Ibadan)
Associate Professor, Department of Zoology,
University of Ibadan,
Ibadan, Nigeria.

.....

Co-Supervisor
G.P. Opit
B.Sc., (Kampala), M.P.M., (Vancouver), Ph.D. (Kansas)
Professor, Department of Entomology and Plant Pathology,
Oklahoma State University,
Stillwater, OK., USA.

DEDICATION

I dedicate my dissertation work to my parents, Isiaq and Basirat Ajao for their love and to my co-supervisor, Professor George P. Opit. A special gratitude to Professor Opit for his mentorship, inspiration and financial grant I enjoyed throughout my study.

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ABSTRACT

Maize is an important staple crop widely used in domestic and industrial processes. It is usually stored in Polypropylene (PP) bags, where it is prone to heavy insect infestations, resulting in high economic losses. A number of recent interventions to prevent insect infestations during grain storage include Diatomaceous Earth (DE) and novel bags such as ZeroFly (ZF) and Purdue Improved Crop Storage (PICS). However, there is paucity of data on their effectiveness and optimal use in insect pest management. Therefore, this study was conducted to determine the effectiveness of different storage bags and DE against stored-insect pests of maize.

The study was conducted between February, 2017 and January, 2018 in a storehouse at Arisekola Market, Bodija, Ibadan. Pristine 50 kg SWAN 2 maize variety was stored using eight different bag treatments comprising: PP (control) and ZF, DE admixed in PP and ZF (PPDE and ZFDE), single and double hermetic liners in PP (PP1L and PICS), single and double hermetic liners in ZF (ZF1L and ZF2L). A stack of three replicates for each treatment was placed on separate pallet and arranged one meter apart. In addition, for each ZF1L, ZF2L, PP1L and PICS, two sets comprising 3 replicates/set were stored for destructive sampling every four-months using standard procedure. Maize in ZF, PP, ZFDE and PPDE were sampled monthly. Insect population count, insect damaged kernel, maize weight loss and insect perforation on bags were determined through standard procedures. Maize quality was determined through standard seed germination method, maize Moisture Content (MC) was measured by MC meter and aflatoxin level by Thin-Layer Chromatography. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

Total insect population of 5,945 in all treatment bags comprised predominantly *Sitophilus zeamais* (2,593), followed by *Tribolium castaneum* (1,298), *Liposcelis* spp. (1,193) and the least occurring *Cryptolestes ferrugineus* (861). Percentage of insect species per bag: ZF2L (0.2) and ZF1L (0.3) were significantly lower than the population in ZFDE (3.2) and PPDE (7.9), but higher in ZF (51.8) and PP (35.4). Insect damaged kernel was significantly low in ZF2L (0.4±0.0%), ZF1L (0.5±0.0%) and ZFDE (0.8±0.3%) compared to ZF (16.9±1.6%) and PP (5.4±0.9%). The maize weight loss was significantly low in ZF2L (0.1±0.0%), PICS (0.2±0.0%) and ZFDE (0.2±0.1%) compared to ZF (6.7±0.8%). The number of insect perforations on ZF2L (0.0±0.0), PICS (1.3±0.4) and ZF (17.0±3.1) were significantly lower compared with the control, PP (51.5±5.7). Seed germination rate (97.5±0.6%) in ZF2L and PICS were significantly higher than PP (78.3±0.1%) and ZF (66.0±2.3%). The initial MC of the maize in treatment bags was 11.4±0.1%, but the final MC in ZF2L (12.2±0.1%) was significantly lower than in PP (13.5±0.1%). Aflatoxin levels in maize in all treatment bags were within the recommended limits (4 µg/kg) of Standards Organisation of Nigeria, except the PP (5.0 µg/kg).

Hermetic storage bags and diatomaceous earth were effective at controlling insect infestations and preserved stored maize quality. Therefore, they could be used in post-harvest storage interventions.

Keywords: Stored maize, Hermetic bags, Diatomaceous earth, Insect infestation, Post-harvest loss

Word count: 485

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LIST OF ABBREVIATIONS

| | |
|--------|---|
| AF | Aflatoxin |
| ASABE | American Society of Agricultural and Biosystems Engineers |
| ASAE | American Society of Agricultural Engineers |
| CGC | Canadian Grains Commission |
| Codex | Codex Alimentarius Commission |
| DE | Diatomaceous Earth |
| EC | European Commission |
| FAO | Food and Agricultural Organization |
| GAC | Grain Analysis Computer |
| HDPE | High Density Polyethylene |
| IDK | Insect Damaged Kernel |
| IITA | International Institute of Tropical Agriculture |
| JD | John Deere |
| MLs | Maximum Limits |
| MRLs | Maximum Residue Levels |
| NAFDAC | National Agency for Food, Drug Administration and Control |
| NSPRI | Nigerian Stored Products Research Institute |
| PACA | Partnership for Aflatoxin Control in Africa |
| PE | Polyethylene |
| PHL | Post-Harvest Loss |
| PICS | Purdue Improved Crop Storage |
| PP1L | Polypropylene single liner |
| PP | Polypropylene |
| ppb | part per billion |
| Pt | <i>Prostephanus truncatus</i> |
| RFB | Red Flour Beetle |
| SH | Storehouse |
| SSA | sub-Saharan Africa |

| | |
|-------|--|
| Sz | <i>Sitophilus zeamais</i> |
| USFDA | United States Food and Drug Administration |
| MWL | Maize Weight Loss |
| ZF | ZeroFly |
| ZF1L | ZeroFly single liner |
| ZF2L | ZeroFly double liners |
| ZFH | ZeroFly Hermetic |

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Maize (*Zea mays* Linnaeus) is amongst major grain crops most widely produced and consumed globally with estimated cultivated area of 197 million hectares (Food and Agricultural Organization Statistics, FAOSTAT, 2021). It is also commonly called corn and as a major staple, its versatility for agricultural and industrial application in sub-Saharan African (SSA), Asia and America is enormous. The importance of maize in the global food agri-system encompasses direct consumption and indirect feed pathways for human and livestock (Erenstein *et al.*, 2022). The upward rise in world production of maize may be attributed to rapid income growth and urbanization, thus fueling demand as regional yield increases and agricultural land expands (Erenstein *et al.*, 2022). Nigeria is arguably the second highest maize producer in Africa after South Africa, and ranks 14th largest producer in the world with an estimated 11 million tons harvested from over 6.8 million hectares of land in 2019 (PricewaterhouseCoopers, PwC, 2021). Smallholder farmers in SSA and especially in Nigeria, deals with scores of storage threats subsequent to their grain leaving the field (Abdoulaye *et al.*, 2016). The desire of most smallholder farmers to keep their harvest in storage to cover for food requirements and future cash needs is often faced with insect pest risks. The risk can be problematic when there are few cost-effective control techniques to protect harvested grains and sometimes forces farmers to immediately sell large portion of their produce when prices are low at bounty periods (Guenha *et al.*, 2014) for fear grain losses to storage pests.

In tropical and sub-tropical regions, huge quantity of maize is harvested and preserved. Ineffective storage techniques which expose maize to unnecessary contamination by insects, microorganisms, chemicals, excessive moisture, fluctuating temperature extremes contribute greatly to food losses (Zorya *et al.*, 2011). Significant postharvest loss up to 50% has been attributed to the lack of adequate knowledge and implementation of sound grain storage management in Nigeria (United States Department of State, DOS, 2013). These losses directly contribute to the food insecurity of millions of smallholder farmers and vulnerable families (Costa, 2014).

The severity of postharvest losses varies and does not depend solely on the farmers' management techniques, but also the prevailing surrounding conditions and the abundance of insect pests which attacks subsequent to harvest (Ransom, 2001). The maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) and larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) are the most damaging insect pests of stored maize in Nigeria and many countries in SSA (Nwaubani *et al.*, 2020). *Sitophilus zeamais* is universally abundant and standing crop may first be infested from the farm and carry on its damage in storage while the larger grain borer is an exotic and store pest of maize. *P. truncatus* and *S. zeamais* are capable of damaging sound grains having moisture content as low as 10.5 % (Meikle *et al.*, 1998), thereby resulting in substantial weight losses. Other important storage insect pests of maize include rice weevil *Sitophilus oryzae* (Linnaeus) (Coleoptera: Curculionidae), red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), lesser grain borer *Rhyzopertha dominica* (Fabricus) (Coleoptera: Bostrichidae), rusty grain beetle *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloidae) (Kumar and Kalita, 2017). Globally, these insect pest damages have been reported to result in major economic losses to farmers (Obeng-Ofori, 2008). It has been estimated that 1 – 5 % and 20 – 50 % of stored grains in developed and developing nations, respectively are wasted and lost because of these insect feeding damage during storage (Ileleji *et al.*, 2007). Abdoulaye *et al.*, (2016) concluded insects as the major factor causing postharvest loss of maize in Nigeria followed by rodents and moulds. Damaged seeds offer prospective openings for proliferation of diseases and fungal infection in grains (Canadian Grain Commission CGC, 2019).

The issue of food contamination is threatening in SSA (Costa, 2014). In cereal grains, poor physical quality, mycotoxins and chemical contaminations are increasingly problems to food safety (Bankole and Adebajo, 2003). Maize typically has natural co-habiting fungi that contaminate the cereal with their various mycotoxins (Carvajal-Moreno, 2022), thereby posing a dangerous health threat to man and animals which feeds on them. Two fungal infections (i.e. aflatoxins and fumonisins) are commonly found associated in mycotoxins contamination of standing and stored maize (Kankolongo *et al.*, 2009). In Nigeria, a projected 10 – 60 % of maize has unacceptably high levels of aflatoxin (AgResults, 2013).

Insect pests of stored grains are fast developing tolerance to commonly used synthetic chemicals and fumigants. Potential substitute control techniques to mitigate insecticide failure, residue, pest resistance, and human exposure have been stressed (Ebeling, 1971). This has prompted the resurgence and development of non-toxic formulations. Diatomaceous Earth (DE) is an inert dust formed from fossilized diatoms which are made up of thin silica film. The action of DE is physical and not chemical. The DE works by abrading the epicuticular wax layer of insect bodies and cause water loss, thus resulting in death by dehydration (Lorini, 2003). Diatomaceous earth has been recognised as a valuable alternative grain protectant to Nigerian farmers because of its long term consistency, low mammalian toxicity, ease of application, local availability and the fact that a single application can be effectively use to protect and disrupt stored insect proliferations as long as the grains are kept dry (Korunic and Fields 2018).

In Nigeria, polypropylene (PP) weaved storage bags remains the established means of maize preservation, this shows that bag technologies are culturally acceptable to many smallholders in SSA (Abdoulaye *et al.*, 2016). The introduction and adoption of new value-added storage solutions are vital to the success of postharvest storage options in SSA. Numerous technologies have been employed to reduce postharvest storage losses and create desired economic, social and environmental impacts (Rockefeller Foundation, 2015). Examples include hermetic bags, insecticide bags and metal or plastic silos which allow smallholder farmers to reduce losses by preventing crop exposure to moisture, heat and pest infestations (Rockefeller Foundation, 2015). Thus, allowing commodities to store for longer period. For example, commercial airtight storage solutions such as three-layered technology–Purdue Improved Crop Storage (PICS) bag and the double layered hermetic bags like GrainPro super grain bags and AgroZ bags have been encouraged and demonstrated as a better substitute to protect commodities from insects and molds in developing countries (Baoua *et al.*, 2013). A novel, non-hermetic pesticide-treated bag designed for grain storage is the insecticide (deltamethrin) incorporated ZeroFly bags capable of preventing insect infestations (Baban and Bingham, 2014). The bag has deltamethrin, a pyrethroid insecticide infused into its individual polypropylene yarn, thus providing a strong killing action against stored product insects before their infestation (Baban and Bingham, 2014). Therefore, the adoption of new storage options by smallholder farmers would result in reduced food

losses, and impact on stable food prices (Bokusheva *et al.*, 2012). These would translate to increased food availability for the increasing population.

Traditionally, loss reduction was seen as a stand-alone intervention for improving food security (Rockefeller Foundation, 2015). However, postharvest loss reduction solutions will not achieve its intended benefits when implemented in isolation. Combined and systemic approach is required to achieve significant reduction in crop losses and promote food safety and security (Rockefeller Foundation, 2015).

1.2 Statement of the problem

The demand of maize for both human and animal consumption has been predicted to rise owing to the projected increase in population over the next 30 years. However, with the intense maize production efforts to cover for such demand, postharvest losses have continued to undermine food security and threaten income of smallholder farmers in many African countries including Nigeria. This has been directly related to inappropriate storage techniques resulting into massive grain deterioration associated with insect pest infestations. The impact of climate change on existing synthetic insecticide practices, and traditional polypropylene storage bags have shortcomings. These methods are often times ineffective, expensive, inappropriate and posed health risks. Consequently, the paucity of data on novel storage interventions in Nigeria especially, diatomaceous earth and various improved storage bags, compared to other countries in sub-Saharan Africa motivated this research. Only one study was recently conducted in Nigeria for maize storage under storehouse or on-farm condition (Nwaubani *et al.*, 2020).

1.3 Justification for the study

Improved storage bags including deltamethrin impregnated bag, double and triple airtight bags and admixture of grain protectant (diatomaceous earth dust) are among novel reduced-risk technologies currently employed for the reduction of storage losses. These novel methods of storage have been demonstrated effective in laboratory and on-farm trial studies in few African countries to protect grains such as maize and paddy from stored-product insect pests. Bag storage for future sale is one of the most popular means of grain preservation by smallholder farmers in Nigeria. Therefore, the forms of bag or hermetic bag technologies tested in this study are comparable to the typical polypropylene bag utilized by farmers and grain merchants or aggregators in Nigeria. However, it is essential to obtain adequate quantitative on-farm storage data on the grain

quality assurance for each of these novel reduced-risk method of storage in Nigeria to encourage their adoption. The data obtained on each of the tested technology could help inform farmers, grain aggregators and other stakeholders of their effectiveness and suitability as a potential storage technique option. This could contribute to the reduction of postharvest losses in maize production, promote safe grain storage and thus, leverage food security in Nigeria.

1.4 Aim and objectives of the study

The main aim of this research is to evaluate the performance and efficient use of improved storage bags and diatomaceous earth dust for maize preservation against stored-insect pest infestations. This major objective was accomplished through the following specific objectives to:

1. Assess the pest status of *P. truncatus* and *S. zeamais* on stored maize for six weeks using insect infestation levels.
2. Assess insect infestation of stored maize in hermetic and non-hermetic bags over 3- month and 12-month storage periods.
3. Evaluate the quality of stored maize grains using insect damaged kernels, weight loss, grain viability, moisture content, aflatoxin, insecticide residue and changes in microclimatic condition as indices after storage duration.
4. Evaluate the relative efficiency of different moisture meters as it affect moisture quality of stored maize over 12-month duration in hermetic and non-hermetic bags.
5. Examine damage by insects on hermetic and non-hermetic bags over storage period.

CHAPTER TWO

LITERATURE REVIEW

2.1 Maize production and demand in sub-Saharan Africa

Maize is a strategic crop planted on over 40 million hectares of land in over half of the countries in sub-Saharan African (SSA) countries to ensure food security and economic stability (Cairns *et al.*, 2021). Maize originated from Mesoamerica and its cultivation has extend diverse agro-ecologies and socio-economic conditions (Shiferaw *et al.*, 2011). Its production is dependent largely on availability of water, however most of Africa countries' agriculture are rain-fed. The bulk of it is produced by smallholder farmers and maize yield in SSA has shown a positive and accelerated trend in the last decade (Abate *et al.*, 2017a). With an annual 36 million hectares harvested, maize is widely grown occupying the largest land mass among all staples in SSA with annual maize production projected at approximately 72 million metric tons (MT) (Abate *et al.*, 2017b). Maize is ranked number one in southern Africa where the quantity of production relative to other harvests is in abundance. On the other hand, maize production in eastern Africa is either classified as first or second and whereas, in West Africa, it may rank first, second or third (Abate *et al.*, 2015). Generally, maize is classified as the first and most important crop across SSA (Abate *et al.*, 2015).

The current maize productivity in SSA is evaluated at 1.8 metric tons per hectare (MT/ha) (Abate *et al.*, 2017b) and a 2.2 % production increase per year is projected to meet the rising future generation needs (Erenstein *et al.*, 2021). In recent years, some countries have recorded notable productivity gains, although more yields are projected with improved genetic cultivars and crop management (Abate *et al.*, 2017b). The popularity of maize as a food source is essentially a direct result of its conversion into assorted forms providing nutrient for both man and animals (Nuss and Tanumihardjo, 2010). In developed countries, maize is chiefly utilized as animal-feed crop with diverse function as industrial and energy crops (Erenstein *et al.*, 2021). Contrastingly in SSA or undeveloped economies, rising incomes together with urbanization have prompted the consumption of animal-based protein, and this has accelerated a sharp demand of maize

as diary feeds, Nigeria being a typical example (USDA, 2022). The demand for maize in SSA and other part of the world is predicted to continue to soar and by the year 2030, maize production will surpass other cereal grains as the most cultivated crop by area globally (Erenstein *et al.*, 2021).

2.2 Maize: origin, production and consumption in Nigeria

Maize is undoubtedly a new World domesticate and the Portuguese are the logical carriers of maize to the coast of West Africa, where there is evidence of its early establishment (Blench *et al.*, 1994). Maize was rapidly adopted into pre-existing cultigens repertoires in Nigeria and its origin forgotten as it spread from one farming community to another (Blench *et al.*, 1994). Maize is cultivated in all of the agro-ecological regions of Nigeria aside from the Sahel Savannah, with a large expanse of cultivation in the Northern Guinea Savannah (Manyong *et al.*, 1996). The land area cultivated to maize is an excess of six million hectares. Its production started off as subsistence farming and over the years rose to the status of both commercial and economic crop (Iken and Amusa, 2004).

Nigeria is arguably the second highest maize producer in Africa after South Africa, and ranks 14th largest producer in the world with an estimated 11 million tons harvested from over 6.8 million hectare of land in 2019 (PwC, 2021). Maize production has continually outperform all other cereals in the country since 2010 and is still growing. Area and yield increased at an annual rate of 4.1% and 2.7% respectively, between 2000 and 2013 (Abate *et al.*, 2015). During the farming year 2016/17, an outbreak of fall armyworm *Spodoptera frugiperda* infestation caused havoc to maize production across the many countries, thus making farmers to increasingly perceive maize farming as a high-risk occupation (Prasanna *et al.*, 2018). Annual maize production in Nigeria has however, surge from 10.1 million tons in 2014 to 11.6 million tons in 2021, a volume that has been predicated to increase by 8% (12.5 million tons) in 2022 (USDA, 2022).

Maize constitute the primary food for most Nigerians in many forms such as baby foods, refreshments and main meals (Ekpa *et al.*, 2018) and an important ingredient used in the manufacture of poultry and aquaculture feeds (USDA, 2022). It accounts for over 30% caloric intake per day (Goredema-Matongera *et al.*, 2021) averaging per capital consumption of over 100 grams per day in SSA (Cairns *et al.*, 2021). Given the high favouritism of maize-based diets among low-resource rural and urban consumers, the

genetic fortification of commercial maize varieties with other micronutrients have further increased its demand (Cairns *et al.*, 2021). Consumption rate for year 2022/23 is projected at 12.5 million tons, an estimated 7.8 % increase relative to 11.6 million tons estimate in 2021/22 (USDA, 2022). Of all maize produced, only about 10 – 15 % could be tagged to household consumption whereas, the poultry sector is the major user of about 60% maize production for dairy feed, causing many poultry farms to increasingly struggle with rising feed costs. The energy content derived from maize is supplied through its nutritional constituent of about 72% starch, 10% protein and 4% lipid (Nuss and Tanumihardjo, 2010).

2.3 Maize harvesting and postharvest handling

The reaping of the standing maize after maturity is the beginning of quality control for the harvested maize. Harvesting is the single conscious activity to isolate the cob from its developed medium. The optimal time of reaping maize is the point at which the stems have become shriveled and dampness of grain is around 17 – 20% (Abate *et al.*, 2015). Maize cobs are collected and moved into storage, and without contact with the soil to prevent contamination.

Subsequent to harvest, the utmost enemy of grain is dampness. Insects and mold are attracted to damp grains in storage. Therefore, grains must be dried as soon as possible prior to harvest. Drying is the efficient method of moisture reduction of yields to safe storage levels, ranging maximally between 13-13.5% moisture levels. This drying activity is an important postharvest processes since all other down-stream tasks rely on it. Drying allows the removal of water from grain to a suitable level, which can tolerate reduced metabolic activity. The grain's enzyme actions and tissue respiration are diminished to an exceptionally low level, thereby preventing germination. The dry air quickly remove moisture from the grain in the course of drying, particularly when there is breeze and low humid atmosphere. Harvested grains may be dehydrated in a granary prior to hulling or placed on polythene sheets following been hulled. Grains that have contact with soil will absorb water, pick up dirt and get infested with insects. The use of plastic spreads for sun-drying is turning into a typical practice by farmers who are attempting to protect their maize from contact with the soil. Shelling is typically accomplished either by whipping the corn cobs contained in a bag or curbed ground area with a cane. The consequence of whipping the maize is physical damage making the

germ easily attacked by insects and fungi. However, the utilization of maize sheller machine is more ideal and been adopted by many farmers.

The vital goal of storage in any container is to preserve the quality of stored commodity in order to prevent loss in amount and value. Grains meant for storage must be kept dried and clean during drying. Grains can be kept in storage for as long as two years with no critical loss in value. Nonetheless, most farmers trade off their harvested grains at a lower price immediately at harvest because of foreseen losses in storage. Improved storage containers which can extend the grain storability in anticipation of increased market values for grains are commercially available.

2.4 Overview of postharvest losses

Postharvest loss of food is characterized by measurable qualitative and quantitative damages of crop commodities through the supply network, beginning from harvest through to the end user. During this period, a variety of activities are usually achieved along the chain before it gets into storage. Nearly thirty-three percent of food production (almost 1.3 billion ton), valued at US \$1 trillion are lost worldwide in the course of postharvest processes yearly (Gustavsson *et al.*, 2011). The issue of food loss has progressively become problematic and especially intense in emerging economies where it cuts revenue by at least 15% for 470 million smallholder farmers and downstream value chain actors (Rockefeller Foundation, 2015).

The losses can comprehensively be considered as weight reduction because of waste, quality, nutritional, germinability and market losses (Boxall, 2001). Various degree of postharvest wastage and damage in supply value network existed for different crops, territories and economies. Nonetheless, large quantity of commodities are usually lost in following postharvest processes as a result of unskilled and deficient technical ability of grain managers as well as improper storage facilities. Postharvest grain loss comprised of direct physical and quality losses which results in the reduction of the monetary and un-utilization of crop. In worst scenario, these losses may range close to 80% of the entire harvest (Fox, 2013). In SSA, grain losses have been reported to vary between 20% and 40%. These losses are significantly great in view of the low agricultural productivity in the continent (Abass *et al.*, 2014). As indicated by a World Bank report, grain loss in only SSA is estimated to value approximately USD 4 billion annually (Zorya *et al.*, 2011). These losses impact lives of large groups of farmers

affecting accessible food capacities and exchange values of the commodities. Despite the monetary and social consequences, losses similarly influence the atmosphere, and in addition, land-use, aquatic supply and vigor employed in the creation of the lost food, all of which are also lost alongside the food. Furthermore, unconsumed food leads to additional carbondioxide productions and in time influencing nature. An FAO report utilizes the life cycle perception estimated a near 3.3 gigatonnes of carbondioxide corresponding to discharges which resulted from cultivated and un-utilized food (FAO, 2013).

2.4.1 Postharvest losses of maize

Maize is more subject to postharvest loss than any other cereals (Zorya *et al.*, 2011). The postharvest loss of maize have been defined by leaky food-pipeline (Figure 2.1). As indicated, losses happen in all phases (farm to market). Conversely, huge losses happen at the farm/harvest and storage phases. As indicated by APHLIS, about 60 - 74% portion of reaped maize finally got to the end user (Abass *et al.*, 2014). A USAID study have reported postharvest storage of maize as one of the limitations affecting the maize sector in West Africa (Boone, 2008).

Annually, huge quantities of reaped and kept maize are destroyed due to insect infestations since control of these noxious organisms remain a major obstacle for several smallholder farmers, mostly in inadequately managed stores. The damaging consequences are provoked as a result of inadequate know-how, inappropriate and ineffective improved grain tools (Baributsa *et al.*, 2014). Thus, causing reduction in food and returns to most farmers occur when stored maize quantity, quality and value are lost to insect infestations and contaminations.

Prostephanus truncatus and *S. zeamais* are major insect pests of preserved maize (Quellhorst *et al.*, 2020). However, few quantitative data have been reported by researchers on the extent of damage due to insect infestations in parts of Africa. Losses resulting from infestation of kept maize have been averaged in the range of 20 and 30 % after a 3 month period of storage (Boxall, 2002). In the case of *P. truncatus* infestation, a 30 % loss was estimated in Togo after a 6 month capacity storage (Pantenius, 1988) while in Tanzania, losses worth between 17.9 and 41.9% were recorded after a 6 – 8 month period (Keil, 1988).

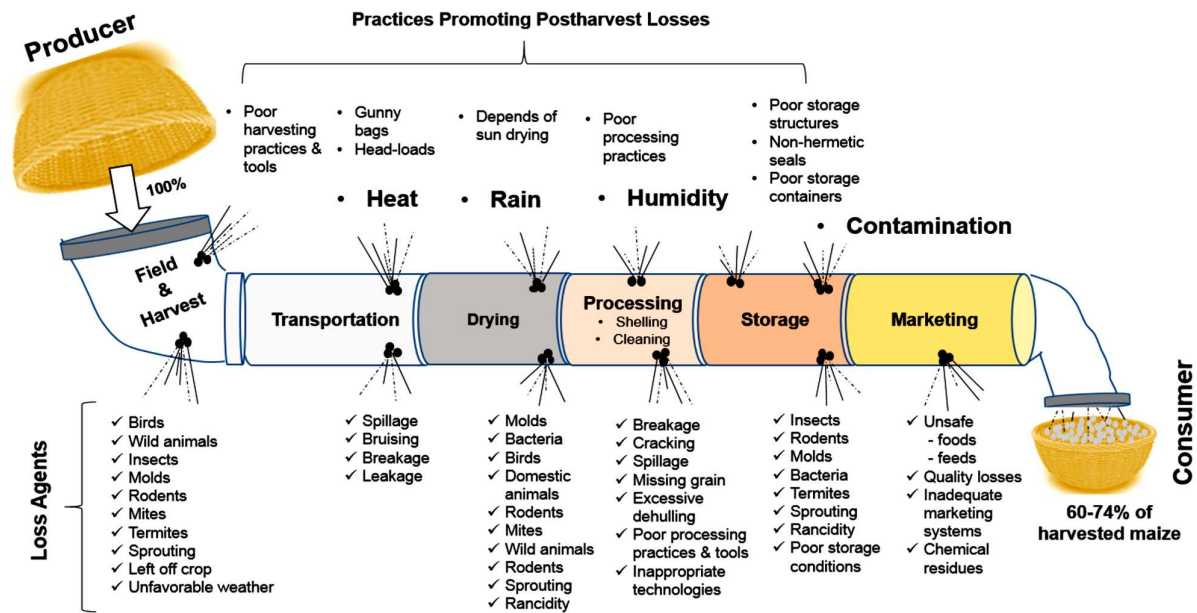


Figure 2.1. Typical maize postharvest chain (Suleiman and Rosentrater, 2015).

For *S. zeamais*, a 17.5% loss was recorded after a 6 month storage period in Tanzania (Mulungu *et al.*, 2007) and losses between 41.2 and 48.5 % after 8 months in an on-farm study trial using conventional storage bags in Kenya (Ng'an'ga *et al.*, 2016) have been reported. In Benin, losses up to 21.5% was reported due to *P. truncatus* and *S. zeamais* infestations in woven polypropylene bag after a 6.5 month capacity storage (Baoua *et al.*, 2014). More recently, Abass *et al.*, (2018) reported losses of 11.6% in Tanzania after 7 months storage period in traditional polypropylene bag.

2.4.2 Category of postharvest losses

About a billion people around the world are affected by malnutrition and nearly one-third of food production for man's utilization is either lost or unused. Postharvest losses is categorized into any of the three sub-group below:

Quantitative loss is the decrement in physical mass of the grain, this could possibly be measured and evaluated. For instance, substantial grain commodities are typically damaged as a result of insect pest activities or lost during transport. This category of loss is typical in developing countries (Kitinoja and Gorny, 1999).

Qualitative loss refer to the reduction in the quality of a grain type and relates to infection or infestation of grains by fungi or insect pest. This comprises of loss in nutritional content, edibility, end-user acceptability as well as the energy value of the commodity (Zorya *et al.*, 2011) and usually typical in developed countries. Most qualitative losses are often based on subjective judgments.

Economic loss refers to the decrement in cash worth of a grain commodity as a result of loss in quality as well as quantity of food (Tefera, 2012).

2.5 Factors affecting postharvest losses of maize

The extent of maize postharvest losses across the world differs from one continents to another. These losses are especially challenging to the smallholders in subsistence farming, where losses negatively affect family livelihood (Ransom, 2001). The harshness of these losses varies significantly and not reliant solitary on the farmer's handling methods, but the environment and the prevalence of postharvest pests. Factors that affect post-harvest losses of maize include physical, mechanical, socio-economic and biological factors.

2.5.1 Physical factors

Temperature and the amount of water content are the two most significant physical factors associated with postharvest losses of stored maize. As a rule, as temperature increases to the maximum level, the higher the rate of organic processes. At increasing temperatures, the rate at which seeds respire and insects reproduction becomes very high, coupled with the introduction of some harmful fungi which could be widespread. Temperature has a great deal of affecting the rate of respiration of any stored commodity, noxious creatures, relative dampness and amount of water contained in food. The climatic conditions in the temperate and tropical regions may offer favourable conditions for insect development and in regions with high humidity, condition are suitable for mould development. The proliferation of insect is incredibly diminished when the temperature of kept grain is less than or equal to 19 degrees celcius. The impacts of temperature in stored grains comprise increment or decline in drying state of commodities, reduced seed germinability due to increasing temperatures and speeding up rate of physiological process by living creatures (Ransom, 2001).

Water is contained in all food in varying extents. This is a major concern with harvested grain, when its water activity level surpasses 12% of the grain mass. Wet grains are good substrate source for storage insect pests and fungi, thus causing damage and contamination of grain with mycotoxin such as aflatoxins. After harvest, the amount of water contained in any stored food begins to alter. Foods containing high amount of water tends to go bad fast. Also, the amount of air enveloping the grain bulk and the amount of water present in the grain itself can define the kind of damaging biological and physiological processes which could occur (Murdock and Baoua, 2014).

In addition, interaction exist between temperature and humid air. Increasing humid air together with increasingly high heat can rapidly decrease kernel germination rates and permit multiplication of insects and fungal diseases. For any maize meant for storage, the water content in the maize must be dried properly to the safest level of 13% for prolonged storage at any given length of time (Baoua *et al.*, 2014). Maize exceeding 15% amount of water content becomes difficult to store as a result of fungal tolerance, irrespective of temperature. A direct correlation existed between the surrounding air and the amount of water contained in stored grain. The amount of water contained in the grains of a growing maize crop can vary significantly in the event of a day as a result of unstable relative humidity. Maize at 13% water content is steady with relative humid air

at 70%. The amount of water contained in any grain can be assessed using hand-held meters or predicted with traditional methods such as biting and admixture with salt.

2.5.2 Mechanical factors

Mechanical factors also have the potential to impact negatively on postharvest losses. Losses can occur during grain transportation as a result of spillage. Also, some postharvest procedures in which farmers engage during shelling operations can lead to substantial breakage. Broken grains are generally more subjected to infestations by insects, mites and microorganisms. Drying facilities such as solar dryers are currently used to reduce the drying time required to attain a safe moisture level for any grain type (Omobowale *et al.*, 2021).

2.5.3 Socio-economic factors

The impact of socio-economic factors on losses may include; the affordability of storage type, volume of grain to be consumed or kept in storage, and also whether the grains will be sold off or utilized by the farmers' family. The farmers' socio-economic condition (for example, the urgency of the need for cash) also plays a critical role in the length of storage of the commodity. The period of storage of the maize generally dictates the price of market at the point of sale, but also the higher the risk of grain losses. In the case of many maize aggregators in Nigeria, most maize harvested from the farm are only kept between 3 to 6 months before sale. At this time, the losses at the time of usage may be minimal. Most of these farmers put their yields up for sale immediately at harvest time, especially when prices are low because of the immediate need for cash. Using this system, grain losses are typically of low importance to the farmer. In the case of maize meant for future planting, the length of storage will be more than 6 months and this emphasize the need for storage to be at optimal conditions in order to maintain seed germinability and value.

2.5.4 Biological factors

Biological agents such as insects, birds, rodents, other wildlife, microorganisms (fungi, and bacteria) and man can have a direct impact on postharvest losses of grains. The comparative significance of these several biotic agents varies from farm to farm. The environmental factors earlier mentioned can greatly affect the predominance and attack initiated by insects and microorganisms. Aside feeding on the grains, they infect them with their excreta, remains etc., and this may lead to the introduction of diseases and

parasites to the grains. Microbes can cause food loss and spoilage. The food values and palatability are altered by their toxins which affect animal and man. In addition, deterioration of storage container may occur as a result of moulds and insect damage. In low water content foods, the reproductive structure and mycelia of the microorganisms are inactive until suitable conditions for their growth is attained. Increasing humid condition of the atmosphere or presence of high water content in a commodity offers the satisfactory conditions needed. Warmer temperature in addition with increasing amount of water contained in the grain influence the rate of postharvest losses of stored grains. Seed hardness contributes to reduction in the damage rate of grains. Also, anti-feeding substances may be present within the kernel which reduces the rate of insect development and damage to the grain.

2.6 Agents of biological deterioration of stored maize

Harvested crops are naturally subjected to biological spoilage, but the extent of this spoilage varies and depend greatly on factors which emanates from individual farming methods and consistency through the supply-value chain activities between harvest and delivery of food to end-users (Costa, 2014). Ineffective management practices which allow crops to be unnecessarily exposed to contamination by microorganisms, extreme moisture, unstable temperature extremes, and insect pests contribute significantly to food losses. Addition to the losses as a result of biotic deterioration are the severe health risks emanating from damaged kernels during pre- and post-harvest stages.

2.6.1 Storage fungi

Storage fungi majorly attack maize kept in storage and requires the maize water content to be in stability with humid atmosphere of 70-90%. Fandohan *et al.*, (2003) in a study reported storage fungi (most common – *Aspergillus* spp) to rank next to insect pests as these microorganisms also contributes to significant losses of maize in tropical countries. After storage molds attack maize kernel, they cause decay, discolouration, fungal poisoning, and ensuing sprout diseases (Williams and McDonald, 1983). In addition, cracked maize, chaffs and dockages encourage the growth of storage molds, since fungi are able to breach cracked kernels than sound and whole kernels (Sone, 2001). Similarly, Dharmaputra *et al.*, (1994) stated that damage of maize grains resulting from using mechanical equipment at harvest and during subsequent postharvest operations can create access points to fungal reproductive structure. The proportion of

grain damage and cracks will determine the extent by which fungi is able to propagate and breach the maize grain (Fandohan *et al.*, 2003).

2.6.2 Storage insect pest

Losses of grains as a result of insect infestation over storage period are particularly problematic in the developing countries. The consumption of kernels as a result of insect attack is the primary loss associated with insect pest, but also the contamination of grains with insect fragments, webbings, and their remains are seen. Grains with high degree of the insect debris may lead to grain that is unpalatable for utilization and loss of produce, in respect of quality and quantity. Insect infestation may generate induced changes in the storage environment causing warm-moist hotspots and offer favourable conditions for storage fungi to cause further losses.

A USAID report (Boone *et al.*, 2008) emphasized the limitations of the maize sector in West Africa to be postharvest storage. A number storage insect pests of maize have been implicated to include *S. zeamais*, *T. castaneum* and *P. truncatus* (Markham *et al.*, 1994).

2.7 Postharvest insect pests of maize

2.7.1. Biology of *Sitophilus zeamais* (Motschulsky 1878)

The adult maize weevil, *S. zeamais* (Coleoptera: Curculionidae) measures 2.4 – 4.5 mm long having an elongated head characteristics protruding into a rostrum-like extension and which carries the mouthparts in a position ideal for penetrating commodities. The adults usually have distinct coloured (yellow blotches) spots on their forewings and the antennae are elbowed in shape while at rest (Plate 2.1), as captured with the aid of a portable LCD digital microscope (G1200, China). The larvae are eruciform and legless and found in holes bored in the kernel. A female weevil lays egg singly in holes made in the grain with its mouthparts and plugs it with a waxy material. The egg is usually whitish and oval-shaped. A single female *S. zeamais* is capable of laying 300 to 400 eggs in grains in the field or during storage which then hatch into small larvae and eat within the kernel (Hill, 1983). Immature developments and pupation occur within a grain and damage is thus not visible visually (Cotton, 1956). The sexuality of developing adult weevil is in the proportion of 1:1, the females have been found to outlive their male counterparts (Tefera *et al.*, 2010). After pupae development, the weevil bores through the outer layer of the grain and leave an irregular rounded hole. The developmental time from ova to adult stage range between one - two months at 30°C on maize having 13.0%



Plate 2.1. Dorsal (up) and lateral (down) views of *Sitophilus zeamais*

water content and this period may vary depending on kinds and condition of substrate being attacked (Tefera *et al.*, 2010). Both the larvae and adult devour stored commodities and are usually long-lived. The weevils utilize their extended rostrum, primarily adapted for penetrating into hard materials, whereas the females use their jaw-like mouthpart for tunneling a narrow cavity into where their eggs are deposited into. As a result of the high reproductive ability of female weevils, population of developing weevils tends to escalate if they are not adequately managed (Tefera *et al.*, 2010). Although, at sub-optimum conditions below 20°C or exceed 32°C and in commodities which have less than 11 % water content. Adult weevil copulation usually occur after three days of insect emergence (Walgenbach *et al.*, 1987). A number of factors may be responsible for the number of generations and longevity of adult weevils. The type of grain and their varietal differences are amongst factors which may impact developmental time and generative rate of *Sitophilus* species (Gomez *et al.*, 1983). Developmental time is particularly longer at low temperature such as 98 days at 18°C and 70% humid air (Darling, 1951).

2.7.1.1 Economic importance of *S. zeamais*

This insect is an economic pest of stored maize, and with little or no adequate control of moisture content coupled with ineffective chemical protectants, losses can be up to 100 %. Reduction in grains by 12 – 20% are widespread, and a near 80% loss was reported in unprotected maize stored using conventional structures in the tropics (Boxall, 2002). Damage caused as a result of this infestation usually manifest as reduction in mass and germinability potential of seeds meant for planting, a practice common amongst smallholder farmers (Boxall, 2002). In addition, weevil-infested maize have been found associated to be contaminated with *Aspergillus flavus*, an aflatoxin agent. The consumption of such grain is regarded harmful and may impact on human health conditions (Tefera *et al.*, 2010).

2.7.2 Biology of *Prostephanus truncatus* (Horn 1878)

The larger grain borer, *Prostephanus truncatus* (Coleoptera; Bostrichidae) is cylindrical, dark brown (Plate 2.2) with adult body length ranging between 3 – 4.5 mm (Birkinshaw and Hodges, 2000) and clubbated antennae with 10 segments each. Adult *P. truncatus* has the capability of flying long distances ranging from 2 to 25 kilometers (Pike *et al.*, 1992).



Plate 2.2. Dorsal (left) and lateral (right) views of *Prostephanus truncatus*

The thorax possess series of teeth on its upper front edge and the head is bowed beneath the thorax such that it is concealed from above. The pronotum which curves downward at the anterior end, robust mandibular structures, and their elongate-shaped body are characteristic of wood boring insects (Tefera *et al.*, 2010). The hood-like projecting prothorax shields the hypognathous head during burrowing and offers strong support for the muscular region of the mouthpart (Li, 1988). *Prostephanus truncatus* has significant potential to burrow into hard surfaces, for example, a plastic with 35 mm thickness have been reported breached by adults *P. truncatus* (Li, 1988). This mandibular prowess can though, merely be functional if the beetle have the ability of getting adequate spaces between grains on a cob which provides the insect to be fixed firmly while it chews it way into the kernel. In *P. truncatus*, the ends of the wing cover are flattened and has a sloping region with two curved ridges at the tips to give it a characteristic square cut-end. This feature distinguishes *P. truncatus* from other bostrichids known to attack stored products in particular *Rhyzopertha dominica* and *Dinoderus* spp. *P. truncatus* infestation may commence with the mature standing maize crop on the farm, whilst maize is drying or once these have been dried and placed in store. Adults penetrate into the kernel or cob by leaving a characteristic circular hole. As they burrow, the adults generate large quantities of frass. Breeding takes place in the tunnels and eggs are deposited in batches, and protected by frass formed by the adults when cultured on uncobbed grain. Female deposits an averaged 5 – 8 eggs in each oviposition cavity at right angles to the main tunnel (Bell and Watters, 1982). The grub hatches subsequently at 3 – 7 days to finally develops into a pupa which then gives rise to the adult. As the juvenile stages grow entirely inside the food substrate, they are normally invisible.

The adult female has a lifespan of 300 fertile eggs when cultured on a yellow maize variety. Extremely dried and hard maize have been reported to reduce the rate of fertility and survival when used (Li, 1988). There are three larval instars with a mean period of 16 days (Tefera *et al.*, 2010). The immature is characterized by reduced head capsule and an enlarged thorax forming a C-shaped body. *Prostephanus truncatus* is tolerant of dry conditions such that development may take place in grain even with humid air of 40% (10% water content for maize). The optimum condition for this insect development is at a moderately high temperature (near 30°C) humid air (near 70% relative humidity equal 13% grain moisture content). Under these conditions, the developmental time from

egg to mature stage can take place as short as 25 days at 32°C, but in somewhat longer period to 167 days at 18°C under cooler conditions (Hodges, 1998).

2.7.2.1 Economic importance of *P. truncatus*

This insect is a serious and primary pest of stored maize. Standing and cobbed maize may be attacked during pre- and post-harvest period. A significantly high weight loss averaged 35 % have been reported in some East Africa cribs after storage time lasted between 3 – 6 months (Muhihu and Kibata, 1985). Adults bore in to wide-ranging food and other commodities such as woods. During severe infestation, storage structures made up of wood may become heavily infested and act as reservoir for future outbreaks.

2.7.3 Biology of *Tribolium castaneum* (Herbst, 1797)

The adult red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae) is reddish-brown with flat curved-sided body and its antennae ending in a three-segmented club (Bousquet, 1990) (Plate 2.3). The beetle measures one-eighth of an inch in length. It principally attacks processed grain and cereal products. The adults may live a year (some almost four years) (Walter, 1990). The males possess a hairy patch on the ventral surface of the anterior femur, while this patch is absent in females. Female lays 2-10 eggs each day throughout most of her adult life. Optimal conditions of temperature of 35°C and 75 % humid air, egg-laying can increase at a rate of 70-100 times a month (Herrman, 1998). The eggs are whitish, tiny and mostly have bits of powder held to their surface, while hatching can occur within 2 weeks. The period by which larvae emerged from egg can be shortened at favourable temperature (Beeman *et al.*, 2012). The delicate grubs are creamy yellow to light brown in colour and attain a length of 0.3 inch when fully grown. They possess two upwardly curved urogomphi on the ninth abdominal segment (Devi and Devi, 2015). There are generally four larval stages, the larval period can last from 22 to 100 days depending on food supply and environmental conditions. The pupae are pale in colour and are immobile except for the ability to flex the body at the junction of thorax and abdomen. Pupation occur in floury commodities and this period could extend between 6 – 9 days (Smith and Whitman, 1992).

The only reliable external sexual characteristic for any stage is found in the pupal stage. When the ventral posterior ends of the male and female pupae are observed under low magnification, the sexual distinction is obvious. On the terminal section, the female has a pair of small appendages which are reduced to indistinct elevations in the male. The



Plate 2.3 Dorsal (left) and ventral (right) views of *Tribolium castaneum*

egg to adult life cycle takes about 30 days. Adults are long-lived and could spend up to three years (Baldwin and Fasulo 2003). Development time of ova to mature insect is between 26 – 30 days during optimum period, but could be extended when conditions of temperature and food are not favourable (Dhaliwal *et al.*, 2006). *T. castaneum* have been found to be able to breed continuously in the presence of adequate substrate.

T. castaneum can reproduce throughout the year given optimum temperature conditions. All *T. castaneum* stages may be found in infested grain products at the same time. Adults possess chewing mandibular structures but do not bite or sting but may also cause sensitized reactions (Alanko *et al.*, 2000). They are ubiquitously found infesting stored processed and whole commodities at home and grocery stores. This pest is widely distributed in temperate and tropical regions of the world where conditions are favourable for their survival (Tripathi *et al.*, 2001).

2.7.3.1 Economic importance of *T. castaneum*

Infestations due to this insect cause significant loss resulting in value reduction of grain products. Their presence also appears to increase temperature and moisture conditions leading to a faster rate of proliferation of moulds comprising harmful species (Magan *et al.*, 2003). The adult and larvae are secondary pest of cereal grains (Bagheri-Zenouz, 1995) and infestation leads to persistent offensive odour of stored commodities. They attack cereal grains with high water level and can cause discolourations to infested processed commodities. Besides consumption and contamination of products (through faeces, shed skin, body parts, secretions, dead insects), these beetles can cause infested products to give off a displeasing odour and taste.

2.7.4 Biology of *Cryptolestes ferrugineus* (Stephens, 1830)

The adult rusty grain beetle, *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae) are flat, small, shiny reddish-brown beetle about 2 mm in length (Plate 2.4). The adult beetle is depressed and elongate, having eleven segmented antenna with the last three segments slightly enlarged (Rilett, 1949). The antenna is nearly as long as the elytra. An adult female lays egg on or amongst food commodities making use of substitutional ovipositor consisting of the contracted caudal abdominal segments. Their eggs are quite large relative to the size of the adult insect which deposit them and are visible physically when placed on a clean dark surface (Rilett, 1949). Hatching occurs in three to four days after oviposition. Before emergence of the immature, its segmentation is faintly noticeable



Plate 2.4 Ventral (left) and dorsal (right) views of *Cryptolestes ferrugineus*

through the chorion (Rilett, 1949). The newly formed elongate larva, which is a bit longer than the egg has pronounced tail horns passes through four instars and pupates in a gelatinous cocoon which is usually covered in food particles. Shortly after emerging, the larva goes in quest of suitable food. Although, cannibalism may occur under crowded conditions (Odeyemi, 2001). Both adult and larvae have similar feeding habits, thereby causing damage to stored grains. The adults feed extensively on the germ of a grain. The larvae also eat through the endosperm, particularly in seriously damaged kernel that has been voraciously de-germed by previous groups of the infesting insect. This way, the whole kernel may become riddled with hole, leaving only the outer seed coats as an empty shell. The insect life cycle can be completed within the range of 20 – 42.5°C. However, development may be completed in 21 days at 35°C and about 100 days at 20°C. The rate of insect development is dependent on humidity as mortality increased with low humidity. The adult flies actively and are usually considered unimportant in storage as a result of their tiny body which permit them to hide in small crevices.

2.7.4.1 Economic importance of *C. ferrugineus*

C. ferrugineus is a major secondary pest which attack and feed on germ and endosperm of stored cereals, thereby resulting in reduced seed germinability. Heavy infestation may also result in heating of grain and spoilage. The beetles are also implicated in the spread of fungal spores.

2.7.5 Biology of *Liposcelis* spp

Stored-product psocids (Psocoptera: Liposcelididae) commonly called booklice belong to the genus *Liposcelis*. The genus has over 120 species recognized globally. Amongst them, *Liposcelis entomophilia* (Enderlein), *L. decolor* (Pearman), *L. paeta* (Pearman) and *L. bostrychophilia* (Badonnel) are most commonly associated species of stored products (Turner, 1994). *Liposcelis bostrychophilia* is likely one of the commonest species of the genus *Liposcelis* (Turner, 1994; Opit *et al.*, 2011). Adults are very minute measuring almost 1 mm, yellowish white, without wing, soft bodies, louse-like with a large head, long antennae and ventrally plane insect (Plate 2.5) (Mockford, 1993; Turner, 1994). Psocids life-cycle include eggs, immature stages and adult females. Eggs are tiny, oval-shaped, shiny, and are attached to food particles (Turner, 1994). The period of development from ova to mature insect is about 3 weeks and the female lay as many as 100 eggs. The back legs are typically robust and flattened (Mockford, 1993). On a diet of whole wheat flour, the mean developmental time ranged from 6 days at 32.5°C

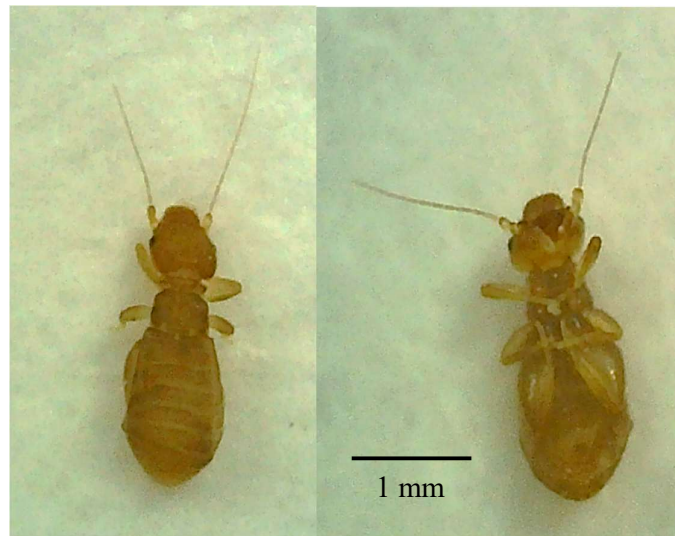


Plate 2.5 Dorsal (right) and ventral (left) views of *Liposcelis* spp

to 14 days at 20°C (Wang *et al.*, 2000). Oviposition is usually dependent on temperature (Wang *et al.*, 2000). The immatures looks like the adults but are lighter in colour and can often be identified to the species. The lifespan of the adult increases with increasing temperature (Wang *et al.*, 2000).

2.7.5.1 Economic importance of *Liposcelis* spp

Liposcelis spp which were formerly referred to as a nuisance pest are now considered a noxious insect of stored produce and a menace to food security worldwide (Ahmedani *et al.*, 2010). These pests have been reported to be resistant to some commonly used insecticides targeted at other stored-product insect pest, thus making their control relatively difficult (Athanassiou *et al.*, 2009). This insect has been found by researchers to infest various kinds of stored grains (Kalinovic *et al.*, 2006), causes weight loss due to feeding on embryo and endosperm (Kučerová, 2002), quality reduction by increasing grain moisture content and contaminations (Yang *et al.*, 2013). Furthermore, allergic reactions have been caused in sensitized individuals (Kalinovic *et al.*, 2006), spread of fungal pathogens and consequently posing threats to human well-being (Opit *et al.*, 2012). These pests have also been reported to be intercepted at entry ports in China.

2.8 Food safety

The production and accessibility to wholesome food is pre-requisite for healthy lifestyle of citizens and the progression of national economies. The quantity of inferior and unsafe yields in the developing world have detrimental influence on the wellness of both animals and humans, and are a major limiting factor to smallholders who trade off their harvests at premium markets (Manjula *et al.*, 2009). There are some factors which threatened food quality and safety, but are mostly comprised of poor physical quality, indiscriminate application of chemicals and mycotoxin contaminations (Bankole and Adebajo, 2003). The issues of contaminated foods in SSA is of serious concern, although the most problematic and of health issues are the unceasing danger of food poisoning caused by pesticide residues and toxic aflatoxin contaminations in food.

2.8.1 Pesticide residues

A pesticide may be a chemical or biological product specifically developed and to be applied for the control of pests, weeds, and diseases while at crop production or during grain storage. The incessant application of pesticides over years in different parts of the world have led to unsafe and persistent degrees of toxic chemical residues in the food

supply chain. There is an increasing dependent on foreign organophosphate insecticides by smallholders to protect harvested grains in storage against stored product insects. A major challenge encountered by the grain industries globally is the safeguarding of grain commodities during storage from noxious insect pests which have tolerance ability against generally used grain protectants (for example, aluminum phosphine) while also complying by harsh limits on chemical remains (Subramanyam and Hagstrum, 1995). The acclaimed effectiveness and safety of the various grain protectants are progressively been questioned by farmers, grain industry experts and several authorities.

The probable risks to end-users from toxification of foods with chemical deposits is presently of great public health concern globally. Residues in food can be accumulated from different sources but there are three major possible sources of residue in grains. These may emanate from the use of chemicals on standing crops, pollution of the surrounding by extremely persistent chemicals formerly used for other purposes and also, the use of chemicals applied to crops to protect infestations while the grain is in storage and during handling.

The major chemical residue found in cereals comes from crops contact with insecticidal deposits or mists which are intentionally used to protect against stored product insect infestations. The kind of chemical compounds used for this purpose are limited. The contact mode of entry of poisons recently employed are majorly either of organophosphorus compounds of low acute mammalian toxicity or pyrethroids. Small amount of unreacted aluminum phosphide which are commonly insignificant remain as a grayish powder after fumigation with phosphine is applied but residue may attain great levels in produce which have been exposed to repeated disinfestation using methyl bromide. The essential factor in applying contact poisons either as spray and fogs in storehouses which contain stacks of bag produce is majorly to limit insect population below sub-economic levels or curtail insect resurgence after fumigation to the barest. Chemical remains from such treated produce are usually of low importance and may be narrowed to the top level of grain bulk in stacks. It is possible to discover substantial pesticide remains in grain bulk mixed with insecticides as it is been uploaded in to storage. The rate by which such insecticides are applied are usually estimated by laboratory protocols to determine the least dosage concentration required to regulate the population of specific pests. The degree of disintegration of this poisonous formulations is calculated and a limit is subsequently set up for residue of a particular compound that

may be probably found when the acceptable treatment rates are applied to a particular produce. Strictest residue levels have been established by many advanced nations for cereal and legumes, including their processed products, using legislative systems which help regulate safety, trade and application of poisonous formulations.

2.8.1.1 Effects of pesticide residue

Severe impact relating to health threats as a result of consumption of pesticide residue foods have been reported to include cancer, nervous and endocrine disorder and irritation to the skin and eyes. Despite assurances of negligible risk, the health effects of exposure to multiple chemicals and their carriers are unknown. Thus the use of pesticides must be done in a wise and safely manner. However, measures have been put in place to guard end-users by enforcing the strictest residue limits allowable in food commodities established by food regulatory laws in various countries.

2.8.2 Aflatoxin contamination

Aflatoxins (AF) are poisonous, secondary metabolites produced essentially by species of fungus *Aspergillus* (*A. flavus* and *A. glaucus*) which occur in nature when they flourish under favourable conditions to produce infections (Gong *et al.*, 2015). These molds are widespread in nature and virtually all air contains mold spores in trace amounts, including air around harvest operations which has especially high concentrations of spores. About one-quarter of all food crop produces globally have been projected to be contaminated by aflatoxins yearly. The countries in the continent of Africa all of which are positioned between tropic of cancer (40 degree north of the equator) and the tropic of capricorn (40 degree south of the equator), are vulnerable to aflatoxin infection (Partnership for Aflatoxin Control in Africa, PACA, 2013). Infection of harvested agro-commodities by species of *Aspergillus* is possible within temperature range of 24 – 35°C and 7 – 10 % air moisture (Williams *et al.*, 2004). In Nigeria, an estimated 10 – 60 % of maize has unacceptably high levels of aflatoxin (AgResults, 2013). Famine, noxious organisms, untimely harvest, inadequate grain aeration and management are responsible for the aggravated occurrence of aflatoxin in tropical countries (Wild and Gong, 2010).

Aflatoxin is largely problematic and maize is vulnerable to its infection (Miller, 1995). They are produced specifically by storage molds which generate four significant aflatoxins; B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2). The grading of toxicity

is in the order of B1 > G1 > B2 > G2. The letters B and G indicates the blue and green fluorescence colours produced when observed under electromagnetic rays. Numbers 1 and 2 refer to major and minor compounds, respectively (Hussein and Brasel, 2001). *Aspergillus flavus* only produces B aflatoxins, while *A. parasiticus* and *A. nomius* produces G aflatoxins (Alcaide-Molina *et al.*, 2009). The most potent and commonly occurring of the aflatoxins is AFB1, recognized as a probable human carcinogen by the International Agency for Research on Cancer (IARC, 1987) and considered as a multiple health risk. World Health Organization (WHO) classifies aflatoxins as grade one cause of cancer, as they are extremely noxious substances (Martinez *et al.*, 2011). The reliance on basic food commodities such as maize and groundnuts which are greatly affected by aflatoxin by the less privileged consumers makes them particularly at risk of infection. Similarly, more affluent consumers who also relish assorted foods are also vulnerable.

Several researchers have reported that aflatoxin infection in cereals rises due to length of storage and atmospheric condition. Aflatoxin infection is determined by extended period of storage under poor sanitary and stuffy environments. Increase in aflatoxin concentration due to time of storage (0.84 ppb in a year to 1.17 ppb at two years) was reported (Liu *et al.*, 2006). Several studies have constantly reported increasing temperature and water activity to be majorly responsible for promoting aflatoxin contamination and fungal growth (Alborch *et al.*, 2011).

Aflatoxin infections are microscopic, thus they are not easily detected by end users and as such the avoidance of not consuming infected commodities cannot be guaranteed (PACA, 2013). However laboratory testing is required to determine its presence.

2.8.2.1 Economic impacts of aflatoxin contamination

Aflatoxin contamination is a complex problem. Aflatoxin-infected commodities are of great danger to the less privileged and smallholder farmers who consume majority of harvested crops. Infection of commodities by aflatoxin has numerous negative impacts ranging on markets, food safety and security across tropical nations (PACA, 2013). Although strictest aflatoxin measures have been established at different international markets, thus safeguarding end users against detrimental effects accumulated from their consumption. On the global level, the Codex Alimentarius Commission (Codex) is the body responsible for formulating maximum limits (MLs) for contaminants such as aflatoxins in food. In general, the FAO and WHO member countries implement and

impose maximum limits (MLs) put in place by the Codex. However, for aflatoxins in main staple foods such as maize, Codex has not been able to formulate an internationally acceptable ML (Gong *et al.*, 2015). Due to the absence of consensus on aflatoxin MLs at Codex for these foods, countries and regions have formulated national and regional MLs. European country members have established tolerable limits for AFB1 in the range of 2.0 and 8.0 µg/kg (ppb) and for the overall total of all four of these toxins between 4.0 and 15.0 ppb in crops including grains (European Commission, 2006). The United State Food and Drug Administration (2000) established a maximum levels of 20 ppb for the overall total of the four aflatoxins in grains as well as other foods. In the developing nations, MLs for total aflatoxins range between 10 – 20 ppb (Gong *et al.*, 2015). About 15 tropical nations including Nigeria have laws governing levels of aflatoxins in foods (FAO, 2004). Lower MLs, such as 4.0 ppb for total aflatoxins set in the EU, can serve as a barrier to trade and incur additional costs for producers, processors and traders (Gong *et al.*, 2015).

2.8.2.2 Health implications of aflatoxins contamination

The health risk related to the ingestion of aflatoxin infected commodities may include bleeding, edema and speedy mortality chiefly as a result severe liver impairment (PACA, 2013). Additionally, the effect may be more problematic when individuals are exposed to numerous mycotoxins infection. For example, commodities which are infected by aflatoxins are also likely to be prone to be infected by other kinds of mycotoxins, this therefore means several mycotoxins have ability to cohabit in one food crop (Bankole and Mabekoje, 2004). In previous years, Kenya have reported hundreds of mortality as a result of severe aflatoxicosis (Shephard, 2008). Prolong human exposure from small to reasonable quantity of these toxins as a result of the ingestion of fungal infested foods and also job-related experiences may result in liver disease (IARC, 2002). Reports have similarly revealed evidence of connection between aflatoxins and stunted growth in youngsters (Turner *et al.*, 2003). Also, a pilot report has projected a relationship between protracted aflatoxin exposure along with chronic aflatoxin exposure and resistant destruction and thus, predisposed to communicable ailments including malaria and human immunodeficiency virus and acquired immune deficiency syndrome (Keenan *et al.*, 2011). Studies involving animal experiment revealed that protracted susceptibility to aflatoxins may prone to slow absorption of mineral elements

from food (Williams *et al.*, 2004). Unfortunately, toxins are unavoidably present in many foods processed from raw and finished oilseed products (Carvajal-Moreno, 2022).

2.9 Stored insect pest control options

Stored grains in the tropical environment have been shown to be subjected to depredation by various pests which can cause severe quantitative and qualitative losses. The pest situation in many tropical countries has sometimes be made more acute with the introduction of new pest e.g. *P. truncatus* in Africa (Hodges *et al.*, 1986; Pike *et al.*, 1992). The methods to be adopted for reducing postharvest due to pests will vary with the pest in question, commodity stored, type of storage container, quantity of grains and storage duration (Lale and Ofuya, 2001).

2.9.1 Application of commercial pesticides

The comprehensive usage of inorganic chemicals for the mitigation of storage insects in storage structures have dated back to the 1950s. Many chemicals are used to manipulate insects in and around stored grain. Insect-control chemicals for stored grains can be grouped into two namely; contact insecticides and grain fumigants.

Contact insecticides such as deltamethrin are applied straight on grain or storage structure for safeguarding them against insect damage for extended period. Insecticide treatment methods have become increasingly more common to protect against insects, particularly Actellic super – a combination of 1.6% Pirimiphos-methyl and 0.3% Permethrin (Kimenju and De Groote, 2010) which is ubiquitous. This method requires insecticide applied to dried maize, then reapplied approximately every three months depending on the dominance of maize beetles, the main destructive organisms of stored agricultural produce in SSA.

Several reports on pesticide resistance used to protect commodities is widespread, involving all classes of chemicals as well as majority of the principal pests. Some of these chemicals applied on grains have become less effective due to their prevalent tolerance ability among insect population. Fumigants such as methyl bromide, phosphine and cyanogens speedily results in the mortality of stages of storage insects in a commodity or in a storage structure. The continuous use of methyl bromide has been stopped as a result of its impact on the ozone layer (WMO, 1999). This has led to its expulsion from the market globally. The use of fumigants remain a prominent method of protection for stored commodities from attack of storage insects.

Even though inorganic insecticides are active, their consistent usage has prone lingered toxicity, contamination of the surrounding and negative impact on food apart from impacts on animal lives. The continual and haphazard usage of these chemicals has brought about the development of tolerable strains and build-up of poisonous remains on commodities meant for human consumption leading to health concerns. Alternative methods having minute or no undesirable effect to both the surrounding and animal life are sought after (Ileke, 2008). Plant and inert materials are some of the environmental friendly substances been used today.

2.9.2 Admixture with inert Diatomaceous Earth (DE) dust

Diatomaceous earth has been applied alternatively to synthetic insecticides for several years as it provide long term protection, hygienic to consume, does not impact negatively on grain quality, and a one-time application can be utilized to interrupt insect development on dried grains (Korunic and Fields, 2018). They are frequently employed to enhance fumigation in order to protect kept grains from chemical tolerant insects (Johnson *et al.*, 2014). Mixing stored products with inert dusts made from clays, diatomite, wood-ash, silicates and sand have been traditionally used and empirically verified to reduce insect populations in storage (Ofuya, 1986; Chinwada and Giga, 1997). Insects are killed by the dust material by scratching their body surface and thereby causing dehydration (Ebeling, 1971). Due to its contact toxicity on insect, the likelihood of insect tolerability is little (Quarles and Winn, 1996). The use of suitable options to inorganic chemicals like diatomaceous earth is likely to be favoured in the future (Zorya *et al.*, 2011).

The application of diatomaceous earths for effective storage of stored commodities against insect attack have been in existence for long time in China, whereas its application is new in Africa (Zorya *et al.*, 2011). Inert dust such as DE is an organic substance (soft whitish powder) obtained from fossilized skeletal remnants of diatoms which colonized aquatic bodies (Vayias and Athanassiou, 2004). After processing into powdery form, it can then be admixed with grain to cause insect mortality. When DE particles adhere to the insect body, it abrades its outer layer and causes dehydration leading to death (Ebeling, 1971). It has been reported to work effectively at par with some potent and commonly used inorganic chemicals in reducing attacks by insect affecting grains for an extended storage length of eight months (Stathers *et al.*, 2008). DE is usually applied as it is been augured, loaded or turned into storage facilities but it can also be used as a

surface treatment (top-dress). Grain buyers may be reluctant to buy grain treated with DE owing to reduced flowability, reduced test weight and increased wear on grain moving equipment (Bridgemann, 1999). Furthermore, its use as an empty bin treatment especially below the slotted floor is promising. DE can also be applied as wettable suspension spray on grains to reduce the trouble of dusts usually encountered.

However, the effectiveness of dust is markedly reduced owing to wet application, requiring increased dosage proportions (McLaughlin, 1994). Despite its marked effectiveness, there are still restricted access to registration and commercialization of DEs in some emerging nations for either imported or locally available DEs. The safe usage of DEs in many of the developed countries have been promoted. The potential usage lies with the research or government institutes in African nations to index DEs for grain protection, discover native deposits of DEs which may be more environmentally suitable options, and purchase from abroad alternatives to inorganic chemicals (Zorya *et al.*, 2011).

In Nigeria, massive deposition of DE have been found in some towns of Yobe State (RMRDCN, 2009). Currently, the Nigerian Stored Products Institute (NSPRI) are working with the National Agency for Food, Drug Administration and Control (NAFDAC) and are at the final stage of getting proprietary rights for the commercial production and sale of the Nigeria-derived DE to farmers.

DEs are safe, chemically inert on grains and are non-toxic to human (Stathers *et al.*, 2004). It is also been widely used as a source of raw material in food and pharmaceutical industries etc.

2.9.3 Modern storage structures

The significance of any storage structure or system is to preserve the wholesomeness of stored commodity for a specific period with less qualitative or quantitative loss (Baributsa and Ignacio, 2020). A number of storage techniques ranging from traditional methods, chemical and hermetic systems have been used over the last decades for postharvest management of grains. However, modern storage structures including bag storage, silo storage, hermetic technologies and controlled atmosphere storage have become alternatives to use of synthetic chemicals and traditional techniques, thereby stimulating significant attention among farmers and other agricultural stakeholders globally.

2.9.4 Innovative bag technologies

Polypropylene storage bags commonly referred to as ‘sacks’ are preferred locally for produce storage in Nigeria. In majority of emerging economies such as in Africa, cereal grains are typically kept as bulk in jute or polypropylene bags and stored inside large storehouses. These bags does not limit insect penetrations into and out of the storage container and thereby, have facilitated researchers to develop improved and suitable bag technologies to tackle insect proliferations. In the last few years, there have been keen interests to employ improved bag storage such as the insecticide infused storage bags and hermetically-sealed bags to control stored grain insect pests. Plastic and flexible containers appropriate for extended storage systems, as well as short-term storage in bags or in large quantity have been developed and applied. The significant feature of any hermetic technology is the effectiveness of their airtight condition produced during storage (Baributsa and Ignacio, 2020). Hermetic bags are modified from the traditional system of seal structures such as drums, underground pits etc. The biological processes of the insects present within the grain bulk results in the exhaustion of oxygen and emission of carbondioxide inside the hermetic container (Murdock *et al.*, 2012). Consequently, this hypoxic environment becomes lethal for insects to thrive, thereby limits or halts grain destruction. Hermetic bags are popularly encouraged for use by farmers in SSA and Asian countries, where it has gained significant acceptability led by the development of Purdue Improved Crop Storage (PICS) bags a decade ago. A number of other kinds of hermetic bag that have been marketed and available in various forms and sizes of single, double and triple layer bags are been produced by numerous licensed flexible firms across the world (Baributsa and Ignacio, 2020). The characteristics of each of the innovative and commercial bags varied in composition (single, double or triple bagging), insecticidal impregnated and liner type (single or multilayered) (Table 2.1). These bags have been well researched and the differences in their efficacies have been shown to be minimal and are more appropriate alternatives to insecticides in minimizing storage losses caused by insect pests (Baributsa and Njoroge, 2020).

2.9.4.1 Improved single layer storage bag

The development of improved non-hermetic and hermetic single bag storage are borne on innovative technique to improve existing polypropylene bag and the awareness on the increase demand for effective and low-cost storage technologies to reduce insect deterioration of stored commodities.

Table 2.1: Features of some commercially available small-scale bag technologies

| Bag composition | Polypropylene (PP) Bag | Polyethylene (PE) Liner | Brand examples |
|------------------------|-----------------------------------|----------------------------------|-----------------------|
| Single bags | 1 PP impregnated with insecticide | None | ZeroFly bag |
| | 1 laminated PP | None | ZeroFly Combi bag |
| | None | 1 multilayer PE liner | SuperGrain bag |
| Double bags | 1 PP | 1 multilayer PE liner | AgroZ |
| | 1 PP | 1 multilayer PE with insecticide | AgroZ Plus |
| | 1 PP | 1 multilayer PE liner | ZeroFly Hermetic |
| Triple bag | 1 PP | 2 high density PE liners | PICS bag |

The first innovative single non-hermetic storage bag is the ZeroFly® storage bag produced by Vestergaard S.A. This product is devised from woven polypropylene incorporated with pyrethroid insecticide on its outer fabric (Vestergaard, 2014). Based on manufacturers' information, the concentration of deltamethrin insecticide infused into the yarns of the woven polypropylene ZeroFly bags during extrusion process is three gram per kilogram or 3000 part per million offering a potent killing action against storage insect pests before they can have access or attack stored commodities. The main killing insecticide can be maintained on the bag for period up to two years such that produce are constantly safeguarded against noxious attacks from insects (Baban and Bingham, 2014). The bag is intended to offer safe storage to kept commodities by inhibiting insect entrance, thus aiding protection of produce. In a pilot study conducted, a hundred % effectiveness and no insect penetrate through the bag fabric was reported (Baban and Bingham, 2014). It has effectively been demonstrated with various storage insects (Vestergaard, 2014). Maximum residue limits (MRLs) of the insecticide on commodities are lower than the allowable tolerable limits globally when subjected to extreme usage conditions for its two years active period from production date (Vestergaard, 2015).

The hermetic principle of the single layer bags exist in various brands. For instance, Grainpro Inc., (Subic Bay, Philippines) began with the production of large-scale storage capacity called cocoons for extended storage period. With time, the company developed intermediate and small-scale structures. Small-capacity containers by GrainPro intended for smallholder storage was SuperGrain™ bags which was first used for rice storage, but has since been extended to all other important agro-commodities devoid of pesticide usage (Ziegler and Truitt Nakata, 2014). Each SuperGrain™ bag is composed of a transparent, thin single ultra-hermetic gas-tight multilayered recyclable polyethylene plastic (PE) with a thickness of about 78 µm (0.078mm) and a high oxygen barrier capability (GrainPro, 2017). The capacity of this bag varies up to 1000 kilogram, but the 60 – 90 kilogram is mostly used (Villers *et al.*, 2008).

Another hermetic single layer brand is the Zerofly Combi bag. This bag was produced as a modification of the existing non-hermetic Zerofly bag. The ZeroFly Combi is a patented hermetic laminated storage bag which is composed of polypropylene (PP) polymer that has pyrethroid insecticide incorporated into its outer fabric (BAGCO,

2020). It is an innovative small-scale capacity bag of 50 – 100 kilogram by Vestergaard, and manufactured in Nigeria by BAGO, a Nigerian bag manufacturing company.

2.9.4.2 Double bag storage

The AgroZ® brand is a typical double airtight bag produced in East Africa by A to Z Textile Mills Limited in Tanzania. It is composed of double distinct bags, the outer woven polypropylene bag and a 90 µm (0.090 mm) multilayer inner liner that is co-extruded combining high density polyethylene (HDPE) and high barrier properties preventing oxygen, carbon dioxide, and water vapor permeation (AgroZ, 2017). Its oxygen transmission rate (OTR) is 2.2 cc O₂/m²/day and the bag claimed to have the lowest OTR of any hermetic bag in the market (AgroZ, 2017). It has a storage capacity of 50 kg – 100 kg. AgroZ bags are fully an organic solution for grain preservation and storage. However, the manufacturer put a disclaimer that the bag should not be intended for use to control *P. truncatus* as the hermetic seal of the bag can be readily compromised by the pest.

Another hermetic bag developed by A to Z is the AgroZ bag Plus. The bag which is composed of insecticidal hermetic treatment portrays superior quality with the precise purpose to control larger grain borer (Baributsa and Ignacio, 2020).

ZeroFly® Hermetic storage is another novel double bagging technique which keeps harvested agricultural produce from deterioration caused by inward or outward movement of insects (Vestergaard, 2016). Its production constitutes an external deltamethrin impregnated polypropylene bag together with an internal 80 micron-meter (µm) impenetrable multilayered reusable plastic lining having a gas tight properties. The bag offers smallholder farmers safe storage for a lifespan up to two years (Vestergaard, 2016). Therefore, it demonstrates that the double protection of the bag prevented the entry of insects as a result of the incorporated deltamethrin on its outer fabric and the inner liner which prevents the exit and multiplication of insects as a result of asphyxiation and death caused by reduced oxygen level within the bag.

2.9.4.3 Triple bag storage

The Purdue Improved Crop Storage (PICS) technology is the first small-scale hermetic and triple bag system produced for grain storage. The bag was developed by Purdue University and initially intended for management of stored insect infestation on cowpea seeds in Africa. It consists of three-layered baggage in airtight conditions. It has been

extensively advocated and employed to store varying commodities by farmers in SSA. PICS consists of double layer HDPE bags within a standard polypropylene woven bags to control major insect pests principally works by modification of oxygen levels generated by breathing and rate of metabolic reactions of insects, moulds and grains to lower levels whereas carbondioxide level increases, thus bringing about the mortality of insects and microorganisms by suffocation (Murdock *et al.*, 2012).

The grains are first kept inside a two-layered 80 µm (0.080 mm) thick HDPE hermetic liners and then held inside a third outer polypropylene bag. Afterwards, the bags are individually sealed tightly using cotton rope or plastic clip. It has a storage capacity of up to 50 – 100 kg of grains. This process of hermetic storage helps inhibit circulation of air to the insects and obstruct their metabolic activities, thus causing dehydration and eventual mortality (Murdock *et al.*, 2012). The PICS technology has been considered affordable and safe, which allows smallholder farmers to protect their commodities with insignificant loss. Compared to other technologies, the bag has been effortlessly adopted for use by farmers and majority of the researches conducted have shown its effectiveness against wide-ranging stored commodities attacked by insect and microorganism infection (Williams *et al.*, 2017). Although, its success as an airtight technique is reliant on some influences comprising type of bag closure, type and condition of conserved produce, environmental conditions, severity and dominance of storage insects as well as the malleability of the bag fabric (Njoroge *et al.*, 2014). As for PICS, the double inner liners is purposed to provide additional protection in case one of the liner is breached by insect penetrations or damaged. However, the second liner of this bag is rarely damaged by insect and so, the bag has the capability of providing continued protection to stored commodities.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site and pre-experimental procedure

The performance and optimal use of novel storage technologies for maize grain protection against storage insect infestations were evaluated both in the laboratory and storehouse. The laboratory study involved internal infestations with two major insect pests (*Prostephanus truncatus* and *Sitophilus zeamais*) separately into stored maize contained in bags and the experiment conducted in the Entomology Research Laboratory, Department of Zoology, University of Ibadan, Ibadan. The field study was located within a storehouse in Arisekola market, Bodija, Ibadan (07° 25 N, 03° 54 E) (GPS Garmin eTrex® 30x, Garmin International Inc., Kansas, USA) and involved external infestations of the two major pests to create added infestation pressure within the storehouse.

3.1.1 Preparation of stock culture of insect pests

Adults of *P. truncatus* and *S. zeamais* were obtained from previous cultures of both species in the laboratory at the outset of the study. Each species was then reared separately on whole and clean maize substrate contained in 1-litre glass kilner jars. The maize grains with the insects were then kept enclosed using muslin material to prevent their outward movement and possible cross-infestation. Emerging adults from the established cultures were subsequently used in the infestation procedure.

3.1.2 Source of maize used

Freshly harvested and dried maize was obtained from a particular farmstead situated at Ijaye Farm (07° 42 N, 03° 44 E), Akinyele LGA, Oyo State to guarantee homogeneity of maize variety used in this study (Plate 3.1). The maize was a yellow cultivar “SWAN 2”. Aflasafe (an innovative bio-control product for aflatoxin mitigation) was however applied to the maize field during crop production. Preliminary moisture content of maize was 11.3% using a John Deere moisture meter. This is to ensure that the harvested maize was at safe moisture level suitable for storage before purchase.



Plate 3.1. Harvested maize from Ijaye Farm, Ibadan

Threshed maize from cobs was done using a locally fabricated thresher with a total of three metric tons (MT) of grains bagged in previously washed and dried jute bags (Plate 3.2) and transported to the rented storehouse in Arisekola market, Bodija, Ibadan.

3.2 Storage technologies used

The storage technologies used in the study included storage bags such as the conventional woven Polypropylene (PP) storage bags, Purdue Improved Crop Storage™ (PICS) bags, deltamethrin incorporated ZeroFly® (ZF) storage bags and, a grain protectant Insecto® Diatomaceous Earth (DE) dust. However, the DE was mixed with grains stored in PP or ZF bag to make up the PPDE and ZFDE. Additionally, single or double hermetic liners were fixed into PP and ZF bags to make the PP1L, ZF1L and ZF2L storage technologies. These technologies (hereafter referred to as treatments) which comprised of eight treatments was categorised into non-hermetic (PP, PPDE, ZF and ZFDE) and hermetic (PICS, PP1L, ZF1L and ZF2L) storage.

3.2.1 Polypropylene (PP) storage bag

Standard woven PP bags of 100 kilograms capacity were obtained from a local supplier within Bodija market, Ibadan for use in the study. A total of twenty PP bags were purchased.

3.2.2 ZeroFly (ZF) storage bag

A total of thirty 50 kilograms capacity ZF bags were obtained from Vestergaard S.A. representative in Nigeria. The bags were handled using standard practices to avoid contact with the deltamethrin insecticide-impregnated on the bag.

3.2.3 Diatomaceous Earth (DE) treatment

Commercialised DE dust, INSECTO® was obtained for use in the study from Insecto Natural Products, Costa Mesa, California, USA. The DE was mixed with the maize and placed in PP and ZF bags to make up the PPDE and ZFDE treatments, respectively.

3.2.4 Hermetic double bags — Polypropylene single liner (PP1L) and ZeroFly single liner (ZF1L)

Hermetic liners of 80 µm thickness were supplied by Vestergaard S.A. (Vietnam) and each single liner was inserted into PP and ZF bags to make up the PP1L and ZF1L bag treatments, respectively.



Plate 3.2. Procured maize grain in jute bags on the farm

3.2.5 Hermetic triple bags — Purdue Improved Crop Storage (PICS) and ZeroFly double liners (ZF2L)

The 50 kilograms capacity PICS bags (Figure 3.1) evaluated in this experiment were produced by Lela Agro (Kano State, Nigeria) and obtained from a local supplier in Bodija market, Ibadan. In the case of ZF2L bag, the two hermetic liners contained in PICS bags were each removed and placed inside ZF bags to make up that treatment.

Each treatment are hereafter referred to by their acronyms. For both laboratory and storehouse study of internal and external infestation of insect pests, batches of maize were weighed and filled individually in bags assigned to each of eight treatments and categorized into two groups namely; non-hermetic Polypropylene (PP), Polypropylene plus Diatomaceous earth (PPDE), ZeroFly (ZF) and ZeroFly plus Diatomaceous Earth (ZFDE) treatments and the hermetic Purdue Improved Crop Storage (PICS), Polypropylene single liner (PP1L), ZeroFly double liners (ZF2L) and ZeroFly single liner (ZF1L) treatment bags for easy interpretation.

3.3 Experimental design

The experimental design involved a preliminary investigation of the insect pest status of both *P. truncatus* and *S. zeamais* infestation on maize kept in Kilner jars by assessing their damage potential during storage. Furthermore, a laboratory study of artificial internal infestation with these two pests were established with maize using eight different storage technologies to investigate insect infestation level, insect damaged kernel, weight loss and insect damage on storage bags over 3 months storage period. The storehouse experiment involved artificial external infestation with both *P. truncatus* and *S. zeamais* around stacks of each storage bag treatment in the storehouse over 12 months storage period and the following response variables were evaluated; insect infestation level, insect damaged kernel, weight loss, seed germinability, moisture content, aflatoxin contamination, pesticide residue on maize stored in ZF bag and insect damage on storage bags.

3.4 Procedure for laboratory study

3.4.1 Insect pest status on maize

Maize samples used was pre-conditioned at -5°C for 3 days inside a deep freezer (Scanfrost freezer SFL250L) to get rid of any hidden insect pest and then placed on normal white paper at ambient temperature for 24 hours to allow the grains to stabilize

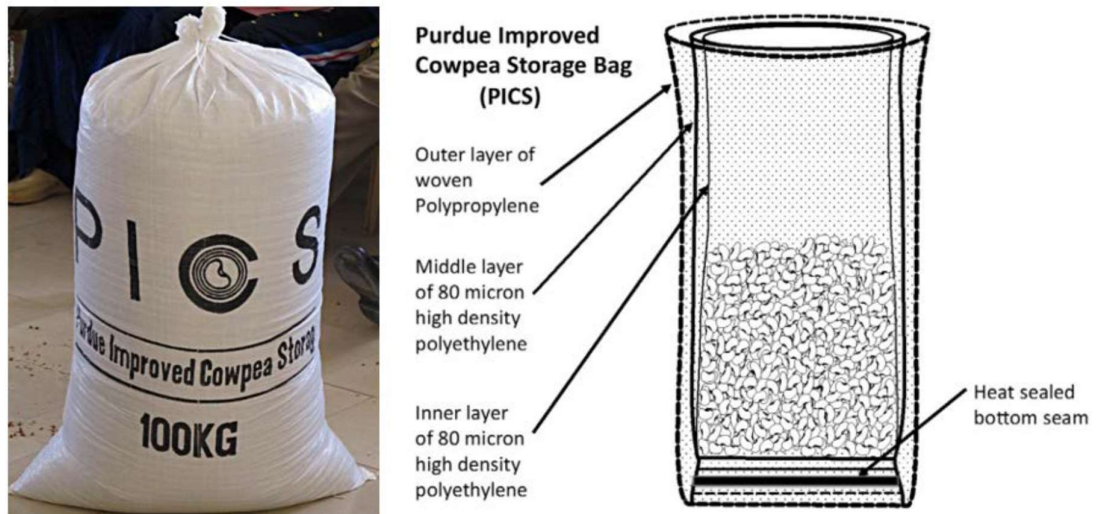


Figure 3.1. Diagrammatic presentation of the three-layered PICS bag (Murdock and Baoua, 2014).

(Ceruti *et al.*, 2008). Ten grams (10 g) of maize grain was then placed into jars with each containing ten (10) unsexed mature individuals of either *P. truncatus* or *S. zeamais*. Each insect served as a treatment and was replicated ten times. The vials were labeled, protected with muslin material and held tight with elastic bands to ensure the insects are kept intact with the substrate. The set up was kept for a period of six weeks at laboratory conditions inside a wooden screen cage (30 x 10 x 20) cm³. The set up was then observed to determine the total of living insects, damaged kernel as well as the mass of lost samples.

3.4.2 Internal insect infestation of bagged maize

Each of the three storage bags (PP, ZF and PICS) and the hermetic liners were cut into a predetermined mini-bag sizes measuring 260 by 160 mm to contain 500 g of maize sample. Untreated 500 g maize was then filled into each of Polypropylene (PP), Purdue Improved Crop Storage (PICS), Polypropylene plus single liner (PP1L), ZeroFly plus double liners (ZF2L) and ZeroFly plus single liner (ZF1L) bags. Furthermore, another 500 g maize was admixed with 0.5 g of DE (that is, at a rate equivalent to 1g/kg of DE) (Nwaubani *et al.*, 2014) and filled into PP and ZF bags to make up the PPDE and ZFDE treatments, respectively. Three replicates of each storage treatment was weighed and 20 adults of *P. truncatus* were artificially infested into the maize contained in the bags, and were individually tied closely using a cotton rope.

The same procedure was repeated for *S. zeamais* infestation. The treatments were set up and stored inside a screen wooden cage and kept for 3 months in the laboratory (Plate 3.3). The ZF bag related treatments were set on top and conversely, PP bagged maize were place in the lower part of the cage to counteract conditions directly around the two bag types. The bags were arranged in completely randomized design and the total experimental units of bagged maize was forty-eight which were destructively sampled after the storage period. Samples were analysed for insect infestation level and measure of insect activity such as insect damaged kernel and weight loss. Insect penetrations on storage bags were also calculated.



Plate 3.3. Laboratory set up of internally infested *P. truncatus* and *S. zeamais* into bagged maize.

3.5 Procedure for field study

3.5.1 Storehouse preparation

A storehouse area (100 x 90 m²) at Arisekola market Bodija, Ibadan was used for the 12 months duration (February, 2017 to January, 2018) of the study. The storehouse was initially cleaned and all potential insect and rodent pest entry openings were sealed before the commencement of the experiment. Vents were also created for proper circulation of air within the store and wooden pallets 1.5 m by 1.5 m on which bags of maize were placed to prevent contact with the floor were used.

3.5.2 Bagging of maize

The maize used in the experiment was not fumigated prior to bagging. Fifty kilogrammes batches of maize were weighed using a Camry dial spring balance™ and filled individually to each of eight storage technologies previously described (Plate 3.4). Treatment for each of the non-hermetic Polypropylene (PP), Polypropylene plus Diatomaceous earth (PPDE), ZeroFly (ZF) and ZeroFly plus Diatomaceous Earth (ZFDE) treatments had 3 bags assigned to them while the hermetic Purdue Improved Crop Storage (PICS), Polypropylene single liner (PP1L), ZeroFly double liners (ZF2L) and ZeroFly single liner (ZF1L), each had 9 nine bags to facilitate quarterly samplings (Table 3.1).

For maize mixed with DE in Polypropylene (PPDE) and ZeroFly (ZFDE) bags respectively, Insecto DE was applied and mixed at a proportion of 50 grams per 50 kilograms bag of maize (that is, at an amount equivalent to 1g/kg) (Nwaubani *et al.*, 2014). Maize contained in individual bags assigned to DE treatment was divided into three equal portions and each portion placed in a big basin. The 50 grams of DE was then shared into three equal lots and each lot was admixed with one portion of maize manually to ensure even distribution of DE within the grains (Plate 3.5). The three portions of maize that were thoroughly mixed with DE were then poured into a single PP or ZF bag.

3.5.3 Bag closure procedure

For all the non-hermetic bag treatments, the bags were simply tied using a bag closure machine. In the case of the hermetic bag treatments, the innermost HDPE liners directly in contact with maize were first pressed down to expel as much air as possible before tying with a cotton rope. The outer HDPE was tied next as is the case with the triple bag



Plate 3.4: Weighed 50 kg lots of maize into storage bags.

Table 3.1. Types of bags and treatment procedure for stored maize in the storehouse

| S/N | Bag/treatment | Number of bags assigned | Bag type |
|------------|---|--------------------------------|-----------------|
| 1. | Untreated maize placed in Polypropylene (PP) bag | 3 | Non-hermetic |
| 2. | Diatomaceous Earth-treated maize placed in Polypropylene bag (PPDE) | 3 | |
| 3. | Untreated maize placed in ZeroFly (ZF) bag | 3 | |
| 4. | Diatomaceous Earth-treated maize placed in ZF bag (ZFDE) | 3 | |
| 5. | Untreated maize placed in Purdue Improved Crop Storage (PICS) bag | 9 | Hermetic |
| 6. | Untreated maize placed in Polypropylene single liner (PP1L) bag | 9 | |
| 7. | Untreated maize placed in ZeroFly double liners (ZF2L) bag | 9 | |
| 8. | Untreated maize placed in ZeroFly single liner (ZF1L) bag | 9 | |



Plate 3.5. Insecto DE dust (A) and maize mixed with DE (B).



Plate 3.6. Bag tied procedure for hermetic bag using cotton rope.

system. Tying of the HDPE was done in such a way that their open ends (mouths) were folded and tucked downwards. The outer woven polypropylene was tied last in a similar fashion.

3.6 Arrangement of treatment bags, infestation and store microclimatic measurements

3.6.1 Arrangement of bagged treatments and replicates

Tightly bagged maize of the various treatments of which three (in the case of non-hermetic treatments) or nine replicates (in the case of hermetic treatments) bags were stacked on individual platforms to avert bags from gaining water from the cemented floor. The platform on which the various treatments were placed were at a minimal two meters span from each other (Plate 3.7). All ZF related bagged maize were arranged across the PP bags in order to counteract conditions directly around the two bag types inside the storehouse. In total, forty-eight 50 kg bagged maize of eight various treatments were stored.

3.6.2 Bag infestation with test insects

Twenty unsexed adults of *P. truncatus* and *S. zeamais* were individually placed into each of eight Kilner jars having 100 g of maize in the laboratory and stocked for six weeks. The bottles holding each species of insect were collected and taken to the storehouse. Each test insect-infested maize was then placed between stacks of each bag treatment on the platform to generate the needed pest burden around bags. This practice took place continually at quarter interval (1st, 4th, 8th months) until the end of the storage period.

3.6.3 Storehouse microclimate measurements

Three temperature and relative humidity sensors (Onset U23-001A-HOBO U23 Pro v2 internal temperature/relative humidity logger) were placed in the storehouse. One was suspended at the eaves' level, the second in the middle of the storehouse and the third was situated at the grain level. The sensors were set up to record data hourly and data were downloaded on a monthly basis. Data were summarized and properly filed through a computer system for the duration of the study period.

3.7 Grain sampling and data collection procedures

In the laboratory study involving internal insect infestation of bagged maize, all three replicates in each of the different treatments were destructively sampled after 3 months storage period. For the storehouse study, three of each bag treatment were sampled



Plate 3.7. Storehouse set up of bagged maize treatments on separate pallet.

at the start of the experiment in February 2017. Subsequently, all three bags each of non-hermetic Polypropylene (PP), Polypropylene plus Diatomaceous earth (PPDE), ZeroFly (ZF) and ZeroFly plus Diatomaceous Earth (ZFDE) treatments were inspected on monthly interval from March 2017 to January 2018. Furthermore, three representative bags from each nine bags assigned to hermetic Purdue Improved Crop Storage (PICS), Polypropylene plus single liner (PP1L), ZeroFly plus double liners (ZF2L) and ZeroFly plus single liner (ZF1L) treatments were subjectively chosen for inspection at quarter interval. Inspected bags were thereafter taken away from the storehouse after 4, 8 and 12 months since they were no longer required and this process is referred to as destructive sampling.

Destructive sampling here means that the sampled bagged maize were marked out and could not be sampled again as a result of a breach in the generated modified atmosphere within the enclosed bag due to the opening of the bag at the time of sampling.

For the non-hermetic treatments, a small opening was made using a razor blade near the seam of each bag that was sealed whereas, bags were simply untied in the hermetic treatments to facilitate sampling. Afterwards, a grain trier (40" brass probe, open-handle, 6 openings) (Seedburo Equipment Company, Des Plaines, IL) inserted into each bag (Plate 3.8) was applied to take sub-sample of approximately 700 g which was then placed into a labeled Ziploc bag for laboratory analysis. Samples of maize were drawn from the middle and the two sides of each the bags. Maize taken with the probe constitutes a composite sample used for analyses. Opened non-hermetic bags were re-sealed with duct tape to prevent spillage of maize and entrance of insects from opened end while the hermetic bags were tied back and marked as sampled.

3.8 Laboratory analysis of samples

Samples collected from bags in the laboratory and those in the storehouse in Ziploc bags were analysed in the laboratory. For each triplicate samples, data on number of insect species present, % insect damaged kernel by number and weight (IDKn and IDKw), % maize weight loss, % seed germinability and moisture content were analysed.

3.8.1 Insect infestation level

Maize samples collected were screened with a United States grade 10 mesh (2 millimeter holes) (Seedburo Equipment) over a stainless platter to retrieve insect species. Each type



Plate 3.8. Bag opened by cutting to facilitate sampling with grain probe.

of species were recognized with the aid of a tripod magnifier and their population were categorized as dead and live.

3.8.2 Determination of pest status of *P. truncatus* and *S. zeamais* using percentage Insect Damaged Kernel (% IDK)

Maize seeds having holes due to insect feeding were removed from the lots and counted. Percentages of insect damaged kernels by numerical and weight (% IDKn and % IDKw) were evaluated according to Quitco and Quindoza (1986).

For the preliminary insect pest status assessment, % IDKn (Formula 3.1) and % IDKw (Formula 3.2) were calculated per 10 grams of maize sample as:

$$\% \text{ IDKn} = \frac{\text{Nd}}{\text{Total grain count}} \times 100 \quad (\text{Formula 3.1})$$

$$\% \text{ IDKw} = \frac{\text{Wd}}{10} \times 100 \quad (\text{Formula 3.2})$$

For the laboratory study of internal pest infestation in storage bags, % IDKn (Formula 3.3) was calculated per 500 grams of maize sample as:

$$\% \text{ IDKn} = \frac{\text{Nd}}{\text{Total grain count}} \times 100 \quad (\text{Formula 3.3})$$

For maize samples collected in Ziploc bag from the storehouse, percentage number of insect damaged kernel (% IDKn) (Formula 3.4) were calculated per 250 grams (FAO, 2004) sub-sample as:

$$\% \text{ IDKn} = \frac{\text{Nd}}{\text{Total grain count}} \times 100 \quad (\text{Formula 3.4})$$

Where, Nd and Wd represent number and weight of damaged kernel, respectively.

3.8.3 Estimation of percentage Weight Loss (%WL) of stored maize in different bags

Damaged and undamaged kernels were identified visually in 10 grams, 250 grams and 500 grams sample of maize as previously indicated. Damaged and undamaged kernels were then totaled and weighed. Weight loss was thus estimated with count and weigh procedure (Formula 3.5) according to Gwinner *et al.*, (1996):

$$\% \text{ WL} = \frac{[(W_u \times N_d) - (W_d \times N_u)]}{W_u \times (N_d + N_u)} \times 100 \quad (\text{Formula 3.5})$$

Where, %WL = weight loss

W_u and W_d represent weights of undamaged and damaged kernels, respectively.

N_u and N_d represent numbers of undamaged and damaged kernels, respectively.

3.8.4 Determination of viability of stored grain in different types of bags

Germination assessments were done by applying procedure used by Rao *et al.*, (2006). From 250 grams maize sub-sample earlier mentioned, one hundred seeds were randomly picked and divided into 25 seeds per four disposable Petri-plates. The bases of the plates were already laid with dampened cotton sheets before the seeds were scattered on them. All the plates were then organized at random on a wooden platform in the laboratory and the seeds moistened every day until after 7 days when germinated grains were recorded on the basis of the number of sprouted seeds.

$$\% \text{ Viability} = \frac{\text{Number of sprouted seed}}{\text{Total seed count}} \times 100 \quad (\text{Formula 3.6})$$

3.8.5 Estimation of moisture content of maize grains stored in different bags

The amount of water contained in each maize sample from each storage bags were determined monthly using four moisture measurement methods (Fig. 3.1); GrainMate meter (Ajao *et al.*, 2018; Sesi Technologies, Ghana), John Deere meter SW08120 (AgraTronix, USA), Grain Analysis Computer GAC 2100 Agri (DICKEY-john, USA) and an oven-dry reference method (ASABE Standards).

3.8.5.1 Determination of the MC, temperature and R.H of stored maize using GrainMate meter

The GrainMate meter (Fig. 3.2A) was operated by positioning the probe into the depth of each treatment bag of maize stored and left to equilibrate within the grain bulk for about 6-minutes. The meter works by applying temperature and air surrounding the grain data to determine the equilibrium moisture content of commodities. The values of moisture content (% wet basis) along with temperature (°C) and relative humidity (% ERH) of maize were digitally displayed and then subsequently recorded manually. Data from three positions (middle and two sides) in each bag treatment were measured and the mean MC was estimated for individual bag treatment.



Figure. 3.2. Moisture meters: GrainMate meter (A), Grain Analysis Computer 2100 (B) and John Deere moisture meter (C)

3.8.5.2 Determination of MC of stored maize using Grain Analysis Computer (GAC) 2100 meter

Maize sample of approximately 200 g was put inside the hopper (upper unit) of the moisture analyzer (Fig. 3.2B). As the load button was push down, grain sample mechanically drops inside the assessment chamber. Within the chamber, a strike-off arm level out the grain and the superfluous was discarded. Grain moisture was then measured for about 15 seconds before the water level together with grain temperature were digitally presented. Three sample data were determined to estimate the mean MC obtained from individual bag treatment. This procedure was conducted in the laboratory.

3.8.5.3 Determination of MC of stored maize using John Deere (JD) moisture meter

Sampled maize of about 100 g was taken with a spoon and placed inside the upper hollow chamber of the meter (Fig. 3.2C) and subsequently clamped tightly with the cover to flatten the grains all over the electrodes. There was a uniform force exerted on the inspected grain sample through a piston in the cover comprising a cylindrical coil spring, supporting the piston bar which extends between the cover as the force on the grain mount. By this, the piston bar is squarely laid and levelled with the cover cap respectively, for every inspected sample. The meter was battery-operated to manage its backlight electronic display and microchip and uses it to relay the degree of grain moisture and heat. Three sample data were determined to estimate the mean MC obtained from individual bag treatment. This procedure was conducted in the laboratory.

3.8.5.4 Determination of MC of stored maize by Oven-dry test

A 100 g whole maize sample replicated 2 times was placed into a previously cleaned and dried crucible and oven dried at 103⁰C for 72 hours (ASABE Standards, 2008) in a Binder ED 56 oven (Binder GmbH, Tuttlingen, Germany). At the completion of heating procedure, the dried specimen were retained in a desiccator to chill off hot air before the moisture content (wet basis) (Formula 3.7) was evaluated for respective bag treatment. Two sample data were taken to estimate the mean MC obtained from individual bag.

$$\% \text{ MC} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \times 100 \quad (\text{Formula 3.7})$$

3.8.6 Determination of Aflatoxin in stored maize samples

Three 100 g sub-samples were taken each from the eight bag treatments in the storehouse for aflatoxin test. All the samples were then taken to the Pathology and Mycotoxin Laboratory of the International Institute of Tropical Agriculture (IITA) for laboratory analyses prior to and following 12-month storehouse duration. Maize specimens were analysed in accordance to standard aflatoxin method of analysis as described below.

Aflatoxin was extracted from stored maize by mixing 20 g ground samples with 100 ml 70% methanol (Atehnkeng *et al.*, 2008), and the suspension was shaken on a Roto-Shake Genie® (Electron Microscopy Sciences, Hatfield, PA) for 30 minutes at 400 revolutions per minute and strained using Whatman No. 1 filter paper (Whatman International Ltd., England). The filtrates were then collected in 250 ml separatory funnels, combined with 100 ml distilled water, and extracted twice with 25 ml methylene chloride. The methylene chloride phase was filtered through a bed of 25 g anhydrous sodium sulfate contained in fluted Whatman No. 4 filter paper, combined and, evaporated to dryness in a fume chamber. Residues were dissolved in 1 ml methylene chloride and subjected to scanning densitometry. Homogenates were directly spotted (4 µl) alongside aflatoxin standards (Supelco, Bellefonte, PA) on TLC aluminium (20 × 10 cm) silica gel 60 F₂₅₄ plates (Merck, Darmstadt, Germany) and developed with diethyl ether-methanol-water (96:3:1) (Probst *et al.*, 2011). The plates were then visualized under ultraviolet light (365 nm) for presence of aflatoxins. Aflatoxins were quantified directly on TLC plates with a scanning densitometer (CAMAG TLC Scanner 3) and quantification software (winCATS 1.4.2, Camag, AG, Muttenz, Switzerland).

3.8.7 Insecticide residue analysis of stored maize in ZeroFly bag

A representative 1000 g sample of maize stored in ZF storage bag was taken for deltamethrin residue test. The test was conducted at SGS Inspection Services Nigeria Limited, Lagos using QuEChERS and BS EN 15662:2009 method of analyses involving Liquid Chromatography with tandem Mass Spectrometry (LCMS) and Gas Chromatography-Mass Spectrometry (GCMS). The standard procedure used for the analysis involved extraction steps to isolate the pesticide from the sample matrix and a clean up steps to isolate compounds of interest from co-extracts. The clean-up is achieved with dispersive solid phase extraction (dSPE) using anhydrous magnesium sulphate and a primary secondary amine sorbent combined with acetonitrile extract to remove polar matrix components. This multimethod for analysing pesticide residues in

foods of plant origin is a European Standard procedure. The method has been studied on varieties of products and pesticides mixtures (European Standard, 2021).

Initial test on samples were conducted at the first month of storage while a final residue test was carried out at the end of 12-month storage duration.

3.8.8 Insect penetration of storage bag

Storage bags were inspected for perforations or penetrations caused by insects. Insect damage on bags characterized by scratches and deep scars on the entrance side of holes (Riudavets *et al.*, 2007) were marked and counted. Mean number of holes was therefore calculated.

3.9 Statistical analysis

Statistical analyses were done using Statistical Package for the Social Sciences (SPSS) (Version 20.0, IBM Chicago, IL, USA). The Randomized Complete Block Design (RCBD) experimental style was used. Differences in the infestation level of *P. truncatus* and *S. zeamais* on stored maize for six weeks was assessed using paired Students't-test. Data to determine the performance of each hermetic and non-hermetic storage bags on insect infestation levels, insect damage kernels, maize weight losses, grain viability and efficiency of different moisture meters were analysed using descriptive statistics and Analysis of Variance (ANOVA). Significance of mean differences were separated using Tukey's test at $\alpha = 0.05$. Differences in aflatoxin level of stored maize between non-hermetic and hermetic bag storages were assessed using paired Students't-test. Mean values were plotted using SigmaPlot 13.0 (Systat Software Inc., USA). Correlations between insect species and maize quality indices were evaluated using Spearman correlation analysis in SigmaPlot at $p < 0.05$.

CHAPTER FOUR

RESULTS

4.1 Pest status of *P. truncatus* and *S. zeamais* on maize stored for six weeks

After six weeks of maize infestation with *P. truncatus*, an average of 3.9 ± 0.2 live insect was recorded. The mean percentage number of Insect Damaged Kernel (% IDKn) due to their feeding activities was $11.7 \pm 0.3\%$ and the percentage weight of Insect Damaged Kernel (% IDKw) was $4.5 \pm 0.3\%$. This insect caused damage resulted in substantial mean weight loss of $7.8 \pm 0.2\%$ (Table 4.1). Regarding *S. zeamais* infestation on stored maize, an average of 3.3 ± 0.1 live insect was found. This consequently resulted in % IDKn and % IDKw values of $8.7 \pm 0.3\%$ and $8.0 \pm 0.3\%$, respectively with mean %WL of $3.9 \pm 0.3\%$ (Table 4.1). The result obtained showed that there was no difference in number of live insect recovered ($t = 0.53$, $p > 0.05$) but a varied differences in weight of damaged kernels ($t = 0.85$, $p < 0.05$) and weight loss (0.11 , $p < 0.05$).

4.2. Insect infestation on bagged maize stored for three months in the laboratory

4.2.1. *P. truncatus* infested stored maize in the laboratory

In bags infested with *P. truncatus*, the pattern for number of live and dead insects were not similar in the different bag treatments (Table 4.2). Apart from *P. truncatus* that was initially infested with the maize, *S. zeamais* was also found infesting the stored maize. The total live *P. truncatus* and *S. zeamais* recovered in PPDE, PP, PP1L and ZF bags were high relative to other bag types. The mean total of *P. truncatus* and *S. zeamais* in PPDE was 82.7 ± 1.9 and 17.3 ± 2.6 , respectively followed by PP bag which had 46.0 ± 11.1 *P. truncatus* and 36.3 ± 5.9 *S. zeamais* (Table 4.2). Also in PP1L bag, mean *P. truncatus* (76.7 ± 12.2) and *S. zeamais* (2.0 ± 0.6) were found. The total number of live insects were much lower in PICS, ZF2L, ZFDE and ZF1L treatment with an average value of below 5 live *P. truncatus* recorded in any of these bags and no live *S. zeamais* recorded after 3 months of storage period (Table 4.2). Regarding dead *P. truncatus*, ZFDE (40.0 ± 13.7), PP1L (33.0 ± 2.9), ZF1L (32.7 ± 9.3) and ZF (31.7 ± 5.9) bags had the highest mean number of dead insects whereas, in PP, PPDE, PICS and ZF2L

Table 4.1. Pest status of *P. truncatus* and *S. zeamais* on stored maize grains

| Variables | <i>P. truncatus</i> | <i>S. zeamais</i> | t-value |
|-------------------|----------------------------|--------------------------|----------------|
| Live insect count | 3.9 ± 0.2a | 3.3 ± 0.3a | 1.53 |
| IDKn (%) | 11.70 ± 0.3b | 8.70 ± 0.3a | 0.27 |
| IDKw (%) | 4.52 ± 0.3a | 7.97 ± 0.3b | 0.85 |
| WL (%) | 7.82 ± 0.2b | 3.89 ± 0.3a | 0.11 |

Values are mean (±SE); Means within the same row followed by different letters are significantly different ($p < 0.05$)

%IDKn = Percentage number of insect damaged kernels; %IDKw = Percentage weight of insect damaged kernels; %WL = Percentage weight loss.

Table 4.2. Mean (\pm SE) of *S. zeamais* and *P. truncatus* in maize initially infested with *P. truncatus* and stored for 3 months.

| Bag types | | Live | Dead | Live | Dead |
|---------------------|-----------------|-------------------|-------------------|---------------------|---------------------|
| | | <i>S. zeamais</i> | <i>S. zeamais</i> | <i>P. truncatus</i> | <i>P. truncatus</i> |
| Non-hermetic | PP | 36.33 \pm 5.90 | 5.67 \pm 1.45 | 46.00 \pm 11.13 | 11.00 \pm 1.15 |
| | PPDE | 17.33 \pm 2.60 | 5.00 \pm 1.73 | 82.67 \pm 1.86 | 12.67 \pm 2.19 |
| | ZF | 0.00 \pm 0.00 | 0.00 \pm 0.00 | 34.33 \pm 5.24 | 31.67 \pm 5.93 |
| | ZFDE | 5.67 \pm 2.85 | 0.33 \pm 0.33 | 2.33 \pm 2.33 | 40.00 \pm 13.65 |
| Hermetic | PICS | 0.00 \pm 0.00 | 0.00 \pm 0.00 | 3.67 \pm 1.45 | 14.00 \pm 0.58 |
| | PP1L | 2.00 \pm 0.58 | 0.00 \pm 0.00 | 76.67 \pm 12.20 | 33.00 \pm 2.89 |
| | ZF2L | 0.00 \pm 0.00 | 0.00 \pm 0.00 | 4.33 \pm 0.88 | 18.00 \pm 1.53 |
| | ZF1L | 0.00 \pm 0.00 | 0.00 \pm 0.00 | 22.33 \pm 1.20 | 32.67 \pm 9.3 |
| | F(7, 16) | 27.12* | 9.21* | 26.52* | 1.33 ^{ns} |

Each datum represent mean total of insects contained in three replicates of maize samples

PP = Polypropylene; PPDE = Polypropylene plus Diatomaceous Earth; ZF = ZeroFly; ZFDE = ZeroFly plus Diatomaceous Earth; PICS = Purdue Improved Crop Storage; PP1L = Polypropylene single liner; ZF2L = ZeroFly double liner; ZF1L = ZeroFly single liner

^{ns} $P > 0.05$; * $P < 0.05$; ns = not significant

bags, dead *P. truncatus* was ≤ 18 insects, respectively. Also, dead *S. zeamais* was recorded only among three bag types; PP, PPDE and ZFDE with values of 5.7 ± 1.5 , 5.0 ± 1.7 and 0.3 ± 0.3 insects, respectively (Table 4.2). In the remaining treatments, no dead *S. zeamais* was recorded.

4.2.2 Infestation of stored maize by *S. zeamais* in improved storage bags and DE-treated maize for 3 months

In bags infested with *S. zeamais*, the insect count varied with the storage bags (Table 4.3). Apart from *S. zeamais* that was initially infested with the maize, *P. truncatus* and *T. castaneum* were also found infesting the stored maize in PP and PPDE bags after three months of storage. The total mean number of live insects recovered from PP (42.7 ± 9.3) and ZF (33.0 ± 4.6) bags were higher than the other treatments. Live *S. zeamais* in PICS, PP1L, ZFDE, ZF2L and ZF1L bags did not exceed a mean of 6, respectively. Live *P. truncatus* and *T. castaneum* were only recovered in PP and PPDE bags, respectively (Table 4.3). ZeroFly recorded the highest number of *S. zeamais* (46.0 ± 12.7) while the number of dead beetles were below 24 in the other treatments. No dead *P. truncatus* and *T. castaneum* in all treatments except PP and PPDE.

4.2.3 Effect of improved storage bags and DE on the number of insect damaged kernel in maize stored in the laboratory

In bags initially infested with *P. truncatus*, the pattern for percentage number of insect damaged kernels (% IDKn) were different in all bag types (Table 4.4). The mean %IDKn in PPDE (35.8%), followed by PP (34.6%) and PP1L (28.1%). However, ZF bag had 12.3 ± 1.2 % while values for PICS, ZFDE, ZF2L and ZF1L bags were low and did not exceed an average of 6.0%, respectively (Table 4.4).

In bags initially infested with *S. zeamais*, the pattern of IDKn were not similar in all bag types (Table 4.4). High values of mean % IDKn were recorded in PPDE ($22.7 \pm 1.6\%$), PP ($21.7 \pm 1.4\%$), ZF ($13.5 \pm 2.1\%$) and PP1L ($10.8 \pm 0.9\%$) bags. The percentage IDKn in PICS, ZFDE, ZF2L and ZF1L bags were relatively low with values of approximately 2.0% and below.

Table 4.3. Effect of hermetic and non-hermetic storage bags on infestation and damage by insect pests

| | Bag type | Live <i>S. zeamais</i> | Dead <i>S. zeamais</i> | Live <i>P. truncates</i> | Dead <i>P. truncatus</i> | Live <i>T. castaneum</i> | Dead <i>T. castaneum</i> |
|---------------------|----------------------------|---------------------------|---------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Non-hermetic | PP | 42.7 ± 9.3 | 12.0 ± 2.1 | 9.7 ± 2.7 | 2.0 ± 1.5 | 5.0 ± 1.0 | 1.7 ± 0.3 |
| | PPDE | 24.0 ± 5.1 | 14.7 ± 4.3 | 5.3 ± 2.6 | 0.7 ± 0.3 | 4.3 ± 2.4 | 0.3 ± 0.3 |
| | ZF | 33.0 ± 4.5 | 46.0 ± 12.7 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | ZFDE | 1.7 ± 0.3 | 19.3 ± 0.3 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Hermetic | PICS | 3.3 ± 0.9 | 18.7 ± 1.2 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | PP1L | 5.3 ± 0.9 | 22.7 ± 1.5 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | ZF2L | 3.7 ± 0.9 | 19.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | ZF1L | 4.3 ± 0.3 | 21.7 ± 1.5 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | F_(7, 16) | 15.33* | 4.54* | 10.66* | 1.53^{ns} | 5.55* | 7.95* |

Each datum represent mean total of insects contained in three replicates of maize samples

PP = Polypropylene; PPDE = Polypropylene plus Diatomaceous Earth; ZF = ZeroFly; ZFDE = ZeroFly plus Diatomaceous Earth; PICS = Purdue Improved Crop Storage; PP1L = Polypropylene single liner; ZF2L = ZeroFly double liner; ZF1L = ZeroFly single liner

^{ns} $P > 0.05$; * $P < 0.05$; ns = not significant

Table 4.4. Effect of infestation by *P. truncatus* and *S. zeamais* on damage and weight loss of stored maize

| | Bag type | <i>P. truncatus</i> infested maize | | | <i>S. zeamais</i> infested maize | | |
|---------------------|----------------------------|------------------------------------|---------------|---------------|----------------------------------|---------------|----------------|
| | | % IDKn | % IDKw | % WL | % IDKn | % IDKwb | % WL |
| Non-hermetic | PP | 34.6 ± 2.3 | 27.8 ± 1.6 | 22.1 ± 2.5 | 21.7 ± 1.4 | 16.5 ± 1.0 | 11.0 ± 0.7 |
| | PPDE | 35.8 ± 3.4 | 27.9 ± 3.2 | 21.1 ± 2.2 | 22.7 ± 1.6 | 17.8 ± 1.4 | 12.7 ± 0.9 |
| | ZF | 12.3 ± 1.2 | 7.3 ± 1.3 | 5.9 ± 0.9 | 13.5 ± 2.1 | 9.5 ± 1.0 | 8.3 ± 0.4 |
| | ZFDE | 3.2 ± 1.1 | 1.1 ± 0.9 | 0.8 ± 0.4 | 1.6 ± 0.4 | 1.1 ± 0.1 | 0.3 ± 0.2 |
| Hermetic | PICS | 4.9 ± 0.1 | 2.5 ± 0.2 | 1.3 ± 0.2 | 2.1 ± 0.3 | 1.4 ± 0.3 | 0.2 ± 0.0 |
| | PP1L | 28.1 ± 2.2 | 20.7 ± 3.3 | 14.8 ± 2.4 | 10.8 ± 0.9 | 7.2 ± 0.8 | 3.6 ± 0.6 |
| | ZF2L | 4.8 ± 0.5 | 1.6 ± 0.4 | 1.7 ± 0.8 | 1.7 ± 0.3 | 0.7 ± 0.3 | 0.2 ± 0.0 |
| | ZF1L | 6.0 ± 1.4 | 3.5 ± 0.7 | 2.5 ± 0.5 | 1.7 ± 0.3 | 1.1 ± 0.7 | 0.2 ± 0.0 |
| | F_(7, 16) | 62.49* | 42.55* | 35.05* | 67.19* | 84.69* | 127.90* |

Each datum represent mean total of insects contained in three replicates of maize sample

PP = Polypropylene; PPDE = Polypropylene plus Diatomaceous Earth; ZF = ZeroFly; ZFDE = ZeroFly plus Diatomaceous Earth; PICS = Purdue Improved Crop Storage; PP1L = Polypropylene single liner; ZF2L = ZeroFly double liner; ZF1L = ZeroFly single liner

In all cases **P* < 0.05

4.2.4 Effect of improved storage bags and DE on the weight loss of maize stored in the laboratory

In the *P. truncatus* infested maize, PP (22.1%), PPDE (21.1%) and PP1L (14.8%) bags had the highest weight loss values (Table 4.4). The ZF bag had a low %weight loss value of $5.9 \pm 0.9\%$. Lower weight losses of maize were found in ZFDE, PICS, ZF2L and ZF1L bags with mean values of $0.8 \pm 0.4\%$, $1.3 \pm 0.2\%$, $1.7 \pm 0.8\%$ and $2.5 \pm 0.5\%$, respectively (Table 4.4).

In the *S. zeamais* infested maize, high weight loss values were recorded in PPDE, PP and ZF bags (Table 4.4). The values were 12.7 ± 0.9 , 11.0 ± 0.7 and $8.3 \pm 0.4\%$ weight losses, respectively. Low weight losses were found in ZFDE, PICS, ZF2L, PP1L and ZF1L bags and ranged between 0.1 to 4.0%, respectively.

4.2.4 Insect damage on bags

The outer PPDE bags of *P. truncatus* infested maize recorded the highest mean insect penetration of 42.7 ± 4.7 , compared to PP (35.1 ± 5.3) and PP1L (21.4 ± 2.1) bags (Table 4.5). No holes were however found on the outer bags of all the other storage bags except ZF1L bag with a single hole on its outer bag. Insect holes on the inner HDPE liners of PP1L and ZF1L bags were highest having 25.3 ± 3.3 and 14.3 ± 3.0 holes, respectively relative to PICS and ZF2L bags with number of holes not exceeding 2.

In the case of *S. zeamais* infestation, insect penetrations were only found in PP and PPDE bags with values of 13.1 ± 0.6 and 11.4 ± 1.0 holes, respectively (Table 4.5). No insect hole was found on all the other bag types. In case of the inner liners, no insect hole was found in any of the hermetic liner bags.

4.3 Storehouse study of stored maize in bags

4.3.1 Insect infestation level

Four insect species namely *S. zeamais*, *T. castaneum*, *C. ferrugineus* and *Liposcelis* spp were found infesting the stored maize contained inside the different storage treatment bags over the 12 months storage period. The total insect species population ($n = 5,945$) featured the predominant and primary pest; *S. zeamais* (2,953), followed by secondary pests; *T. castaneum* (1,298), *Liposcelis* spp. (1,193) and *C. ferrugineus* (861) (Fig. 4.1).

Table 4.5. Mean (\pm SE) insect holes in the inner HDPE liners and outer woven mini bags of stored maize

| Bag type | | <i>P. truncatus</i> infested maize | | <i>S. zeamais</i> infested maize | |
|---------------------|-------------|------------------------------------|-------------------------|----------------------------------|-------------------------|
| | | Holes on outer woven bag | Holes on inner HDPE bag | Holes on outer woven bag | Holes on inner HDPE bag |
| Non-hermetic | PP | 35.1 \pm 5.3c | N/A | 13.1 \pm 0.6b | N/A |
| | PPDE | 42.7 \pm 4.7c | N/A | 11.4 \pm 1.0b | N/A |
| | ZF | 0.0 \pm 0.0a | N/A | 0.0 \pm 0.0a | N/A |
| | ZFDE | 0.0 \pm 0.0a | N/A | 0.0 \pm 0.0a | N/A |
| Hermetic | PICS | 0.0 \pm 0.0a | 1.0 \pm 0.5a | 0.0 \pm 0.0a | 0.0 \pm 0.0a |
| | PP1L | 21.4 \pm 2.1b | 25.3 \pm 3.3b | 0.0 \pm 0.0a | 0.0 \pm 0.0a |
| | ZF2L | 0.0 \pm 0.0a | 0.0 \pm 0.0c | 0.0 \pm 0.0a | 0.0 \pm 0.0a |
| | ZF1L | 1.0 \pm 1.0a | 14.3 \pm 3.0b | 0.0 \pm 0.0a | 0.0 \pm 0.0a |

Means within the same column followed by different letters are significantly different ($p < 0.05$).

PP = Polypropylene; PPDE = Polypropylene plus Diatomaceous Earth; ZF = ZeroFly; ZFDE = ZeroFly plus Diatomaceous Earth; PICS = Purdue Improved Crop Storage; PP1L = Polypropylene single liner; ZF2L = ZeroFly double liner; ZF1L = ZeroFly single liner.

N/A = value not applicable

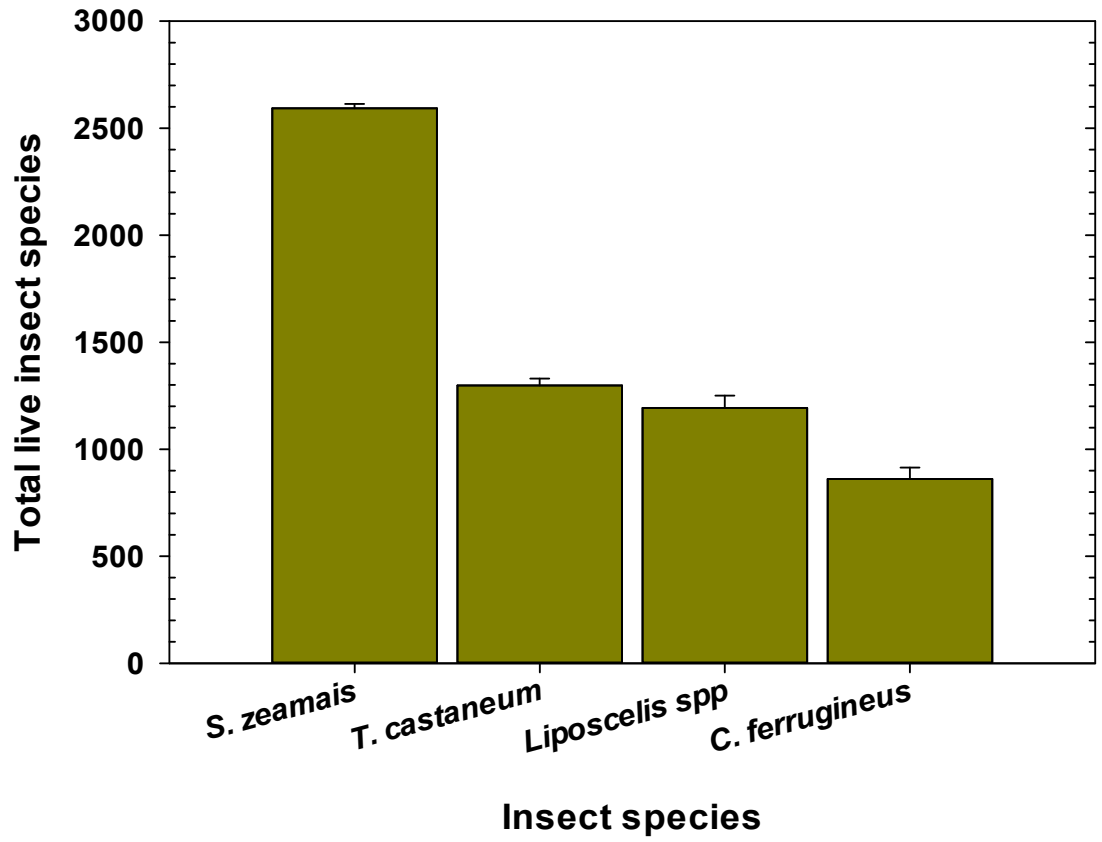


Figure 4.1. Density of insect population recovered by species at 12-month storage period

4.3.1.1 Infestation by *Sitophilus zeamais*

The infestation levels recorded for *S. zeamais* in the different treatments were different during the months when sampling was conducted. Significant differences were recorded in the population of live insects ($F = 12.99$, $df = 7,184$, $p < 0.05$) and dead insects ($F = 8.96$, $df = 7,184$, $p < 0.05$) among all treatments.

In the non-hermetic bags, live *S. zeamais* was not found in maize samples collected during the first 2 months of storage (Fig. 4.2). Initial detection of live *S. zeamais* in PP and ZF bag treatments was during the third and fourth months of storage, respectively (Fig. 4.2), but the number inside ZF bags increase significantly than in PP at 5 months of storage. Number of live weevil in the PP bag treatment increased consistently from the third month (0.7 ± 0.3) to the 12th month (52.0 ± 2.6) (Fig. 4.2). In ZF bag treatment, weevil infestation increased from a mean value of 0.3 ± 0.3 to 124.0 ± 15.5 over 4th and 9th month of storage but this number decreased to 54.3 ± 6.9 after 12th month (Fig. 4.2). Inside the PPDE and ZFDE maize inspected monthly, no weevil was found throughout the initial eight months of storage. However in ZFDE bags, population of living weevil was minimal and an average of 18.1 ± 3.6 insect was recorded after 12 month. In the case of PPDE treatment, the population increased to 30.7 ± 3.7 on termination of the experiment after storage time of 12 months.

Conversely, the numerical population of dead weevil was the highest in ZeroFly (ZF) bags amongst all the treatments assessed except during the 9th month in PP bag, where a greater amount of dead insects were recorded (Fig. 4.3). Between 8th and 12th months of storage, dead *S. zeamais* increased from an average of 11.0 ± 1.2 to 33 ± 3.2 per sample in ZF bags, respectively.

Among the hermetic bags, very few number of live *S. zeamais* were collected and did by no means exceeded an average of 4 insects in any of the four treatments (Fig. 4.4). For PICS bags, a mean of 3.7 ± 1.0 alive weevil per inspected maize were collected in 12 months for which storage lasted (Fig. 4.4). Mean population of dead weevil recorded was insignificant having the value of ≤ 1 in all hermetic bags inspected for duration of storage (Fig. 4.5).

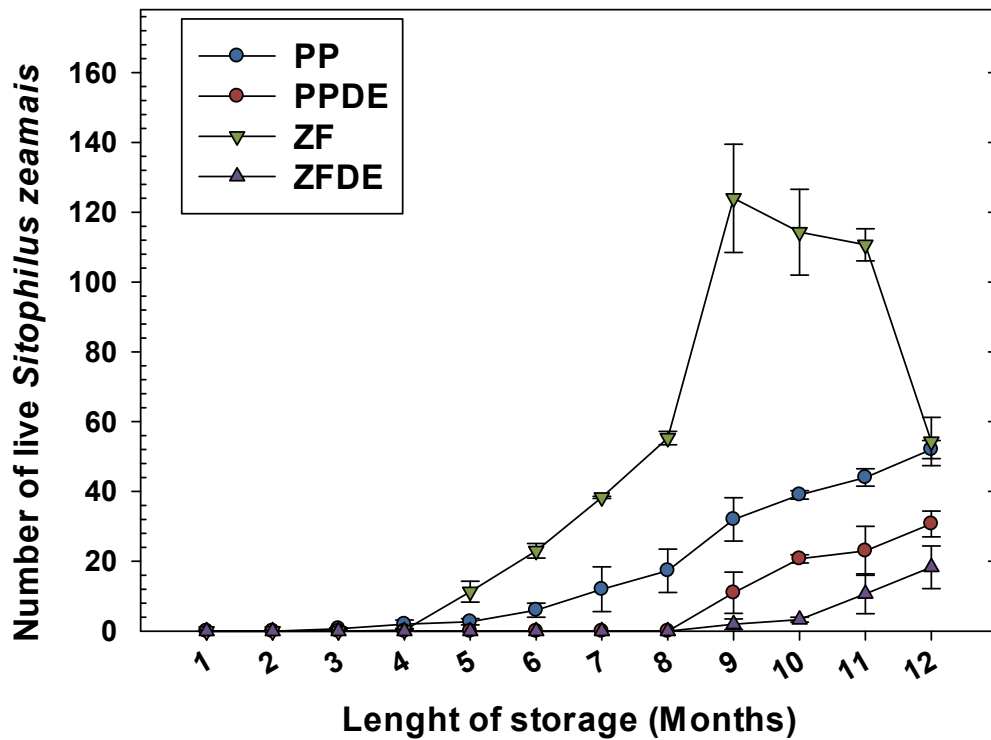


Figure 4.2. Monthly mean (\pm SE) of live *Sitophilus zeamais* per bag

PP = Polypropylene; PPDE = Polypropylene plus Diatomaceous Earth; ZF = ZeroFly; ZFDE = ZeroFly plus Diatomaceous Earth.

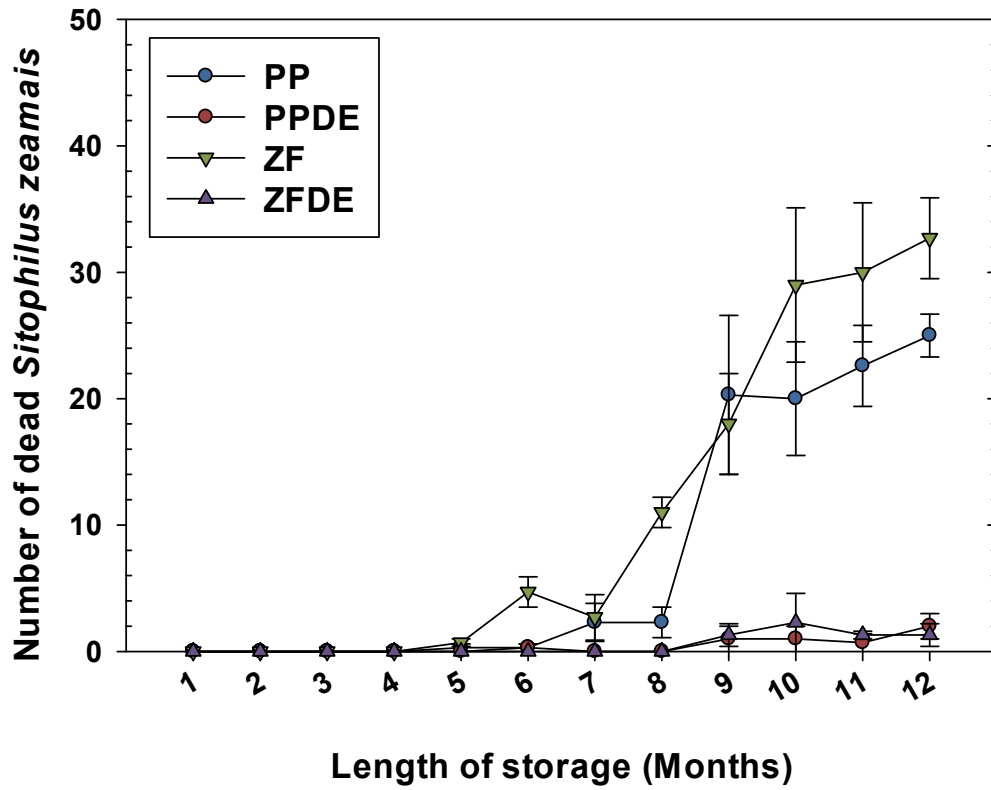


Figure 4.3. Monthly mean (\pm SE) of dead *Sitophilus zeamais* per bag

PP = Polypropylene; PPDE = Polypropylene plus Diatomaceous Earth; ZF = ZeroFly; ZFDE = ZeroFly plus Diatomaceous Earth.

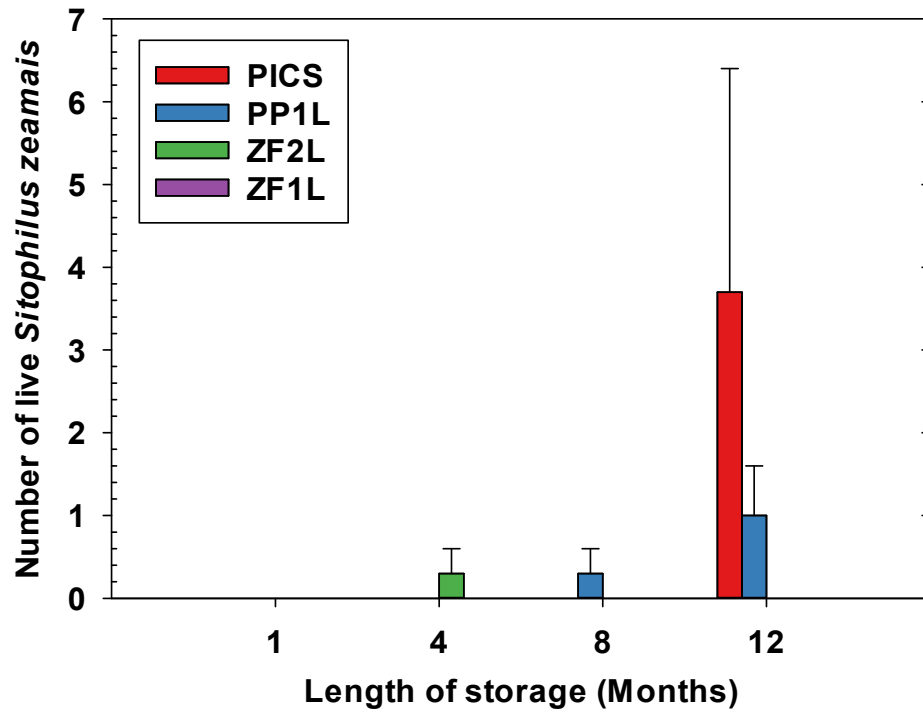


Figure 4.4. Quarterly mean (\pm SE) of live *Sitophilus zeamais* per bag

PICS = Purdue Improved Crop Storage; PP1L = Polypropylene single liner; ZF2L = ZeroFly double liner; ZF1L = ZeroFly single liner.

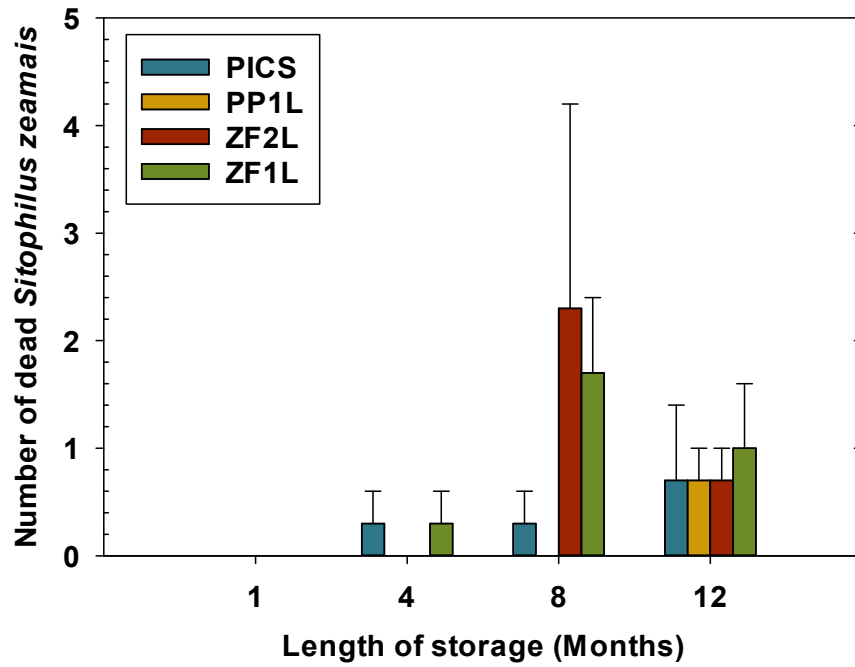


Figure 4.5. Quarterly mean (\pm SE) of dead *Sitophilus zeamais* per bag

PICS = Purdue Improved Crop Storage; PP1L = Polypropylene single liner; ZF2L = ZeroFly double liner; ZF1L = ZeroFly single liner.

4.3.1.2 Infestation by *Tribolium castaneum*

Adults of *T. castaneum* and their population pattern found in the different treatments were different during the months when sampling was conducted. Significant differences were found in the population of live insects ($F = 18.48$, $df = 7,18$, $p < 0.05$) and dead insects ($F = 5.62$, $df = 7,18$, $p < 0.05$) among all the treatments.

Inside the PP treatment, mean number of live flour beetles increased with storage period from the third month (1.0 ± 0.6) to the 8th month (30.2 ± 4.8) (Fig. 4.6). However, 9th to 12th month period recorded higher population of live *T. castaneum* from 57.3 ± 17.4 to 61.7 ± 9.8 in PP bag treatment. For the PPDE bag treatment, fewer live insects were found in the 6th month, having mean population of 5.3 ± 4.8 (Fig. 4.6). This low number was maintained during the months of storage and after 12 months, where mean live *T. castaneum* reduced to 3.0 ± 1.2 in PPDE treatment. For ZF bag, average population of live insects between the first and 11th month period was below 1.0 but population increased to 2.0 ± 2.0 after 12th storage month (Fig. 4.6). ZFDE bag treatment recorded no *T. castaneum* per sample during the entire storage months (Fig. 4.6).

The number of dead *T. castaneum* in PP bags consistently increased until 6th month where 5.7 ± 3.0 insects were recorded but decreased to 4.7 ± 2.4 in the 8th month (Fig. 4.7). After 12 months storage period, dead insects increased to 11.0 ± 1.7 . Fewer dead insects were recorded in the PPDE, ZF, and ZFDE bag treatments between the 6th and 12th months with 2.7 ± 1.8 , 0.3 ± 0.3 , and 0.3 ± 0.3 , respectively (Fig. 4.7).

However for hermetic bags, live population of red flour beetles (RFB) were low and was not more than a mean of 6 in any of the four treatments all through the period which storage lasted (Fig. 4.8). The average sum of live flour beetle in PICS bags rose from 3.7 ± 0.3 in the 8th month to 6.0 ± 2.7 in each inspected bag at the last quarter of sampling (Fig. 4.8). In PP1L, ZF2L and ZF1L, mean live number were 2.0 ± 0.6 , 1.0 ± 0.6 and 2.0 ± 1.5 species, respectively after 12 months of storage.

There was no dead *T. castaneum* found in the entire set up through the first month of sampling in February, however the numerical mean of dead flour beetles per PP1L and PICS bags were highest out of all hermetic treatments during the 8th month with 10.3 ± 5.8 and 6.7 ± 5.2 , respectively (Fig. 4.9). After duration of storage, dead insect insect number was low and did not exceed a mean of 6.7 *T. castaneum* in any of the bag treatment.

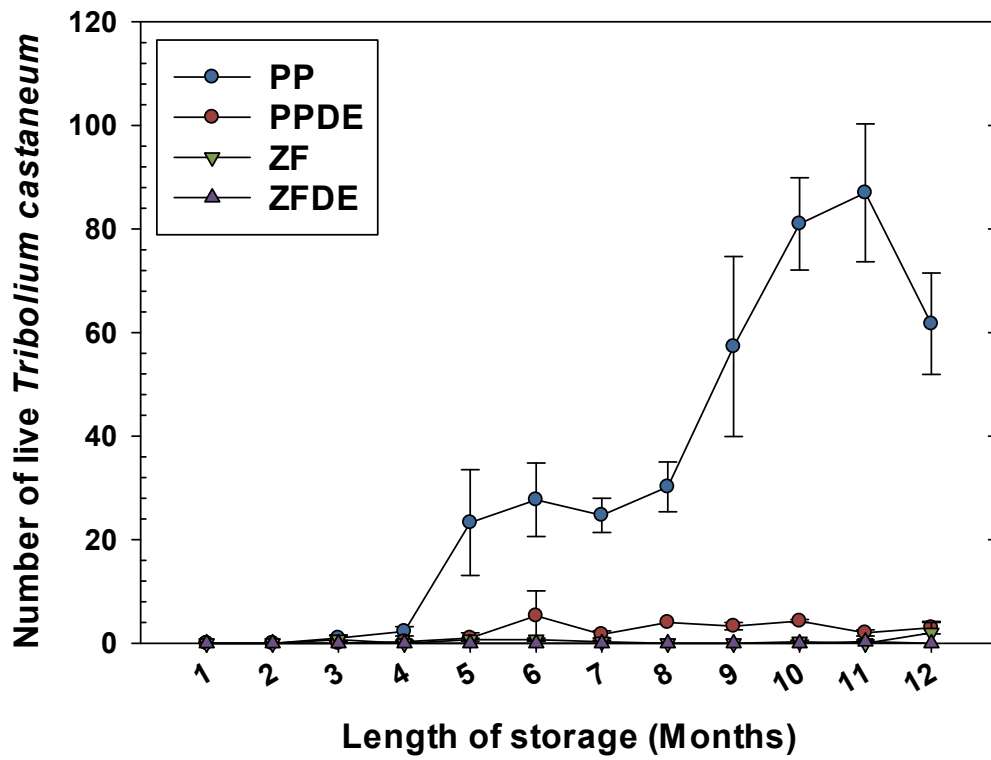


Figure 4.6. Monthly mean (\pm SE) of live *Tribolium castaneum* per bag

PP = Polypropylene; PPDE = Polypropylene plus Diatomaceous Earth; ZF = ZeroFly;
 ZFDE = ZeroFly plus Diatomaceous Earth

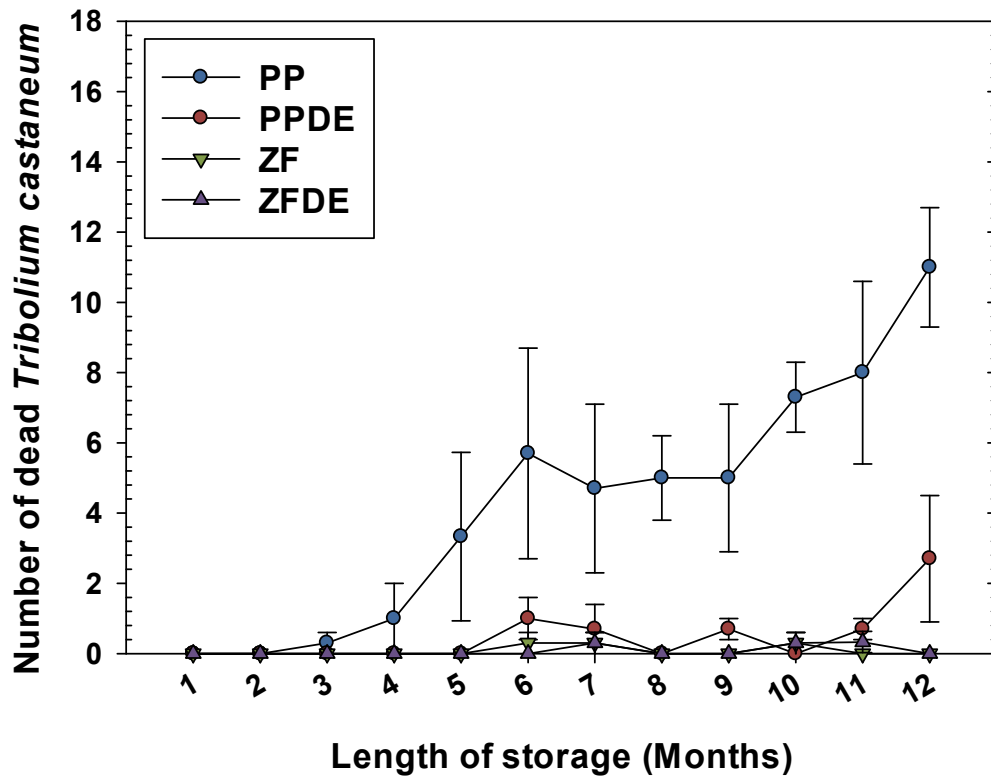


Figure 4.7. Monthly mean (\pm SE) of dead *Tribolium castaneum* per bag

PP = Polypropylene; PPDE = Polypropylene plus Diatomaceous Earth; ZF = ZeroFly; ZFDE = ZeroFly plus Diatomaceous Earth.

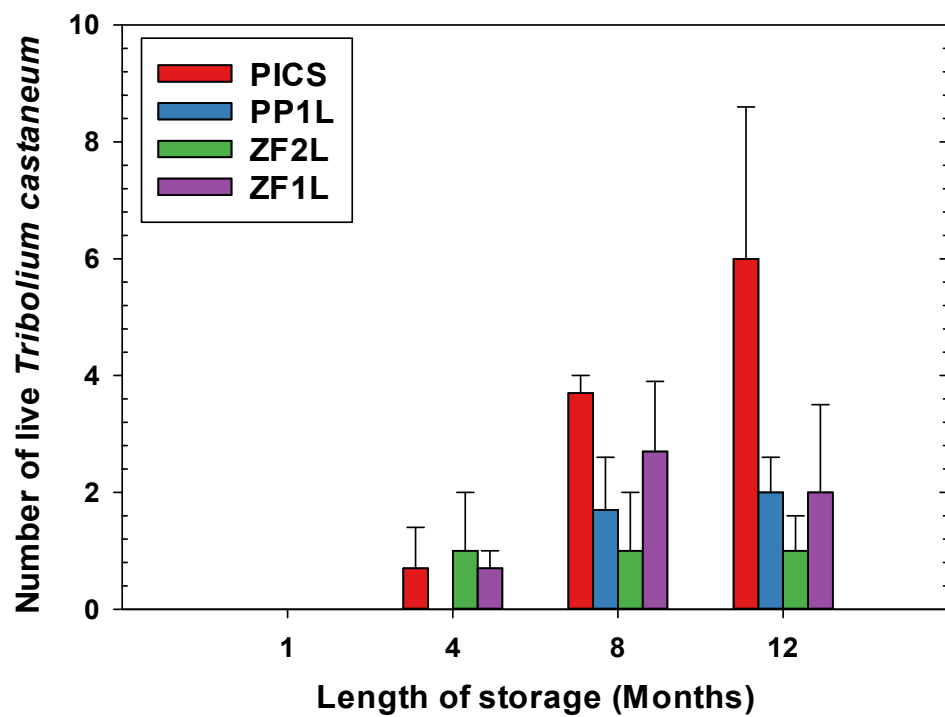


Figure 4.8. Quarterly mean (\pm SE) of live *Tribolium castaneum* per bag

PICS = Purdue Improved Crop Storage; PP1L = Polypropylene single liner; ZF2L = ZeroFly double liner; ZF1L = ZeroFly single liner.

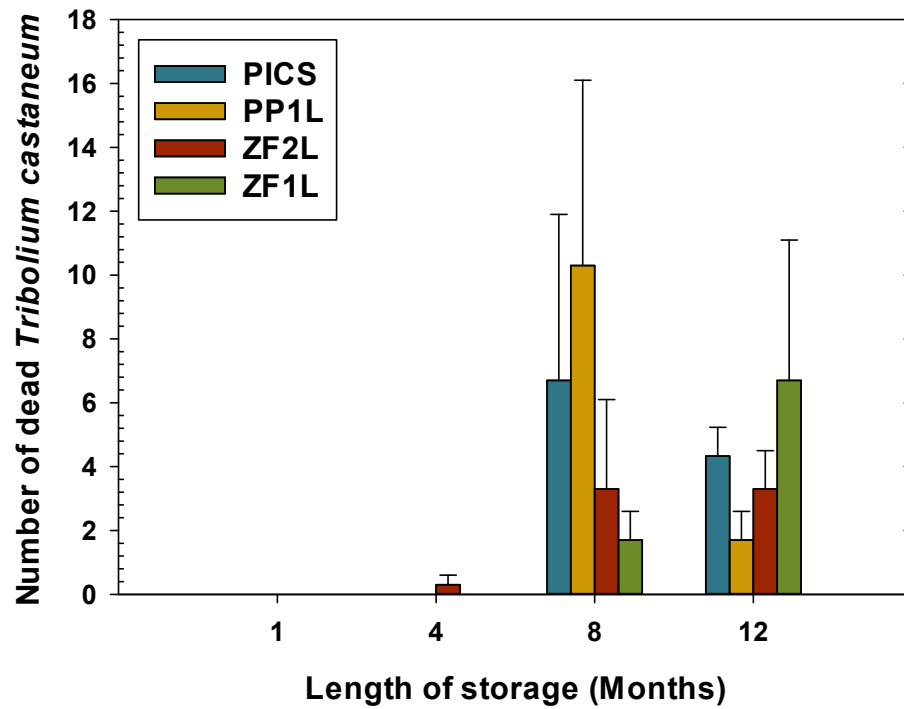


Figure 4.9. Quarterly mean (\pm SE) of dead *Tribolium castaneum* per bag

PICS = Purdue Improved Crop Storage; PP1L = Polypropylene single liner; ZF2L = ZeroFly double liner; ZF1L = ZeroFly single liner

4.3.1.3 Infestation by *Liposcelis* spp

The population of psocids collected in the different treatments varied with storage bags and length of storage during the months when sampling was conducted (Table 4.6). Significant differences were detected in the number of live insects ($F = 15.2$, $df = 7,18$, $p < 0.05$) among the non-hermetic treatments. In the non-hermetic treatments, number of live insects were not consistent and there were constant fluctuations in their mean population (Table 4.6). The mean number of psocids seen within ZF bag-stored maize decreased in the 4th month (30.3 ± 10.2) to 12th month (19.7 ± 7.9) when the study terminated (Fig. 4.10). For PPDE bag treatment, the mean number of live psocids increased consistently from 1.7 ± 1.7 in 3rd month to 20.3 ± 3.5 in the 7th month but decreased to 12.0 ± 6.2 after 12 month (Fig. 4.10). The mean total of psocids in the ZFDE bag treatment reduced from 29.0 ± 1.2 to 15.3 ± 3.5 between 6th and 12th months of storage (Table 4.6) and whereas in the PP bag treatment, the mean population of live psocids increased during the 3rd and 6th months from 4.7 ± 2.9 to 6.0 ± 2.6 insects (Table 4.6).

On the contrary, there was no dead *Liposcelis* spp reported in all the non-hermetic treatment throughout storage months.

Similarly, no live or dead *Liposcelis* spp was found in any of the hermetic bag treatments throughout storage.

4.3.1.4 Infestation by *Cryptolestes ferrugineus*

The monthly mean total of live adult *C. ferrugineus* is presented in Fig. 4.10. Significant differences were found in the number of live insects ($F = 9.84$, $df = 7,18$, $p < 0.05$) and dead insects ($F = 4.04$, $df = 7,18$, $p < 0.05$) among the non-hermetic bag treatments. There was absence of live *C. ferrugineus* in any of the non-hermetic treatment samples at the initial 4 months of storage (Fig. 4.10).

The mean population of live *C. ferrugineus* within ZF bag-stored maize increased significantly from the 5th month (1.7 ± 1.2) to 12th month (69.3 ± 10.7) (Fig. 4.10). The ZF bag had the highest population of *C. ferrugineus* recorded. In the PP bag treatments, fewer live *C. ferrugineus* was recorded with 6.0 ± 1.2 after the 12th month when the study was terminated.

Table 4.6: Monthly mean (\pm SE) of live *Liposcelis* spp per bag

| Storage month | <i>Liposcelis</i> spp population per bag type | | | |
|---------------|---|----------------|-----------------|----------------|
| | PP | PPDE | ZF | ZFDE |
| 1 | 0.0 \pm 0.0 | 0.0 \pm 0.0 | 0.0 \pm 0.0 | 0.0 \pm 0.0 |
| 2 | 0.0 \pm 0.0 | 0.0 \pm 0.0 | 0.0 \pm 0.0 | 0.0 \pm 0.0 |
| 3 | 4.7 \pm 2.9 | 1.7 \pm 1.7 | 5.7 \pm 1.2 | 0.7 \pm 0.3 |
| 4 | 0.0 \pm 0.0 | 8.0 \pm 3.1 | 30.3 \pm 10.2 | 0.7 \pm 0.7 |
| 5 | 1.0 \pm 1.0 | 10.7 \pm 1.5 | 16.3 \pm 0.9 | 11.3 \pm 0.9 |
| 6 | 0.0 \pm 0.0 | 18.0 \pm 3.8 | 11.0 \pm 1.2 | 29.0 \pm 1.2 |
| 7 | 0.0 \pm 0.0 | 20.3 \pm 3.5 | 3.7 \pm 2.0 | 24.3 \pm 3.8 |
| 8 | 0.0 \pm 0.0 | 8.0 \pm 8.0 | 0.0 \pm 0.0 | 20.3 \pm 3.7 |
| 9 | 1.0 \pm 1.7 | 17.3 \pm 3.7 | 10.7 \pm 3.7 | 12.3 \pm 1.9 |
| 10 | 0.0 \pm 0.0 | 10.7 \pm 5.6 | 5.3 \pm 3.2 | 12.7 \pm 1.5 |
| 11 | 0.0 \pm 0.0 | 14.7 \pm 2.3 | 15.3 \pm 2.7 | 7.7 \pm 1.2 |
| 12 | 6.0 \pm 2.6 | 12.0 \pm 6.2 | 19.7 \pm 7.9 | 15.3 \pm 3.5 |

PP = Polypropylene; PPDE = Polypropylene plus Diatomaceous Earth; ZF = ZeroFly; ZFDE = ZeroFly plus Diatomaceous Earth.

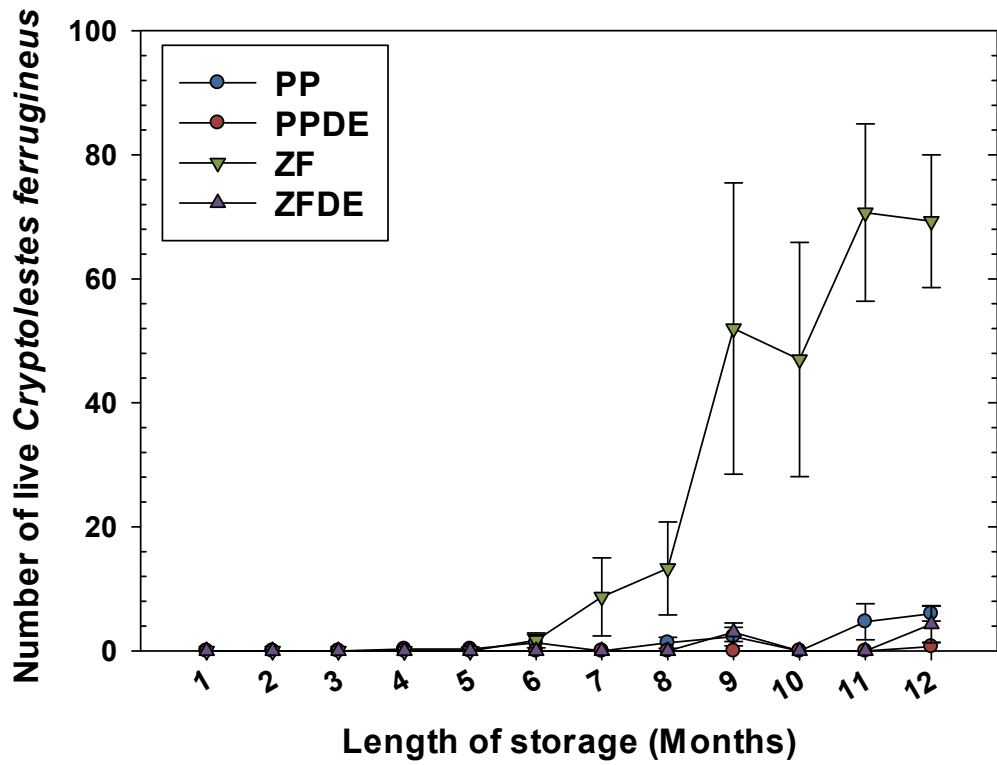


Figure 4.10. Monthly mean (\pm SE) of live *Cryptolestes ferrugineus* per bag

PP = Polypropylene; PPDE = Polypropylene plus Diatomaceous Earth; ZF = ZeroFly; ZFDE = ZeroFly plus Diatomaceous Earth.

As for mean number of dead *C. ferrugineus*, population was generally low in all the non-hermetic treatment bags. At 11th month of storage, the ZF bag treatment recorded the highest mean value of 10.0 ± 1.2 which also decreased to 5.7 ± 0.7 at the final month of storage (Fig. 4.11). The PP treatment had a mean population of 3.0 ± 3.0 during the 10th and 11th months, respectively while the other months in which sampling was conducted, no dead *C. ferrugineus* was found. There were no dead *C. ferrugineus* found in the ZFDE and PPDE during storage months, respectively, until the 12th month when 3.3 ± 3.3 insects were found in PPDE (Fig. 4.11).

On the other hand in each hermetic treatments, neither live nor dead *C. ferrugineus* were present in all inspected bags throughout the duration of storage.

4.3.2 Effect of hermetic and non-hermetic bags on the percentage insect damaged kernels of stored maize

Figure 4.13 shows the pattern for percentage Insect Damaged Kernel by number (% IDKn) found in the different treatments. The %IDKn varied widely during the months when sampling was conducted. Significant differences were found in % IDKn ($F = 9.35$, $df = 7,184$, $p < 0.05$) among all the treatments. In the ZF bag treatment, % IDKn increased consistently and significantly from the second month of storage ($0.2 \pm 0.1\%$) to the 12th month ($16.9 \pm 1.6\%$) end of storage duration (Fig. 4.12). With regards to Polypropylene (PP) bag treatment, a steady rise in % IDKn from $0.1 \pm 0.0\%$ to $5.4 \pm 0.9\%$ between the 2nd and 12th months was recorded. In the PPDE treatment, mean % IDKn decreased from its highest value of 1.9 ± 0.2 in the 9th month to 1.0 ± 0.4 after 12th month, and while for ZFDE bag treatment, % IDKn did not exceed a mean of 0.8 ± 0.3 after 12th month of storage (Fig. 4.12).

In the hermetic bag treatments, mean % IDKn values were below 1% in any of the four treatments throughout the storage months (Fig. 4.13). However, after 12th month, PICS recorded the highest IDK having $0.9 \pm 0.1\%$ compared to PP1L ($0.6 \pm 0.2\%$), ZF2L (0.4 ± 0.0) and ZF1L (0.5 ± 0.0) (Fig. 4.13).

4.3.3 Effect of hermetic and non-hermetic storage bags on the percentage weight loss of stored maize

The extent of weight losses in stored maize showed significant differences among the bag treatments ($F = 6.19$, $df = 7,184$, $p < 0.05$). Amongst the non-hermetic treatments,

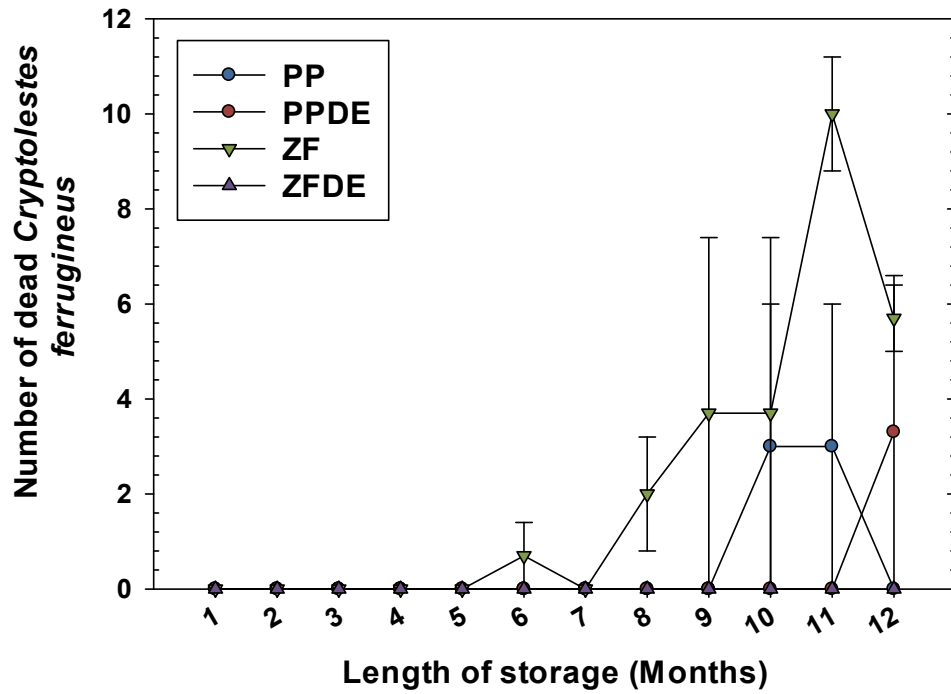


Figure 4.11. Monthly mean (\pm SE) of dead *Cryptolestes ferrugineus* per bag

PP = Polypropylene; PPDE = Polypropylene plus Diatomaceous Earth; ZF = ZeroFly; ZFDE = ZeroFly plus Diatomaceous Earth.

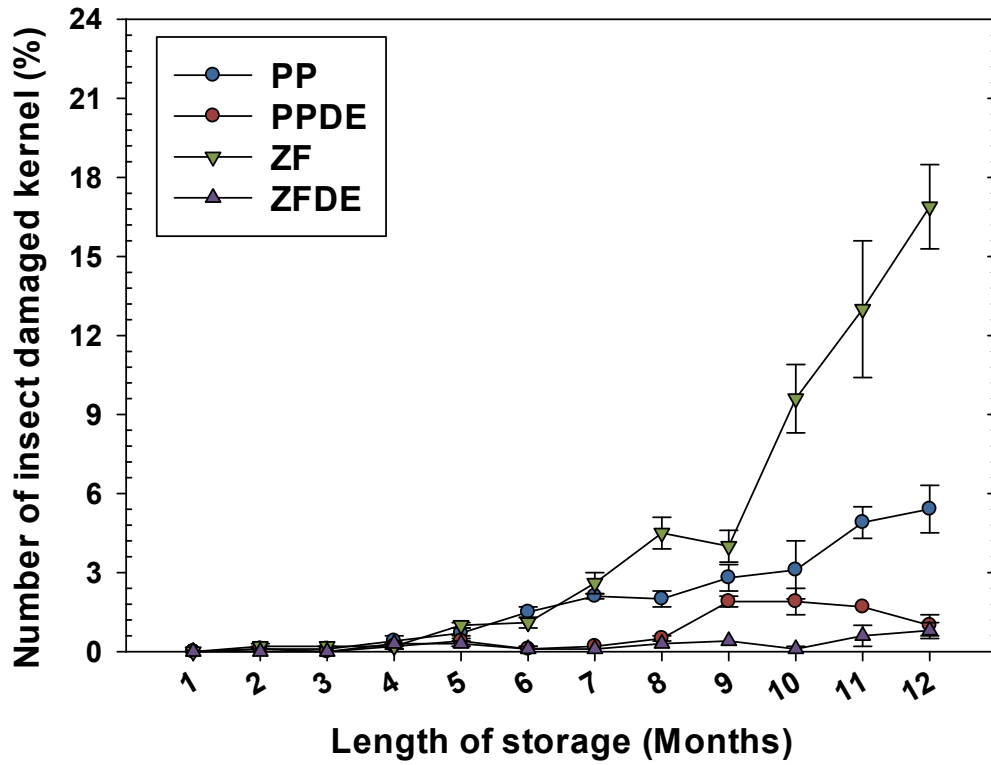


Figure 4.12. Monthly percentage mean (\pm SE) insect damaged kernel per bag

PP = Polypropylene; PPDE = Polypropylene plus Diatomaceous Earth; ZF = ZeroFly; ZFDE = ZeroFly plus Diatomaceous Earth

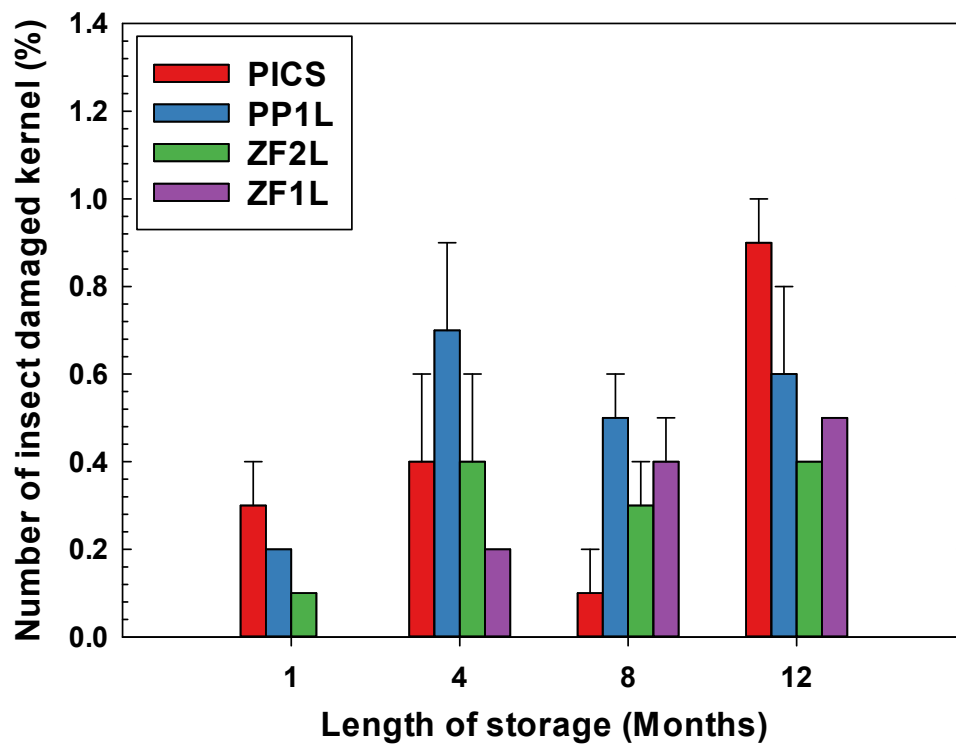


Figure 4.13. Quarterly percentage mean (\pm SE) insect damaged kernel per bag

PICS = Purdue Improved Crop Storage; PP1L = Polypropylene single liner; ZF2L = ZeroFly double liner; ZF1L = ZeroFly single liner.

ZF and PP bags had more % WL; this was clearly observed from the 6th to 12th month of storage. The mean percentage weight loss rose considerably from the 3rd month ($0.1 \pm 0.0\%$) to the last month of storage ($6.7 \pm 0.8\%$) in ZF bags (Fig. 4.14). In the PP bag treatment, mean percentage weight loss increased steadily from 0.1 ± 0.1 (4th month) to 1.4 ± 0.2 % on completion of storage (Fig. 4.14). For the PPDE treatment, mean % WL was very low until the 9th month when $0.8 \pm 0.1\%$ was recorded and this value decreased to 0.2 ± 0.1 % at the end of storage duration. Similarly, significantly low mean % WL which did not exceed the value of $0.2 \pm 0.1\%$ was recorded throughout the storage months for ZFDE bag treatment (Fig. 4.14).

In the hermetic treatments, significantly low % WL was found in all the treatment bags during sampling month (Fig. 4.15). The PP1L, ZF2L and ZF1L bags mostly had mean % WL value as low as $0.1 \pm 0.0\%$ throughout the storage months whereas loss was $0.2 \pm 0.0\%$ on the average in the PICS bag treatment at 12th months of preservation (Fig. 4.15).

4.3.4 Effect of hermetic and non-hermetic bags on percentage grain viability of stored maize

Significant differences existed in the percentage maize viability among all treatments ($F = 3.33$, $df = 7,184$, $p < 0.05$) after storage period. Proportion of germinating grains in ZF bags progressively decreased during storage months and a mean value of $87.0 \pm 2.3\%$ was recorded at the 12th month (Table 4.7). This value was relatively low compared to PP, PPDE and ZFDE which had 91.3 ± 0.3 , 92.7 ± 1.9 and $93.3 \pm 0.9\%$ viability rates, respectively after 12 months of storage (Table 4.7). In the non-hermetic treatments, the mean germination rate after 12 months storage period was $91.1 \pm 1.0\%$. This mean value was low compared to the average value of $97.5 \pm 0.2\%$ obtained for the preliminary mean viability for the entire treatments in at the first month of storage.

In the hermetic bag treatments, the average viability rate after 12 months of storage was $97.0 \pm 0.0\%$. This value was not significantly different from the viability value obtained at the beginning of the experiment (Table 4.8). The minimum average percentage grain viability recorded was not below 96.3% in any of the hermetic treatments, respectively (Table 4.8).

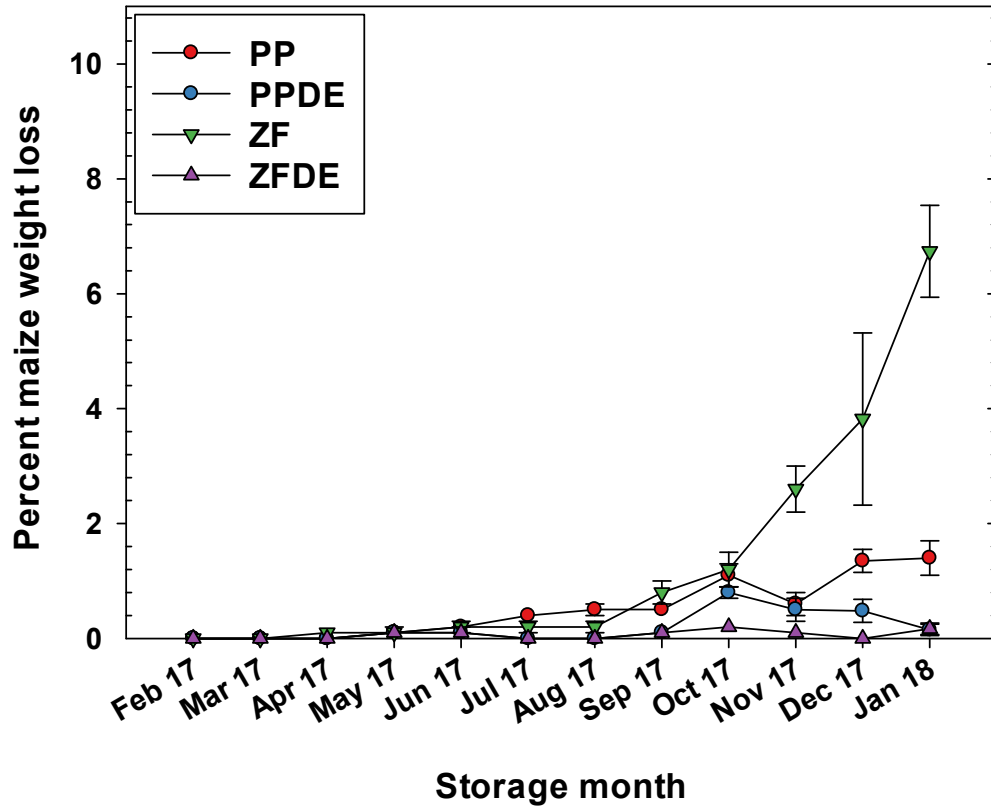


Figure 4.14. Monthly percentage mean (\pm SE) maize weight loss per bag

PP = Polypropylene; PPDE = Polypropylene plus Diatomaceous Earth; ZF = ZeroFly; ZFDE = ZeroFly plus Diatomaceous Earth.

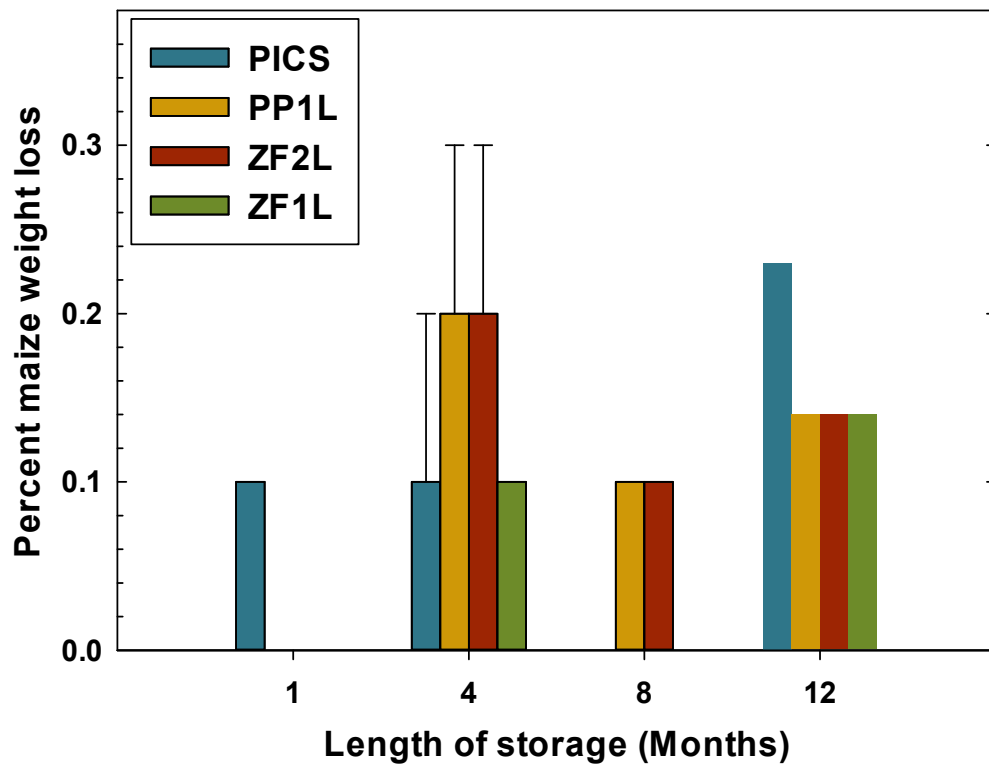


Figure 4.16. Quarterly mean (\pm SE) of percentage maize weight loss per bag

PICS = Purdue Improved Crop Storage; PP1L = Polypropylene single liner; ZF2L = ZeroFly double liner; ZF1L = ZeroFly single liner.

Table 4.7: Monthly percentage mean (\pm SE) grain viability per non-hermetic bag.

| Storage month | Grain viability (%) per bag type | | | |
|---------------|----------------------------------|-----------------------------|------------------------------|-----------------------------|
| | PP | PPDE | ZF | ZFDE |
| 1 | 97.9 \pm 0.9 ^a | 97.9 \pm 0.9 ^a | 97.9 \pm 0.9 ^a | 97.9 \pm 0.9 ^a |
| 2 | 100.0 \pm 0.0 ^a | 99.7 \pm 0.3 ^a | 99.3 \pm 0.7 ^a | 98.0 \pm 1.0 ^a |
| 3 | 99.0 \pm 0.6 ^a | 96.3 \pm 0.9 ^a | 98.3 \pm 0.6 ^a | 96.3 \pm 0.3 ^a |
| 4 | 96.0 \pm 0.6 ^a | 91.0 \pm 2.0 ^b | 96.3 \pm 1.5 ^a | 96.3 \pm 1.2 ^a |
| 5 | 99.7 \pm 0.3 ^a | 97.0 \pm 1.2 ^a | 98.3 \pm 0.9 ^a | 98.0 \pm 1.0 ^a |
| 6 | 95.3 \pm 0.9 ^a | 94.0 \pm 2.5 ^a | 95.7 \pm 0.3 ^a | 97.3 \pm 0.9 ^a |
| 7 | 99.3 \pm 0.3 ^a | 97.3 \pm 0.7 ^a | 98.3 \pm 0.3 ^a | 97.3 \pm 0.7 ^a |
| 8 | 96.3 \pm 1.3 ^a | 96.7 \pm 1.3 ^a | 95.4 \pm 1.2 ^a | 95.0 \pm 0.6 ^a |
| 9 | 93.0 \pm 0.1 ^b | 94.7 \pm 0.3 ^a | 93.7 \pm 0.9 ^b | 94.7 \pm 0.3 ^a |
| 10 | 93.7 \pm 0.7 ^b | 95.3 \pm 0.3 ^a | 92.3 \pm 1.7 ^b | 94.3 \pm 0.7 ^a |
| 11 | 93.3 \pm 0.7 ^b | 94.0 \pm 0.6 ^a | 91.7 \pm 0.9 ^{bc} | 93.3 \pm 0.5 ^b |
| 12 | 91.3 \pm 0.3 ^b | 92.7 \pm 1.9 ^b | 87.0 \pm 2.3 ^c | 93.3 \pm 0.9 ^b |

Means within the same column followed by different letters are significantly different ($p < 0.05$). PP = Polypropylene; PPDE = Polypropylene plus Diatomaceous Earth; ZF = ZeroFly; ZFDE = ZeroFly plus Diatomaceous Earth.

Table 4.8: Quarterly percentage mean (\pm SE) grain viability per hermetic bag

| Storage month | Grain viability (%) per bag type | | | |
|---------------|----------------------------------|-----------------------------|-----------------------------|------------------------------|
| | PICS | PPIL | ZF2L | ZF1L |
| 1 | 97.9 \pm 0.9 ^a | 97.9 \pm 0.9 ^a | 97.9 \pm 0.9 ^a | 97.9 \pm 0.9 ^a |
| 4 | 96.7 \pm 2.8 ^a | 97.3 \pm 0.3 ^a | 97.7 \pm 0.7 ^a | 97.7 \pm 0.7 ^a |
| 8 | 99.0 \pm 0.0 ^a | 99.0 \pm 0.6 ^a | 98.3 \pm 0.9 ^a | 100.0 \pm 0.0 ^a |
| 12 | 98.3 \pm 0.3 ^a | 96.7 \pm 0.9 ^a | 96.3 \pm 0.9 ^a | 97.7 \pm 0.7 ^a |

Means within the same row followed by different letters are significantly different ($p < 0.05$). PICS = Purdue Improved Crop Storage; PPIL = Polypropylene single liner; ZF2L = ZeroFly double liner; ZF1L = ZeroFly single liner.

4.3.5 Effect of aflatoxin levels on maize stored in hermetic and non-hermetic bags

The aflatoxin (AF) levels in the maize tested varied from undetectable (zero) to 11 µg/kg per sample of a treatment after 12 months of storage (Table 4.9). At the initial aflatoxin test conducted, aflatoxin was not detected in any of the maize sample tested (Table 4.9). Afterwards at 12th month of storage, aflatoxin was found in PP, PPDE, PICS and ZF bag treatments. The average aflatoxin levels in PP was the highest with a value of 5.0 µg/kg and, followed by PPDE and ZF bags with values of 3.7 and 3.0 µg/kg, respectively. Among the hermetic treatments, PICS bag had 2.7 µg/kg whereas aflatoxin was not detected in PP1L, ZF1L and ZF2L bags after storage duration (Table 4.9). The results therefore indicates that there was no significance differences in the aflatoxin level among all the treatment (hermetic and non-hermetic) bags ($t = 1.45$, $df = 11$, $P > 0.05$).

4.3.6 Storehouse and maize microclimatic conditions during storage months

The microclimatic conditions at different positions within the storehouse which comprised within stack, above stack and vent during the 12 months of storage was recorded. For temperature, the value ranged between 26.4 – 30.7, 26.2 – 30.2 and 26.4 – 30.4°C, respectively. These values tallied to averages of 28.8, 28.5 and 28.7°C, respectively. The values for relative humidity varied between 53.3 – 80.8, 53.2 – 81.8 and 52.7 – 81.1 %, respectively. These corresponded to average values of 72.4, 71.6 and 70.6 %, respectively (Table 4.10).

In the non-hermetic treatments, the microclimatic conditons (MC, temperature and RH) of stored maize varied throughout storehouse duration (Fig. 4.16). Mean maize moisture content and relative humidity appears to increase during the period of 4th and 10th month and then decrease at the 11th month while, mean maize temperature was 29.6°C throughout storage months but appears to increase during the 7th to 10th month in ZF treatment compared to other bags (Fig. 4.16). However, the mean temperature for PP (29.1°C), PPDE (29.8°C), ZF (30.8°C) and ZFDE (28.7°C) were recorded in all storage months. For relative humidity among treatments PP (65.6%), PPDE (64.5%), ZF (66.3%) and ZFDE (64.8%), these corresponding values were recorded in all months (Fig. 4.16). Similarly, the corresponding mean moisture content of maize among treatments PP (13.1%), PPDE (13.0%), ZF (13.2%) and ZFDE (13.1%) were recorded in all storage months (Fig. 4.16).

Table 4.9. Initial and final concentrations of aflatoxin in stored maize

| Bag type | Treatment | Initial conc. ($\mu\text{g}/\text{kg}$)^{1,2} | Final conc. ($\mu\text{g}/\text{kg}$)^{1,2} |
|---------------------|------------------|---|---|
| Non-hermetic | PP | 0.0 \pm 0.0 ^a | 5.0 \pm 2.5 ^a |
| | PPDE | 0.0 \pm 0.0 ^a | 3.7 \pm 3.7 ^a |
| | ZF | 0.0 \pm 0.0 ^a | 3.0 \pm 3.0 ^a |
| | ZFDE | 0.0 \pm 0.0 ^a | 0.0 \pm 0.0 ^a |
| Hermetic | PICS | 0.0 \pm 0.0 ^a | 2.7 \pm 2.7 ^a |
| | PP1L | 0.0 \pm 0.0 ^a | 0.0 \pm 0.0 ^a |
| | ZF1L | 0.0 \pm 0.0 ^a | 0.0 \pm 0.0 ^a |
| | ZF2L | 0.0 \pm 0.0 ^a | 0.0 \pm 0.0 ^a |

¹ Zero means the aflatoxin level is below detection limit of the analytical method (1 $\mu\text{g}/\text{kg}$)

² Values are means of three subsamples of each sample.

Means within the same column followed by same letters are not significantly different ($p < 0.05$). PP = Polypropylene; PPDE = Polypropylene plus Diatomaceous Earth; ZF = ZeroFly; ZFDE = ZeroFly plus Diatomaceous Earth; PICS = Purdue Improved Crop Storage; PP1L = Polypropylene single liner; ZF2L = ZeroFly double liner; ZF1L = ZeroFly single liner.

Table 4.10. Monthly mean (\pm SE) storehouse climatic conditions

| Storage Month | SH/Temp (within stack) | SH/Temp (above stack) | SH/Temp (vent) | SH/RH (within stack) | SH/RH (above stack) | SH/RH (vent) |
|----------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| 1 | 30.2 \pm 0.1 | 30.2 \pm 0.1 | 30.5 \pm 0.1 | 59.0 \pm 0.5 | 59.0 \pm 0.5 | 59.1 \pm 1.2 |
| 2 | 30.2 \pm 0.1 | 30.2 \pm 0.1 | 30.4 \pm 0.1 | 58.7 \pm 1.7 | 58.8 \pm 2.7 | 58.2 \pm 2.7 |
| 3 | 30.0 \pm 0.1 | 29.7 \pm 0.1 | 29.9 \pm 0.1 | 69.5 \pm 0.7 | 70.9 \pm 0.7 | 70.6 \pm 0.7 |
| 4 | 29.7 \pm 0.1 | 29.4 \pm 0.1 | 29.8 \pm 0.1 | 71.4 \pm 0.5 | 73.3 \pm 0.3 | 71.9 \pm 0.5 |
| 5 | 29.2 \pm 0.1 | 29.0 \pm 0.1 | 29.2 \pm 0.1 | 74.4 \pm 0.4 | 76.1 \pm 0.4 | 75.3 \pm 0.4 |
| 6 | 27.8 \pm 0.1 | 27.6 \pm 0.1 | 27.9 \pm 0.1 | 77.5 \pm 0.4 | 78.9 \pm 0.5 | 78.0 \pm 0.4 |
| 7 | 26.9 \pm 0.1 | 26.7 \pm 0.1 | 26.9 \pm 0.1 | 80.6 \pm 0.2 | 81.8 \pm 0.3 | 80.7 \pm 0.3 |
| 8 | 26.4 \pm 0.1 | 26.2 \pm 0.1 | 26.4 \pm 0.1 | 80.8 \pm 0.3 | 82.1 \pm 0.3 | 81.1 \pm 0.3 |
| 9 | 27.3 \pm 0.1 | 29.3 \pm 0.1 | 29.5 \pm 0.1 | 79.4 \pm 0.4 | 80.4 \pm 0.4 | 79.4 \pm 0.4 |
| 10 | 31.2 \pm 0.1 | 31.0 \pm 0.1 | 31.3 \pm 0.2 | 75.1 \pm 0.7 | 75.6 \pm 0.8 | 74.4 \pm 0.8 |
| 11 | 32.0 \pm 0.1 | 31.7 \pm 0.1 | 32.0 \pm 0.1 | 69.0 \pm 0.8 | 69.6 \pm 0.9 | 68.4 \pm 0.8 |
| 12 | 31.0 \pm 0.1 | 30.9 \pm 0.1 | 31.0 \pm 0.1 | 53.3 \pm 2.4 | 53.2 \pm 2.5 | 52.7 \pm 2.4 |
| Avg. | 29.3 \pm 0.1 | 29.3 \pm 0.1 | 29.6 \pm 0.1 | 75.2 \pm 0.8 | 71.6 \pm 0.6 | 70.8 \pm 1.0 |

SH – storehouse, Temp – Temperature, RH – relative humidity

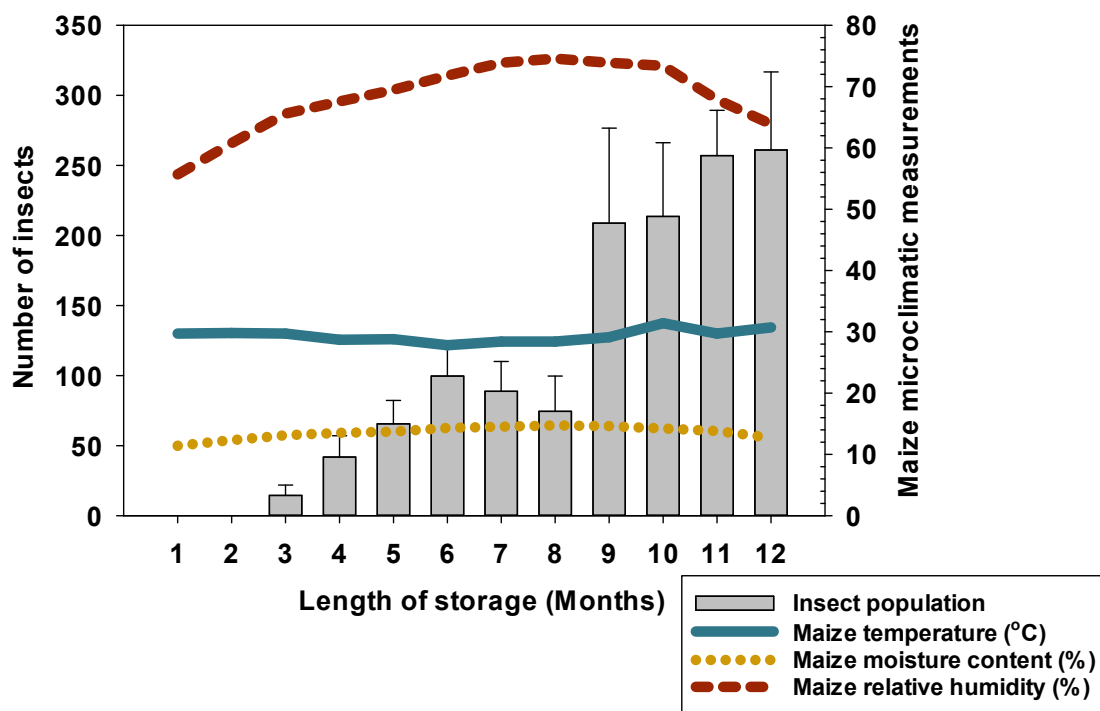


Fig 4.16. Monthly insect population alongside maize moisture content, relative humidity and temperature in the non-hermetic treatments.

In the hermetic treatments, MC, temperature and RH values of stored maize varied throughout the storage time (Fig. 4.17). The mean moisture and air surrounding the stored maize appears to be slightly stable in all storage months while, the mean maize temperature was 28.0°C throughout storage months but appears to decrease in the 8th month and increase again in the 12th month (Fig. 4.17). However, the mean temperature for stored maize in PICS (28.0°C), PP1L (28°C), ZF2L (27.9°C) and ZF1L (28.0°C) were similar in during storage months. For relative humidity of stored maize in PICS (58.9%), PP1L (59.5%), ZF2L (58.3%) and ZF1L (59.2%), these corresponding values were low compared to data obtained in non-hermetic bags which range between 64.5-66.3%. Similarly, the mean moisture content of maize in hermetic treatments PICS (12.0%), PP1L (12.1%), ZF2L (12.0%) and ZF1L (12.2%) were low throughout storage months.

4.3.7 Pesticide residue of maize contained in ZF bag

The initial deltamethrin residue level in maize contained in ZF bags at the start of the experiment was below 0.10 mg/kg. This value was however lower during the final residue test at 12 months of storage to a value of 0.02 mg/kg.

4.3.8 Correlation of insect species and indices measured

Using combined hermetic and non-hermetic bag treatments data and months of storage, there were no relationship between maize micro-climatic conditions; temperature (T), relative humidity (RH), and moisture content (MC) with total insect population (Table 4.8). However, *S. zeamais* had significant positive correlation with temperature ($r = 0.50$, $p < 0.05$) and the number of *Liposcelis* spp correlated significantly with RH ($r = 0.66$, $p < 0.05$) and MC ($r = 0.67$, $p < 0.05$) while, *T. castaneum* and *C. ferrugineus* populations showed no correlation with maize microclimatic conditions (Table 4.8).

Percentage number of insect damaged kernel (% IDKn) tended to increase and correlated significantly with total population of insects ($r = 0.91$, $p < 0.05$) and numbers of *S. zeamais* ($r = 0.88$, $p = 0.05$), *T. castaneum* ($r = 0.89$, $p < 0.05$) and *C. ferrugineus* ($r = 0.95$, $p = 0.05$) (Table 4.11). Also for percentage weight loss (% WL), relationship with all of total population of insects ($r = 0.89$, $p < 0.05$) and numbers of *S. zeamais* ($r = 0.84$, $p < 0.05$), *T. castaneum* ($r = 0.85$, $p = 0.05$) and *C. ferrugineus* ($r = 0.95$, $p < 0.05$) occurred and were positively significant. However, in no case was insect population of *Liposcelis* spp correlated with % IDKn ($r = 0.44$, $p = 0.15$) and % WL ($r = 0.43$, $p =$

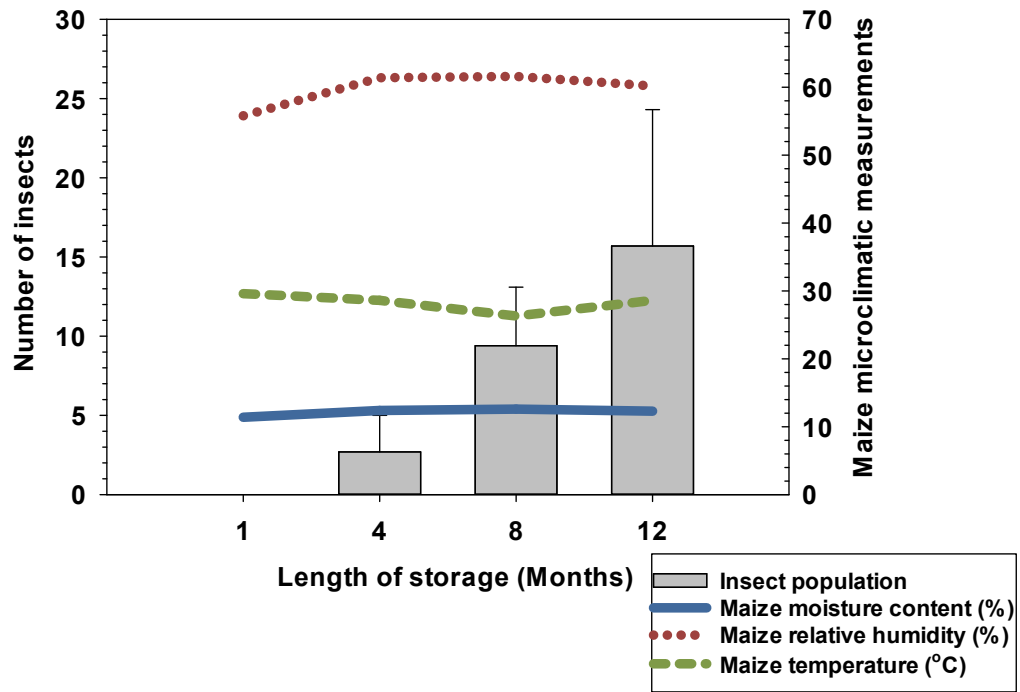


Figure 4.17 Quarterly insect population alongside maize moisture level, relative humidity and temperature in the hermetic treatments.

Table 4.11. Correlation of insect populations and indices estimated

| Indices/Insect population | TEMP | RH | MC | % IDKn | % WL | % VIAB | AF |
|----------------------------------|-------------|-----------|-----------|---------------|-------------|---------------|-----------|
| Sz | 0.50 | 0.40 | 0.39 | 0.88* | 0.84* | -0.87* | 0.34 |
| Tc | 0.35 | 0.46 | 0.44 | 0.89* | 0.85* | -0.85* | 0.38 |
| Cf | 0.38 | 0.18 | 0.18 | 0.95* | 0.95* | -0.86* | 0.57 |
| Lp | -0.40 | 0.66* | 0.67* | 0.44 | 0.47 | -0.51 | 0.32 |
| Total insect | 0.29* | 0.43* | 0.42* | 0.91* | 0.89* | -0.87* | 0.49* |

Sz = *Sitophilus zeamais*; *Tc* = *Tribolium castaneum*; *Cf* = *Cryptolestes ferrugineus*; *Lp* = *Liposcelis* spp; TEMP = Temperature; RH = Relative humidity; MC = Moisture content; %IDKn = Percentage number of insect damaged kernel; % WL = Percentage weight loss; % VIAB = Percentage viability; AF = Aflatoxin. * indicate significance at $p < 0.05$

0.43) (Table 4.11). In addition, correlation between % IDKn and % WL was greatly significant ($r = 0.99, p < 0.05$) (Table 4.11). Percentage grain viability (% Viability) tended to decrease and correlated significantly with total numbers of insect ($r = -0.87, p < 0.05$), numbers of *S. zeamais* ($r = -0.87, p < 0.05$), *T. castaneum* ($r = -0.85, p < 0.05$) and *C. ferrugineus* ($r = -0.86, p < 0.05$) but was not significant with *Liposcelis* spp ($r = -0.51, p > 0.05$) (Table 4.11). Similarly, % seed viability tended to decrease with % IDKn ($r = -0.78, p < 0.05$). In no case were correlations between aflatoxin (AF) and total insect population, numbers of *S. zeamais*, *T. castaneum*, *C. ferrugineus* and *Liposcelis* spp occurred (Table 4.8). However, there was significant correlation between AF and % IDKn ($r = 0.68, p < 0.05$) (Table 4.11).

4.3.9 Variation in moisture contents measured by different methods

The amount of moisture in the bagged maize measured using GrainMate, JD and GAC 2100 meters had a difference of approximately positive 3.0 % when compared to the oven-dry method. Moisture measurements among the entire treatments recorded varied values for GrainMate (11.4 – 15.0%), JD (12.5 – 15.5%), GAC 2100 (11.0 – 14.4%) and oven-dry (10.6 – 13.0%), these values are derived from data obtained through the duration of storage. Moisture content assessment by the oven-dry procedure provided consistent and lower measurements than the other three moisture meters employed during storage duration (Figs 4.18 and 4.19). Out of the meters used, the GAC 2100 meter provided lower measurements relatively to GrainMate and JD meters.

Differences existed in the assessments of moisture in the bagged maize throughout the months of storage (Figs 4.18 and 4.19). For non-hermetic bags where measurements were assessed on monthly basis, stored maize progressively gained moisture over duration of storage as showed by the four moisture determinants. MC of stored maize in non-hermetic bags increased during the wet period (3rd – 9th month) but the values declined during hot weather (10th – 12th month) (Fig 4.18).

The hermetic bags however, showed marginal change in humid accumulation as indicated in storage time when moisture assessment took place (Fig 4.19). Pooled moisture data for all treatment bags revealed that measurements exhibited a mean positive variances of $2.3 \pm 0.1\%$, $1.6 \pm 0.2\%$ and $1.1 \pm 0.1\%$ moisture content respectively, relative to the oven-dry method ($F = 13.39, df = 7,18, p < 0.05$).

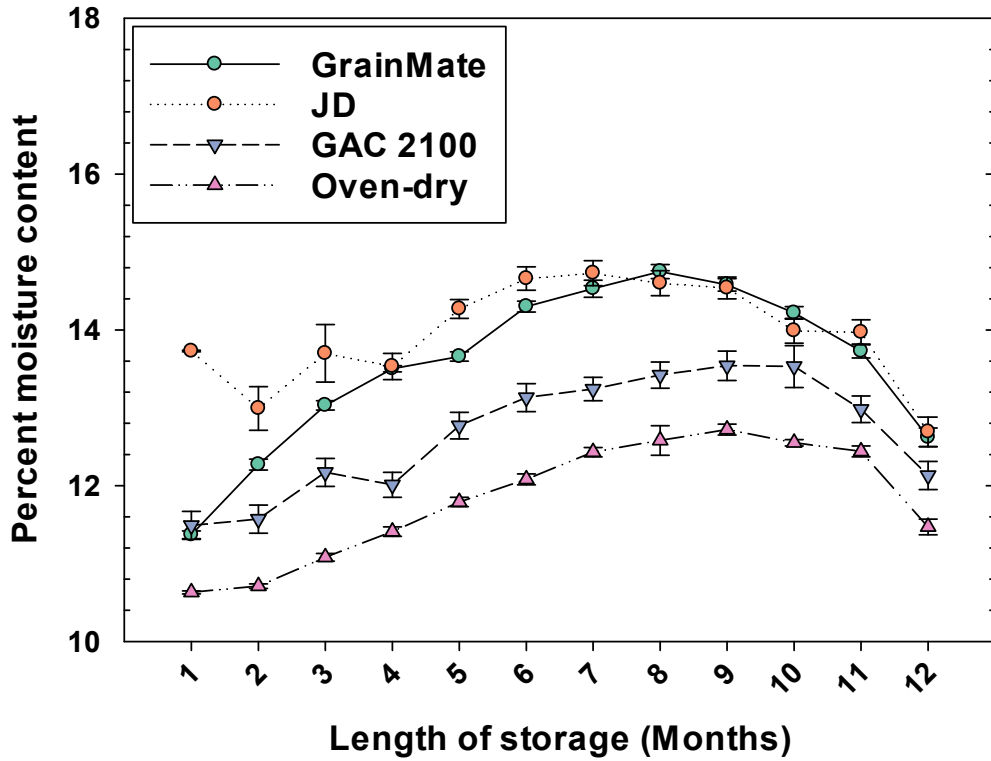


Figure 4.18. Monthly moisture measurements of maize by different methods in non-hermetic bags

PP = Polypropylene; PPDE = Polypropylene plus Diatomaceous Earth; ZF = ZeroFly; ZFDE = ZeroFly plus Diatomaceous Earth

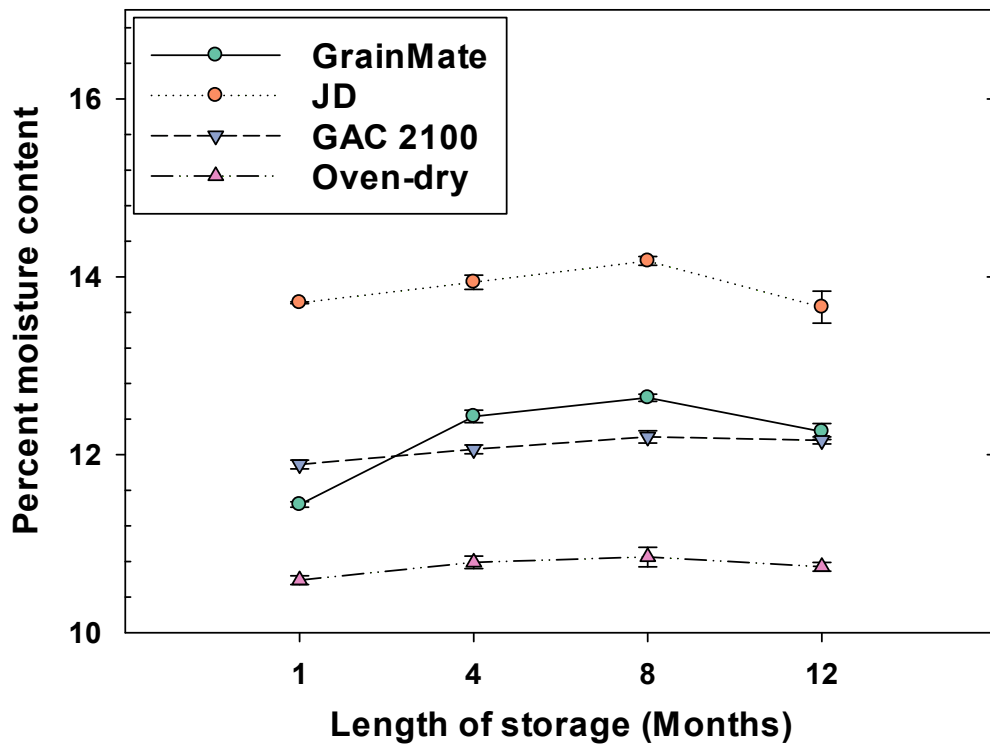


Figure 4.19. Quarterly moisture measurement of maize by different methods in hermetic bags

PICS = Purdue Improved Crop Storage; PP1L = Polypropylene single liner; ZF2L = ZeroFly double liner; ZF1L = ZeroFly single liner

Comparing the moisture meters, GrainMate and JD have mean positive differences of 0.6 ± 0.2 and 1.3 ± 0.2 % moisture content, respectively relative to GAC 2100 meter.

4.3.10 Insect bore on storage bags

The outer woven bag and inner high density polyethylene (HDPE) liners (that is, the hermetic treatments) of some bags used were either breached by small circular holes bored by resident insects within the bags or those attempting to penetrate the bags from outside. Among the non-hermetic treatment bags, the mean number of insect holes bored on the outer PP bags was the highest having 51.5 ± 5.7 and this value ranged from the minimum of 46 to the maximum value of 57 holes per bag, this was followed by PPDE bag treatment with a mean value of 34.9 ± 2.3 and having a range between 33 and 37 insect holes per bag, respectively (Table 4.12). The ZF bag treatment had a mean of 17.0 ± 3.1 ranging between 11 to 21 insect holes per bag whereas the ZFDE bag treatment did not record any insect hole per bag (0.0 ± 0.0) on its outer bag (Table 4.12).

In the hermetic bag treatments, some outer woven bags did not record any insect hole, as in ZF1L and ZF2L bags. However, only one hole was found on PP1L (Table 4.13). For PICS bag treatment, mean insect hole on its outer bag ranged from one to two holes per bag (Table 4.13). Furthermore in the inner HDPE liners contained in those hermetic bags, fewer number of insect penetrations were made. Insect holes created per bag ranged from two to six in PICS bags and the lowest number of insect penetration observed on ZF1L bag was one (Table 4.13).

Table 4.12. Mean (\pm SE) insect holes in the outer woven bags of maize stored for 12 months.

| Treatment | Number of bags | Damage by insect on outer woven bags after 12 months of maize storage | |
|-----------|----------------|---|---------|
| | | Holes in outer woven bags | |
| | | Mean \pm SE | Range |
| PP | 3 | 51.5 \pm 5.7 ^c | 46 – 57 |
| PPDE | 3 | 34.9 \pm 2.3 ^b | 33 - 37 |
| ZF | 3 | 17.0 \pm 3.1 ^{ab} | 11 - 21 |
| ZFDE | 3 | 0.0 \pm 0.0 ^a | 0 |

Each datum represent the mean (\pm SE) of replicate bags, Means within the same column followed by different letters are significantly different ($p < 0.05$).

PP = Polypropylene; PPDE = Polypropylene plus Diatomaceous Earth; ZF = ZeroFly; ZFDE = ZeroFly plus Diatomaceous Earth

Table 4.13. Mean (\pm SE) insect holes in the inner HDPE liners and outer woven of maize stored for 12 months.

| Treatment | Number of bags | Damage by insect on outer woven and inner HDPE liner bags after 12 months of maize storage | | | |
|-------------|----------------|--|-------|----------------------------|-------|
| | | Holes in outer woven bags | | Holes in inner HDPE liners | |
| | | Mean \pm SE | Range | Mean \pm SE | Range |
| PICS | 9 | 1.3 \pm 0.4 ^a | 1 - 2 | 4.0 \pm 1.1 ^a | 2 - 6 |
| PP1L | 9 | 0.5 \pm 0.4 ^a | 0 - 1 | 1.7 \pm 0.4 ^a | 1 - 2 |
| ZF2L | 9 | 0.0 \pm 0.0 ^a | 0 | 3.2 \pm 0.8 ^a | 2 - 4 |
| ZF1L | 9 | 0.0 \pm 0.0 ^a | 0 | 0.8 \pm 0.3 ^a | 0 - 1 |

Each datum represent the mean (\pm SE) of replicate bags; Means within the same column followed by different letters are significantly different ($p < 0.05$).

PICS = Purdue Improved Crop Storage; PP1L = Polypropylene single liner; ZF2L = ZeroFly double liner; ZF1L = ZeroFly single liner

CHAPTER FIVE

DISCUSSION

5.1 Insect pest status

Based on findings from this study, the *P. truncatus* and *S. zeamais* introduced into maize grains readily caused substantial insect damage to kernels and associated weight losses. This corroborated Quellhorst *et al.*, (2020) who reported that *P. truncatus* and *S. zeamais* are important pests of stored maize and are the most occurring pests of farm-stored maize in Africa. *Prostephanus truncatus* feeds voraciously compared to *S. zeamais*, this is evident in the extent of damage caused as a result of their feeding activities. The feeding damage in the *P. truncatus* infested maize was high, resulting in reduced weight of grains compared to *S. zeamais* infested maize. Damage by *P. truncatus* has been noted to be twice that of *S. zeamais* (Borgemeister *et al.*, 2003). A visible quantitative loss in term of hollowed grain kernels and frass, and which therefore resulted in substantial weight loss over period of storage was observed.

5.2 Laboratory (internal) insect infestation of bagged maize

The three months duration for the laboratory study was sufficient for at least 1 - 2 generations of each of *P. truncatus* and *S. zeamais* to develop and for insect population to increase in number and be able to damage both stored maize and storage bags, respectively. In this study, insect populations in PP, PPDE and ZF bags containing artificially introduced *S. zeamais* or *P. truncatus* were high after storage period compared to PICS, ZFDE, ZF2L and ZF1L bag treatments which had few number of insects. In maize initially infested with *S. zeamais*, insect damage and weight losses after 3 months storage period ranged from 1.7 – 22.7% and 0.2 – 12.7%, respectively. Regarding maize initially infested with *P. truncatus*, insect-caused damage and weight losses were higher and ranged from 3.2 – 35.8% and 0.8 – 22.1%, respectively. Similar results were reported by Boxall (2002), in which 20 to 30 % losses were estimated due to postharvest pests of maize after 3 months of storage. Damage and weight losses due to *P. truncatus* considerably surpasses *S. zeamais* infestation. Specifically, *P. truncatus* is reported as a noxious and destructive insect species of on-farm stored maize (Ng'ang'a *et al.*, 2016). Substantial damage resulting in over 30% weight loss in grains stored

between 3 – 6 months was reported in a dominant *P. truncatus* experiment (Mutambuki and Ngatia, 2012). Weight losses due to maize weevil have also been reported to result in 10 – 20% and may increase to 80% if maize is stored using conventional storage containers after a 3 – 6 months period (Mutiro *et al.*, 2002). In Tanzania, 20 – 40% of maize are lost to storage losses (Ragumamu, 2005). Although, Vendi *et al.*, (2018) have found that both *P. truncatus* and *S. zeamais* have robust mandibular structure which allows their mouthpart to attack compact materials, a distinctive characteristics of insects which feeds on solid commodities. The molars found in *P. truncatus* appears to be finely developed and this may be relative to the amount of frass produced on damaged grains (Kumar, 2002), alongside with number of insect holes on bag fabric (Otitodun *et al.*, 2019).

The increase in number of insect during the 3 months storage period in PP and PPDE bags was probably due to the type of bag used which permit freely the entrance and exit of insects and therefore, resulting in increased populations and possible spread of infestations. This corresponded to the observations of Baoua *et al.*, (2014) who reported that PP bags were somewhat easy for adult insects to pass through by boring into the spaces between the fabric, in a way that insects exit and enter the storage bag freely. In addition, PP bags have been demonstrated to allow proliferations of insect population with stored grain (Ng'ang'a *et al.*, 2016) and thus, tallies with results obtained in this study.

In the case of non-hermetic ZF bag, this bag did not perform satisfactorily to prevent insect population substantially. Although, ZF bag has deltamethrin insecticide fixed into the fibers of its outer fabric, the bag was not successful in mitigating insect proliferation within the bag. Abass *et al.*, (2018) noted that ordinary ZF bags were not quite effective in protecting stored grains from resident stored product beetles and this submission is similar to the findings in this study. However, a 100% mortality of insects were reported when introduced to the outer and inner surfaces of ZF bags (Kavallieratos *et al.*, 2017). Marked number of insect populations inside the ZF bags were probably due to rapid increase of insect number infested with maize at the beginning of the 3 month storage. As expected, secondary pests such as *T. castanuem* was found after *S. zeamais* and *P. truncatus* infestations on maize grains in some bags. This correlate to the observations of Ng'ang'a *et al.*, (2016) who found *T. castaneum* infestation in their numbers after primary damaged by *S. zeamais* jute and PP bags, respectively. The cutting and sealing

of the mini-bags used for the 3-month study could have compromised the amount of insect presence, leading to observed high cross contamination of insect species and damage losses.

Insect holes bored on storage bags used were made either by insects artificially infested into the bags or those developing from the stored grains. Higher number of holes were found in the *P. truncatus* infested bagged maize than the *S. zeamais*. The findings of the laboratory study revealed that *P. truncatus* and *S. zeamais* have the ability to create hole through storage bags, but the extent depended on bag type. The PP and PPDE bags were relatively easier to penetrate by *P. truncatus* than *S. zeamais*. The ability of *P. truncatus* to bore readily has been reported to correspond to its mouthpart morphology (Vendi *et al.*, 2018). *Prostephanus truncatus* has strong and well developed mouthparts for attacking of storage materials (Popoola, 2012) whereas *S. zeamais* mandibles are inferiorly developed to cause such damage (Ragumamu, 2005). There was no insect penetration on ZF bags, compared to PP and PPDE bags which had 35 and 43 holes, respectively. These results indicated that no insect hole on ZF bags is in agreement with findings of Kavallieratos *et al.*, (2017), who reported that *P. truncatus*, *S. zeamais* and *T. castaneum* infestations on ZF bags did not result in insect penetration of the bag. The ZF bag has deltamethrin insecticide infused into it which are usually steadily released on its outer fabric in a sustained way (Vestergaard, 2014). However, storage insects that have developed resistance or are able to tolerate deltamethrin insecticide may potentially penetrate into the material of the bag (Paudyal *et al.*, 2017). In the case of PICS and PP1L bags, results showed that *P. truncatus* were able to bore readily into some outer woven and inner hermetic bags compared to *S. zeamais* which were unable to create any penetration. Similar results were reported by Chigoverah and Mvumi (2018) stating that hermetic liners of double and triple storage bags were susceptible to perforations from internal *P. truncatus* infestations. Specific concerns have been raised about the ability of *P. truncatus* to create holes on storage bags produced from flexible materials, resulting in loss of efficacy of the hermetic technology (Hodges and Stathers, 2015). The PICS bags have been reported by Hell *et al.*, (2014) to be penetrated by *P. truncatus*. However, typically for PICS or any triple airtight brand, the second plastic liner is design to create additional protection in the occurrence that one of the two liners is damaged. Although, the integrity of the second plastic film in the three-layered bag is rarely

compromised, therefore, the bag have the ability to offer continued safe storage of commodity (Baributsa and Ignacio, 2020).

5.3 Storehouse study

5.3.1 Insect infestation level

Sitophilus zeamais (2593), *T. castaneum* (1298), *Liposcelis* spp (1193) and *C. ferrugineus* (861) were the only insect species collected and therefore, responsible for the kernel damages and weight losses of maize stored in the various bag treatments. Despite the external infestation of *P. truncatus* in the storehouse around each bag treatment stack, only one live *P. truncatus* was found in PP bag during the month of July throughout the sampling period of 12 months. This is possibly as a result of the sporadic mode of occurrences of this insect pest (Hodges, 2002). A number of farm-stored trial studies carried out in Tanzania (Abass *et al.*, 2018), Ghana (Danso *et al.*, 2017) and Kenya (Ng'ang'a *et al.*, 2016) have reported the non-occurrence of *P. truncatus* but the presence of a predominant *S. zeamais* during storage. Birkinshaw *et al.*, (2002) reported that *Sitophilus* species are widespread causing a high level attack on stored commodities; while *P. truncatus* attack may be unpredictable due to a number of biological causes which may include environmental conditions, climatic variability, food availability, such that their incidence may be insignificant for some period and then unexpectedly increase at a particular time (Borgemeister *et al.*, 2003).

In PP and ZF bags used alone, *S. zeamais*, *T. castaneum* and *C. ferrugineus* were the predominant pests. In storage, *S. zeamais* infestations are more economically important than *T. castaneum* and *C. ferrugineus* because they primarily damage stored maize. In the untreated maize stored in ZF bag, high densities of *S. zeamais* (1,595) and *C. ferrugineus* (788) were found over months of storage while regarding the PP bag, the most abundant species was *T. castaneum* (1,141), but a consistent and gradual increase in *S. zeamais* (623) infestation was observed along storage period. This is likely possible because of the favorable surrounding air environment which is most suited for insect development and particularly, the high oxygen concentrations generated within the bags. This agrees with findings of Anankware *et al.*, (2013) who stated that suitable environmental conditions together with ease of air interaction between grain environment and the surrounding storage environment contributes to high losses in PP and jute bags. Thus, allowing unrestricted development and multiplication of biotic organisms within the enclosed bag.

In the case of the non-hermetic ZF treatments (untreated maize in ZF bag), the bags were only effective in preventing insect infestations until 5 months into storage (February – June) and subsequently, population of beetles for instance *S. zeamais* and *C. ferrugineus* obviously rose as storage duration progresses. An increased beetle intensity inside ZF bags following a 4 month period of maize storage in Ghana has been reported by Paudyal *et al.*, (2017). Furthermore, on-farm studies across Africa countries on effectiveness of the non-hermetic ZF bag showed high occurrence of *S. zeamais* and substantial kernel damage in untreated maize stored in ZF bag in Zimbabwe (Mlambo *et al.*, 2017), Tanzania (Abass *et al.*, 2018) and Malawi (Singano *et al.*, 2019). The huge infestations observed in ZF bags in this study is possibly due to the (internal) infestation of stored maize by the immature forms of the insects at the maize maturation phase on the farm. Infestation by *S. zeamais* may start from the field and continues through storage. Maize stored were untreated until bagging and storage. However in Nigeria, most grains stored at smallholder level are usually not fumigated or treated before bagging since the grains are not held in storage for longer period than 3 – 6 months subject on the economic standing of the farmer. The abundance of live *S. zeamais* in the ZF bag shows that the insects multiply at high rates and may not be in contact for long periods with the exterior of the deltamethrin incorporated ZF bag enough for their population levels to be significantly reduced. The ZF bags have been reported to be effective in killing stored product insect species when they make contact with the bag fabric for a minimum period of 24 hours (Paudyal *et al.*, 2017). Although, a large number of dead *S. zeamais* possibly trying to enter into or exiting the bags are usually seen on the outer ZF bags at sampling periods. Moreover, with the inclusion of the introduced *S. zeamais* around the bag treatments, introduced populations have the potential to spread new adverse biological traits including insecticide resistance (Fragoso *et al.*, 2003). Therefore, the increasing *S. zeamais* population may be those that have survived deltamethrin exposure. Also, the frequent closing and opening of the ZF bags during sampling have been reported to possibly compromise the lethal effect of deltamethrin wall and thus, permitted a stress-free passage of insect species into the bagged maize (Paudyal *et al.*, 2017).

Since harvested grains are presumed to be infested from the farm or during storage (Hagstrum, 2001), the ZF bag used in this study did not result in effective protection and therefore demonstrates the importance of storing to store pre-fumigated grains in them. Nwaubani *et al.*, (2020) showed that ZF bags were moderately effective against

coleopterans in pre-fumigated maize stored in Nigerian storehouses for a period of 11 months. Similar results from field trials using pre-fumigated or insecticide-treated grains have showed that ZF bags are significantly protective in mitigating both incoming and resident infestations which has find their way into the commodities from the farm and continue to manifest in storage (Baban and Bingham, 2014). The use of ZF bags for storage of un-fumigated grains have been concluded not to be effective (Mlambo *et al.*, 2017). This submission is in line with the findings of this present study. Therefore, for effective use of ZF bags, maize must first be fumigated or admixed with natural insecticides to exclude internal insect infestations. This practice is recommended by the production company indicating that for best storage efficacy, grains meant for further processing must be insect-free prior to storage (Vestergaard, 2014). By this recommendation, the production of novel ZeroFly hermetic storage bag having impenetrable multilayered reusable composite plastic lining with gas tight properties to complement the fabric insecticidal activity of outer bags has been subsequently produced.

For diatomaceous earth-treated maize in Polypropylene (PPDE) and diatomaceous earth-treated maize in ZeroFly (ZFDE) treatments, both were respectively effective against beetle pests and resulted in lower damage levels compared to the PP and ZF bag treatments used alone (untreated maize in PP and ZF bags). Insecto brand of DE admixed with maize presented desirable preservation properties on the stored maize from insect attacks for a period of 8 months before insects were noticeable. Stathers *et al.*, (2008) reported similar result for DE been effective as grain protectant for a period of 8 months in Tanzania and Zimbabwe. In this study, the PPDE treatment had a sum total of *Liposcelis* spp (364) and *S. zeamais* (256), whereas ZFDE treatment had *Liposcelis* spp (403) and *S. zeamais* (103) over period of storage, this shows that the DE had relative efficacy against beetle pest compared to psocids. Nwaubani *et al.*, (2020) reported similar result of high incidence of *Liposcelis* spp in maize treated with a localized Nigerian raw DE (Bularafa DE) during storage. However, the application of DEs singly will not offer adequate control of *Liposcelis* spp (Athanassiou *et al.*, 2009) as their population is also dependent on other grain quality variables such as grain moisture. *Liposcelis* spp occurrence might be seen as irrelevant to smallholder farmers as they are secondary insect pests and very tiny creatures but these species have been found to frustrate international trades. The use of DEs have been considered highly appropriate

to replace synthetic chemicals (Korunic *et al.*, 2020). The DEs may serve as a good substitute for smallholder farmers in Nigeria as its application does not require any apparatus or knowledge and thus, offers extended period of effectiveness which stems from their high persistency (Nwaubani *et al.*, 2020). Insect populations build up from a very low number of *S. zeamais* after 8 months storage period is possibly as a result of the reduction in the toxicity of the DE caused by gradual increase in moisture content of bagged maize from initial 12.0 to 14.4 % (moisture level at 8 months of storage). Grain moisture content above 14.0 % tolerates insect recovery from water lost through dehydration. DEs are not effective in a humid surrounding, not because water saturates the absorptive surface, but because insects can constantly replenish their water loss by ingesting moist grain (Quarles, 1992).

In contrast to all the non-hermetic treatment used in this study, the performance of all the hermetic bag treatments Purdue Improved Crop Storage (PICS), Polypropylene single liner (PP1L), ZeroFly double liners (ZF2L) and ZeroFly single liner (ZF1Liner) used demonstrated minimal amount of insect infestation. Regarding ZeroFly hermetic bags (ZF2L and ZF1L), the total number of *S. zeamais* and *T. castaneum* over months of storage was 1 and 25 insects, respectively whereas PICS had 11 *S. zeamais* and 31 *T. castaneum*, and PP1L contained 4 *S. zeamais* and 11 *T. castaneum*, respectively. Hermetic bag systems have been shown to successfully preserve stored commodities and sometimes outperform chemical use to prevent storage losses (Baributsa and Njoroge, 2020). A possible reason for the high efficacy of these hermetic technologies is the generation of hypoxic environment within the grain bulk generated by the biological processes of those insects. The absence of oxygen and increased carbon-dioxide within the grain bulk is responsible for mitigation of insect infestations in the airtight storage bags (Baributsa and Njoroge, 2020).

5.3.2 Maize damage and weight loss in hermetic and non-hermetic bags

Grain damage is one of important quality parameters which influences consumers' purchasing and pricing abilities. The presence of insect pests (live or dead) on grain products have been observed to affects consumers buying decision (Okori *et al.*, 2022). Conversely, grain weight loss is the loss of edible food which is related to a number of factors such as mass and moisture loss. Mass weight loss is attributed to pests feeding on grains, fungal infection and grain metabolic activity (Covele *et al.*, 2020).

The total population of insects and individual species of *S. zeamais*, *T. castaneum* and *C. ferrugineus* were highly correlated with each of % IDKn and % WL of bagged maize but not with *Liposcelis* spp. A significant correlation was similarly reported for insect species with each of % IDK and % WL (Nwaubani *et al.*, 2020; Manu *et al.*, 2019). These high and positive correlations obtained emphasized how insect populations during storage can be detrimental to maize quality (Tefera *et al.*, 2011). Mean % IDKn (0.2 – 16.9 %) and % WL (0.1 – 10.9 %) in all the bags were recorded after 12 months of storage. The ZF bag had the highest percentage damage and weight losses followed by the PP bags. These high rates of grain damage in ZF and PP bags could be ascribed to the type of insect species encountered.

The findings from this study showed that *S. zeamais* was the most abundant and primary feeder encountered. This maize weevil most probably contributed to the quantity of damaged kernels recorded. The presence and activities of other stored-maize secondary insect pests like *T. castaneum*, *C. ferrugineus* and *Liposcelis* spp may also have contributed to seed damage. This observation agrees with Mutambuki and Likhayo (2021) who reported that *P. truncatus* and *S. zeamais* infestations resulted in high kernel damage of stored maize over 9 months. Although damage and losses observed due to *S. zeamais* was low, this was consistent with previous studies which have showed that *S. zeamais* infestations are generally low (Borgemeister *et al.*, 2003). The findings on percentage damaged kernel and weight losses in this study were low compared to an average of 1.1 – 53.9 % and 8.1 – 11.6 % reported in Benin and Tanzania, respectively (Baoua *et al.*, 2014; Abass *et al.*, 2018). Moderately low damage and weight loss values were similarly reported in maize storehouse studies mostly infested by *S. zeamais* in Nigeria (Nwaubani *et al.*, 2020). The relatively low percentage damage and weight losses found from all the bags may be attributed to adequately dried and un-infested or near un-infested maize which were store in the various bag treatments at the start of the experiment in February 2017 and which likely caused delayed insect development and attack.

Damage and losses were less than 1 % in Purdue Improved Crop Storage (PICS), Polypropylene single liner (PP1L), ZeroFly double liners (ZF2L) and ZeroFly single liner (ZF1L) bags over 12 months storage period. The findings of Costa (2014) stated that the usage of hermetic bags achieved significant insect reduction and grain losses to

below 1 % after long storage months supported the results from this study. The quality of conserved maize in those bags were highly maintained in contrast to maize kept in ordinary PP and ZF bags. Findings from this present study is similar to previous studies focused on the usage of hermetic bag storage such as PICS bags, which demonstrated better outcomes and effective suppression of all stored insect species relative to other bag types (Nwaubani *et al.*, 2020; Abass *et al.*, 2018). The optimal use of ZF bags with either one or two hermetic liners (ZF1L and ZF2L) in this study also demonstrated that ZF hermetic bags could provide more secured protection than any of the bags used. In fact, the findings of this study has showed that the two categories of ZF hermetic (ZF1L and ZF2L) bags used performed better than PICS bags. The performance of these bags could be related to its double action of protection; the insecticidal property on it outer woven bag preventing entrance of insects and the airtight closure provided by it inner hermetic liner causing asphyxiation and death of resident insect pests compared to other hermetic bags with untreated outer woven fabric and non-hermetic storage bags.

5.3.3 Maize grain germinability in hermetic and non-hermetic bags

The desirable quality of any stocked seed is to guarantee viability during planting. Owing to improper storage, farmers have over time lost own saved hybrid to insect deterioration (Mutambuki and Likhayo, 2021). A number of factors including storage container and insect infestation have been attributed to germinability potential of grains. The results from this work showed an overall 96% germinability potential in the hermetic bags ZF2L, ZF1L, PICS and PP1L treatments through storage duration. Although, a near 98% germination rate was found in PICS bags after an 11-months storage time (Nwaubani *et al.*, 2020). Several studies on grain storage have showed that hermetic containers have the capacity to protect stored grains from fluctuating external climatic conditions that could otherwise affect seed germination potential (Okori *et al.*, 2022), leading to a tolerable or insignificant viability reduction (Villers, 2017). The reduced oxygen level within hermetic bags is highly responsible for maintaining grain quality by suppressing insect attacks and prevent their feeding on the germ and endosperm of stored maize (Williams *et al.*, 2017).

However among the non-hermetic treatments, maize germination rate was reduced from the initial rate by 6.6% in PP bag and 11.0% in ZF bag for the period of storage. The values obtained from this study were low compared to a 30% germination drop reported for maize storage over 9 months in Kenya (Mutambuki and Likhayo, 2021). Stored-

product insect pest feeds chiefly on grain germ and endosperm. Any severely infested kernel will not sprout since the seeds are randomly selected from each treatment bag as sample. By this action, a drop in seed viability potential and vigor of such planted seed is witnessed (Kuyu *et al.*, 2022). Insects, together with grain microflora and ambient temperature have been observed to likely cause germination differential on stored grains (Mutambuki and Likhayo, 2021). Seeds stored in non-hermetic structures are exposed to changing environmental variables whereas, conditions are relatively constant in hermetic containers (Odjo *et al.*, 2022). However, seeds meant for planting must meet an 85% germination potential after at least 12 months of storage (Fufa *et al.*, 2020). Since most Nigerian farmers depend on own-saved seeds for crop production, the results from this study suggests that reasonable germination potential could be achieved when maize is stored using PICS, ZeroFly hermetic and DE-treatment.

Individual species and total insect population were negatively correlated with grain viability (% viability). This negative correlation between insect population and % viability showed how the feeding activities of these pests reduce germination rates of stored grains. This is consequently because of high insect population of *S. zeamais*, *T. castaneum*, *C. ferrugineus* and *Liposcelis* spp found associated with the maize contained in those bags. Both the developing larva and adults of these insect species have been implicated to devour the germ and endosperm of maize kernels. Therefore, application of hermetic technologies would be reasonable to minimize the relative abundance of stored product insects that can markedly affects the viability of maize meant for future planting.

5.3.4 Aflatoxin levels in stored maize

Maize is among crops that can be largely liable to damage by toxigenic fungi in the tropical and temperate climates. Earlier investigations carried out in Nigeria reported high concentrations of aflatoxin levels in maize during production and in storage (Udoh *et al.*, 2000). Aflatoxins are secondary metabolites produced by a number of *Aspergillus* species and are highly toxic to humans and animals when ingested at high concentrations (Perrone *et al.*, 2014). It is prevalent and constitute the main mycotoxin found in maize and groundnut (Suleiman *et al.*, 2013). Findings from this study showed that the initial and final aflatoxin (AF) levels from all treatments were within the 10 part per billion (ppb) (1 ppb = 1 µg/kg) acceptable standard set by the Standards Organization of Nigeria for maize (PACA, 2021). Average values detected in maize samples stored in PP, PPDE,

PICS and ZF treatment bags varied between 2.7 to 5.0 ppb, respectively over 12 months of storage whereas the ZF2L, ZF1L, ZFDE and PP1L bags had undetectable levels of aflatoxin after storage period. The internationally set standard for aflatoxins in food is between 4 - 20 ppb (Codex, 2017). In 2016, 31% of Nigeria's maize samples had aflatoxin levels above the European limits (4 ppb) whereas 16% of the samples exceeded the United States standard (20 ppb) (PACA, 2021).

The undetectable and risk-free levels of aflatoxin obtained from the stored maize is probably due to the bio-control agent (aflasafe) applied to crop field during maize production and the efficacy of each of the postharvest storage techniques employed in this study. Nwaubani *et al.*, (2020) also reported a less than 5.0 ppb aflatoxin level for all stored maize initially planted with aflasafe and then stored in different bags located in Nigerian storehouses for a period of 11 months. The application of aflasafe to mitigate toxigenic fungi at risk-free levels on the farm is effective, this can further be maintained during storage using good management practices (Ortega-Beltran, 2017).

Regarding Polypropylene (PP), ZeroFly (ZF) and diatomaceous earth-treated maize in PP (PPDE) bags where low levels of aflatoxin were found, these bag treatments were found to permit internal infestations of stored-products insect pests. Insects have been linked with the dispersal of fungus *A. flavus* and maize infection by aflatoxin (Setamou *et al.*, 1998). Specifically, the damage caused by *S. zeamais* on maize has been described to greatly aid *Aspergillus flavus* contamination and successive aflatoxin formation (Udoh *et al.*, 2000). This weevil infestation help to enlarge the kernel area and moisture content of the maize attributable to metabolic activity (Udoh *et al.*, 2000). Undetectable level of aflatoxin in the ZF hermetic bags and lower contamination in the PICS bag than in the non-hermetic bags was possibly because of the lowered oxygen level and the increasing carbondioxide levels connected with air-sealed storage which inhibits the growth of fungal pathogens (Baoua *et al.*, 2014). In addition, Namusalisi *et al.*, (2018) concluded that hermetic storage performed better than non-hermetic treatments in limiting mycotoxin levels. Aflatoxin level has been reported to increase with storage period (Liu *et al.*, 2006) and handling practices (Setamou *et al.*, 1997).

5.3.5 Pesticide residue level in stored maize

The deltamethrin residue level of 0.02 milligram per kilogram (mgkg^{-1}) found in maize contained in the ZF bag after 12-month storage was found to be below the regulatory

maximum residue limits (MRLs) of 2.0 mgkg⁻¹ of cereal (Codex and EU country standards), 1.0 mgkg⁻¹ of cereal (US EPA) and 0.5 mgkg⁻¹ of cereal (Indian standards). In a multi-country studies conducted, the MRLs in maize stored remain under threshold levels after 12 months storage period in Kenya (0.1 mg/kg) and Zambia (0.1 mgkg⁻¹) while in Ghana (0.2 mgkg⁻¹) after a 3 month storage period (Vestergaard, 2015). Although there is no Nigeria standards for MRLs, the Nigerian Agency for Food, Drug Administration and Control (NAFDAC) adopts the Codex limits (Keri, 2009). The low residue level obtained from this result indicates that ZF storage bag does not leave harmful residue on stored grains or seeds, therefore the stored maize could be safe and suitable for consumption.

5.3.6 Maize temperature, relative humidity and moisture content

The degree of hotness within bagged maize varied over the 12 months period of storage but the mean temperature values were in the ideal range (27.4 to 31.4°C) for developing insect population to increase in number. There was no relationship between temperature and number of insects recovered. Similar lack of correlation was obtained in Nigeria (Nwaubani *et al.*, 2020) and Ghana (Manu *et al.*, 2019) for relationship between temperature and number of insects. This lack of relationship with insect recovered was anticipated as temperatures obtained all through storage months were at ideal values suitable for development of storage insect pests (Fields, 1992).

The number of *Liposcelis* spp was correlated with each of RH and MC but in no case was other insect species correlated. Nwaubani *et al.*, (2020) reported total population of insect to correlate with RH and MC. However, no correlations were reported for insect population and either MC or RH (Danso *et al.*, 2018). In Ibadan, between the storage periods of April–October marks the rainy season. The findings of this study showed that the month of April was the period when initial detection of insect species were found, most especially in ZF and PP bags, and their numbers increased along the months of storage with exceptions of all the hermetic bag treatments. Although, the maize used at the onset of the study was well dried, the increasing humid air in the bagged maize during the rainy period was expected to increase grain MC and to be favourable to insect development. This corresponded to findings of Nwaubani *et al.*, (2020). The relatively high number of *Liposcelis* spp in the ZF bags used alone and DE–treated maize (PPDE and ZFDE) during the wet season may possibly be due to the high humid air found in those bags coupled with the maize also having relatively high water amount (Haines,

1991) and the fact that these pests are well tolerated to deltamethrin insecticide infused into the yarn of ZF bags (Ahmedani *et al.*, 2010) and DEs when used singly as a protectant. *Liposcelis* spp are now considered a serious storage pest globally (Ahmedani *et al.*, 2010).

The preliminary water content of grains used ranged from 11.3 to 13.5% (mean 12%). This suggests that farmers have the potential to dry standing maize cobs sufficiently during the hot weather to a safe storage level of below 13% moisture content. Maize kept in hermetic bags neither gain nor lose significant moisture during storage as small moisture gain was showed in months when measurements were recorded. Thus, hermetic bags such as PICS and ZF with hermetic liners (ZF1L and ZF2L) bags were consistent in maintaining the preliminary maize water content and therefore, protected the maize against fluctuations in seasonal humidity conditions. Similar occurrence was reported in Republic of Benin in a comparative study on maize storage using hermetic bags (Edoh Ognakossan *et al.*, 2013). The hermetic systems were invented to offer adequate airtight and moisture barrier characteristics. In contrast, maize in the non-hermetic bags gained an average of 20% moisture (initial mean 12%, final mean 14.4%) during storage. The interplay between maize stored in bags along with the ambient environmental condition will result in grain moisture change regarding atmospheric humidity (Williams *et al.*, 2017). Several studies on hermetic bags have revealed that the effect of ambient climatic change on these technologies are minimal (Baributsa and Njoroge, 2020). The non-hermetic (PP) bags in this study are quite more exposed to humid air in storage such that maize kept in those bags gained considerable amount of moisture as a result of absorption. Increasing atmospheric humidity witnessed during the wet season and reduced humid air through the arid weather could explain the early build-up and afterwards drop in moisture of bagged maize (Ajao *et al.*, 2018). This present study which began at the near end of the hot weather (February) and spanned through the humid weather during which transition ambient atmospheric humidity of the storehouse where the maize was stored tends to rise from a mean monthly range of 50 - 60 % into the humid 70 - 80 % range. By this, moisture would have exchanged through the non-hermetic bags until the moisture level of maize reached an equilibrium with the atmospheric condition of the storehouse. Bagged maize have been shown to responds easily to variations in monthly environmental conditions (Armstrong *et al.*, 2017; Ajao *et al.*, 2018).

Hermetic bags such as PICS and ZF hermetic bags have the ability to maintain grain moisture better than non-hermetic PP bags, which in fact are opened to surrounding air and in time gradually equilibrate with it. This agrees with other studies which reported that hermetic bags have ability to maintain a fixed relative humidity environment compared to other bag types (Njoroge *et al.*, 2014). A relatively steady grain moisture environment is an important management operation as it creates an expectation that the grain will maintain its primary grain moisture state as much as the bag is tightly sealed (Williams *et al.*, 2017). Other factor aside environmental conditions which may promote moisture gain in stored grain is insect infestation. Heavy insect infestations in PP and ZF bags appears to contribute to moisture gain compared to the hermetic bags which had no insect infestation.

5.3.7 Efficiency and comparison of moisture meters

The determination of moisture level in stored grains is one of critical quality assessments carried out to ensure agricultural produce are in good conditions. The oven dry procedure is the standard and earliest method used for assessing the degree of moisture in agricultural commodities (ASAE, 2002), however the lengthy duration and unstable energy supply for drying in developing countries has required the evolution of moisture meters (Ajao *et al.*, 2018). Two moisture meters, JD and GAC 2100 are commercially available and quite expensive for smallholder farmers in emerging economies. On the other hand, the GrainMate meter is cheap (Armstrong *et al.*, 2017) and was developed to ease affordability by farmers.

Combined moisture data for hermetic and non-hermetic treatment bags revealed a less than 3.0% positive difference of the meters comparative to oven dry technique. When comparing the meters, GrainMate and JD meters had a mean positive differences of 0.57 and 1.28% MC, respectively relative to GAC 2100 meter. Armstrong *et al.*, (2017) stated a lower GrainMate moisture readings relative to JD meter revealing an average positive variances of 0.45% (GrainMate) and 2.12% (JD) comparative to GAC 2100 meter. Based on findings from this study, approximately 1.0% variance from readings of GrainMate and JD meters were obtained. In contrast, Paudyal *et al.*, (2017) observed a near 2.0 % variation from readings of GrainMate and JD meters. The average highest moisture recorded was $14.8 \pm 0.1\%$ in the PP bag during the September period using the JD meter. In addition, the highest moisture measured in any bag was 15.5% in PP and

ZF bags. Overall, the amount of water present in all bagged maize after the period of storage was low ($12.9 \pm 0.1\%$), thus maize was at safe moisture level.

Although, the price of each method or meter used did not correspond to accuracy or precision, the relatively low cost GrainMate meter appears to read moisture content readings which were somewhat close to the oven-dried method. This indicate that the meter may be used as alternative to the more expensive ones or the time consuming oven-dried method as it is well suitable for bagged grain bulk in stores at smallholder level as well as for laboratory and field use.

5.3.8 Insect damage on storage bags

Data obtained on inspection of storage bags upon conclusion of this study showed that insect bore on bags or liners were as a result of emerging insects from stored maize or those outside the bags. The population of *S. zeamais*, a known major pest could be responsible for insect bore on the bags. The feeding behavior of stored-product insects had been reported to relate to morphological structures of their mouthparts (Stejskal *et al.*, 2018) which enables them to bore into solid materials. The mean number of insect perforation in the bag wall ranged up to 6 in the inner HDPE liners and ranged up to 125 in the outer woven bags of all the storage bags used. Insect bore made on the outer bag of PP and ZF bags used alone were higher compared to those found on the outer hermetic bags. The number of penetrations associated with *S. zeamais* infestation is usually lower compared to extent of damage on bag fabric by *P. truncatus* when the latter is more prevalent (Li, 1988). *Sitophilus zeamais* mouthparts are less developed for such damage (Ragumamu, 2005) compared to *P. truncatus* which has well adapted mandibles for boring on hard substances. *Prostephanus truncatus* breach storage materials more readily than *S. zeamais* (Otitodun *et al.*, 2019).

Previous studies reported that *S. zeamais* was unable to create insect hole on ZF fabrics (Anankware *et al.*, 2014; Kavallieratos *et al.*, 2017). The ZF bags have deltamethrin insecticide which exert powerful killing effects on insects and thereby prevents the entry and exit of stored-product insect pests. A few number of insect penetrations were seen on ZF bags used for a 4-month storage of maize (Otitodun *et al.*, 2019). Similarly, quite high number of insect bore on the ZF bags after storage time may be attributed to *S. zeamais* that have developed resistance to deltamethrin and have the ability to bore through the bags. Due to introduced *S. zeamais* infestation in the storehouse, Fragoso

et al., (2003) stated that introduced populations have the potential to spread new adverse biological traits including insecticide resistance. Mortality of insects may perhaps be possible if the attacking insects are exposed to the ZF fabric sufficiently enough to the deltamethrin insecticide when they try to penetrate into the bags (Kavallieratos *et al.*, 2017). This is evident as large number of dead insects are usually seen on the outer bag of ZF bags placed in the storehouse. Insects that are tolerant to deltamethrin likely have the potential to breach ZF bag (Paudyal *et al.*, 2017). In contrast, PP bags are more readily bored by storage insects. PP bags permit insect penetration as a result of the spaces between the bag mesh, thus allowing insects to push through and infest stored maize.

Fewer holes were seen on the outer hermetic bags and it can be suggested that the external insect infestations were responsible for such breach. The low number of holes may possibly be result of the infrequent occurrences of *P. truncatus* (Hodges, 2002) and inability of *S. zeamais* to bore readily into storage bags (Ragumamu, 2005). Also, it appears that these insects attacked the storage bags soon after they were introduced and might have died of starvation when their entrance into the stored maize have been denied by the nature of hermetic storage. Hence, infestation depends primarily on insect density (Hodges, 2002).

As for the plastic liners, the material has good barrier properties which hinders the emission of volatile substances from the stored maize to the external surrounding, thereby lessening the probabilities of attack from outside the bag when insects migrate and in search of food. However, the few holes found on the liners could have been generated by insects trying to escape the hypoxic environment created by the hermetic technology. Similar studies in Benin have reported that PICS bags were perforated by insects when maize and cowpea were stored, but this breach did not affect the preservation of the grains as the grain bulk itself forms a wall which prevent the movement of air into and within the bags (Baoua *et al.*, 2014). *Prostephanus truncatus* capability to breach PICS bags when cassava chips were stored have been documented (Hell *et al.*, 2014). This appears to show that mature *P. truncatus* will have the chance to perforate bag that has not been chemically treated (Kavallieratos *et al.*, 2017). From observation during this study, it was found that some PICS hermetic liners were either torn or have busted thereby, breaching the level of oxygen and promoting build-up of

live insects. The integrity of PICS liners may have been compromised by the sharp edges of maize, and consequently resulting in inadequate control of storage insect pests.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The importance of maize in the global food agri-system is diverse and dynamic. However, its storage losses have continued to threaten food security and livelihood of low-resource farmers in Nigeria, and many parts of sub-Saharan African countries. The traditional practice of synthetic insecticide use and ordinary polypropylene bags to assure supply and availability between harvests have not been effective and appropriate. Reduced-risk technologies involving hermetic (double and triple systems) and non-hermetic (insecticide incorporated) bags, and diatomaceous earth have been commercialized to minimally reduce insect infestation of commodities stored for long periods. Therefore, the efficacy of these available technologies including ZeroFly hermetic, PICS and ordinary ZeroFly bags, and diatomaceous earth against stored-maize insect infestations were investigated. Data obtained were based on insect infestation level, insect-damaged kernels, weight loss, seed germination rate, moisture levels, aflatoxin contamination and insect bore on storage fabric. The results for the laboratory study showed that simulated internal infestation activities of *P. truncatus* and *S. zeamais* accelerated maize deterioration from popular polypropylene (PP). This type of storage bags readily permits *P. truncatus* attack than *S. zeamais*. For the storehouse study, the predominant insect species were *S. zeamais*, *T. castaneum*, *C. ferrugineus* and *Liposcelis* spp. Insect damage kernels in ZF (16.9%) compared to PP (5.4%) was high, so also is the reduction in grain viability rate at 66.0% and 78.3%, respectively. This was not the case for all the hermetic treatments ZF2L, ZFIL, PICS, PP1L and DE-treated maize where kernel damage and weight loss were below 1%, respectively with little or no drop in germination potential over 12-month period of storage. Furthermore, aflatoxin contamination of the stored maize in all technologies were at the barest minimum, below 10 ppb SON standards. The hermetic storage and DE storage techniques used in the study preserved and maintained maize quality, and therefore, provides evidence to support their adoption in order to increase accessibility to safe and nutritious food supply in Nigeria.

6.2 Conclusion

The laboratory (internal) simulation of both *P. truncatus* and *S. zeamais* into bagged maize has demonstrated that both insects are damaging pest of stored maize and are capable of boring through improved storage bags but at varying degrees. In the storehouse study, *S. zeamais* was the predominant insect pest responsible for most of the initial damage and weight loss recorded over storage period. The application of hermetic technologies (ZF2L, ZF1L, PICS and PP1L bags) were most suitable and effective at keeping insect infestation and moisture content in check throughout storage months. In addition, the combined treatment with diatomaceous earth dust provided long term storage and can be incorporated to perform a significant function in integrated pest management of storehouse/on-farm storage. However, batches of grains are rarely uniform in quality when stored and this may relate to immense number of insect infestation observed inside ordinary ZeroFly bag (an insecticidal storage bag) compared to the conventional polypropylene bag (control). The ZF bag may be well managed if grains were treated before storage, however, the maize used were un-fumigated. Due to the efficacy of nearly all treatments used, seed germination potential of stored grains were generally protected. The moisture meters used in this study provided reliable moisture measurements relative to the conventional oven-dry meter, suggesting that these meters may be used as substitute to provide fast and predictable moisture measurements of grains. The moisture levels of stored maize tended to respond to monthly seasonal variations, as maize in the non-hermetic bags recorded high moisture level during the wet season but fell during the dry season. Maize in the hermetic bags maintained stable moisture level throughout the storage period. Aflatoxin contamination in stored maize was low and ranged from undetectable level (zero) to 5.0 ppb at the initial and end of storage. Levels obtained were within the maximum limits set by the Standards Organization of Nigeria (SON). Pesticide residue analysis conducted on the maize stored in the ordinary ZF bags showed that the bag do not leave harmful residue on stored maize, that is, there is no migration of toxic deltamethrin residue on commodities. Levels obtained at the initial and end of storage period were below standards sets by US EPA and Codex, this thus indicate the food safety potential of the bag. All data obtained on effective and optimal use of postharvest and improved storage bag options has provided quantitative information on their potential for long term storage of maize using on low-cost and adaptable storage techniques readily managed

by poor and unexperienced farmers, bulk aggregators and other maize storage experts in Nigeria.

6.3 Recommendations

The following recommendations were suggested based on the conclusions from this study:

- a. Maize meant for storage inside PP and ordinary ZF storage bags should first be treated with a grain protectant such as diatomaceous earth to ensure long term preservation.
- b. ZF hermetic and PICS bag technologies readily mitigate insect infestations and therefore should be generally encouraged to better food security.
- c. The GrainMate meter used which is cost-effective appeared to provide reliable and predictable MC readings, this meter should be adopted by farmers to allow them make quick decisions on the condition of stored grains.
- d. Government agencies and organizations should ensure that farmers are aware of current storage technologies for successful adoption and effective use for better and increased food security.

6.4 Contributions to knowledge

The data obtained from this study will provide maize aggregators and value chain stakeholders with reliable information on optimal usage of improved storage technologies (hermetic bags and diatomaceous earth) for minimizing maize postharvest losses arising from insect infestations, increased moisture content and aflatoxin contamination within market storehouses.

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APPENDICES

Appendix I: Storehouse study - Monthly mean of live and dead insect types per storage treatments

| Month | Bags | Insect types | | | | | | |
|--------------------------|------|------------------|------|---------------------|------|-----------------------|------|-----------------------|
| | | <i>S. oryzae</i> | | <i>T. castaneum</i> | | <i>C. ferrugineus</i> | | <i>Liposcelis</i> spp |
| | | Live | Dead | Live | Dead | Live | Dead | Live |
| FEB. 17 | PP | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | PPDE | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | PICS | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | PP1L | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | ZF | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | ZFDE | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | ZF2L | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | ZFIL | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MAR. 17 | PP | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | PPDE | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | ZF | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | ZFDE | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| APR. 17 | PP | 2 | 0 | 2 | 1 | 0 | 0 | 14 |
| | PPDE | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| | ZF | 0 | 0 | 2 | 0 | 0 | 0 | 17 |
| | ZFDE | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| MAY 17 | PP | 6 | 0 | 7 | 3 | 1 | 0 | 0 |
| | PPDE | 0 | 0 | 1 | 0 | 0 | 0 | 24 |
| | PICS | 0 | 1 | 2 | 0 | 0 | 0 | 0 |
| | PP1L | 0 | 0 | 0 | 0 | 0 | 0 | 7 |
| | ZF | 1 | 0 | 0 | 0 | 0 | 0 | 2 |
| | ZFDE | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | ZF2L | 1 | 0 | 3 | 0 | 0 | 0 | 0 |
| | ZF1L | 0 | 1 | 2 | 0 | 0 | 0 | 0 |
| JUN. 17 | PP | 8 | 1 | 70 | 10 | 1 | 0 | 3 |
| | PPDE | 0 | 0 | 3 | 0 | 0 | 0 | 32 |
| | ZF | 34 | 2 | 2 | 0 | 0 | 0 | 49 |
| | ZFDE | 0 | 0 | 0 | 0 | 0 | 0 | 34 |
| JUL. 17 | PP | 18 | 1 | 83 | 17 | 4 | 0 | 0 |
| | PPDE | 0 | 1 | 16 | 3 | 0 | 0 | 54 |
| | ZF | 69 | 14 | 2 | 1 | 5 | 2 | 33 |
| | ZFDE | 0 | 0 | 0 | 0 | 0 | 0 | 87 |
| | PP | 26 | 2 | 74 | 14 | 0 | 0 | 0 |

| | | | | | | | | |
|--------------------------|------|-----|----|-----|----|-----|----|----|
| AUG. 17 | PPDE | 0 | 0 | 5 | 2 | 0 | 0 | 61 |
| | ZF | 115 | 8 | 1 | 26 | 26 | 0 | 11 |
| | ZFDE | 0 | 0 | 0 | 1 | 0 | 0 | 73 |
| SEP. 17 | PP | 52 | 7 | 43 | 6 | 4 | 0 | 0 |
| | PPDE | 0 | 0 | 16 | 0 | 0 | 0 | 24 |
| | PICS | 0 | 1 | 10 | 39 | 0 | 0 | 0 |
| | PP1L | 1 | 0 | 5 | 31 | 0 | 2 | 0 |
| | ZF | 116 | 33 | 0 | 0 | 40 | 6 | 0 |
| | ZFDE | 0 | 0 | 0 | 0 | 0 | 0 | 71 |
| | ZF2L | 0 | 7 | 3 | 10 | 0 | 0 | 0 |
| | ZF1L | 0 | 5 | 8 | 5 | 0 | 0 | 0 |
| OCT. 17 | PP | 96 | 71 | 172 | 15 | 7 | 0 | 30 |
| | PPDE | 33 | 3 | 10 | 2 | 0 | 0 | 52 |
| | ZF | 372 | 54 | 0 | 0 | 156 | 11 | 32 |
| | ZFDE | 6 | 4 | 0 | 0 | 9 | 0 | 37 |
| NOV. 17 | PP | 117 | 26 | 243 | 0 | 0 | 9 | 0 |
| | PPDE | 62 | 3 | 13 | 0 | 0 | 0 | 32 |
| | ZF | 343 | 87 | 1 | 1 | 141 | 11 | 16 |
| | ZFDE | 10 | 7 | 0 | 1 | 0 | 2 | 38 |
| DEC. 17 | PP | 132 | 24 | 261 | 24 | 14 | 9 | 0 |
| | PPDE | 69 | 8 | 6 | 2 | 0 | 0 | 44 |
| | ZF | 432 | 90 | 0 | 0 | 212 | 30 | 46 |
| | ZFDE | 32 | 4 | 1 | 1 | 0 | 0 | 23 |
| JAN. 18 | PP | 156 | 75 | 185 | 33 | 18 | 0 | 18 |
| | PPDE | 92 | 6 | 9 | 8 | 2 | 10 | 36 |
| | PICS | 11 | 2 | 18 | 13 | 0 | 0 | 0 |
| | PP1L | 3 | 2 | 6 | 5 | 0 | 0 | 0 |
| | ZF | 163 | 98 | 6 | 0 | 208 | 17 | 59 |
| | ZFDE | 55 | 4 | 0 | 0 | 13 | 0 | 46 |
| | ZF2L | 0 | 2 | 3 | 10 | 0 | 0 | 0 |
| ZF1L | 0 | 3 | 6 | 15 | 0 | 0 | 0 | |

Appendix II: Storehouse study - Monthly average of IDK, WL and viability per storage treatments

| Month | Treatment | % IDKNb | % WL | % Viability |
|----------------|------------------|----------------|-------------|--------------------|
| FEB. 17 | PP | 0 | 0 | 96.3 |
| | PPDE | 0 | 0 | 98.3 |
| | PICS | 0 | 0 | 98.5 |
| | PP1L | 0 | 0 | 97.0 |
| | ZF | 0 | 0 | 97.0 |
| | ZFDE | 0 | 0 | 97.5 |
| | ZF2L | 0 | 0 | 97.0 |
| | ZF1L | 0 | 0 | 97.0 |
| MAR. 17 | PP | 0.1 | 0 | 100.0 |
| | PPDE | 0.1 | 0 | 99.5 |
| | ZF | 0.2 | 0 | 99.0 |
| | ZFDE | 0 | 0 | 98.0 |
| APR. 17 | PP | 0.1 | 0 | 98.5 |
| | PPDE | 0 | 0 | 96.5 |
| | ZF | 0.2 | 0.1 | 97.0 |
| | ZFDE | 0 | 0 | 96.0 |
| MAY 17 | PP | 0.4 | 0.1 | 96.0 |
| | PPDE | 0.2 | 0 | 91.0 |
| | PICS | 0.4 | 0.1 | 96.7 |
| | PP1L | 1.0 | 0.2 | 97.5 |
| | ZF | 0.1 | 0.0 | 96.3 |
| | ZFDE | 0.3 | 0.1 | 96.3 |
| | ZF2L | 0.3 | 0.1 | 97.6 |
| | ZF1L | 0.2 | 0.1 | 97.0 |
| JUN. 17 | PP | 0.7 | 0.2 | 99.5 |
| | PPDE | 1.0 | 0.1 | 97.0 |
| | ZF | 0.7 | 0.1 | 98.4 |
| | ZFDE | 0.2 | 0.1 | 98.6 |
| JUL. 17 | PP | 1.6 | 0.4 | 95.4 |
| | PPDE | 0.0 | 0.0 | 94.0 |
| | ZF | 1.1 | 0.1 | 95.5 |
| | ZFDE | 0.0 | 0.0 | 97.6 |
| AUG. 17 | PP | 2.1 | 0.5 | 99.0 |
| | PPDE | 0.2 | 0.1 | 97.3 |
| | ZF | 2.6 | 0.7 | 98.2 |
| | ZFDE | 0.1 | 0.0 | 97.4 |
| SEP. 17 | PP | 2.0 | 0.5 | 96.2 |

| | | | | |
|----------------|------|------|-----|------|
| | PPDE | 0.5 | 0.1 | 96.7 |
| | PICS | 0.1 | 0.0 | 99.0 |
| | PP1L | 0.5 | 0.1 | 99.0 |
| | ZF | 4.5 | 0.8 | 93.7 |
| | ZFDE | 0.3 | 0.1 | 95.0 |
| | ZF2L | 0.3 | 0.1 | 98.4 |
| | ZF1L | 0.4 | 0.1 | 100 |
| OCT. 17 | PP | 2.8 | 1.5 | 93.0 |
| | PPDE | 1.9 | 0.8 | 94.7 |
| | ZF | 4.0 | 1.2 | 92.3 |
| | ZFDE | 0.4 | 0.2 | 94.4 |
| NOV. 17 | PP | 3.2 | 0.6 | 94.6 |
| | PPDE | 1.9 | 0.4 | 95.4 |
| | ZF | 9.6 | 3.0 | 91.4 |
| | ZFDE | 0.2 | 0.1 | 94.2 |
| DEC. 17 | PP | 4.9 | 1.4 | 90.0 |
| | PPDE | 1.7 | 0.4 | 93.4 |
| | ZF | 13.0 | 3.8 | 81.0 |
| | ZFDE | 0.6 | 0.2 | 93.4 |
| JAN. 18 | PP | 5.4 | 1.4 | 89.0 |
| | PPDE | 1.0 | 0.1 | 91.3 |
| | PICS | 0.9 | 0.2 | 96.7 |
| | PP1L | 0.4 | 0.1 | 96.4 |
| | ZF | 16.9 | 6.8 | 83.4 |
| | ZFDE | 0.8 | 0.3 | 92.0 |
| | ZF2L | 0.7 | 0.2 | 96.4 |
| | ZF1L | 1.0 | 0.1 | 96.4 |

Appendix III: Initial and final levels of aflatoxin in three replicates of maize samples

| Sample | Initial Aflatoxin ($\mu\text{g}/\text{kg}$) ^{ab} | | | | Final Aflatoxin ($\mu\text{g}/\text{kg}$) ^{ab} | | | | Mean (ppb) |
|--------|---|----|----|----|---|----|----|----|------------|
| | B1 | B2 | G1 | G2 | B1 | B2 | G1 | G2 | |
| PP 1 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 5.0 |
| PP 2 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | |
| PP 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| PPDE 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.7 |
| PPDE 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| PPDE 3 | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 0 | |
| PICS 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.7 |
| PICS 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| PICS 3 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | |
| PP1L 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| PP1L 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| PP1L 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| ZF 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.0 |
| ZF 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| ZF 3 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | |
| ZFDE 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| ZFDE 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| ZFDE 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| ZF2L 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| ZF2L 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| ZF2L 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| ZF1L 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| ZF1L 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| ZF1L 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |

Appendix IV: Storehouse study - Monthly average of maize temperature, humidity and moisture level per storage treatment.

| Month | Treatment | PHL meter | | | JD meter | GAC meter | Oven-dry |
|----------------|-----------|-----------|------|------|----------|-----------|----------|
| | | T °C | RH % | MC % | MC % | MC % | MC % |
| FEB. 17 | PP | 29.5 | 55.8 | 11.2 | 13.7 | 11.4 | 10.6 |
| | PPDE | 29.6 | 55.8 | 11.3 | 13.7 | 11.6 | 10.7 |
| | PICS | 29.6 | 55.7 | 11.3 | 13.7 | 11.6 | 10.7 |
| | PP1L | 29.7 | 55.8 | 11.4 | 13.7 | 11.6 | 10.5 |
| | ZF | 29.7 | 55.7 | 11.5 | 13.7 | 11.4 | 10.7 |
| | ZFDE | 29.9 | 55.7 | 11.5 | 13.7 | 11.4 | 10.6 |
| | ZF2L | 29.6 | 55.8 | 11.5 | 13.7 | 11.6 | 10.5 |
| | ZF1L | 29.7 | 55.7 | 11.5 | 13.7 | 11.4 | 10.6 |
| MAR. 17 | PP | 30.0 | 61.7 | 12.4 | 13.7 | 12.0 | 10.6 |
| | PPDE | 29.6 | 59.7 | 12.3 | 12.9 | 11.1 | 10.8 |
| | ZF | 29.6 | 60.1 | 12.3 | 13.8 | 12.2 | 10.8 |
| | ZFDE | 29.8 | 60.2 | 12.1 | 11.6 | 11.0 | 10.7 |
| APR. 17 | PP | 30.0 | 64.7 | 12.9 | 13.9 | 12.3 | 11.0 |
| | PPDE | 29.6 | 66.0 | 13.1 | 12.4 | 11.9 | 11.1 |
| | ZF | 29.6 | 66.5 | 13.1 | 15.5 | 12.7 | 11.2 |
| | ZFDE | 29.8 | 65.3 | 12.9 | 12.9 | 11.7 | 11.2 |
| MAY 17 | PP | 29.2 | 68.0 | 13.5 | 14.0 | 12.5 | 11.5 |
| | PPDE | 28.8 | 66.5 | 13.3 | 13.1 | 11.5 | 11.4 |
| | PICS | 28.4 | 61.4 | 12.3 | 13.6 | 11.8 | 10.5 |
| | PP1L | 28.6 | 63.4 | 12.8 | 14.2 | 12.3 | 11.1 |
| | ZF | 28.5 | 68.4 | 13.6 | 14.0 | 12.5 | 11.3 |
| | ZFDE | 28.4 | 68.3 | 13.6 | 13.0 | 11.6 | 11.3 |
| | ZF2L | 28.6 | 60.2 | 12.2 | 14.0 | 12.1 | 10.8 |
| | ZF1L | 28.6 | 60.6 | 12.3 | 14.0 | 12.1 | 10.8 |
| JUN. 17 | PP | 28.8 | 68.5 | 13.6 | 14.7 | 12.7 | 11.6 |
| | PPDE | 28.5 | 68.5 | 13.6 | 13.8 | 11.9 | 11.7 |
| | ZF | 29.2 | 70.8 | 13.9 | 14.5 | 13.3 | 11.9 |
| | ZFDE | 28.7 | 68.1 | 13.5 | 14.0 | 12.9 | 12.0 |
| JUL. 17 | PP | 27.5 | 70.5 | 14.1 | 15.0 | 13.0 | 11.8 |
| | PPDE | 27.0 | 71.7 | 14.3 | 14.3 | 12.6 | 12.1 |
| | ZF | 29.3 | 73.1 | 14.4 | 15.1 | 14.0 | 12.3 |
| | ZFDE | 27.2 | 72.2 | 14.3 | 14.1 | 12.9 | 12.0 |
| AUG. 17 | PP | 27.6 | 73.0 | 14.5 | 15.2 | 13.5 | 12.1 |
| | PPDE | 27.1 | 73.2 | 14.7 | 14.2 | 12.8 | 12.5 |
| | ZF | 30.9 | 76.9 | 15.0 | 15.1 | 13.8 | 12.6 |

| | | | | | | | |
|----------------|------|------|------|------|------|------|------|
| | ZFDE | 27.8 | 70.8 | 14.1 | 14.4 | 12.9 | 12.6 |
| SEP. 17 | PP | 27.3 | 75.5 | 15.0 | 15.3 | 13.5 | 12.4 |
| | PPDE | 27.6 | 72.5 | 14.4 | 14.1 | 13.3 | 12.9 |
| | PICS | 26.3 | 61.7 | 12.7 | 14.2 | 12.3 | 10.7 |
| | PP1L | 26.4 | 60.9 | 12.6 | 14.3 | 12.1 | 10.7 |
| | ZF | 31.9 | 76.6 | 14.9 | 14.7 | 13.4 | 12.3 |
| | ZFDE | 26.9 | 73.7 | 14.7 | 14.3 | 13.5 | 12.7 |
| | ZF2L | 26.2 | 60.8 | 12.6 | 14.0 | 12.1 | 10.7 |
| | ZF1L | 26.3 | 62.7 | 12.8 | 14.2 | 12.4 | 11.3 |
| OCT. 17 | PP | 27.5 | 73.0 | 14.7 | 15.0 | 13.7 | 12.5 |
| | PPDE | 28.2 | 73.2 | 14.5 | 14.2 | 12.8 | 12.6 |
| | ZF | 31.3 | 78.9 | 14.9 | 15.0 | 14.5 | 13.0 |
| | ZFDE | 28.9 | 70.8 | 14.3 | 14.1 | 13.3 | 12.7 |
| NOV. 17 | PP | 31.2 | 72.0 | 14.0 | 14.8 | 13.4 | 12.5 |
| | PPDE | 31.4 | 72.5 | 14.1 | 13.6 | 13.4 | 12.4 |
| | ZF | 32.9 | 74.5 | 14.3 | 14.0 | 14.6 | 12.7 |
| | ZFDE | 30.0 | 73.2 | 14.3 | 13.6 | 13.0 | 12.6 |
| DEC. 17 | PP | 29.7 | 67.9 | 13.7 | 14.3 | 13.2 | 12.3 |
| | PPDE | 29.9 | 66.3 | 13.5 | 13.8 | 12.2 | 12.3 |
| | ZF | 28.6 | 69.3 | 13.8 | 14.4 | 13.6 | 12.8 |
| | ZFDE | 30.5 | 65.6 | 13.8 | 13.4 | 12.9 | 12.4 |
| JAN. 18 | PP | 30.6 | 63.2 | 12.6 | 13.3 | 12.5 | 11.4 |
| | PPDE | 30.7 | 63.0 | 12.5 | 12.5 | 11.5 | 11.2 |
| | PICS | 28.7 | 62.0 | 12.6 | 13.7 | 12.2 | 10.7 |
| | PP1L | 28.7 | 58.4 | 11.9 | 14.0 | 12.1 | 10.8 |
| | ZF | 31.5 | 65.1 | 12.8 | 12.7 | 12.8 | 11.9 |
| | ZFDE | 27.9 | 62.6 | 12.5 | 12.3 | 11.7 | 11.3 |
| | ZF2L | 28.4 | 60.1 | 12.2 | 13.2 | 12.1 | 10.6 |
| | ZF1L | 28/5 | 60.5 | 12.3 | 13.6 | 12.2 | 10.9 |

Appendix V: Storehouse study - Average environmental conditions (temperature and humidity) within the storehouse at different level

| Storage month | Within stack | | Above stack | | Vent | |
|----------------------|---------------------|-------------|--------------------|-------------|-------------|-------------|
| | T °C | RH % | T °C | RH % | T °C | RH % |
| FEB. 17 | 30.0 | 68.1 | 30.0 | 57.9 | 30.0 | 56.1 |
| MAR. 17 | 30.7 | 69 | 30.2 | 58.8 | 30.4 | 58.1 |
| APR. 17 | 30.0 | 69.5 | 29.7 | 70.9 | 29.9 | 70.6 |
| MAY 17 | 29.7 | 71.4 | 29.3 | 73.4 | 29.8 | 71.9 |
| JUN. 17 | 29.2 | 74.7 | 29.0 | 76.1 | 29.2 | 75.3 |
| JUL. 17 | 27.8 | 77.5 | 27.6 | 78.9 | 27.9 | 78.0 |
| AUG. 17 | 26.9 | 80.6 | 26.7 | 81.8 | 26.9 | 80.7 |
| SEP. 17 | 26.4 | 80.8 | 26.2 | 82.1 | 26.4 | 81.1 |
| OCT. 17 | 27.3 | 79.4 | 27.1 | 80.4 | 27.3 | 79.4 |
| NOV. 17 | 28.9 | 75.1 | 28.7 | 75.6 | 29.0 | 74.4 |
| DEC. 17 | 29.6 | 69.0 | 29.4 | 69.6 | 29.6 | 68.4 |
| JAN. 18 | 28.7 | 53.3 | 28.6 | 53.2 | 28.7 | 52.7 |

APPENDIX VI



Typical insect exit holes observed on inner HDPE liners and outer woven bags marked with solid circle