

Assessing and controlling bio-deterioration of maize in Tanzania

by

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A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY

Major: Industrial and Agricultural Technology

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2016

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DEDICATION

I would like to dedicate this thesis to my father the late *Ali Suleiman Abdallah* and my mother *Moza Massoud Seif* for their endless love, sacrifices, patience, care, and most importantly giving me strength to achieve higher goals in life. I also dedicate this work to my beautiful wife *Mchanga Massoud Msabah* and my daughter *Khadija Rashid Suleiman* for their enduring patience, support, encouragement and whose sacrifices, which were realized by our loss of precious time together, were for me the most painful and humbling of all.



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NOMENCLATURE

AA	Ambient Aeration
AGRA	Alliance for a Green Revolution in Africa
ANOVA	Analysis of Variance
APHIL	African Postharvest Losses Information
ASAE	American Society of Agricultural Engineers
ASDP	Agricultural Sector Development Program
CA	Chilled Aeration
CAST	The Council for Agricultural Science and Technology
CH ₄	Methane
CIMMYT	International Maize and Wheat Improvement Center
CO ₂	Carbon dioxide
COT	<i>Cryptolestes-Oryzaephilus-Tribolium</i>
DKT	Total Damaged Kernels
DML	Dry matter loss
DNA	Deoxyribonucleic Acid
EA	East Africa
Eqn.	Equation
EU	European Union
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agricultural organization of the United Nations-Statistics division
FU	Function unit
GAPs	Good agricultural practices
GDP	Gross domestic product
GHS's	Greenhouse gases emissions
GLM	General Linear Model
H ₂ O	Water
IARC	The International Agency for Research on Cancer
IITA	The International Institute of Tropical Agriculture
IPCC	Intergovernmental Panel on Climate Change
IPMP	Integrated Pest Management Plan

ISO	International Standard Organization
Kwh	Kilowatt-hour
LCA	Life Cycle Assessment
LGB	Larger Grain Borer
LPD	Lag phase duration
LSD	Least Significant Differences
M.C	Moisture contents
MRLs	Maximum residual levels
MSD	Maize streak disease
N A	No aeration control
NB	Nforya-Bamenda farm
NBS	National Bureau of Statistics
NFRA	National Food Reserve Agency
NMRP	The national maize research program
NOEL	The no-observed-effect level
NO _x	Nitrous oxide
O ₂	Oxygen
PHLs	Postharvest Losses
PICS	Purdue Improved Crop Storage
PMTDI	Provisional Maximum Tolerable Daily Intake
R.H	Relative Humidity
RST	<i>Rhizopertha-Sitophilus-Tribolium</i> system,
SAS	Statistical Analysis System
SSA	Sub-Saharan Africa
TBS	Tanzania Bureau of Standards
TEA	Techno-Economic Analysis
TFDA	Tanzania Food and Drugs Authority
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
WFP	The World Food Programme
WHO	World Health Organization
WHR	World Health Ranking

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation and profound gratitude to my advisors Dr. Kurt Rosentrater and Prof. Bernard Chove, for their excellent guidance, caring, patience, and encouragement throughout my time as his student. I especially want to thank my advisor Dr. Rosentrater has been a great mentor and inspired me to work as a team and independently. He always showered me with valuable suggestions and experience which significantly impacted the overall quality of the research. He constantly guided me throughout my PhD program and equipped me with important technical skills that will be an integral part of my career, in the future. I am also grateful to my committee members Dr. Carl J. Bern, Dr. Thomas J. Brumm, Dr. Gretchen A. Mosher and Dr. Joel R. Coats for their invaluable assistance, encouragement, insight, and counsel throughout my program of study. I really appreciate valuable discussions and feedback throughout the course of this research, which helped guide me to finishing this work.

I would also like to sincerely thank the United States Agency for International Development (USAID), under the Feed the Future programme through the Innovative Agricultural Research Initiative (iAGRI) programme, for financial support, which enabled this study to be done. I would like to express my sincere gratitude to my employer, the Sokoine University of Agriculture, for granting me permission to pursue the graduate studies. In addition, I would also like to offer my heartiest thanks to Denise Bjelland and Eduarda Becerra from Global Programs for their willingness to help and support whenever I was in need.

Furthermore, I would like to thank all my friends, my colleagues, department faculty and staff at the Iowa State University for their support. In fair honesty, it is impossible to mention everyone. I am immensely indebted to for their help in and outside of the study, but to those I didn't mention, thank you and may you be blessed with only the best in your lives. Last but certainly not least, I would like to thank my parents, wife, daughter, and siblings for their unconditional love, encouragement, prayers and support during the last four years. Thank you, doesn't seem enough.

ABSTRACT

Agriculture is the backbone of the Tanzanian economy. It accounts for about one-third of the gross domestic product (GDP), provides 85 percent of all exports and serves as a livelihood to over 80 percent of the total population. Maize or corn (*Zea mays* L.) is the primary staple crop; it's grown in nearly all agro-ecological zones in the country. Tanzania is a major maize producer in Sub-Saharan Africa. In the last four decades, Tanzania has ranked among the top 25 maize producing countries in the world. Despite the steady production of maize over the past three decades, post-harvest losses of maize remained significantly high, especially for small-holder farmers. Post-harvest handling, poor infrastructure, and weather variability, bio-deterioration brought about by pest organisms such as insects, molds, and fungi, rodent, bacteria, pathogens, and viruses often aggravate such losses.

In tropical countries, a large proportion of the maize is harvested and stored under humid and warm climatic conditions, which subsequently results in rapid deterioration of the grains, mainly because of growth of molds and pests. Deterioration of maize is mainly affected by moisture content, temperature (grain and air), relative humidity, storage conditions, fungal growth, and insect pests. Fungal growth, especially *Aspergillus flavus* and *Fusarium sp* in maize, facilitated by hot and humid conditions, poses a major health risk through production of mycotoxins. Mycotoxins are toxic secondary metabolites of fungi that frequently contaminate the maize in the field and/or during storage. The most important mycotoxins in maize are the aflatoxins, Fumonisin, deoxynivalenol, and ochratoxin. In order to maintain high quality maize for both short- and long-term storage, maize must be protected from weather, the growth of microorganisms, and insect pests.

Stored product pests such as *Sitophilus zeamais* (Motschulsky), the maize weevil, are serious pests of economic importance in stored products in tropical and subtropical countries. Infestation often starts in the field, but serious damage is done during maize storage. This study determined the resistance of flint corn and dent corn to infestation by *S. zeamais*. Improved King Philip hybrid flint corn and Fontanelle 6T-510 hybrid dent corn were used. Two temperature conditions (10 and 27°C) and two storage times (15 and 30 days) were used. Results showed flint corn was more resistant to insect damage than dent corn at 27°C and 30 days storage time. After 30 d storage time and 27°C, death rate of the weevils was significantly higher in flint corn ($R^2 = 0.945$) compared to dent corn ($R^2 = 0.634$). Likewise, the damaged seed was 10% higher in dent corn than in flint corn at 27°C and 30 days. However, no significant difference was observed for seed weight loss between flint corn and dent corn at the same storage conditions.

Further, the study evaluated *S. zeamais* infestation on seven varieties of maize. Seven commercial maize varieties (white dent, yellow dent, orange flint, Indian flint, white and yellow popcorn, and sweet corn), two temperature conditions (10 and 27 °C) and three storage times (30, 60, and 90 days) were used. The moisture contents of all maize samples were adjusted to $15.5 \pm 0.5\%$ (wet basis) prior to initiating storage trials. Numbers of live weevils, seed damage, weight loss, and weight of powder produced were assessed at the end of each storage time. As expected, severe damage was observed at 27°C and 90 d for all maize varieties. Exponential growth rates of *S. zeamais* were observed in almost all maize varieties. Among seven varieties evaluated, orange flint corn, yellow, and white popcorn show resistance to *S. zeamais*. Sweet and dent corn were most susceptible to maize weevil infestation. Higher numbers of live *S. zeamais* were observed on Indian flint corn and sweet

corn. Consequently, there was a higher seed weight damage and weight loss. In addition, seed damaged, percentage seed weight loss and weight of powder produced was significantly and positively correlated with a number of live *S. zeamais* ($r = 0.91, P < 0.05$), ($r = 0.88, P < 0.05$), and ($r = 0.89, P < 0.05$) respectively. Thus, some varieties of flint corn and popcorn can be considered as potential maize varieties to be used to reduce postharvest loss of maize in tropical countries due to their natural resistance to *S. zeamais* infestation.

Moreover, the study also determined the techno-economic analysis (TEA) and life cycle analysis (LCA) of maize storage for middle class farmers in developing countries. Maize is the most widely cultivated cereal crop worldwide. It is produced on a seasonal basis, usually harvested once per year. To maintain a constant supply throughout the year, maize should be properly stored. But this entails high cost and high-energy consumption, which can contribute significant amounts of greenhouse gas emissions. Three storage capacities (25,000 bu, 250,000 bu and 2,500,000 bu) per year were evaluated for economic analysis and environmental impact. The result shows the total storage cost per kilogram decreased as storage capacity increased (3.69\$/bu, 1.89\$/bu, and 0.42\$/bu). Likewise, energy consumption (electricity, diesel and liquid propane) increased as storage capacity increased. Consequently, more greenhouse gas emissions (CO_2 , CH_4 , and NO_x) were emitted to the environment. Thus, to obtain an optimal balance between economics and the environment, it is important for the farmers to understand the concepts of techno-economic analysis and life cycle assessment. Furthermore, the study also determined the measured and predicted temperature of maize under hermetic conditions. Three different storage conditions (room at 25°C, cooling at 4°C, and freezing at -20°C) were investigated. Yellow dent corn variety Blue River 571136 from Iowa, harvested in 2011 was used. Maize was stored in two hermetically

sealed bins (50-cm diameter x 76-cm height). Five logger sensors were installed inside the bin to measure temperature and relative humidity of the air and maize grain. The sensors were located at the top, center, bottom, left and right at about 12 centimeter apart. After placing each barrel into storage, temperature and relative humidity values were measured every minute for 9 days throughout the duration of the experiment. Model validation was carried out by comparing predicted with measured maize grain temperature data in the radial and vertical directions. The temperature in the hermetically sealed cylindrical bins varied, mostly in the radial direction and very little in the axial vertical directions. No noticeable change in temperature was observed in the room condition. Moreover, the temperature in the grain changed more rapidly in the freezing conditions than in the room temperature and cooling conditions. Furthermore, the lag time between the center temperature and the side (right, left, top, and bottom) was greater in the radial direction compared to in the vertical direction. The maximum difference between predicted and measured temperature was $\pm 1.5^{\circ}\text{C}$. The predicted and measured values of maize grain temperature at radial and vertical directions were found to be in good agreement. The model shows a good potential application to predict the temperature of maize grain stored at room, cooling and freezing conditions under hermetic storage.

In addition, the study determined the impact of moisture content and *S. zeamais* on maize quality during hermetic and non-hermetic storage conditions. Commercially commingled maize kernels were conditioned to target moistures 14, 16, 18, and 20% moisture content (wet basis), and then three replications of 300 grams of maize grain were stored in glass jars or triple Ziploc® slider 66 μm (2.6-mil) polyethylene bags at four conditions: hermetic with weevils, hermetic no-weevils, non-hermetic with weevils, non-hermetic no-weevils. All jars

and bags were stored in an environmental chamber at 27°C and 70% relative humidity for either 30 or 60 days. At the end of each storage period, jars and bags were assessed for visual mold growth, mycotoxin levels, CO₂ and O₂ concentrations, pH level, the numbers of live and dead *S. zeamais*, and maize moisture content. The maize stored in non-hermetic conditions with weevils at 18 and 20% exhibited high levels of mold growth and aflatoxin contamination (>150 ppb). Although mold growth was observed, there were no aflatoxins detected in maize stored in hermetic conditions. The CO₂ and O₂ concentrations were directly related to the maize moisture contents and storage times. In general, CO₂ increased and O₂ gradually decreased as storage time increased. No significant difference in pH was observed in any storage conditions ($P < 0.05$). Total mortality (100%) of *S. zeamais* was observed in all hermetically stored samples at the end of 60 days storage. The number of *S. zeamais* linearly increased with storage time for maize stored in non-hermetic conditions. Moisture content for hermetically stored maize was relatively constant. Moreover, a positive correlation between moisture content and storage time was observed for maize stored in non-hermetic conditions with weevils ($r = 0.96$, $P < 0.05$). The results indicate that moisture content and the number of *S. zeamais* play a significant role in maize storage, both under hermetic and non-hermetic conditions.

The study also determined whether there is a synergistic interaction between *P. truncatus* and *S. zeamais* during storage. The interaction between the two insects was evaluated in terms of the numbers of the live population, percent damaged grain, the weight of powder (flour) produced, and percentage seed weight loss. Higher damage was observed in non-hermetic storage with *P. truncatus* and in mixed treatments (*P. truncatus* and *S. zeamais*). A significant difference ($P < 0.05$) and positive correlation were observed between the number

of live population, percentage grain damage, the weight of powder produced, and percentage seed weight loss on infestation by *P. truncatus*, *S. zeamais*, and mixed treatments. *S. zeamais* dominate populations in the early stage, but were outnumbered by *P. truncatus* after 60 d of storage in the individual species as well as in mixed treatments. The high percentage grain damage was observed in non-hermetic storage after 60 days in *P. truncatus* (58%) and mixed treatments (54%). The weight of powder produced ranged from 0-30 grams per 250 grams of maize. Percentage seed weight loss decreased after 60 days for *P. truncatus* and mixed treatments, but increased onward for *S. zeamais*, a low synergistic interaction between *P. truncatus* and *S. zeamais* was observed. However, *P. truncatus* plays a significant role when two insects coexist and cause more severe damage than *S. zeamais* in maize under non-hermetic storage conditions.

Furthermore, the study determined the practicability of periodic physical disturbance on *S. zeamais* mortality and adaptation by smallholder farmers in developing countries. *S. zeamais* is the most widely occurring and important cosmopolitan postharvest insect pest of stored maize in tropic and sub-tropical regions. Preventing infestation of this pest without using chemicals remains a huge challenge for smallholder farmers in the developing countries. Physical control methods are effective and attractive alternative methods to prevent, and control stored product pests in grain handling and storage facilities. Physical techniques are based on the application of some kind of force to manipulate the storage environments. They can provide unfavorable conditions for insect pests to multiply or damage to the grain. In this experiment, disturbed and stationary/control treatments were arranged in a Completely Randomized Design (CRD) with three replications and three-storage times (30, 60, and 90 days) in three regions of Tanzania. A total of 108 clean 20L

(L284 x W234 x H391) millimeter plastic containers were each loaded with 10 kilograms of fresh white dent corn and 0.50 kilograms of maize infested with *S. zeamais*. The initial numbers of *S. zeamais* were determined. For the turned treatment, containers were disturbed or turned twice a day, whereas for the controls, the containers were not disturbed until the end of storage. The overall percent mortality after 30, 60, and 90 days of storage were 88, 96, and 98% respectively. A statistically significant difference ($P < 0.05$) was observed for the number of live *S. zeamais* in the control treatments. While the number of live *S. zeamais* in the turned treatment significantly decreased as storage time increased. The study shows the potential of a feasible, simple, affordable, safe and effective method of protecting maize grain for small-holder farmers in developing countries without using chemicals.

Lastly, the study assessed the postharvest practices and awareness of mycotoxins contamination in maize grain. Maize is a major cereal crop in Tanzania and it is grown in diverse agro-ecological zones. Like other sub-Saharan countries, postharvest losses of maize during storage in Tanzania remain significantly high, especially for smallholder farmers. Unpredictable weather and poor postharvest practice contribute significant to rapid deterioration of grain and mold contamination, and subsequent production of mycotoxins. The purpose of this study was to assess the postharvest practices and awareness and knowledge of mycotoxin contamination in maize grain in three agro-ecological zones (Eastern, Central, and Northern) of Tanzania between November 2015 and February 2016. A survey using semi-structured questionnaires was administered to farmers, traders, and consumers of maize. A total of 90 people (30 from each zone) were surveyed with a response rate of was 96% (87). In addition, several samples of maize were collected and analyzed for aflatoxin, fumonisin, and Zearalenone contamination to validate the awareness and

knowledge of mycotoxin contamination of maize. The result shows a high level of postharvest losses of maize mainly through insect infestation. Moreover, over 80% of the farmers, traders, and consumers of maize were unaware of mycotoxins contamination. All maize samples collected contained detected levels of mycotoxins. The maximum concentration of aflatoxins, fumonisin, and Zearalenone in maize samples was 19.20 ppb, 7.60 ppm, and 189.90 ppb respectively. Education intervention is necessary to decrease the disconnect observed between actual mycotoxin contamination and the awareness and knowledge of farmers, traders, and consumers of maize in Tanzania. Enhancing awareness and knowledge provide the opportunity to educate on post-harvest practices that reduce postharvest losses of maize in Tanzania.

CHAPTER 1. GENERAL INTRODUCTION

1.1. Research problem

Despite the great effort from researchers, farmers, governmental and nongovernmental organizations, post-harvest losses of maize in Sub-Saharan Africa (SSA) have significantly increased in the past decade (FAO, 2011), mainly due to climatic conditions, bio-deterioration brought about by pest organisms such as insects, molds, and fungi, rodent and birds, as well as poor post-harvesting practices (Hodges et al., 2013). This has resulted in food shortage, and labor losses. Postharvest loss is considered a big challenge for development, food security, malnutrition, and poverty alleviation in SSA (Jones and Thornton, 2003). Food security and poverty alleviations are two major development challenges in Tanzania. Over 85% of the population depends on subsistence agriculture for livelihood (Kaliba et al., 1998). Post-harvest damage contributes significant losses of maize cultivated by these subsistence farmers, whose farmers are small scale. In Tanzania, more than 20% (Figure 1) of stored maize is lost due to post-harvest loss (Rembold et al., 2011) mostly by bio-deterioration specifically by insects such as the maize weevil *Sitophilus zeamais* and larger grain borer (*Prostephanus truncates*). Biodeterioration of cereal grain is defined as the loss of physical and nutritional qualities of cereal grain caused by organisms such as insect pests, rodent, mold, and bacteria, which render grains unsuitable for human consumption (Hodges, 2013; Sreenivasa et al., 2011). The *S. zeamais* and *P. truncates* are the primary or major pests due to their ability to destroy a whole grain kernel (Kanyamasoro et al., 2012). A recent study in the Rungwe district in Tanzania observed a loss up to 80% of untreated harvested maize due to insect infestation (Mujila, 2013).

The potential loss of maize due to molds and fungi in tropical and sub-tropical countries is believed to be higher than the sum of the potential losses due to animal pests, since they can contaminate of maize with mycotoxins (Pitt, 2000; Kaaya and Kyamuhangire, 2006). Maize has been incriminated as a leading food vehicle of mycotoxicosis outbreaks in tropical countries (Lewis et al., 2005; Probst et al., 2007). Mycotoxins attract worldwide attention because of the significant economic losses associated with their impact on human health, animal productivity and trade (Wagacha and Muthomi, 2008). Other consequences include reduced human labor productivity, socioeconomic loss, and death. The Food and Agricultural Organization (FAO) estimated that between 25 and 50% of agricultural crops worldwide are contaminated with mycotoxins (Lewis et al., 2005; Wagacha and Muthomi, 2008). Furthermore, the estimated value of maize lost due to mycotoxins per year is around \$932 million in the United States (Betran and Isakeit, 2003). Mycotoxicosis is a broad spectrum of diseases, both acute and chronic in nature. The main toxic effects are carcinogenicity, genotoxicity, teratogenicity, nephrotoxicity, hepatotoxicity, impaired growth, immunosuppression, and deaths in humans and farm animals (Desjardins et al., 2000; Satish et al., 2007). A strong synergistic effect between mycotoxin exposure and some important diseases in Africa such as malaria, kwashiorkor, malnutrition and HIV/AIDS have been suggested (Wagatha and Muthomi, 2008).

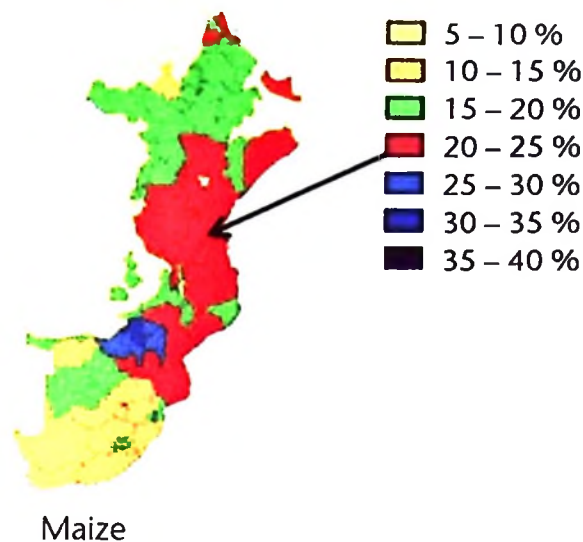


Figure 1. Estimated Post-Harvest Loss of Maize in Eastern and Southern Africa (Rembold et al., 2011).

1.2. Problem Justification

Maize accounts for over 30% of the total food production and constitutes more than 70% of the cereal consumption in Tanzania (Msuya et al., 2008). The majority of farmers cultivate different varieties of maize (hybrid and conventional) and most of them enjoy the highest yields, especially during periods of sufficient rain (Tewele, 2012). The problem facing them is that they cannot store their crops from one season to another due to post-harvest losses; the main cause of post-harvest losses during storage is bio-deterioration mainly by an infestation of insects, mold, and fungi. The problem is worse for newly introduced varieties (hybrid maize) due to less resistance to insect infestation. Post-harvest storage of maize and other cereal grains is the most critical part. If improperly handled these can be damaged resulting from bio-deterioration by insects and molds. Often insect infestation causes an unpleasant smell and taste while molds affect the taste and lead to mycotoxins contamination which

reduces quality and quantity, causes discoloration, and decreasing the viability of grain (Magan et al., 2003). For longer storage many farmers rely on the application of synthetic insecticides such as actellic super. These chemicals have led to pesticide and fungicide resistant strains, bioaccumulation, poisoning of staff and of useful insect pollinators (e.g. bees), domestic animals, and environmental degradation (Fredrick, 2007). Moreover, most of these farmers are poor and they cannot afford the commercial pesticides. For those affording it, they have little or no knowledge of using it, and as a result often harm of themselves, polluting the environment, and contaminating maize with poisons which threaten the health of the end user. Several health disorders such as miscarriages, memory disorders, and birth defects have been related to pesticide application in developing countries (Carvalho, 2006).

Due to increases in post-harvest losses of maize in Tanzania, it is essential to understand the effect of bio-deterioration of maize and find an easy available, and affordable way to control it while, at the same time, reducing chemical residue, ensuring food security, reducing malnutrition, and increasing income of small scale farmers. Because most of the farmers are illiterate, there is a great need for the development of affordable and simple techniques that will be easily integrated into the society structure since most of the developed modern post-harvest techniques have often bypassed the rural community (Kitinoja et al., 2010). Moreover, although many studies have been conducted on post-harvest loss, this study aims to understand the effect of bio-deterioration of maize by looking at postharvest practices and storage conditions, as well as by reviewing literature on the current maize production, postharvest losses and the risk of mycotoxin contamination in Tanzania. Additional, goals are to determine the effects of deterioration parameters on the storage of maize, evaluate the natural resistance of different varieties of maize to storage pests, and evaluation of maize

weevil infestation on seven varieties of maize. Another goal is to techno-economic analysis and life cycle assessment of stored maize. Also, the study determines measured and predicted temperature of maize grain (*Zea mays* L.) under hermetic storage conditions. The impact of maize moisture content and maize weevils on maize quality during hermetic and non-hermetic storage was also evaluated. Moreover, the study determines the synergistic interaction between maize weevil (*Sitophilus zeamais*) and the larger grain borer *Prostephanus truncatus* (Horn) on stored maize in hermetic and non-hermetic storage. In addition, the study also evaluates periodic physical disturbance, an alternative method to control *Sitophilus zeamais*, the maize weevil infestation, and lastly, the study assesses the postharvest practices related to mycotoxin contamination of maize in three agro-ecological zones in Tanzania.

1.3. Research Objectives

The overall primary objective of this research was to evaluate susceptibility and control of bio-deterioration of maize in Tanzania. The specific objectives of the research were:

1. To review current maize production, postharvest losses and the risk of mycotoxin contamination in Tanzania.
2. To review the current literature on effects of deterioration parameters on storage of maize in tropical countries.
3. To determine if flint corn is naturally resistant to *Sitophilus zeamais* infestation.
4. To evaluate maize weevils *Sitophilus zeamais* Motschulsky infestation on seven varieties of maize.

5. To determine the techno-economic analysis (TEA) and the life cycle assessment (LCA) of maize storage for middle scale farmers.
6. To determine measured and predicted temperature of maize grain (*Zea mays* L.) under hermetic storage conditions.
7. To determine the impact of moisture content and maize weevils on maize quality during hermetic and non-hermetic storage.
8. To determine the synergistic interaction between maize weevil (*Sitophilus zeamais*) and the larger grain borer *Prostephanus truncatus* (Horn) on storage of maize in hermetic and non-hermetic storage.
9. To determine the practicability of periodic physical disturbance as an alternative method to control *Sitophilus zeamais*, the maize weevil infestation.
10. To assess postharvest practices and mycotoxin contamination of maize in three agro-ecological zones in Tanzania.

1.4. Research Hypotheses

The overall hypothesis of this dissertation was to evaluate susceptibility and control of bio-deterioration of maize in Tanzania. A hypothesis is a statement you make and then try to prove or disapprove. This dissertation is divided into several chapters depending on the specific objectives.

Chapter 1.

General introduction, research problems, and justification.

Chapter 2

To review the current literature on the current maize production, postharvest losses and the risk of mycotoxins contamination in Tanzania.

Chapter 3

To determine effects of deterioration parameters on storage of maize.

Chapter 4

Ho: Temperature and time will not have effects on maize storage and maize weevil infestation.

Ha: Temperature and time will have effects on maize storage and maize weevil infestation.

Ho: Maize varieties will not have effects on maize storage and maize weevil infestation.

Ha: Maize varieties will have effects on maize storage and maize weevil infestation.

Chapter 5

Ho: Maize varieties will not have effects on maize weevil infestation and storage conditions.

Ha: Maize varieties will have effects on maize weevil infestation and storage conditions.

Chapter 6

Ho: TEA will not help middle scale farmers understand the cost of maize storage.

Ha: TEA will help middle scale farmers understand the cost of maize storage.

Ho: LCA will not help middle scale farmers understand environmental impacts.

Ha: LCA will help middle scale farmers understand environmental impacts.

Chapter 7

Ho: The measured temperature will be different from the predicted temperature of maize grain (*Zea mays* l.) under hermetic storage conditions.

Ha: The measured temperature will be close to the predicted temperature of maize grain under hermetic storage conditions.

Chapter 8

Ho: Moisture content will not have effects on maize quality during hermetic and non-hermetic storage.

Ha: Moisture content will have effects on maize quality during hermetic and non-hermetic storage.

Ho: Maize weevils will not have effects on maize quality during hermetic and non-hermetic

Ha: Maize weevils will have effects on maize quality during hermetic and non-hermetic

Chapter 9

Ho: There is no synergistic interaction between maize weevil and larger grain borer on storage of maize in hermetic and non-hermetic storage conditions.

Ha: There is a synergistic interaction between maize weevil and larger grain borer when introduced simultaneously into maize in hermetic and non-hermetic storage conditions.

Chapter 10

Ho: Periodic physical disturbance will not control *Sitophilus zeamais*, the maize weevil, infestation during storage of maize.

Ha: Periodic physical disturbance will control *Sitophilus zeamais*, the maize weevil, and infestation during storage of maize.

Chapter 11

Ho: People are not aware of postharvest practices and effects of mycotoxin contamination of maize in three agro-ecological zones in Tanzania.

Ha: People are aware postharvest practices and effects of mycotoxin contamination of maize in three agro-ecological zones in Tanzania.

1.5. Thesis Outline

This thesis follows the journal paper format. Chapter one contains research problems, research justification, objectives of this research, research hypothesis, and thesis outline. Chapter two through ten are either published journal articles or manuscripts formatted for submission to specified journals. Chapter two is the review of the current literature on maize production, postharvest losses and the risk of mycotoxin contamination in Tanzania. Chapter three looks at the effects of deterioration parameters on the storage of maize. Chapter four determines the natural resistance of flint and dent corn to *Sitophilus zeamais* infestation. Chapter five presents evaluation of maize weevil *Sitophilus zeamais* Motschulsky infestation on seven varieties of maize. Chapter six focuses on the techno-economic analysis (TEA) and the life cycle assessment (LCA) of maize storage for middle-class farmers. Chapter seven presents a published article on measured and predicted temperature of maize grain (*Zea mays* L.) under hermetic storage conditions. Chapter eight looks at the impact of maize moisture content and maize weevils on maize quality during hermetic and non-hermetic storage. In addition, Chapter nine determines the synergistic interaction between *Sitophilus zeamais*, the maize weevil, and *Prostephanus truncatus*, larger grain borer on storage of maize in hermetic and non-hermetic conditions. Moreover, Chapter ten presents a field research article on periodic physical disturbance, an alternative method to control *Sitophilus zeamais*, the maize weevil infestation. Chapter eleven focuses on postharvest practices and awareness of mycotoxin contamination of maize in three agro-ecological zones in Tanzania. Lastly, chapter twelve contains general conclusions based on the conclusions of all chapters and outcomes from this research.

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CHAPTER 2. CURRENT MAIZE PRODUCTION, POSTHARVEST LOSSES AND THE RISK OF MYCOTOXINS CONTAMINATION IN TANZANIA

Modified from a paper to be submitted to *African Journal of Agricultural Research*

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Abstract

Agriculture is the backbone of the Tanzanian economy. It accounts for about one-third of the gross domestic product (GDP), provides 85 percent of all exports and serves as a livelihood to over 80 percent of the total population. Maize is the primary staple crop; it's grown in nearly all agro-ecological zones in the country. Tanzania is a major maize producer in Sub-Saharan Africa. In the last four decades, Tanzania has ranked among the top 25 maize producing countries in the world. In Tanzania, in the 2013/14 marketing season, over a half billion metric tons of maize was produced majority (<85%) by smallholder farmers. Despite the steady production of maize over the past three decades, post-harvest losses of maize remained significantly high, up to 30-40% in some rural areas. Post-harvest handling, poor infrastructure, and weather variability along with biotic factors such as insect pests, bacteria, pathogens, viruses, and fungi, often aggravate such losses. Mycotoxin-producing fungi pose a major risk. Mycotoxins are toxic secondary metabolites of fungi that frequently contaminate maize in the field and/or during storage. Mycotoxin contamination of maize poses a health risk to humans and animals if not properly managed. The most important mycotoxins in Tanzania are the aflatoxins, Fumonisin, and ochratoxin. The objective of this chapter was to review the current literature on the production trends, consumption, post-harvest losses, and mycotoxin contamination of maize and to provide strategies to control and prevent postharvest losses and mycotoxin contamination in Tanzania.

Keywords. Tanzania; maize; post-harvest losses; mycotoxins; aflatoxins; Fumonisin.

2.1. Introduction

Agriculture is the backbone of the Tanzanian national economy. It accounts about one-third of the gross domestic product (GDP), provides 85 percent of all exports and serves as a livelihood to over 80 percent of the population (CIA World Fact book, 2014). Maize (*Zea mays* L.) is a primary staple crop; it's grown in nearly all agro-ecological zones in the country (USAID, 2010). Maize together with wheat and rice are the three most cultivated cereal crops in the world (Suleiman et al., 2013). Current world annual maize production is about 10.14 billion metric tons (De Groote et al., 2013). The United States (US) are the largest producer, producing over 30%, followed by China 21% and Brazil 7.9% (Table 1). Africa produces around 7% of the total world production. Two-thirds of all African maize come from eastern and southern Africa (Verheye, 2010; FAOSTAT, 2014). In sub-Saharan Africa, (SSA) maize is the most important cereal crop and staple food for about 1.2 billion people (IITA, 2009) and occupies a third of the cultivated area (Blackie, 1990). Maize accounts for over 30% lower-house income and contributes 60% of dietary calories and 50% of protein intake (IITA, 2009; Amani, 2004). Tanzania is a major maize producer in Sub-Saharan Africa. In the last five decades, Tanzania has ranked among the top 25 maize producing countries in the world (Barreiro-Hurle, J. 2012). Currently is ranked 1, 4, and 19 among top maize producing countries in East Africa (EA), Africa and in the world respectively ([http://www. indexmundi.com](http://www.indexmundi.com), FAOSTAT, 2014; McCann, 2001).

Mycotoxins are toxic secondary metabolites of fungi that frequently contaminate the maize in the field and/or during storage (Smith et al., 2012). Mycotoxin contamination of maize poses a health risk to humans and domesticated animals (Mboya et al., 2012; Suleiman

et al., 2013). The most important mycotoxins in maize are the aflatoxins, Fumonisin, deoxynivalenol, and ochratoxin (Kimanya et al., 2012). Aflatoxins are a group of mycotoxins produced as secondary metabolites by the action of two fungi species *Aspergillus flavus* and *A. parasiticus* (Marin et al., 2013; Feng et al., 2011). Fumonisin are mycotoxins synthesized mainly by *Fusarium verticilloides* and *Fusarium proliferatum* (Garrido et al., 2012). Deoxynivalenol (DON) is a common type of mycotoxins produced by pink mold *F. graminearum* (Garrido et al., 2012). Ochratoxin is another type of mycotoxin mostly produced by *Penicillium verrucosum*, *A. ochraceus*, and *A. niger* species (Lai et al., 2014). The objective of this chapter was to review the current literature on the production and consumption, postharvest losses and mycotoxins contamination of maize and to provide strategies to control and prevent postharvest losses and mycotoxin contamination in Tanzania.

2.2. Background

The United Republic of Tanzania is situated on the east coast of Africa and lies at longitudes 29° and 41° east and latitude 1° and 12° south of the equator (www.nationsencyclopedia.com). Tanzania consists of a mainland and offshore islands of Zanzibar (Unguja and Pemba) and Mafia in the Indian Ocean (Ak'habuhaya and Lodenius, 1988). It is the largest of the East African countries with a total area of 945,078 km² (364,900 sq. mil). Bordered by Kenya and Uganda to the north, Rwanda, Burundi, and the Democratic Republic of the Congo to the west, and Zambia, Malawi, and Mozambique to the south. The country's eastern borders lie on the Indian Ocean. Tanzania is administratively divided into thirty regions (Figure 2). The population in 2014 was 50.76 million and increasing by an average of about 3% per annum (FAOSTAT, 2015).

Table 1. Top 25 World Maize Producing Countries.

Rank	Country	Production (million tons)	Yield (tons/acre)	Area harvested (million Ha)
1	United States	367.68	12	33.63
2	China	271.00	6	36.80
3	Brazil	75.00	5	15.00
4	EU-27	71.02	7	9.57
5	Ukraine	25.00	5	4.60
6	Argentina	23.00	7	3.25
7	Mexico	22.50	3	6.90
8	India	21.00	2	8.60
9	South Africa	13.50	4	3.20
10	Russian Federation	12.00	5	2.60
11	Canada	11.50	9	1.25
12	Indonesia	0.92	3	3.12
13	Philippines	0.79	3	2.63
14	Nigeria	0.75	2	4.25
15	Serbia	0.69	0.9	1.28
16	Ethiopia	0.65	3	2.15
17	Egypt	0.58	8	0.71
18	Vietnam	0.54	5	1.20
19	Tanzania	0.50	1.5	4.00
20	Pakistan	0.50	4	1.14
21	Thailand	0.50	4	1.10
22	Turkey	0.46	8	0.55
23	Malawi	0.39	2	1.75
24	Zambia	0.34	3	1.21
25	Paraguay	0.31	4	0.70

FAOSTAT (2014) and Indexmundi (2014).

Geographically and topographically, Tanzania has diverse and complex climatic and environmental conditions. Tanzania includes both the highest (Mt. Kilimanjaro-5, 895 m

high) and lowest (the floor of Lake Tanganyika, 358 m below sea level) parts of the African continent (Ak'habuhaya and Lodenius, 1988). It has a sub-tropical climate with seven agro-ecological zones (BEFS, 2013). Climatic condition varies considerably from tropical at the coast to temperate in the highlands (Rowhani et al., 2011). The coastal areas are warm and humid. They have an average temperature of 25 °C and they receive about 1500mm of rainfall per year (Ak'habuhaya and Lodenius, 1988).

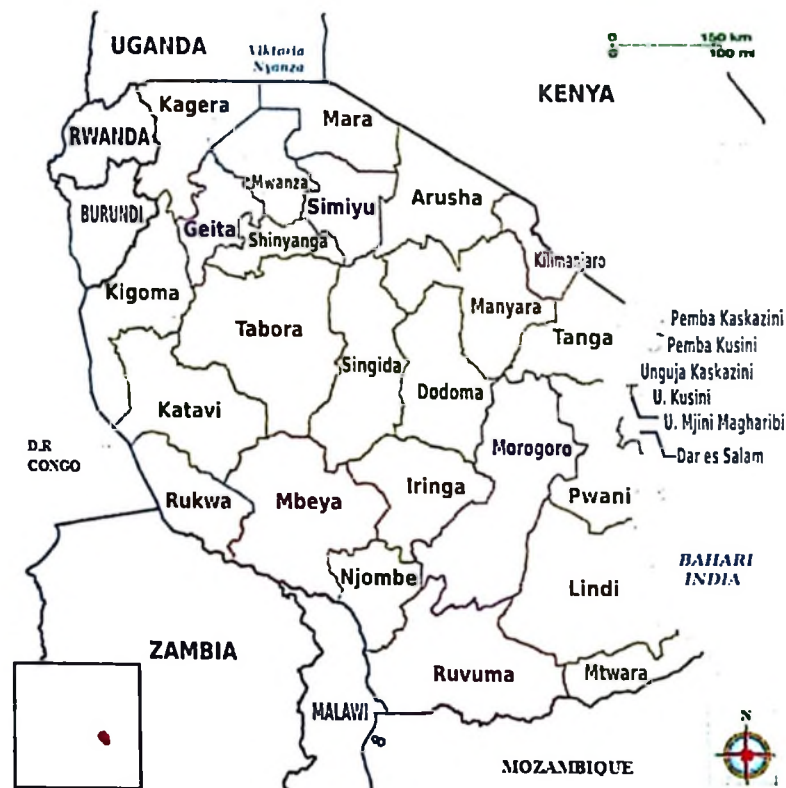


Figure 2. Map of Tanzania (Modified from Wikipedia).

The Country receives two predominant rainfall/precipitation events. One is unimodal (December-April) and the other is bimodal (*Vuli*) October-December and (*Masika*) March-May (www.tanzaniatrade.co.uk). Average monthly rainfall and temperature from 1900-2009 are shown in Figure 3 (www.worldbank.org).

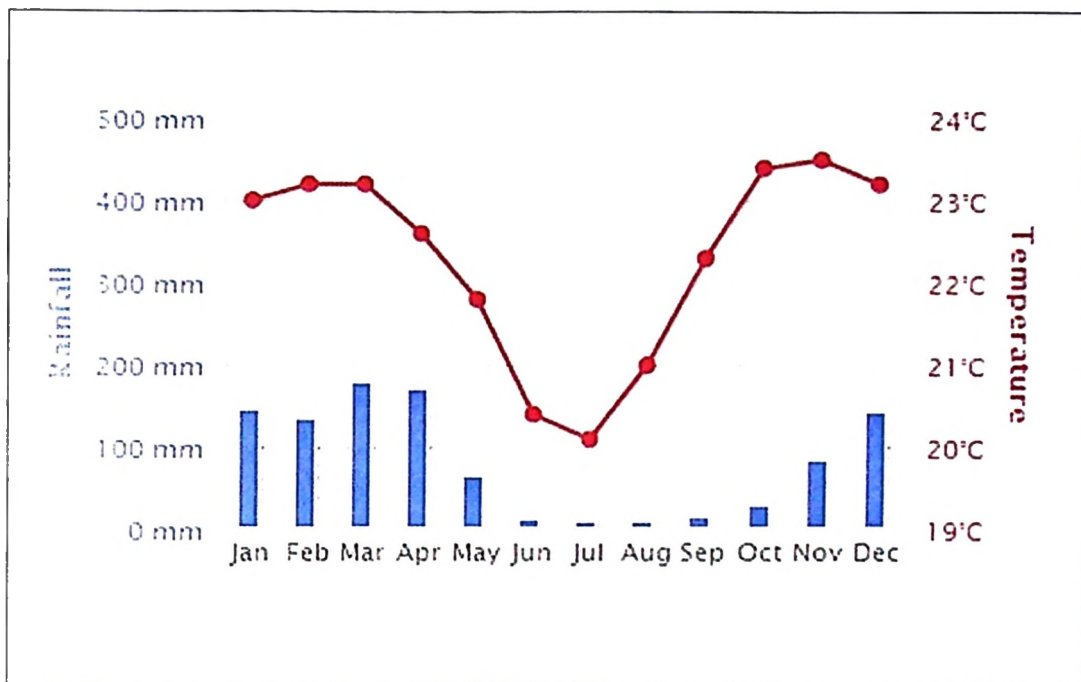


Figure 3. Average Monthly Temperature and Rainfall for Tanzania from 1900- 2009 (www.worldbank.org/climateportal/index.cfm).

2.3. The History of Maize in Tanzania

Maize was introduced to Africa along the western and eastern coasts in the 16th century (Miracle, 1966) as part of global ecological and demographic transformation by the Portuguese and Arab explorers to provide the slave trade (McCann, 2001; Smale and Jayne, 2003). According to Wright (1949) cited by McCann (2001) maize was first received in the coastal area (Pemba Island). The island was used by Portuguese planters in the 16th century to raise foodstuffs, including maize, to supply their coastal battalion. Maize was introduced in Tanzania mainland (Tanganyika) in the 17th and spread inner parts by mid-19th century (Ashimogo, 1995). It soon established itself as an important cereal crop all over the country and was accepted by most of the ethnic groups (Urassa, 2010).

2.4. Maize Production in Tanzania

2.4.1. Overview of Maize Production Trends

Agriculture is the most important economic activity for the majority of the Tanzanian population of all staples and cash crops cultivated, maize is the major and most preferred staple crop (USAID, 2010). It has been identified as a key crop to enhance food production, income, poverty alleviation and food security (Homann-Kee et al., 2013). More than half of cultivated land in Tanzania is allocated to cereal crops (FAOSTAT, 2014). Around 45% or over 4.9 million hectares used for maize production (Pauw and Thurlow, 2011). Average national yield varies between 1.0 and 1.5 t/h, compared to the estimated potential yields of 4-5t/h (Barreiro-Hurle, 2012; Mbwanga and Massawe, 2002). Overall maize production has grown at an annual rate of 4.6% over the last 25 years.

Compared to other SSA countries, Tanzania produces maize throughout the year thanks to two rainfall seasons (*Masika* and *Vuli*) and adaptation of a shorter maize growing season (Verheye, 2010). About 41% is grown during the *Masika* season and around 47% grown on *Vuli* season. This allows the constant domestic production of maize around the year (WFP, 2010). Figure 4 shows maize cropping seasons in Tanzania. Maize together with rice and sorghum are the three most important cereal crops in Tanzania and is grown on nearly all agro-ecological zones in the country and in all twenty-five of Tanzania's mainland, although at different levels (Figure 5). For research, management and production purposes the national maize research program (NMRP) and the ministry of agriculture, food security and cooperatives divide the maize production area into three main agro-ecological zones: the southern highlands, the Lake zone and the northern zone (Nkonya et al., 1998).

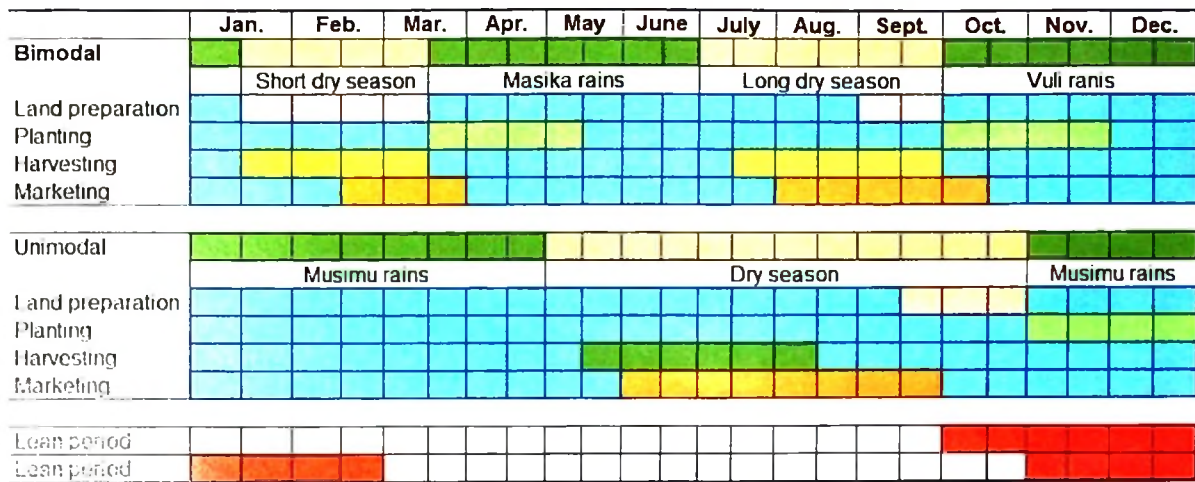


Figure 4. Tanzania Maize Crop Calendar (WFP, 2010; FAO/GIEWS, 2014).

The southern zones include Iringa, Rukwa, Ruvuma, Njombe, and Mbeya. These regions are the largest producers of maize. This so-called “breadbasket” accounts for over 45% of the total annual maize production (USAID, 2010). The Lake zone includes Mwanza, Simiyu, Mara, Geita, and Kagera and collectively these regions produce around 25-30% of the total maize output. The Northern zone consists of three regions, Arusha, Kilimanjaro, and Manyara, it accounts for about 10% of the total maize production. Figure 5 shows maize production in Tanzania for the market year 1990-2014 (FAOSTAT, 2014; <http://www.indexmundi.com>).

Maize production in Tanzania is categorized into four main groups. The first group is comprised of smallholder farms with less than 10 ha (2-3 ha each). This is the most important group and contributes about 85% of total production. Another group is a community farm with around 50 – 100 ha and contributes 5%. The third group includes large farms with over 100 ha which contributes 5% and the remaining 5% is produced by large private and public farms (<100 ha) (Croon, 1984).



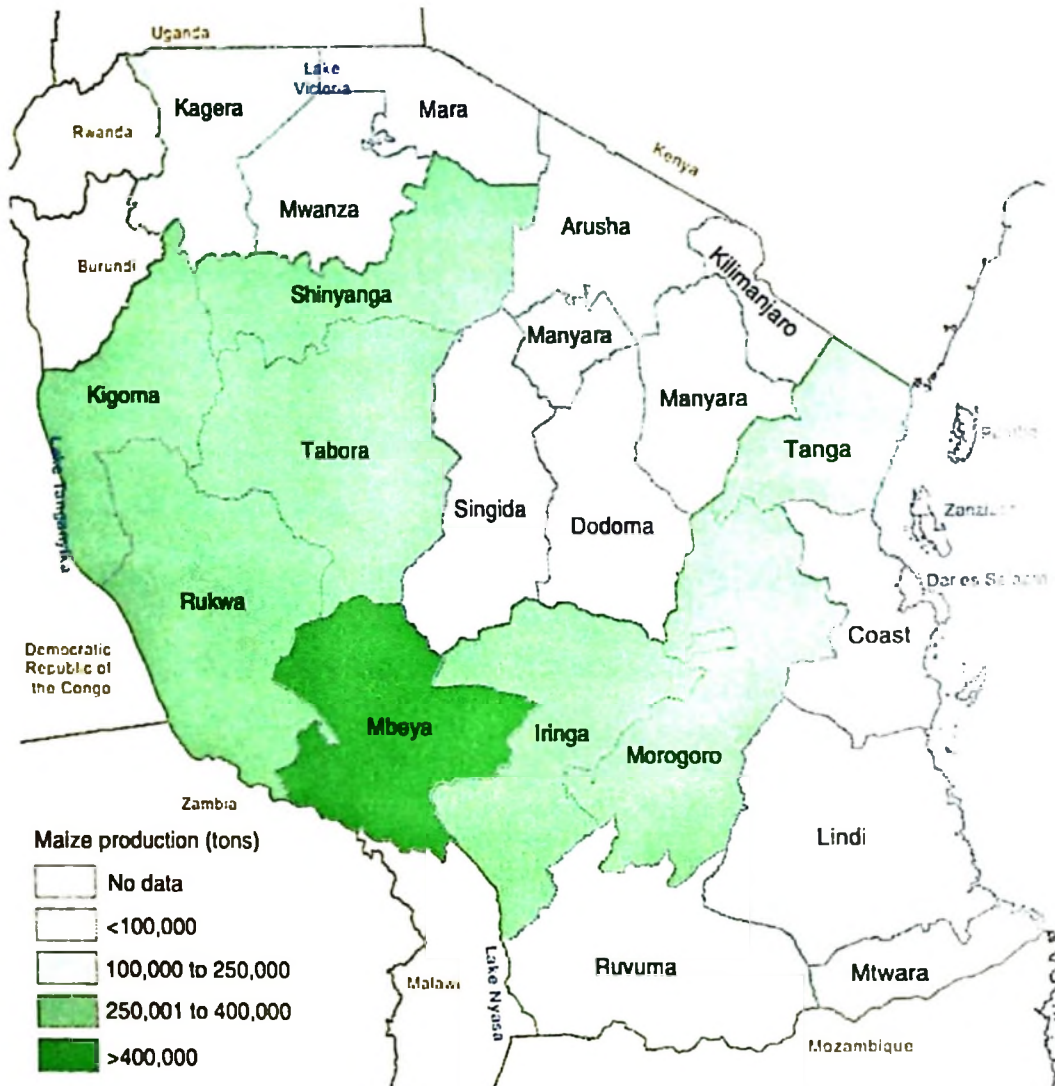


Figure 5. Maize Production Area in Tanzania (Cochrane and D'Souza, 2015).

According to FAOSTAT (2014) and the ministry of agriculture, food security and cooperatives, the total area of maize production has increased gradually from 1630 thousand hectares in 1990 to over 4000 thousand hectares in 2012 (Figure 6).

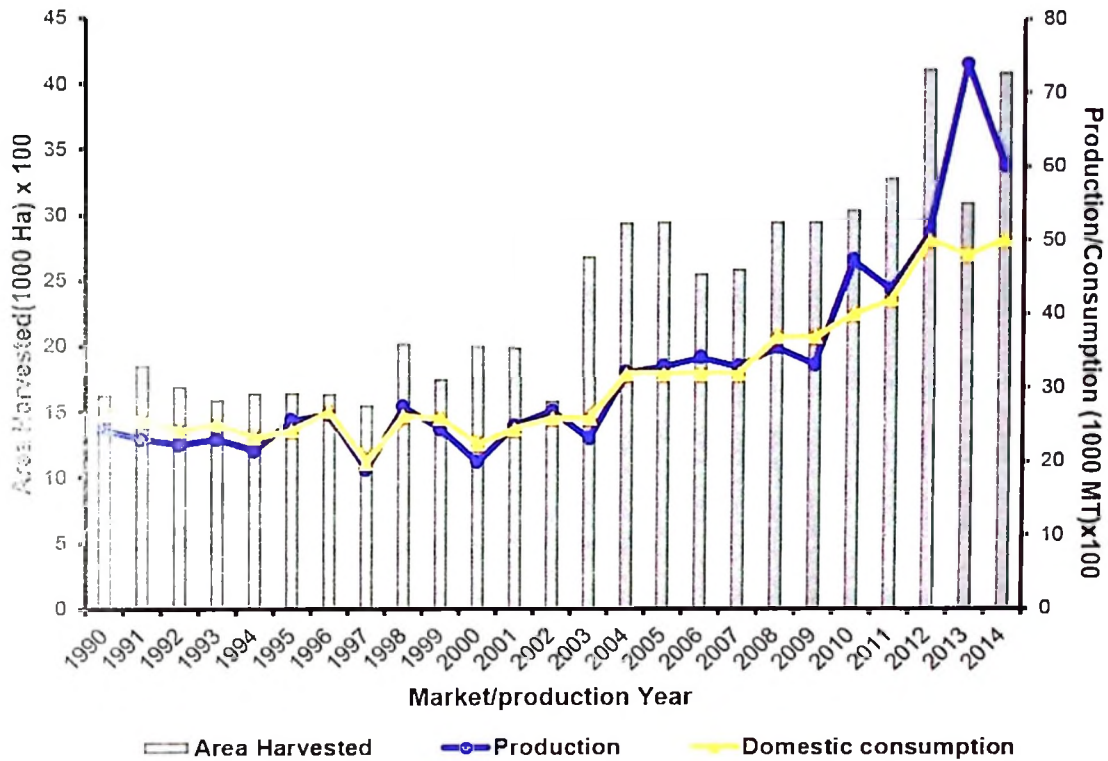


Figure 6. Tanzania Maize Production, Consumption and Area Harvested for 25 years (FAOSTAT, 2014; <http://www.indexmundi.com>).

Generally, the cultivated area, per capita production and consumption of maize in Tanzania (Figure 5) have been consistently increasing over the past four decades (CIMMYT, 1990; FAOSTAT, 2014). Nevertheless, maize yields remain very low at 1.0-1.5 t/ha, against 12 t/ha, in US or 4 t/ha, in South Africa. The main constraints of low yield and sporadic production are drought stress (shortage of rainfall) and infestation by insects, molds, and other pests. Other factors include weeds and diseases, low agricultural inputs such as fertilizer and crop protection chemicals, low levels of technology and poor infrastructure and storage facilities (Cairns et al., 2013; Kaliba et al., 2000; Homann-Kee et al., 2013). In addition, low price and poor market channels contribute. For instance, in the crop season

2013/14 farmers were enjoying a bumper harvest with a total production of around 6 million metric tons, but the government or National Food Reserve Agency (NFRA) could afford to buy only 5% of the total output (EABW, 2014). This resulted in causing the market price to drop drastically to about 50% below the record level of \$33 to \$9 for one 90 kg sack of maize (EABW, 2014). Furthermore, other constraints include poor agricultural practices, farm size, low fertilizer usage, lack of improved seed and inadequate access to information and extension service. Also, inadequate institutional support (credit), lack of credit to purchase inputs and reliance on unpredictable and irregular weather conditions (Lyimo et al., 2014; Otunge et al., 2011; Chauvin et al., 2012).

2.4.2. Consumption Trends

Worldwide consumption of maize in 2013/14 was around 950 million metric tons (FAOSTAT, 2014). Africa consumes over 30% and SSA around 21%. Eastern and Southern Africa use larger portions of approximately < 85% of its production as food (IITA, 2009) and about 5% as animal feed ([www. asareca.org](http://www.asareca.org)). Unlike other cereal crops that are consumed mainly by human as food (wheat and rice), maize is a multipurpose crop used as food, feed, fuel, and as raw materials for industry (Morris and López-Pereira, 1999). Tanzania is like other developing country's maize is mainly used for human consumption. It's a single most important staple food both in rural and urban areas (Oladejo and Adetunji, 2012). Maize accounts for about 31% of the total food production and constitutes more than 75% of the cereal consumption in the country. It is estimated that the annual per capita consumption of maize is around 128kg. According to Nyoro et al. (2004) and Peter et al. (2013) nearly 400 grams of maize are consumed per day per person in Tanzania; average national consumption

is estimated to be over three million metric tons per year (FAOSTAT, 2014). Maize contributes about 34- 36% of the average daily calorie intake (Amani, 2004; BEFS, 2013; Zorya et al., 2011).

According to FAOSTAT, (2014) food balance sheet, 60.8% of the total maize produced in 2013 was used for human consumption with the average waste of around 20.6%. Feed represents 16.1% and 0.5% was used for food manufacturing (Figure 7). Maize is consumed in a variety of forms; ground maize flour is prepared by mixed with water to make thin porridge or stiff porridge (“*Ugali*”) (Morris et al., 1999). Green (fresh) maize is boiled or roasted on its cob and served as a snack as well as popcorn which is also a popular snack (IITA, 2009).

2.5. Postharvest Losses of Maize

Post-harvest losses (PHL) is defined “as grain loss which occurs after separation from the site of growth or production to the point where the grain is prepared for consumption” (Boxall, 1986 cited by Nyambo, 1993). Other authors describe PHL as measurable quantitative, qualitative, and economics of grain loss across the supply chain or the post-harvest system, from the time of harvest till its consumption (Aulakh and Regmi, 2013; Tefera, 2012).

The Food and Agriculture Organization (FAO) of the United Nations and World Bank data revealed that PHL of cereal in SSA ranged between 5-40%, worth around \$4 billion (Zorya et al, 2011). Which was stated in a recent report of a joint FAO/World Bank report (Zorya et al., 2011).

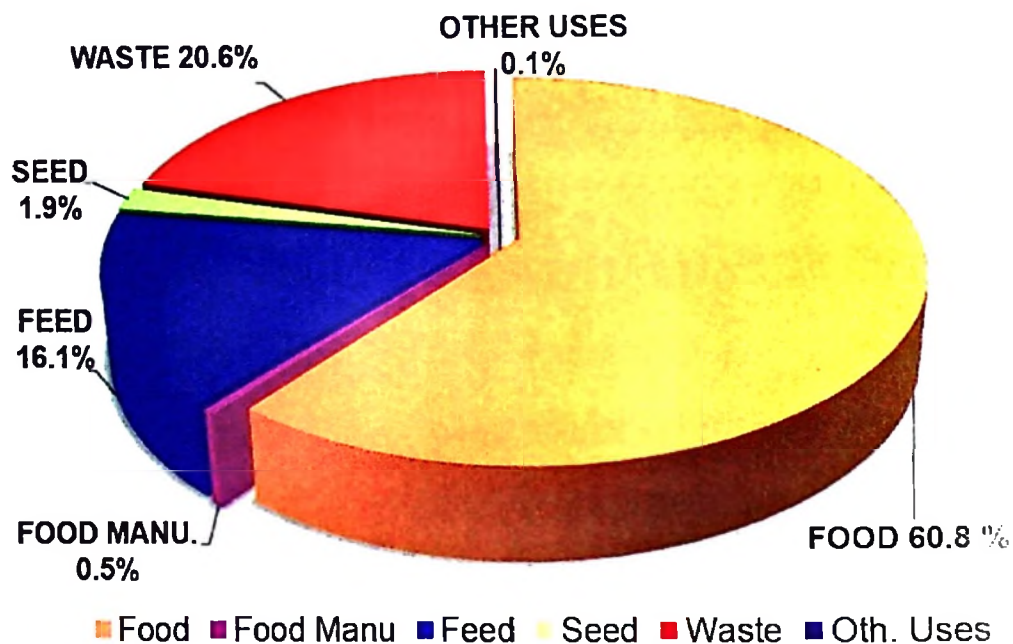


Figure 7. Utilization of Maize in Tanzania- Average for 2012-2013 (FAOSTAT, 2014).

In addition, the report shows PHL of cereal in Eastern and Southern Africa account for over 40% of the total PHL in SSA countries. This represents losses of about \$1.6 billion in value each year. Such losses are equivalent to the annual caloric requirement for at least 20 million people (FAO, 2013) or more than half of the value of total food aid received by SSA in a decade (Zorya et al., 2011). Furthermore, it has been reported by Meronuck (1987) that post-harvest losses of maize in various storage facilities in undeveloped tropical countries ranged from 15-25%.

The PHL of maize can be described by the leaky food-pipeline (Figure 8) modified from Bourne (1977) and Abass et al. (2014). As indicated in the pipeline, losses occur at all stages (from the field to market). However, higher losses occur at the field/harvest and storage. According to APHLIS, only 60-74% of the harvested maize reaches the final consumer (Abass et al., 2014). Figure 9 shows a typical storage condition of maize during a bumper harvest.

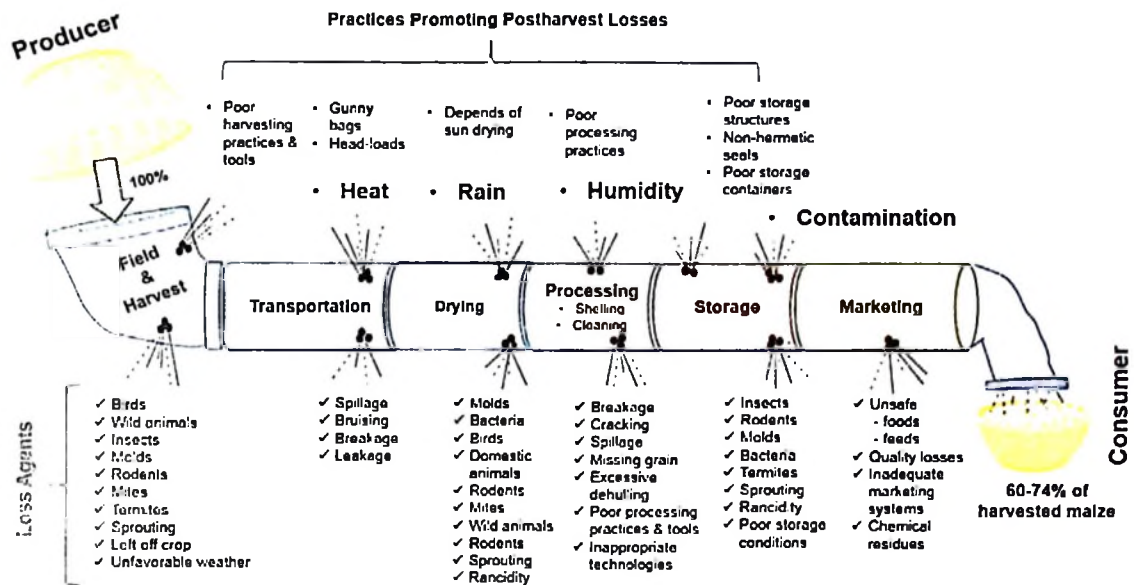


Figure 8. Postharvest Losses Pipeline for Maize (Modified from Bourne, 1977 and Abass et al., 2014).

2.5.1. Types of Losses

Post-harvest losses can be classified into three main categories: quantitative loss, qualitative loss, and economic or commercial loss. Also, they can be classified as direct and indirect losses. Quantitative loss indicates the reduction in physical weight, and can be readily quantified and valued. For example, a portion of grain damage by pests or lost during transportation. A qualitative loss is contamination of grain by molds and includes loss in nutritional quality, edibility, consumer acceptability of the products and the caloric value (Zorya et al., 2011; Kader, 2005). Economic loss is the reduction in monetary value of the product due to a reduction in quality and or/ quantity of food (Tefera, 2012).



Figure 9. Pile of Maize Stored outside House (<https://busiweek.com/index>).

2.5.2. Weight Loss

Weight loss (WL) is the standard international measure of grain loss (De Lima, 1979), generally regarded as a loss of food. WL is expressed as a loss in the dry matter or dry weight basis (Tefera, 2012). According to APHLIS, WL is estimated in two ways: first, a scattering of grain due to poor post-harvesting handling practices includes harvesting, threshing, drying, poor packaging, and transport. Second, from bio-deterioration brought by pest organisms such as insects, molds, and fungi, rodents and birds (Hodges, 2013). It is agreed by many researchers that WL is due to the persistent action of pests that can occur along post-harvest activities (De Lucia and Assennato, 1994). Weight loss is the common and most convenient way of defining and expressing post-harvest losses of cereal grain. Many researchers use WL when reporting post-harvest losses of maize. For instance, the study conducted by Rugumamu in (2004) reported post-harvest losses of maize to be around 20-30% and as high

as 40% in a traditional storage structure. A similar result has been reported by APHLIS as shown in Figure 10 (APHLIS, 2014). The weight loss can be calculated by the count and weight method (Eqn.1) developed by Adams and Schulten (1978). Other methods include standard volume and weight method (SVM) equation 2 (Reed, 1987) and by the thousand grain mass (TOM) equation 3 and converted percentage damage method (Dick, 1988).

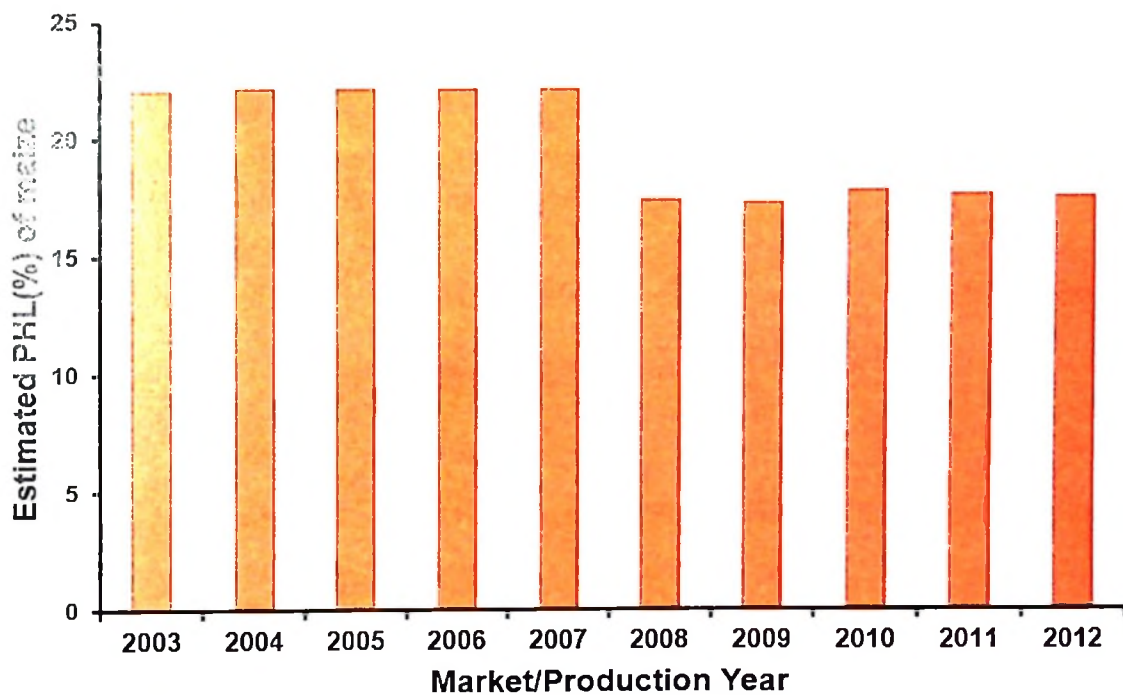


Figure 10. Estimated Percentage (%) Weight Losses of Maize in Tanzania (2003-2012). (APHLIS, 2014).

$$\text{Percentage (\%)} \text{ weight loss} = \frac{(W_u \times N_d) - (W_d \times N_u)}{W_u \times (N_d + N_u)} \times 100 \quad 1$$

Where, W_u = weight of undamaged grains, N_u = number of undamaged grains, W_d = weight of damaged grains, and N_d = number of damaged grains.

$$\text{Percentage weight loss (\%)} = \frac{\text{Initial dry weight} - \text{Final dry weight}}{\text{Initial dry weight}} \times 100 \quad 2$$

$$\text{Percentage weight loss (\%)} = \frac{M_1 - M_x}{M_1} \times 100 \quad 3$$

Where M_1 = grain mass before attack and M_x = grains mass after attack. mass = dry matter weight.

2.6. Major Pests of Maize in Tanzania

The major constraints of maize production in the field and in storage are insect pests, diseases, weeds, rodents, fungi, pathogens, and viruses. Maize is attacked by many insect pests during all stages of growth from seedling to storage (Shiferaw et al., 2011). Insects and other pests are a major threat to maize production (Ak'habuhaya, and Lodenius, 1988) and responsible for direct and indirect losses of maize on the farm and during storage (Bankole and Mabekoje, 2004). According to Mihale et al. (2009) insects are responsible for 15-100% and 10-60% of the pre- and post-harvest losses of grains in developing countries.

The most economically important insect pests of maize in Tanzania can be categorized into two main groups: (1) field pests such as stalk borer (*Busseola fusca*), maize leafhoppers (*Cicadulina mbila*) and mole crickets (*Gryllotalpidae*). Also, African bollworm (*Helicoverpa armigera*), African armyworm (*Spodoptera exempta*) and black cutworms (*Agrotis ipsilon*) and (2) storage pests like the maize weevil (*Sitophilus zeamais*), larger grain borer (*Prostephanus truncatus*) (Hon), red flour beetle (*Tribolium castaneum*) and dried bean beetles (*Callosobruchus maculatus*) and Indianmeal moth (*Plodia interpunctella*) (ASSP, 2004). Table 2 shows common field pests of maize in Tanzania. The major diseases of maize

include leaf rusts (*Puccinia sorghi* and *P. polysora*), leaf blights (*Helminthosporium turcicum* or *Setosphaeria turcica*). Others include Maydis leaf blight (*Helminthosporium maydis*), maize streak disease (maize streak virus), grey leaf spot (GLS) (*Cercospora zaeae-maydis*) and Gibberella Ear Rot caused by Fusarium- *Gibberella zaeae* fungus (ASSP, 2004).

2.6.1. Maize Stalk Borers

The maize stalk borer, *Busseola fusca* (Fuller) belongs to a group Lepidoptera: Noctuidae. *B. fusca* is considered the most damaging field insect pests of maize and sorghum in sub-Saharan Africa (Onyango, 1994). *B. fusca* is an endemic species across a wide geographical distribution (Sezonlin et al., 2006). However, it is mostly adapted to middle-and high altitude conditions- above 1500 m and annual mean temperature below 30°C (Sezonlin et al., 2006). In Tanzania, the main damage of *B. fusca* occurs during the early stages of plant growth (Katinila et al., 1998). Young larvae cause foliar damage and older larvae feed inside the stem, panicle, and do direct damage to grain (Onyango, 1994), resulting in the production of 'dead hearts' and a consequent loss of crop stand (Haile and Hofsvang, 2002). The extent of damage and average yield loss vary considerably from region to region, season to season, the infestation of the pest and the growth stage of the crop (Haile and Hofsvang, 2002). Haile and Hofsvang (2002) and Chabi-Olaye et al. (2005) reported that *B. fusca* can reduce maize yield by 20–100%. The life cycle of *B. fusca* is about 66 days in the rainy season and as long 200 days during the dry season (Unnithan, 1987).

Table 2. Common Field Pests of Maize in Tanzania. ASSP (2004).

Insects	Scientific name	Agricultural zone
Maize Stalk Borer	<i>Busseola fusca</i>	South Highlands, Lake, Northern, Western, Eastern, Central
Africa armyworm	<i>Spodoptera exempta</i>	Northern, Western, Eastern, Central
Maize leafhopper	<i>Cicadulina mbila</i>	South Highlands
Mole crickets	<i>Gryllotalpidae</i>	
Africa bollworm	<i>Helicoverpa armigera</i>	
Black cutworms	<i>Agrotis ipsilon</i>	
Spotted Stemborer	<i>Chilo partellus</i>	Northern

2.6.2. African Armyworm

African army worms are the caterpillars of the noctuid moth (*Spodoptera exempta*, Walker), and are one of the most devastating lepidopteran pests of graminaceous crops and grasses in sub-Saharan African (Tanzubil and McCaffery, 1990). The caterpillar is about 2 to 3 cm long, grey at first, then changing to greenish black when fully grown (HDRA, 2014). It is the larval stage that causes serious damage, voraciously feeding on young stages of maize (Figure 11) and other major cereal crops (Armyworm Network, 2000). “The extent of damage is illustrated by the facts that two larvae can destroy 10-day old maize plant with 6-7 open leaves. A single larva can consume about 200mg dry mass of maize leaves in the course of the sixth instar” (Odiyo, 1979).

The armyworm outbreaks usually begin in Tanzania or Kenya in November – December and then spread to other countries over a relatively short period. This is achieved by rapid growth of larvae and migratory behaviour of the adult moths. The adult moths are highly mobile, capable of achieving displacement of hundreds of kilometres each generation, flying with the wind at altitudes of several hundred meters (Gun and Gatehouse, 1985; Vilaplana et al., 2010; Boer, 1978). In Tanzania, armyworm outbreaks are usually severe and extensive during the rainy season following droughts (Gunn and Gatehouse, 1985). According to Rose et al. (1995) “outbreaks of armyworm occur sporadically and caterpillars are generally not noticed until they change color from green to black at their third stars”.



Figure 11. African Armyworms (www.lancaster.ac.uk).

2.6.3. Weeds

Weeds are plants that grow where they are not wanted or are a plant that is hazardous to crops, people and animals (Bubl, 2010). Weeds competes with the crops for water, soil nutrients, CO₂, space and light (Rajcan and Swanton, 2001). Besides direct competition with the plant for nutrients, weeds also cause indirect damage by harboring insect pests, rodents, diseases, and crop pathogens, as well as reducing wildlife habitat and crop quality (Bubl, 2010). Likewise, weeds increase the cost of crop production and interfere during harvest and cleaning or separation of crops (Tefay et al., 2014). According to FAO, worldwide, 13% losses of agricultural products is credited to weeds. In Africa, more than 50% of crop losses are due to weeds (Sibuga, 1997). According to Sibuga (1997), that weeds are the most important crop pests in SSA. According to Chikoye et al. (2005) a significant amount of crop production cost (40 – 80%) in SSA is used for weed management.









In addition, over 50% of the farming time in SSA is devoted to weed management (Tefay et al., 2014). The estimated loss due to weeds is higher than the sum of the potential losses due to insect, pathogens, and viruses (Oerke, 2006). The recent study conducted in Tanzania shows weeds deny over production of 1.7 million metric tons of maize per year (Kitabu, 2013). Weed management is an important aspect of crop production. It reduces crop yields and can lead to total crop failures if uncontrolled (Steiner and Twomlow, 2003). Weed competition greatly reduces crop yields. It is often a greater problem in a single crop or in simple crop associations than in the multi-crop associations. Some report of yield losses in maize due to weed ranges between 20 to 100% (Tadiou and Bogale, 1994). Furthermore, it has been reported by Chikoye et al. (2005) that in West Africa weeds contribute maize yield

losses of about 50 to 90%. Tesfay et al. (2014) concluded that proper control of weeds in maize can increase yield up to 96%. The common weeds of maize in Tanzania are summarized in Table 3.

2.6.4. *Striga* (witchweed)

Striga (*Orabanchaceae*) also known as witchweed is a genus of obligate root parasitic flowering plants. *Striga* is the serious biotic pest to crop production in sub-Saharan Africa (Menkir et al., 2004). It is estimated that over 60% loss in crop production in SSA is due to *Striga* species. This accounts for an annual loss of agricultural revenue of about \$7 billion (Robson and Broad, 1989). According to FAO, over 100 million people globally lose over half of their crop production to witchweed (Kanampiu et al., 2004). In addition, it was revealed by Odongo et al. (2004) that around 21 million hectares of cereals (maize and sorghum) with an estimated yield of nearly 4.1 million metric tons are infested by *Striga* each year in Africa. Yield losses on cereals attributable to infection by *Striga* parasites could be as high 100% under a high infestation season (Lagoke et al., 1991). Furthermore, the study conducted by Massawe et al. (2002) in Tanzania show the yield loss due to *Striga* of maize crops ranged from 18 to 42%. A major reason that these parasites are so pernicious is their highly efficient mechanism of seed production. Seeds of *Striga* are among the smallest of any known seed, measuring only 0.20–0.50 mm long, and they are often dispersed with planting material of which they are common contaminants (Berner et al., 1999).

Table 3. Common Weeds of Maize in Tanzania (ASSP, 2004).

Common name	Scientific name	Agricultural zone	Picture
Wild lettuce	<i>Lactuca virosa</i>	South Highlands, Lake, Northern, Western, Eastern, Central	
Wandering Jew	<i>Tradescantia pallid</i>		
Witch weed	<i>Striga</i> spp	Lake	
Simama (Mbigili Nyamwezi)	<i>Oxygonum simiatum</i>		
Bristly Starbur weeds	<i>Acanthospermum hispidum</i>	Lake,	
Star grass	<i>Heteranthera zosterifolia</i>	Eastern, South Highlands	
Crabgrass	<i>Digitaria</i> spp.		
Mexican poppy	<i>Argemone mexicana</i>		

Further, in a single growing season, each *Striga* plant can produce over 500,000 seeds (Saunders, 1933) which may remain viable for 14 years in the soil (Bebawi et al., 1984). Seed germination requires an exogenous stimulant that initiates ethylene production within the *Striga* seed (Babiker et al., 1993; Logan and Stewart, 1991) or directly provides ethylene (Eplee, 1975). Without adequate moisture and an external germination stimulant, the seeds remain dormant. Once germinated, the *Striga* seedling must attach to a host root within 3–5 days or the seedling dies (Worsham and Musselma, 1987). The *Striga* species of economic importance in Tanzania include *Striga hermonthica*, *S. asiatica* (L.) and *S. forbesii* (Massawe et al., 2002). Moreover, the problems of *Striga* in sub-Saharan Africa are fueled by many factors such as poor farming practices, deterioration of soil fertility, and expansion of production to marginal lands (Menkir et al., 2004). Most studies show the phytotoxic effect of *Striga* to its host is the main cause of yield losses (Ransom et al., 1990). The recommended approaches to control *Striga* include hand pulling, use of herbicides, application of high rates of fertilizers, and adoption new resistant maize cultivars. Other tactics include crop rotation, ethylene gas, mixed cropping of cereals with legumes such as maize and cowpea (Kabambe and Kanampiu, 2002; Massawe et al., 2002).

2.7. Maize Diseases of Maize in Tanzania

The main diseases of maize in Tanzania include: leaf rusts (*Puccinia sorghi* and *P. polysora*), leaf blights (*Helminthosporium turcicum*), Maydis leaf blight (*Helminthosporium maydis*), maize streak disease (maize streak virus), grey leaf spot (GLS) (*Cerospora zaea-maydis*), and Gibberella Ear Rots (ASSP, 2004). Table 4 shows some common diseases of maize in Tanzania.

Table 4. Common Diseases of Maize in Tanzania (ASSP, 2004).

Common name	Scientific name	Agricultural zone
Maize Steak Virus (MSV)		South Highlands, Lake,
Leaf rust	<i>Puccinia sorghi</i> and <i>P. Polysora</i>	Northern,
Leaf blights	<i>Helminthosporium turcicum</i> and <i>maydis</i>	Lake, Northern,
Corn smut	<i>Ustilago maydis</i>	Lake
Grey leaf spot (GLS)	<i>Cercospora zea-maydis</i>	
Northern leaf blight	<i>Setosphaeria turcica</i> or <i>Exserohilum turcicum</i>	South Highlands

2.7.1. Maize Streak Disease

Maize streak disease (MSD) is a disease caused by maize streak geminivirus. It is recognized as one of the most serious virus diseases of monocotyledonous plants in sub-Saharan Africa (Bock et al., 1974). “Globally, MSD is regarded as the third most serious disease of maize after northern corn leaf and grey leaf spot” (Martin and Shepherd, 2009). MSD causes an annual loss of around 120 to \$480 million dollars with estimated yield loss of 6-10% (Martin and Shepherd, 2009). This is equivalent to loss of over one million metric tons of maize grain (Karavina, 2014). MSD is spread by several species of leafhoppers that belong to the genus *Cicadulina* (Rose, 1978) but as many by *C. mbila* and *C. storeyi* (Shepherd et al., 2010).

Furthermore, the first symptom of MSD is the appearance of pale, spherical; chlorotic spots 0.5-2.0 mm in diameter on the lowest exposed portions of the youngest leaves (Rose, 1978). Lesion color generated by streak disease varies from whitish to pale yellow (Figure 12). This yellow streaking reduces photosynthesis and increases respiration rate, leading to a reduction in leaf length and plant height (Shepherd et al., 2010). MSD is more severe in younger plants and irrigated crops (Owor, 2008). The control methods of MSD include cultural control (crop rotation, field hygiene, timely planting, barriers, and cultivar choice), chemical control (systemic insecticides like aldicarb, carbofuran, dimethoate, endosulfan and others), and host plant resistance (plant resistant hybrids) (Karavina, 2014; Shepherd et al., 2010).

2.8. Storage Pests

Insect pests are the principal cause of grain losses in the field and storage (Suleiman et al., 2013). In general, smallholder farmers store maize for three main purposes: as food until next season; as seed and for selling when attractive prices become available. However, storage pests damage significant portions of their stored maize (Rugumamu, 2004). The most serious insect pests that cause severe economic damage to maize in the storage are the maize weevils, *Sitophilus zeamais*, and the larger grain borer (LGB), *Prostephanus truncatus* (Suleiman et al., 2015). Others include the Angoumois grain moth (*Sitotroga cerealella*), the lesser grain weevil (*Sitophilus oryzae*), red flour beetle, and dried bean beetle (Gitonga et al., 2015). Most of the maize grain harvested in Tanzania is traditionally stored on the farm where post-harvest pest management is inadequate (Rugumamu, 2004). This leads to huge amounts of maize grain losses (Sori and Ayana, 2012). Table 5 shows common storage pests of maize in Tanzania.



Figure 12. Maize Plants Infected by MSD (Karavina, 2014).

2.8.1. The Larger Grain Borer (*Prostephanus truncatus*)

The larger grain borer, *Prostephanus truncatus* (Hon) (Coleoptera: Bostrichidae) also termed “Scania beetle”, or “*Dumuzi*” meaning robber in Tanzania is the most destructive pest of farm-stored maize grain and dried cassava roots (Nansen and Meikle, 2002) causing weight losses of 9 to 45% after 5- 8 months of storage (Golob, 1988). *P. truncatus* is native to Mesoamerica (Stathers, 2002), where it is found infesting maize grain and wood (Hill et al., 2003). It’s described as a dual existence insect as both in storage pest and forest insect (Nansen et al., 2004). The adult *P. truncatus* have a cylindrical bostrichid shape (Figure 13), the body is 3 to 4.5 mm long and dark brown in color (Hodges, 1985).



Figure 13. The Adult, Larger Grain Borer, *Prostephanus truncatus* (Hon) <http://www.infonet-biovision.org>.

P. truncatus was accidentally introduced from Central America to Tanzania in the early 1980's (Dunstan and Magazini, 1981) and then Togo in 1984 (Harnisch and Krall, 1984). Since then, *P. truncatus* has become a serious threat to stored maize and dried cassava (Key et al., 1994), reducing the storage period of these commodities in the granaries of small-scale farmers. First recognized outbreaks were reported in the western regions of Tanzania (Tabora, Shinyanga, and Mwanza) in 1981 (Dales and Golob, 1997). It has now spread to most of the countries in sub-Saharan Africa, and more recently it has been identified in 17 countries (Figure 14) in Africa (Schneider et al., 2004). *P. truncatus* can also infest and cause damage to bamboo, plastic, soap, stored timber and timber products (Cabi, 2015).

Life cycle of *P. truncatus*

Adult *P. truncatus* tunnel the hole through the stored maize grain, dried cassava or other foodstuffs, creating large quantities of dust (Cabi, 2015). *P. truncatus* is a long-lived species- the life cycle in about 4-6.5 weeks. The female lives 16 days longer than the male (Shires, 1980). Adult females lay small yellow ovoid (ellipsoidal) shape eggs in chambers at right

angles to the main tunnels (BioNet-Earfinet. 2011). Larvae hatch from eggs after 3 to 7 d at 27-32°C and about 50-80% relative humidity (Cabi. 2015).

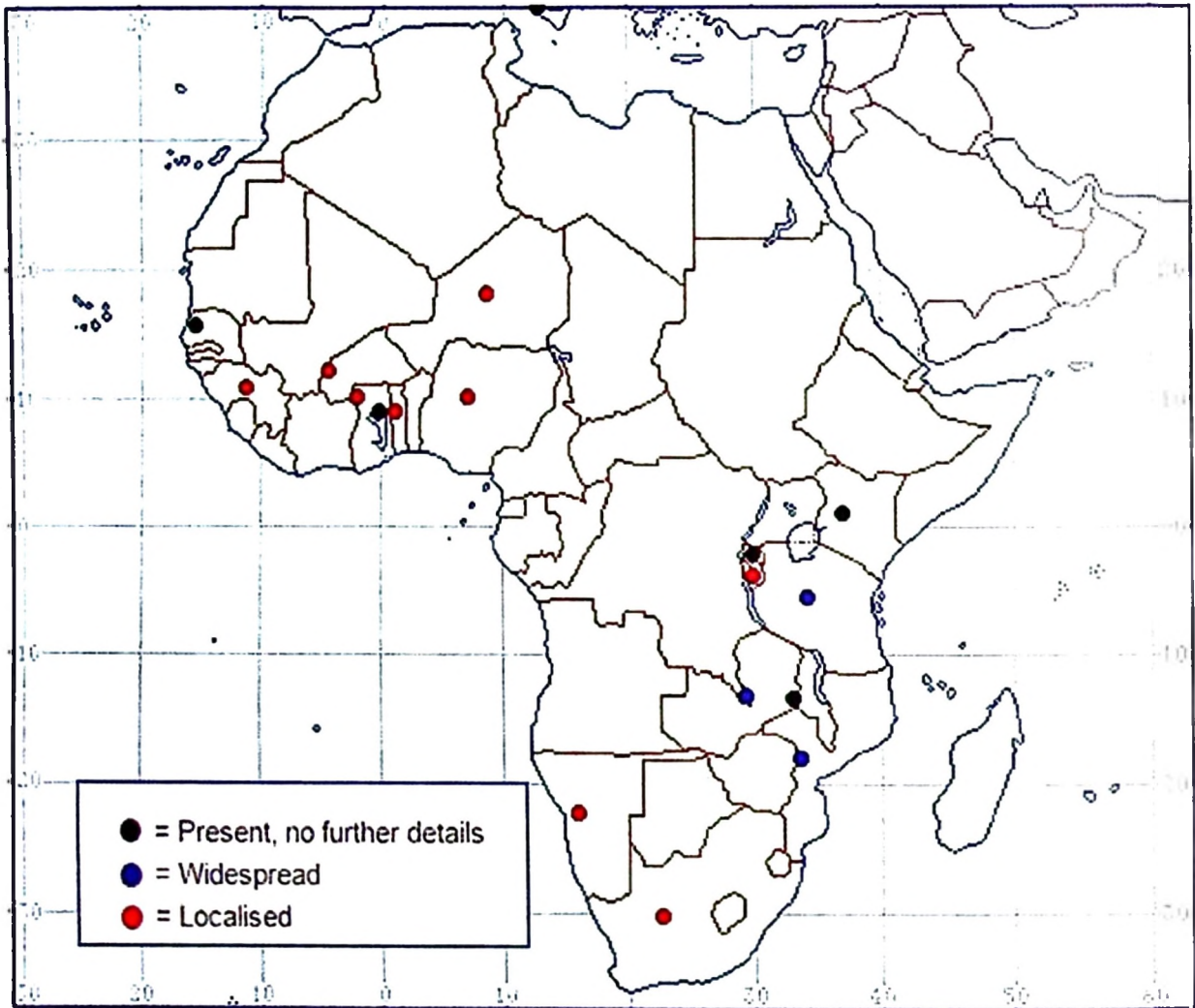


Figure 14. Distribution Map of *P. truncatus* in Africa (www.cabi.org).

The control strategies of *P. truncatus* include good store hygiene, cleaning the warehouses or stores between harvests and burning infested maize grain. Others include harvesting maize soon after maturity, the use of resistant varieties, and traps employing chemical attractant (pheromone) produced by the male beetle to attract females (Cabi, 2015;

www.infonet-biovision.org; Bergvinson and Garcia-Lara, 2011). Other methods include: immersing used sacks in boiling water to eliminate residual infestations, addition of inert dust (ash and clay), storing the grain in hermetic containers and removing any wood materials from stores (Markham et al., 1994; Schneider et al., 2004; Bergvinson and Garcia-Lara, 2011; www.infonet-biovision.org). In addition, fumigation with phosphine and application of synthetic pyrethroid insecticides such as permethrin and deltamethrin (Golob, 1988) show positive results against *P. truncatus*.

Table 5. Common Storage Pests of Maize in Tanzania (ASSP, 2004).

Insect species	Scientific name	Agricultural zone
Larger grain borer (LGB)	<i>Prostephums truncates</i>	South Highlands, Lake, Northern, Western, Eastern, Central
Maize weevil	<i>Sitophilus zeamais</i>	
Red flour beetle	<i>Tribolium castaneum</i>	Lake
Dried bean beetle	<i>Callosobruchus maculatus</i>	Lake
Indian moths	<i>Plodia interpunctella</i>	Eastern, South Highlands

2.8.2. The Maize Weevil (*Sitophilus zeamais*)

The maize weevil (Figure 15) *Sitophilus zeamais* Motschulsky, is a small reddish-brown to black snout beetle (Suleiman and Abdulkarim, 2014). It is described as one of the most destructive stored and primary grain pests of maize and other cereal grain in tropical and

subtropical regions (Suleiman et al., 2015). *S. zeamais* is so devastating and capable of multiplying to large populations, causing tremendous damage to the stored grain (Cosmas et al., 2012). It has been estimated that 5-30% of the total grain weight of the stored product is lost due to infection by *S. zeamais* (Ojo and Omoloye, 2012). Other studies cite as high as 80% losses may occur in untreated maize grain stored in traditional structures (Tefera et al., 2011). Infestation by *S. zeamais* often begins in the field, but the most serious damage is done in storage (Fikremariam et al., 2009; Suleiman et al., 2015).



Figure 15. Adult Maize Weevil, *Sitophilus zeamais* (<http://keys.lucidcentral.org>).

Life Cycle of *S. zeamais*

Sitophilus zeamais is regarded as an internal feeder of grains. Typically they range from 2.5 to 4.5mm in length (Kasozi, 2013). The average life span of *S. zeamais* ranges from 3 to 6 months up to one year (Rees, 2003; Kranz et al., 1997). Female weevils releases sex pheromones to attract the males (Mason, 2003). Once fertilized the female uses the snout to excavate a small hole in a maize kernel and then lays eggs (ovipositing) and plugs the hole

with a waxy secretion (Kasozi, 2013). At optimal conditions, each female can lay up to 150 eggs in her lifetime (Gewinner et al., 1996). Eggs hatch into small larvae in about 6 days; the larva feeds (Figure 16) and develops inside the maize kernels for about 25 days (Kasozi, 2013; Throne 1994; Kossou and Bosque-Perez, 1998). Total development periods depend on environmental conditions and range from 35 to 110 days (Kossou and Bosque-Perez, 1998). The adults emerge by eating their way towards the testa causing rugged exit holes resulting in a damaged kernel and reduced grain weight (Mwangangi and Mutisya, 2013; Suleiman et al., 2015).

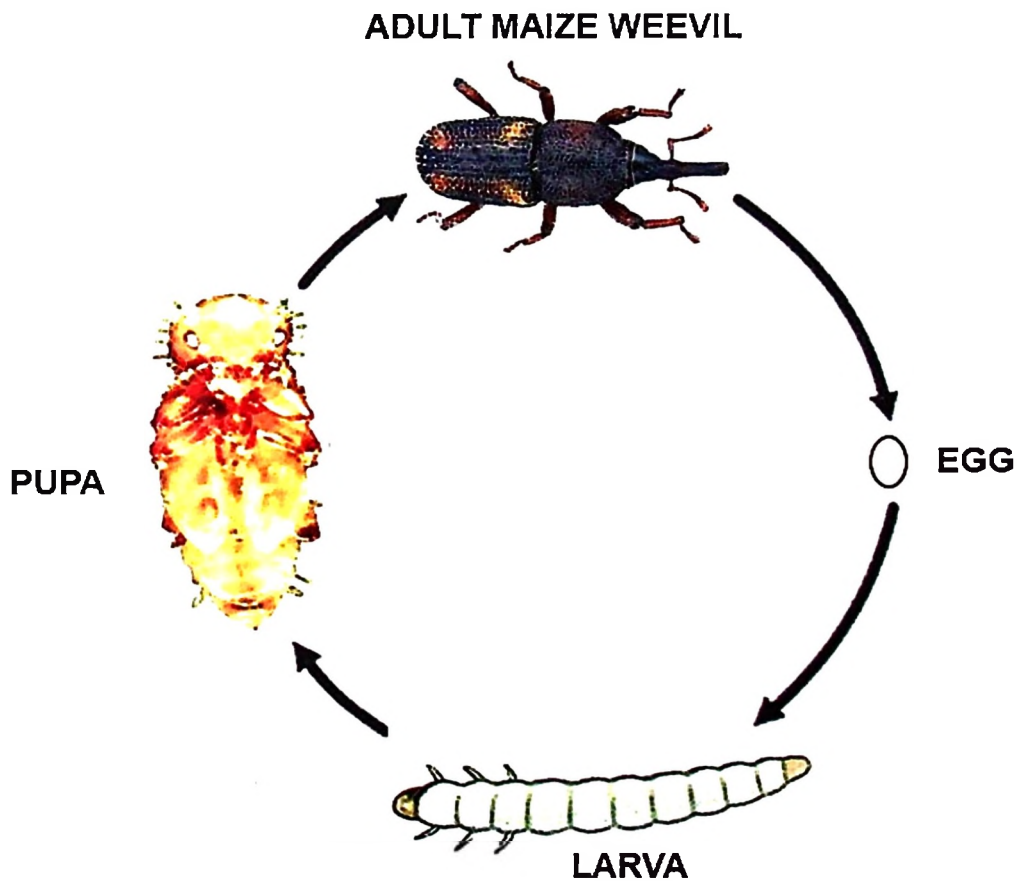


Figure 16. Life Cycle of Maize Weevil, *Sitophilus zeamais*.

2.9. Rodents

Rodents are a significant pest problem worldwide. Rodents are a major pest in cereal grains, causing both qualitative and quantitative damage (Mdangi et al., 2013). Qualitative losses occur through the decreased value of grain due to spoilage caused by grain discolouration, physical contamination, and spillages such as feces, hairs, and urine (Brown et al., 2013c). Quantitative losses arise through grain wastage between farmer and to the end-user (Brown et al., 2013c). In the literature, estimates of damage losses vary widely. The data show between 5 to 15% yield loss of maize in Tanzania (Makundi et al., 1991) and 20% of annual maize loss in Kenya (Oguge et al., 1997). Likewise, 10-20% annual loss of rice in Indonesia, 6-7% in Thailand, 5-10% in India and over 10% in Vietnam (Leirs, 2003). The average rodent damage to stored maize in developing countries is around 35%, and even higher in certain cropping seasons during rodent outbreaks (Mdangi et al., 2013; Mulungu et al., 2011). This is equal to an annual loss of about \$141 million (\$11.1/100 kg bag of maize), corresponding to food grain to feed approximately 7 million people (0.5 kg/day/person) per year (Mulungu, 2003).

Rodents are known to cause damage at all stages of crop production. By digging up newly sown seed, by attacking the developing grain maize in the field, matured grain just before harvesting and in storage (Segerbäck, 2009; Brown et al., 2007b). Besides crop damage, rodents also have serious implications for public health and animal husbandry. They act as a vector carrying numerous zoonotic diseases, including Lassa fever, hemorrhagic fever, Lymphocytic choriomeningitis, *Leptospira*, Scrub typhus, Toxoplasmosis, Murine typhus and Lyme disease (Meerburg, et al., 2009). In addition, rodents also transmit plague

diseases by carrying several protozoa and bacteria like *Salmonella* spp., *Listeria* spp., *E. coli* O157: H7, *Campylobacter*, *Giardia* spp. and others (Meerburg et al., 2009). An excellent review of various diseases associated with rodents is explained by Meerburg and others (Meerburg et al., 2009). Moreover, rodents cause spoilage and contamination of food with hair, urine, and faeces, biting people, killing chicks and leading to structural damage to storage buildings in developing countries (Makundi, 2009).

2.9.1. Rodents in Tanzania

The most destructive rodent pests in Tanzania and other SSA countries is the multimammate shamba rat, *Mastomys natalensis* (Makundi et al. 1991; Leirs et al., 1996). Damage due to *M. natalensis* in Tanzania causes an estimated annual yield loss of 5-15% of maize, equivalent to about \$45 million or 400,000 tons of maize. To put into context, such losses are estimated to be equal to the annual caloric requirement to feed about 2 million people (Odhiambo et al., 2005; Leirs et al., 1996; and Makundi et al., 1991). The main characteristics of *M. natalensis* are an enormous breeding capacity and ability to coexist both as field and house rats (Sluydts et al., 2009; Brooks and Fielder, 2013). This makes huge challenges to control (Odhiambo et al., 2005) and remains a chronic problem for many countries in sub-Saharan Africa (Mwanjabe et al., 2002). As reported by Keener (2007) *M. natalensis* are very smart and once a population is established, it may be difficult to control.

M. natalensis is a small rat. The body length measures 1.0-1.5 cm, with a tail approximately the same length. They weigh about 50-120 g (Brooks and Fielder, 2013). “The dorsum is grey to brownish-grey, brown, or reddish-buff, the venter is lighter coloured” (Brooks and Fielder, 2013). Females have up to 8-12 pairs of mammies or about twice that of

most rodents (Fiedler, 1994). They have a mean gestation period of around 23 days and females mate multiple times during the breeding season and have a litter size of 9-13 (Figure 17) (Fiedler, 1994; Kennis et al., 2008). The young are weaned often about 3 weeks and siblings reach sexual maturity after 3.0-3.5 months (Brooks and Fielder, 2013; Fiedler, 1994). The maximum lifespan ranges between 339-487 days (Coetzee, 1975). The population dynamics depends on food availability and rainfall (Julliard et al., 1999; Massawe et al., 2011).



Figure 17. Litters of Multimammate Shamba Rat, *Mastomys natalensis* (<http://www.biolib.cz>).

2.10. Strategies to Reduce Postharvest Losses

Reducing PHL has positive consequences for poverty alleviation, food security, nutrition status, and increases household income for the smallholder farmer in developing countries (Shiferaw et al., 2011; Affognon et al., 2015). Also, has significant impacts on the

environment, increases the amount of food available for consumer and reduces utilization of production resources (Affognon et al., 2015; Zorya et al., 2011). Figure 18, show repaired postharvest losses leaky pipeline for maize. For instance, by introducing simple strategies like improved varieties, harvest at the right time, improved storage structures, and increases drying efficiency. As well as using of moisture and temperature meters, proper hygiene and sanitation, and access to market information save a significant portion of maize harvested.

According to Affognon et al. (2015), likely strategies to mitigate PHL in developing countries is to look each stage rather than concentrate all effort on the storage activities. Other potential strategies include better government policies like reduction of taxes for materials, a public-private partnership that enable dissemination of new technologies, and extension services such as farm field school and precision agriculture. As well as promotion of newly innovated technologies, communication and market information and investments in infrastructure (Shiferaw et al., 2011).

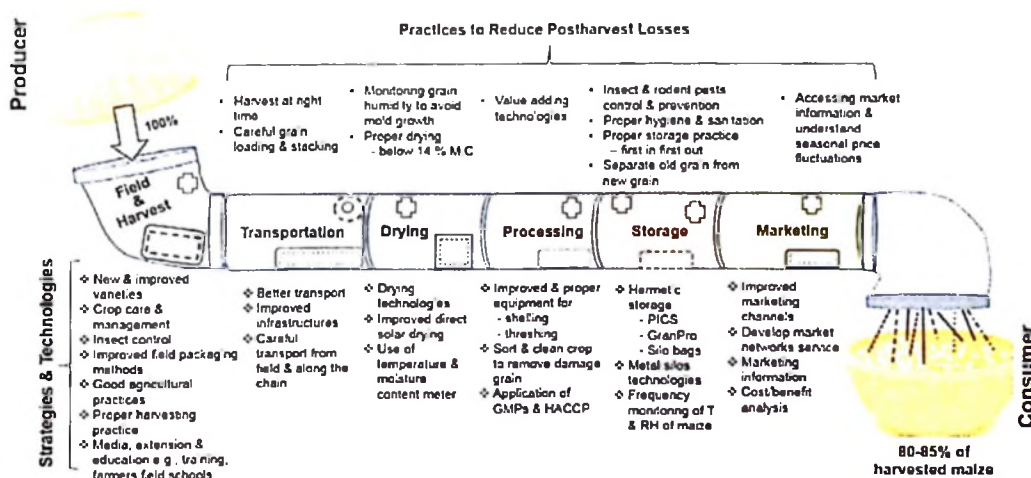


Figure 18. Repaired Postharvest Losses Leaky Pipeline for Maize (Suleiman and Rosentrater, 2015).

2.10.1. Technology and Infrastructure

Multidisciplinary approaches and several technologies have been developed to reduce PHL in developing countries. However, the potential gains from adopting these technologies have been challenged, particularly in rural areas (Rosegrant et al., 2015). Cited by Greely (1982) the main constraints of the PHL reduction in developing countries is most of the smallholder farmers are reluctant to change unless the losses are considerably higher than average. In addition, these technologies turn up to be inappropriate for smallholder farmers, and unavailable at the right price and the right time. Also, inadequate knowledge of the biological and environmental factors on product deterioration and adopt technology only when is offered free of charge (Shiferaw et al., 2011).

Further, quoted by Meena et al. (2009) the main setback of PHL reduction in less developed countries is a huge gap between agricultural technologies developed at research institutions and its adoption by smallholder farmers. Gamon et al. (1994) the limited adoption of technologies in rural areas is due to the lack of disseminating information. He continued and add that most of the technologies offered or developed by researchers and development patterns are unsuited and perceived as irrelevant by most smallholder farmers. Moreover, the factors such as socioeconomic status, education background, economic motivation, and training received have a positive correction with technology adoption (Atibioke et al., 2012). Nevertheless, hermetic storage technology is considered the best solution to combat PHL in developing countries.

2.10.2. Hermetic Storage Technology (HST)

Hermetic storage (HS) is an ancient method to control insect infestation and preserve the quality of grain (Quezada et al., 2006). HS also termed as “hermetic silo storage”, “sealed storage”, “airtight storage”, “sacrificial sealed storage” has emerged as an alternative and cost-efficient methods for minimizing PHL and increases food security in developing countries (Navarro et al., 1994; Villers et al., 2008; Jonfía-Essien et al., 2010). The basic principle of HS based on the simultaneous depletion of oxygen and accumulation of carbon dioxide in the storage container (Sanon et al., 2011). This is achieved by the aerobic respiration of grain, insects, and molds (Quezada et al., 2006). The lack of O₂ inside the container causes insects to suffocate, become inactive and eventually die of asphyxiation or desiccation (Njoroge et al., 2014). The main advantages of hermetic storage are simple, feasible, eliminate the need for toxic chemical (insecticides) or fumigations, climate control and environmentally friendly (Navarro et al., 1994; Villers et al., 2008). HS is a technology that enables farmers to store their grains with negligible loss of quality and quantity.

2.10.3. Types of Hermetic Storage

Hermetic storage is categorized according to the amount of grain being stored, small quantity usually employs the use of bags and small containers, while huge or bulk storage employs larger storage facilities (Yakubu, 2009). For small quantity, two types of hermetic storage container (bags) have been developed, Purdue Improved Crop Storage (PICS) bags (Murdock and Baoua et al., 2014) and GrainPro Super Bags (Villers et al., 2010). Other HS includes metal silo technology and silo or grain bags.

2.10.3.1. Purdue Improved Crop Storage (PICS)

PICS bags (Figure 19), also known as the triple-layer bags consisting of three plastic liners. Two 80-micron high-density polyethylene plastic bags, one surrounded by the second; both are enclosed by a third bag made of woven polypropylene bag for reinforcement (Murdock and Baoua et al., 2014). This technology was created in late 1980's under the USAID project for the preservation of cowpea grain in sub-Saharan Africa (Murdock et al., 2003). The technology was named "Purdue Improved Cowpea Storage" (PICS) bags and served as protection against *Callosobruchus maculatus* (F.) a destructive cowpea seed (bruchids) beetles (Murdock et al., 2003).

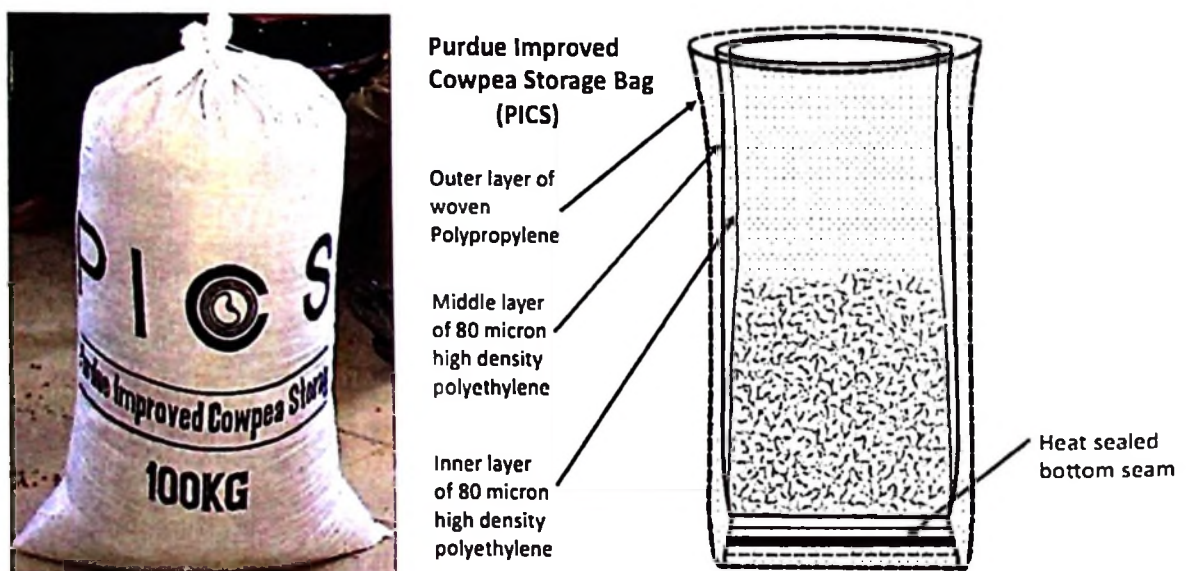


Figure 19. PICS-Schematic Presentation of three Plastic Liners (Murdock and Baoua et al., 2014).

PICS bags are based on the principle of the bio-generated modified atmosphere, where oxygen environment low inhibits the growth and development of insect pests (Sanon et al., 2011). It takes advantage of an airtight seal where oxygen concentration dramatically

decreases (Figure 20). While carbon dioxide levels proportionally increase within a few days after sealing through respiration of insect, fungal, and grains/seed (Quezada et al., 2006).

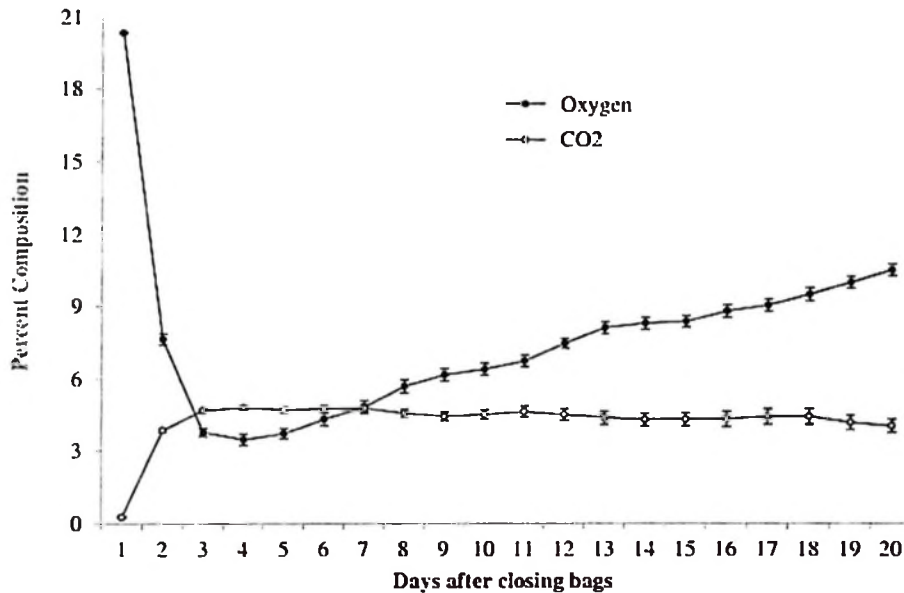


Figure 20. Oxygen and CO₂ Concentration within PICS Bags for 21 days (Murdock and Baoua et al., 2014).

Further, the PICS technology has been considered low-cost non-chemical technology that enables smallholder farmers to store their seed and grains with minimal loss. Unlike other technologies, PICS has been easily accepted by farmers and many studies prove to be effective storage systems for a variety of crops, including cowpeas, maize, peanuts, sorghum, wheat, and common beans against insect infestation, fungal growth and aflatoxin accumulation (Zorya et al., 2011; Williams et al., 2014). However, the effectiveness of the hermetic technology depends on several factors such as airtightness of the seal, the commodity stored, agro-climatic conditions, type and prevalence of insect pests and mechanical strength of the barrier material (Njoroge et al., 2014).

2.10.3.2. The GrainPro Super Bags

The SuperGrain™ bag is a portable hermetic sack suitable for the small-scale farmer to store maize and other commodities up to 1000 kg. It consists of a single reusable layer of 0.078 mm thick plastic film made from 2 plain polyethylene films between which is sandwiched a plastic layer that acts as a gas and moisture barrier (Baoua et al., 2013). Then the sealed bag is placed in a protective woven outer bag (Bern et al., 2013). The technology is based on the principle of hermetic storage systems. There are a number of GrainPro Super bags (Figure 21) include GrainPro SuperGrainbag III™ used to store a range of dry agricultural commodities such as maize, wheat, sorghum, millet, paddy, coffee, and others (<http://www.grainpro.com>).



Figure 21. Different GrainPro Super Bags (www.grainpro.com).

Other products of GrainPro Inc. include Cocoon Cargo and TranSafeLiner™ (Figure 22) that can accommodate up to 1000 tons of grain (<http://www.grainpro.com>). GrainPro bags have proven effective for storage of wheat in several Asia countries attacked by insect pests such as *Tribolium castaneum*, *Rhizopertha dominica*, and *Sitophilus oryzae* (Baoua et al., 2013). Some advantages of GrainPro Super bags as mentioned on their website are;

affordable and reusable, and environment-friendly. Also, prevent commodities against insect infestation, contamination, moisture, oxidation, fungi, and mold growth and damage of larger grain borers and cowpea weevils (<http://www.grainpro.com>). Furthermore, these technologies are available in more than 100 countries include Mali, Burkina Faso, Ghana, Niger, Rwanda, Kenya, Malawi, Uganda, Ivory Coast, India, Costa Rica, Sri Lanka, Philippines, Pakistan, Guatemala, Zambia, Afghanistan to mention a few (www.grainpro.com). “These technologies can provide a sustainable and affordable solution for the prevention and reduction of post-harvest loss, and thus increase global food and nutrition security” (Maier and Cook, 2014).



Figure 22. GrainPro Cocoon™ and TransSafeliner™ (www.grainpro.com).

2.10.3.3. Metals Silo Technology

The metal silo technology is an effective method for reducing grains PHL for small and medium scale farmers in developing countries. This technology provides grains protection for both short and long time storages against insect pests, pathogen, birds, molds, rodent, theft, and other domestic animals (Yusuf and He, 2013; Tefera et al., 2011; Gitonga et al., 2015). A metal silo is a cylindrical (Figure 23), square or rectangular prism structure,

constructed from a high quality galvanized iron sheet and hermetically sealed with a top inlet and a smaller bottom lateral outlet (Bokusheva et al., 2012). The main advantage of the metal silo is hermetically sealed. Eliminates or reduces oxygen and increases CO₂ concentration inside. Consequently, suffocate, and killing any insect pests inside (Quezada et al., 2006; Tefera et al., 2011).

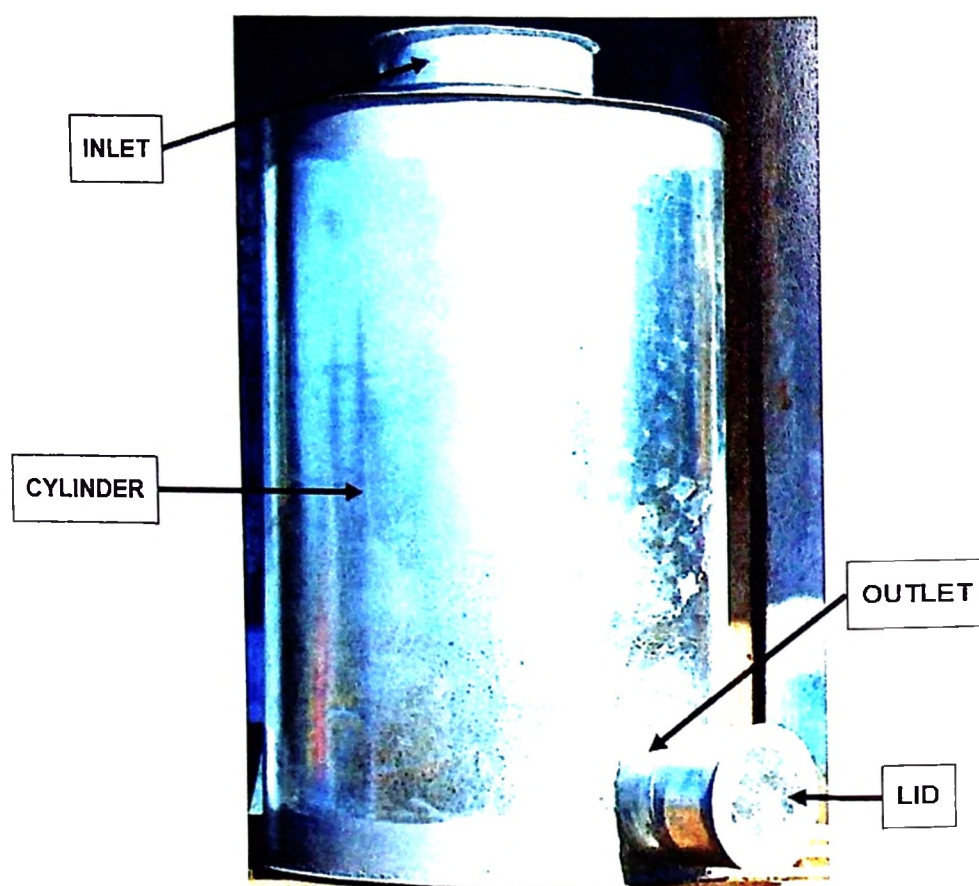


Figure 23. Different Parts of Metal Silo.

The metal silo is a key and a promising technology for effective post-harvest management of grains for small-scale farmers in the developing countries (Tefera et al., 2011). In addition, metal silo improved food security, maintained grain quality, reduce

women's workload, improved family health, and reduce usage of storage pesticides. Also, improve hygiene and welfare and creates jobs for artisans/metalworkers (Bokusheva et al., 2012; Bravo, 2009). As well as reducing macroeconomic fluctuations in grain price and increases farmer's flexibility to sell their grains in the lean season (Shiferaw et al., 2011). Metal silo technology program or Postcosecha program considered most successfully PHL program in Central American countries (SDC, 2008). According to Raboud and others cited by Bokusheva et al. (2012) about 380,000 tons of maize is saved annually in Honduras, Guatemala, Nicaragua, and El Salvador, corresponds to 13% of the region annual production of maize. This is equivalent to food for over 50,000 families and worth more than US\$12 million (Bravo, 2009). Metal silo's technology is getting popular in many developing countries like Kenya, Malawi, Tanzania, Mozambique and others. For more information on metal silos, see an excellent review by Tefera et al. (2011) and a research paper by Gitonga et al. (2015).

2.10.3.4. Silo Bags

The silo bag or "grain bag" was originally developed as a temporary storage system for chopped grain silage (Abalone et al., 2011). Nowadays has emerged as the best alternative for bulk grain storage in Argentina, Australia, Canada, and the US (Ward and Davis, 2012; Maier and Cook, 2014). Silo bags are hermetically sealed to prevent the growth and development of insect pests and molds, consequently, reduce postharvest losses, storage cost and maintains the quality of grain (Barbosa, 2008; Maier and Cook, 2014). Typically, silo bags (Figure 24) consist of three-ply of 0.250 mm thick polyethylene films. The outer layer is painted white to reflect solar radiation while the inner layer is black to block sunlight. It is

constructed from a high quality galvanized iron sheet and hermetically sealed with a top inlet and a smaller bottom lateral outlet (Bokusheva et al., 2012). The main advantage of the metal silo is hermetically sealed. Eliminates or reduces oxygen and increases CO₂ concentration inside. Consequently, suffocate, and killing any insect pests inside (Quezada et al., 2006; Tefera et al., 2011).

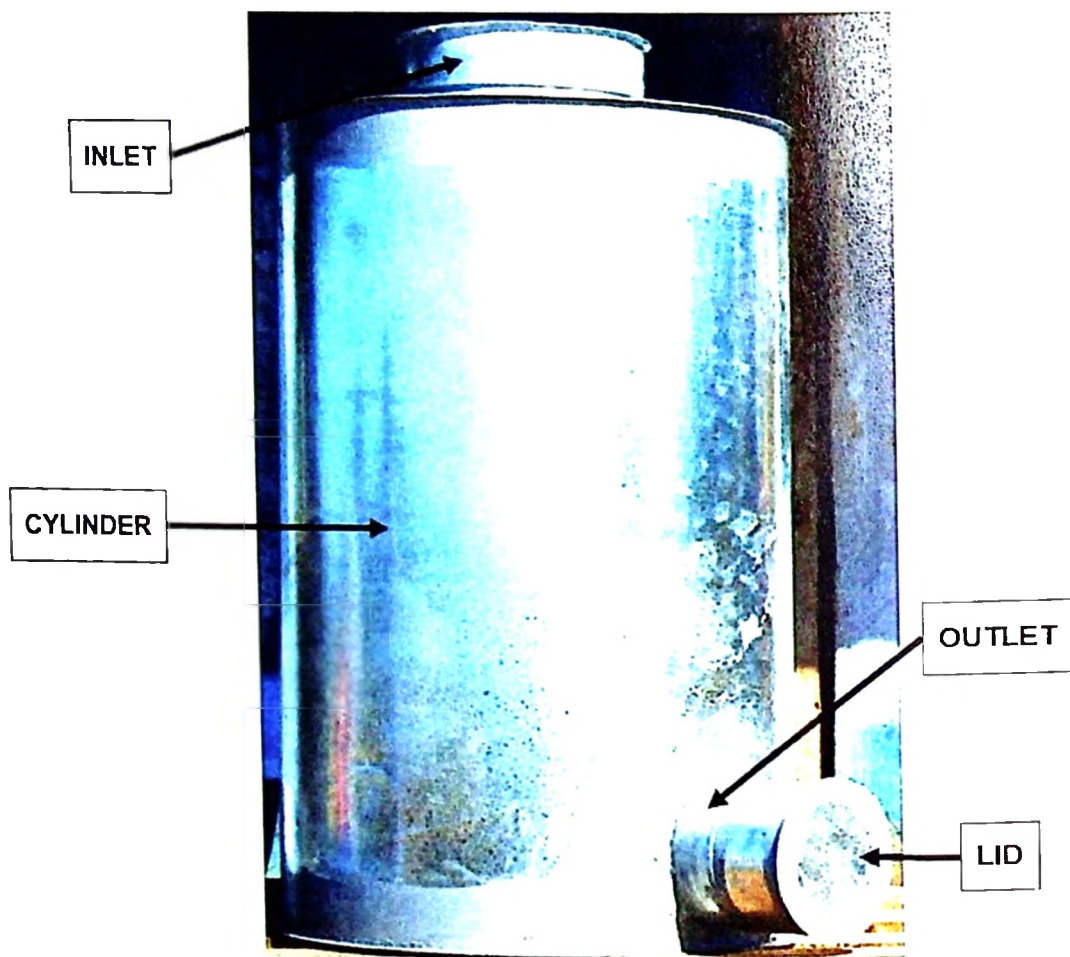


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women's workload, improved family health, and reduce usage of storage pesticides. Also, improve hygiene and welfare and creates jobs for artisans/metalworkers (Bokusheva et al., 2012; Bravo, 2009). As well as reducing macroeconomic fluctuations in grain price and increases farmer's flexibility to sell their grains in the lean season (Shiferaw et al., 2011). Metal silo technology program or Postcosecha program considered most successfully PHL program in Central American countries (SDC, 2008). According to Raboud and others cited by Bokusheva et al. (2012) about 380,000 tons of maize is saved annually in Honduras, Guatemala, Nicaragua, and El Salvador, corresponds to 13% of the region annual production of maize. This is equivalent to food for over 50,000 families and worth more than US\$12 million (Bravo, 2009). Metal silo's technology is getting popular in many developing countries like Kenya, Malawi, Tanzania, Mozambique and others. For more information on metal silos, see an excellent review by Tefera et al. (2011) and a research paper by Gitonga et al. (2015).

2.10.3.4. Silo Bags

The silo bag or "grain bag" was originally developed as a temporary storage system for chopped grain silage (Abalone et al., 2011). Nowadays has emerged as the best alternative for bulk grain storage in Argentina, Australia, Canada, and the US (Ward and Davis, 2012; Maier and Cook, 2014). Silo bags are hermetically sealed to prevent the growth and development of insect pests and molds, consequently, reduce postharvest losses, storage cost and maintains the quality of grain (Barbosa, 2008; Maier and Cook, 2014). Typically, silo bags (Figure 24) consist of three-ply of 0.250 mm thick polyethylene films. The outer layer is painted white to reflect solar radiation while the inner layer is black to block sunlight. It is

about 60 m by 3 m length to diameter and can store up to 200 tons of maize, soybean or wheat (Maier and Cook, 2014). The silo bags can play an important role as temporary on-farm grain storage during bumper harvest in Mid-West and Mid-South US and eliminate the immediate need to transport grain to the elevator (Barbosa, 2008; Ward and Davis, 2012) as well as increase harvesting efficiency and reduce farming cost (Barbosa, 2008). Like other hermetic bags, when proper airtight the silo is water-resistant and achieved a high degree of oxygen and carbon dioxide level that attained hermetic storage environment (Maier and Cook, 2014).

Moreover, the main shortcoming of silo bag is vulnerable to damage from birds, wild animals, insect pests, and rodents and silo bags can only be used once. In addition, it is difficult to monitor temperature and moisture movement within a grain mass, the grain conditions are influenced by the external climatic conditions, and moisture migration can occur within the bags (Barbosa, 2008; Abalone et al., 2011; Ward and Davis, 2011; Ileleji, no date). Likewise, an overload of silo bag can result in bag breaking, needs special loading and unloading equipment and the bag should be inspected regularly for leaks or damage by vermin (Maier and Cook, 2014).

2.11. Mycotoxins

2.11.1. Fungi deterioration

Insects, birds, mice, and rodents cause the more noticeable damage, but the role of storage fungi in the loss of stored grain cannot be ignored (Dunkel, 1988). Some storage insects are disseminators of storage fungi, while others are the exterminators (Sinha, 1971). Fungi are well-known to cause a variety of deteriorating changes in grains and fresh produce

before and after harvest (Sauer, 1988). It has been reported by many researcher's fungi grow faster under warm conditions than under cool conditions. As a rule of thumb, deterioration is increasing about 10 times faster at 25°C than at 3°C (Sauer, 1988; Suleiman et al., 2013). Contamination of maize to fungi can be categorized into two main classes: the field and storage fungi (Bankole and Mabekoje, 2004).



Figure 24. The Picture Silo-Bag Hermetic Storage System (INTA, 2014).

The field fungi or pathogenic are those that predominate in the field and invade the developing or mature seed of cereal plant at moisture contents of about 20% (Christensen, 1957; Meronuck, 1987). Field fungi do not compete well under normal and dry storage conditions, but may grow extensively in improperly preserved maize at high moisture (Meronuck, 1987). Normal has insignificant consequences in the storage. The *Fusarium spp.*

Alternaria, *cladosporium*, *Pullularia*, and *Helminthosporium* are a common genus of fungi that infect maize in the field (Bankole and Mabekoje, 2004). These fungi usually do not continue to grow after harvest (Christensen and Kaufmann, 1965) because most grains stored at moisture contents below 20%. On the other hands, the storage fungi (saprophytic) are those that develop on and within seeds at moisture contents often encountered in storage, principal species are *Aspergillus* and *Penicillium* (Christensen, 1957). The major effects of storage fungi on grain, including discoloration, losses in germination, caking, nutritional changes, heating, and mustiness, musty odors. Also, cause, dry matter loss, mycotoxins production, nutrition and chemical changes and reduction in processing quality (Meronuck, 1987; Sauer, 1988). The storage fungi do not invade grains before harvest (Christensen and Kaufmann, 1965). However, it is unknown what factors determine why field fungi primarily develop on the standing crop while storage species became dominant in store. Nevertheless, fungi are well-known for their role to produce secondary metabolites or mycotoxins (Magan and Lacey, 1984).

Mycotoxins are a heterogeneous group of toxic secondary metabolites that are produced by several fungal genera and exert toxic effects (mycotoxicosis) on human and domesticated animals (Peraica, 1999). Contaminate a range of agricultural commodities such as grains and their derived processed products (Njumbe et al., 2014). Mycotoxins contamination is unavoidable and unpredictable can occur throughout the food chain from the field or pre-harvest, during harvest, drying, during processing and storage (Lopez-Garcia et al., 1999). Which makes it an enormous challenge to manage and control, particularly in developing countries (Anukul et al., 2013). The production of mycotoxins depends on various factors, such as the commodity, poor agricultural and harvesting practices, improper drying,

handling, storage conditions, climatic conditions and seasonal variations (Marin et al., 2013; Leslie et al., 2008) often times most factors are beyond human control (Hussein and Brasel, 2001).

Mycotoxins contamination attracts worldwide attention due to the huge economic losses incurred and their impact on human, domestic animals and trade (Wu, 2006; Chilaka et al., 2012). May be detrimental to the health of humans and animals. Dietary exposure to mycotoxins can result in serious health affect both acute and chronic. Ranging from sudden death to deleterious effects upon the central nervous, induction of hepatocellular carcinoma, effects on the cardiovascular, reproductive, pulmonary, and gastrointestinal systems to mention a few (Burger et al., 2013; Suleiman et al., 2013). In addition, it is well established in several clinical trials that mycotoxins in animals cause decreases in productivity, damage vital organs, reduce animal weight, cause growth retardation, immune suppression, and interference with reproductive systems (CAST, 2003).

Mycotoxins may also be carcinogenic, teratogenic, tremorogenic, haemorrhagic, and immune-toxic, oestrogenic, effects dermatitis and nephrogenic to a lot of organisms (Burger et al., 2013; Leslie et al., 2008). Likewise, a synergistic effect between mycotoxins exposure and some important common diseases in sub-Saharan Africa such as malaria, kwashiorkor, protein energy malnutrition, decrease resistance to infection such as diarrhea, and HIV/AIDS have been suggested (Wagatha and Muthomi, 2008; Rustom, 1997).

Further, an increasing awareness of the deleterious effects of mycotoxins on the health and productivity of human and animals has persuaded many countries around the world to implement regulations for maximum tolerable levels to control occurrence of these

compounds in human food and animal feed (Coker, 1991; Garrido et al., 2012). A recent report by the FAO on mycotoxins shows over 100 countries worldwide had set regulatory limits on allowable mycotoxins levels in human and animal feeds (Warth et al., 2012; Wu and Guclu, 2012). Current regulations encompass about 13 different groups of mycotoxins (Van Egmond et al., 2007). However, despite sporadic outbreaks of mycotoxins incident in sub-Saharan African and Asian countries. Regulatory limits are rarely in place or not properly implemented due to improper testing equipment, lack of monitoring and surveillance system, and poor management of grains and oilseeds (Wild and Gong, 2010; Wu and Guclu, 2012). Table 6 shows maximum acceptable limits of mycotoxins in maize for some selected countries.

Furthermore, mycotoxins can occur both in temperate and tropical regions of the world. However, the impact of the problem is higher in tropical and sub-tropical climatic regions of the world (Suleiman et al., 2013) between 40° North and 40° south of the equator. Currently, over 300 different mycotoxins have been identified; In general, mycotoxins are categorized by fungal species, structure, and mode of action (Darwish et al., 2014). The most important and frequently encountered mycotoxins in maize include the aflatoxin (AFs), fumonisin (FUM), ochratoxins (OT), trichothecenes (TCT), deoxynivalenol (DON) and zearalenone.

2.11.2. Aflatoxins (AFs)

Aflatoxins are a group of secondary metabolites produced by two main strains of fungi, *Aspergillus flavus*, and *Aspergillus parasiticus* (Marin et al., 2013; Feng et al., 2011). These fungi resist a wide range of conditions and contaminate several agricultural commodities. AFs are of great concern due to their detrimental effects on the health of humans and animals

(Zinedine and Manes, 2009). AFBs are the most common and probably the most significant mycotoxin in terms of human and animal health risk (Sauer, 1988; Bluma and Etcheverry, 2008). In addition, due to the potent of AFBs, several studies have been conducted to look at nature, identification, classification, biosynthesis, metabolism, and detoxification of these toxins. Toxicity of AFBs can be categorized into two main groups: acute toxicity and chronic toxicity. Structurally, AFBs are related to difuranocoumarins compounds and classified into four main chemotypes (Figure 25): AFB₁, AFB₂, AFG₁, AFG₂ and two more minor, M₁ and M₂, usually found in milk and milk product (Jolly et al., 2008).

Likewise, these AFBs are pentaheterocyclic and highly conjugated compounds. Like many others heterocyclic fluoresce compounds, AFBs also are distinguished by native fluorophore characteristics. AFB₁ and AFB₂ fluoresce blue color while AFG₁ and AFG₂ are endowed with yellow-green fluoresce under ultraviolet light (Hussein and Brasel, 2001; Vazquez et al., 1991). The abbreviations B and G show **B**lue and **G**reen color, while 1 and 2 represent the relative migration distance, 1 (higher) and 2 (lower) of the compounds as seen on a thin-layer chromatographic plate under ultraviolet light (Klich, 2007).

Moreover, AFB₁ are known to be highly toxic and several studies have shown to be carcinogenic, teratogenic, mutagenic, hepatotoxic, genotoxic, immune suppression, growth retardation, and inhibit several metabolic systems in humans and other animal species (Zinedine and Manes, 2009; Bluma and Etcheverry, 2008; Shephard, 2003). AFB₁ classified by the International Agency for Research on Cancer (IARC) as a class 1 carcinogen to humans (IARC, 1993).

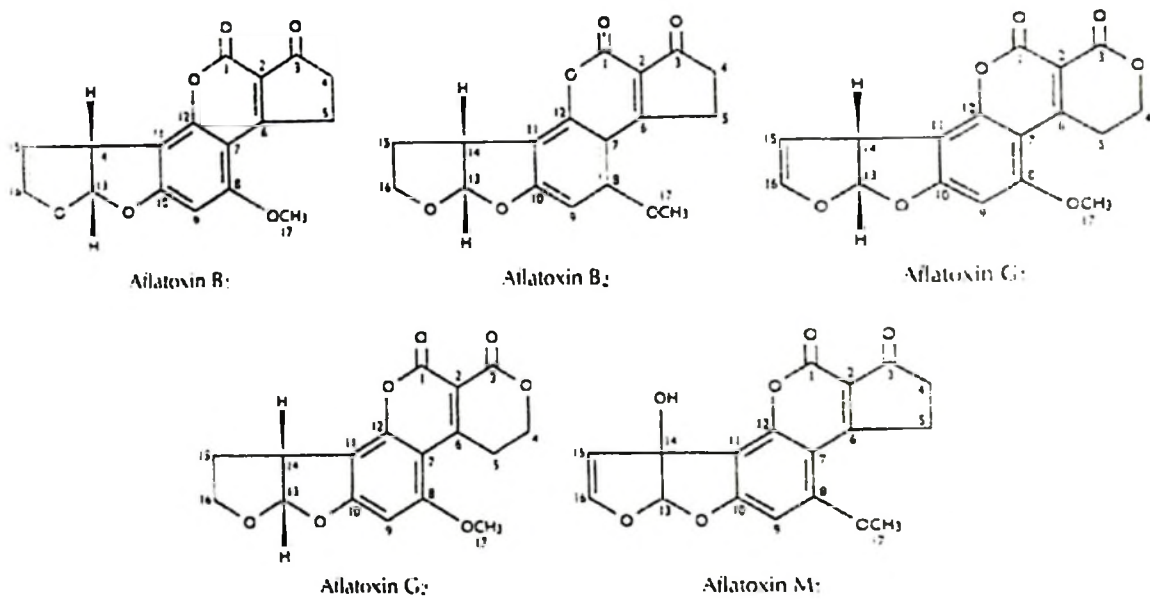


Figure 25. Chemical Structures of Aflatoxins B (B₁ and B₂), G (G₁ and G₂) and M₁.

It is considered to be the primary cause of hepatocellular carcinoma in mammals (Barkai-Golan and Paster, 2011). The risk of hepatocellular carcinoma is elevated in areas where hepatitis B virus infection is endemic (Lewis et al., 2005). Table 6 represents an association between food intake (cereals) and risk of AFB₁ that cause liver cancer in the human. Further, the incidence of aflatoxins in maize is a perennial threat in warm and humid subtropical and tropical conditions (Kaaya and Kyamuhangire, 2006). The warm and humid conditions provide a favorable environment for the growth of the molds and production of toxins both in field and storage (Rustom, 1997; Suleiman et al., 2013). In the field, the optimum thermal condition for fungal growth is 36 °C to 38 °C. While aflatoxin production occurs at 25 °C to 27 °C and 0.99 water activity and about 85% relative humidity (Pitt, 1993; Shephard, 2003). In storage, *A. flavus* requires at least 85% relative humidity and grows fastest at fairly high temperatures (Sauer, 1988).

2.11.3. Fumonisin (FUM)

Fumonisin is a group of mycotoxins produced by some *Fusarium* species, primarily *F. verticillioides* (syn. *F. moniliforme*) and *F. proliferatum* (Marin et al., 2013). FUM was first isolated and identified in South Africa in the late 1980s (Gelderblom et al., 1988).

Structurally, FUM is the diester of propanoic acid and can be classified into four main groups; A (A₁ and A₂), B (B₁, B₂, B₃ and B₄), C, and P. Moreover, FB₁, FB₂, and FB₃ (Figure. 26) are highly toxic and occur naturally in maize and maize-based products (Shephard et al., 2005). The most potent form of Fumonisin is FB₁ and classified by the IARC as a group 2B, a possible human carcinogen (IARC, 2002).

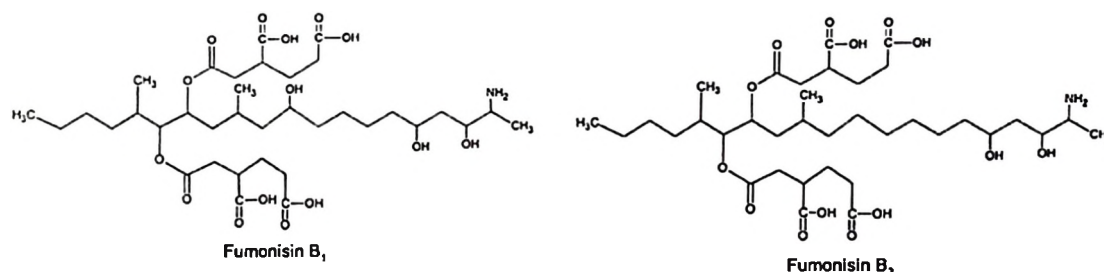


Figure 26. Chemical Structures of Fumonisin B (B₁ and B₂).

Fumonisin is known to exhibit toxic effects on a number of animal species. Several ecological and clinical studies have shown Fumonisin to cause equine leukoencephalomalacia and neurotoxicity in horses (Marasas et al., 1988). Also, pulmonary edema in swine brain, hepatitis and nephrosis in sheep and promote tumor in rats, mice, and rabbits (Hussein and Brasel, 2001). In addition, Fumonisin has been found to produce a broad range of pathological effects in mammals (Shephard et al., 2000) such as interference with cellular foliate uptake (Stevens and Tang, 1997). Likewise, Fumonisin has detrimental effects on the

central nervous system, liver, pancreas, kidney, heart, and lung to several domesticated and other animal species (Bucci and Howard, 1996). These effects are associated with decreases in food intake, inhibits ceramide synthesis and disruption of sphingolipid metabolism (Merill et al., 1996; Smith et al., 2012).

2.11.4. Ochratoxins (OT)

Ochratoxins are mycotoxins produced by several fungal strains of *Aspergillus* and *Penicillium*. Three main types of ochratoxin are: A, B, and C (Figure 27). Ochratoxin-A (OTA) is the most toxic of the three compounds. OTA is a frequent natural contaminant of many commodities such as coffee, dried fruit, grapes, raisins, red wine and beer (Erkekoğlu et al., 2008). In addition, OTA also occurs in wheat, barley, rye, corn, soy, peanuts, rice, oats, and cassava flour (Zain, 2011; CAST, 2003) and in several foods of animal origins (Peraica et al., 1999). Chemically ochratoxin is described as 3, 4-dihydromethylisocoumarin derivatives linked by an amide bond to the amino group of L- β -phenylalanine (Anli and Alkis, 2010).

OTA toxin is responsible for nephrotoxic, teratogenic, mutagenic, genotoxic, immunotoxic complications, as well as reproductive toxicity and other detrimental effects to several animal species (Erkekoğlu et al., 2008). OTA has been classified by the IARC as a Class 2B carcinogen, possible carcinogen to human (Murphy et al., 2006). Ochratoxin A toxin has been shown to be weakly mutagenic by its induction of oxidative DNA damage (Bennett and Klich, 2003). Several studies show OTA causes renal adenomas and carcinomas in male mice and rat (Schwartz, 2002). In addition, OTA has been suggested as an aetiological agent to interstitial nephritis, urothelial, and testicle tumors in human (Anli and Alkis, 2010). Also,

OTA is associated with a chronic disease called Balkan Endemic Nephropathy (BEN) (Schwartz, 2002). BEN is a fatal chronic kidney disease affecting rural populations, in Romania, Bulgaria, and the former Yugoslavia (Schwartz, 2002).

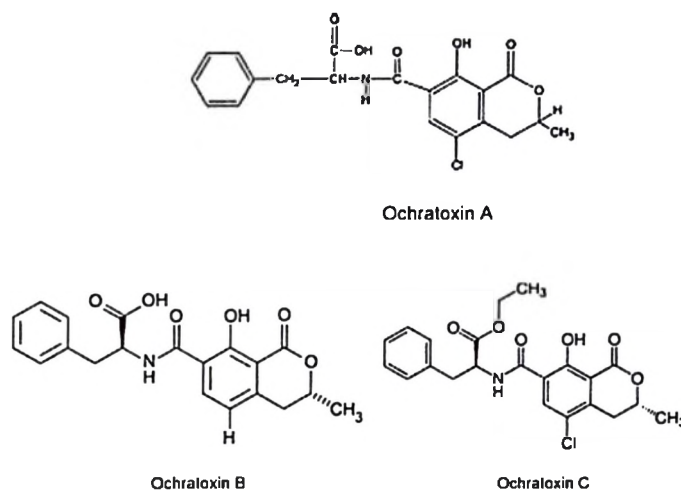


Figure 27. Chemical Structure of Ochratoxin A, B, and C.

2.11.5. Trichothecenes (TCT)

Trichothecenes are a group of mycotoxins which are produced by several fungal genera, most notably *Fusarium* species. TCT is a toxic tricyclic sesquiterpenoid compound with as a 12, 13-epoxy ring and a variable number of hydroxyl or acetyl groups (Eriksen et al., 2004; Sweeney and Dobson, 1998; WHO, 1990). At present over 150 TCT toxins are known, TCT is chemically classified based on the presence or absence of characteristic functional groups and their producer fungi (Sudakin, 2003). There are four subtypes of the TCT; Type A (Figure 28) has a functional group other than a ketone at position C-8, include T-2 toxin (T-2) and HT-2 toxin (HT-2) produced by *F. sporotrichoides* and *F. poae*.

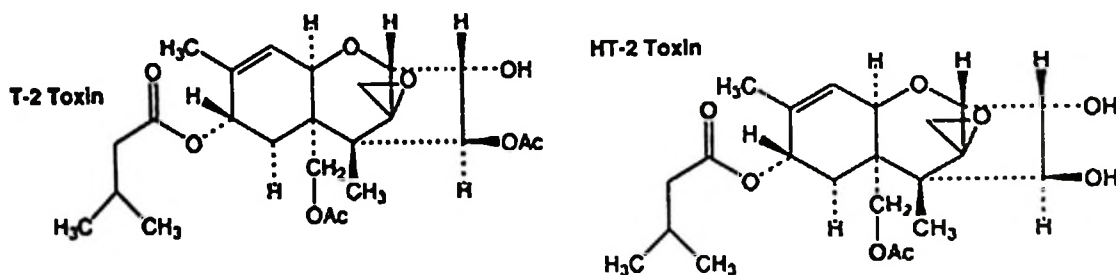


Figure 28. Chemical Structures of Type A, T-2 Toxin, and HT-2 Toxin.

Moreover, type B (Figure 29) TCT has a ketone at position C-8, include nivalenol (NIV) and deoxynivalenol (DON) produced by *F. culmorum* and *F. graminearum*. Type C (Figure 30) has a second epoxy group at C-7, 8 or C-9, 10, include crotocin and baccharin produced by *cephalosporium crotocingigenum*. Type D TCT is potent compound has a macrocyclic ring linking C-4 and C-15 with two ester linkages, produced by *S. alternans*. Type D TCT is not produced by *Fusarium* species (Sweeney and Dobson, 1998; Foroud and Eudes, 2009; Moss, 2002; WHO, 1990). Trichothecenes are common mycotoxins occur worldwide in agricultural commodities such as maize, wheat, barley, rye, rice, oats and vegetables (Eriksen, 2004) as well as in animal feed (WHO, 1990). However, Type C and D are rarely found in human food.

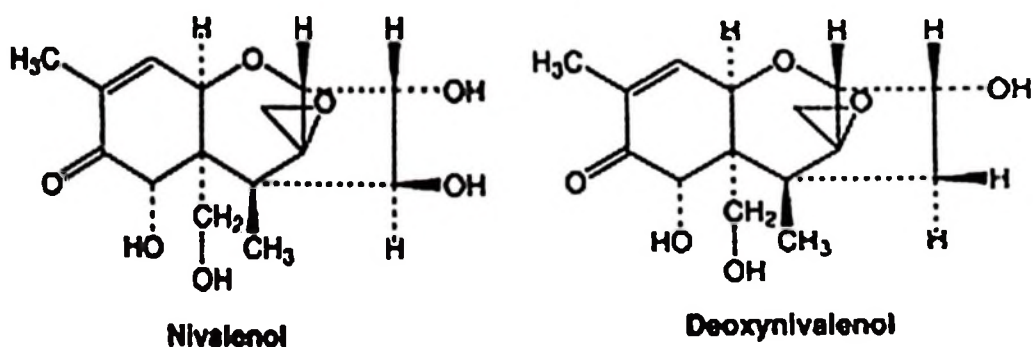


Figure 29. Chemical Structures of Type B, Nivalenol, and Deoxynivalenol.

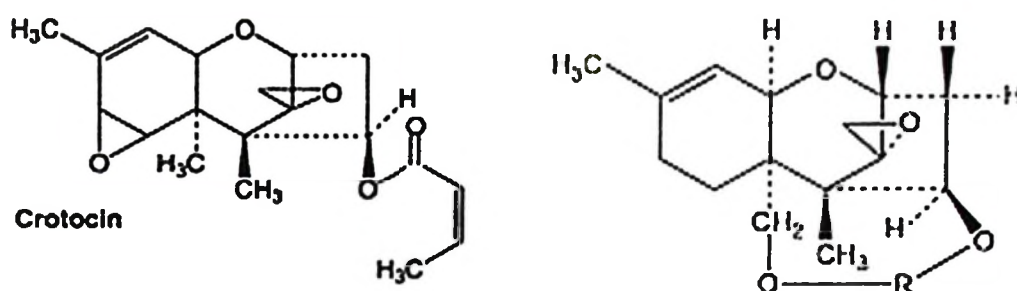


Figure 30. Chemical Structures of Type B, Nivalenol, and Deoxynivalenol.

Furthermore, the exact metabolic toxicity of TCT to the vertebrate body are poorly understood but is related to the inhibition of protein and DNA synthesis at the ribosomal level (Fink-Grenmels, 1999). In addition, to their inhibition activity, they have a wide range of gastrointestinal, dermatological and neurological effects such as vomiting, diarrhea, and bowel inflammation. Likewise, TCT has been previously associated with anemia, digestive disorders, leukopenia, and skin irritation. Also, feed refusal, decreased bone marrow, reduced ovarian function and cause growth retardation in several animal species (Erkekoğlu et al., 2008; Zain, 2011; Quiroga et al., 1995; Sudakin, 2003). Moreover, TCL is recognized for their phytotoxic properties, and at very low-level cause wilting, chlorosis, necrosis and other symptoms in a variety of plant (Sudakin, 2003; Muhitch et al., 2000). The phytotoxic effects of TCL on plants include inhibiting seed germination, growth retardation, and green plant regeneration to both mono and dicotyledonous plant (Sudakin, 2003; Masuda et al., 2007).

2.11.6. Deoxynivalenol (DON)

DON is a trichothecene and non-fluorescent mycotoxin produced by *F. graminearum* and *F. culmorum* (Anukul et al., 2013). DON (Figure 31), also known as “vomitoxin” is the most well-studied group of mycotoxins contaminating many cereal grains, especially maize, and wheat, in both tropical and temperate regions (Foroud and Eudes, 2009). Likewise, DON found in rye, rice, oat, barley as well as in safflower seeds and mixed feeds (Pestka et al., 2005). DON exposure has been linked to incidences of acute gastrointestinal diseases, kidney problems and immunosuppressive in animals (Pestka et al., 2005; Richard, 2007).

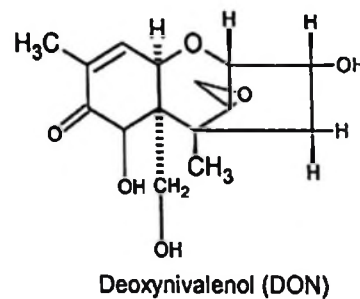


Figure 31. Chemical Structure of Deoxynivalenol.

Moreover, a short-term exposure of DON causes a condition is known as “anorexia”, decreased food intake or refusal to eat, thus the lower weight gain and decreased nutritional efficiency (Anukul et al., 2013; Pestka et al., 2005). Whereas a long-term exposure elicits “emesis” acute effects vomiting, abdominal distress, rectal bleeding, increased salivation, diarrhea, malaise and inhibiting reproductive performance in several monogastric animal species (Anukul et al., 2013; Pestka et al., 2005). Also found to reduce the milk in dairy cattle (Akande et al., 2006). The International Agency for Research on Cancer (IARC) placed DON in Group III, not classifiable as to its carcinogenicity to humans (CAST, 2003).

Table 6. Maximum Acceptable Limits of Mycotoxins in Maize for Some Selected Countries.

Country	Maximum regulatory limits ($\mu\text{g/kg}$)										References
	AFB1	AFB2	AFG1	AFG2	FB1	FB2	FB3	DON	ZEA	OTA	
Argentina	20	20			--			--	--	10	Zinedine et al., 2007; FAO, 2004
Australia/ New Zealand	15	--			500-5000			100- 5000	150	--	Suleiman et al., 2013; Vicam, 2010
Brazil	20	20			1000			1000	150- 400	5-10	Suleiman et al., 2013; Souza et al., 2013; FAO, 2004
Canada	15	15			4000			1000	--	2000	Suleiman et al., 2013; Wu, 2007; Kubo, 2012
China	20	--			500			1000	500	5	Suleiman et al., 2013; Kubo, 2012; Li et al., 2014
Egypt	5	10			--			--	--	--	Darwish et al., 2014
EU	2	10			2000			1750	100	5	Garrido et al., 2012; Marin et al., 2013; Souza et al., 2013
India	30	30			--			1000	10-10	20	Suleiman et al., 2013; Kubo, 2012
Japan	10	--			--			1100	1000	--	Suleiman et al., 2013; Wu, 2007; Kubo, 2012
Kenya	20	20			--			--	--	5	Lewis et al., 2005
Malaysia	35	--			--			--	--	0.5- 10	Suleiman et al., 2013
Mexico	20	20			--			--	--	--	Suleiman et al., 2013; Guzmán-de-Peña & Peña-Cabriales, 2005
Nigeria	15	--			--			--	--	20	Ezekiel et al., 2012.
Russia	5	--			--			700	1000	5	Rai & Bai, 2014; Kubo, 2012; Zinedine et al., 2007; FAO, 2004.
South Africa	5	10			--			--	50- 8000	--	Suleiman et al., 2013; van Egmond, 2007.
Tanzania	5	10			--			--	--	--	TFDA, 2012; Kimanya et al., 2010; FAO, 2004
USA	20	20			2000			1000 (advisory limit)	114- 3000	2-80	Suleiman et al., 2013; Roben & Cardwell, 2003; Mamsa et al., 2008; Wu, 2007; Kubo, 2012; Rai & Bai, 2014.

Note: -- no information available.

The worst effect of DON toxicity in human depends on the extent of contamination in the food ingested. Several studies show a strong association between DON and outbreaks of acute diseases such as gastrointestinal upset, nausea, dizziness, vomiting, headache, abdominal pain and diarrhea after red mold intoxication in India and China, Korea, and rural Japan (WHO, 2011; Kpodo et al., 2008; Robert et al., 2010). The no-observed-effect level (NOEL) for adult is 0.5-mg/ kg body weight /day. Likewise, the NOAEL for fetal toxicity on based on impaired fetal development is 2.5-mg/kg body weight/per days and considered to be a teratogen at 5-mg/kg body weight /days (Pestka, 2010).

Table 7. Food Intake at Different AFB₁ Levels of Contamination and Risk of Liver Cancer (cancers per 100,000 populations).

Food Intake (g/person (60 kg) per day)						
AFB ₁ (ng/g)	10	50	100	150	200	400
1	0.01	0.07	0.14	0.21	0.28	0.55
2	0.03	0.14	0.28	0.41	0.55	1.10
5	0.07	0.34	0.69	1.00	1.40	2.80
10	0.14	0.69	1.40	2.10	2.80	5.50
20	0.28	1.40	2.80	4.10	5.50	11
50	0.69	3.40	6.90	10	14	28
100	1.40	6.90	14	21	28	55

The shaded area represents region of risk in excess of 1 per 100,000 (Adapted from Shephard, 2008b).

2.11.7. Zearalenone (ZEA)

Zearalenone is classified as an estrogenic mycotoxin synthesized by several *Fusarium* species, including *F. graminearum*, *F. culmorum*, *F. cerealis*, *F. equiseti*, and *F. crookwellense* (Anukul et al., 2013). Contamination of ZEA occurs mainly in cereals such as maize, wheat, and barley fields, but also in sorghum, soybean, oats, hay, rice, rye, sesame seed, and silages (Peraica et al., 1999; Zinedine et al., 2007). *Fusarium* species are common soil fungi and mostly grow in moist, warm, and temperate conditions (Richard, 2007). Chemically, ZEA (Figure 32) is described as a phytoestrogenic compound of a 6-(10-hydroxy-6-oxo-trans-1-undecenyl)- β -resorcylic acid μ -lactone (Hussein and Brasel, 2001).

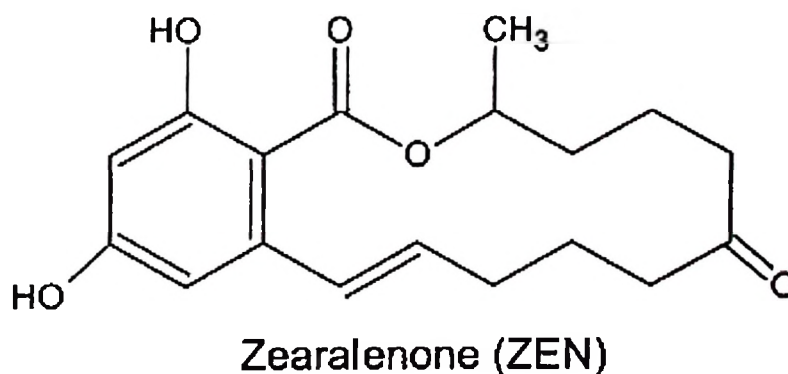


Figure 32. Chemical Structure of Zearalenone.

ZEA is renowned for its detrimental effect on the urogenital system in animal species as well as neuroendocrine disruption by binding to estrogen receptors (Richard, 2007). The interaction of ZEA with estrogen receptors, resulting in apparent hyperestrogenism, reduced fertility, vulval edema, vaginal prolapse, macromastia, and gigantomastia or mammary hypertrophy in females (Peraica et al., 1999; Zinedine et al., 2007). In addition, ZEA has

been associated to induce feminization such as enlarged nipples, testicular atrophy and swollen prepuce in young male pigs (Peraica et al., 1999; Richard, 2007; Whitlow and Hagler, 2002). Moreover, ZEA has been known to causes depress serum testosterone, weights of testes, spermatogenesis, fetal reabsorption, aborted pregnancies, reduced litter sizes and low birth weights, in swine. Likewise, in cows, ZEA has been linked to infertility, reduced milk production and hyperestrogenism (D'Mello et al., 1999; Zinedine et al., 2007).

2.11.8. Mycotoxins Contamination in Tanzania

Maize and cassava are the two major staple foods in Tanzania and are essential components of complementary foods for infants and young children (Sulyok et al., 2014; Kimanya et al., 2008). Nevertheless, these two crops are the most prone to mycotoxins contamination (Manjula et al., 2009; Sulyok et al., 2014). Mycotoxins contamination of maize is considers the greatest public health threat due to their detrimental effects on human health (TFDA, 2012). In addition, to health concerns, mycotoxins can restrict maize trade and limits income of smallholder farmers, because of food safety concern and trade restrictions (WHO, 2006). The most frequently encountered mycotoxins in Tanzania are aflatoxins and Fumonisin (Kimanya et al., 2008). However, other types of mycotoxins such as zearalenone, deoxynivalenol, ochratoxins, and T-2 toxins, HT-2 toxins have also been reported (Doko et al., 1996; Mboya et al., 2012; Kimanya et al., 2014; Srey et al., 2014; Kamala et al., 2015).

The recent economic assessment conducted by Abt Associates in collaboration with Tanzania food and drug authority (TFDA) observed the significantly higher prevalence of AFB₁ in multiple regions around the country (TFDA, 2012). AFB₁ is the most potent types of

mycotoxins responsible for liver toxicity. As shown in Figure 33, all regions assessed AFB₁ level was well above 5 µg/kg (5 ppb) maximum acceptable limits for maize grain set by the Tanzania Bureau of Standards (TBS). The report concluded that lack awareness about mycotoxins among the communities (farmers, traders, and consumers) and policy makers exacerbated the problem of mycotoxins in Tanzania (TFDA, 2012).

Table 2. Relationships between Food Intake (maize) and Fumonisin Contamination.

FB (µg/g)	Maize intake (g/60 kg person/day)						
	10	50	100	150	200	400	500
0.2	0.0	0.2	0.3	0.5	0.7	1.4	1.7
0.5	0.1	0.4	0.8	1.3	1.7	3.4	4.2
1	0.2	0.8	1.7	2.5	3.3	6.6	8.3
2	0.3	1.7	1.3	5.0	6.7	13	17
3	0.5	2.5	5.0	7.5	10	20	25
4	0.7	3.3	6.7	10	13	27	33
5	0.8	4.2	8.3	13	17	33	43
10	1.7	8.3	17	25	33	67	83
12	2.0	10	20	30	40	80	100

White area= Provisional Maximum Tolerable Daily Intake (PMTDI) - tolerable daily intake levels; lightly shaded area= risk of hepatocarcinogenicity; Medium shaded region= risk of nephrotoxicity; Dark shaded region= above maximum PMTDI tolerable daily intake levels (Adapted from Marasas et al., 2008).

Further, the results obtained from this assessment concurred with several studies. The research conducted by Kimanya et al. (2008; 2010) found 12% of all samples of maize collected exceeded the maximum limit for total aflatoxins (10 ppb). In addition, the study conducted to assess the occurrence of mycotoxins exposure for the stunting of infants and

young children in rural Tanzania. The result revealed a high percentage of mycotoxins exposure, particularly fumonisin and aflatoxins were significantly higher than provisional maximum tolerable daily intake (PMTDI) (Kimanya et al., 2010). On the other hand, a cross-sectional study conducted in Morogoro, Tanzania found 68% of all feed samples collected were contaminated by AFB₁ (Kajuna et al., 2013).

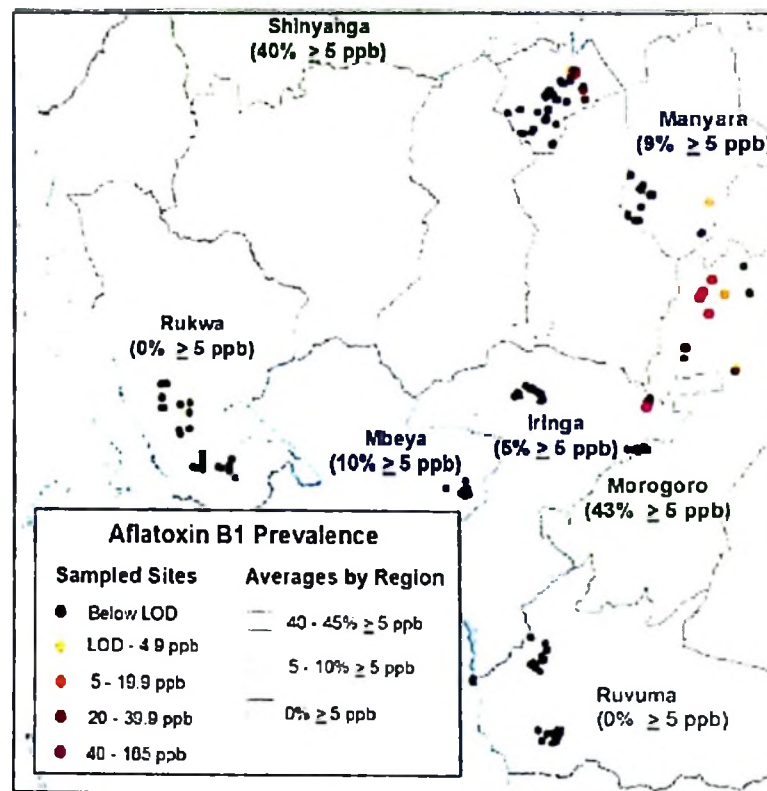


Figure 33. Aflatoxin B1 Contamination in Maize in Tanzania (TFDA, 2012).

Likewise, the study conducted by Srey et al. (2014) shown young children in Tanzania is frequently exposed to DON due to consuming contaminated maize related food. Thus, when all these findings, taken together revealed that majority of Tanzanian population were at risk of exposure to different types of mycotoxins (Kimanya et al., 2008, 2009, 2011; 2014; Mboya and Bogale, 2012; TFDA, 2012; Magoha et al., 2014; Kamala et al., 2015). The

contamination levels were alarming in respect to food safety and regarded most of these toxins were found on main staples food eating by the majority of the rural population. Thus, we wonder if these results relate to the recent WHO and WHR (World Health Ranking) report that shows an enormously increased in oesophagus and liver cancer in Tanzania. According to WHR website, oesophagus cancer is the second leading cause of death in terms of cancer in Tanzania and ranked 14 in the world (WHR, 2015).

Moreover, our speculation is because several studies have directly linked to those types of cancer with consumption of maize and mycotoxins contamination (Marasas et al., 1981; Sydenham et al., 1990; Mohanlall et al., 2013; Shephard et al., 2000; Van Der Westhuizen et al., 2003; Zhang et al., 1997; Barkai-Golan and Paster, 2011). In addition, the majority of the Tanzanian population was frequently exposed to these toxins at an early age (Kimanya et al., 2008, 2009) and exposure levels increased as the children grew older (Avakian, 2014). Moreover, most reported cases of oesophagus cancer were related to young age and people from rural areas (Mchembe et al., 2013) where maize and cassavas are the main dietary staple food (Kimanya et al., 2008; Manjula et al., 2009; Kamala et al., 2015). However, extensive studies are needed to address these issues before jumping to any conclusions.

2.11.9. Mycotoxin Economic Aspects

Tanzania is an agricultural country, as explained in the previous section, agriculture played a vital role in Tanzanian economy, contributing around 33% of the total GDP and over 80% of export by value (CIA World Factbook, 2014). Tanzania's economy mainly depends on the export of its major agricultural commodities such as coffee, cashew nut, cereals, oilseed and grains for foreign earning (FAOSTAT, 2015). Grain export like the

maize has a significant driving force for overall economic growth, increase farmers' income and poverty reduction (Diao et al., 2013). However, maize in most parts of the country is contaminated with mycotoxins well above acceptable levels (TFDA, 2012), thus, posse's greater economic losses and risk to agricultural export and trade.

In general, the economic consequences of mycotoxin contamination are profound (Leslie et al., 2008). The economic losses associated with mycotoxin have been reported by many authors, although most of them agreed, difficult to assess in a consistent and uniform way. As well as a general formula to quantify the economic impact of mycotoxin contamination (Dohman, 2003; Zain, 2011). "Thus, most reports on the economic impact of mycotoxins are on a single aspect of mycotoxin exposure or contamination" (Hussein and Brasel, 2001). The Food and Agriculture Organization of the United Nations estimates around 25% of the world crops and up to 50% in developing countries are affected by mycotoxins each year (Miller, 1995). In the USA, the estimated crop losses from mycotoxins are about \$932 million per year (CAST, 2003). Similarly, the estimated cost due to management and testing of mycotoxins in the US ranged between \$500 million-1.5 billion per year (Robens and Cardwell, 2003). Likewise, the estimated economic losses due to mycotoxins in Africa is around \$670 million in terms of export per year (Hell, 2004).

Mycotoxins have a significant impact on economic and trade. The main criteria used to assess economic impact due to mycotoxins are categorized into five main groups; crop value losses due to contamination, yield losses due to diseases, losses in animal productivity, human health costs, and cost due management and prevention (Schmale and Munkvold, 2015). Plus, regulatory, and research costs related to mycotoxins (Hussein and Brasel, 2001).

Other researchers categorized economic losses into two main groups: direct and indirect economic losses. Direct economic losses are those related to reducing crop yields for growers and animal performance (morbidity and mortality) and rejection of crops by the international market (PACA, 2013). While indirect economic losses are those costs related to reduce the marketable value of the product, and costs associated with monitoring, research, loss of consumer confidence and increased processing costs (PACA, 2013). Further, the economic losses of mycotoxins have both domestic and international trade effects. In domestic, economic losses occur at all stages of the product value chain from the producers (farmers) to the final consumer (WHO, 2006). On the other hand, in the international market, products that exceed the maximum tolerance level of aflatoxin B₁ (mycotoxins) are either quarantined and confiscated at the port-of-entry, assigned a lower price or diverted to animal feeds (PACA, 2013).

2.11.10. Strategies for prevention of mycotoxins in maize

The strategies to reduce mycotoxins in maize can be grouped into two main categories: pre-harvest and post-harvest strategies, it also termed 'primary' or agricultural interventions. It is described as strategies or technologies that can be applied either in the field, drying, storage, transportation or processing to reduce mycotoxins contamination in maize (Wu and Khlangwiset, 2010).

2.11.11. Pre-harvest Strategies

It is well established that most of the mycotoxin contamination of maize start in the field and continue during storage (Kabak et al., 2006). Thus, prevention at this stage is crucial to prevent the development of mycotoxins during drying and storage (Strosnider et al., 2006).

As cited by Magan and Aldred (2007) pre-harvest factors are critical for effective post-harvest prevention of mycotoxins from contaminated maize entering the food supply chain. Several strategies have been investigated to manage, prevent, and reduce mycotoxins contamination in crops include biological, chemical and cultural control practices (Cleveland et al., 2003; Kabak et al., 2006; Strosnider et al., 2006; Wu and Khlangwiset, 2010; Yin et al., 2008; Dorner, 2004; Brown et al; 1991a; Wagacha and Muthomi, 2008; Magan and Aldred, 2007).

Biological control (BC): it is considered a promising strategy for reducing mycotoxins contamination in maize. BC referred as the use of organisms to reduce the incidence of pests, diseases, or toxins (Wu and Khlangwiset, 2010). Strategies include the application of atoxigenic fungal strains and antagonistic microorganisms (bacteria and yeasts) (Cleveland et al., 2003). Atoxigenic applications rely upon the ability of atoxigenic strains to competitively exclude toxigenic strains from infecting the crop (Cleveland et al., 2003; Wu and Khlangwiset, 2010; Strosnider et al., 2006). Atoxigenic fungal strains include the application of competitive nontoxigenic strains of *A. flavus* and/or *A. parasiticus* (Dorner, 2004; Yin et al., 2008; Brown et al., 1991a). The best examples of atoxigenic fungal are AF36 (cottonseed), Afla-Guard™ (groundnuts) and AflaSafe™ (maize) (Wu and Khlangwiset, 2010; <http://www.aatf-africa.org>). A detailed review of biocontrol of mycotoxins can be found in Cleveland et al. (2003).

Chemical control: Another important factor which is known to increase the susceptibility of mycotoxins to crops is damage due to insect pests and fungal contamination (Kabak et al., 2006; Magan et al., 2010). Revealed by Magan et al. (2003) that pre-harvest insect damage can lead to increased post-harvest production of mycotoxins in crops. Thus, insect damage

and fungal infection must be controlled in the vicinity of the crop by proper application of insecticides and fungicides (Kabak et al., 2006). For instance, application of itraconazole and amphotericin B fungicides to control *Aspergillus* species (Wagacha and Muthomi, 2008). Also, application of tridemorph on T-2 toxin and diacetoxyscirpenol (DAS) to inhibit growth and development of *F. sporotrichioides* in vitro (Pirgozliev et al., 2003).

Cultural control: CC is the practices designed to reduce mycotoxin contamination of crops have their roots in plant disease epidemiology (Munkvold, 2003). Cultural control strategies include crop rotation, tillage practices, appropriate application of fertilizers, weed control. As well as tillage practices, plant density, irrigation, insect control, planting, and harvesting dates, genotypes of seed planted, competitive exclusion and good agricultural practices (Wagacha and Muthomi, 2008; Munkvold, 2003; Pirgozliev et al., 2003; Strosnider et al., 2006). The main principles of cultural control are to alter the conditions under which the crop is grown so that infection by the offending fungus is avoided and discourage disease development (Munkvold, 2003; Battilani et al., 2008). However, many cultural practices require decisions to be taken before planting (Munkvold, 2003; Battilani et al., 2008).

In addition, other pre-harvest strategies include plant resistance to insects, integrated management programs, prevention of invasion of mycotoxigenic fungi through the incorporation of antifungal resistance into crops that comprised. Development of aflatoxin resistance screening assays, identification of resistance-associated proteins and natural products in corn, which inhibit *A. flavus* growth and aflatoxin contamination. Also, plant breeding strategies for enhancing resistance to mycotoxigenic fungi, genetic engineering strategies to enhance resistance in crops to mycotoxin contamination (Cleveland et al., 2003).

2.11.12. Post-harvest Strategies

Post-harvest strategies for mycotoxins must be implemented to maintain proper storage conditions, including insect and mold control (Munkvold, 2003). Poor post-harvest management can lead to a growth of spoilage fungi, especially mycotoxigenic fungi as well as the rapid loss of maize quality (Aldred and Magan et al., 2004). Post-harvest strategies to reduce mycotoxins contamination include proper storage (hermetic storage), and drying conditions, thermal treatment, grain milling, chemical treatment such as inactivation with ammoniation and ozonation, and adsorbents/binders. Others include minimizing the time between harvesting and drying, sanitation, efficient dry to below 14% moisture content, physical separation of damaged grains and processing such as dehulling (Jouany, 2007; Magan and Aldred, 2007; Lopez-Garcia et al., 1999; Suleiman et al., 2013). Table 9 summarized pre and post-harvest strategies to reduce mycotoxins in maize and other cereal grains.

2.12. Conclusions

Agriculture is the backbone of the Tanzanian national economy. It accounts for about one-third of the gross domestic product, provides 85 percent of all exports and saves as a livelihood to over 80 percent of the total population. Maize is a primary staple crop; it's grown in nearly all agro-ecological zones in the country. Tanzania is a major maize producer in SSA. However, despite being the highest producer of maize in the EA region, post-harvest losses of maize remained significantly higher. Such loss often aggravated by inappropriate handling, poor storage facilities, insects, and other pests, and contamination by spoilage

fungi. The major effects of fungi on maize are discoloration, reduce quality and contaminate maize with mycotoxins. Mycotoxins are toxic secondary metabolites of fungi that frequently contaminate the maize in the field and/or during storage.

Table 9. Summary of Pre and Post-Harvest Control Strategies to Reduce Mycotoxins in Maize.

Stage	Strategies
Pre-harvest	Choice of suitable cultivars
	Timing of planting and crop planted
	Field management: Soil cultivation, Irrigation, crop rotation, fertilization.
	Transgenic or conventional breeding for resistance
	Competitive exclusion
	Biocontrol
	Time of harvest
	Chemical control (insecticides, fungicides)
	Good agricultural practices (GAPs)
	Antioxidants (caffeic acid, gallic acid)
Post-harvest	Cleaning
	Sorting and segregation
	Improved storage (hermetic storage)
	Improved drying (solar drying) and transportation
	Chemical control (insecticides, fungicides)
	Processing; Crushing, Dehulling, Nixtamalization, Acidification, Chemoprotectant, Ammoniation

Mycotoxins contamination of maize poses a health risk to humans and domesticated animals if not properly managed because of their acute and chronic effects. The most important mycotoxins in maize are the aflatoxins, Fumonisin, deoxynivalenol, and ochratoxin. Furthermore, postharvest losses are a major factor negatively affecting smallholder farmers in Tanzania. The major constraints to maize production include insect pests (maize weevils and LGB), diseases, weeds, pathogens, and viruses. In addition, reducing PHL has positive consequences for the society like poverty alleviation, increase food security, improving nutrition status, and increases household income of smallholder farmer. Also, impacts on the environment, and reduces the utilization of production resources. The main strategies to reduce PHL include improving varieties, harvest at the right time and improve storage structures like metal silos, PICS bags. As well as improving drying efficiency, uses of moisture and temperature meters, proper hygiene and sanitation and access to market information.

Moreover, mycotoxins contamination of maize is considered the greatest public health threat due to their detrimental effects on human health. In addition, to health concerns, mycotoxins can restrict maize trade and limit the income of smallholder farmers, because of food safety concern and trade restrictions. The strategies to reduce mycotoxins in maize include pre-harvest and post-harvest strategies. Likewise, pre-harvest strategies include the application of atoxigenic fungal strains and antagonistic micro-organisms, crop rotation, tillage practices, appropriate application of fertilizers, weed control, irrigation, insect control, genotypes of seed planted. On the other hand, post-harvest strategies to reduce mycotoxins contamination include proper storage (hermetic storage), improve drying conditions and grain milling. Minimizes times between harvesting and drying, sanitation, efficient dry to below 14% moisture content and physical separation of damaged grains.

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CHAPTER 3. EFFECTS OF DETERIORATION PARAMETERS ON STORAGE OF MAIZE

Modified from a paper published in the *Journal of Natural Science Research*

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Abstract

Maize (*Zea mays* L), commonly known as corn in the United States, is the third most important cereal grain worldwide, after wheat and rice. It is a basic staple grain for large groups of people in Africa, Latin America, and Asia. In tropical countries, a large proportion of the maize is harvested and stored under humid and warm climatic conditions, which subsequently results in rapid deterioration of the grains, mainly because of growth of molds and pests. This study reviewed the main factors that lead to deterioration of maize in tropical countries and suggests ways of preventing the identified causes. This chapter also reviews world production, varieties, climatic and storage conditions of maize. Deterioration of maize is mainly affected by moisture content, temperature (grain and air), relative humidity, storage conditions, fungal growth, and insect pests. Fungal growth, especially *Aspergillus flavus* and *Fusarium sp.* in maize, facilitated by hot and humid conditions, poses a major risk through production of mycotoxins. In order to maintain high quality maize for both short- and long-term storage, maize must be protected from weather, growth of microorganisms, and pests.

Keywords. Maize, corn, relative humidity, temperature, fungal growth, storage.

3.1. Introduction

Maize (*Zea mays* L) commonly known as corn in the United States and Canada, is the third most important cereal grain worldwide after wheat and rice (Golob et al., 2004). It is referred to as the cereal of the future for its nutritional value and utilization of its products and by-products (Lee, 1999). The demand for maize has been estimated to increase by 50%, from 558 million metric tons in 1995 to over 837 million metric tons in 2020 (Martinez et al., 2011), fueled by diverse uses, from food processing, animal feed, to ethanol production (FAO, 2006). It is a basic staple food grain for large parts of the world, including Africa, Latin America, and Asia (Yaouba et al., 2012). In tropical and subtropical countries, a large proportion of the grain (such as maize) is harvested and stored under hot and humid conditions, and most farmers lack proper knowledge, equipment and methods of drying grain (Weinberg et al., 2008). Subsequently, the maize is stored while still relatively moist and warm; both warmth and high moisture contents can result in rapid deterioration of the grains and promote the growth of microorganisms (e.g. fungi and bacteria) and insects in the grains (Ekechukwua and Norton, 1999). Maize, like other stored products, is hygroscopic in nature and tends to absorb or release moisture. Even if properly dried after harvest, exposure to moist and humid conditions during storage will cause the kernel to absorb water from the surroundings (Devereau et al., 2002), leading to increased maize moisture contents, which results in enhanced deterioration.

To maintain high quality maize during storage, maize should be protected from weather (including relative humidity and temperature), growth of microorganisms, and insects (Oyekale et al., 2012). According to Campbell et al. (2004), the current estimates of the cost

of grain loss due to insect and microorganism damage of grain stored in developing countries each year ranged from \$500 million to \$1 billion. Reported by Tuite and Foster (1979) that insects in grain enhance mold development because they increase moisture content and temperature, and open areas of the grain for attack. Fungal growth in maize is facilitated by hot and humid conditions (Egal et al. 2005). It has been reported by several researchers that fungal infestation in maize results in color change, decreases in nutritional values and reduction of overall quality and quantity of the maize. Major fungi associated with grain storage, including maize are *Aspergillus flavus* and *Fusarium sp* and others. Fungal growth in maize presents a major risk for humans and animals, through the production of mycotoxins (especially Aflatoxins). According to Manoch et al. (1988), aflatoxin production by the fungi in the grain depends on the storage conditions, including relative humidity, temperature, and storage period. The objective of this chapter was to review the published literature and discuss the main factors that lead to deterioration of maize in tropical countries and to suggest ways of preventing the identified causes.

3.2. Maize World Production

Maize is among a few crops grown on almost every continent. According to FAO (2006), global maize production has increased by nearly 50 percent over the last ten years (Figure 34). The total global production for 2011/12 decline due to severe drought in some part of the US, which is the biggest producer of maize (Hoff and O'Kray, 2012). The total world production for 2011/12 was 0.8 billion metric tons; the US contributed 36.19% of the overall world's total. Other major producers of maize are China (22.1%), EU-27 (7.44%), Brazil (7.15%), Argentina (2.54%), India and Mexico (2.48% and 2.36%, respectively), Ukraine (2.59%), South Africa (1.38%), and other (15.77%). (USDA, 2012 and USGC, 2012).

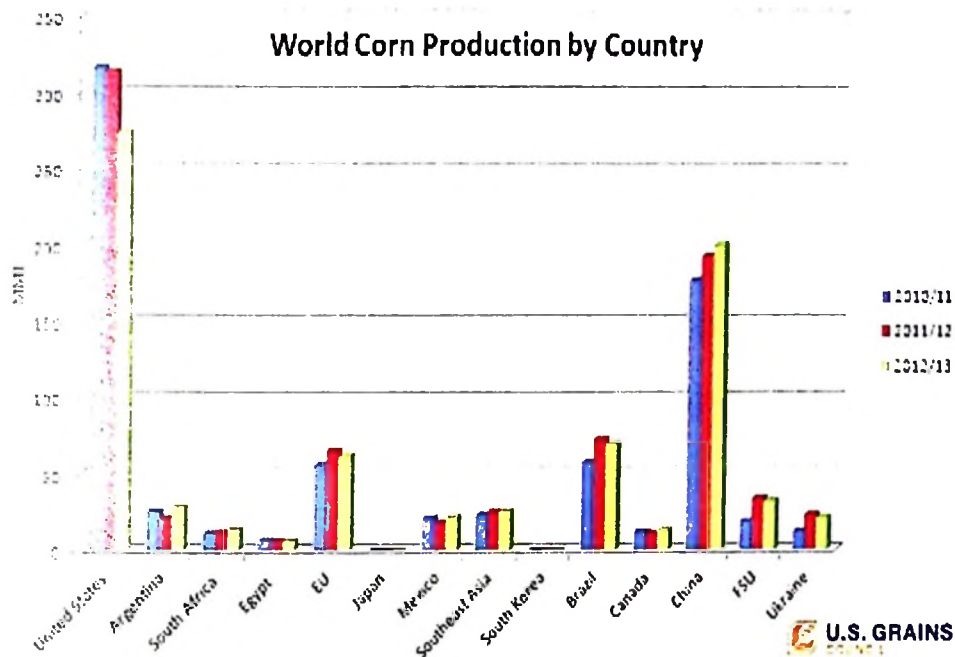


Figure 34. World Corn Production by Country (USGC, 2012).

3.2.1. Origin of Maize Grain

Maize is one of the oldest human-cultivated crops. The center of origin is believed to be the Mesoamerica region, at least 7000 years ago when it was grown as a wild grass called *teosinte* in the Mexican highlands (FAO, 2006). Maize spread around the globe after the European discovery of the Americas in the 15th century (OGTR, 2008). Maize has tremendous variability in kernel color, texture, composition and appearance. Botanically, maize belongs to the grass family *gramineae* (*Poaceae*); it is an annual plant with an extensive fibrous root system. It is a diploid species, with a chromosome number of $2n = 2x = 20$ (Cai, 2006).

3.2.2. Maize Varieties

The kernel, or seed, of a maize plant consists of three main parts (Figure 35): the pericarp, endosperm and embryo (Belfield and Brown, 2008). Maize grain is subdivided into distinct types based on endosperm and kernel composition, kernel color, the environment in which it is grown, maturity, and its use (Paliwal et al. 2000). There are six major varieties that are commercially grown specifically for human consumption, including *Zea mays* var. dent (*indurate Sturt*), flint (*indurate Sturt*), popcorn (*everta Sturt*), waxy, and sweet (*saccharata Sturt*) (Nuss and Tanumihardjo, 2010).

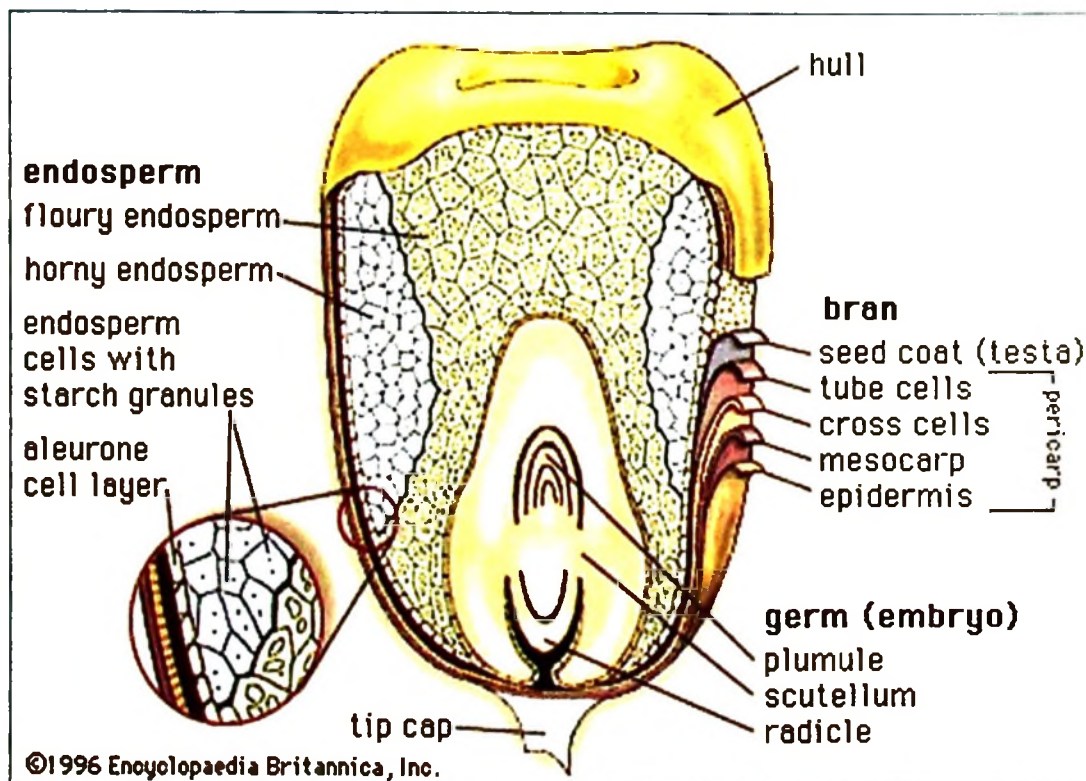


Figure 35. Layers and Internal Structure of the Maize Kernel (Merriam-Webster Inc. 2006).

3.2.2.1. Dent Corn (*Indurate Sturt*)

Dent corn (*indurate Sturt*) also referred as "field" corn, is the most common type of corn grown for grain, silage, and biofuel in the United States and around the World. The main features that distinguish this from other types of corn are the presence of corneous, horn endosperm at the sides and back of the kernels; generally, the central part is soft and floury (Johnson, 1991). During drying, the soft endosperm collapses to form an indentation; this central core or crown is unique in the dent types and originated the name "dent" corn. Dent corn is generally higher yielding compared to other types (PE/AI, 2012). Due to the soft endosperm dents, this type of corn is more susceptible to grain insects and molds, both in the field and in storage (Paliwal et al., 2000). Two common types of dent corn have been identified as yellow and white (Figure 36); normally white is more preferred in the food processing industry.

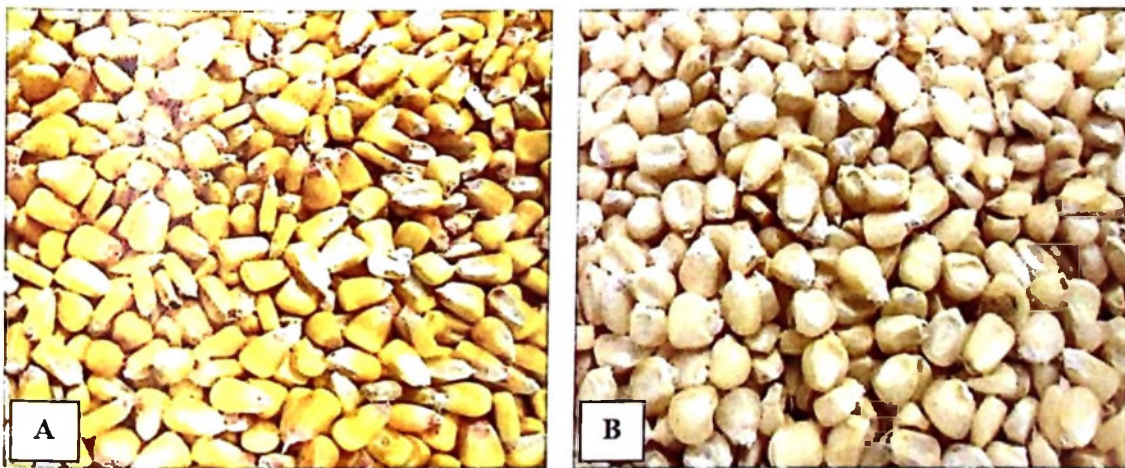


Figure 36. Yellow Dent (A) and White (B) Dent Corn.

3.2.2.2. Flint Corn (*Indurate Sturt*)

Flint corn is a type of corn with short, rounded or flat kernels, surrounded by a hard outer layer (hull), starchy and soft endosperm in the middle. Other features that distinguish flint from other corns are long, slender ears with few rows, relatively high protein and lipid contents, and the ability to produce high-quality flour (Gangaiah, 2008; Ruiz de Galarreta and Álvarez, 2010; <http://www.ogtr.gov.au>). The hardness of the flint corn outer layer makes it less prone to damage by grain mold and insects, both in the field and in storage (Paliwal et al., 2000). It is a multicolored grain, ranging from pale orange to dark red (Figure 37). Flint corn is extensively grown in Central and Southern America, Asia, and Southern Europe for human consumption and industrial purposes (OGRT, 2008). It is not grown extensively in the US.

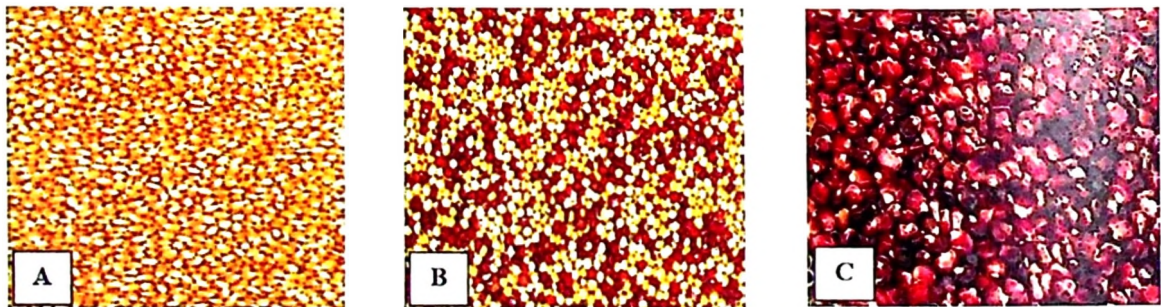


Figure 37. Orange (A), Indian (B), and Red (C) flint corn.

3.2.2.3. Popcorn (*Everta Sturt*)

Popcorn is a popular type of corn (Figure 38); it is characterized by a very hard outer layer, corneous endosperm and a small portion of soft starch (reviewed in Brown and Dallah, 1995). The shape of popcorn is either pointed (rice-like) or round (pearl-like) (Johnson, 1991). Compared to other types of corn (such as dent), popcorn is a minor crop. It is used to make popped corn, or as the basis of popcorn snacks (Brown and Dallah, 1995).

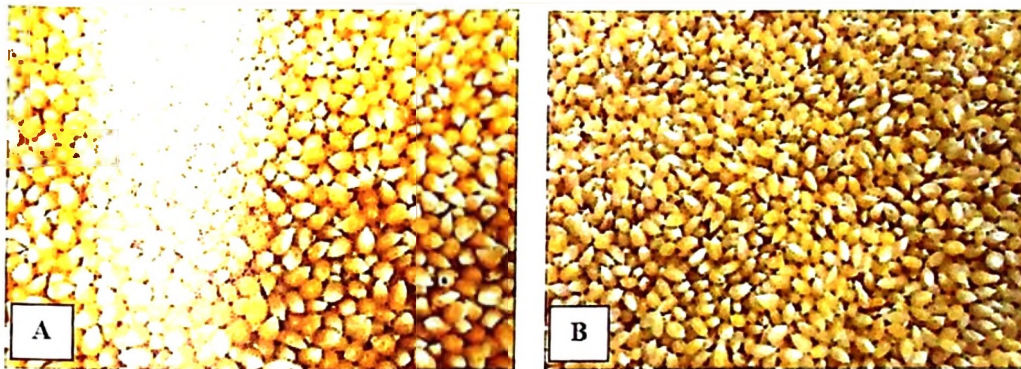


Figure 38. Yellow (A), and White (B) Popcorn.

3.2.2.4. Waxy Corn (Waxy Maize)

Waxy (corn) maize looks like flint corn in appearance, except it has a thick, transparent waxy endosperm (Kereliuk and Sosulski, 1996). Research has shown that waxy corn starch resembles potato starch in properties (Boutard, 2012). It contains approximately 99% amylopectin, very small quantities of amylose, high transmittance and low retrogradation properties (Zhou et al., 2013). Nutritionists have found that waxy corn may be a suitable source of carbohydrate for maintaining glucose control in insulin sensitive individuals (Sands et al., 2009). Waxy corn is extensively used in food processing as thickening and emulsifying agent, as well as remoistening adhesives on the paper, gummed tape, and the textile industry (Sandhu et al., 2007).

3.2.2.5. Sweet Corn (*Saccharata Sturt*)

The production and consumption of sweet corn have increased dramatically in the past decade in the US, Brazil, Canada, and Europe, for both fresh vegetables and for food processing (Williams, 2012). Sweet corn (Figure 39) originated from a mutation in the Peruvian race Chullpi. The entire endosperm in sweet corn is translucent, and the starch has been partially converted to sugar (Boutard, 2012; Najeb et al., 2011). They are white or yellow in color, but yellow sweet corn is more preferred by the consumer because of the high amount of vitamin A and C (Gangaiah, 2008). According to Coskun et al. (2006), sweet corn contains approximately 221g of carbohydrates, 3.35g of protein and about 10g of oil. It is an attractive crop for many farmers because these plants can grow very quickly and harvest can be mechanized (Johnson, 1991).



Figure 39. White Sweet Corn.

3.3. Chemical Composition and Nutritional Value of Maize

The importance of cereal grains in human nutrition is widely recognized, as they provide substantial amounts of energy and protein to a millions people, especially in developing countries (FAO, 2011). According to Nuss and Tanumihardjo (2010), cereal provides an estimated 10% and 15% of the world's calories and protein, respectively. Maize is a multipurpose grain. It can be used directly as a human food, but provides even greater nutritional values when used as an ingredient in the food processing industry and the animal feeding industry (Ullah et al., 2010).

Typical proximate compositions of the main parts of the maize kernel (yellow dent corn) are shown in Table 10. Chemically, dried maize kernel contains about 10.4% moisture, 6.8% to 12% protein, 4% lipid, 1.2% ash, 2.0% fiber and 72% to 74% carbohydrate (Katz, 1974; Kulp and Joseph, 2000). It also contains macro and micronutrients such as calcium, phosphorus, iron, sodium, potassium, zinc, copper, magnesium, and manganese, with 7mg/100g, 210mg/100g, 2.7mg/100g, 35mg/100g, 287mg/100g, 2.2mg/100g, 0.3mg/100g, 127mg/100g, and 0.45 mg/100g each, respectively on dry matter basis (db) (Nuss and Tanumihardjo, 2010). Maize also contains important vitamins such as thiamine 0.38 mg/100g, riboflavin 0.20 mg/100g and niacin 3.63 mg/100g, pantothenic acid 0.42 mg/100 g and folate 19 µg/100 g (Nuss and Tanumihardjo, 2010). However, the values vary greatly due to variety, hybrid, growing seasons, and soil conditions.

Table 10. Proximate chemical composition of main parts of maize kernels (% db) (Nuss and Tanumihardjo, 2010).

Chemical component	Pericarp	Endosperm	Germ
Protein	3.7	8.0	18.4
Fat	1.0	0.8	33.2
Crude fiber	86.7	2.7	8.8
Ash	0.8	0.3	10.5
Starch	7.3	87.6	8.3
Sugar	0.34	0.62	10.8

3.4. Factors Affecting Storage

Temperature and moisture content of the cereal grains are the two key features affecting the resulting quality of the grain, biochemical reactions, dry matter losses, allowable storage times and overall storage management of the grain (Gonzales et al., 2009; Lawrence and Maier, 2010).

3.4.1. Moisture Content

Biological and biochemical activities occur only when moisture is present. Hence, for safe storage of grain, both the moisture content of the grain and that of the surrounding air should be reduced and monitored (Jayas and White, 2003). Maize grains, like other stored products, are hygroscopic materials (i.e. they absorb and release water). They consist of a constant amount of dry matter, but water content will vary (Devereau et al., 2002). Moisture content plays a significant role in the storage of grain; when the grain has more moisture, it heats up and can have mold spoilage (Brewbaker, 2003). Living organisms, such as molds and insects, and thermal heat produced by the respiration of the grain itself will enhance water vapor, which in turn will lead to further deterioration of the grain (Freer et al., 1990;

Wimberley, 1983). As a general expression, the higher the moisture content, the more susceptible the maize grain is to mold and insect deterioration. Grain moisture content can be expressed as a percentage of moisture, based on wet weight (wet basis, eq. 4) or dry matter (dry basis, eq. 5). Wet basis moisture content is generally used (ACDI/VOCA, 2003).

$$\text{M. C. (wet basis)} = \frac{\text{weight of water in sample}}{\text{weight of water} + \text{dry matter}} \times 100\% \quad 4$$

$$\text{M. C. (dry basis)} = \frac{\text{weight of water in sample}}{\text{dry sample}(\text{weight of dry matter})} \times 100\% \quad 5$$

3.4.2. Relative Humidity

Relative humidity can be described as the amount of water vapor that is contained in the air as a proportion of the amount of water vapor required to saturate the air at the same temperature (Lawrence, 2005). It can also be expressed as the ratio of the actual water vapor pressure (e) to the equilibrium vapor pressure over a plane of water (e_s) (often called the “saturation” vapor pressure).

$$\text{RH} = \left[\frac{p_w}{p_{ws}} \right] \times 100\% \quad 6$$

Where p_w = partial pressure of the water vapor, p_{ws} = partial pressure of pure water at saturation, or

$$\text{RH} = \frac{e}{e_s} \times 100\% \quad 7$$

Where e = vapor equilibrium, and e_s = saturation vapour pressure

3.4.3. Interactions Between Temperature and Relative Humidity

Several studies have been conducted to examine the relationship between temperature and relative humidity in grain storage in the tropics, and results have revealed a direct relationship between them (Figure 40), that is, as temperature increases, grain will lose moisture to the surrounding air, thereby increasing the relative humidity (Devereau et al., 2002). Has been observed that in most cereal grains, every 10°C rise in temperature causes an increase of about 3% in relative humidity (ACDI/VOCA, 2003). Changing temperature and relative humidity not only promotes mold growth but also causes considerable nutrient losses of grain (Shah et al., 2002). Reported by Rehman et al. (2002) that protein, soluble sugars will decrease to over 20% after six months of maize storage at 45°C and 12% R.H.

Moreover, according to Samuel et al. (2011), even after drying, maize grain harvested in tropical countries retained a certain amount of moisture, and when exposed to air, exchanges of moisture between the maize grains and surrounding occur until the equilibrium is reached (Samuel et al., 2011). Also, the fluctuation of temperature and relative humidity in tropical countries accelerates rapid multiplication of molds and insects, which facilitate further spoilage of grain (Yakubu, 2009). Likewise, according to White and Sinha (1980) the survival and multiplication of molds and insects in the grain greatly dependent on the temperature and moisture levels.

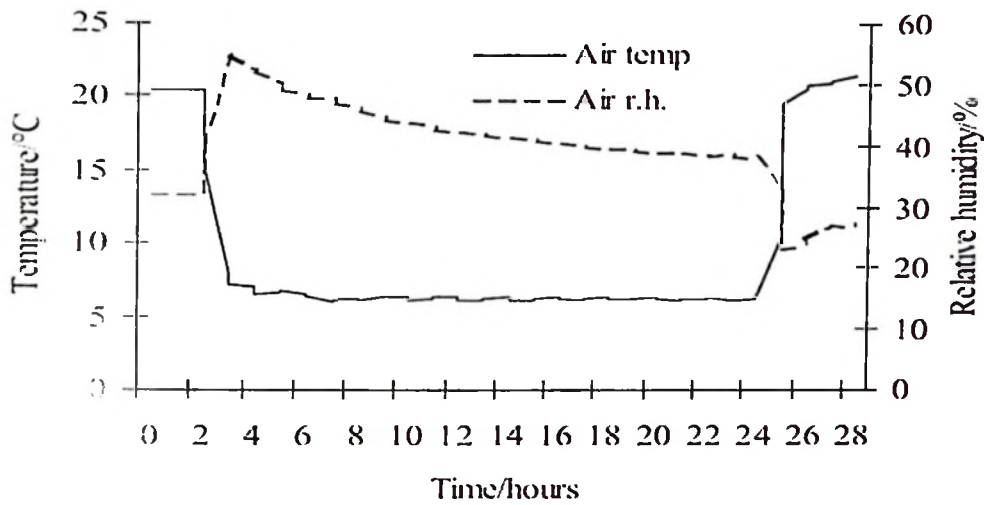
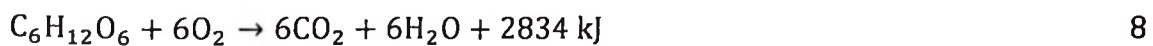


Figure 40. Relationships between Temperature and Relative Humidity (Devereau et al., 2002).

3.4.4. Maize Storage Losses and Deterioration

3.4.4.1. Respiration and Dry Matter Loss

The viable grain kernels, insects, molds, mites and other organisms in the stored grain are living things and they are respiring; during the respiration process (eq.8), oxygen is consumed and carbon dioxide, water, and heat are produced (Bern et al., 2013; ACDI/VOCA, 2003). As the moisture content of the grain increases, the respiration rate also increases (Hayma, 2003) Hence, for safe storage of grain, moisture contents of the grain and of the surrounding should be properly controlled (Hayma, 2003).



The carbon dioxide, moisture, and heat produced through respiration of the grain causes an increase in temperature and dry matter loss of grain (Lee, 1999). A two-month trial conducted by Reed et al. (2007), at three different levels of moisture content (low 15.0%,

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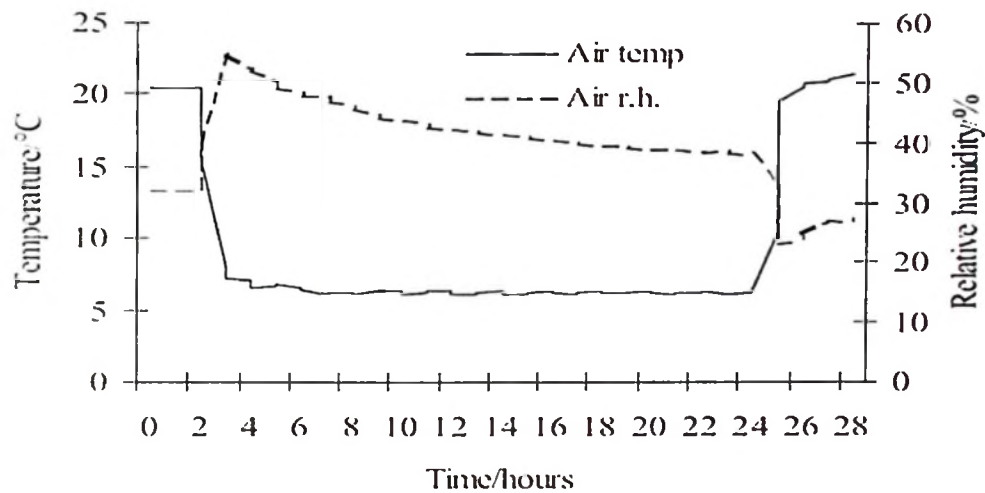
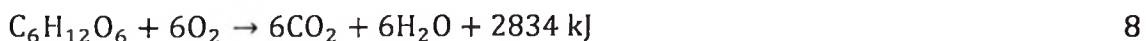


Figure 46. Relationships between Temperature and Relative Humidity (Devereau et al., 2002).

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The carbon dioxide, moisture, and heat produced through respiration of the grain causes an increase in temperature and dry matter loss of grain (Lee, 1999). A two-month trial conducted by Reed et al. (2007), at three different levels of moisture content (low 15.0%,

medium 16.6% and high 18.0%) showed gradual increases in moisture content of $15.1 \pm 0.01\%$, $16.6 \pm 0.04\%$, and $18.2 \pm 0.03\%$, for low, medium, and high moisture content maize, respectively. Results also showed a greater reduction of the mean oxygen concentration and gradually increases in carbon dioxide level; as expected maize with high moisture contents had a higher rate of oxygen consumption, as show in (Figure 41). the respiration rate increased steadily until the end of the experiment (Lee, 1999). The respiration activity of stored grain is also considerably influenced by the condition, or soundness, of the product. Carbon dioxide has been used by many researchers as one way of quantifying the deterioration of maize grain over time (Muir et al., 1985).

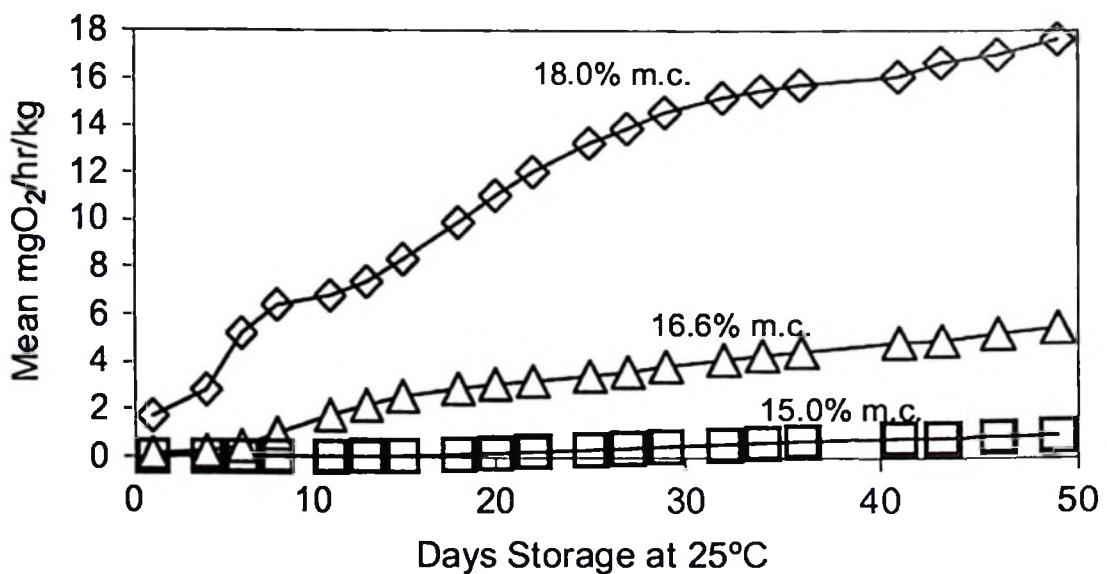


Figure 41. The Respiration Rate in the Storage (Reed et al., 2003).

3.4.4.2. Molds and Fungi

Mold and fungal species can develop on grains, in the field as well as in storage (Table 11); contamination of maize grain with mold and fungi is regarded as one of the most serious safety problems in the tropical countries and throughout the world (Kaaya and Kyamuhangire, 2006). Toxigenic fungi invading maize are divided into two distinct groups, field fungi and storage fungi (Barney et al., 1995). Field fungi invade maize and produce toxins before harvest or before the grains are threshed, and can develop under high relative humidity of over 80%, with moisture content of 22 to 33% and wide range of temperature ($10 \pm 35^{\circ}\text{C}$) (Williams and Macdonald 1983; Montross et al., 1999). These usually dies out in storage, but some can live under storage conditions (Sanchis et al., 1982), cause significant damage, reducing the yield and quality, especially in warm humid climates (Moturi, 2008). Conversely, storage fungi invade grain, primarily during storage and require moisture content in equilibrium with a relative humidity of 70 to 90%. Storage molds replace field molds that invade/ contaminate the maize before harvest (Reed et al., 2007).

Table 11. Conditions for growth of common storage molds on cereals and grain at 25 to 27°C (Montross et al., 1999).

	Relative humidity (%)	Moisture content (% wb)
<i>Asperigullus halophilieus</i>	68	12-14
<i>A. restrictus</i>	70	13-15
<i>A.glaucus</i>	73	13-15
<i>A.candidus, A. ochraeus</i>	80	14-16
<i>A.flavus, parssiticus</i>	82	15-18
<i>Penicillium spp</i>	80-90	15-18

Furthermore, there are several key fungal species associated with stored grains, including *Fusarium spp*, *Penicillium spp*, *Rhizopus spp*, *Aspergillus spp* and *Tilletia spp*. (Williams and MacDonald, 1983; Barney et al., 1995). Infection of maize grain by storage fungus results in discoloration, dry matter loss, chemical and nutritional changes and overall reduction of maize grain quality (Chuck-Hernández et al., 2012). It has been reported by Fandohan et al. (2003) that storage fungi contribute to the losses of more than 50% of maize grain in tropical countries, and ranks second after insects as the major cause of deterioration and loss of maize. According to Williams and McDonald (1983), when storage molds invade maize grain they cause rot, kernel discoloration, loss of viability, vivipary, mycotoxin contamination, and subsequent seedling blights. It was revealed by Sone (2001) that broken maize and foreign materials promote development of storage molds because fungi more easily penetrate broken kernels than intact kernels. Similarly, Dharmaputra et al. (1994) reported that mechanical damage during or after harvesting of maize grains can provide entry points to fungal spores. Likewise, Fandohan et al. (2006) reported that increases in grain damage and cracking create an opportunity for fungi to grow and penetrate the maize grain.

Moreover, moisture content and temperature are the two key environmental factors that influence the growth of molds and fungi (Alborch et al., 2011). Maize grain is generally harvested with a moisture content of around 18 to 20% and then dried. If inadequately dried the conditions are favorable for molds and fungi to grow, which can result in a significant decrease in grain quality and quantity (Marin et al., 1998). Barney et al. (1995) and Rees (2004), report that fungal growth in stored grain in the tropical countries is mainly associated with increases in grain moisture contents, and fluctuation in temperatures, resulting in unsafe storage of high-moisture grain and moisture migration and condensation. Furthermore, a

study conducted by Reed et al. (2007) on the effect of moisture contents and temperature of storage molds, found that the higher the initial moisture contents the greater the infection of maize kernels. According to Miller (1995), the growth and development of storage fungi in grain are governed by three main factors, crop (nutrients), physical (temperature, moisture) and biotic (insects) factors.

3.4.4.3. Mycotoxins

Molds growing on maize grains present a great threat, especially through the production of secondary metabolites (mycotoxins) (Weinberg et al., 2008). Mycotoxins are a chronic problem for maize grown in warm, humid, tropical, and subtropical regions of the world (Kaaya and Kyamuhangire, 2006). Molds and fungal infections can result in mycotoxin contamination in all stages from growing, harvesting, storage to processing (Chulze, 2010). The most important mycotoxins that frequently occur in cereal grains are aflatoxins, ochratoxins, Fumonisin, trichothecenes, and zearalenone (Pitt, 2000). The two most common and toxic mycotoxin compounds encountered on maize in tropical and subtropical regions are aflatoxins and Fumonisin (Krska, 2008). According to Miller (1995), aflatoxin is predominantly a problem in cereal grains, particularly in maize; it is produced by three main species of fungi, *Aspergillus flavus*, *A. parasiticus*, and *A. nomius*. These fungi tolerate and resist a wide range of conditions, and can be found everywhere such as in soil, in plant and animal remains, milk, and in grains and seeds such as peanuts and maize (Pitt, 2000). They generate four significant aflatoxins: B₁, B₂, G₁, and G₂ (Figure 42), and they can produce toxin during storage, transportation, and during processing. The hierarchy of toxicity is in the order of B₁>G₁>B₂>G₂. At present, aflatoxin B₁ is considered to be among the strongest

natural known carcinogens (Widstrom, 1996), and regarded as a quadruple threat, i.e., as a potent toxin, carcinogen, teratogen, and mutagen (Waliyar et al., 2003). World Health Organization (WHO) categorizes aflatoxins as class I carcinogens, as they are highly poisonous, toxic substances (Martinez et al., 2011).

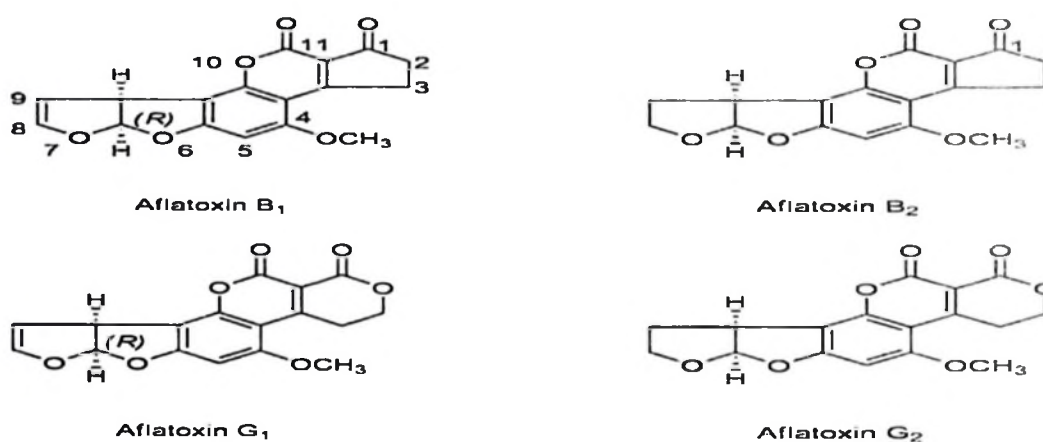


Figure 42. Structures of Aflatoxins B₁, B₂, G₁, and G₂ (Fujimoto, 2011).

Aflatoxin contamination has been associated with stunting in children, immune suppression, micronutrient deficiencies, and higher prevalence of cancers in sub-Saharan Africa, East Asia, and China (Smith et al., 2012; Hell, 2010; Moturi, 2008). Wu and Guclu (2012) found a strong relationship between aflatoxin exposure and liver cirrhosis. Due to the carcinogenic properties of aflatoxins, many countries around the world have set regulatory limits on allowable aflatoxin levels in foods and feeds (Liu et al., 2006) (Table 12).

Table 12. Maximum Amount of Aflatoxins Allowed in Foodstuffs in Different Countries ($\mu\text{g}/\text{kg}$) (Liu et al., 2006).

Australia/New Zealand	Brazil	Canada	China	EU	India	Japan	Malaysia	Mexico	South Africa	UK	USA
15	30	15	20	2	30	10	35	20	15	10	20

Several researchers report that aflatoxin contamination in grain increases with storage period and environmental conditions. Aflatoxins contamination is facilitated by long-term storage under unhygienic and unventilated conditions (Egal et al., 2005). Research conducted Liu et al. (2006) in China showed a significant increase in aflatoxins in storage length (i.e. $0.84 \mu\text{g}/\text{kg}$ in twelve months to $1.17 \mu\text{g}/\text{kg}$ in twenty-four months). Aflatoxin contamination and *A. flavus* infection are often associated with high temperature and drought conditions. Kaaya and Kyamuhangire (2006), found higher levels of aflatoxins in the moist regions of Uganda than in dry regions (Table 13).

Table 13. Aflatoxin Contaminations of Maize Kernels Stored for two to Six Months in three Agro-Ecological Zones of Uganda (Kaaya and Kyamuhangire, 2006).

Agro-ecological zone	No of samples	% positive	Aflatoxin content (ppb)	
			Range	Mean
Mid-Altitude (moist)	80	87.5	0-32	20.54
Mid-Altitude (dry)	80	77.5	0-22	18.02
High land	80	68.8	0-15	12.35
LSD ($P \leq 0.05$)				5.022
CV (%)				22.4

In addition, many researchers consistently found the high temperature to be a major factor influencing aflatoxin contamination and fungal growth (Widstrom, 1996; Kaaya and Kyamuhangire, 2006; Tubajika and Damann, 2001). Alborch et al. (2011) revealed that temperature and water activity (a_w) influence not the only rate of fungal spoilage, but also the production of mycotoxins. Mycotoxins produced by *Fusarium moniliforme* and closely related species, growing of maize and other grains are serious problems throughout the world (Pitt, 2000). There are widespread in tropical and subtropical regions (Afolabi et al., 2006), cause symptomless infections throughout the plant and in maize grain, and its occurrence is mostly ignored because it does not cause visible damage to the plant (Fandohan et al., 2003). The U.N Food and Agriculture organization (FAO) estimated that about 25% of the world food crops are lost due to mycotoxin contamination with *Fusarium* (Fareid, 2011). *Fusarium* is considered field fungi as it invades over 50% of maize grains before harvest (Fandohan et al., 2003). It is regarded as most prevalent fungi associated with maize, and can cause asymptomatic infection (Scott, 1993).

There are many reports which suggest that *Fusarium* toxins (Fumonisin) could affect livestock and humans (Miller et al., 1983). It has been statistically associated with an increased risk of esophageal cancer in humans who consumed contaminated maize in the Transkei part of South Africa, North East Italy, Iran and Central China (Doko et al., 1996; Kimanya et al., 2009); it is also associated with a possible cause of neural tube defects in newborns along the Texas-Mexico border (Stack, 1998). It is also reported by Pitt (2000) that Fumonisin are a major cause of leukoencephalomalacia, a fatal brain disease of horses, donkeys, mules, and rabbits, and pulmonary edema in swine. However, research conducted by Kimanya et al. (2010) in rural Tanzania, showed that the exposure of Fumonisin to

infants negatively affected growth. There are six common types of Fumonisin; A₁, A₂, B₁, B₂, B₃, and B₄ (Figure 43). According to Cawood et al. (1991), fumonisin B₁, B₂, and B₃ are the most important ones found in naturally contaminated maize and in maize fungal cultures, and produce the highest amounts of toxins (up to 17900 µg/g) (Fandohan et al., 2003).

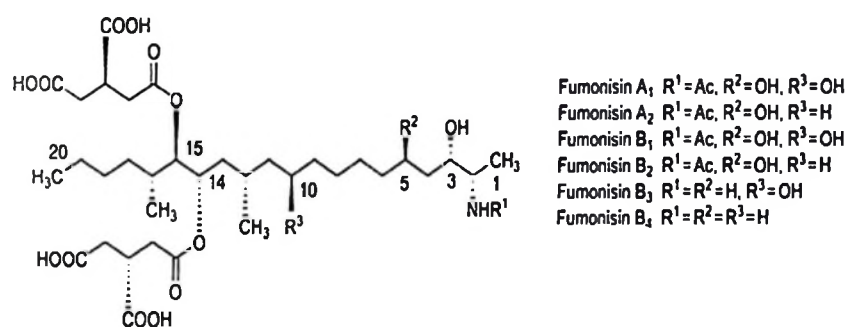


Figure 43. Chemical Structures of Fumonisins (Fujimoto, 2011).

Toxins from *Fusarium moniliforme* is categorized as Class 2B, possibly carcinogenic to humans (Munkvold and Desjardins, 1997). Even if the effects are not well established/understood in humans, many countries, including the USA have set the maximum level of fumonisin in maize and maize-based foods (Fandohan et al., 2003). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) set maximum tolerable daily intake (PMTDI) of 2µg/g for B₁, B₂, and B₃, while The US Food and Drug Administration (FDA) set 4 µg/g for all types of Fumonisins (WHO 2002; Marasas, 2001). Moreover, Afolabi et al. (2006) report fusarium contamination and growth are favored by warm and dry conditions. Typical symptoms of maize kernels infected by fusarium are white or pinkish-white color of maize kernels (Figure 44). The optimum conditions required for fumonisin production are still unknown (Robertson-Hoyt et al., 2007), but the occurrence of *F. moniliforme* is related to drought stress and climatic conditions (Scott, 1993).



Figure 44. Fusarium Infections on Maize Kernels.

Furthermore, a study conducted by Fareid (2011), revealed that temperature and water contents are key factors for the growth and mycotoxinogenesis of *Fusarium species*, the results show linear relationships between temperature and levels of fumonisin B₁ production; maximum production was observed at 25°C. Similar research conducted by Marín et al. (1998) showed growth rates of *Fusarium species* and other fungal species are critically dependent on water activity and temperature; research found a higher growth rate of *Fusarium species* at 0.995 water activity. Likewise, Marín et al. (1998), found the best temperature for the production of fumonisin B₁ in maize is 30 °C and 0.98 a_w. On the other hand, Fumonisin are only concentrated in the pericarp and the germ of the maize grain, so removing those outer parts can significantly reduce the level of toxin in the maize (Charmley and Prelusky, 1994). Similarly, research conducted by Fandohan et al. (2006), showed significant decreases in Fumonisin after dehulling (removing hulls), as shown in (Figure 45).

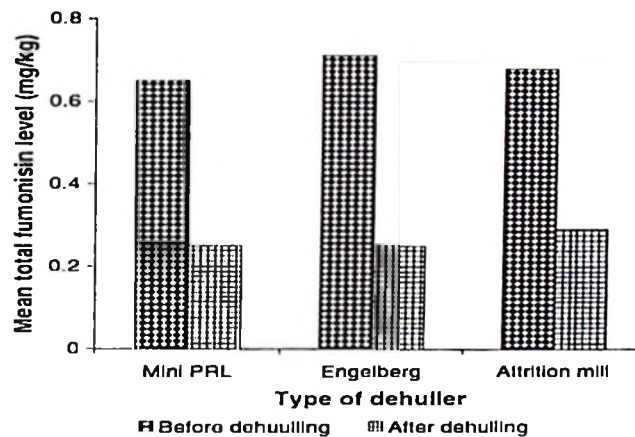


Figure 45. Mean Fumonisin Level in Maize Before and after Dehulling using Different Dehulling Methods (Fandohan et al., 2006).

There is a close relationship between storage fungi and insect infestation, Jian and Jayas (2012) report that some storage fungi attract insects and promote their growth, but other prevent through secretion of toxic metabolites. Similarly, Burns (2003) found a direct association between insect feeding activity, fungal growth and mycotoxin production. Likewise, Setamou et al. (1997), detected low levels of mycotoxin for less damaged maize (2%) than in higher damaged maize.

3.4.4.4. Insects and Pests

It has been observed globally that the greatest losses of stored grains are due to insect infestation. Grain storage provides the ideal environment for several insects to flourish, consume grain nutrients, and contaminate it with insect fragments and feces (Boxall, 1991; Paliwal, 2000). According to White and Sinh, (1980), grain storage systems are ecologically unstable, containing varieties of species with high reproductive potential that can damage the grain over a short period of time. It is estimated that 1 to 5% of stored grain in developed countries and 20 to 50% of stored grain in developing countries are lost due to insect damage

(Ileleji et al., 2007; Nukenine, 2010). More than 500 insect species are reported to be associated with grain, among which 250 are directly linked to maize grain both in the field and in storage conditions (Jian and Jayas, 2012; Mathur, 1987). The stored-grain insects are classified into two main groups; internal and external feeders. The internal feeds are those insects developed inside the kernels, while, external are those whose eggs hatch and live on the surface of grain kernels (Montross et al., 1999). Among the key insects in maize storage is the maize weevil *Sitophilus zeamais*. *S. zeamais* is classified as a primary or major pest of maize grain in tropical regions due to its ability to destroy a whole grain kernel (Kanyamasoro et al., 2012). Table 14 show other major grain insect pests. When maize grains are stored they are exposed to a broad range of complex ecological factors; the most important factors that affect grain quality and pest developments are temperature and moisture (Maier et al., 1996).

Moreover, according to Maier and others the grain storage conditions such as temperature and moisture content, and environmental conditions, such as temperature and relative humidity, play an important part in how fast insects and pests develop and threaten the quality and quantity of stored grain. Revealed by Montross et al. (1999), that propagation and development of insects depend on several factors such as moisture content and temperature of the grain, the level of damage and foreign material of the grain, and atmosphere around the grain. Likewise, Hayma (2003), found that favorable conditions for most grain storage insects to develop are between 25 to 30°C and relative humidity between 70 and 80%. Conversely, research conducted by Yakubu et al. (2011), shows that insect infestation problems can be controlled under hermetic storage at moisture and temperature of 6 and 16% and 10 and 27 °C, respectively.

3.5. Maize Storage

Stored grains are considered an ecological system. Jian and Jayas (2012) described it as an approach by which grain integrated with other factors such as relative humidity and temperature to promote protection of grain and environments to deliver good quality grain at the end of storage time. The practice of grain storage has direct effects on quality of stored grain. According to Nukenine (2010), “storage is a way or process by which agricultural products or produce is kept for future use”. In maize storage ecosystems, the most important factors that influence molds and insect infestation are water activity, temperature and air (Montross et al., 1999). In addition, grain temperature and moisture content affect grain quality in storage and promotes growth and development of molds, insects, mites and dry matter losses (Maier et al., 1996). Maize and grain storage systems are classified into three main types; crib, bags, and bulk storage (Yakubu, 2009; Montross et al., 1999).

The allowable storage time for maize is the time until 0.5% of dry matter decomposition is reached (Hellevang, 2005). The dry matter loss of corn is directly related to the carbon dioxide (CO₂) production (eq.8). Bern et al. (2002), found about 7.33g of CO₂ per kg of dry matter was required to lose 0.5% of the dry matter. According to Steele (1967) and Thompson (1972) cited by Bern et al. (2002), the amount of CO₂ can be easily predicted under certain conditions ($T = 15.6^{\circ}\text{C}$, $M = 25\%$ and $D = 30\%$) using equation 9, where t_s is the time in hours, and for non-reference conditions can be computed by equation 10, where M_M , M_T and M_D are multipliers for moisture, temperature and mechanical damage respectively.

Table 14. Common Insect Species Found in Grain Storage and Optimal Growth Conditions (Montross et al., 1999).

Insects species	Relative Humidity (%)	Temperature (°C)
<i>Sitophilus zeamais</i> (maize weevil)	70	27-31
<i>Sitophilus oryzae</i> (rice weevil)	70	26-31
<i>Prostephanus truncatus</i> (larger grain borer)	80	25-32
<i>Rhyzopertha dominica</i>	50-60	32-34
<i>Sitotroga cerealella</i> (Angoimois grain moth)	75	26-30
<i>Plodia interpunctella</i> (Indian meal moth)	70	26-29
<i>Tribolium castaneum</i> (red flour beetle)	70-75	32-35
<i>Cryptolestes ferrugineus</i> (rusty flour beetle)	70-80	33
<i>Oryzaephilus surinamensis</i> (sawtoothed grain beetle)	31-34	90
<i>Trogoderma granarium</i> (khapra beetle)	33-37	25

$$Y = 1.3(e^{0.006t_s} - 1) + 0.015t_s \quad 9$$

$$t_n = t_s M_M M_T M_D \quad 10$$

Allowable storage time is cumulative term and functions of temperature and corn moisture contents; maize at 20% moisture content and 60°F has an allowable storage time of 29 days.

If after five days, the maize is dried to 18%, the allowable storage time at 18% and 60°F will

be $\frac{(29-5)}{29} \times 56 = 46$ days (Hellevang, 2005; Bern et al., 2013). Bern et al. (2002) formulated the allowable storage time for shelled corn for different temperature and moisture combinations (Table 15).

3.6. Conclusions

In conclusion, for the proper storage of maize grain, environmental factors such as temperature and moisture content must be controlled. Such factors are the major influences of maize deterioration because they affect molds, and insect pests, which can result in significant losses of maize grain in a very short time. To prevent mycotoxin contamination, maize should be monitored regularly to assure safe storage conditions, hence, maize contaminated by fungi and molds not only render grains unfit for human consumption by discoloration but can also lead to toxin production such as aflatoxins and Fumonisin.

3.7. Recommendations

Based on the findings, the following recommendations are made:

- ① Proper monitoring of temperature and relative humidity of maize grain and the surrounding atmosphere on storage, especially in the initial stage of storage to maintain the highest possible quality of stored grain; in general, the lower the temperature and moisture content the longer it can be stored without being infected with mold and insects;
- ① To avoid deterioration of maize in tropical and subtropical regions, maize should be dried to moisture contents below 14% immediately after harvest;
- ① Hygiene and sanitation from harvest to storage are key factors in eliminating sources of infection and reducing levels of contamination;

Table 15. Shelled Corn Storage Time (SCST) for 0.5% DM Loss in days* (Bern et al., 2002).

Temp		Corn Moisture (%)									
°C	°F	16	18	20	22	24	26	28	30	32	34
1.7	35	1144	437	216	128	86	63	50	41	35	31
4.4	40	763	291	144	85	57	42	33	27	24	21
7.2	45	509	194	96	57	38	28	22	18	16	14
10.0	50	339	130	64	38	26	19	15	12	10	9
12.8	55	226	86	43	25	17	13	10	8	7	6
15.6	60	151	58	29	17	11	8	7	5	5	4
18.3	65	113	43	22	13	9	7	5	4	4	3
21.1	70	85	32	16	10	7	5	4	4	3	3
23.9	75	63	24	12	8	5	4	3	3	2	2
26.7	80	47	18	9	6	4	3	3	2	2	2
29.4	85	35	14	7	5	3	3	2	2	2	1
32.2	90	26	10	5	4	3	2	2	2	1	1
35.0	95	20	8	4	3	2	2	2	1	1	1
37.8	100	15	6	3	2	2	2	2	1	1	1
40.6	105	11	4	3	2	2	2	1	1	1	1
43.3	110	8	3	2	2	2	1	1	1	1	1
46.1	115	6	2	2	2	1	1	1	1	1	1
48.9	120	5	2	1	1	1	1	1	1	1	1

*D = 30%, M_D = M_H = M_F = 1

- Sorting or separating foreign materials and broken corn kernels produced during harvesting from clean maize; those promoting development of grains pest and molds;
- ① Maize should be stored in a sealed, airtight container or structure, to reduce oxygen concentration, which will limit the presence of aerobic organisms.
- ① Clean, fumigate, or separate maize grain immediately after discovery of insects and molds.
- ① Remove or separate old grain from new grain, and maize should be placed on pallets above the floor to avoid cold conditions that may lead to mold contamination.

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Table 16. Summary of Articles Investigating Effects of Moisture Content (M.C.) and Relative Humidity (R.H.) *.

Table 16 (Continued)						
Storage Temperature	Storage or M.C.	R.H. storage	Other parameter(s)	Key results	Conclusions	References
	12.2-16.9% M.C. (wet basis)		(1) Drying temp= 95°C for 60 min, and (2) ambient temp for 25 min two samples were tested: (1) Fresh, wet (20 - 25% M.C.) grain from harvest, and (2) samples of dry (12.2-16.9% M.C.)	(1) Hybrid The results for hybrid showed the storage changes with time (both at harvest and in storage), some are more prone to deterioration at harvest and after long-term at low-moisture bin storage. (2) Drying method For the case of drying methods, after 3 and 7 months the results revealed that sample dried at (>80°C) drying methods has lower storage time similar to that of the on-farm. Systems, at low temperature storage. (3) Previous storage history (moisture and time)	The study showed storage of maize is directly related to moisture contents, and found that the higher the moisture contents the lower storage time and the lower the moisture contents the higher the storage time.	Marks and Stroshine (1995).
(1) Below 5°C for trial 1 and 2, storage and (2) for trial 3, two temperatures were used (a) 21.1°C for ambient aeration, and (b) 15.6°C chilled storage.			Three year trials at three temperatures to determine survival, reproduction and suppression of <i>Sitophilus zeamais</i> . (1) No aeration control (NA) and	Trial 1 The result showed that it is undesirable to store maize under control conditions and no activity was observed at the first month, but considerably has	The study showed for proper and safe storage of maize should store at temperature below 15°C, since at this temperature most of the <i>S. zeamais</i> will be suppressed	Ileleji et al. (2007).

Table 16 (Continued)

Storage Temperature	Storage or M.C.	R.H. storage	Other parameter(s)	Key results	Conclusions	References
			(2) ambient aeration (AA) ($\leq 23^{\circ}\text{C}$).	detected in the 3rd, 4th and 5th months of storage time. In other hand.		
			(3) Chilled aeration (CA) ($\leq 18^{\circ}\text{C}$).	significant (higher) changes were observed at AA than in CA.		
				Trial 2		
				Similar trends were observed in the NA for the first month, but the number of insects increased significantly in the 2nd, 3rd, 4th and 5th months, high progenies were detected in NA and AA than in CA in the 2nd, 3rd, and 4th months. For the 5th month's higher changes were observed in AA than in NA and CA.		
				Trial 3.		
				The results show that progeny of <i>S. Zeamais</i> differ significantly for the first three months from one treatment to another and no significant changes were observed in the 4th and 5th months. Higher numbers of progenies were observed for the NA and AA in the first three months and same similar results for other		

Table 16 (Continued)

Storage Temperature	Storage or M.C.	R.H. storage	Other parameter(s)	Key results	Conclusions	References
				treatments in the last two months.		
Maize stored at 10, 25, and 45°C for 6 months	grains	Moisture content of maize 12% (wet basis)		<p>(1) No change in pH and titratable activity for the maize stored at 10 °C for 6 months</p> <p>(2) High changes were observed for the sample store at 25 and 45°C. no change was observed for the maize stored at 10 °C for six months. Moisture contents decreased by 24% at 25°C and 37% at 45°C during six months' storage.</p> <p>(3) Results show decreased in total soluble sugars at 45°C and gradually increased at 10 and 25°C</p> <p>(4) Lysine contents decreased significantly during six months' period.</p> <p>(5) For thiamine contents the results show decreased from 9.26 and 20.4 % for maize grains stored at 25 and 45 °C respectively, after six months</p> <p>(6) For protein and starch digestibility, only change was observed at 45°C and remained</p>	Results show that storage of maize at higher temperature affects overall nutritional quality of maize including protein, starch digestibility, total insoluble sugar, as well as amino acids such as lysine and thiamine. The best (optimum) temperature to retain the quality of maize was between 10 to 20 °C.	Rehman et al. (2002).

Table 16 (Continued)

Storage Temperature	Storage or M.C.	R.H. storage	Other parameter(s)	Key results	Conclusions	References
				unchanged for other treatments.		
Corn storage temperature of 40 and 20°C	Moisture contents in the grain 18, 15 and 10% (wet basis)		All grain samples were stored up to 180 days.	<p>The study showed that albumin and globulin of stored corn at 40°C and 20°C with 18, 15 and 10% moisture content has higher change in enthalpy values than those stored at control condition.</p> <p>Study also revealed that at 40 °C, enthalpy changes were independent in the moisture contents, while at 20°C the moisture content had significant effects on enthalpy changes.</p>	<p>At all moisture contents and both temperatures, enthalpy decreased about 80% as compared to the control samples, and temperature had more often effect than moisture content on corn protein fractions.</p> <p>At 40°C shows negative correlation between corn protein fractions and enthalpy for all three moisture contents. For 20 °C sample and 10% moisture contents decreases enthalpy for the corn stored no longer than two months.</p>	Del-Angela et al. (2003).
Temperature of the stored maize 30 ± 1°C.	The moisture contents of the maize were 14, 16, 18, 20, and 22% (wet basis)		At the beginning of the hermetic storage period the M.C. of the maize in the 14, 16, 18, 20 and 22% moisture categories were 13.7 ± 0.1, 16.1 ± 0.0, 18.4 ± 0.1, 20.4 ± 0.1 and 22.8 ± 0.2% M.C. respectively.	<p>The results showed that under hermetic storage moisture contents increased up to 17 g/kg due respiration reactions. The pH remained constant (i.e., 6) for most moisture contents, except for 22% where it decreased from 5.8 to 5.5%</p> <p>No mold growth at any treatment at 14</p>	<p>Maize with intermediate and high moisture contents (16-22 %) can be stored without spoilage under hermetic sealed jars due anaerobic conditions created by sealed containers. On other hand, significant change in dry matter losses and high number of</p>	Weinberg et al. (2008).

Table 16 (Continued)

Storage Temperature	Storage or M.C.	R.H. storage	Other parameter(s)	Key results	Conclusions	References
				- 18% moisture contents and numbers of bacteria and mold decreased substantially during storage.	colonies of yeast and bacteria for sample stored at 20 - 20% moisture contents.	
	Moisture contents (wet basis) 18, 22 and 26 %		Temperature conditions 15°C, 20 and 25°C	<p>For all three moisture levels, corn reached 0.5 % dry matter loss faster when stored at 25°C prior to storage at 15°C compared to storage at 15°C prior to storage at 25°C.</p> <p>This can be explained by the fact that fungi grow disproportionately faster at 25°C than they do at 15°C</p> <p>When corn was stored at warmer temperatures before cooler temperatures, there was probably a greater initial build-up of fungal mycelia during the warm storage period and thus, greater respiration and dry matter loss during both the warm storage period and the cool storage period that followed</p> <p>The predicted allowable storage time values for corn exposed to temperatures that cycled between 15</p>	<p>The general shapes of dry matter loss vs. storage time curves for predicted values were similar to those for measured values for both step changes and cyclical changes in storage temperature.</p> <p>This indicates that current methods for predicting allowable storage time for changing temperature conditions are generally adequate.</p> <p>Corn stored at 25 °C before storage at 15 °C reached 0.5% dry matter loss much sooner than did corn stored at 15°C before storage at 25°C.</p>	Wicklow et al. (1998).

Table 16 (Continued)

Storage Temperature	Storage or M.C.	R.H. storage	Other parameter(s)	Key results	Conclusions	References
				°C and 25°C were fairly close to allowable storage time values for corn stored at a constant temperature of 20 °C (the average of 15 °C and 25°C).		

*All moisture contents in wet basis unless noted otherwise

Table 17. Summary of Articles Investigating Effects of Insects, Fungi, and Molds.

Table 17 (continued)

Storage Temperature	Storage R H or storage M.C.*	Other parameter(s)	Key results	Conclusions	References
Seven different temperature were 10, 15, 20, 25-30 35, or 40°C)	Grain moisture contents (9.4to 17.5 %) (wet basis)	The shelled maize used in this study were harvested from Furman, South Carolina in 1992. and were treated at seven different temperatures for 348 and 751 days of continuous storage.	Twenty different fungal species were recorded from these conditioned grain treatments. About 50-95% of the kernel were infested by <i>Eurotium chevalieri</i> No fungi growth at temperature 30-40 °C and moisture contents 9.4-14.2 %	The results of this study showed that most of the fungi grew in the storage maize were directly dependent on the presence of pre-harvest fungal colonists and their potential replacement by <i>E. chevalieri</i> .	Wicklow et al. (1998).
Temperature (5 – 45°C)	Water activity (a_w) (0.92 – 0.98)	Incubation time (5 – 60 days) for growth and <i>ochratoxin A</i> (OTA) production by <i>Aspergillus niger</i> and <i>Aspergillus carbonarius</i> on maize kernels	The growth of fungi was highly influenced by both water activity and temperature. Higher colonies of <i>A. niger</i> were observed at temperature range of 25 – 40°C.	In <i>A. niger</i> the temperature ranges for <i>ochratoxin A</i> production (15–40°C) was slightly narrower than that for growth (15 – 45 °C), but in <i>A.</i>	Alborch et al. (2011).

Table 17 (continued)

Storage Temperature	Storage R H or storage M.C.*	Other parameter(s)	Key results	Conclusions	References
			For the case of <i>A. carbonarius</i> optimum growth temperature for <i>A. carbonarius</i> were observed at 20–35 °C, and water activity at 0.92	<i>carbonarius</i> the range was the same (15–35°C).	
Temperature (15–25°C)	Water activity (0.85–0.995)		<p>The results show that at 25°C, <i>Fusarium</i> species were dominant at higher water activity than at low (<0.95 aw), and dominance index increase as water activity increased (0.85 to 0.99).</p> <p>For other species such as <i>A. niger</i> higher index of dominance were observed in between 0.90–0.995 aw, while <i>A. tamaritii</i> were more dominant at 0.95–0.85 aw, irrespective of temperature.</p>	In general, fungus species such as <i>Aspergillus</i> , <i>Fusarium</i> and <i>Trichoderma</i> grew fast under certain combinations of water activity and temperature, while other species like <i>Penicillium</i> grow very slowly irrespective of any water activity and temperature combination.	Marin et al. (1998).
Storage temperature (10, 20, 30 and 37°C)	Water activity (0.88, 0.92 and 0.96)		Maize at a storage temperature of 30 °C was particularly vulnerable to contamination, as the specific growth rate is high. Variation of growth rate between 10°C and 30°C was higher at a water activity of 0.92, with an increase of about 18 times.	<p>Results showed that specific growth rates and logarithmic population increased as the temperatures and water activities increased, except at lag phase, which showed a decreased trend.</p> <p>For temperatures between 10°C and 30°C, and the three water activity levels, the relationships</p>	Galati et al. (2011).

Table 17 (continued)

Storage Temperature	Storage R H or storage M.C.*	Other parameter(s)	Key results	Conclusions	References	
				between parameters maximum specific growth rate (μ), and lag phase duration (L.P.D) with temperature and water activity.		
				By knowing the initial count, and the grain temperature and water activity histories at one or more positions inside a bin, the model was able to predict viable mold count as a function of time.		
Storage temperature °C	25	Desired M.C. 15, 16.5, and 18.0% (wet basis).	Maize was stored for 2 months in chambers maintained at 25 °C	<p>The wettest grain heated rapidly and became semi-anaerobic. The hot grain then dried rapidly, with the amount of moisture loss influenced by the ratio of water vapor pressures inside and outside the grain.</p> <p>The hot grain cooled and became more aerobic over time. New infections by storage molds, disappearance of viable field molds, development of kernel damage, and changes in atmospheric gases within the grain masses were correlated with the</p>	<p>This simulation of stored maize carried into warm weather after winter storage demonstrated that grain containing 16–18% M.C. should be expected to heat, and that the heating may stop or the hot spot may change position as moisture is driven to cooler grain.</p> <p>Within the hot spot, the atmosphere may become quite anaerobic, and the mold population may change rapidly as field molds die and storage molds grow, principally</p>	Reed et al. (2007).

Table 17 (continued)

Storage Temperature	Storage R H or storage M.C.*	Other parameter(s)	Key results	Conclusions	References
			<p>grain moisture or temperature and the rate at which the moisture and temperature changed.</p> <p>The rate of increase in new kernel damage was as high as 3.3% per week.</p>	<p>in the maize embryo.</p> <p>The mold activities caused loss of weight, damaged kernels, and often reduce the energy content of the maize.</p>	
<p>Corn samples were stored under two different conditions (nominally 18 moisture and 2% moisture at 20°C) to five different levels of dry matter loss (0, 0.25, 0.50, 0.75, and 1.0%).</p>		Two different storage conditions	<p>Grain storage fungi grow more slowly at lower moisture contents.</p> <p>The total damaged kernels (DKT) analysis showed that corn have no mechanical damage, at 18% moisture and 1.0% loss of its initial dry matter, the sample was still U.S. Grade No. 1</p> <p>Corn with greater mechanical damage lost grade at lower levels of DML.</p> <p>In all cases, the corn samples stored at 22% moisture had higher DKT than corn at 18% moisture.</p> <p>Colony forming units were isolated from corn kernels after the storage tests. <i>Aspergillus glaucus</i> and several species of <i>Penicillium</i> were the main species detected.</p> <p><i>Fusarium</i> species</p>	<p>The level of DKT for a given level of dry matter loss (DML) increased with moisture and mechanical damage.</p> <p>As mechanical damage increased to 30%, the permissible DML fell to about 0.5 for 18% moisture corn and to about 0.2 for 22% moisture corn.</p>	Gupta et al. (1999).

Table 17 (continued)

Storage Temperature	Storage R H or storage M.C.*	Other parameter(s)	Key results	Conclusions	References
			<p>were also detected, but in very small numbers compared to <i>Penicillium</i>. Other species detected in smaller amounts during plating were <i>A. candidus</i> and species of <i>Mucor</i>, <i>Cladosporium</i>, <i>Alternaria</i>, <i>Phoma</i>, <i>Cephalosporium</i>, and <i>Nigrospora</i>.</p>		
<p>Minimum temperatures were 14 to 17°C, and maximum temperatures were slightly cooler in NB (21°C in August to September, and 24 to 26°C in October to January) than in D (25 to 29°C).</p>		<p>Triplicate samples of kernels were shucked from freshly harvested maize in August 2010, after two weeks, two months and five months of storage.</p>	<p>Bacterial counts on maize decreased generally between harvest and two months of storage, probably because the decreasing moisture contents in the maize were unfavorable for survival of these organisms.</p> <p>Yeast counts on both farms decreased from harvest time until two months later, and then increased again in the five month samples.</p> <p><i>Fusarium</i> sp. commonly infect maize in the field, where <i>F. verticillioides</i> can produce <i>fumonisin</i> and <i>F. meridionale</i>. These species were also present on farm NB, but infection with <i>Fusarium</i> was less severe. On both farms, <i>Fusarium</i></p>	<p>Mold infections of the maize samples at harvest and during storage varied between the two farms, and probably were affected by cultivar and individual storage conditions.</p> <p>Potentially toxigenic <i>Fusarium</i> spp., typically infecting maize kernels in the field, was present at harvest and during the early stages of storage.</p> <p>Among the yeasts, <i>M. guilliermondi</i> was dominant during late storage; however, the biocontrol yeast (<i>W. anomalus</i>) was not naturally present.</p>	<p>Leong et al. (2012).</p>

Table 17 (continued)

Storage Temperature	Storage R H or storage M.C.*	Other parameter(s)	Key results	Conclusions	References
			infection decreased during storage, whereas more xero-tolerant (dry-tolerant) genera such as <i>Penicillium</i> were common after two months.		
Mid-Altitude (dry) zone- Temperatures 25 °C		Three treatments (regions) were used to determine aflatoxin contamination in maize kernels in Uganda.	The result showed that average moisture contents of maize for treatment were with in normal range of $\leq 15\%$	Aflatoxin contamination in Uganda is highly influenced by environmental conditions and storage time.	Kaaya and Kyamuhangire (2006).
Mid-Altitude (moist) zone temperatures 18 °C			Higher aflatoxin contamination was found in the Mid-Altitude regions and lowest at High- Altitude zone, main reasons were high temperature and high relative humidity in Mid-Altitude regions than in High-Altitude zone.		
Highland altitude Temperatures 28 °C					
Samples were sealed in plastic bags and stored at $22 \pm 1^\circ\text{C}$ for 9-11 months.		Temperature, moisture contents, and CO ₂ were monitored continuously through the study.	After 9-11 months of storage of wheat, rapeseed, barley and corn, results indicate that out of 39 grain bins used, 34 showed 87% increases in levels of CO ₂ (i.e. above 0.03%); in these 34 bins 30 were contaminated with storage pests.	The concentration of CO ₂ is a good indication of storage pests, and the higher the CO ₂ level the higher the storage pests and higher rate of deterioration and lower germination rate of cereal and grain.	Muir et al. (1985).
			Higher levels of CO ₂ were detected at the center of the bins. In the other bins with lower deterioration		

Table 17 (continued)

Storage Temperature	Storage R H or storage M.C.*	Other parameter(s)	Key results	Conclusions	References
			indicate lowered germination.		
The temperature setting was (15, 22, 29, or 36°C)	Initial moisture content 9.7 or 12.3% (Wet basis)	Pioneer hybrids 3378 and 3320 were used in the study.	In this study, 17 fungi species were detected, but only 4 were more abundant (i.e. <i>Fusarium sp.</i> , <i>Penicillium sp.</i> , <i>Rhizopus sp.</i> and <i>A. glaucus</i>), and other fungi found was <i>A. flavus</i> . The study revealed no significant differences in levels of fungi (especially, <i>Fusarium</i> or <i>Penicillium</i> spp.) between hybrids. <i>A. glaucus</i> were higher in 3320 than in 3378.	Changing temperature and moisture contents of the corn has major effects on fungi; at 36°C and 9.7% inhibit the growth of fungi in corn. Also hybrid selection is very important, some hybrids resist fungi growth while other were less resistant. The presence of insects also has higher influence on fungi growth some and some suppresses.	Barney et al. (1995).
		Review	The field and storage fungi that invade maize grain can cause significant damage, especially in the tropics and humid areas; causes cob rot, discoloration, loss of viability, vivipary, mycotoxin contamination, and subsequent seedling blights, which result in reduction in grain quantity and quality, and seed value. The problems of mycotoxins are	Preventing grain from mycotoxin contamination should be done both at the field and storage. In most cases only concentration is emphases in storage places, where most of fungi originate from the field, effective prevention should involve all stakeholders along the chain from grower, to consumer.	Williams and Macdonald (1983).

Table 17 (continued)

Storage Temperature	Storage R H or storage M.C.*	Other parameter(s)	Key results	Conclusions	References
			higher in tropics than in temperate region because of main factors such as (1) higher temperatures and humidity; (2) low capital for effective and rapid drying; (3) lower awareness of the problem; (4) poor quality control systems; (5) poverty and hunger lead people to buy and eat low quality grain; and (6) the prevalence of many diseases that may increase or accelerate the effects of mycotoxins		
		Review	Infection of maize grain with <i>Fusarium</i> species and contamination by fumonisin are influenced by two main factors: (1) abiotic factors (i.e. environmental conditions, temperature, and humidity), insect infestation, and (2) pre-biotic factors (i.e. storage conditions and Fungal interactions).	In order to overcome the problem of fumonisin contamination in Africa and other parts of the world, the following should be considered (i) Awareness; (ii) information regarding environmental and agroecological influences of fumonisin toxicity with respect to humans; (iii) more research and documentation on aflatoxins and fumonisin.	Fandohan et al. (2003).
		Review	In this study	Mycotoxins	Pitt (2000).

Table 17 (continued)

Storage Temperature	Storage R H or storage M.C.*	Other parameter(s)	Key results	Conclusions	References
			<p>reviewed that there are four main types of toxicity caused by mycotoxins (i.e. acute, chronic, mutagenic and teratogenic). The most common acute effect of mycotoxin is liver or kidney function or cancer.</p> <p>The most significant toxigenic species and mycotoxins were: Aflatoxins, Ochratoxin A, Fumonisin, Trichothecene toxins (i.e. deoxynivalenol and nivalenol) and Zearalenone.</p>	<p>contaminations are much and more serious problem than last decade, due to poor quality control in developing country, and climate change.</p> <p>The problems are accelerated by the presence of other diseases such as hepatitis B, and food-borne bacteria in some parts of Africa and Southeast.</p>	
		<p>A total of 98 samples of maize growing in Europe and Africa were used in the analysis of <i>Furasium</i>.</p>	<p>The results show in both Europe and Africa levels of <i>Furasium</i> (FB1 and FB2) were high, but level in Africa was much higher as compare to Europe, for the case of contamination also the levels were higher in Africa 80-100%, as compared to 50% in Europe.</p>	<p>Higher occurrences of <i>Furasium</i> in Africa might be due to climatic conditions, which influence the growth of molds and also could due to poor agricultural practices as both fungi start from field to storage.</p>	<p>Doko et al. (1995).</p>
		<p>This study was conducted in four villages in northern Tanzania.</p> <p>215 infants were involved, 52% were male and 48 % were females.</p>	<p>The results showed possibility of fumonisin exposure, which negatively affected growth of infants.</p>	<p>The authors concluded that further study should be conducted</p>	<p>Kimanya et al. (2010).</p>

Table 17 (continued)

Storage Temperature	Storage R H or storage M.C.*	Other parameter(s)	Key results	Conclusions	References
		The study was conducted in Kilimanjaro region. Northern Tanzania.	<p>This research focused on three main areas:</p> <p>(1) Occurrence and exposure of fumonisin in freshly harvested unsorted maize.</p> <p>For the cases of occurrence in the fresh maize the study found higher levels of the FB3 (60%), than FB1 and that of FB2.</p> <p>(2) Relationships between fumonisin exposure and agronomic practices.</p> <p>The results revealed fumonisin contamination was three times higher for the farmer. used local variety without using any fertilizer than those who used hybrids and fertilizer.</p> <p>(3) For the case of sorting, the results show fumonisin level lower for sorted maize than unsorted (freshly harvested) maize, with mean $486 \pm 691 \mu\text{g}/\text{kg}$ and $1718 \pm 4538 \mu\text{g}/\text{kg}$, respectively.</p>	This study showed that agricultural practices as well as selection of good varieties have an important role in reduction of fumonisin contamination, and initial sorting of maize after harvesting could help to reduce fumonisin toxin.	Kimanya et al. (2010).
		Isolation of Fumonisin B1 (FB1) and B2 (FB2) in com.	The results show high recovery rate of FB1 and FB2, around 98 to	Although the study was developed mainly to purify FB1 and	Cawood et al. (1991).

Table 17 (continued)

Storage Temperature	Storage R H or storage M.C.*	Other parameter(s)	Key results	Conclusions	References
		Extraction of the fumonisin were carried out using CH ₃ -OH/H ₂ O at the ratio of 3:1.	97.5% respectively. Also revealed two new types of <i>Furasium</i> (i.e. FB3 and FB4) The study revealed most effective ways of separation of fumonisin from other materials by using silica gel of two different mobile phases, which results in approximately 72 % recovery of FB1	FB2 in com. small modifications of the methods can be used to purify FB1, FB2 FB3 and FB4.	
Temperature of 30 ± 2°C. and moisture contents 15.5%.		The study was conducted for 60 weeks in three different storage systems	The results show increases in CO ₂ level in the first 12 weeks, and then level decreased to the end of the experiments. During study periods, highest mean CO ₂ level was about 11% in the control system, 18% in the RST system, and 14.5 % in the <i>Cryptolestes-Oryzaephilus-Tribolium</i> (COT) system. Also higher level of detected at the top than at the bottom. For the moisture contents, at control fell steadily up the ends, but for the RST, the moisture rose up to 20.5% at week 51 at the top and 18.5% at the bottom, for the case of COT	The study showed significant relationships between temperature, moisture contents, CO ₂ production, and mold growth; the higher the moisture contents the more growth of fungus and more deterioration.	White and Sinha (1980).

Table 17 (continued)

Storage Temperature	Storage R H or storage M.C.*	Other parameter(s)	Key results	Conclusions	References
			<p>system the moisture content remained stable at the top 15.5% and increased steadily at the bottom to 19% at week 51.</p> <p>For microbial changes, at the end of week 15, around 26 to 47% of the seeds were infected by fungus especially <i>Aspergillus glaucus</i> gr.in all systems.</p>		
		Review	<p>A stored grain ecosystem consists both living and non-living factors: 32 variables were included in the study; important variables monitored were time, temperature, moisture contents, pests, fungi and molds and geographic location.</p> <p>Main study showed the above factors were the main causes of deterioration of grain in the storage systems.</p>	The authors conclude by suggesting the proper management of grain in the store is complex, and involves monitoring of many factors to achieve proper standards by regulatory bodies.	Jian and Jayas (2012).

*All moisture contents in wet basis unless noted otherwise

CHAPTER 4. IS FLINT CORN NATURALLY RESISTANT TO *SITOPHILUS ZEAMAI*S INFESTATION?

A paper published in the *Journal of Stored Product Research*

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Abstract

Sitophilus zeamais (maize weevil) is one of the most destructive pests of maize stored in tropical and subtropical regions. This study determined the resistance of flint corn and dent corn to infestation by *S. zeamais* (Motschulsky), the maize weevil. Improved King Philip hybrid flint corn and Fontanelle 6T-510 hybrid dent corn were used in this experiment. Two temperature conditions (10 and 27 °C) and two storage times (15 and 30 d) were used. Results showed that flint corn was more resistant to insect damage than dent corn at 27 °C and 30 d storage time. After 30 d storage time and 27 °C death rate was significantly higher in flint corn ($R^2 = 0.945$) compared to ($R^2 = 0.634$) in dent corn. The damaged seed was 10% higher in dent corn than in flint corn at 27°C and 30 days. However, no significant difference was observed for seed weight loss between flint corn and dent corn at the same storage conditions. Both dent and flint corn were extensively cultivated in developing countries. It appears that storage of flint corn may be one promising solution to reducing corn damage, infestation problems in the tropics and in developing countries, but more research is needed.

Keywords: *Sitophilus zeamais*, flint corn, dent corn, corn damage, corn storage

4.1. Introduction

Corn (*Zea mays* L.) is a unique crop in its versatility; it is the only food grain that is eaten from flower to flour (Boutard, 2012). It is the principal staple food and a major source of calories in many developing countries (FAO, 2009), and the biggest source of feed, biofuel, and raw material for many industries in developed countries. It is the third most important cereal crop globally after wheat and rice (Adarkwah et al., 2012). According to the United Nations Food and Agriculture Organization (FAO), in 2010/2011 over 800 million metric tons of corn was produced (FAO, 2011); this is predicted to double by 2025, and corn is predicted to become the greatest crop in terms of production by 2050 (Rosegrant et al., 2008). Nearly half is produced in North America, with over 35% of total world production occurring in the United States Corn Belt, followed by China, European Union (EU-27), Brazil, and Argentina (USDA, 2012). Despite increases in production, post-harvest losses due to biotic factors such as insects and molds remain a huge challenge worldwide (FAO, 2009). It is estimated that 14 to 50% of the total corn produced each season in developing countries is lost due to insect infestation, compared to only 1 to 2% in developed countries (Ojo and Omoloye, 2012).

Corn is classified into groups based on endosperm characteristics, kernel color, maturity, and final uses (Paliwal et al., 2000). There are six main varieties of corn grown worldwide for commercial and human consumption: dent corn, flint corn, flour or soft corn, sweet corn, waxy corn and popcorn (Singh et al., 2009). Dent corn is the most widely grown corn in the United States (US) Corn Belt, and most parts of the world (Boutard, 2012). The kernel contains both corneous and soft starches, characterized by very hard, vitreous, horny

endosperm at the sides and back (Singh et al., 2009). The central core extends to the top, or crown of the kernel, which collapses on drying, resulting in the distinctive indentation (dent) (Paliwal et al., 2000). Dent corn has a fairly wide range of colors, from yellow to white, but yellow is the most common and is extensively grown for seed, silage, biofuel, and other commercial uses in the US. Dent corn is susceptible to grain insect infestation by insects such as *Sitophilus zeamais* (Paliwal et al., 2000).

Flint corn is less popular than dent corn. The kernels of flint corn range from small (11 mm long) in size are rounded on the top, smooth, hard and thick with no indentation of the crown at maturity (Boutard, 2012). Flint corn exhibits an extended range of colors from white through yellow, orange, red, mahogany, blue, purple, and black (Boutard, 2012), and it is widely grown in Latin America, Northern Europe, and some parts of Asia for commercial purposes (Gujral et al., 2001). The endosperm of flint corn is primarily vitreous, with less soft starch, and is enclosed by a corneous outer layer. Starch is more concentrated at the periphery than in the center, which gives the endosperm hard external layers (Haros et al., 2001). The hard outer layer of flint corn may make it less prone to insect damage (Paliwal et al., 2000) and less water absorbent than dent corn (Haros et al., 2001). In terms of nutrients, flint corn typically contains more protein than dent corn, (9.2 versus 7.0% dry basis, respectively), while flint corn contains less starch (63%) than dent corn (76%), but its quality is good and the ratio of amylose -amylopectin is about the same as that of dent corn (Haros et al., 2003; White and Johnson, 2003). Compared to dent corn, flint has a lower yield, is less cultivated, and farmers normally receive a higher price from millers and brokers (Cirilo et al., 2011).

Corn and other cereal grains account for over 70% of the total crops produced in developing countries. Smallholder, subsistence farmers produce most of these grains; unfortunately, significant amounts are often lost after harvest, resulting in increased hunger and human labor (FAO, 2011). Africa Postharvest Losses Information System (APHLIS) statistics showed that nearly 17% of the total corn produced in Africa was lost in 2011/2012 (APHLIS, 2012). FAO estimates about \$4 billion lost each year in sub-Saharan Africa due to post harvest grain losses (FAO, 2011). The biggest cause of grain loss is infestation by insects such as *S. zeamais* during storage (Ukeh et al., 2012). *Sitophilus zeamais* Motschulsky, the maize weevil is among the most destructive pests in stored grain, especially corn in tropical regions (Paes et al., 2012). *Sitophilus zeamais* are regarded as internal feeders of grains. Adult female *S. zeamais* cause damage by boring into the kernel and laying eggs (ovipositing). Then, larvae and pupae eat the inner parts of the kernel, resulting in a damaged kernel and reduced grain weight (Ojo and Omoloye, 2012). Apart from weight losses, the feeding damage caused by weevils leads to severe reductions in nutritive and economic values, reduced seed viability, as well as contamination by chemical excretions (silk) and insect fragments (Ukeh et al., 2012). The infestation also elevates temperature and moisture content in the stored grain mass, which can lead to mold growth, including toxigenic species such as *Aspergillus flavus* (Chu et al., 2013). *Sitophilus zeamais* cause extensive losses in quality and quantity of the grain in the field as well as in storage (Sabbour, 2012). Several studies have examined storage infestation in dent corn; little work, however, has been reported on the infestation of flint corn by *S. zeamais*. Therefore, the objective of this research was to determine the resistance of flint and dent corn to *S. zeamais* infestation.

4.2. Materials and Methods

4.2.1. Experimental Design

In this experiment, three replications of two corn varieties (dent and flint), and twenty-four glass jars with screened lids were used with two temperature conditions (10 and 27 °C) and two storage/opening times (15 and 30d) (Table 18). The moisture content of each corn variety was determined with samples of 30g in three replications at 103 °C for 72 h, following ASAE Standard S352.2 (ASAE, 2001).

Table 18. Experimental Design.

Treatment	Corn type	Time (days)	Temp (°C)
1	Dent	15	10
2	Dent	15	27
3	Flint	15	10
4	Flint	15	27
5	Dent	30	10
6	Dent	30	27
7	Flint	30	10
8	Flint	30	27

4.2.2. Treatment and Storage Trials

The dent corn was a commercial hybrid (Fontanelle 6T-510) harvested during 2012, and flint corn was Improved King Philip hybrid from the crop year 2009-2010. The moisture contents of all corn samples were adjusted to $13.5 \pm 0.5\%$ (wet basis) prior to initiating the storage trials. Two identical environmental chambers with different temperature settings (10 and 27°C) were used (Model 23-988 126 GW, Fisher Scientific Inc., Waltham, MA 02454). *Sitophilus zeamais* used in these experiments were obtained from the stock of *S. zeamais*

already feeding on dent corn in the Department of Agricultural Biosystems Engineering at Iowa State University (Yakubu et al., 2011). Twenty-four 246-mL glass jars, with screened lids to allow air flow (i.e., 12 each of the dent and flint) was each loaded with 230g of corn; then 20 unsexed adult *S. zeamais* were introduced into each jar, based on Yakubu et al. (2011). The 12 jars for each hybrid were then stored in each experimental chamber.

4.2.3. Data Collection and Analysis

Mortality was assessed after 15 and 30 days of storing the weevil-infested maize. All weevils were separated and removed (by hand) from the corn at the end of these two periods. Numbers of live and dead weevils were recorded at this time. By visual inspection, the number of damaged and undamaged kernels (seeds) in each treatment was recorded, as were the weights of damaged and undamaged kernels. Damaged kernels meant that visible physical damage caused by *S. zeamais* was present. Percent (%) kernel weight loss was determined by using the count and weigh method developed by Adams and Schulten (1978). The factorial design consisted of three main effects, two corn types, two temperatures, and two storage times. Analysis of variance (ANOVA) was performed using the Statistical Analysis System (SAS) version SAS 9.3, with a general linear model (GLM), using PROC GLM (2011) at an of 5%, to determine the main and interaction effects and least significant differences (LSD) between treatment means. Additionally, treatment effects were examined at an of 0.05%.

4.3. Results and Discussion

The results for the main effects (Table 19), show that all independent variables had significant effects ($P < 0.05$) on *S. zeamais* infestation parameters, except for live *S. zeamais* (LSZ), dead *S. zeamais* (DSZ) at 10 °C and 27 °C, and seed weight loss (SWL) for dent and flint corn. For the interaction effects (Table 3), the results show significant effects due to corn type and time, but mixed results for the other independent variable interactions. No significant effects were observed for the three-way interaction (corn by time by temperature). Furthermore, all independent variables showed significant effects of treatment combinations, except for the LSZ (Table 4).

Table 19. Main Effects of Corn Types, Temperature and Time on *Sitophilus Zeamais* Infestation.*

	LSZ	DSZ	DS	UDS	WD	WUD	SWL (%)
Corn							
Dent	16.2 ± 2.4 ^a	4.1 ± 2.1 ^b	56.7 ± 15.1 ^a	639.1 ± 19.2 ^a	14.3 ± 3.5 ^a	207.8 ± 6.2 ^b	1.8 ± 1.0 ^a
Flint	11.0 ± 7.6 ^b	10.3 ± 7.9 ^a	36.3 ± 13.7 ^b	708.9 ± 17.2 ^b	7.7 ± 3.1 ^b	215.4 ± 6.2 ^a	1.5 ± 0.8 ^a
Temp (°C)							
10	13.8 ± 6.1 ^a	6.5 ± 5.8 ^a	39.9 ± 14.8 ^b	675.6 ± 38.8 ^a	10.3 ± 4.1 ^b	216.1 ± 4.5 ^a	1.1 ± 0.9 ^b
27	13.4 ± 6.4 ^a	7.9 ± 7.2 ^a	53.0 ± 18.2 ^a	672.4 ± 42.6 ^b	11.7 ± 5.3 ^a	207.1 ± 6.6 ^b	2.1 ± 0.7 ^a
Time (d)							
15	17.8 ± 1.8 ^a	3.1 ± 1.7 ^b	35.7 ± 14.8 ^b	688.3 ± 35.6 ^a	8.3 ± 3.4 ^b	214.8 ± 4.9 ^a	1.3 ± 1.1 ^b
30	9.3 ± 5.9 ^b	11.7 ± 6.6 ^a	57.3 ± 13.0 ^a	659.7 ± 40.2 ^b	13.6 ± 4.4 ^a	208.4 ± 7.7 ^b	2.0 ± 0.5 ^a

*The values in the table are mean ± standard deviation, values with the same letter for a given property, within each independent variable, are not significantly difference ($P < 0.05$) for the dependent variable. LSZ= live *S. zeamais* (counts), DSZ=dead *S. zeamais* (counts), DS = damaged seed (counts), WD = weight of damaged seed (g), WUD = weight of undamaged seed (g), SWL (%) = percentage seed weight loss.

4.3.1. *Sitophilus zeamais* Mortality

There were significant ($P < 0.05$) differences seen with corn type and time for mortality, i.e. LSZ and DSZ (Table 20). However, there were no significant effects on mortality between 10 and 27°C. The numbers of LSZ were significantly higher in flint corn at 15 d storage time. This concurred with a study by Paliwal et al. (2000), who examined dent corn susceptibility to grain insect infestation. Likewise, as expected, there was a higher number of DSZ observed in flint corn with the 30 d storage time; this was attributed to the end of life cycle of *S. zeamais*, the hardness of kernel and anti-feedants compounds such as phenolic acids that caused damage to midgut cells of the insects (Kevin, 2002). Kernel hardness was found to be the biggest factor contributing resistance to *S. zeamais* infestation on flint corn. Several studies reported results that concurred with our study (see, for example, Golob, 1984; Kossou et al., 1993; Dombrink-Kurtzman and Knutson, 1997). Many of these reported that maize kernel hardness has a strong correlation with insect damage during harvesting, handling, and storage and concluded that establishing maize varieties with higher kernel hardness is necessary for reducing insect infestation and improving protein quality of maize. Moreover, similar results were reported by Kossou et al. (1993) who reported that grain kernel hardness has a significant effect upon *S. zeamais* infestation, and Serratos et al. (1987) who described that out of four varieties they studied, two varieties were less susceptible to weevils. These were found to be those with harder kernel structure. The higher correlation between kernel hardness and pericarp cell wall of maize on *S. zeamais* resistance was observed by García-Lara et al. (2004) (see Figures. 46 and 47).

Table 20. Interaction Results (*P*-Values) for Corn Types, Temperature and Time on *Sitophilus zeamais* Infestation. [†]

Variable	LSZ	DSZ	DS	UDS	WD	WUD	SWL (%)
Corn	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0547
Time	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0011
Temp	0.7187	0.1398	0.0001	0.3874	0.0295	0.0001	0.0001
Corn * Time	0.0001	0.0001	0.4388	0.2981	0.3683	0.8677	0.1103
Corn * Temp	0.7187	0.0919	0.0586	0.7132	0.5253	0.8077	0.0146
Time * Temp	1.0000	0.9063	0.9188	0.0024	0.0060	0.0001	0.0002
Corn * Time *Temp	1.0000	0.0232	0.4008	0.9266	0.1034	0.3690	0.2552

[†] The values in the table are mean \pm standard deviation, values with the same letter for a given property, within each independent variable, are not significantly difference ($P < 0.05$) for the dependent variable. LSZ= live *S. zeamais* (counts), DSZ=dead *S. zeamais* (counts), DS = damaged seed (counts), WD = weight of damaged seed (g), WUD = weight of undamaged seed (g), SWL (%) = percentage seed weight loss.



Figure 46. Flint Corn Damage Caused by *Sitophilus zeamais* during 30 days of Storage.



Figure 47. Dent corn Damage Caused by *Sitophilus zeamais* during 30 days of Storage.

In addition, significant effects ($P < 0.05$) were observed for the time and the interaction of corn type and time (Table 20) for LSZ and DSZ. However, no significant effects were detected for temperature, temperature-time interaction, corn type temperature interaction, or the three-way interaction (i.e. corn type by time by temperature). Moreover, no significant differences were found for the treatment combination effects for LSZ (Table 21), while there were some higher significance differences for DSZ, amongst treatments. Results also show that the growth of *S. zeamais* in dent corn (Figure 48) follows a fairly linear growth curve ($R^2 = 0.574$), while different results were observed for flint corn (Figure. 48) whereby *S. zeamais* growth decreased exponentially with time ($R^2 = 0.945$); this was believed due to shortage of food due to the hard structure of flint corn. Furthermore, the first derivative of the death curves in dent and flint (Equations (11) and (12)) respectively, show that death rates increase

over time for both types of corn, and after 30 d storage time death rates for *S. zeamais* in flint corn are almost three times higher than those of dent corn (Figure 49 and Figure 50).

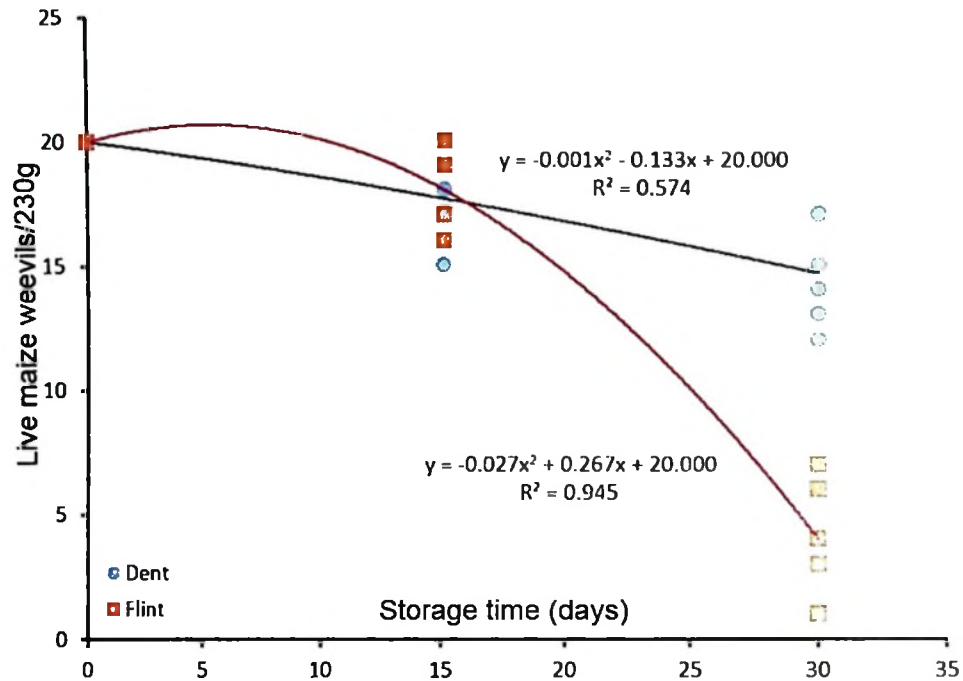


Figure 48. *Sitophilus zeamais* Mortality (number live) over time (days).

Furthermore, the results also revealed that growth rate decreased over time as shown in Figure 48. The rate seems higher on flint corn ($R^2 = 0.945$) than in dent corn. The main reasons believed to be structural differences between flint and dent corn as flint corn exhibits hard endosperm (Maiorano et al., 2010) which makes them harder for *S. zeamais* to bore into the kernel and oviposit and also due to decreased food as the weevil population increased in dent corn.

$$\frac{d \text{ Dead(dent)}}{dt} = (-0.002t + 0.233) \quad 11$$

$$\frac{d \text{ Dead(flint)}}{dt} = (0.052t - 0.189) \quad 12$$

4.3.2. Damaged and Undamaged Seed

For the case of damaged seed (DS) and undamaged seed (UDS), there were significant differences between all three main effects (Table 19). The highest DS was observed in dent corn while the lowest DS was observed in flint corn. As time and temperature increased, DS increased, and UDS decreased. Examining treatment effects, dent had a greater DS for all times and temperatures. Higher temperature led to greater insect activity. As described by Monstross et al. (1999) the main factors influencing propagation and development of insects are temperature and moisture content. Hayma (2003) found that favorable conditions for most grain storage insects to develop are between 25 and 30 °C.

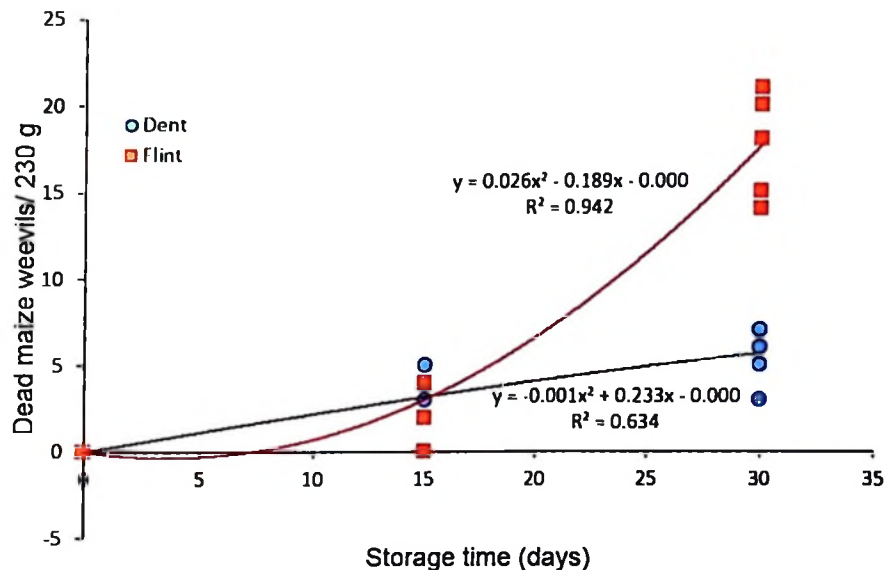


Figure 49. *Sitophilus zeamais* Mortality (number dead) over time (days).

Likewise, stated by Gudrups et al. (2001) that factors like kernel hardness, husk protection, kernel size, and texture, plays a significant role in maize protection on insect attack, and these agreed with our findings. As shown in Table 21, damaged seed (DS) on dent corn were higher compared with flint corn both at 15 and 30 days storage times as well on 10 and 27 °C temperature conditions.

Table 21. Treatment Combination Effects Due to Corn Types, Temperature and Time on *Sitophilus zeamais* Infestation.*

Trmt	Corn	Time	Temp	LSZ	DSZ	DS	UDS	WD	WUD	SWL (%)
1	Dent	15	10	17.8 ± 2.5a	2.3 ± 2.5d	36.7 ± 4.5e-d	649.7 ± 1.5d	10.8 ± 1.6c	214.3 ± 0.7c	0.5 ± 0.2d
2	Dent	15	27	17.8 ± 2.5a	4.0 ± 1.0d-ca	57.0 ± 9.5b	661.0 ± 1.1c	11.8 ± 1.5a	208.1 ± 1.4e-d	2.7 ± 0.8a
3	Flint	15	10	18.3 ± 2.1a	2.7 ± 2.3d	21.3 ± 1.5e	717.3 ± 4.7b-a	6.3 ± 0.4e-d	220.9 ± 1.9a	0.1 ± 0.1d
4	Flint	15	27	17.7 ± 2.1a	3.3 ± 1.2d	27.2 ± 2.1e-f	725.3 ± 1.2a	4.4 ± 0.7e	216.2 ± 0.7b-c	1.7 ± 0.0b-c
5	Dent	30	10	14.7 ± 2.1a	6.7 ± 0.6c	58.6 ± 7.2b	630.3 ± 6.0e	15.8 ± 2.6b	210.6 ± 2.6d	1.6 ± 0.3c
6	Dent	30	27	14.6 ± 2.5a	4.6 ± 1.5d-c	74.3 ± 4.9a	615.3 ± 4.2e	18.5 ± 0.6a	198.6 ± 1.1f	2.4 ± 0.8b-a
7	Flint	30	10	4.3 ± 2.5b	15.6 ± 2.1b	43.0 ± 7.5c-d	705.0 ± 1.5b	8.0 ± 1.9d	218.7 ± 2.1b-a	2.3 ± 0.1b-a-c
8	Flint	30	27	3.6 ± 2.5b	19.7 ± 1.5a	53.0 ± 5.3c-d	688.0 ± 7.5c	12.0 ± 1.1c	205.7 ± 0.9e	1.7 ± 0.3c

*The values in the table are mean ± standard deviation, values with the same letter for a given property, within each independent variable, are not significantly difference ($P < 0.05$) for the dependent variable. LSZ= live *S. zeamais* (counts), DSZ=dead *S. zeamais* (counts), DS = damaged seed (counts), WD = weight of damaged seed (g), WUD = weight of undamaged seed (g), SWL (%) = percentage seed weight loss.

The numbers of DS were directly related to LSZ. With an increasing number of LSZ, there was an increase in DS. Similar results were observed by Singh and McCain (1963), who found positive correlations between kernel nutrient contents, reproduction, and weights

of weevils (i.e., as nutrients of kernels increased, weevil reproduction rate and weevil weights increased, and thus seed damage increased).

Clearly, significant differences ($P < 0.05$) were observed for all three main effects (Table 20) for DS, while only two main effects (corn and time) exhibited significant differences for UDS while opposite results were observed for their interaction.

4.3.3. Weight of Damaged and Undamaged Seed

There were significant differences ($P < 0.05$) in the weight of the damaged (WD) and undamaged (WUD) seed (Table 19). Higher WD was observed in dent corn than in flint corn for both 27°C and 30 days storage time. As expected, more DS and LSZ were found in dent corn than in flint. Corn type and time were the only significant effects (Table 20) on *S. zeamais* infestation

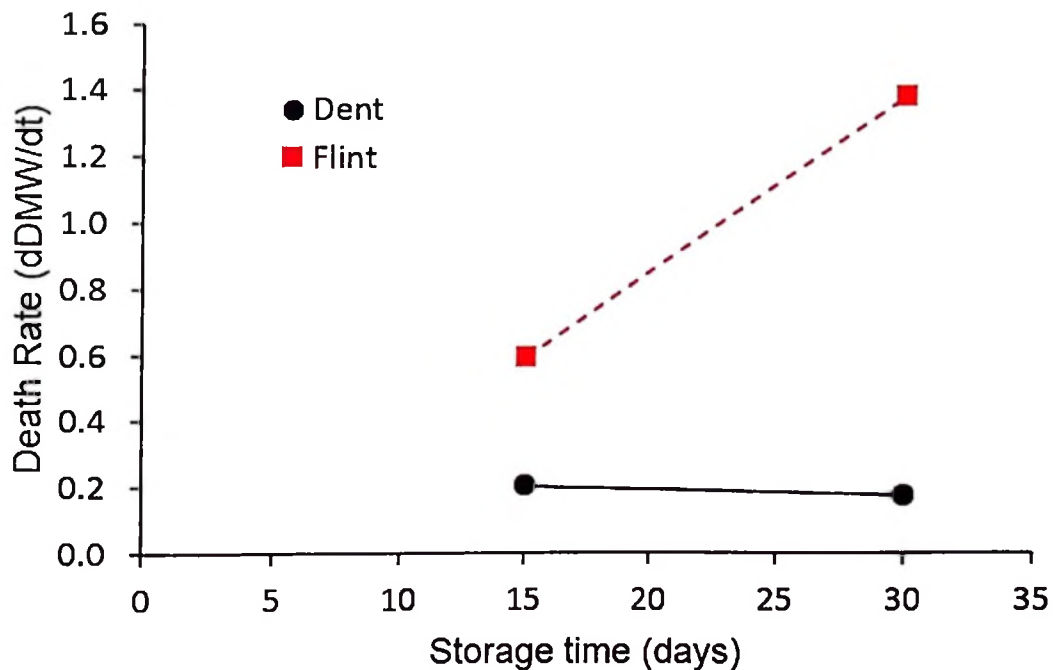


Figure 50. First Derivatives (Increase in Death Rate) for *Sitophilus zeamais* Mortality over Time.

Similarly, temperature and all other interactions were not actors influencing WD. In the case of WUD, significant effects were observed for corn type, time by temperature, and the interaction of time and temperature. However, no significant effects were detected for corn by time, corn by temperature or the three-way interaction of corn by time by temperature (Table 20).

4.3.4. Seed Weight Loss (SWL)

Results showed few significant differences between dent and flint corn: the only significant differences ($P < 0.05$) detected were due to temperature and storage time. The highest percentages of SWL were recorded at 27°C and 30 days storage time, for both dent and flint corn. It is suspected a higher number of LSZ corresponds with high SWL in dent corn, according to a study conducted by Abebe et al. (2009), that found direct relationships between seed damage and weight loss with the number of weevils emerged, for different maize varieties. For this study, mixed results were observed in the interaction results, ranging from highly significant to no significance for some factors (such as the type of corn, corn by time, and corn by time by temperature) (Table 20). Treatment combinations showed that dent corn at 10 °C and 15-day storage time had similar results to flint corn under the same conditions (Table 21). The results also showed that dent corn at 10°C and 15 days' storage time were similar ($P < 0.05$) to flint corn at 27°C and 30 days' storage time. The resistance of stored grain insects such as *S. zeamais* to protectants has recognized as an increasingly important problem in tropical countries. Studies conducted by Samson et al. (1988) and Arnason et al. (1992), shows that most of the chemicals used to protect corn against stored

product insects in tropic climates have low effectiveness and insects build resistance to them. To avoid creating stronger pests and reduce postharvest losses of corn in developing countries, the uses of plant resistance varieties like flint corn remain the best option, and many scientists considered it as a sustainable way of integrated pest management strategy (GarcíaLara et al., 2010; Arnason et al., 1992; Abebe et al., 2009).

4.4. Conclusions

This experiment was conducted to determine the resistance of flint and dent corn to *S. zeamais* infestation. The results suggest that dent corn is more susceptible to *S. zeamais* than flint corn. Other factors, such as time and temperature, play large roles in corn infestation, as this study revealed that most of the damage occurred at 27°C and 30 days storage time. Therefore, flint corn, or a hybrid of flint and dent, could be a viable approach to reduce the problem of infestation and damage in developing countries. Further study is needed to look at different varieties of flint, especially for longer storage times. These studies are in progress at the moment, and results will be forthcoming soon.

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CHAPTER 5. EVALUATION OF MAIZE WEEVILS *SITOPHILUS ZEAMAI*S MOTSCHULSKY INFESTATION ON SEVEN VARIETIES OF MAIZE

A paper published in the *Journal of Stored Products Research*

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Abstract

Sitophilus zeamais (Motschulsky), the maize weevil, is a serious pest of economic importance in store products in tropical and subtropical countries; infestation often starts in the field, but serious damage is done during maize storage. This study evaluated *S. zeamais* infestation on seven varieties of maize. Seven commercial maize varieties (white dent, yellow dent, orange flint, Indian flint, white and yellow popcorn, and sweet corn), two temperature conditions (10 and 27 °C) and three storage times (30, 60, and 90 d) were used. The moisture contents of all maize samples were adjusted to 15.5 ± 0.5 % (wet basis) prior to initiating storage trials. Numbers of live weevils, seed damage, weight loss, and weight of powder produced were assessed at the end of each storage time. As expected, severe damage was observed at 27 °C and 90 d for all maize varieties. Exponential growth rates of *S. zeamais* were observed in almost all maize varieties. Among seven varieties evaluated, orange flint corn, yellow and white popcorn show resistance to *S. zeamais*. Nevertheless, sweet and dent corn were most susceptible to maize weevil infestation. Higher numbers of live *S. zeamais* were observed on Indian flint corn and sweet corn. Consequently, there was a higher seed weight damaged and weight loss. Further, seed damaged, percentage seed weight loss and weight of powder produced was significantly and positive correlated with a number of live *S. zeamais* ($r = 0.91, P < 0.05$), ($r = 0.88, P < 0.05$), and ($r = 0.89, P < 0.05$) respectively. Thus, some varieties of flint corn and popcorn can be considered as potential maize varieties to be used to reduce postharvest loss of maize in tropical countries due to their natural resistance to *S. zeamais* infestation.

Keywords. Maize weevil; dent corn; flint corn; sweet corn; popcorn; maize damage; maize storage.

5.1. Introduction

Maize (*Zea mays L.*) together with rice and wheat are three most important cereal crops worldwide. In Sub-Saharan Africa (SSA) maize is the staple grain, cash crop, food security crop, and major source of calories (Jones et al., 2011; Smale et al., 2011). It is a primary source of energy in developing countries and contributes up to 60 and 30% of the diet's energy and protein respectively (Mlyneková and Čerešňáková, 2013). Based on kernel characteristics, maize can be classed into five main groups: dent, flint, popcorn, sweet, and floury (Boutard, 2012). The hardness of the flint corn outer layer makes it less prone to damage by grain mold and insects, both in the field and in storage (Paliwal et al., 2000). It is a multicolored grain, ranging from pale-orange to dark red (Suleiman et al., 2013). Flint corn is extensively grown in Central and South America, Asia, some parts of Africa, and Southern Europe for human consumption and industrial purposes (OGRT, 2008).

Popcorn (*Zea mays everta Sturt*) is the most popular snack in the United States (US) and around the world. The US is the largest producer and consumer of popcorn in the world; over 230 million metric tons of popcorn (Hansen et al., 2013). It is estimated that over 54 million metric tons of popcorn are consumed every year in the US. This enormous consumption of popcorn may be partially due to claims made by the Dietary Guidelines for Americans and MyPyramid as one among whole-grain food/snacks (Grandjean et al., 2008). Popcorn is described as a special type of flint corn with small ears and small pointed or rounded kernels and a structure characterized by hard starch, and very hard pericarp and outer layers of endosperm (Karababa, 2006; Yang et al., 2005). Production and consumptions of sweet corn have increased dramatically over the past 30 years in the US, Brazil, Canada, China, Australia, and Europe for both consumption as a fresh vegetable and for food processing

(Williams, 2012). According to Hansen et al. (2013), in 2012 approximately 3.4 million metric tons of sweet corn, valued at over US \$ 1.1 billion were produced, a 10 percent increase from the previous year. This is expected to increase in upcoming years (NASS, 2013). Suleiman et al. (2013) reported that sweet corn originated from a genetic mutation of field corn in the Peruvian race Chullpi. It differs from dent (field corn) by only one recessive gene (*su1*) or sugary that prevents some of the sugars from being converted to starch (Najeeb et al., 2011).

Sitophilus zeamais (Motschulsky), the maize weevil, is a serious pest of economic importance in store products in tropical countries; infestation starts in the field, but serious damage is done during of maize storage (Fikremariam et al., 2009). Muzemu et al. (2013) and Giga et al. (1991a) reported grain weight loss of 20-90% due to maize weevil for untreated maize in tropical countries. In developed countries, maize is stored in commercial structures, with proper monitoring of temperature and moisture content to control pests. However, in tropical countries, maize is often stored in traditional structures with no environmental control and usually without chemical protectants (Dhliwayo and Pixley, 2003). Maize damage by weevils causes food loss, increased poverty, and lower nutritional values of grain, increased malnutrition, reduced weight and market values (Keba and Sori, 2013). Similarly, *S. zeamais* increased malnutrition, reduced the germination percentage, and maize production as most farmers in developing countries store grain and seed together (Pingali and Pandey, 2001). According to Renkow et al. (2004), most of the smallholder farmers (83%) in SSA sell their maize within two months of the harvest to avoid loss from insect infestation and thus miss opportunities of getting the highest price at the lean season. To reduce such problems a strategy mentioned by many researchers is to use weevil resistant

varieties like flint corn (Suleiman et al., 2015). The use of weevil resistant varieties is safe, environmentally friendly, effective, acceptable by farmers, economically feasible and can be incorporated into existing programs like the integrated pest management (IPM) approach (Keba and Sori, 2013). We found no published research on the infestation of popcorn or sweet corn by *S. zeamais*. Hence, this study seeks to evaluate infestation of *S. zeamais* on seven varieties of maize include popcorn and sweet corn for their resistance on long term storage.

5.2. Materials and Methods

5.2.1. Experimental Design

In this experiment, the treatments were arranged in a Completely Randomized Design (CRD) with three replications for each maize variety, two temperature conditions (10 and 27 °C) and three storage times (30, 60, and 90 d). The moisture content of each maize variety was determined with samples of 30 g with three replications at 103 °C for 72 h, following ASAE Standard S352.2 (ASAE, 2001).

5.2.2. Treatments and Storage Trials

Maize varieties used in this experiment were yellow dent corn (commercial hybrid Fontanelle 6T-510 harvested in 2012), white dent corn (hybrid PI-570679), orange flint corn (CIMMYTMA-006442/Ames 26579 varieties), Roy's Calais, Indian Flint Corn (2390-PTO variety), sweet corn (variety H3A-368-732, Stowell's Evergreen from Seed Savers Exchange Decorah, Iowa), and yellow popcorn (variety SH 2865) and white popcorn (variety SH 2662) from ISU Seed Science Center, Iowa State University. The moisture content of all maize was regulated to $15.5 \pm 0.5\%$ (wet basis). According to CIMMYT (2001), maize is most

susceptible to insect damage if it is stored at moisture contents above 15% (wet basis). Two identical environmental chambers with different temperature settings (10 and 27 °C) were used (Model 23-988 126 GW, Fisher Scientific Inc., Waltham, MA 02454). Weevils used in these experiments were obtained from the stock of *S. zeamais* maintained in the Department of Agricultural Biosystems Engineering at Iowa State University. One hundred twenty-six 246-mL glass jars with screened lids to allow airflow were each loaded with 230 g of maize; then 20 unsexed adults weevils were introduced into each jar, based on Suleiman et al., 2015, giving *S. zeamais* populations of 87 weevils/kg maize. The 9 jars for each variety were then stored in each environmental chamber.

5.2.3. Data Collection and Analysis

Stored samples were assessed after 30, 60, and 90 d. All weevils were separated and removed (by hand) from the maize at the end of these three periods and numbers of live weevils were recorded. By visual inspection, the numbers of damaged kernels (seeds) in each treatment were recorded, as well as the weights of damaged. Also, powders produced due to insect feeding were weighed on an electronic balance. Damaged kernels were those with visible physical damage (characteristic hole) caused by *S. zeamais*. Percent kernel weight loss was determined by using equation 13 and the count and weigh method developed by Adams and Schulten (1978).

$$\text{Weight loss (\%)} = \frac{(W_u * N_d) - (W_d * N_u)}{(W_u * (N_d + N_u))} * 100 \quad 13$$

Where W_u = Weight of undamaged seed, N_u = Number of undamaged seed, W_d = Weight of damaged seed, and N_d = Number of damaged seed.

The data collected were subjected to the Statistical Analysis System (SAS) software version 9.4. The statistical analysis was performed using a PROC GLIMMIX procedure (SAS Institute, Cary, NC, 2011) with a type I error (α) of 0.05. An ANOVA model was used and least significant differences (LSD) between treatment means were determined. Also, Pearson correlation coefficients were obtained using SAS software.

5.3. Results

5.3.1. *Sitophilus zeamais* Mortality and Progeny Emergence

Exponential growth rates were observed for all varieties except yellow popcorn and a population exceeding 2000 live *S. zeamais* per kg of maize occurred in Indian flint corn, yellow dent corn and sweet corn (Figure 51 and 52). There were significant differences seen among maize varieties, temperatures, and times for mortality, i.e. LSZ (Table 22), the number of Live *S. zeamais* counts for Indian flint corn, yellow dent corn and sweet corn were significantly higher than for the other varieties, and LSZ for yellow popcorn was significantly lower than, and only about 18% as high, as the group values mean at 27 °C were significantly higher than at 10°C. Likewise, for a time, significance for LSZ divided the same way. LSZ means for 90 days were significantly higher than those for 60 days, which were significantly higher than those for 30 days. The major parameters associated with *S. zeamais* infestation and maize varieties were analyzed for multiple interactions during 90 days of storage (Table 23). There was a significant interaction ($P<0.05$) between maize varieties and among all the sources of variation.

Table 22. Mean of Number of Live *S. Zeamais*, Seed and Weight Damaged Seed, Percentage Seed Weight Loss and Weight of Powder Produced on *S. Zeamais* Infestation on Seven Maize Varieties by Two Temperatures, and Three Times.

Effect	LSZ (count)	DS (count)	WD (g)	SWL (%)	PW (g)
Maize variety					
White dent corn	320 ± 582 ^c	75 ± 137 ^d	67 ± 114 ^b	3 ± 6 ^b	1 ± 3 ^b
Yellow dent corn	705 ± 1315 ^{a-b}	159 ± 234 ^c	138 ± 200 ^a	8 ± 14 ^a	3 ± 6 ^b
Orange flint corn	197 ± 377 ^{d-c}	46 ± 76 ^c	48 ± 74 ^c	1 ± 2 ^b	0 ± 0 ^c
Indian flint corn	793 ± 1552 ^a	205 ± 276 ^b	133 ± 167 ^a	0 ± 0 ^c	7 ± 11 ^a
White popcorn	215 ± 407 ^d	93 ± 145 ^d	43 ± 62 ^c	1 ± 3 ^b	0 ± 0 ^c
Yellow popcorn	135 ± 194 ^c	61 ± 80 ^{d-c}	37 ± 49 ^d	1 ± 1 ^b	0 ± 0 ^c
Sweetcorn	728 ± 1373 ^{a-b}	289 ± 329 ^a	136 ± 190 ^a	3 ± 5 ^b	1 ± 3 ^b
Temperature (°C)					
10	48 ± 32 ^b	17 ± 19 ^b	13 ± 16 ^b	1 ± 1 ^b	0 ± 0 ^b
27	836 ± 1298 ^a	231 ± 258 ^a	159 ± 169 ^a	6 ± 11 ^a	2 ± 5 ^a
Time (d)					
30	75 ± 26 ^c	76 ± 111 ^c	58 ± 79 ^c	1 ± 1 ^c	0 ± 0 ^b
60	484 ± 574 ^b	168 ± 187 ^b	133 ± 153 ^b	4 ± 6 ^b	0 ± 0 ^b
90	1199 ± 1678 ^a	251 ± 314 ^a	154 ± 188 ^a	9 ± 13 ^a	4 ± 6 ^a

⁺ Values followed by the same letter are not significantly different ($P < 0.05$) among varieties. LSZ (count) = Live *S. zeamais* per 1000 g maize, DSZ (count) = Dead *S. zeamais* per 1000 g maize, SWL (%) = Percentage Seed Weight loss, PW= Weight of powder produced (g). Mean ± SD (n= 126).

5.3.2. Damaged and Undamaged Seed

The trends for damaged seed (DS) means follow somewhat those for live weevils (LSZ). DS means for sweet corn were significantly higher than for any other varieties. Means for yellow popcorn were significantly lower than for any other varieties. A means for other varieties were between these two. Furthermore, for temperature, the trends were reversed. DS means for 27°C were significantly higher than for 10°C. This is intuitive, one expects more damage and less undamaged at the higher temperature. For a time, the order of means was intuitive. DS means at 90 days were significantly higher than for 60 or 30 days.

5.3.3. Weight of Damaged and Undamaged Seed

Damaged seed weight means (WD) for yellow dent corn, Indian flint corn, and sweet corn was significantly higher than those for the other varieties while yellow popcorn means were significantly lower than those of the other varieties. Trends for temperature were intuitive. WD means at 27°C were significantly higher than those at 10°C. Moreover, the damage level is following the activity level of the weevils. WD trends in time are also intuitive. WD means after 90 days are significantly higher than for 60 or 30 days.

5.3.4. Percentage Seed Weight Loss (SWL%)

Significant differences ($P<0.05$) were observed on SWL% between maize varieties; the results show time and temperature also had a large influence on a SWL% (Table 22). The highest percentage of seed weight loss was observed in yellow dent corn at 27°C and 90 d of storage. Likewise, lower % of SWL was observed on Indian flint corn and two varieties of popcorn (yellow and white). The results of SWL% of yellow dent corn were similar to kernel weight damaged. Overall, there were significant differences between SWL% and maize varieties ($P<0.05$); the results show time and temperature had a larger influence on SWL%. The highest SWL% was observed on yellow dent corn at 27 °C and 90 d of storage (Table 22). In general, the results of SWL% were similar to kernel weight damaged.

Table 23. Analysis of Variance for *S. zeamais* Infestation on Seven Maize Varieties*.

Source	df	LSZ (count)		DS (count)		WD (g)		SWL (%)		PW (g)	
		F	P	F	P	F	P	F	P	F	P
Maize ^a	6	33	<0.001	26	<0.001	28	<0.001	30	<0.001	16	<0.001
Time ^b	2	309	<0.001	205	<0.001	168	<0.001	142	<0.001	108	<0.001
Temp ^c	1	436	<0.001	392	<0.001	468	<0.001	186	<0.001	129	<0.001
Maize* Time	18	26	<0.001	13	<0.001	13	<0.001	18	<0.001	13	<0.001
Maize * Temp	6	30	<0.001	20	<0.001	19	<0.001	17	<0.001	16	<0.001
Time * Temp	3	314	<0.001	176	<0.001	138	<0.001	122	<0.001	108	<0.001
Maize * Time * Temp	18	25	<0.001	11	<0.001	11	<0.001	14	<0.001	13	<0.001

^a Seven maize varieties, ^b Three storage times, ^c Two storage temperatures, df = degree of freedom, F = F-value, P = P-value. *Significant difference ($P < 0.05$).

5.3.4. Weight of Powder Production

A strong significant difference was observed for the weight of powder produced ($P < 0.05$). The highest weight of the powder was observed on yellow dent corn and Indian flint corn (Table 22). This result is consistent with the number of LSZ, seed weight damaged and percentage seed weight loss. More powder weight was detected on yellow dent corn and Indian flint corn. Results were consistent with the number of insects, weight damaged and percentage seed weight loss.

5.3.5. Simple Correlation Coefficient of the Variables

Simple linear correlation coefficients between variables like LSZ and DS, WD, SWL% and PW are summarized in Table 24. Number of live *S. zeamais* are positively correlated with seed damaged ($r = 0.91$, $P < 0.05$), weight damaged ($r = 0.84$, $P < 0.05$), percentage seed weight losses ($r = 0.88$, $P < 0.05$) and weight of powder produced ($r = 0.89$, $P < 0.05$) (Table 24).

Table 24. Pearson Correlation Coefficient of *S. Zeamais* Infestation on Maize Varieties.

Variables	LSZ	DS	WD	SWL	PW
LSZ	1.00				
DS	0.91*	1.00			
WD	0.84*	0.95*	1.00		
SWL	0.88*	0.81*	0.80*	1.00	
PW	0.89*	0.72*	0.66*	0.90*	1.00

LSZ= Number of live *S. zeamais* per 1000g (count) maize, DS (count) = damaged seed. Weight of damaged seed (g), SWL (%) = Percentage Seed Weight loss, PW = Weight of powder produced (g). *Correlation is significant at 0.05 level.

5.4. Discussion

Maize varieties were significantly different with respect to the number of live *S. zeamais*, the number of damaged, weight damaged, percentage seed weight and weight of powder produced. Orange flint corn and yellow and white popcorn exhibited minimum kernel damage and percentage seed weight loss, high mortality and a low number of *S. zeamais* emergence. Likewise, the highest *S. zeamais* population was observed in Indian flint corn, sweet corn and yellow dent corn, and all three exceed 2000 live *S. zeamais* /kg of maize after 90 days (Figure 51 and 52). The growth rate in yellow popcorn was near zero. Moreover, the exponential growth rate was observed in all varieties except yellow popcorn. The main effect due to maize varieties, temperature, and time on *S. zeamais* infestation is shown in Table 22. A significant effect was observed for storage time, temperature, maize by time, and time by temperature on all dependent variables (Table 22).

Further, orange flint corn and two varieties of popcorn show some resistance to *S. zeamais*. This is believed due to be the kernel hardness of these varieties. A similar result was

reported by Suleiman et al. (2015) and Abebe et al. (2009). It has been reported by Keba and Sori (2013) that small kernels like popcorn are hard and compact, thus more resistant to the *S. zeamais* attack. Moreover, it has been reported by several authors that the resistance of grain to stored grain insect attack is attributed to a number of factors include antibiosis, kernel hardness, husk protection, kernel size and pericarp surface texture, starchy amylose content, antifeedants compounds such as phenolic, presence of toxic alkaloids, and grain temperature and moisture contents (Gudrups et al., 2001; Kevin, 2002; Abebe et al., 2009; Keba and Sori, 2013; Gofishu and Belete, 2014; Suleiman et al., 2015). According to Gofishu and Belete (2014) these factors acting alone or in combination to reduce stored grain insect damage. Seed and weight loss was highly correlated with maize varieties and LSZ (Table 24). The highest seed and weight loss was recorded in maize varieties with high progeny emergence compared with those with low progeny emergence or high mortality. This was also related to storage time. Less damage was observed in 30 d than in 60 days. According to Gofishu and Belete (2014) *S. zeamais* development required less development time in soft kernel varieties (31days) while the longer developmental period for the resistant varieties (42 days). Further, according to Abraham (1991) cited by Gofishu and Belete (2014), the extent of damage during grain storage is highly related to two main factors. One is the number of emerging adults during each generation and the other is higher levels of adult emergence. This suggests that maize varieties with high adult *S. zeamais* emergence and a low percentage of mortality were more damaged than those with low progeny emergence and high mortality.

In addition, a high significant difference ($P<0.05$) was observed for temperature in all maize varieties, high damaged was observed at 27 than 10 °C (Table 23). Hagstrum et al.

(1988) found high temperature (25°C) increased the developmental times of eggs and larvae of *S. oryzae* (L). Similar observation was also made by Maier et al. (1996) who reported that the female *S. zeamais* lay few eggs when storage temperature drops below 20°C. Similarly, the study conducted by Burges and Burrell (1964) concluded that reducing storage temperatures to below 17 °C will slow most insect development enough to limit pest damage in grain storage. Another, factor that increases susceptibility to *S. zeamais* to maize is the nutritional quality of variety such as sugar, protein, and amino acid (García-Lara et al., 2004). This concurred with our result as many LSZ was observed on Indian flint and sweet corn (Figure 51 and 52). A significant difference ($P<0.05$) was recorded among maize varieties with respect to percentage seed weight losses and weight of powder produced (Table 23). The highest SWL% was observed on yellow dent corn (42%) and Indian flint corn (33%). However, no significant difference on weight powder produced on observed on yellow dent corn and Indian flint corn (18/1000 g and 17/ 1000 g respectively).

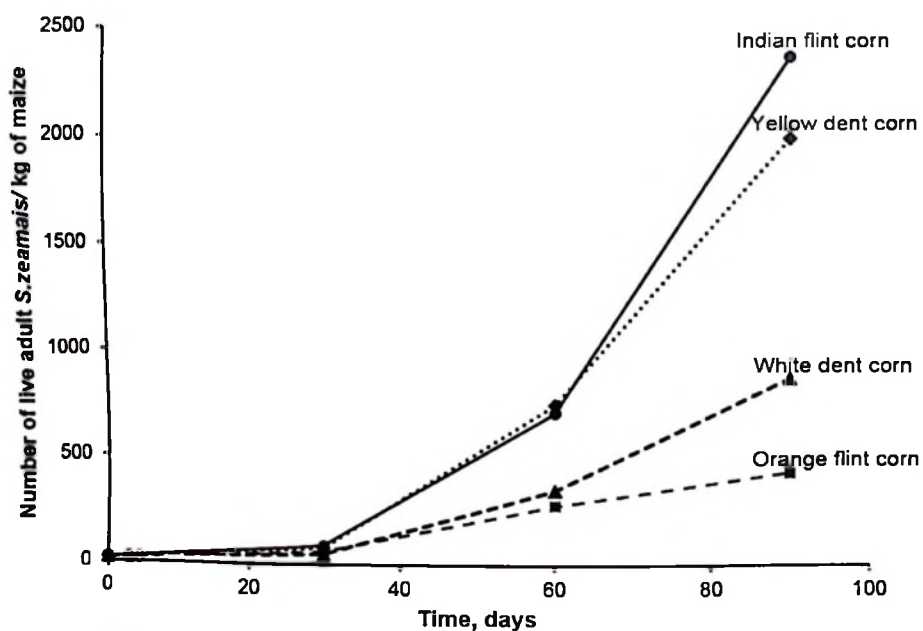


Figure 51. Growth of *S. zeamais* over time on Flint and Dent Corn Varieties at 27 °C.

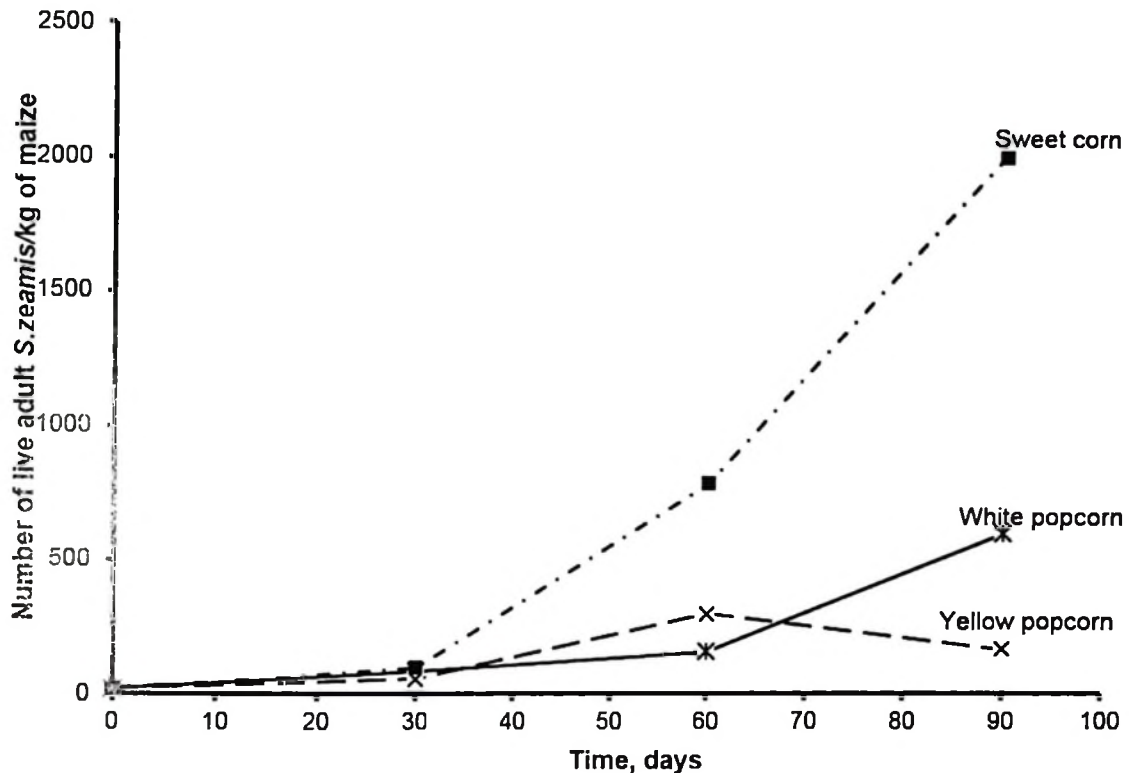


Figure 52. Growth of *S. zeamais* over time on Sweet and Popcorn Varieties of Maize at 27 °C.

5.5. Conclusions

In summary, this research shows that among seven maize varieties evaluated for *S. zeamais* infestation, three varieties (orange flint corn, yellow popcorn and white popcorn) were found resistant to *S. zeamais* infestation based on the number of live *S. zeamais*, seed weight damaged, percentage seed weight losses and weight of powder produced. Thus, orange flint corn and popcorn may be potential maize varieties to be used to reduce the postharvest loss of maize in tropical countries due to *S. zeamais*. Other factors such as yield potential also need to be considered as varieties are related.

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CHAPTER 6. TECHNO-ECONOMIC ANALYSIS (TEA) AND LIFE CYCLE ASSESSMENT (LCA) OF MAIZE STORAGE FOR MIDDLE SIZED FARMERS

Modified from a paper to be submitted to *African Journal of Agricultural Research*

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Abstract

Maize is the most widely cultivated cereal crop worldwide, currently ranked the third most important crop globally after wheat and rice. It is a key staple food in many developing countries. However, maize is produced on a seasonal basis, usually harvest once per year. To maintain a constant supply throughout the year, maize should be properly stored. But this entails high cost and high-energy consumption, which can contribute significant amounts of greenhouse gas emissions. In this study, three storage capacities (25,000 bu, 250,000 bu and 2,500,000 bu) of maize were evaluated for economic analysis and environmental impact. The result shows that the total storage cost per bushel decreased as storage capacity increased (3.68\$/bu, 1.89\$/bu, and 0.40\$/bu). Likewise, energy consumption (electricity, diesel and liquid propane) increased as storage capacity increased. Consequently, more greenhouse gas emissions (CO₂, CH₄, and NO_x) were emitted to the environments. Thus, to obtain an optimal balance between economics and the environment, it is important for the middle-sized family farms to understand the concepts of techno-economic analysis (TEA) and life cycle assessment (LCA).

Keywords: Maize storage, techno-economic analysis, life cycle analysis, greenhouse gasses emissions, engineering economic analysis.

6.1. Introduction

The maize crop is the mostly widely cultivated cereal crop worldwide, together with wheat and rice are the three most important cereal crop in the world. Over 800 million metric tons were produced in 2012/13. The production is expected to double by 2025 and to be the number one cereal crop by 2050 (M'mboyi et al., 2010). Maize is produced on a seasonal basis; usually once per year (FAO/GIEWS, 2014), but consumption is evenly spaced throughout the year (Benirschka and Binkley, 1995). Thus, to maintain a constant supply throughout the year, maize should be properly stored. Grain storage plays a significant role to ensure a constant supply, and in stabilizing the food supply at the household level by smoothing seasonal food production (Tefera et al., 2011).

In addition, proper storage help to minimize post-harvest losses of maize, acts as guarantor for inflation-proof saving banks and improve agricultural income (Tefera et al., 2011). For the government maize grain is stored as a food security reserve, a price stabilization stock, a national storage reserve or strategic reserve, buffer stocks, and production controls (Proctor, 1994). There are two main costs associated with maize storage: fixed and variable costs. Fixed costs are incurred regardless of whether the grain is actually stored in the storage facilities or not, whereas variable costs those that increase or decrease and incurred only when maize is stored (Edwards and Johanns, 2015).

6.2. Methodology

The Life Cycle Assessment (LCA) is a tool that is common used to evaluate the environmental impact or effect of a product, process or systems throughout its life cycle (Roy et al., 2005). In this study, the LCA has been used to evaluate the environmental profile of maize storage. The input data were obtained from different sources. Moreover, the techno-economic analysis (TEA) is as a systematic analysis used to evaluate the economic feasibility aimed to recognize opportunities and threats of projects or product taking into account the capital, variable (operational), and fixed costs (Simba et al., 2012). The Microsoft Excel was used to model LCA and TEA of maize storage for middle- sized family farmers. Table 25 and 26 shows general assumptions and storage scenarios used to build LCA and TEA of maize storage. The information from Table 26 was obtained from multiple sources (www.extension.iastate.edu; www.extension.purdue.edu; Johams, 2016; Uhrig and Maier, 1992; Edwards, 2014; www.electricitylocal.com; www.waterandenergyprogress.org). The length of the harvest period depends on main factors such as the size of the operation, combine speed and capacity, and weather (McNeill and Montross, 2003). In this study, combine ground speed was assumed to be 2.5 miles per hour and combine operate for 12 hours. The total operational time (harvesting, transporting, drying and material handling) varies from one scenario to another. The total operation time were assumed to be 300, 600, and 1000 hours for scenario I, II, and III respectively.

6.2.1. Life Cycle Assessment (LCA)

An LCA comprises four main stages include (Figure 53): goal and scope definition, life cycle inventory, life cycle impact assessment, and interpretation of the results (ISO 14040,

2006; Blengini and Busto 2009). The goal and scope are an essential component of an LCA since the analysis is carried out according to the statements made in this phase, which defines the purpose of the study (Roy et al., 2009). This establishes the functional unit, system boundaries, and quality criteria for inventory data. The goal of this study is to estimate the life cycle assessment of maize storage for middle-sized family farmers. The middle-sized family farmer are those farmers with 50 to 100 ha of land or annual sales between \$100,000 and \$250,000 (USDA, 1997).

Table 25. General Assumptions used for TEA and LCA.

Maize are harvested, dried and stored on farm		
Brand new facility, include combine and transport truck		
Corn (yield)	1 acre	164 bushels
Corn harvested	21	% M.C (wet basis)
Target moisture content	16	%
Bins & dryer service life	25	years
Combine, track service life	15	years
Corn storage time	6	months
Capacity of flight conveyor	80	m ³ /h
Total length of conveyor	10	m
Interest rate (I)	8	%
Electricity cost (1kWh) *	8.01	cents/kWh
All vehicles use gasoline (1 gallon) *	1.99	\$
Liquid propane (1 gallon)	0.995	\$
Fuel consumption for combine	2.24	gallons/ acre
Fuel consumption for truck	4.25	mpg
Liquid propane consumption	0.02	gallons/bu/ per % MC
Flight conveyor size	12 x 34	ft
Dryer size	42"	diameter (9 rings)
Facility (bins & dryer) installation: completed at beginning of year 0		
Capital, fixed and variable costs were only for the first year after installation		

* Price in State of Iowa.

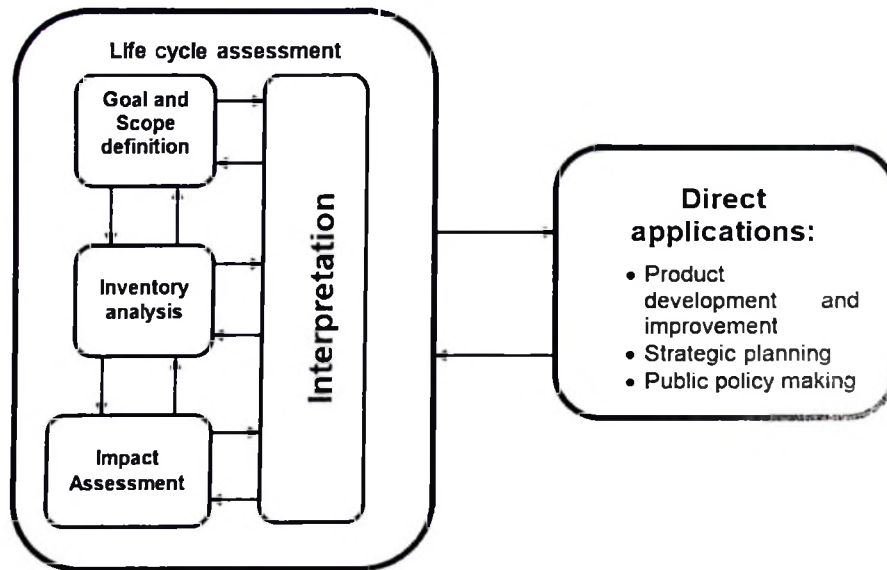


Figure 53. Stages of Life Cycle Assessment (ISO, 2006).

The functional unit (FU) is described as the functional outputs of the product system. It is important for the result of an LCA and depends on the environmental impact category and the aims of the investigation (Schau and Fet, 2008). The purpose of FU is to provide a reference unit to which the inputs and outputs can be related. According to Cederberg and Mattsson (2000), the functional unit is often based on the mass of the product under study. In this study, the functional unit is defined as 1kilogram of maize grain stored. Moreover, the definition of system boundaries affects the outcome of an LCA. The system boundary includes all operations that contribute to the life cycle of the product or process and any activities that fall within the system boundaries (Roy et al., 2009). The system boundaries can be illustrated by a general input and output flow diagram (Schau and Fet, 2008). This includes all input processes to the maize grain storage system, as shown in Figure 54. In this study, farm infrastructure and agricultural input such as fertilizers were not included in the

system boundary. The inventory analysis includes a detailed description of the functions and boundaries of the system, data collection, calculation and assessment of sensitivities and uncertainties.

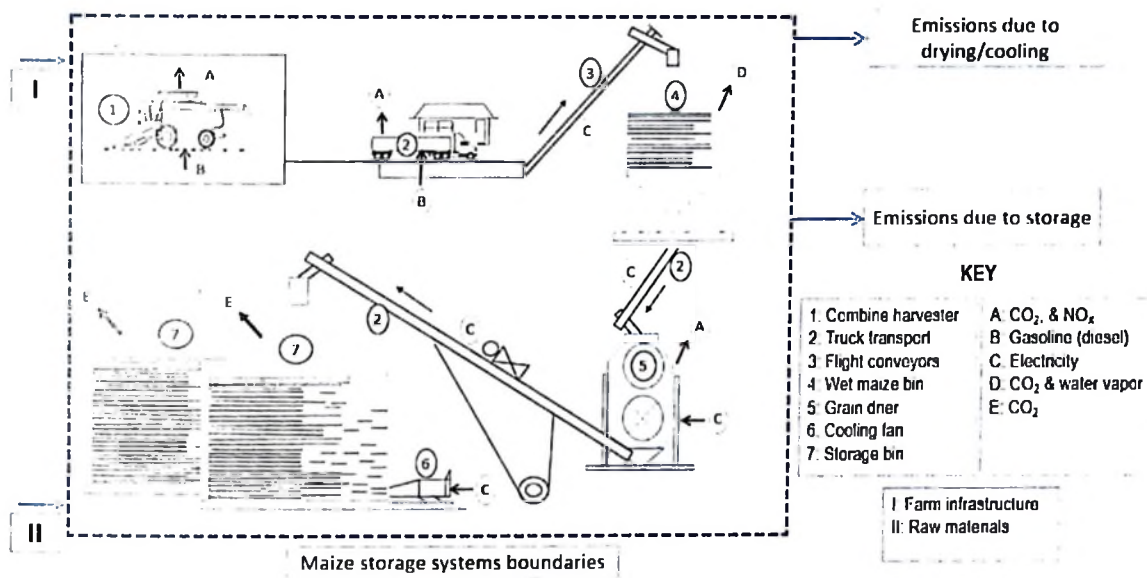


Figure 54. Process Flow Diagram for Farm Scale Maize Storage.

6.3.2. Techno-Economic Analysis (TEA)

The investment costs of grain storage can be divided into two main categories. The first category includes the cost due to equipment, this is the largest cost of storage facilities it combines the costs of storage bins, dryers, conveyance equipment, grain carts, and the truck. The second category is the cost due to building it comprised the costs of space, concrete floor, and bin erection. The equipment cost data was collected from several manufacturers and vary by size. The cost of a concrete floor and erection was estimated according to Dhuyvetter et al. (2007). Storage capacity has a significant effect on investment cost. In general, the larger the storage facility the lower the investment cost per unit (\$/bu/y).

Table 26. Production Scenarios used for TEA.

	Scenario		
	I	II	III
Daily storage input (bu/d)	50	500	5,000
Total storage capacity (bu)	25,000	250,000	2,500,000

In addition, the fixed costs are costs related to storage facilities and equipment ownership. Typical fixed costs in grain storage facilities include depreciations, interest, overhead, taxes, handling, repairs and insurance cost. Conversely, variable costs are the main cost of grain storage, it includes the costs that are only incurred if grain is stored (Brennan and Lindner, 1991). It is a parameter that change and depends on the amount of grain stored and the length of the storage period (Pardey et al., 2001; Dhuyvetter et al., 2007). Include costs such as labor, management, trucking in and out of storage, insecticides, interest in the grain, and cost of energy (e.g. liquid propane and electricity) for grain drying (Reff, 1983).

6.3. Results and Discussion

6.3.1. Techno-Economic Analysis (TEA)

In this study, three main storage scenarios were evaluated. An outline of the farm structure and material flows are shown in Figure 54 and 55. Scenario one was baseline and assumed 25,000 bu of maize. The second and third scenarios were 250,000 bu and 2,500,000 bu respectively. The maximum storage time of maize was six months.

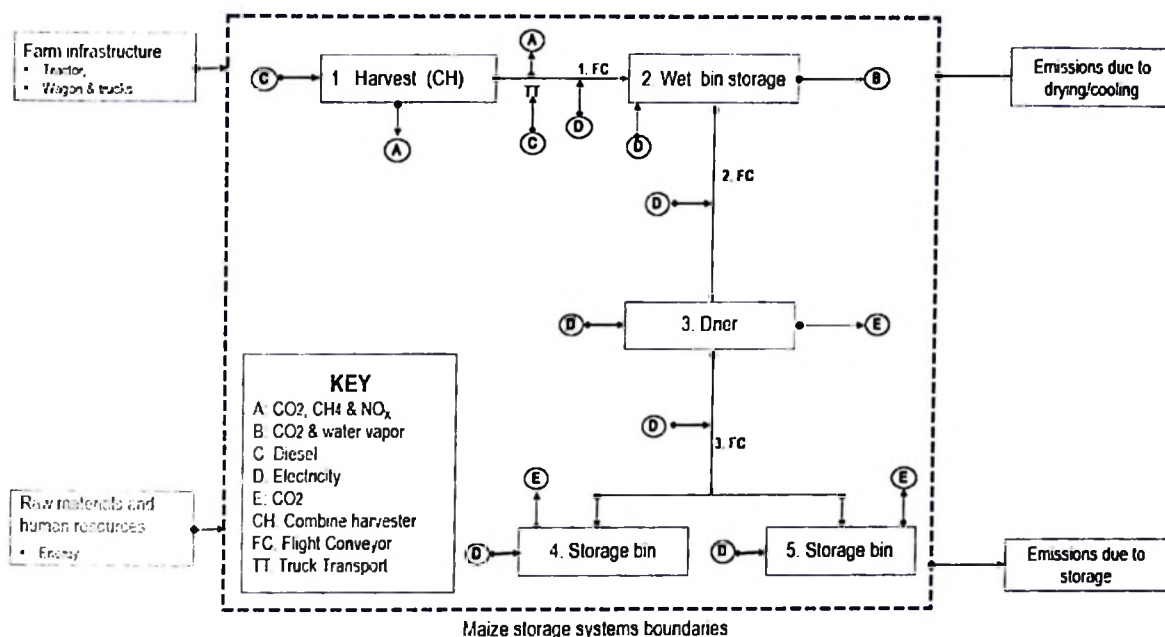


Figure 55. Process Flow and Systems Boundaries for Farm Scale Maize Storage.

Another important cost associated with grain storage is the interest cost. The interest fixed cost is the major part of total storage cost and it is the combination of the interest due to investment (equipment and building) and interest due to maize being stored. The interest cost of grain is the largest cost because it includes the rate of existing loans and the rate of return on investment (Reff, 1983). According to Wright (2011), when the interest rate is falling or if it remains low, it will encourage greater storage or higher stocks and subsequently stabilize and lower the grain prices. Moreover, the supply and demand also have significant influence on prices of corn. According to Westcott and Hoffman (1999), the prices of wheat and corn are determined by the interaction of the supply and demand functions.

Furthermore, the fixed costs contribute a large component of the total costs in commercial grain operation (Kenkel, 2008). In general, the fixed costs comprised about 64% of the total operation costs in grain storage facilities (Schnake and Stevens, 1983). In this

study, investment interest rate was calculated as 8% of the total equipment and building cost. For simplicity, straight-line depreciation (i.e. purchase price, minus salvage value divided by its estimated useful life) was used. As shown in Figure 56, the fixed cost per kg decreased as the storage capacity increased. This concurred with the surveys conducted by Baumel (1997) in Iowa between two crop years (1993 to 1995) show that as crop production increasing handling and storage costs, decreasing from \$0.152 per bushel for 2 million bushel to \$0.103 for 4.4 million bushel.

The variable costs include the operating cost such as utilities (electricity) for drying, lighting, and conveyance; it also contains labor and management costs as well as the cost of insecticides, turning and aeration, liquid propane and others related costs of operations (Kenkel, 2008; Pardey et al., 2001). The cost for electricity and liquid propane depends on the initial and final moisture contents of maize, airflow rate, and time of drying. In addition, the cost of electricity for aeration, augers, and conveyance; differs from one place to another and mainly depend on the cost of electricity per kilowatt-hour (kWh), motor size, and time of aeration. Another important parameter to incorporate in variable costs were shrinkage and handling losses. Maize like other grain, loses moisture during storage, so it loses weight as well. The weight loss is called “shrinkage”, hence maize is sold based on weight shrinkage should be considered (Alexander and Kenkel, 2012). Moisture shrinkage is calculated by using equation 14. Likewise, the handling losses or “invisible shrink” is the weight loss due to dry mater. It include mechanical losses from broken kernels and foreign material, and loss of volatile compounds (oil), (Hicks and Cloud, 2001). The handling loss of grain or corn depends on several factors such as method of drying, the handling processes during drying, physical quality of the corn, and how long the corn is dried (SDSU, 2014). According to

Iowa State University researcher the handling loss for on-farm ranged from 0.22 to 1.71% (Hicks and Cloud, 2001). In this study, the handling loss was assumed to be 0.5%.

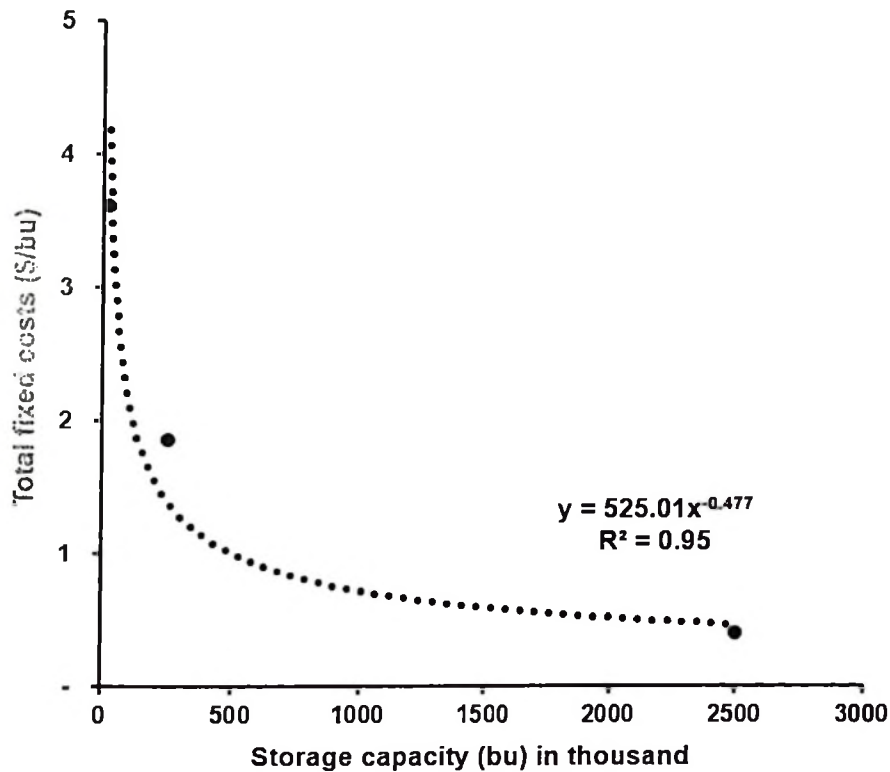


Figure 56. Total Fixed Cost of Maize Storage for Middle Scale Farmers.

$$\text{Percentage moisture shrinkage(\%)} = \frac{M_i\% - M_f\%}{100 - M_f\%} \times 100 \quad 14$$

Where M_i and M_f = initial and final moisture content respectively, for our case initial moisture content was assumed to be 20% and final moisture content to be 14%, hence the moisture shrinkage = 6.97%.

$$M.S(\%) = \frac{20 - 14}{100 - 14} \times 100 = 6.97\%$$

The variable cost per bushel decreased as the amount of grain stored increased (i.e. 0.16\$/bu, 0.07\$/bu, and 0.04\$/bu). This contributed by many parameters like decrease in cost of electricity. Normal the overall cost of electricity decrease when exceeding a certain amount of kilowatt-hour per month. Furthermore, the total storage cost was by adding up the the operational and fixed cost. In this study, the total storage cost per kg decreased as storage capacity increased. The estimated total storage costs per bu were 3.68\$/bu, 1.89\$/bu, and 0.42\$/bu for the scenario I, II, and III respectively (Figure 57). The result concurred with those reported by Valente et al. (2011), that higher reduction storage costs and economic viability occurred when the amount of stored product increased. However, the values were for scenario I and II seem higher than those estimated by Edwards (2015) who reported cumulative storage costs for corn to be around 0.45 cents and 0.70 cents per bushel for on-farm storage and commercial rental storage respectively.

6.3.2. Life Cycle Assessment (LCA)

The results of LCA are summarized in Table 27. The results indicate the environmental impact generated from maize storage increased as storage capacity increased. Energy was main parameter determined in an LCA of maize storage. In this study, the energy usage was divided into two main parts: electricity and fossil fuel (diesel and liquid propane). The electricity used for drying, lighting and other operations in maize storage ranges from 0.33kWh/bu to 0.78kWh/bu (Figure 58). Electricity was primary energy used in almost all activities except on trucks. The total fuel consumption (diesel) used for combine and transport trucks increased as storage capacity increased from around 605 gallons for scenario one to 34,410 gallons for scenario three.

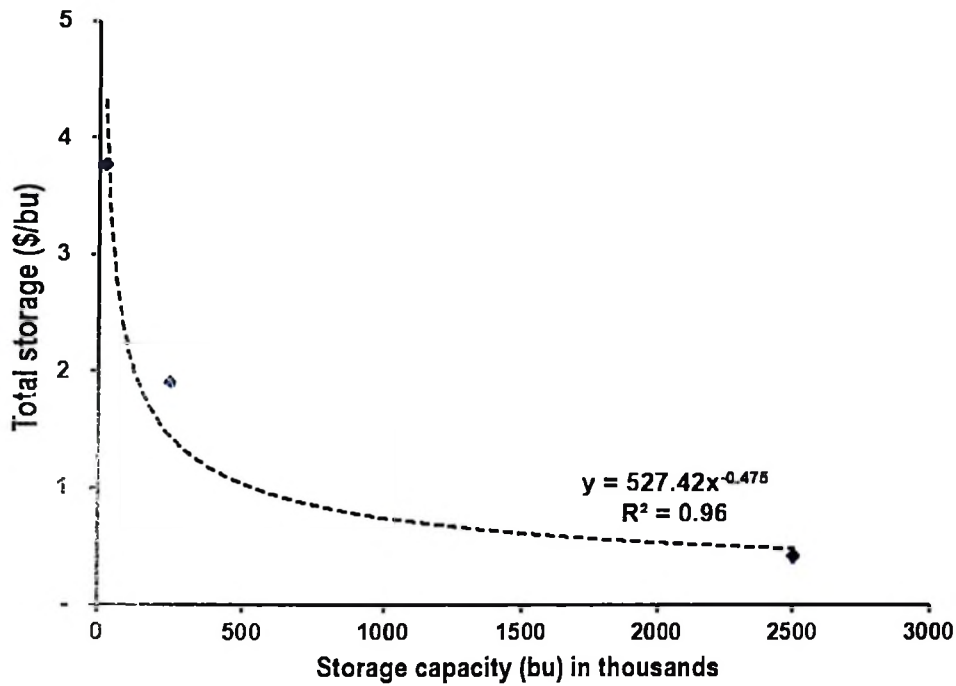


Figure 57. Annual Total Maize Storage Cost Per \$ Per kg/y for Middle- Sized Farmers.

In addition, liquid propane was also used in the dryer. The emission was calculated based on assumption made earlier. The result shows energy usage is proportional to storage capacity and emission production increased and this agreed by many authors (Searchinger et al., 2008; Norman et al., 2006; Kim and Dale, 2005).

Table 27. Distribution of the Emissions of three Maize Storage Scenarios for Middle-Sized Farmers.

Scenario	Capacity (bu)	Energy (kWh/bu)	Kg-CO ₂ (Mg/y)	Kg-Nox (Mg/y)	Kg-H ₂ O vapor (Mg/y)	Kg-CO ₂ eqv (Mg/y)	Kg-CO ₂ eqv (Mg/y)
I	25,000	0.33	231.33	0.02	16.12	0.00	3.54E ⁻¹¹
II	250,000	0.51	357.10	2.72	24.18	13.66	5.46E ⁻⁰⁵
III	2,500,000	0.78	546.01	4.15	32.24	208.84	8.35E ⁻⁰⁵

6.3.2.1. Greenhouse Gasses Emissions

Many studies agreed that GHG's emission, especially CO₂ emissions as leading causes of climate change or global warming (Soytas et al., 2007; Zhang and Cheng, 2009; Halicioglu, 2009). According to the World Bank reports CO₂ is held responsible for over 50% of the total global GHG emissions (World Bank, 2007). Outlined in the Intergovernmental Panel on Climate Change (IPCC) guidelines that CO₂ emissions data are based on estimates.

Emissions from different sources such as agricultural production and grain storage can be measured directly or continuously depend on applications (Bastianoni et al., 2004). In the maize storage study, CO₂ emissions were calculated by adding together all main sources of CO₂. The results show CO₂ emissions were the highest contributor of GHS's emissions. Similar results have been reported by Roy et al. (2005) in the production and post-harvest of rice in Japan, and Carlsson-Kanyama (1998) in the storage and transportation of tomato, imported from Israel. The system boundary in this study started at harvest, hence, CO₂ emissions from the field were not included in the calculation, and CO₂ emissions due to human respiration were considered negligible compared to another source of CO₂ emissions such as trucks. The emission varied from the scenario I to scenario III. Higher CO₂ emissions were observed in scenario III (Table 27). Additionally, the results indicated that the CO₂ emissions have a significant impact on maize storage and it is directly proportional to energy consumption. This result supported by other authors. For instance, Zhang and Cheng (2009) found a strong tie between carbon emissions, energy consumption, and economic growth in China. According to Roy et al. (2009), greenhouse gas emission increased remarkably due to increasing in energy use. Likewise, Acaravci and Ozturk (2010) show a positive relationship between energy usage, CO₂ production and economic growth in several European countries.

In addition, the study conducted by Soyta et al. (2006) in the US found in the long run the main causes of carbon dioxide emissions is energy consumption.

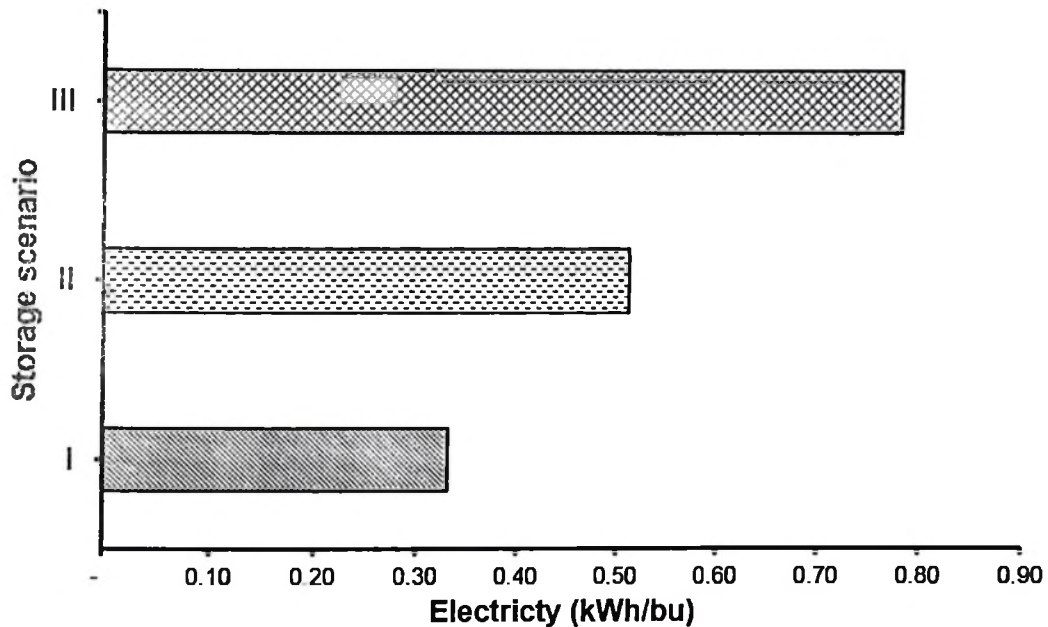


Figure 58. Electricity Usage in three Different Scenario for Middle-Sized Farmers.

6.3.2.3. CH₄, NO_x and CO₂ Equivalent Emissions

Many governments around the world have implemented strong policies to reduce GHG emissions from agriculture, especially CH₄ and NO_x (Boadi et al., 2004). Research conducted by Beauchemin et al. (2010) revealed that collectively CH₄ and NO_x accounting for over 30% of the total global GHG emissions. Methane is generated in the atmosphere through anaerobic activities of microorganism like *Methanobacterium Omelianskii* bacteria, the many sources of CH₄ to the atmosphere from agriculture activities are paddy rice production fertilized with urea, animal wastes, biomass burning, and enteric fermentation in

ruminant animals (Duxbury, 1994). However, in this study, no CH₄ gas was emitted to the environment because we only focused on storage of maize. In addition, N₂O emissions from agriculture, mostly coming from nitrogen fertilizers and manure application (Popp et al., 2010; Kim and Dale, 2005). Likewise, another major source of NO_x identified by many scientists is fossil fuel combustion (Delmas et al., 1997). In the case of NO_x in this study all comes from fossil fuel. The results of NO_x emissions show a direct relationship between storage capacity and NO_x production. As expected, the highest NO_x emissions were observed at scenario III. Furthermore, to determine GHGs emissions, all emissions were converted to CO₂ equivalents, this was done by adding CO₂, NO_x, and H₂O vapor. The highest CO₂ equivalent was observed at scenario III, followed by scenario II and scenario I. The result shows CO₂ equivalent per kg increased as storage capacity increased (Table 27).

6.4. Conclusions

In this chapter, the techno-economic analysis and the life cycle analysis of maize storage were evaluated with three different storage scenarios. The result shows as storage capacity increased, the total storage cost per bushel decreased. Similar results were obtained for fixed costs. Conversely, for the LCA, the study found a direct relationship between energy usage and storage capacity. As storage capacity increases more energy is required to operate the equipment. Likewise, higher carbon dioxide emissions were found on scenarios three. Therefore, the higher storage capacity, the lower the total storage cost per kilogram, the higher energy consumption, the more CO₂ produced. Consequently, more GHG's emissions were emitted.

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CHAPTER 7. MEASURED AND PREDICTED TEMPERATURE OF MAIZE GRAIN (*Zea mays* L.) UNDER HERMETIC STORAGE CONDITIONS

A paper published in the *Journal of Stored Products Postharvest Research*

Suleiman, R., Rosentrater, K. A

Abstract

The physical properties of grain such as temperature and moisture content are two key factors in grain storage. In this study, three different storage conditions (room at 25°C; cooling at 4°C; and freezing at -20°C) were investigated. Yellow dent corn (*Zea mays* L.) variety Blue River 571136 from Iowa, harvested in 2011 was used. Maize grain was stored in two hermetical sealed bins (50-cm diameter x 76-cm height). Five logger sensors were installed inside the bin to measure temperature and relative humidity of the maize grain. The sensors were located at the top, center, bottom, left and right at about 12 cm apart. After placing each barrel into storage, temperature and relative humidity values were measured every minute for 9 days throughout the duration of the experiment. Model validation was carried out by comparing predicted with measured maize grain temperature data in the radial and vertical directions. The temperature in the hermetically sealed cylindrical bins varied, mostly in the radial direction and very little in the axial vertical directions. No noticeable change in temperature was observed in room condition. Moreover, the temperature in the grain changed more rapidly in the freezing conditions than in the room and cooling conditions. Furthermore, the lag time between the center temperature and the side (right, left, top, and bottom) was greater in the radial direction compared to vertical temperature. The maximum difference between predicted and measured temperature was $\pm 1.5^{\circ}\text{C}$. The predicted and measured values of maize grain temperature at radial and vertical directions were found to be in good agreement. The model shows a good potential application to predict the temperature of maize grain stored at the room, cooling and freezing conditions under hermetic storage.

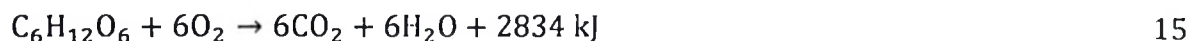
Keywords. Maize, Grain temperature, Grain storage, Hermetic storage, modeling.

7.1. Introduction

The knowledge of physical and thermal properties of grain is essential to the food engineers, processors, and grains ecologists for the effective designing of machine, storage structures, heat transfer optimization, and bulk storage (Amin et al., 2004; Mohamed, 2009). The physical properties of grain such as moisture content and temperature are two key factors in grain storage. Temperature and moisture content are the main causes of grain spoilage in stored-grain ecosystems (Manickavasagan et al., 2006). As reported by many researchers that moisture content and temperature are the two key factors in maintaining grain quality during handling, storage and are the major sources of grain deterioration because encourage the growth of mold and infestation of insects (Parry, 1985; Jin, 1996; Flinn et al., 1997). Likewise, mentioned by Hong et al. (1997) and Ng (1994) that temperature, moisture content, and relative humidity are three major factors influence storage conditions of grain. Furthermore, revealed by Jayas and White (2003) that the temperature and moisture content are two physical factors that control deterioration and quality of grain in storage. In addition, cited by White (1995) that the growth and multiplication of biological agents such as insect and mold in grains are highly dependent on the presence of temperature and moisture content.

Moreover, temperature and moisture content can be used to predict grain drying and deterioration potential (Iguaz et al., 2000). Temperature is regarded as a physical variable in grain storage, the variability comes from two main sources internal and external. Internal are those comes from grain respiration (Eqn. 15), insects, mites, rodents and other microorganisms.

While, external sources are mainly coming from the solar radiation and surrounding area around the storage bin (Jia et al., 2000; Andrade et al., 2002).



In addition, grain kernels like other living substances continue to respire after harvest releases CO₂, water vapor and heat (Iguaz et al., 2004). Consequently, increased the temperature of grain and moisture migration creates favorable conditions for insects and mold to nourish (Suleiman et al., 2013; Iguaz et al., 2004). Carbon dioxide released by grain and other organisms is used as a parameter of grain deterioration and directly related to dry matter loss of grain (Sharp, 1982). Temperature is a single most important non-biological factor controlling the rate of deterioration of grain in storage (Muir and Viravanichai, 1972) and can be easily measured and simulated mathematically (Yaciuk et al., 1975). Moreover, temperature and moisture content can be modeled mathematically to optimize storage conditions and efficient control measured at any point of a storage bin (Lawrence et al., 2013; Iguaz et al., 2000). Further, Sutherland et al. (1971) show mathematical models based upon physical and thermal properties of grain as a useful tool to predict grain conditions in a storage bin.

Furthermore, mathematical models have been used as initial tools for predicting physical factors like temperature and moisture content in grain storage (Jin, 1996; Yaciuk et al., 1975). In addition, mathematical simulation can be used to predict the temperature distribution in grain storage structure with different shape and sizes, grain varieties, and locations (Jia et al., 2000). The main advantages of the mathematical model outline by Andrade et al. (2002) and others include lower cost and it takes less time than it is needed in

the experimental investigations. It allows analyzing systems that are impossible to accomplish by experimental investigations and it allows the complementation experimental investigations with more detailed information (Andrade et al., 2002; Jian et al., 2005; Franca et al., 1995). Several three-dimensional heat transfer models have been developed for simulating grain storage temperature in the cylindrical bin (Andrade et al., 2002; Jian et al., 2005; Lawrence et al., 2013; Jayas et al., 1995). Thus, the consequences of variations in the dimensions, geometry, properties of the materials and external conditions can be easily studied by using computer simulation (Franca et al., 1995). Although, several 3D heat transfer models have been established. However, the 3D heat transfer model in grain storage under hermetic conditions has not yet been developed. Therefore, the aim of this study was to develop a mathematical model to optimize storage condition of maize in a cylindrical bin in the room, freezing and cold temperature under hermetic condition.

7.2. Materials and methods

7.2.1. Experimental Setup and Procedure

A temperature equilibrium experiment was conducted at a water quality, laboratory - Iowa State University; the laboratory was fitted to Norlake Scientific RSF5 compartments chamber (cold room at 4°C and freezer at -20°C). Maize of commercial hybrid Blue River 57436 with initial temperature and moisture content of 21°C and $14.5 \pm 0.5\%$ (wet basis) respectively was used. Two cylindrical plastic barrels, 50 cm diameter by 76 cm by height, were filled with maize to a height of 45 cm and airtight (hermetic) to maintain uniform conditions, each was fitted with five sensors from omega engineering, Inc. models OM-EL-USB-2-LCD and OM-EL-USB-2-LCD-PLUS placed about 12 cm apart (top, center, bottom,

right, and left) as shown in Figure 1 to measure internal temperature, dew point, and relative humidity of maize. The barrels were stored at room temperature for 72h, then move to the cooler for 72h, move back to the room for another 72h. The same procedures were repeated for freezer condition. Censors were set to record the data after every 5 min for 9 days of each condition. Then censors were carefully removed and the data were downloaded to the computer and analyzed.

The following assumptions were made while developing this model:

1. Maize was assumed to be free of arthropod populations and mold growth.
2. Conduction where the only heat transfer between the grain bulk (in the horizontal and vertical direction) and the sides of the bin.
3. Properties of maize grain remain constant.
4. Initial maize temperature, T_o , is at a specified temperature.
5. The maize temperature at the center of the bin ($r = 0$) is a finite.
6. Ambient temperature, T_a , or grain temperature at the wall, ($r = R$)

7.2.2. Model Development

The partial differential equation describes the heat transport inside the grain storage bin under the cylindrical coordinate system (Figure 59) follow Fourier's law (Incropera and De Witt, 1996; Andrade et al., 2002; Mills, 1995). The heat conduction equations are

$$q_r = -k \frac{\delta T}{\delta r}; \quad q_\phi = -\frac{k}{r} \frac{\delta T}{\delta \phi}; \quad q_z = -k \frac{\delta T}{\delta z}; \quad 16$$

Where q_r is the component of the heat flux in the r direction, $\frac{\delta T}{\delta r}$ is the partial derivative of $T(r, \phi, z, t)$ with respect to r, same for ϕ and z directions.

$$q = k\nabla^2 T \quad 17$$

$$\begin{aligned} \frac{1}{r} \frac{\delta}{\delta r} \left(kr \frac{\delta T}{\delta r} \right) + \frac{1}{r^2} \frac{\delta}{\delta \phi} \left(k \frac{\delta T}{\delta \phi} \right) + \frac{\delta}{\delta z} \left(k \frac{\delta T}{\delta z} \right) + \frac{\dot{q}}{\rho c_p} \\ = \frac{\delta T}{\delta t} + v_r \frac{\delta T}{\delta r} + v_\phi \frac{\delta T}{\delta \phi} \\ + v_z \frac{\delta T}{\delta z} \end{aligned} \quad 18$$

For a stationary materials like maize grain ($v_r = v_\phi = v_z = 0$), hence equation (18) will simplifies to

$$\begin{aligned} \frac{1}{r} \frac{\delta}{\delta r} \left(kr \frac{\delta T}{\delta r} \right) + \frac{1}{r^2} \frac{\delta}{\delta \phi} \left(k \frac{\delta T}{\delta \phi} \right) + \frac{\delta}{\delta z} \left(k \frac{\delta T}{\delta z} \right) + \dot{q} \\ = \rho c_p \frac{\delta T}{\delta t} \end{aligned} \quad 19$$

Where r, ϕ , and z are the cylindrical coordinates, k is the thermal conductivity of maize ($\text{W/m}^\circ\text{C}$), ρ is the specific mass or density of maize (kg/m^3), C_p denotes the specific heat of maize in ($\text{kJ/kg}^\circ\text{C}$), t is the time in s, T is the temperature of the maize kernel ($^\circ\text{C}$), and \dot{q} is the rate of generation of heat as function of (r, ϕ, z , and t) in W.

The boundary and initial conditions for the equation (19) are:

$$\begin{aligned} \text{at } t = 0, \quad 0 \leq r \leq R: T \\ = T_{in} \end{aligned} \quad 20$$

$$\begin{aligned} 0 \leq r \leq L: \\ = T_{in} \end{aligned} \quad 21$$

Where, T_{in} – initial temperature, T = temperature at time (t) and point (x), F_o = Fourier number = $(\alpha t/L^2)$, with δ_n being roots of the Bessel function $J_0(R \delta_n) = 0$.

The validation of the simulated model was done by comparing the simulated temperatures with measured temperatures.

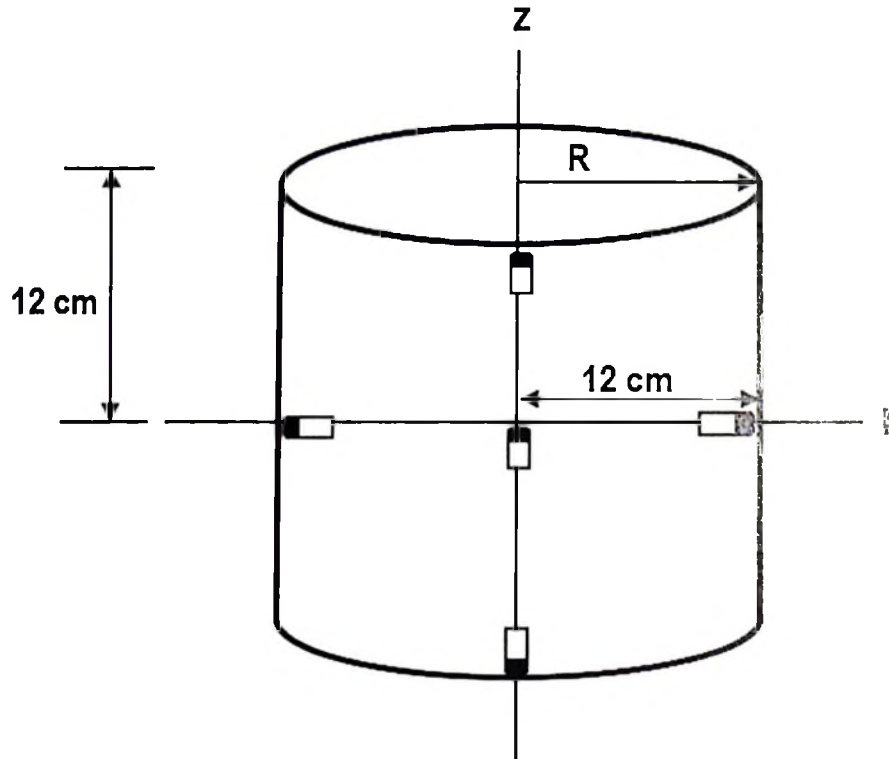


Figure 59. The Cylindrical Geometry Shows Temperature/Relative Humidity Sensor Arrangement.

7.2.3. Physical Properties of Maize Grains

The value of the thermal and physical properties of the maize grains and plastic cylindrical bins used in the simulation is presented in Table 28. Thermal properties of maize grain (the thermal conductivity and the diffusivity) were determined by a thermal properties meter (KD2, Decagon Devices, Pullman, Wash).

Table 28. Thermal and Physical Properties for Corn and Plastic Barrel Bin.

Property	Maize	Plastic barrel (bin)*
Specific heat (J/kg/C)	1851.5	16700
Thermal conductivity (W/m/K)	0.1618	0.50
Thermal diffusivity (m ² /s)	1.21 x 10 ⁻⁷	---
Density (kg/m ³)	1247	---
Initial moisture content (% w. b.)	12.7	---

ASAE, (2000; <http://www.engineeringtoolbox.com/>).

7.3. Results

The measured and predicted maize grain temperatures at five different positions (top, center, right, left and bottom) in the cylindrical bin filled with maize grains were being determined. Temperatures were monitored at three different conditions (room, cooler, and freezer) in vertical and radial directions.

7.3.1. Room and Cooling Condition in the Vertical and Radial Directions

The predicted and measure grain temperature in a vertical direction at room temperature are shown in Figure 60. The grain temperature at the bottom of the bin was first to change followed by top grain temperature and the center grain temperature was last to change. One possible explanation is due to the fact that at the bottom of the grain temperatures was too close contact with the ground concrete floor, thus why it was first to change. The predicted temperatures were closely matched to the measured temperature.

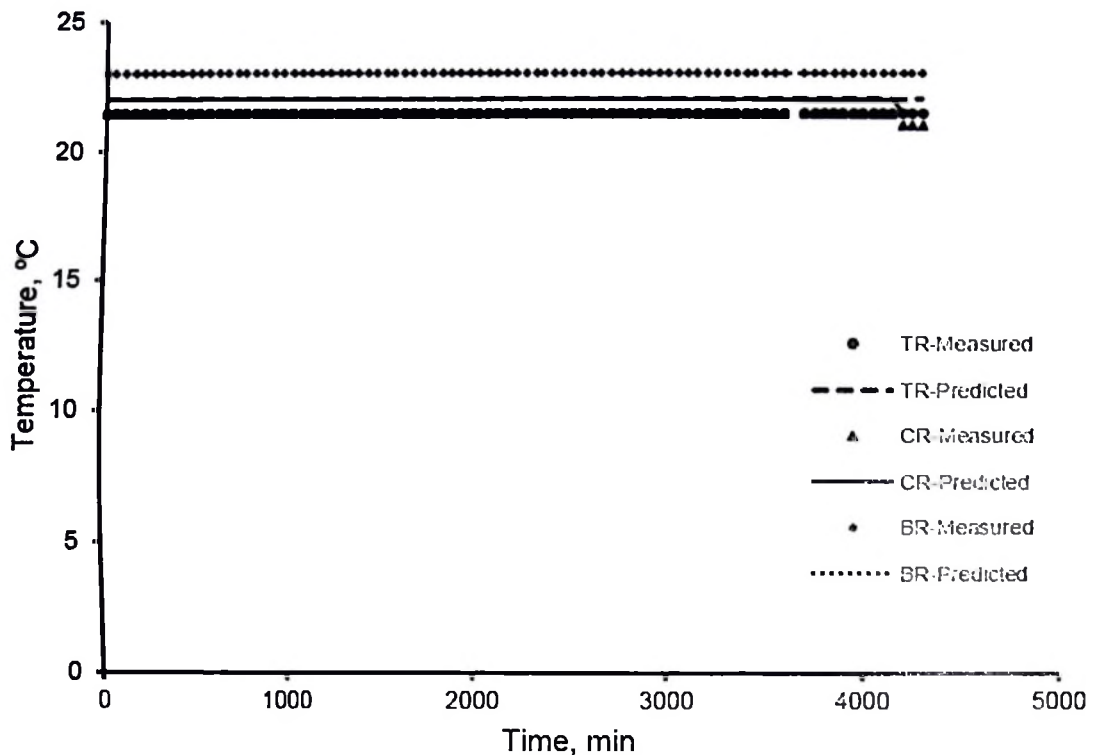


Figure 60. Measured and Predicted Maize Grain Temperature over Time at Room Temperature in the Vertical Direction (Before to the Cooling Experiments). *TR*= *Top Room*, *CR*= *Center Room*, *BR* = *Bottom Room*.

Furthermore, Figure 61 shows the grain temperature at the cooler condition in vertical directions. The grain temperature at the bottom decreases faster at the first 500 min and slowly afterward. For the center, grain temperature was lagging and it takes approximately 600 min before start to drop. Similarly, the top grain temperature was between bottom and center as shown in Figure 61. The predicted temperatures are in excellent agreement with the measured temperature. Moreover, Figure 61 indicates warm temperature condition when grain bins were taken out the cooler. Like in cooler conditions, the bottom temperature was first to change follow a smooth curve as shown in Figure 61. For the top, the temperature it takes approximately 300 min to change and then it the take same path as bottom temperature.

Likewise, the center temperature was lagging it take almost 500 min before the center temperature to respond as seen in Figure 61. All predicted temperatures were in agreement with the measured temperature.

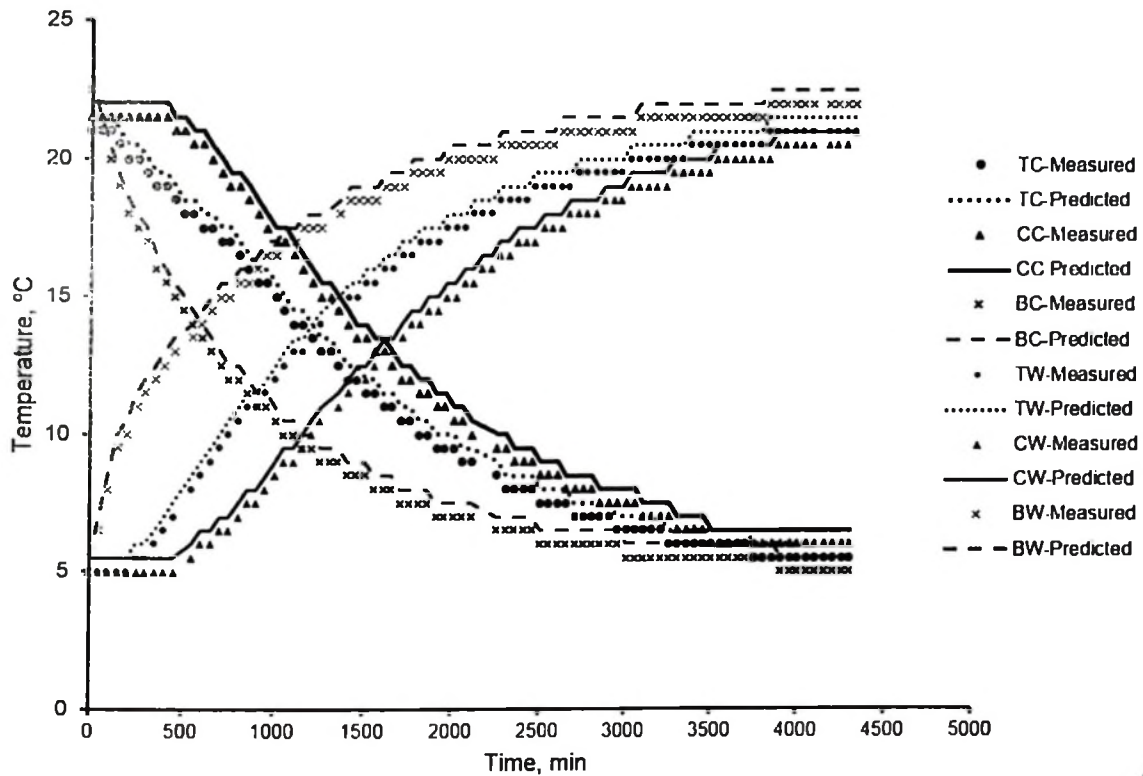


Figure 61. Measured and Predicted Maize Grain Temperature over Time from the Room to Cooler and from Cooler to Back Room Temperature in the Vertical Direction. *TC = Top Cooling, CC= Center Cooling, BC= Bottom Cooling, TW = Top Warming, CW= Center Warming, BW= Bottom Warming.*

In addition, the predicted and measured temperatures in a radial direction (left, center, and right) at room temperature are illustrated in Figure 62. The result shows the measured temperature on the right side was the first one to change followed by left while the center temperature was between right and left temperature. The predicted temperatures were in the same path as measured temperatures. Furthermore, Figure 63 shows the combined results of

the temperature changes from room to cooler and from the cooler back to the room. The result indicates that the right temperature was first to change followed by right and center (Figure 63). The predicted temperatures were similar to the measured temperature as shown in Figure 63.

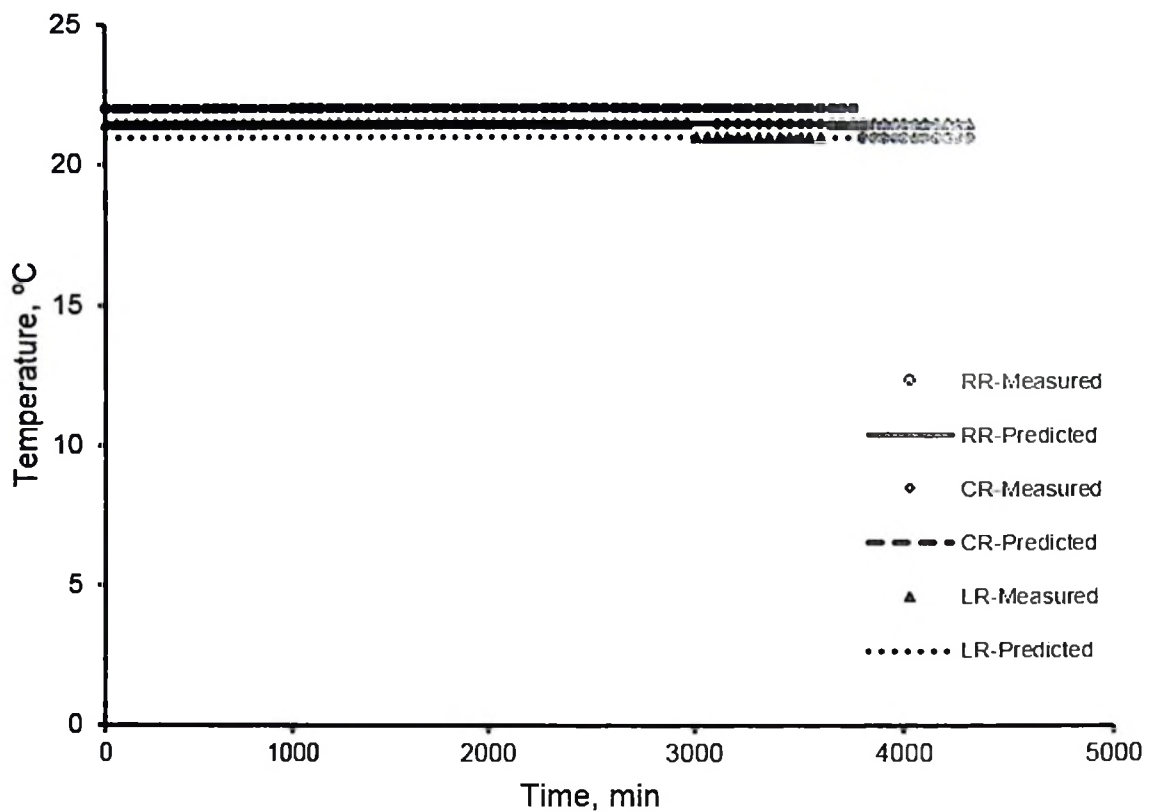


Figure 62. Measured and Predicted Maize Grain Temperature over time at Room Temperature in the Radial Direction (Before to the Cooling Experiments). *RR= Right Room, CR= Center Room, LR= Left Room.*

Furthermore, for the cooler condition, the result shows the temperatures in the boundaries (left and right) were dropped at the same time and reached a steady state after about 4000 min. While the temperature at the center was lagging for about 500min and drops sharply afterward and relatively constant at a lower temperature (Figure 63). In warming conditions,

the same trend was observed for the right, left, and center temperature as shown in Figure 63. These results also show that the predicted temperatures were similar to the measured temperature.

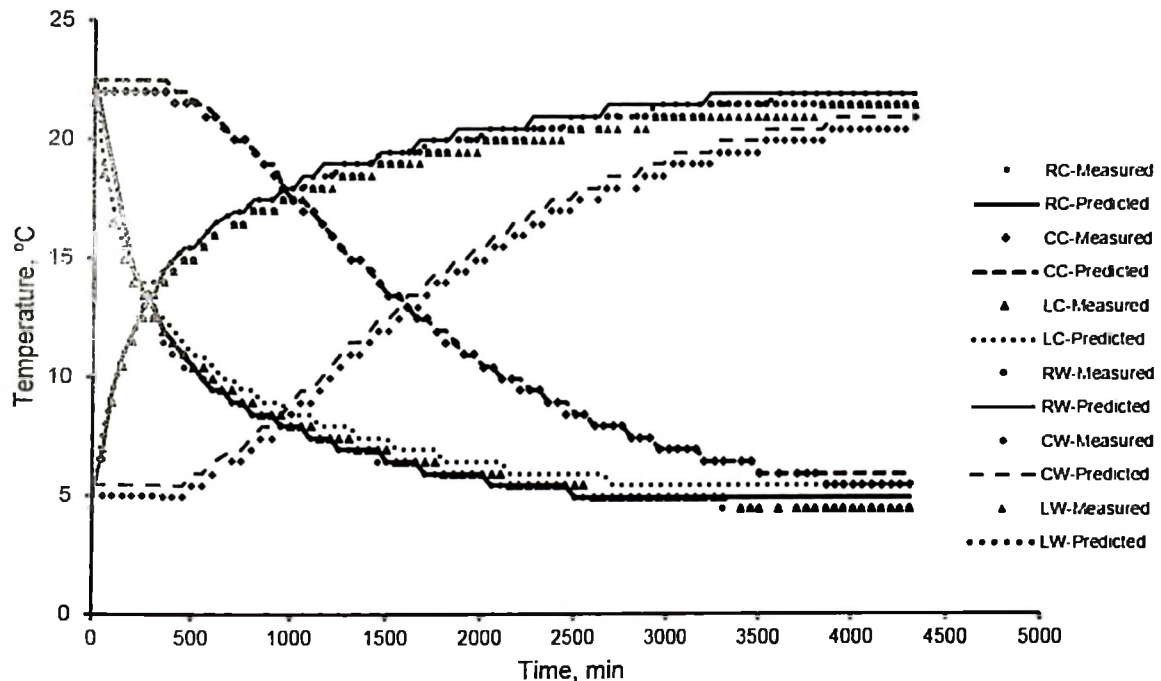


Figure 63. Measured and Predicted Maize Grain Temperature over Time from the Room to Cooler and from Cooler to Back Room Temperature in the Radial Direction. *RC= Right Cooling, CC= Center Cooling, LC= Left Cooling, RW= Right Warming, CW= Center Warming, LW=Left Warming.*

7.3.2. Room and Freezing Condition in the Vertical and Radial Directions

The temperature change in freezing conditions in the vertical direction at room temperature is shown in Figure 64. Slight variation between the top, bottom and center temperature was observed. Bottom temperature was first to respond and center was last to change. Moreover, the predicted temperature was a close tie with measured temperature (Figure 64). The temperature change from room to the freezing condition is shown in Figure

64. The bottom temperature was sharply decreased at the initial 600 min and slowly afterward. The top was second to change and was laid between bottom and center temperature. Likewise, the center temperature was last to change as shown at Figure 64. The predicted temperatures for the room and freezing to room temperature were very close to measure temperature.

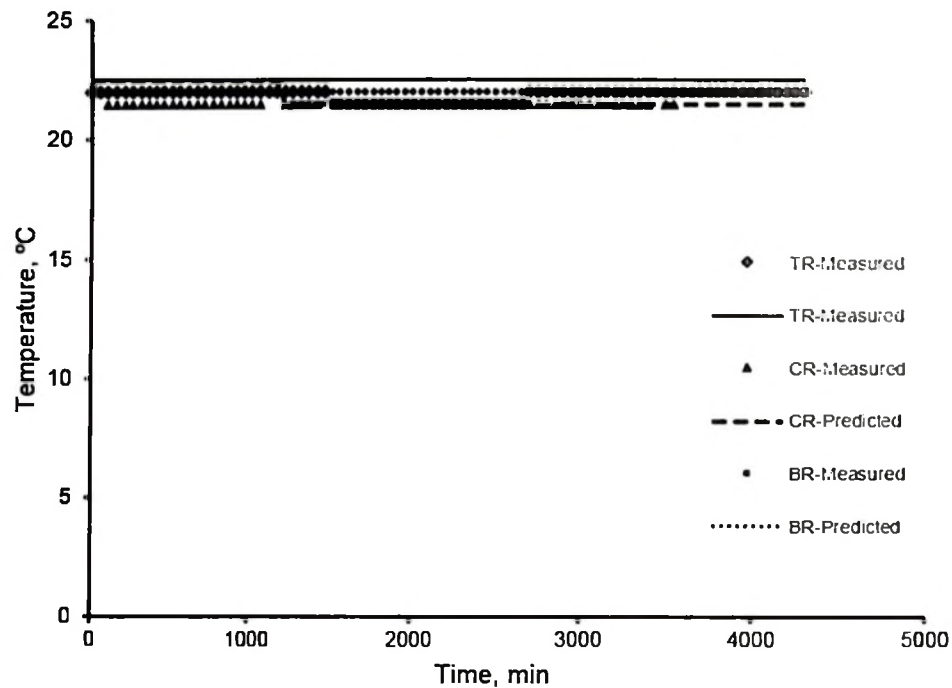


Figure 64. Measured and Predicted Maize Room Temperature Changes over Time at Room Temperature in the Vertical Direction (Before to the Freezing Experiments). *TR* = *Top Room*, *CR* = *Center Room*, *BR* = *Bottom Room*.

Furthermore, for the temperature change from freezing conditions to room at vertical direction is shown in Figure 65. The top and the bottom temperature were first to change, although at different rates, the top temperature was the first to response followed by the bottom and the center was the lagging (Figure 65). Moreover, in a radial direction for the room temperature, the right measured temperature was first to change followed by left and

center temperature (Figure 66). For the temperature change from room to freezing conditions, the left and right were responding simultaneously as shown in Figure 67. Similarly, the center temperature was lagging for about 500 minutes. All measured and predicted temperatures follow the similar trend. In addition, the temperature change from freezing to room temperature is shown in Figure 67. The right and left temperature was moving on the same path, the temperature starts to rise sharply after -19°C and begin to decrease when the temperature reaches about 5°C as shown in Figure 67. There are close agreements between the measured and predicted temperatures at radial direction.

7.4. Discussion

A comparison of predicted and measured temperatures of maize grain was made for radial and vertical directions. The result in the radial direction in the room condition shows small variations between predicted and measured temperatures. However, the temperatures at the right and left-hand sides or at the boundaries of cylindrical bins were either increase for warming conditions or decrease for the cooling conditions at a higher rate after 500 min and do follow the same path, while the temperature for center positions was lagging behind. These were observed throughout the study.

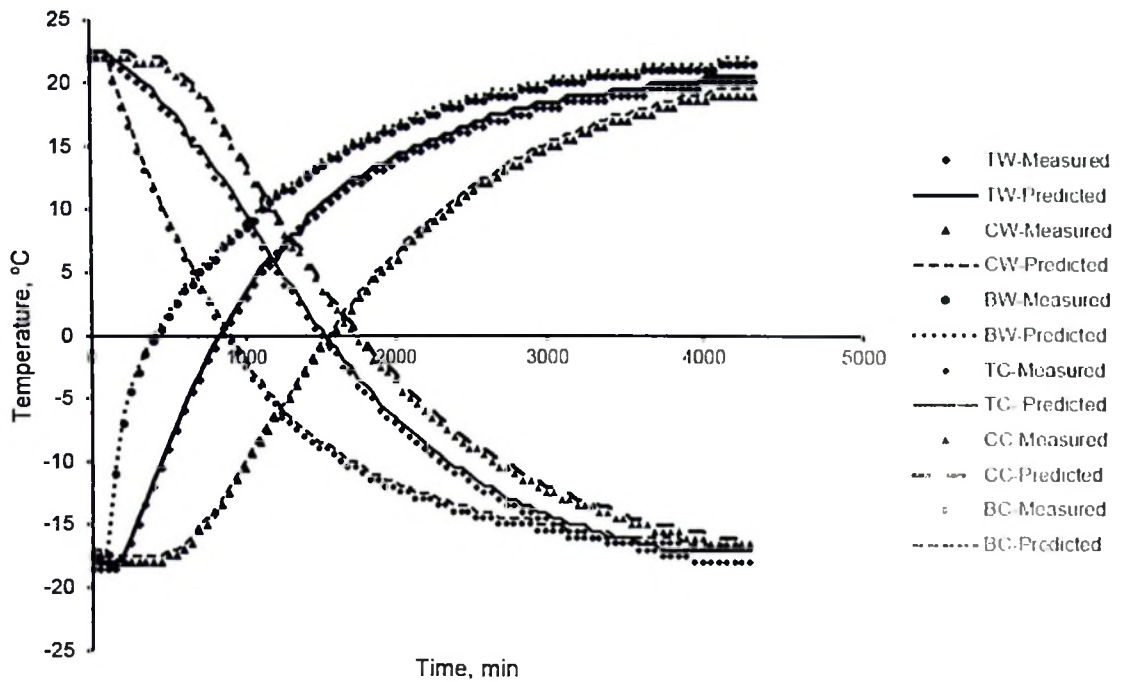


Figure 65. Measured and Predicted Maize Grain Temperature Change over Time from Room to Freezing Temperature and from Freezing to Room in the Vertical Direction. *TW= Top Warming, CW= Center Warming, BW= Bottom Warming, TC= Top Cooling, CC= Center Cooling And BC= Bottom Cooling.*

The results concurred with the finding of Zhang et al. (2013) who reported that the temperature of maize grain increase at the points near the boundary and proceeds at a faster rate than does the temperature increase at the center points. Moreover, the study found the temperature in the hermetic seal cylindrical bins varied, mostly in the radial direction and very little in the axial vertical directions as seen in the Figures 61 and 65. The similar results were reported by Khankari et al. (1994) and Jia et al. (2011) this means heat transfer in the cylindrical hermetic sealed bins occurred mainly due to conduction process. In addition, the maximum difference between predicted and measured temperature of maize grains inside the bins in vertical and radial directions was oscillated around $\pm 1.5^{\circ}\text{C}$ (as seen in the Figures 60 to 67).

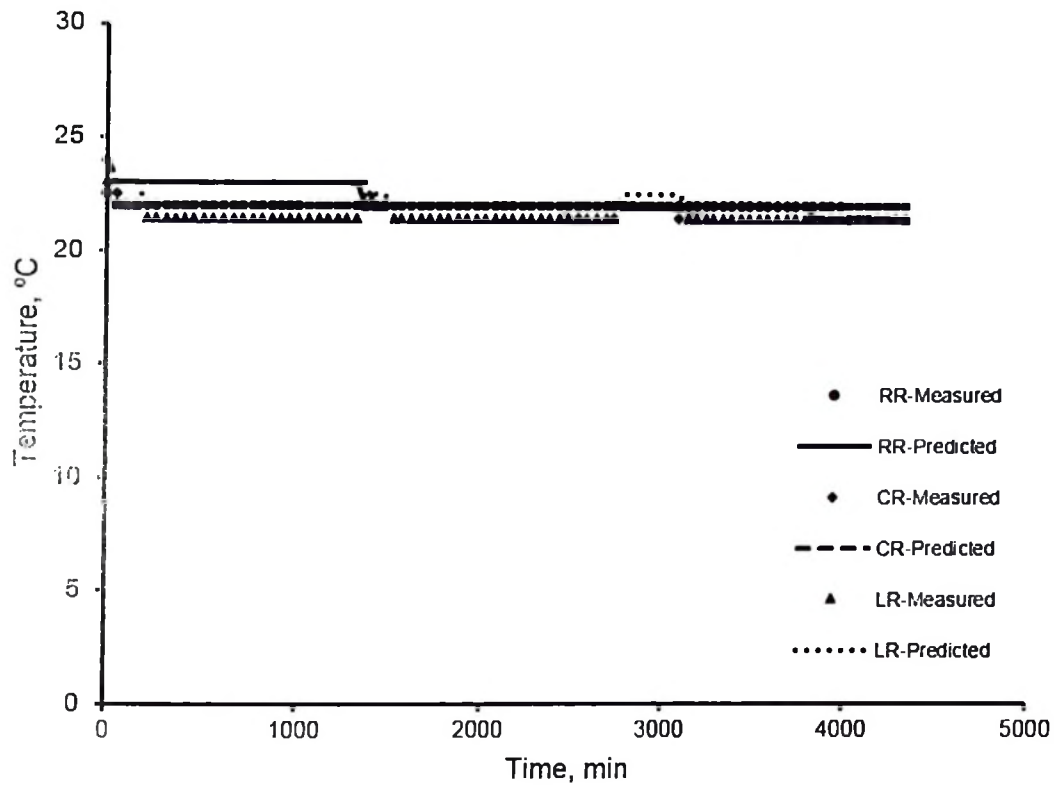


Figure 66. Measured and Predicted Maize Grain Room Temperature Change over Time at Room Temperature in the Radial Direction (Before to the Freezing Experiments). *RR*= *Right Room*, *CR*= *Center Room*, *LR*= *Left Room*.

These results were inconsistent with the results presented by Yaciuket al. (1975). They found the maximum difference in the simulation model between predicted and measured temperature of wheat inside the storage bins to be 3°C. The errors and a small deviation between the predicted and measured temperatures in the model can be attributed to the combined errors in experimentation, in sensors reading, in numerical computation and in some assumptions made during model development. According to Lawrence et al. (2013) the physical and the thermal properties of the grain are the important parameters that affect the accuracy of model development and temperature prediction. For instance, the properties of maize grains were assumed to be constant throughout the experiment, but in the actual sense,

the thermal conductivity of maize grain will change as the temperature changes. Hence, affect the accuracy of model development. This assumption was made because no actual methods of monitoring thermal properties of maize grain inside the bins during an experiment. Another factor to consider is the presence of fines and foreign particles in the maize grain this has a significant effect on the thermal properties of grain, especially thermal conductivity as reported by (Lawrence et al., 2013) thus reduce the accuracy of model development.

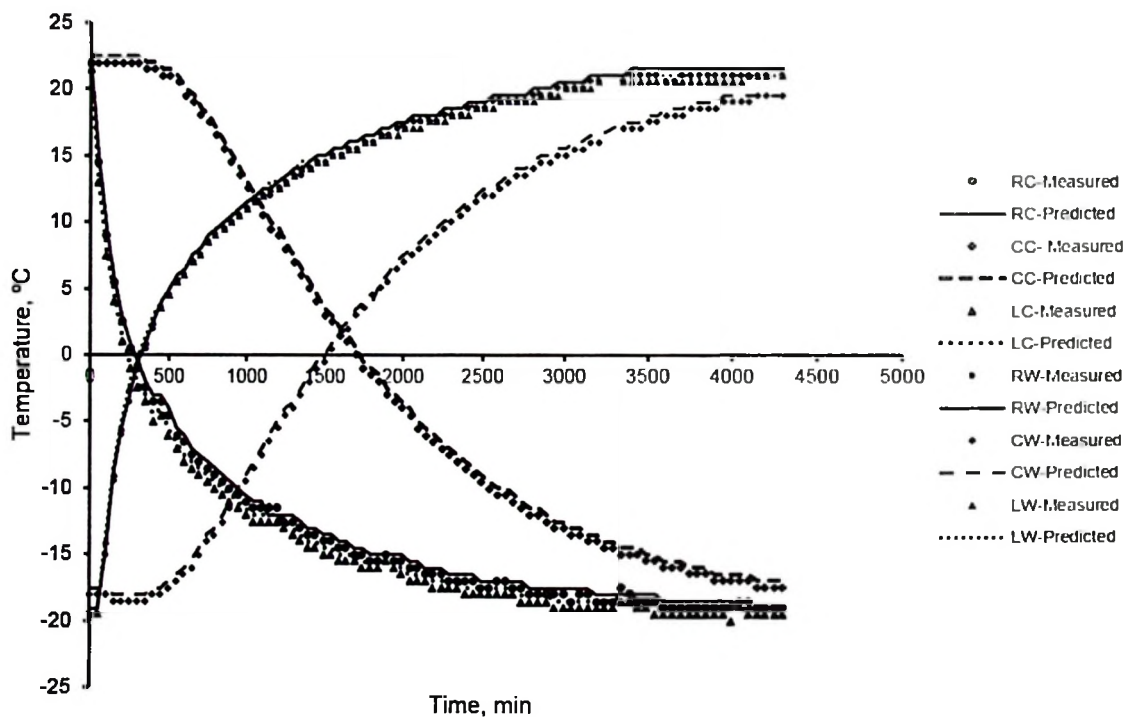


Figure 67. Measured and Predicted Maize Grain Temperature Change over Time from Room to Freezing Temperature and from Freezing to Room in the Radial Direction. *TW= Top Warming, CW= Center Warming, BW= Bottom Warming, TC= Top Cooling, CC= Center Cooling And BC= Bottom Cooling.*

7.5. Conclusions

In general, maize grain temperature oscillated greatly on the boundary of the bin and slightly at the center. Likewise, the predicted temperatures were closely matched with measured values throughout the experiments.

1. Temperature in the hermetically sealed cylindrical bins varied, mostly in the radial direction and very little in the axial vertical directions.
2. No noticeable change was observed in room condition.
3. The lag time between the center temperature and the side (right, left, top and bottom) was greater in the radial direction compared to vertical temperature.
4. The temperature in the grain changed more rapidly in the freezing conditions than in the room and cooling conditions.

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CHAPTER 8. IMPACT OF MAIZE MOISTURE CONTENT AND MAIZE WEEVILS ON MAIZE QUALITY DURING HERMETIC AND NON-HERMETIC STORAGE

Modified from a paper to be submitted to *Journal of Stored Products Research*

Suleiman, Rashid, Rosentrater, K. A, Bern, C. J, Thomas, B

Abstract

The objective of this study was to determine the impact of moisture content and *Sitophilus zeamais* Motschulsky on maize quality during hermetic and non-hermetic storage conditions. Commercially commingled maize kernels were conditioned to target moistures 14, 16, 18, and 20% moisture content (wet basis), and then three replications of 300 g of maize grain were stored in glass jars or triple Ziploc® slider 66 µm (2.6-mil) polyethylene bags at four conditions: hermetic with weevils, hermetic no-weevils, non-hermetic with weevils, non-hermetic no-weevils. All jars and bags were stored in an environmental chamber at 27°C and 70% relative humidity for either 30 or 60 d. At the end of each storage period, jars and bags were assessed for visual mold growth, mycotoxin levels, CO₂ and O₂ concentrations, pH level, the numbers of live and dead *S. zeamais*, and maize moisture content. The maize stored in non-hermetic conditions with weevils at 18 and 20% exhibited high levels of mold growth and aflatoxin contamination (>150 ppb). Although mold growth was observed, there were no aflatoxins detected in maize stored in hermetic conditions. The CO₂ and O₂ concentrations were directly related to the maize moisture contents and storage times. In general, CO₂ increased and O₂ gradually decreased as storage time increased. No significant difference in pH was observed in any storage conditions ($P < 0.05$). Total mortality (100%) of *S. zeamais* was observed in all hermetically stored samples at the end of 60 days storage. The number of *S. zeamais* linearly increased, with storage time for maize stored in non-hermetic conditions. Moisture content for hermetically stored maize was relatively constant. Moreover, a positive correlation between moisture content and storage time was observed for maize stored in non-hermetic with weevils ($r = 0.96$, $P < 0.05$). The results indicate that moisture content and the number of *S. zeamais* plays a significant role in maize storage, both under hermetic and non-hermetic conditions.

Key words: Maize, hermetic storage, moisture content, maize weevil, mycotoxins.

8.1. Introduction

Maize is among the major cereal crops in the world. It is estimated that in 2014, the total world production of maize was over 100 million metric tons, with the United States, China, Brazil, and Africa producing 34, 21, 7.8, and 7% of the total production of maize, respectively (FAO, 2015). Maize is a preferable staple and cash crop in sub-Saharan Africa (SSA). However, despite huge increases in maize production, postharvest losses (PHLs) of maize during storage remain a significant challenge for many farmers in developing countries (Abass et al., 2014). PHLs of cereal grain and oilseeds are an important factor in the world food supply chain and may represent about 5-10% of the total global production of grains and oilseeds (Tipples, 1995). In SSA, postharvest losses have been estimated to be around 5-18% (APHLIS, 2015) and as high as 40% for untreated maize stored in traditional storage structures (Rugumamu, 2004).

The PHLs of maize in tropical countries is fueled by biotic and abiotic factors. The biotic factors include insect pests and molds (FAO, 2009) while abiotic factors that influence the rate of the PHLs are moisture content and temperature (Giorni et al., 2008). The interactions between these factors can determine the level of PHLs during storage (Cairns-Fuller et al., 2005). The most important insect pests in SSA are the maize weevil, *Sitophilus zeamais* and the larger grain borer, *Prostephanus truncatus* (Hon). The *S. zeamais* Motschulsky is the major postharvest pest of maize in tropical and subtropical countries (Baoua et al., 2014; Suleiman et al., 2015). A six-month study conducted by Mulungu et al. (2007) revealed postharvest losses of maize due to *S. zeamais* in Tanzania to be around 17.5%. The devastation of *S. zeamais* relies on its ability to multiply in a short period of time; a female *S.*

zeamais can lay 300- 400 eggs (Cosmas et al., 2012; Baoua et al., 2014) and its ability to migrate between field and storage (Suleiman and Rosentrater, 2015). Furthermore, while most losses result from infestation by insects, rodents, and birds, another significant proportion of total loss results from fungal contamination (Pomeranz and Zeleny, 2009). In tropical countries fungi contamination ranked second after insects as the most important cause of maize grain loss (Fandohan et al., 2003). In addition to direct economic losses, fungal growth causes deterioration of the maize grain, reduces the weight of grain, produces off flavors and several potent mycotoxins (Hell et al., 2000; Pomeranz and Zeleny, 2009; Olstrop et al., 2010). Mycotoxin contamination such as aflatoxins may be detrimental to the health of humans and animals (Burger et al., 2013). Insect and mold infestation are highly influenced by abiotic factors, mainly temperature and moisture content (Pomeranz and Zeleny, 2009). According to Sauer (1988), the most important factors influencing the rate of mold and insect growth and deterioration of grain are moisture content and temperature. Tipples (1995) showed that grain moisture content and temperature affect the rates at which grain will respire and molds and insects will flourish. If these factors are maintained at a sufficiently low level, maize can be stored for several months with few adverse effects (Tipples, 1995).

Hermetic storage or airtight storage is the promising storage system that protects grains from damage caused by insect pests (Navarro et al., 1993). Hermetic storage works under the principle of a bio-generated modified atmosphere (Sanon et al., 2011), where oxygen (O₂) concentration dramatically decreases while carbon dioxide (CO₂) levels proportionally increase (Quezada et al., 2006). This is attained by the aerobic respiration of the grain, insects, and molds (Moreno-Martinez et al., 2000). Low oxygen environment or anaerobic

conditions inhibit the growth and development of insect pests, mold, and aerobic yeast (Sanon et al., 2011), which are the major source of grain deterioration during storage (Weinberg et al., 2008). Moreover, several changes occur during grain storage even under suitable storage conditions. Chemical, biochemical, physiological, quality and nutritional changes occur in grain because the seeds are living, respiring organisms that age (Tipples, 1995; Fleurat-Lessard, 2002). The respiration of seeds, fungi, and insect releases heat, CO₂, and water vapor. This causes the grain to increase in temperature and moisture, which makes insect pests and fungi to grow much faster (Sauer, 1988). Consequently, the grain quality may be deteriorating, resulting in qualitative and quantitative losses. Qualitative losses include poor appearance, discoloration, nutritional degradation, loss of seed viability, off-odors, rancidity, presence of insect fragments and infection (Weinberg et al., 2008), as well as a reduction in processing quality and dry matter, heating, caking, mold contamination and production of secondary metabolites such as mycotoxins (Sauer, 1988; Suleiman et al., 2013). The acids formed include fatty acids, acid phosphates, and amino acids (Pomeranz and Zeleny, 2009). The objective of this chapter was to determine the impact of moisture content and *S. zeamais* on maize quality during hermetic and non-hermetic storage conditions.

8.2. Material and methods

8.2.1. Experiment Design

A complete randomized design was used for this experiment (Table 29). Three replications, four storage conditions hermetic with weevils (HW), hermetic no weevils (HNW), non-hermetic with weevils (NHW), and non-hermetic no weevils (NHNW), two storage times (30 days and 60 days), and four target levels of moisture content (14, 16, 18, and 20%) were used. Samples were stored in a Forma environmental chamber at 27 °C and 70% relative humidity (Model 3940 series, Thermo Scientific Inc., Marietta, OH 45750). The *S. zeamais* used in these experiments were obtained from the stock of *S. zeamais* maintained in the grain quality laboratory, Department of Agricultural and Biosystems Engineering at Iowa State University.

8.2.2 Moisture Content and Sample Preparations

The maize used in this experiment was a conventional dent corn variety harvested during 2014. Maize was cleaned on a Carter-Day dockage tester with a 12/64-inch round-hole screen to remove broken corn and foreign material (BCFM). Six samples of maize were drawn randomly to determine moisture content. The initial moisture content of maize was determined to be 13.5% with samples of 30g in three replications at 103°C for 72 h (ASAE, 2001). To obtain the desired target moisture content (14, 16, 18, and 20%) maize was rewetted by adding distilled water mixed thoroughly and then hermetically sealed in polyethylene bags and stored at 10°C for 48 h to allow moisture equilibrium. Maize was mixed well and about 300g was randomly drawn for each treatment. The four actual levels of adjusted moisture content of maize were 14.01 ± 0.12 , 15.91 ± 0.29 , 18.18 ± 0.37 , and 20.15

± 0.09% wet basis.

Table 29. Experimental Design*

Storage system		Moisture content % (wet basis)			
		14	16	18	20
Hermetic	W	1	2	3	4
	NW	5	6	7	8
Non-hermetic	W	9	10	11	12
	NW	13	14	15	16

* Similar design was used for 30 and 60 days storage time. Each experimental unit were set of three replications. W= Weevils, NW= No-weevils.

8.2.3. Maize Storage Conditions

For the hermetic storage condition, triple Ziploc® slider 66 microns (2.6-mil) polyethylene freezer bags (SC Johnson, Racine, WI 53403) were used. For hermetic storage 246-mL glass jars with screened lids were used. All glass jars were sanitized at 120°C for 30 min in a PRIMUS PSS5-A-MSSD- Autoclave (PRIMUS Sterilizer Company, Inc., Omaha, NE, USA). Lids and screens were soaked in bleach overnight, rinsed, dried and sanitized with 95% ethanol before use. Each jar and Ziploc bag was loaded with 300g of maize, then 20 mixed age unsexed *S. zeamais* were introduced for the weevil treatment, based on (Suleiman et al., 2015). The total number of experimental units was 48 for the hermetic (with and without weevils) and 48 for the non-hermetic (with and without weevils). Three jars and Ziploc® slider bags for each treatment were then stored in a Forma environmental chamber for either 30 or 60 days.

8.3. Data Collection

8.3.1. Visual Mold Assessment and Mycotoxins Determination

Maize in each treatment was visually observed for signs of fungal growth and a picture was taken daily for each treatment to monitor fungi growth. After analyzing all parameters, three replications of each treatment were mixed together and analyzed for aflatoxins using lateral-flow test strips. Aflatoxin was analyzed using ROSA[®]FAST for Feed and Grain (Charm, Sciences, Inc., Lawrence, MA, USA) according to package instructions. Briefly, maize samples were ground using a Romer series II mill (Romer Labs, Inc, Union, MO, USA), mixed well, and stored in the cooler chamber (4°C) overnight stored in non-hermetic conditions. Fifty-gram sub-samples of each treatment were taken, and each sub-sample was extracted with 100 mL of 70% methanol by shaking by hand for one minute. One mL of sample extract was pipetted into a clean micro-centrifuge tube and labeled. The extract was then centrifuged in a mini-centrifuge for 10 seconds to remove solids. One hundred µL of the filtrate was transferred into a micro-centrifuge tube and 1 mL of the AFQ dilution buffer solution was mixed with the filtrate to yield diluted extract. Then, each diluted extract was drawn into a 3 ml syringe and passed through a syringe filter cellulose membrane (0.45 µm pore size) collected in a clean micro-centrifuge tube, and labeled appropriately. Three hundred µL of the filtered dilute extract solution was transferred onto the ROSA[®]FAST Aflatoxin Quantitative Test strip and the strips were incubated at 45 ± 1°C on the ROSA incubator for 5 minutes. After the incubation, the test strip was read using the ROSA-M-reader, configured according to package instructions. Positive and negative controls were verified before experimental sample reading.

8.3.2. Moisture Content and Insect Populations

Moisture content at each level for each treatment was determined using three 30g samples at the end of each storage time at 103°C for 72 h (ASAE, 2001). A count of live and dead *S. zeamais* was obtained by hand sieving (# 10; USA Standard Testing Sieve E-11, Sceburo Equipment Company, Chicago IL, USA). All *S. zeamais* were separated and removed by hand, chilled at 4°C for 1-3 min to reduce their movement and counted by visual inspection. *S. zeamais* showing any sign of movement was considered a live weevil.

8.3.3. pH Determination

The pH was determined by weighing a 10 g maize sample and placing in a blender with 100 mL of distilled water for two minutes. The contents were allowed to settle and stand for five minutes and then filtered using Whatman filter paper number one (90 mm diameter size). About 25mL of the supernatant was pipetted into a 50 mL beaker (Serna-Saldivar, 2012). The pH was measured using a FiveEasyPlus™ pH meter FEP20 (Mettler-Toledo, AG, Analytical, Schwerzenbach, Switzerland). The pH meter was calibrated according to manufacturing information and the electrode was rinsed with distilled water and dried with Kimwipes (Kimwipes, Kimtech, Roswell, GA 30076-2199, USA) after each pH measurement.

8.3.4. Gas Concentration Measurements

CO₂ and O₂ concentrations for hermetically sealed containers were determined by using a CheckPoint II handheld gas analyzer (Dansensor A/S, a Mocon Company, DK-4100 Ringsted, Denmark). Measurements were taken at the end of each storage time. For each hermetic sealed sample, the Dansensor A/S septum (15mm diameter) was placed in each

hermetic sealed container in a location with free space underneath. Then, the needle probe of the gas analyzer was pierced through the Dansensor A/S septum and through three layers of polyethylene.

8.3.5. Data Analysis

Data were analyzed by SAS software (version 9.4) using a general linear model (PROC GLM) procedures with a significance level of $P < 0.05$. Least significant differences (LSD) were performed to determine statistical significance between treatment means. In addition, the Person correlation coefficients were determined using PROC CORR. Data are typically presented as the mean \pm standard deviation of the mean. Data for moisture content were analyzed separately.

8.4. Results

8.4.1 Visible Mold and Mycotoxins Determination

There was a positive relationship between fungal growth and moisture content in the non-hermetic with weevil treatments. A clear visible mold growth was observed in all treatments except maize samples stored at 20% hermetic condition and 14% NHNW (Figure 68). Very little or no mold growth was visible for maize stored in hermetic conditions (HW and HNW) and for maize stored in NHNW conditions (Figure 69). However, a clear sign of mold growth and musty grain was observed for maize, particularly at intermediate and higher moisture contents (16, 18, and 20%) (Figure 70). The smell of fermentation was detected in maize stored at 20% HW and HNW. Likewise, a whitish yeast structure was observed for some maize samples stored in hermetic conditions.

Further, aflatoxin accumulation was detected in the high moisture maize and in maize treatments with weevil, but was absent in control, in all hermetic conditions and in maize stored at 14% moisture content. The results show that aflatoxin accumulation was related to mold growth and storage time. High levels of aflatoxins (90 ppb and <150 ppb) was detected in maize samples stored at high moisture content (18 and 20%) respectively in NHW treatment after 60 days storage. Also, high levels of aflatoxins (88 ppb and 81 ppb) were detected in maize samples stored at 20% in NHNW treatments after 30 and 60 days respectively. However, we did not surface-sterilize maize and did not add initial inoculums of *Aspergillus flavus*. Thus, the presence of *A. flavus* and resulting aflatoxins production was believed due to the complex interaction of several factors such as high moisture maize, the length of storage, and the presence of *S. zeamais*, as well as the environmental conditions, especially the combination of high temperature and relative humidity.

8.4.2. Gas Concentration

CO₂ and O₂ concentrations were directly related to maize moisture contents and storage times. The average onset conditions of O₂ and CO₂ under hermetic treatments were 20.9 ± 0.0% and 0.2 ± 0.1% for HW and 20.9 ± 0.0% and 0.1 ± 0.0% for HNW treatments. The statistical analysis showed a significant difference ($P < 0.05$) between 30 and 60 days of storage for oxygen concentration in HW treatments. The highest level of O₂ (5.43%) was observed at 14% and the lowest percent (0.30%) was detected at 20% after 30 days of storage for maize stored in the HW condition (Figure 71a). In addition, after 60 days of storage, O₂ concentration in the HW treatment was entirely depleted for maize grain stored at 18 and 20% moisture and fell below 3% for those stored at 14 and 16% moisture. Moreover, no

significant difference was observed in % O₂ between 18 and 20% moisture after 60 days of storage in HW treatments (Figure 71b). Likewise, a strong significant difference was observed for percent O₂ in HNW treatment ($P < 0.05$). The oxygen level was significantly higher in HNW treatments than those stored at HW. A similar trend was of decreasing observed for O₂ concentration was observed as moisture content and storage time increased (Figure 71c & 71d). Likewise, a significant difference was observed with and between treatments in CO₂ concentration after 30 and 60 days of storage (Figure 72). However, the level of CO₂ seems to increase as storage time increased. The highest levels of CO₂ were detected at 20% HW and HNW after 30 days storage. Similarly, the lowest concentration of CO₂ was detected at 14% HNW after 30-days storage.

8.4.3. Insects Population

One hundred percent mortality of *S. zeamais* was observed for maize grain adjusted to 16, 18, and 20% moisture content and stored in hermetic conditions after 30 days storage. However, only 50% mortality was observed for the maize grain adjusted to 14% after 30 days (Table 30). Likewise, total mortality (100%) of *S. zeamais* was observed for maize stored in hermetic conditions for all levels of moisture content at the end of 60 days storage. Moreover, a significant difference ($P > 0.05$) was observed between 30 and 60 days of storage for *S. zeamais* in a non-hermetic storage condition. There was an increase in the number of live *S. zeamais* as moisture content increased in the first thirty days of storage. (Table 30). But, higher numbers of maize weevils were observed at 14% moisture than at 20% after 60 days storage.

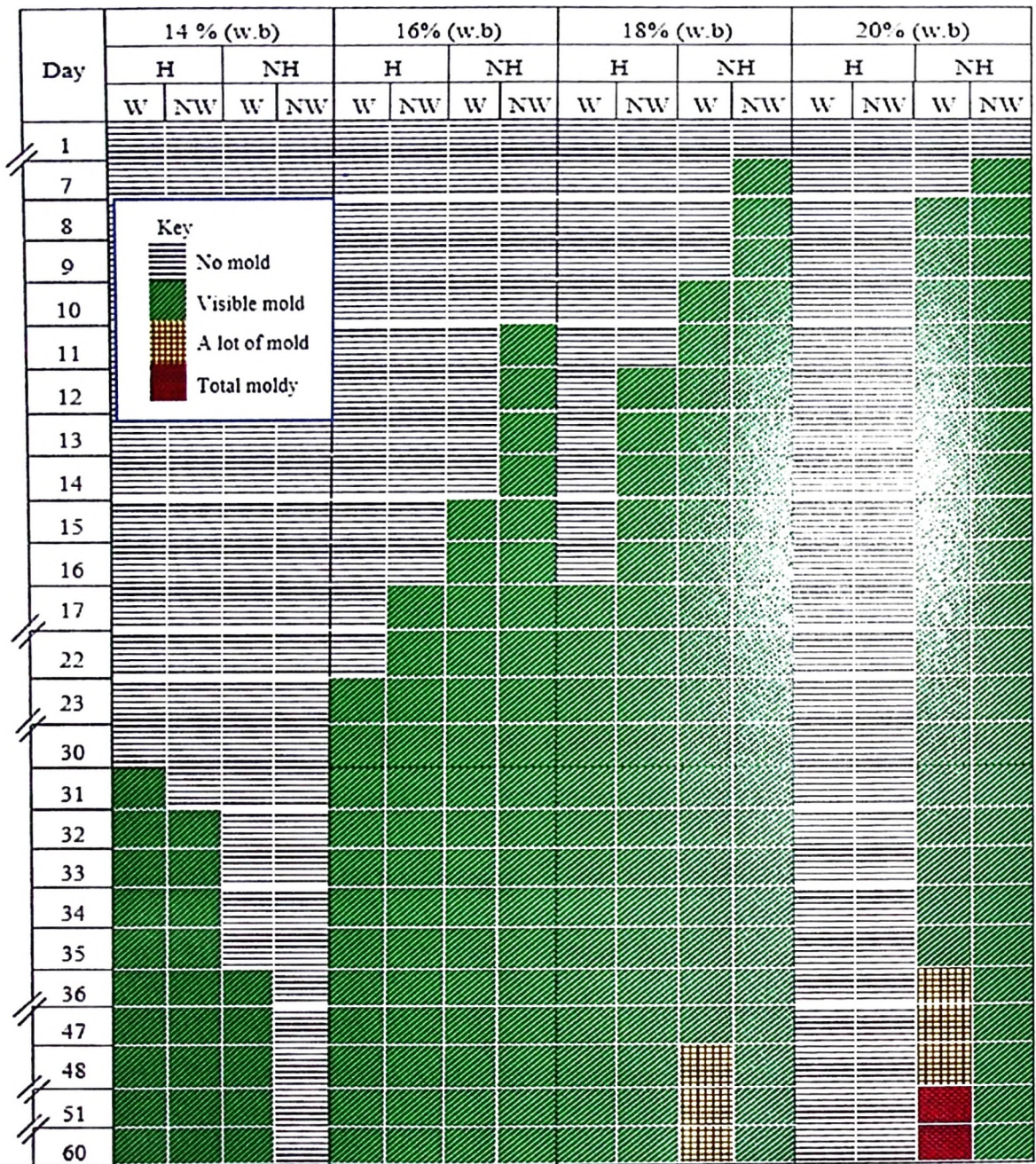


Figure 68. Daily Visual Mold Growth in Hermetic and Non-Hermetic Conditions Stored at 27 °C And 70% Relative Humidity. H= Hermetic, NH= Non-Hermetic, W= Weevils, NW= No Weevils, // = Break.

8.4.4. pH Determination

The results of this study revealed that across all moistures, there were no significant differences between hermetic and non-hermetic, weevils and no-weevils as well as after 30 d and 60 days of storage ($P < 0.05$). The results show that maize grain with no-weevils both in hermetic and non-hermetic had lower pH than the treatments with weevils (Table 31). The pH for control sample was 6.42; the highest pH (6.39) was detected in maize samples stored at hermetic with weevil treatment at 18% moisture content after 60 d storage. On the other hand, the lowest pH (5.73) was measured on maize grain stored in the hermetic condition with no-weevils at 18% moisture content after 60 d storage.

8.4.5. Moisture Content Effects on Hermetic and Non-Hermetic Storage

The adjusted initial grain moisture contents were close to the target moisture values (Table 31). Maize stored in non-hermetically conditions with weevils had a significantly higher moisture than those stored hermetically conditions ($P < 0.05$). The highest level of moisture content (40.49%) was detected in NHW treatments after 60 days storage. Moreover, the moisture content of maize stored in hermetic conditions was relatively constant (Table 31). Moreover, the moisture content for maize stored in NHNW treatments decreases as adjusted to the environmental chamber as shown in Table 31.

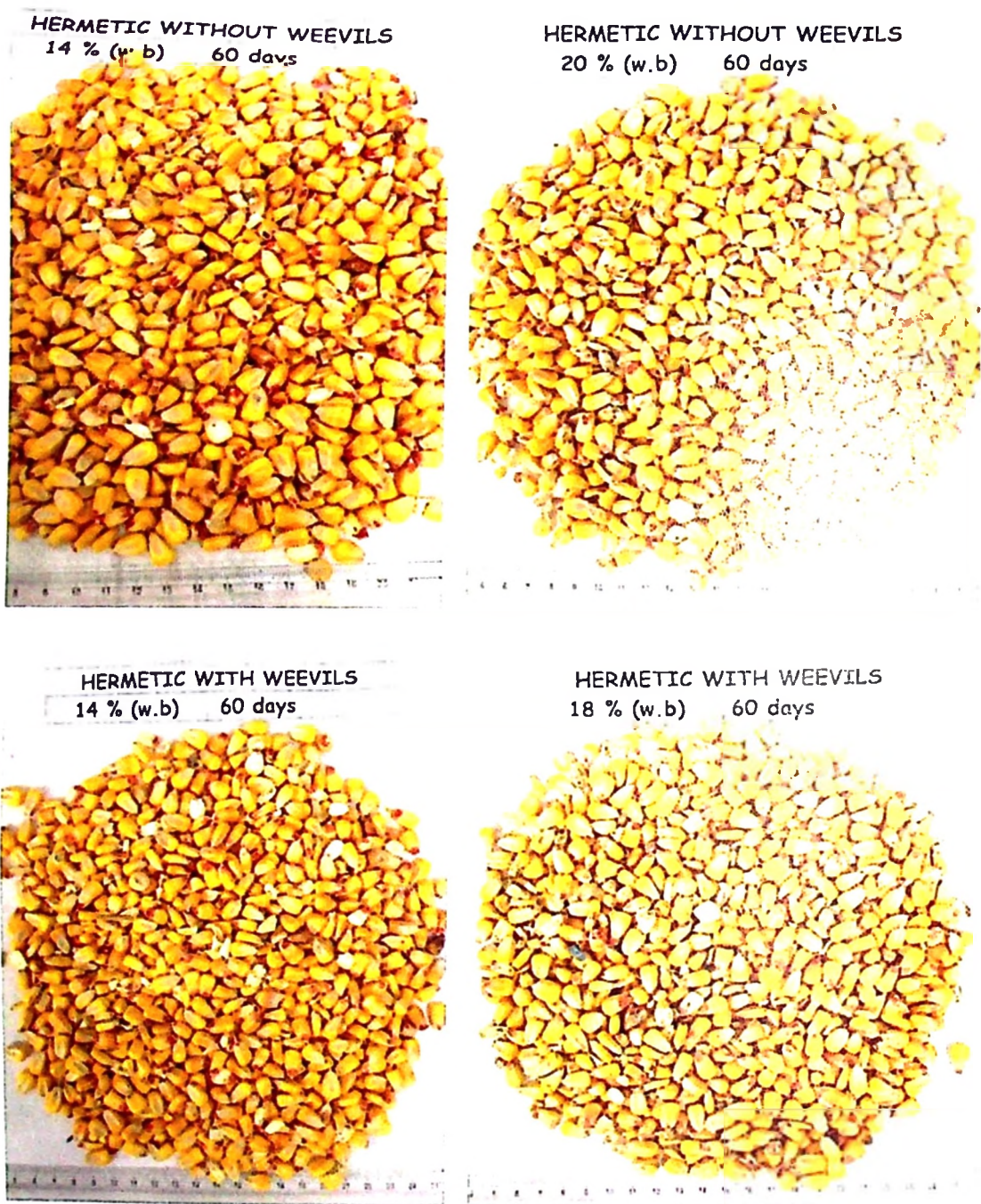


Figure 69. Visible Mold Growth under Hermetic Without Weevils at Different M.C % at 60 Days of Storage.



Figure 70. Visible mold growth under non-hermetic without weevils at different M.C % at 60 days of storage.

Table 30. Population of *S. Zeamais* in Maize Grain Stored in Hermetic and Non-Hermetic Conditions at 27°C and 70% Relative Humidity with Different Moisture Contents.

Storage period (days)	Storage conditions	Moisture content (%) w.b.	<i>S. zeamais</i> mortality (%)	Number of live <i>S. zeamais</i> (count)
30	HW	14	50	-**
		16	100	-
		18	100	-
		20	100	-
	NHW	14	-*	24
		16	-	48
		18	-	80
		20	-	102
60	HW	14	100	-
		16	100	-
		18	100	-
		20	100	-
	NHW	14	-	947
		16	-	748
		18	-	585
		20	-	559

HW = hermetic with weevils, NHW= non hermetic without weevils, *No mortality in HW treatments, ** No live *S. zeamais* in HW conditions, (n= 48).

8.5. Discussion

The mold growth was first clearly visible at day seven in NHNW at 20% moisture content. The predicted shelled corn storage time chart for 0.5% dry matter loss for this condition is nine days. This time often corresponds to the first appearance of visible mold (Bern et al., 2002). The finding also agreed with Wilson and Jay (1975) who found visible mold after one week of storage. However; this somewhat contrasts the finding of Seitz et al. (1982) who reported no visible mold was found in maize kernels until day 15 of the study. While Rohlfs (2005) found a hyphal tissue in *A. niger* after day three of inoculation.

Moreover, maize grain stored at low moisture content (14%) in both hermetic and non-hermetic conditions retained their original quality and very little mold growth were observed after 30 days of storage and none in NHNW (Figure 68). A similar result was reported by Weinberg et al. (2008) who found no biological activity in maize stored at 14% moisture content.

The maize grain stored at the intermediate moisture content (16%) presented a moderate fungal growth. Likewise, high fungal growth with a strong musty off-odor and an objectionable color was observed in maize stored at the higher moisture contents (18 and 20%) in HNW treatments (Figure 68). A similar result was reported by Quezada et al. (2006). Also, a strong, pleasant aromatic odor or smell of fermentation was detected from the 20% hermetic treatments. Beginning on day 14, the smell decreased as storage time increased. In addition, a whitish yeast structure was observed on maize kernels for the hermetically stored samples. Similar observations have also been reported by Wilson and Jay (1975) and Williams et al. (2014). Whereas Weinberg and others (2008) found large numbers of yeasts and molds in maize stored at high moisture levels (20 and 22%) during the initial stage of storage that led to higher dry matter losses.

Furthermore, no accumulations of aflatoxins were detected in maize stored in hermetic conditions and in any maize stored at low moisture content (14%). The result concurred with the findings of Williams and others (2014) who found no aflatoxin B1 in maize stored in hermetically sealed (PICS) bags and in samples stored in woven bags at low moisture content (12 and 15%).

Table 31. Moisture Content, % (W. B.) And pH Of Maize Stored in Hermetic and Non-Hermetic Conditions at 27 °C and 70% Relative Humidity.

Storage conditions	Moisture content, (%) wet basis				pH	
	Target	Initial	30. d	60. d	30. d	60. d
HW	14	14.0 ± 0.1	15.2 ± 0.1 ^d	15.9 ± 0.9 ^f	6.2 ± 0.0 ^a	6.2 ± 0.1 ^a
	16	15.9 ± 0.3	17.1 ± 0.3 ^c	17.3 ± 0.1 ^e	6.4 ± 0.1 ^a	6.4 ± 0.0 ^a
	18	18.2 ± 0.4	19.2 ± 0.1 ^b	19.4 ± 0.2 ^d	6.4 ± 0.0 ^a	6.4 ± 0.1 ^a
	20	20.2 ± 0.1	21.9 ± 0.4 ^a	21.7 ± 0.2 ^c	5.9 ± 0.1 ^b	6.2 ± 0.2 ^a
HNW	14	14.0 ± 0.1	14.8 ± 0.6 ^d	15.4 ± 0.1 ^f	5.9 ± 0.0 ^b	6.0 ± 0.1 ^a
	16	15.9 ± 0.3	16.5 ± 0.1 ^c	17.5 ± 0.1 ^e	5.8 ± 0.1 ^b	6.3 ± 0.1 ^a
	18	18.2 ± 0.4	18.3 ± 0.0 ^b	19.5 ± 0.1 ^d	6.2 ± 0.1 ^a	6.2 ± 0.0 ^a
	20	20.2 ± 0.1	21.9 ± 0.3 ^a	21.8 ± 0.2 ^c	5.8 ± 0.1 ^b	5.7 ± 0.4 ^b
NHW	14	14.0 ± 0.1	14.9 ± 0.5 ^d	22.2 ± 4.3 ^e	6.1 ± 0.0 ^b	5.9 ± 0.2 ^b
	16	15.9 ± 0.3	15.7 ± 1.0 ^d	24.7 ± 9.6 ^{b-c}	5.9 ± 0.2 ^{a-b}	5.8 ± 0.1 ^b
	18	18.2 ± 0.4	16.9 ± 0.6 ^c	29.9 ± 9.7 ^b	5.8 ± 0.1 ^b	5.8 ± 0.1 ^b
	20	20.2 ± 0.1	20.8 ± 1.2 ^a	40.5 ± 3.7 ^a	5.8 ± 0.3 ^b	6.0 ± 0.1 ^a
NHNW	14	14.0 ± 0.1	13.9 ± 0.5 ^e	13.4 ± 0.5 ^e	6.1 ± 0.1 ^a	6.2 ± 0.1 ^a
	16	15.9 ± 0.3	15.0 ± 0.2 ^d	13.9 ± 0.5 ^e	6.2 ± 0.1 ^a	6.2 ± 0.1 ^a
	18	18.2 ± 0.4	15.9 ± 0.1 ^d	14.5 ± 0.3 ^e	6.1 ± 0.1 ^a	6.1 ± 0.1 ^a
	20	20.2 ± 0.1	17.3 ± 0.5 ^c	15.9 ± 0.8 ^f	5.9 ± 0.1 ^a	6.1 ± 0.2 ^a

*HW = hermetic with weevils, HNW= hermetic no weevils, NHW= non-hermetic with weevils, NHNW = non-hermetic no weevils, d = days, means with different letters within the column are significantly different at P<0.05, mean ± SD, (n = 96).

According to Gardisser et al. (2006), the best way to control mold growth and mycotoxins contamination is to store maize grain with low moisture content. Also, as reported by Magan and Aldred (2007), maize grain can be stored at or below 14% moisture content without fear of fungal growth such as *A. flavus*. The main reasons for no aflatoxin contamination in hermetic conditions and at low moisture content are believed to be the limited biological

activity along with limited production of volatile compounds. These volatile compounds together with anaerobic conditions inhibit growth and development of fungal and production of secondary metabolites such as aflatoxin (Moon, 1983; Weinberg et al., 2008). In addition, a study conducted by Olstorpe et al. (2010) found some yeast species have positive effects as inhibiting mold growth like *A. flavus* and others in grain storage systems.

Moreover, significantly higher levels of aflatoxins (>150 ppb) were found in maize samples stored in non-hermetic conditions with weevil treatments. This is due to mutualistic associations between insect infestation and fungal growth as described by many researchers (Setamou et al., 1997; Burns, 2003; Raghavender et al., 2007; Fourar-Belaifa et al., 2011; Jian and Jayas, 2012). We believe the higher incidence of *A. flavus* and mycotoxins production was the result of higher insect infestation. Although insect infestation is not necessary for mycotoxins development, the presence of mold growth and mycotoxin contamination can be greater in insect-damaged kernels (Gardisser et al., 2006). According to Lynch and Wilson (1991) some insects act as vectors by carrying fungal spores and contaminating cereal grain as they move around in the field or storage. A study conducted by Sinha and Sinha (1991) found higher levels of aflatoxin contamination in wheat grain infested by *S. oryzae* than in uninfected grain. Likewise, a six-month study conducted in four agro-ecological regions in Benin found a positive correlation between aflatoxin contamination and maize cob damage by *S. zeamais* (Hell et al., 2000). Another study conducted by Beti et al. (1995) found significantly higher levels of aflatoxin B1 in maize kernels infested with *A. flavus* and contaminated with *S. zeamais* than *A. flavus*-inoculated maize without *S. zeamais*. She concluded that *S. zeamais* is an effective vector of *A. flavus* spores. In addition, Sétamou et al. (1998) reported a significant association between maize infested with *Carpophilus* sp. and aflatoxin B1 in the field.

Table 30 shows *S. zeamais* mortality and the number of live *S. zeamais*. Total mortality (100%) was observed for HW treatments at 60 days of storage. Garcia-Perea et al. (2014) reported similar results for the common bean weevil, *Acanthoscelides obtectus*. However, only 50% mortality was observed for maize stored at 14% moisture content after 30 days (Table 30). It appears that the O₂ concentration was the main reason for fifty percent mortality in hermetic storage at 14% moisture content. As described by Mbata et al. (2001) and Quezada et al. (2006), the critical levels of oxygen required for insect disinfection are below 3%. As expected, the number of live *S. zeamais* increased as moisture contents increased after 30 days of storage in the NHW treatments. However, the study observed a remarkable negative effect on insect growth as moisture content and storage time increased. A higher number of *S. zeamais* population was observed at 14 and 16% moisture than at 18 and 20% moisture content after 60 days of storage (Table 30). This decline of *S. zeamais* was believed due to depletion of food and high fungal presence that may inhibit insect growth and development in maize grain stored at high moisture content (18 and 20%). However, the reduction in the number of weevils as moisture contents increased in relation to fungal growth needs further study.

Further, the oxygen was depleted to below 4% after 30 days of storage in HW treatments for all levels of moisture content, except at 14% moisture content (5.43%) and this was believed to be the main reason for 50% mortality at 14% moisture as described in the previous section. Many researchers (Moreno et al., 2000; Yakubu et al., 2011; Garcia-Perea et al., 2014) have found that insects will perish if the oxygen concentrations fall below a critical level (3%). Also, reported by Genkawa et al. (2008) that the respiration of grain, insects, and growth of molds are inhibited by decreased O₂ and increased CO₂ levels within

storage systems. This is in agreement with our results showing that the all *S. zeamais* were dead when the oxygen level reduced to 2% (Figure 71, Table 30). In addition, 100% mortality of *S. zeamais* was recorded after 60 days of storage in hermetic storage conditions. A higher drop of oxygen and the build-up of carbon dioxide were observed in the maize samples stored at higher moisture contents. The results of this study were in closed agreement with Weinberg et al. (2008) who reported that it takes very short time for the oxygen to be replaced by carbon dioxide in higher moisture corn (16, 18, 20, and 22%). They concluded that the respiration rate increased with increasing maize moisture content. Also, a similar trend of oxygen decrease and the CO₂ increase was observed in the hermetic without weevil treatments, although, the rate of drop and increase were not higher than that of the hermetic with weevil treatments (Table 30). This is due to the fact that *S. zeamais* was the main consumer of oxygen in the HW treatments. Similar observations have also been reported by Moreno et al. (2000) during one-month storage of maize under hermetic conditions. The treatments that contained insects consumed more oxygen compared to treatments with fungus and grains alone.

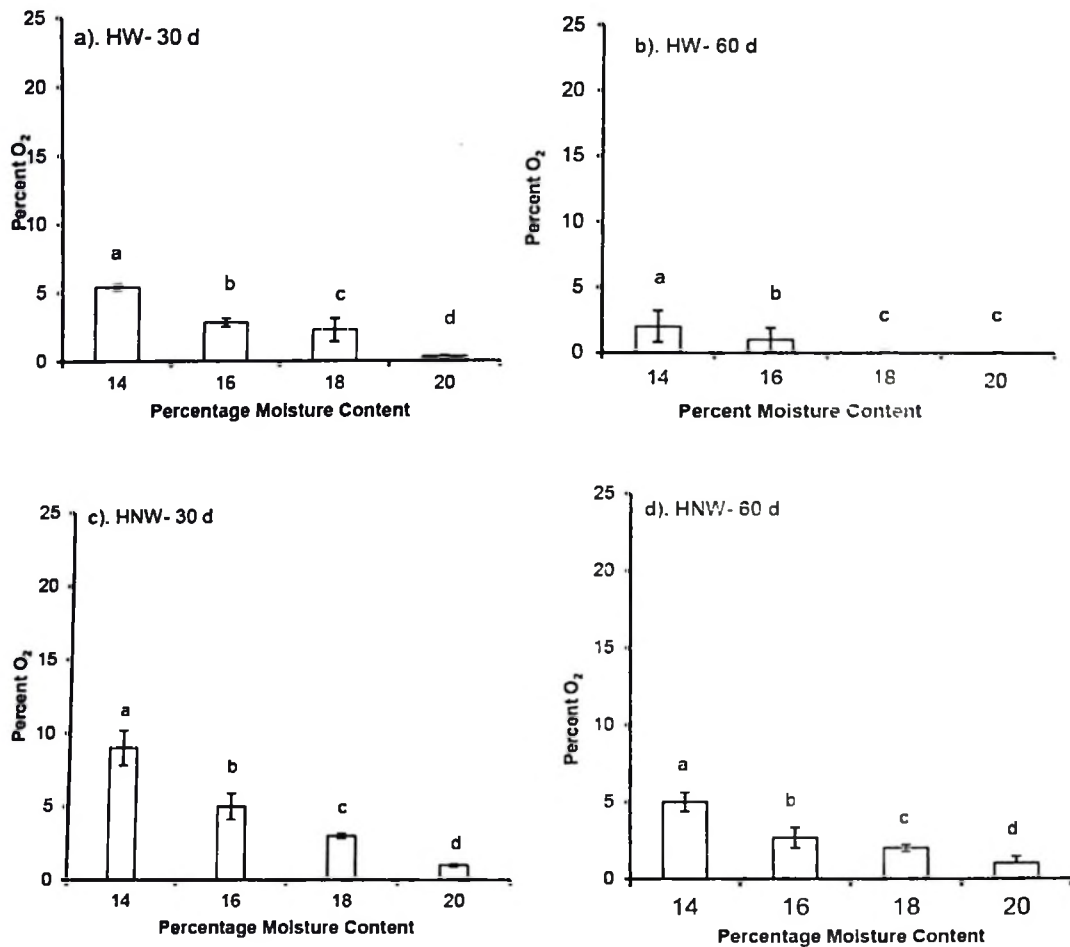


Figure 71. Effect of Moisture Content and *S. Zeamais* on Percent Oxygen (O₂) in Maize Grain Stored In Hermetic Conditions with and with-No Weevils for 30 (A, B) and 60(C, D) Days. HW= Hermetic With Weevils, HNW = Hermetic No Weevils, D = Day(S). Letter above Each Indicates the Significant Difference between Treatment Groups ($P < 0.05$, N= 48).

For the pH level no significant differences were found in hermetic and non-hermetic, weevils and no-weevils and after 30 days and 60 days of storage at all levels of moisture ($P < 0.05$). The average initial pH of maize grain was 6.42 ± 0.07 and did not change much during storage, except for maize samples stored at 20% moisture content in hermetic conditions where there was a decrease from 6.42 to 5.73 after 60 days of storage. A similar

result was also reported by Olstorpe et al. (2010), who found that during the entire storage period, grain pH on most farms decreased only slightly. In addition, Weinberg et al. (2008) found little change in pH when maize was stored at hermetic conditions, except at higher moisture contents (22%). According to Olstorpe et al. (2010) the decrease in pH reflects aerobic metabolism of lactic acid caused by some yeast species and other organic acids thereby disrupting the storage stability of grain.

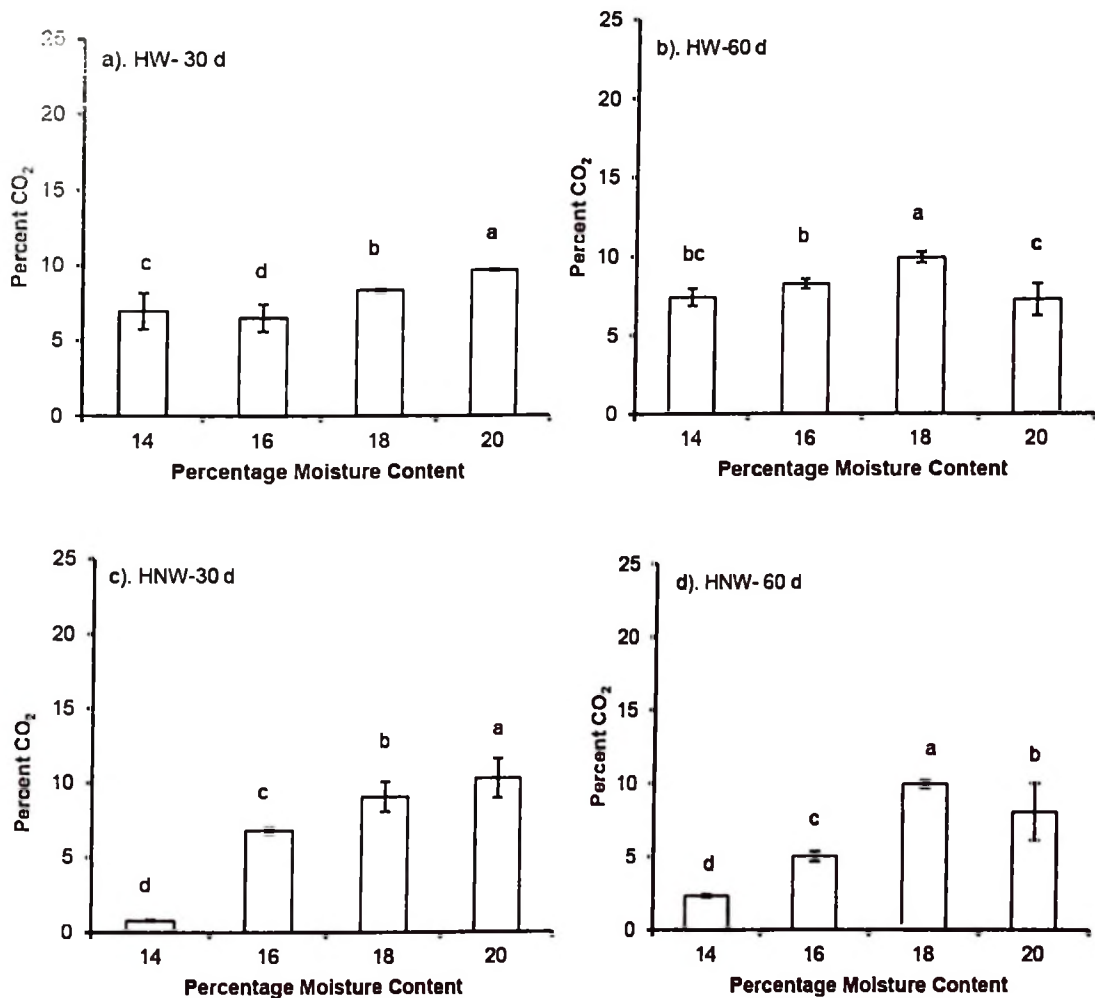


Figure 72. Effect of Moisture Content and *S. Zeamais* on Carbon Dioxide (CO₂) in Maize Grain Stored in Hermetic Conditions With and With-No Weevils for 30 (A, B) And 60(C, D) Days. HW= Hermetic With Weevils, HNW = Hermetic No Weevils, D = Day(S). Letter above Each indicates the Significant difference between Treatment Groups ($P < 0.05$, $N = 48$).

A strong significant difference was observed for moisture contents in hermetic and non-hermetic storage conditions ($P>0.05$). The moisture content for hermetically stored maize was relatively constant. The same finding was also reported by Moreno et al. (2000) and Williams et al. (2014). However, the moisture content of maize grain stored under the non-hermetic conditions with weevils increased from 14.01 ± 0.10 to $22.20 \pm 4.28\%$ after 60 days of storage. This was believed due to high fungal growth and weevil growth, especially at intermediate and high moisture contents as shown in the Table 31. A similar relationship was reported by Beti et al. (1995) who found higher *A. flavus* and high moisture content in corn samples contaminated with *S. zeamais*. Moreover, the moisture content of maize grain stored under non-hermetic without weevil decreased as storage time increased from initial of 14.01 ± 0.12 to $13.43 \pm 0.51\%$ after 60 days of storage. The maize grain lost moisture to the atmosphere to equilibrate with storage chamber conditions. The results of this study were similar to those reported by Moreno et al. (2000). In addition, a strong positive correlation between the moisture content and storage time was observed. The correlation coefficient correlation between moisture content and storage time (Table 32) was ($r = 0.93$, $P<0.05$) for maize stored in a non-hermetic with weevil treatments after 30 days and ($r = 0.96$, $P<0.05$) after 60 days of storage.

Table 32. Pearson Correlation Coefficient of Moisture Content with Storage Time in NHW Treatments.

Storage time	Initial	30 d	60 d
Initial	1		
30 d	0.93*	1	
60 d	0.96*	0.99*	1

Initial = day zero, d = day (s). *correlation at $\alpha = 0.05$.

8.6. Conclusions

In conclusions the study was conducted to determine the impact of moisture content and *S. zeamais* on maize quality during hermetic and non-hermetic storage conditions. The results show that *S. zeamais* and moisture content play significant roles in maize quality during storage. A positive and significant relationship between fungal growth and aflatoxin accumulation was observed in maize grain stored at high moisture content with weevil treatments. However, no aflatoxin accumulation was detected in low moisture maize and under hermetic conditions. Likewise, CO₂ was increasing and O₂ was decreasing as gas storage time increased. No significant difference was observed for pH in any treatments. Total mortality (100%) of *S. zeamais* was obtained at the end 60 d storage of hermetic storage, but the number of insects increased as moisture content and storage time increased for NHW treatments. Furthermore, the moisture content of maize grain stored in hermetic conditions was relatively constant, but linear relationship between storage time and moisture content was in a non-hermetic with weevil treatment. Further study is needed to determine the effects of high fungal presence in *S. zeamais* populations.

8.7. References

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CHAPTER 9. THE SYNERGISTIC INTERACTION BETWEEN *SITOPHILUS ZEAMAI*S, THE MAIZE WEEVIL AND *PROSTEPHANUS TRUNCATUS*, LARGER GRAIN BORER ON STORAGE OF MAIZE IN HERMETIC AND NON-HERMETIC CONDITIONS

Modified from a paper to be submitted to *Journal of Stored Products Research*

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Abstract

Sitophilus zeamais Motschulsky, the maize weevil, and *Prostephanus truncatus* (Horn), the larger grain borer are two notorious insect pests of farm-stored products in sub-Saharan Africa. The goal of this study was to determine whether there is a synergistic interaction between *P. truncatus* and *S. zeamais* during storage. The interaction between the two insects was evaluated in terms of the numbers of the live population, percent damage grain, weight loss, and weight of powder (flour) produced. Higher damage was observed in non-hermetic storage with *P. truncatus* and in mixed treatments (*P. truncatus* and *S. zeamais*). A significant difference ($P < 0.05$) and positive correlation were observed between the number of live population, percentage grain damage, the weight of powder produced, and percentage seed weight loss on infestation by *P. truncatus*, *S. zeamais*, and mixed treatments. *S. zeamais* dominate populations in the early stage, but outnumbered by *P. truncatus* after 60 days of storage in the individual species as well as in mixed treatments. The high percentage grain damage was observed in non-hermetic storage after 60 days in *P. truncatus* and mixed treatments 58 and 54% respectively. The weight of powder produced range from 0-30 g per 250 g of maize. The low synergistic interaction between *P. truncatus* and *S. zeamais* was observed. However, *P. truncatus* play a significant role when two insects coexist and cause more severe damage than *S. zeamais* in maize at non-hermetic storage condition.

Key words: Maize, corn, maize weevil, larger grain borer, hermetic storage.

9.1. Introduction

Maize is the most important food security crop and a major source of income for smallholder farmers in sub-Saharan Africa (SSA) (Smale et al., 2011). It is the main source of calories and protein for the larger population in developing countries (IITA, 2009). Over the past decade, SSA has experienced notably increased in maize production almost doubled from approximately 36 million metric tons in 2000 to over 70 million metric tons in 2014 (FOASTAT, 2016). Nevertheless, significant proportions of these (5- 40%) were lost during storage mainly due to poor postharvest handling practices or by biodeterioration brought by insect pests (Hodges et al., 2013). Biodeterioration of cereal grain is described as the loss of physical and nutritional qualities of cereal grain caused by organisms such as insect pests, rodent, mold, and bacteria, which eventually render grains unsuitable for human consumption (Hodges, 2013; Sreenivasa et al., 2011). The dominant insect pests in farm-stored products in SSA are the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), and the larger grain borer, (LGB) *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) (Tefera et al., 2011a; Abass et al., 2014; Affognon et al., 2015). *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is a serious cosmopolitan pest of stored cereal grains, especially maize (Markham et al., 1994) in tropical and sub-tropical countries (Baoua et al., 2014). *S. zeamais* gain notoriety as a pest of stored maize grain by their ability to proliferate into large populations in a very short period (Cosmas et al., 2012) and infestation is facilitated by their ability to fly (Suleiman and Rosentrater, 2015). In a humid, warm regions infestation of *S. zeamais* often starts in the field. However, severe damage is done during storage (Fikremariam et al., 2009). According to Walgenbach et al. (1987), *S. zeamais* are attracted to the maize grain by the kairomones (odors) produced from maize during

storage (Honda and Ohsawa, 1990). It has been estimated that 10 to 20% of the total weight of the stored product in SSA is lost due to infection by the *S. zeamais* (Boxall, 2002).

Moreover, *Prostephanus truncatus* (Horn) is an invasive insect species native from Mesoamerica that damages farm-stored products and wood (Stathers, 2002). *P. truncatus* was accidentally introduced from Central American in East Africa (Tanzania) in the late 1970's and West Africa (Togo) in the early 1980's (Dunstan and Magazini, 1981; Harnish & Krall, 1984). Since then, *P. truncatus*, has become the most serious post-harvest insect pest of rural stored maize and dried cassava chips in SSA (Key et al., 1994). The larger grain borer has spread dramatically to most of the maize and cassava growing areas of SSA (Omondi et al., 2011; CABI, 2015). The most recent surveillance report on the distribution of *P. truncatus* indicates that the pest currently occurs in at least 18 African countries (Gueye et al., 2008; Nukenine et al., 2010; Bergvinson & García-Lara, 2011; CABI, 2015).

The success of *P. truncatus* as an invasive species in tropical Africa relied on climatic conditions, diversity, and suitability of food sources. Also, the insect live as dual existence insect as storage pest and forest insect (Farrell, 2000; Nansen et al., 2004; Gueye et al., 2008) and make it huge challenge to manage. It is known as a pest of field and stored maize (Bell and Watters, 1982). In addition, *P. truncatus* is a highly polyphagous insect, feeding on wider range of food from grain, dry cassava, woody substrates, timber products, bamboo, plastic, to wooden frames of traditional storage structures (Gueye et al., 2008; Mautinte et al., 2014; CABI, 2015). According to (Tefera et al., 2011a; Bergvinson and García-Lara, 2011; Mautinte et al., 2014) the aggressiveness of *P. truncatus* are contributed by flight behavior of insect (long-distance movement) and lifespan of insect, the female *P. truncatus* live four

times longer than other stored product insects. The weight loss of 9 to 48% of maize are credited to *P. truncatus* on traditional granaries within three to six months of storage in Sub-Saharan Africa (Golob, 1988; Nboyine et al., 2015). This is almost five to six times higher than the damage caused by indigenous storage pests (Omondi et al., 2011). *P. truncatus* causes qualitative and quantitative grain losses by feeding on the maize kernels and cassava chips (Mwololo et al., 2012), turn them into powder after very short time and become unsuitable for food and trade (Richter et al., 1997). Due to the devastating nature of these two insects, it is important to determine their interaction and the distribution when both combined together during storage. Thus, the goal of this chapter was to determine a synergistic interaction between *P. truncatus* and *S. zeamais* during storage under hermetic and non-hermetic conditions.

9.2. Materials and methods

9.2.1. Experiment Design and Insect Culture

The experiment includes three main treatments, storage time (30, 60, and 90) days, two types of insects (*S. zeamais*, *P. truncatus*, and mixed treatments), and two storage conditions (hermetic and non-hermetic). These treatments were arranged in a 3 x 3 x 2 factorial design with three replications. The storage and insect bioassays were conducted at the Department of Food Technology Nutrition and Consumer Sciences (DFTNCS), Sokoine University of Agriculture, Morogoro Tanzania. The insects used in the experiment were obtained from the stores of the local milling machine and bred on maize (250 g) for 21 d at the laboratory of DFTNCS. The insects were reared in four separate plastic jars with screen lids that allow air flow. After three weeks, the original colonies were removed by sieving. When new insects

emerged were transferred into new plastic jars until sufficient numbers were obtained. All maize was conditioned to $13.5 \pm 0.5\%$ moisture content. The mean temperature and relative humidity were 26.6 ± 1.4 °C and $73 \pm 1.7\%$, respectively.

9.2.2. Maize Preparation and Storage Conditions

White dent maize harvested in the 2015 season was used. Prior to the experiment, the maize was cleaned to remove dust, broken kernels, and other foreign materials. The maize was then sun dried for forty-eight (48) hours, to kill any live insects (Laswai et al., 2013). Then fifteen mixed aged unsex *S. zeamais* and *P. truncatus* were introduced into each one-liter (1000 ml) plastic jars of 250g of maize grain (Suleiman et al., 2015). The jars were covered with a screen lid to allow airflow and prevent the escape of the insects for non-hermetic and airtight for hermetic treatments.

9.2.3. Data Collection

At the end of each storage time (30, 60, and 90) days, the maize grain was sieving using mesh sieves (#10) to separate maize, insects and powder produced. The number of live and dead insects and the number of damaged and undamaged kernels were recorded, whereas the weight of damaged and undamaged kernels and the weight of the powder (flour) produced were weighed on the analytical electronic scale (Contech® Instruments Ltd, Model CA-224, 301, Punit Indl. Premises, Turbhe, Navi Mumbai – 400705, India) with 0.0001 g precision. The grain weight loss was determined using the count and weight method described by Adams and Schulten (1978). As shown in equation (23). The percentage of damage grain was determined using equation outlined by Holst et al. (2000).

$$\text{Weight loss (\%)} = \frac{(W_u * N_d) - (W_d * N_u)}{(W_u) * (N_d + N_u)} * 100 \quad 23$$

Where W_u = weight of undamaged kernel, N_u = number of undamaged kernels, W_d = weight of damaged kernel, and N_d = number of damaged kernel.

9.2.4. Statistical Analysis

The data were analyzed using the PROC GLIMMIX using SAS software version 9.4 (SAS Institute, Cary, NC, USA, 2011), an ANOVA model was used and Pearson correlation coefficients were determined using PROC CORR. The difference between the means was compared by Tukey-Kramer HSD test at $\alpha = 0.05$. Data are typically presented as the mean \pm standard deviation of the mean.

9.3. Results

9.3.1. Number of Live Populations

The result shows significant differences on time and storage conditions ($P < 0.05$). Also, significant difference was observed on interaction between insects and storage conditions (Table 33). The number of live populations (NLP) of *P. truncatus* and *S. zeamais* increase as storage time increased in non-hermetic conditions (Table 34). The highest populations of *P. truncatus* and *S. zeamais* (234 and 91 insects per 250 g of maize respectively) were observed at 90 days of storage in non-hermetic conditions (Table 35).

Table 33. Analysis of Variance (ANOVA) for *P. Truncatus* and *S. Zeamais* on Hermetic and Non-Hermetic Condition after 30, 60, and 90 days of Storage.

Source	df	NLP(count)		DG (%)		PW(g)		SWL (%)	
		F	P	F	P	F	P	F	P
Insects ^a	2	5	0.0150	22	<0.0001	11	0.0002	6	0.0063
Time ^b	2	19	<0.0001	53	<0.0001	34	<0.0001	12	<0.0001
Storage ^c	1	38	<0.0001	48	<0.0001	29	<0.0001	4	0.0423
Insects*time	2	2	0.0739	6	0.0011	2	0.1537	4	0.0058
Insects *storage	2	12	<0.0001	0.8	0.4310	2	0.1895	2	0.1537
Time* storage	2	5	0.0158	14	<0.0001	18	<0.0001	3	0.0785
Insects*time* storage	4	0.9	0.4240	5	0.0018	0.4	0.8148	3	0.0384

df = degree of freedom, F = F-value, P = P-value, NLP(count) = number of live population, DG(%) = percentage damage grain, PW = powder weight (g), SWL(%) = percentage seed weight loss, Significant difference ($P < 0.05$), ^a Insects (*P. truncatus*, *S. zeamais*, *P. truncatus* & *S. zeamais*), ^b Time (30, 60, & 90) days, ^c Storage (hermetic & non-hermetic).

The *S. zeamais* populations were growing rapidly in the early stage of the experiment (30 days), however, they were outnumbered by *P. truncatus* after 60 and 90 days of storage (Figure 73A). In hermetic conditions, no live *S. zeamais* was observed after 30, 60, and 90 days of storage. Conversely, some live *P. truncatus* was observed in hermetic conditions at the end of 30, 60, and 90 days of storage. In addition, in mixed populations (*P. truncatus* and *S. zeamais*), *S. zeamais* dominate storage in the 30 days, but were surpassed by *P. truncatus* after 60 and 90 days of storage (Table 35).

Table 34. Mean of Number of Live Insect Populations, Percent Damaged Grain, Powder Weight and Percent Seed Weight Loss on *P. truncatus*, *S. zeamais*, and Combination of *P. truncatus* and *S. zeamais*.

Main Effects	NLP(count)	DG (%)	PW(g)	SWL (%)
Insect				
<i>P. truncatus</i>	69 ± 92 ^a	34 ± 23 ^a	11 ± 11 ^a	14 ± 12 ^a
<i>S. zeamais</i>	28 ± 35 ^b	14 ± 20 ^b	3 ± 5 ^b	5 ± 8 ^b
<i>P. truncatus</i> & <i>S. zeamais</i>	65 ± 89 ^a	29 ± 19 ^a	10 ± 11 ^a	12 ± 13 ^a
Storage condition				
Hermetic	16 ± 30 ^b	17 ± 15 ^b	4 ± 5 ^b	8 ± 11 ^a
Non-hermetic	91 ± 92 ^a	34 ± 25 ^a	11 ± 12 ^a	13 ± 12 ^a
Storage time (days)				
30	12 ± 12 ^c	8 ± 3 ^b	2 ± 1 ^c	3 ± 3 ^b
60	48 ± 34 ^b	32 ± 22 ^a	6 ± 4 ^b	16 ± 15 ^a
90	103 ± 115 ^a	37 ± 22 ^a	16 ± 14 ^a	11 ± 9 ^a

NLP (count) = number of live population, DG (%) = percentage damage grain, PW = powder weight (g), SWL (%) = percentage seed weight loss, means with same letters within the column are not significantly difference at ($P < 0.05$).

Moreover, no live *S. zeamais* was observed in hermetic storage condition when two insects mixed together. Surprisingly, low, but gradually populations of live *P. truncatus* were observed in hermetic storage treatments at the end of each storage time (Table 35). A clear positive correlation between NLP and all other parameters were observed (Table 36).

Table 35. Treatment Combination Effects due to Storage and Time on *P. truncatus* and *S. Zeamais*.

Trmt	Insect	Time (d)	Storage	NLP	DG (%)	PW	SWL (%)
1	PT	30	H	3 ± 2 ^c	8 ± 0 ^g	2 ± 1 ^{e-d}	2 ± 1 ^e
2	PT	60	H	42 ± 7 ^{b-c}	36 ± 15 ^{c-d}	7 ± 19 ^{c-e-b-d}	29 ± 19 ^{a-b}
3	PT	90	H	57 ± 78 ^{b-c}	40 ± 10 ^{b-c}	12 ± 5 ^{c-b}	13 ± 5 ^{c-d-e}
4	SZ	30	H	0 ± 0 ^c	3 ± 1 ^g	0 ± 1 ^e	1 ± 1 ^e
5	SZ	60	H	0 ± 0 ^c	11 ± 4 ^{e-f-g}	3 ± 2 ^{c-e-d}	3 ± 2 ^e
6	SZ	90	H	0 ± 0 ^c	2 ± 1 ^g	0 ± 0 ^c	1 ± 0 ^e
7	PTSZ*	30	H	4 ± 5 ^c	11 ± 1 ^{e-f-g}	2 ± 3 ^{e-d}	8 ± 3 ^{d-e}
8	PTSZ	60	H	29 ± 24 ^{b-c}	24 ± 13 ^{d-c}	4 ± 6 ^{c-e-d}	14 ± 6 ^{c-d-e}
9	PTSZ	90	H	14 ± 9 ^c	21 ± 4 ^{d-e-f}	6 ± 3 ^{c-e-d}	3 ± 3 ^e
10	PT	30	NH	9 ± 1 ^c	8 ± 0 ^{f-g}	2 ± 4 ^{e-d}	5 ± 4 ^{d-e}
11	PT	60	NH	68 ± 11 ^{b-c}	58 ± 19 ^a	10 ± 4 ^{c-b-d}	18 ± 4 ^{b-e-d}
12	PT	90	NH	234 ± 109 ^a	55 ± 11 ^a	30 ± 10 ^a	17 ± 10 ^{b-c-d}
13	SZ	30	NH	27 ± 10 ^{b-c}	5 ± 2 ^g	0 ± 1 ^e	1 ± 1 ^e
14	SZ	60	NH	47 ± 11 ^{b-c}	10 ± 6 ^{e-f-g}	1 ± 3 ^e	4 ± 3 ^e
15	SZ	90	NH	91 ± 17 ^b	55 ± 12 ^a	16 ± 10 ^b	23 ± 10 ^{a-b-c}
16	PTSZ	30	NH	27 ± 6 ^{b-c}	12 ± 3 ^{e-f-g}	4 ± 2 ^{c-e-d}	3 ± 2 ^e
17	PTSZ	60	NH	99 ± 17 ^b	54 ± 14 ^{a-b}	12 ± 21 ^{e-b}	32 ± 21 ^a
18	PTSZ	90	NH	220 ± 126 ^a	50 ± 8 ^{a-b-c}	29 ± 1 ^a	11 ± 1 ^{c-d-e}

PT = *P. truncatus*, SZ = *S. zeamais*, H = hermetic condition, NH = hermetic condition, NLP (count) = number of live populations, DG (%) = % damaged grain, PW (g) = powder weight, SWL (%) = % seed weight loss. Means with same letters within the column are not significantly difference at ($P < 0.05$). *Any parameter involved PTSZ is the combined effects of *P. truncatus* and *S. zeamais*.

9.3.2. Percentage Damage Grain

The trends of percentage damaged grain (%DG) was related to NLP (Table 34). The %DG of maize due to *P. truncatus* and *S. zeamais* infestation ranged from 2 to 58% (Table 35). A significant difference was observed between *P. truncatus* and *S. zeamais* on %DG in all storage conditions ($P < 0.05$). However, no significant difference was observed between *P. truncatus* and *S. zeamais* at 90 days of storage in non-hermetic storage. The result shows

higher %DG caused *P. truncatus* after 60 and 90 days of storage in non-hermetic storage condition (Figure 73B). In addition, a positive correlation between NLP and % DG ($r = 0.71$, $P < 0.05$) was observed. Typical damage caused by *P. truncatus* and *S. zeamais* is shown in Figure 74A and 74B respectively.

Table 36. Pearson Correlation Coefficient of *P. truncatus*, *S. zeamais*, and Combination of *P. truncatus* and *S. zeamais* on Maize Storage.

Variables	NLP	DG (%)	PW	SWL
NLP	1.00			
DG (%)	0.70*	1.00		
PW	0.95*	0.76*	1.00	
SWL (%)	0.42*	0.77*	0.45*	1.00

NLP (count) = number of live population, DG (%) = percentage damage grain, PW = powder weight (g), SWL (%) = percentage seed weight loss, means with same letters within the column are not significantly difference at ($P < 0.05$). *Correlation is significant at 0.05 level.

9.3.3. Weight of Powder

The weight of powder produced (PW) was proportional to the number of live populations (Table 34). The amount of powder produced ranged from (0-30) g. A significant difference was observed between storage conditions and storage time with respect to the weight of powder produced by *P. truncatus* and *S. zeamais* ($P < 0.05$). However, no significant was found on interaction of insects and storage conditions (Table 33). The result shows more powder was produced in treatments infested by *P. truncatus* or in a combination of the *P. truncatus* and *S. zeamais* (Table 35 and Figure 73C) in non-hermetic storage. A strong correlation ($r = 0.95$, $P < 0.05$) was found between the number of live populations and weight of powder produced (Table 36).

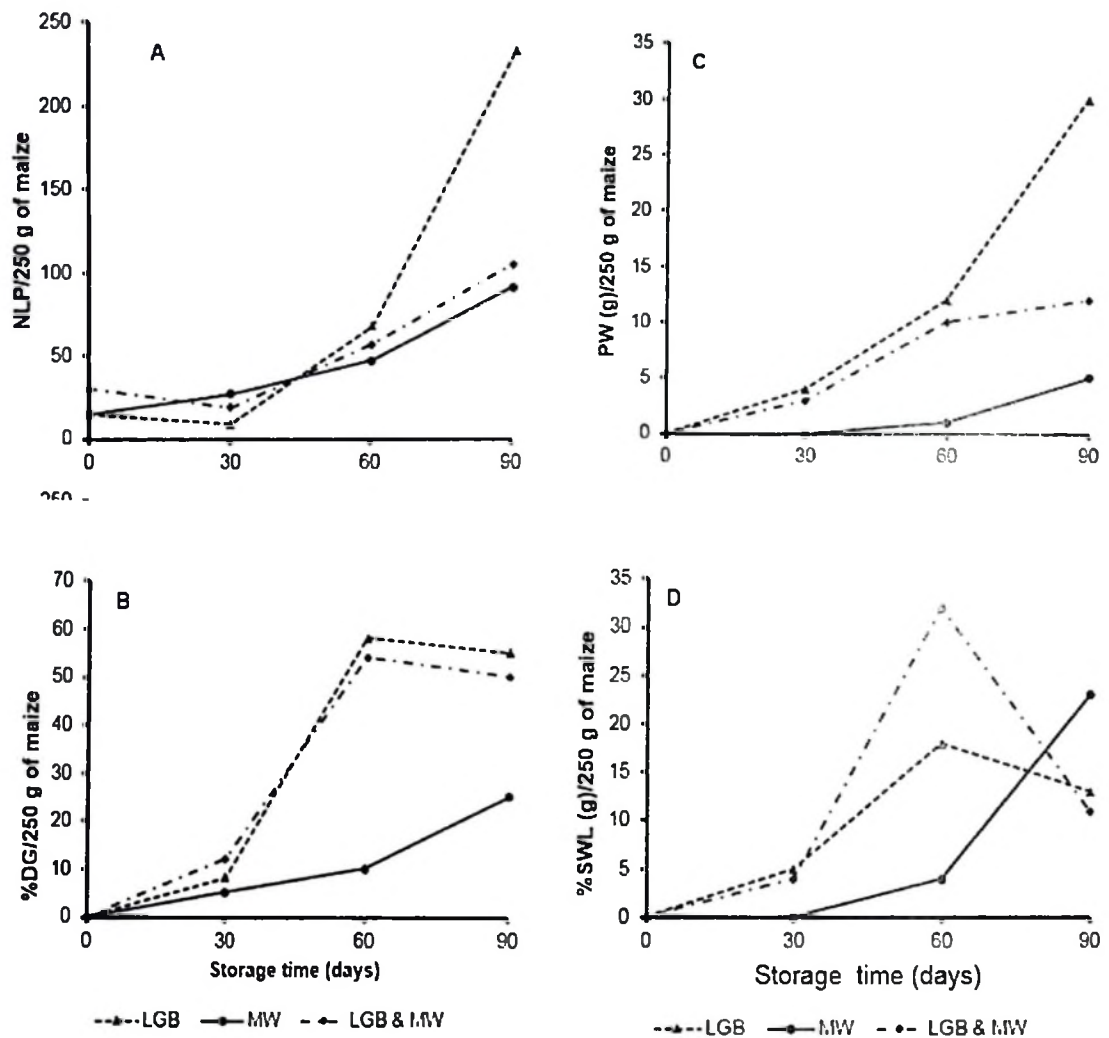


Figure 73. Effect of *P. truncatus* and *S. zeamais* on Maize Grain During Storage in Non-Hermetic Conditions (A) Number of Live Population (Count), (B) % Damaged Grain, (C) Powder Weight (G), and (D) % Seed Weight Loss. NLP (Count) = Number of Live Population, DG (%) = Percentage Damage Grain, PW = Powder Weight (G), SWL (%) = Percentage Seed Weight Loss.

9.3.4. Percentage Seed Weight Loss

Table 34 shows the mean value of percentage seed weight loss (% SWL). The % SWL after 30, 60, and 90 days of storage ranged from 1 to 32%. A significant difference was observed between times and insect type for %SWL. However, no significant was observed between

storage conditions (Table 33). The highest %SWL was observed at 60 days in hermetic and non-hermetic as well as *P. truncatus* and *S. zeamais*, except for treatment combination of *S. zeamais* in non-hermetic at 90 days (Figure 73D and Table 35). A weak correlation was observed between % SWL, NLP, and PW (Table 36).

9.4. Discussion

This study demonstrated differences between *P. truncatus*, *S. zeamais*, and the mixed combination of two insects with respect to the number of live populations, percentage grain damage, the weight of powder production, and percentage seed weight over a three storage time 30, 60, and 90 days. A significant difference and positive correlation were observed between the number of live population, percentage grain damage, the weight of powder production, and percentage seed weight loss on infestation by *P. truncatus* and *S. zeamais*. The results show *S. zeamais* dominate populations in the early stage of the experiment, but densities declined as storage time increased (Table 35). The same finding was observed by Vomotor et al. (2005) that *S. zeamais* colonized the stores first, but overtaken by *P. truncatus* as densities and storage time increases. Also, Meikle et al. (1998) reported *S. zeamais* densities built up much faster than *P. truncatus* during storage. A similar result was reported by Borgemeister et al. (1994). This may be caused by an initial infestation of *S. zeamais* in the field, as reported by many authors that *S. zeamais* infestation usually starts in the field and continue during storage (Borgemeister et al., 1994; Fikremariam et al., 2009).

Moreover, a sharp increase in *P. truncatus* populations was observed after 60 and 90 days and outnumbered *S. zeamais* populations in the individual species treatment as well as in the mixed combination (Figure 73A). According to Hodges et al. (1996), *P. truncatus* is a good

competitor in relation to other storage pest insects in storage. Likewise, Vomotor et al. (2005) found after sampling 3 to 5 times, densities of *S. zeamais* and *P. truncatus* increased after 4 weeks of storage. However, *P. truncatus* populations were significantly higher than that of *S. zeamais*. He also observed *S. zeamais* species are sensitive to the density of conspecifics. In the same study, Vomotor et al. (2005) observed that after sampling 6 occasions, both densities of *S. zeamais* and *P. truncatus* decreasing, but *S. zeamais* decrease much faster than that of *P. truncatus*. Similarly, revealed by Throne (1994) that *S. zeamais* species are very sensitive to the number of populations and net reproduction will decrease abruptly when crowding.

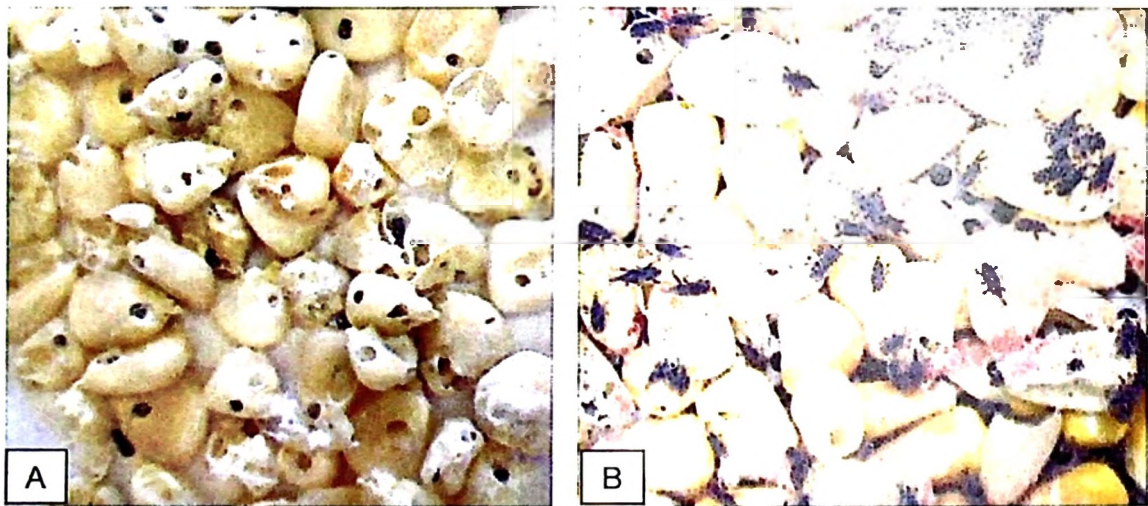


Figure 74. Typical Damage Caused by *Prostephanus truncatus* (A) and *Sitophilus zeamais* (B).

Likewise, the study conducted by Golob and Hanks (1990) found after 4 months of storage in most of the maize cob and grain trials *P. truncatus* populations grew much faster than those of *S. zeamais*. Also, Meikle et al. (2001) observed very high densities of *P. truncatus* (over 3500 insects/kg) in all artificially infested treatments within four months of

storage. They also found very low densities of *S. zeamais* approximately 30 insects/kg in all infested treatments at the end of the experiment. In addition, De Groote et al. (2013) found very few live *P. truncatus* in the control samples in the first month of storage, but number increase gradually to approximately 30 beetles per 450g of maize in fourth months of storage. Nevertheless, a laboratory study conducted by Makundi et al. (2010) found a high number of the population in the treatment infested with *S. zeamais* than those infested with *P. truncatus* at the end of storage trial.

The highest %DG was recorded in maize samples infested with *P. truncatus* and combination of *P. truncatus* and *S. zeamais* after 60 and 90 days in all storage conditions. As well as in *S. zeamais* after 90 days in non-hermetic storage (Table 35). High correlation between % DG and the number of live populations was observed (Table 36). In general, high % DG was observed in maize treatments infested with *P. truncatus*. This concurred with the finding of Ayertey et al. (1999) who found grain damage increased with increased insect populations, particularly when *P. truncatus* present. He also observed strong association ($r^2 = 0.83$) between percentage grain damage and densities of *P. truncatus*. The result of this study is also consistent with previous studies (Golob and Hanks, 1990; Borgemeister et al., 1994; Vomotor et al., 2005; Meikle et al., 2001; Makundi et al., 2010; Bergvinson and García-Lara, 2011; De Groote et al., 2013) which had shown that when *S. zeamais* and *P. truncatus* present in the maize stores cause a significant damage. However, *P. truncatus* play a significant role and cause severe damage. The weight of powder produced (PW) was highly correlated with a number of live population ($r = 0.95$, $P < 0.05$). The results agreed with the finding of Tefera et al. (2011b) who found lower percentages of dust/flour in treatment with a fewer number of insects. The results show a significant amount of PW was produced in the

treatment infested with *P. truncatus* or in a mixed combination in non-hermetic storage condition (Table 35). According to Bell and Watters (1982), destructiveness and significant quantities of powder produced by *P. truncatus* are contributed by insect's behavior. He found that as one adult *P. truncatus* tunnel hole in the maize kernel opens the ways for other adults to follow through the same opening and for the extension of a tunnel into nearby kernels. Adult *P. truncatus* cause approximately four times as much grain damage as larvae (Holst et al., 2000). Cited by Tefera et al. (2011a) that the typical characteristic of *P. truncatus* is the ability to convert huge quantities grain into powder for a very short time. Furthermore, the percentage seed weight loss (% SWL) for *P. truncatus*, *S. zeamais*, and mixed combination are illustrated in a Figure 73D. The % SWL increases up to 60 days for *P. truncatus* and mixed treatments, but sharply decreases after 60 days of storage. On the other hand, for the *S. zeamais*, % SWL was proportionally increased up to 60 days and sharply increases after 60 days of storage (Figure 73D).

The same result was reported by Tefera et al. (2011a). The mean percentage weight losses of 32 % were recorded after 90 days of storage in the mixed combination treatment. The result was similar to those reported by Gueye et al. (2008) that up to 35% of stored maize grain loss caused by mixed infestations of *P. truncatus* and *Sitophilus zeamais* in Senegal. Another field study conducted in Kenya found cumulative percentage weight losses caused by primary storage pests (*S. zeamais* and *P. truncatus*) to be over 20% in the untreated maize stored in the house. Which is equivalent to losses of over 300 thousand bags of 90 kg per year, equal to losses of approximately US\$6.3 million in one district every season (Mutambuki and Ngatia, 2012). Likewise, a study conducted by Keil (1988) in Tanzania observed losses of over 40% in improved maize varieties after eight months of

storage. Concurrently, the field trial conducted by Golob et al. (1982) recorded percentage weight losses of 34% in maize stored on the cob with husks in traditional storage structures after 3-6 months of storage.

9.5. Conclusions

The study observed a low synergistic interaction between *P. truncatus* and *S. zeamais* in terms of number live populations and weight of powder. A higher number of *S. zeamais* was observed at the end of 30 day but was outnumbered by *P. truncatus* after 60 and 90 days of storage. The percentage grain damage and the weight of powder produced was higher in *P. truncatus* and mixed treatments than *S. zeamais* treatments. Percentage seed weight loss was decreased after 60 days for *P. truncatus* and mixed treatments, but was sharply increased for *S. zeamais*. Also, *P. truncatus* was less affected by hermetic storage compared to *S. zeamais*. When both insects cause significant damage when coexisting during storage. However, *P. truncatus* was the major contributor of grain damage both in the individual treatments as well as in the mixed treatments in the hermetic and non-hermetic storage. This study can provide information for the control of two insects in the storage.

9.6. References

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CHAPTER 10. PERIODIC PHYSICAL DISTURBANCE: AN ALTERNATIVE METHOD TO CONTROL *SITOPHILUS ZEAMAI*S, THE MAIZE WEEVIL INFESTATION

Modified from a paper to be submitted to *Journal of Insects Special issue "Alternatives to chemical control of Stored-Product Insects"*

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Abstract

Sitophilus zeamais Motschulsky, the maize weevil is the most widely occurring and important cosmopolitan postharvest insect pest of stored maize in tropic and sub-tropical regions. Preventing infestation of this pest without using chemicals remains a huge challenge for smallholder farmers in the developing countries. Physical control methods are effective and attractive alternative methods to prevent, and control stored product pests in grain handling and storage facilities. Physical techniques are based on the application of some kind of force to manipulate the storage environments. They can provide unfavorable conditions for insect pest to multiplication or damage to the grain. The objective of this study was to determine the practicability of periodic physical disturbance on *S. zeamais* mortality and adaptation by smallholder farmers in developing countries. In this experiment, disturbed and stationary/control treatments were arranged in a Completely Randomized Design (CRD) with three replications and three-storage times (30, 60, and 90 days) in three regions of Tanzania. A total of 108 clean 20 L (L284 x W234 x H391) mm plastic containers were each loaded with 10 kg of fresh white dent corn and 0.50 kg of maize infested with *S. zeamais*. The initial numbers of *S. zeamais* were determined. For the turned treatment, containers were disturbed or turned twice a day, whereas for the controls the containers were not disturbed until the end of storage. The overall percent mortality after 30, 60, and 90 days of storage were 88, 96, and 98% respectively. A statistically significant difference ($P < 0.05$) was observed for the number of live *S. zeamais* in the control treatments. While the number of live *S. zeamais* in the turned treatment significantly decreased as storage time increased. This study shows the potential of a feasible, simple, affordable, safe and effective method of protecting maize grain for small-holder farmers in developing countries without using chemicals.

Key words: Maize, corn, maize weevil, physical disturbance, insects mortality.

10.1. Introduction

Maize is the most important cereal and cash crop in sub-Saharan Africa (SSA) and is part of the staple diet for over 1.2 billion people in developing countries (IITA, 2016). Current maize production in SSA is about 7 million metric tons (FAOSTAT, 2014), which is an increase of three percent from 2012/13 maize production year. Nevertheless, post-harvest losses (PHL) of cereal grain in SSA remain significantly higher (5 – 40%) (World Bank, 2011). However, the exact magnitude of losses varies greatly from region to region and country to country and depends on several factors such as length of storage, drying and storage methods, storage structures, and pest damage (APHLIS, 2014). In Tanzania, PHL of maize has been estimated to be between 15 and 26% (APHLIS, 2014). The greatest portions of these losses occur in the field and during storage and are mainly due to insect infestation. The most economically important and widely occurring PHL insect pests of stored maize in Tanzania include *Sitophilus zeamais* Motshulsky, the maize weevil and *Prostephanus truncatus* (Hons), the larger grain borer (Rugumamu, 2012). Preventing infestation from these pests remains a huge challenge for small-holder farmers in most countries in SSA, including Tanzania (Suleiman et al., 2015). In addition, the problems have significantly increased in the recent years due to the replacement of local varieties by improved varieties which mostly are not pest resistant. This is increasing the demand for synthetic insecticides (Demissie et al., 2008) which are commonly used to control insect pests of stored products (Dal Bello et al., 2000; Nwosu et al., 2015). However, inadequate education, haphazard application, lack of protective equipment, overuse, lack of proper regulations and inadequate or non-existent of storage facilities of insecticides in developing countries (Wilson and Tisdell, 2004) have resulted in a number of serious drawbacks, such as persistence in the

environment, development of insecticide-resistant insect species, chemical residues in foodstuffs, and adverse health consequences to humans and animals (Khan and Selman, 1989; Ngowi et al., 2007). Currently, national governments globally have set maximum residual levels (MRLs) of insecticides in food products including maize. Farmers are seeking alternatives to chemical insecticides to meet such demands. Physical control methods have been described as effective and alternative methods to pesticides to prevent and control pests during grain handling and storage (Jayaprakash et al., 2010).

Mechanical or physical techniques for control of stored-grain pests are based on the application of some kind of force or activities that manipulate the storage environment to provide conditions unfavorable to pests (Banks, 1987; Paliwal et al., 1999). Physical control methods are not a new technique in grain protection and actually were the main techniques before synthetic insecticides come into use (Banks, 1987). It is predicted that in a near future, physical control methods will again be the predominant process in grain handling and storage (Banks, 1987, White et al., 1997) because of increased consumer awareness of the health risks of pesticide use and the demand for products free synthetic insecticides. In addition, restrictions on the use of chemical insecticides such as methyl bromide are becoming more common. The physical control methods can be simple, affordable, and safe methods of controlling stored insect pests in grain facilities (Facknath, 1993; White et al., 1997). They include the use of techniques like heat, cold, inert dust, aridity, physical exclusion, removal, and impact or physical disturbance (Banks, 1987). Furthermore, the study conducted by Quentin et al. (1991) by the tumbling of beans in half-filled buckets every morning and evening reduced *Acanthoscelides obtectus*, bean weevil populations by 97% relative to controls without significant damage of the beans. A recent laboratory study which involved

rolling coffee cans half-filled with maize one circumference twice a day reduced *S. zeamais* populations by 81% compared to the controls (Bbosa et al., 2014). Similarly, Muir et al. (1977) observed that “during grain movement, insects infesting grain are subject to shaking, jarring, vibrations, and centrifugal forces which can be fatal to insects, and reduce grain temperatures to unfavorable levels for insect development”. In another study conducted by Joffe and Clarke (1963) found that rice weevils, *Sitophilus oryzae* (L.), are sensitive to pouring and many insects were eliminated during turning of the grain in a grain elevator. According to Joffe (1963) turning or physical disturbance of grain from one bin to another can reduce live grain weevil infestation to a significant level. The objective of this chapter was to determine the practicability of periodic physical disturbance on *S. zeamais*, the maize weevil mortality by subsistence farmers in developing countries as an alternative method to synthetic pesticides.

10.2. Materials and methods

10.2.1 Study Area

The study was conducted in maize producing regions of Manyara, Dodoma and Morogoro in Tanzania between November 2015 and February 2016 (Figure 75). These regions are each located in different agro-ecological zones (Northern, Central, and Eastern) and represent different patterns of maize production in the country. The Northern zone produces large quantities, the Central zone produces low quantities and the Eastern produces substantial quantities of maize. All regions have the history of high post-harvest losses (APHLIS, 2014). For each region, one major maize-producing district was purposely selected by Babati district representing Manyara, Chamwino district representing Dodoma and Kilosa

district representing the Morogoro region (Figure 75). From each district, one ward was selected to conduct the study. The wards selected are shown in Table 37. From each ward, three maize farmers were randomly chosen for this study.

Table 37. Sampling Plan for Physical Disturbance Study.

Region	District	Ward	Village	Number of farmer (s)	Number of treatment
Dodoma	Chamwino	Ikawa	Makoja	3	36
Morogoro	Kilosa	Mabwerebere	Muongano	3	36
Manyara	Babati	Gallapo	Gallapo Mjini	1	12
			Gallapo Kati	1	12
			Chalo B	1	12

10.2.2. Experimental Design

The study employed a farmer participatory research approach. The method attempts to incorporate farmers, agricultural extension officers, and researchers. The study consisted of two treatments: disturbed and stationary /control and each treatment were performed in triplicate. The trial was conducted for three months in three districts from three different regions (Babati in - Manyara, region, Chamwino in- Dodoma region and Kilosa in – Morogoro region). A total of 108 clean 20-L (L284 x W234 x H391) mm plastic containers (36 per region) was used and each replicate was loaded with 10 kg of fresh white dent corn (Figure 77A) and 0.50 kg of infested maize with *S. zeamais*. The initial numbers of *S. zeamais* were determined (Table 38). To avoid asphyxiation of *S. zeamais* a small hole was drilled at the top of each container to allow airflow. Each container was loaded to about half

capacity with 10 kg of maize was chosen so that thorough physical disturbance could readily be achieved. All containers were sealed properly to avoid re-infestation. For the disturbed treatment, containers were disturbed or turned twice a day (early in the morning and late in the evening) whereas the control containers were not disturbed or touched until the end of storage.

Table 38. Initial Numbers of *S. Zeamais* in Each Region per 0.5 kg of Infested Maize.

Storage time (days)	Initial number of <i>S. zeamais</i>					
	Dodoma		Morogoro		Manyara	
	Control	Disturbed	Control	Disturbed	Control	Disturbed
30	89	53	28	21	75	30
60	52	54	25	27	73	41
90	74	51	23	20	120	86

10.2.3. Determination of Live and Dead Insects (Mortality Rate)

At the end of the first, second, and third month, four containers from each farmer were opened and poured into a clean dry surface (Figure 77B). After thorough mixing, about one-fourth (2.5 kg) of maize was randomly drawn from the mixture and then divided using a quartering technique (Figure 77C and 77D) to determine the number of live and dead *S. zeamais* by visual inspection (Schuler et al., 2014). The percentage insect mortality was calculated by using equation 24 (Omotoso and Oso, 2005).

$$\% \text{ Mortality} = \frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100 \quad 24$$

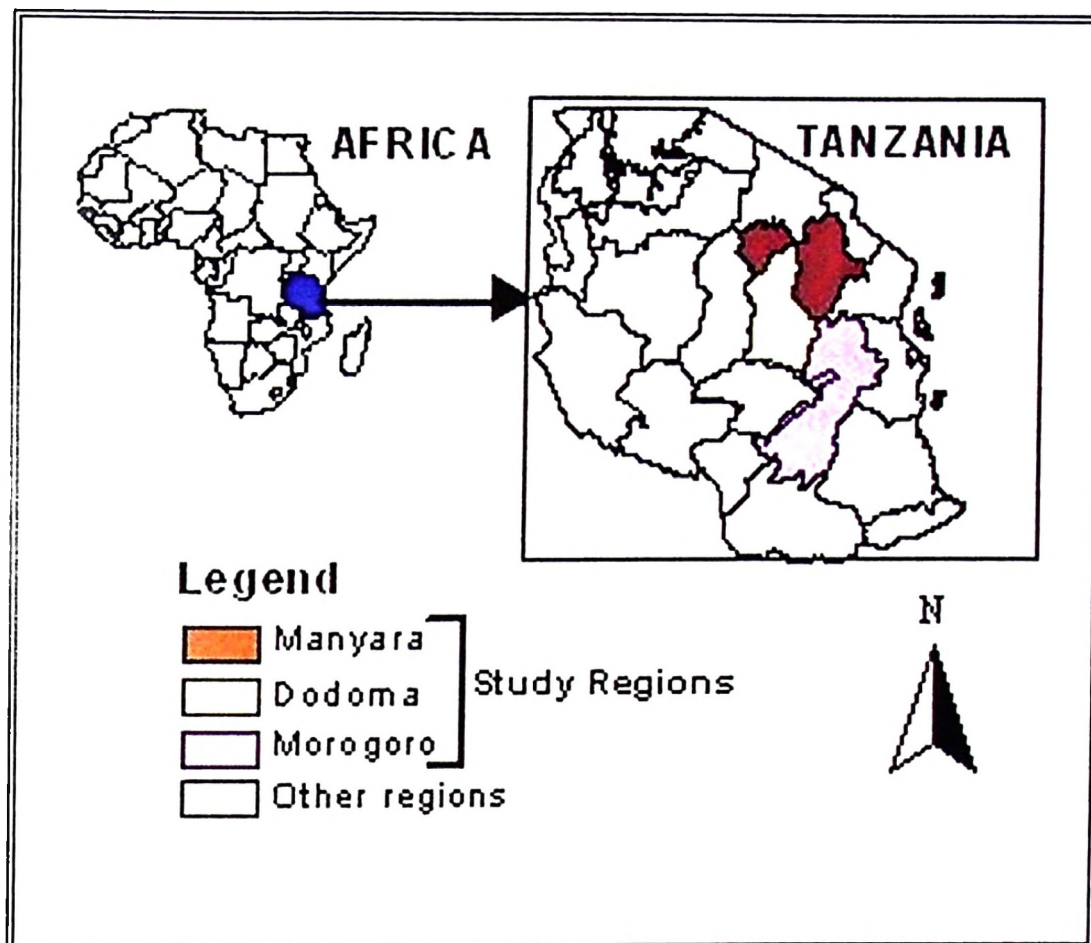


Figure 75. Map of Tanzania Showing Study Regions.

10.2.4. Data Analysis

The data collected were subjected to one-way analysis of variance (ANOVA) using the Statistical Analysis System (SAS) software version 9.4 for Windows, with a general linear model PROC GLM (SAS Institute, 2011) at α of 0.05. Tukey's HSD test was performed to determine statistical differences among the means. The values in the tables mean of three replicates plus minus standard deviation. Microsoft Excel[®] 2016 for Windows was used to calculate percent insect mortality and to draw bar charts.

10.3. Results

10.3.1. Insect Mortality

The statistical analysis showed a significant difference ($P < 0.05$) between control and disturbed treatment on percent mortality rate of *S. zeamais* (Table 39). Compared to the control, a significant increase of *S. zeamais* percentage mortality rate was observed in the disturbed treatments. Overall percentage mortality rates of *S. zeamais* were 88% after the first month, 96% after the second month and around 98% after the third month (Figure 78). Conversely, declines trend in mortality rate was observed in the control treatments. The overall percentage mortality rate in the control treatment was less than 50% (Table 39). The percentage mortality rate for the Chamwino district (Dodoma region) increased from 91% in the first month to 99% in the third month (Figure 76a). For the Morogoro region (Kilosa district), percentage mortality rate of *S. zeamais* after 1, 2 and 3 months were 96, 89, and 98% respectively (Figure 76b). However, the percentage mortality rate in the second month was slightly lower than in the first and third month. Moreover, no significant difference ($P < 0.05$) was observed in the percentage mortality rate in Manyara region (Babati district, Figure 76c).

Table 39. Effect of Physical Disturbance on % Mortality Rate of *S. zeamais*, per 2.5 kg of the Maize Samples.

Storage time (days)	Control (stationary)			Disturbed		
	Dodoma	Morogoro	Manyara	Dodoma	Morogoro	Manyara
30	10 ± 12 ^a	43 ± 13 ^a	32 ± 9 ^a	91 ± 4 ^a	96 ± 4 ^a	98 ± 4 ^a
60	8 ± 2 ^b	24 ± 17 ^b	6 ± 1 ^b	95 ± 1 ^a	89 ± 6 ^b	100 ± 1 ^a
90	6 ± 5 ^b	21 ± 11 ^b	10 ± 17 ^b	99 ± 1 ^a	98 ± 3 ^a	100 ± 0 ^a

Each value inside the table is the mean mortality rate ± standard deviation of three replicates. Mean followed by the same letter in a column indicates no significant difference ($P < 0.05$, for each region $n = 36$).

10.3.2. Number of Live Insects

Table 4 indicates the number of live insects. The result shows a significant difference ($P < 0.05$) in the number of live insects among the control treatments. However, no significant difference ($P < 0.05$) was observed for the disturbed treatments (Table 40). The number of live *S. zeamais* in the control treatments increased significantly with as storage time for all study sites in the three regions. As expected, a high number of *S. zeamais* was found at the end of storage time (90) days for the unturned control samples. For the control, the number of live *S. zeamais* were almost triple in the second month and quadrupled in the third month. On the other hand, for the disturbed treatments, the number of live insects decreased as the storage time increased. For instance, the number of live adult *S. zeamais* in Dodoma region was 10, 2, and 0 after 30, 60, and 90 days of storage respectively. Also, the result shows that at the end of the study (90 days) there were no live insects in any of the three regions (Table 40). The number of live *S. zeamais* live related to the percentage mortality rate in the disturbed treatments.

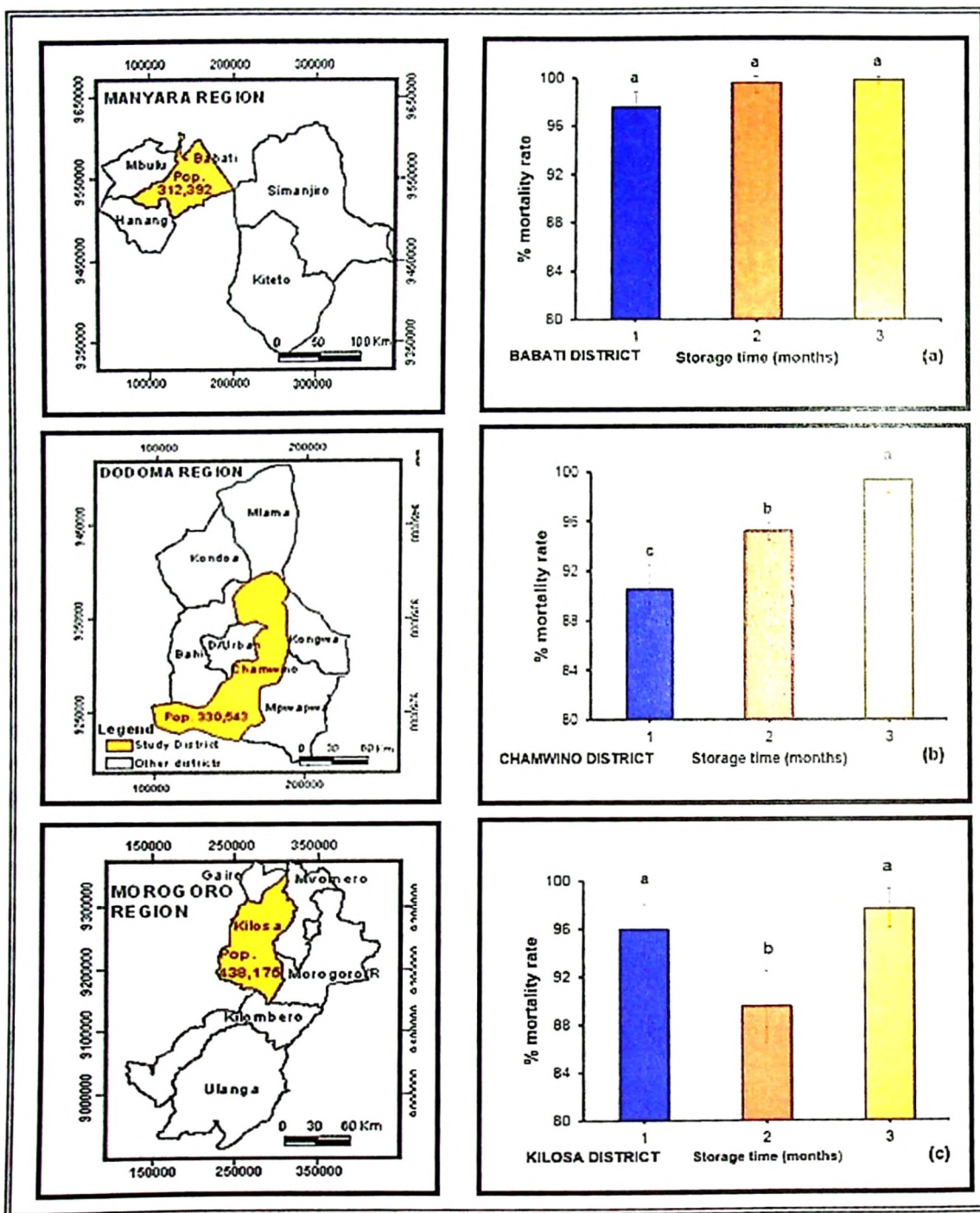


Figure 76. Map of Regions (Districts) and Level of Percent Mortality of *S. Zeamais* in Each District. Letter above Each Bar Indicates the Significant Difference between Treatment Groups (Note: Bar with the Same Letter Are No Significant Difference, $P < 0.05$, $N = 36$).

Table 40. Number of Live *S. Zeamais* for the Control and Disturbed Treatments after 30, 60, and 90 days, Per 2.5 kg of the Maize Samples.

Storage time (days)	Control (stationary)			Disturbed		
	Dodoma	Morogoro	Manyara	Dodoma	Morogoro	Manyara
30	20 ± 8 ^c	9 ± 2 ^c	12 ± 4 ^c	10 ± 2 ^a	2 ± 1 ^a	3 ± 1 ^a
60	68 ± 31 ^b	49 ± 35 ^b	77 ± 44 ^b	2 ± 1 ^b	5 ± 1 ^a	0 ± 0 ^a
90	109 ± 22 ^a	119 ± 35 ^a	152 ± 36 ^a	0 ± 0 ^b	0 ± 0 ^a	0 ± 0 ^a

Each value inside the table is the mean ± standard deviation of three replicates. Mean followed by the same the same letter in a column indicates no significantly different ($P < 0.05$, for each region $n = 36$).

10.4. Discussion

The higher percentage mortality rate and the lower adult emergence of *S. zeamais* in the disturbed treatments were due to physical disturbance of the containers. The physical disturbance (turning) has been previously studied as an alternative method to reduce insect infestations in stored grain (Bailey, 1962; Joffe, 1963; Bailey, 1969; Bryan and Elvidge, 1977; Loschiavo, 1978; Ungsunantwiwat and Mills, 1979; Banks, 1987; Quentin et al., 1991; Facknath, 1993; Plarre and Reichmuth, 2000; Facknath, 2006; Bbosa et al., 2014). The method is known to significantly reduce insect populations in all stages of development (from eggs, larvae, pupae, to adults) as during the mechanical agitation of the grain (Facknath, 2006; Banks, 1987; Bahr, 1990). It is believed that because the insects are disturbed and will not be able to bore the hole and lay eggs, this would end up in starving the insects which ultimately results in death. This study confirms this belief as almost all *S. zeamais* were dead (98% mortality rate) at the end of the study. Moreover, no grain damage from insect infestation was observed in any of the three regions after 30, 60, and 90 days of storage for the disturbed treatments.

According to Facknath (2006), turning of grain kills the insects outside of the grains as well as those inside the grains. The turning not only keeps stored product cool, but also reduces the risk of insect infestations (Joffe, 1963). The percentage mortality rate of *S. zeamais* in the third months were 99, 98, and 100% in Dodoma, Morogoro and Manyara regions respectively (Table 39). These results were consistent with the findings of the other previous studies. For instance, a study conducted by Loschiavo (1978) found 96% mortality for adults *S. oryzae* when small wheat sacks dropped several times a day. Furthermore, the visual observation shows minimum mechanical damage of the maize grain. A similar finding was reported by Quentin et al. (1991). This could be one of the reasons for low adult emergence in the turning treatments. Many studies reported mechanical damage as the most important factor in grain storability because kernel damage facilitates insects and fungal invasion (Ng et al., 1998). High mortality in the control treatments in the first month (30 days), since mixed age *S. zeamais* were introduced in the maize samples, high mortality rate observed at 30 days could be the end of the life cycle of *S. zeamais*.

According to Sharifi and Mills (1971), the average life-cycle of *S. zeamais* from egg to adult is about 36 days. As reported in previous studies (Terefa et al., 2011; Gofishu and Belete, 2014; Suleiman et al., 2015), this study observed a significant difference ($P < 0.05$) on a number of live insects for the control treatments (Table 40). However, the number of live insects was slightly lower in Morogoro region compared to other regions. Likewise, the numbers of live *S. zeamais* in the disturbed treatments were decreased with time (Table 40). This could be due to the physical turning of the maize grain as physical turning or moving of grain from one bin to another reduces the risk of insect infestations Paliwal et al. (1999). According to Bailey, (1969) physically disturbing the grain at least twice or more times a

week might significantly prevent insect development and reduce grain infestations.



Figure 77. Sampling and Data Collection of Physical Disturbance Study.

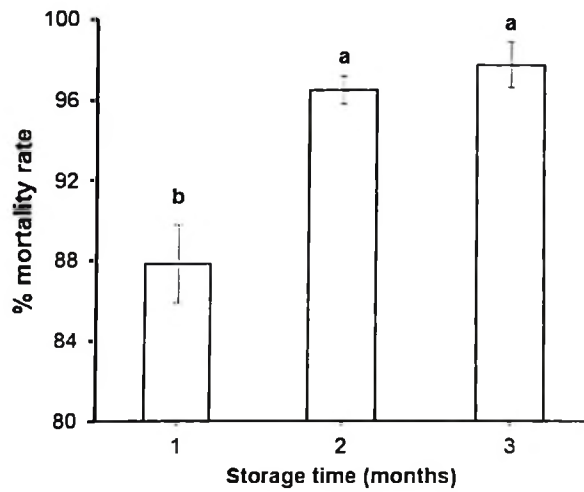


Figure 78. The Overall Effect of Physical Disturbance on *S. Zeamais* Mortality Rate for Dodoma, Morogoro, and Manyara Regions (Letter above Each Bar Indicates the Significant Difference between Treatment Groups (Note: Bars with the Same Letter Signify No Significant Difference, $P < 0.05$, $N = 36$), Per 2.5 kg of the Maize Samples.

10.5. Conclusions

This field was conducted to determine the practicability of periodic physical disturbance on *S. zeamais*, the maize weevil mortality. The physical disturbance was a very effective and may be a feasible method to protect maize grain from *S. zeamais* infestation. The result has shown that disturbed containers twice a day could reduce *S. zeamais* infestation significantly with minimum mechanical damage of the maize grains. After three months of storage percentage mortality rate of *S. zeamais* were 98%. After three months of storage of maize, there was no live of *S. zeamais* in any container in any of the three regions. Hence, this study demonstrates the potential of a simple, affordable, feasible, safe, and effective method of protecting maize grain for small-holder farmers in developing countries who cannot afford modern and costly methods to control maize grain from insect infestation. Also, may be a possible solution to reduce maize damage and infestation problems without using chemicals. This will minimize chemical contamination of maize grain.

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CHAPTER 11. POSTHARVEST PRACTICES AND MYCOTOXINS OF MAIZE IN THREE AGRO-ECOLOGICAL ZONES IN TANZANIA

Modified from a paper to be submitted to *African Journal of Agricultural Research*

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Abstract

Maize is a major cereal crop in Tanzania and it is grown in diverse agro-ecological zones. Like other sub-Saharan countries, postharvest losses of maize during storage in Tanzania remain significantly high, especially for smallholder farmers. Unpredictable weather and poor postharvest practice contribute significant to rapid deterioration of grain and mold contamination, and subsequent production of mycotoxins. The purpose of this study was to assess the postharvest practices and awareness and knowledge of mycotoxin contamination in maize grain in three agro-ecological zones (Eastern, Central, and Northern) of Tanzania between November 2015 and February 2016. A survey using semi-structured questionnaires was administered to farmers, traders, and consumers of maize. A total of 90 people (30 from each zone) were surveyed with a response rate of was 96% (87). In addition, several samples of maize were collected and analyzed for aflatoxin, fumonisin, and Zearalenone contamination to validate the awareness and knowledge of mycotoxin contamination of maize. The result shows a high level of postharvest losses of maize mainly through insect infestation. Moreover, over 80% of the farmers, traders, and consumers of maize were unaware of mycotoxins contamination. All maize samples collected contained detected levels of mycotoxins. The maximum concentration of aflatoxins, fumonisin, and Zearalenone in maize samples was 19.20 ppb, 7.60 ppm, and 189.90 ppb respectively. Education intervention is necessary to decrease the disconnect observed between actual mycotoxin contamination and the awareness and knowledge of farmers, traders, and consumers of maize in Tanzania. Enhancing awareness and knowledge provide the opportunity to educate on post-harvest practices that reduce postharvest losses of maize in Tanzania.

Key words: Maize, postharvest practices, mycotoxins, aflatoxins Fumonisin, Tanzania.

11.1. Introduction

Maize (*Zea mays*, L.) is the major and most cultivated cereal crop in Sub-Saharan Africa (SSA) with over 70 million metric tons grown on more than 34 million hectares in 2014/15 (Macauley, 2015; FAOSTAT, 2016). It is the third most important cereal crop in the world and serves an important food source for over one billion people (IITA, 2009). It accounts for over half and one-fifth of the calories and protein consumed in East and West Africa, respectively (Macauley, 2015). In Tanzania, maize is considered the major staple food for a large proportion of (< 75%) the population, and is grown in diverse agro-ecological zones (Suleiman and Rosentrater, 2015). Maize contributes 36% of the total daily calorie intake, with an estimated annual per capital consumption of about 128 kg (Smale et al., 2011; BEFS, 2013). This is equivalent to around 400g per person per day, with average annual national consumption of three million metric tons (Kimanya et al., 2008; Peter et al., 2013).

Unfortunately, despite its importance as the main staple and commercial crop, many smallholder farmers in SSA, including Tanzania have continued to experience problem post-harvest losses (PHL) of maize during storage. These losses are mainly due to storage insect pests, lack of proper storage structures, and poor handling practices (Demissie et al., 2008). The most significant PHL pests to maize in storage are maize weevil (*Sitophilus zeamais*), larger grain borer (*Prostephanus truncates*), Angoumois grain moth (*Sitotroga cerealella*: Olivier) and rodents (Abass et al., 2014; Kaminski and Christiaensen, 2014; Affognon et al., 2015). The estimated PHL of maize in SSA ranged ranges 10 and 40% (APHILIS, 2015) and can be as high as 50% in a traditional storage structure (Rugumanu, 2004). According to Abdoulaye et al. (2016) the current PHL of maize in Tanzania is around 7.5%. The

postharvest losses of maize and other cereal grains has a significant impact on the food security and the economy of the smallholder farmers (Jones et al., 2015). In SSA, smallholder farmers are more affected by PHL than middle and larger scale farmers. A survey conducted by the World Bank in Tanzania between November and December 2008 shows PHL for smallholder farmers is almost twice (11%) compared to large scale farmers (6%), which corresponds to 19.9 and U\$10.8 per ton respectively (AGRA, 2013). According to Rosegrant et al. (2015) PHL of cereal grain not only pose a threat to the sustainable food security, but also to the nutritional status of the population, especially to the women and children under five in developing countries. Postharvest losses also increases food price by removing a portion of the maize from the supply chain and as well as loss of revenue from producers and traders (Mhlanga et al., 2010; Tefera, 2012). Therefore, reducing PHL will have a significant impact on smallholder farmers by increasing their incomes, food security, reduces malnutrition (Arends-Kuenning et al., 2015), and counteracts the issues of poverty and hunger in developing countries (de-Schutter, 2016).

Furthermore, the poor postharvest practices can lead to rapid deterioration of grain quality, dry matter losses and mold growth (Tangi and Pussemier, 2006; Magan and Aldred, 2007). Mold growth in grain is associated with the production of toxic metabolic by-products or mycotoxins (Hell et al, 2004; Magan et al., 2003). Besides the postharvest losses, mycotoxin contamination is another huge burden on smallholder farmers in SSA (Merck, 2006). It attracts much attention because of its significant impact on the economy and its potential hazard to human health, animal productivity, and trade (Wu, 2004; Wagacha and Muthomi, 2008; Darwish et al., 2014). Mycotoxins are a major problem in SSA countries where climatic conditions, agronomic and storage practices are favorable for insect

infestation, fungal growth and toxin production (Fandohan et al., 2004; Kumar et al., 2008). They are described as ‘silent killers’ since they are hard to detect and some are extremely toxic to both humans and animals (Haladi, 2014; Alimi and Workneh, 2015) due to damage they cause to by damaging the immune system (Mboya et al., 2012). The most important groups of mycotoxins that often occur in agricultural products such as maize grain and of public concerns are aflatoxins, zearalenone, deoxynivalenol (vomitoxin), fumonisins, and ochratoxin (Owaga et al., 2011; Kimanya et al., 2014). However, in SSA, the most prevalent classes of mycotoxins are aflatoxins and fumonisins (Lewis et al., 2005; Kimanya et al., 2008).

Aflatoxins are secondary metabolites primarily produced by spoilage fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Williams et al., 2004; Marin et al., 2013). Aflatoxin contamination is a major contributor to PHL of maize, especially when stored above 12% moisture content (Hell et al., 2010). Most of the maize grain in SSA is poorly handled and stored in local traditional structures (Rugumanu, 2004). Storing maize in these structures exposes them to the environment which leads to insect infestation and invasion by storage fungi (Hell et al., 2000), subsequently increasing the risk of aflatoxin contamination (Borgemeister et al., 1998).

Another important class of mycotoxins is Fumonisins, which are produced by several *Fusarium* species (Bennett and Klich, 2003), notably by *Fusarium moniliforme* (Bruns, 2003). Fumonisins have been related to several fatal diseases in animals such as leukoencephalomalacia in horses, donkeys, and rabbits, pulmonary edema and hydrothorax in swine, hepatotoxic and apoptosis in sheep. They also promote tumors in several animals

such as rats and mice (Hussein and Brasel, 2001; Bennett and Klich, 2003; Fandohan et al., 2004). In humans, fumonisins have been linked to carcinogenic effects such as oesophageal cancer in different regions of the world such as South Africa, China, Italy and Iran (Bennett and Klich, 2003; Fandohan et al., 2004) and impaired growth in young children (Shirima et al., 2014; Kimanya et al., 2008).

Zearalenone (ZEA) is another type of mycotoxin produced by *Fusarium* species, primarily by *Fusarium graminearum* (Doko et al., 1996). Like other types of mycotoxins Zearalenone has been associated with a number of detrimental effects to animals. There affects include hyperestrogenisms, increased incidence of pseudopregnancy, infertility, change in libido, abnormal lactation, feminization, vaginal prolapse, vulval edema and others in pigs (Kuiper-Goodman et al., 1987; Peraica et al., 1999; Zinedine et al., 2007). In the dairy cows, Zearalenone has been associated with milk reduction (Suleiman and Rosentrater, 2015). In humans, the primary symptoms of Zearalenone include nausea, vomiting, and diarrhea (Lombard, 2014). It has also been linked with pubertal changes of young children in Puerto Rico (Kuiper-Goodman et al., 1987). The objective of this study were to assess the postharvest practices and awareness of and knowledge of mycotoxins contamination in maize grain in three agro-ecological zones (Eastern, Central, and Northern) of Tanzania.

11.2. Materials and methods

11.2.1. Study Area

This study was conducted in three districts in Tanzania: Babati (located below the equator between latitude 3° and 4' south, and between longitude 35° and 36° east), Chamwino (located below the equator between latitude 7° and 5' south, and between

longitude 36° and 13° east) and Kilosa (locate between latitude 6° and 42' South, and between longitude 367° and 48' East) for the Manyara, Dodoma, and Morogoro regions respectively (Figure 79). These locations were purposefully selected due to different agro-ecological zones and previous reports of high postharvest losses and mycotoxins contamination of maize and other cereal grains (TFDA, 2012; APHLIS, 2015; Kamala et al., 2015).

11.2.2. Assessment of Postharvest Practices and Awareness of Mycotoxins Contamination

The study was conducted to attempt to seek answers to the key questions about postharvest losses, awareness, and knowledge of mycotoxin. What are the main causes of postharvest losses of maize? At what level do you discard your maize grain? How long do you store your maize grain? In the maize value chain where does the major losses occur? Any knowledge or awareness of mycotoxin contamination (Table 41).

A semi-structured questionnaire was developed and used to collect the data. After written informed consent was obtained from the Institutional Review Board (IRB 15-528 Suleiman), the study was conducted in three districts (Kilosa, Chamwino and Babati) of Tanzania between November 2015 and February 2016. A total of 90 participants (30 farmers, 30 traders and 30 consumers) have participated in the study with a response rate of 98% (89).

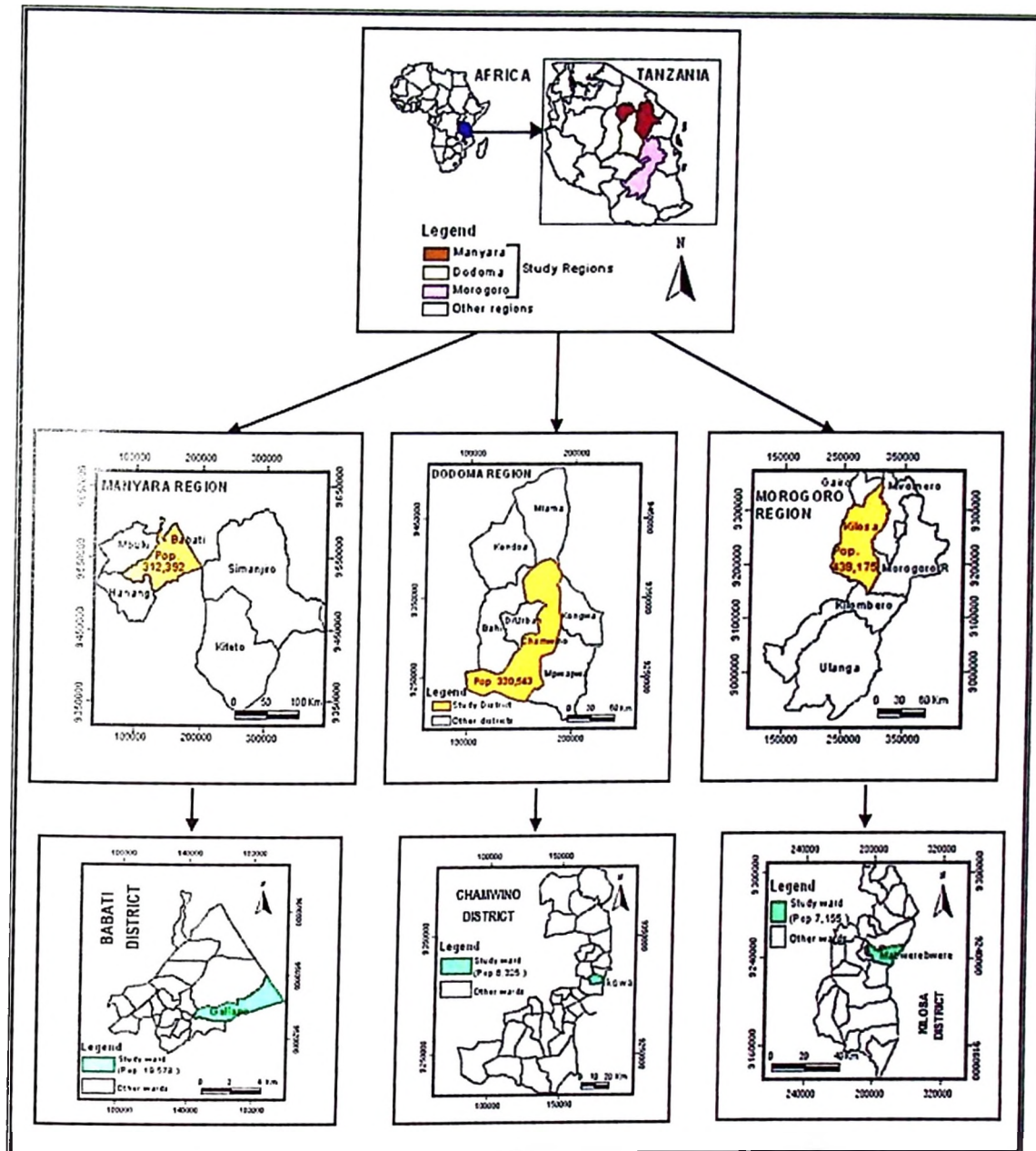


Figure 79. Map of Tanzania Showing Study Regions, Districts and Wards Sampled.

The survey was pre-tested with farmers, traders, and consumers of maize in Morogoro municipality December 2014 ($n = 10$). Farmers, traders and consumers of maize were chosen because they were stakeholders in maize production process. The questions were written in English and was then translated to Swahili to make it easy for the participants to understand.

For those participants that unable to read, the investigator read each question and the participants responded verbally. Each participant was given an honorarium of \$2 for their participating in the study.

Moreover, maize samples for mycotoxins analysis were sampled according to the procedures described by Kimanya et al. (2008) and Kamala et al. (2015). About 1kg of maize was drawn from randomly from farmers and traders for mycotoxin analysis. A total of 30 samples (10 per district) from all regions were collected and stored in airtight plastic bags at 4°C until analyzed for aflatoxins, fumonisin, and zearalenone. The samples of maize were collected to analyze various mycotoxin to validate the survey on awareness and knowledge of mycotoxins of maize in Tanzania.

11.2.3. Sample Preparation and Mycotoxins Determination

The aflatoxin, fumonisin, and zearalenone content of maize samples was analyzed by using Reveal Q⁺ kits (Neogen® Corporation, Lansing, MI, USA) as per manufacturer's instructions. Briefly, the 1kg of maize samples collected from farmers and traders were mixed well and about 500g was ground using a high-speed universal grinder (Great Wall Instruments Co., Ltd, Huang Cheng, Mainland, China), thoroughly mixed and stored in Ziploc® slider (6.8 µm) one-quarter polyethylene freezer bags (SC Johnson, Racine, WI 53403) stored at 4°C until analyzed. Then, 10g of a well-homogenized ground sample was weighed using an electronic balance (Contech® Instruments Ltd, Model CA-224, 301, Punit Indl. Premises, Turbhe, Navi Mumbai – 400705, India).

Table 41. Types of Information Collected in the Study.

Type of information	Specific data collected in the questionnaire
General information	Biodata (gender, age, education level)
	Name of district
	Source of income (daily activity)
Postharvest practices	Total area cultivated (ha)
	Amount of maize harvested (last season)
	Sorting criteria after harvest
	Storage structures, practices and losses
	Main cause of losses (postharvest losses) of maize
Mycotoxin contamination	Knowledge on moldy maize
	How moldy maize is handled(discard, sell, as food/feed)
	Have you heard the word mycotoxin before?
	Awareness of mycotoxin (aflatoxin) contamination
	Effects of mycotoxins contamination on humans and animals

Mycotoxin extractions were performed by adding 50 mL of 65% ethanol to the sub-samples followed by handshaking for three minutes. The mixture was allowed to settle for about two minutes, then the supernatant was drawn by uses of a three mL syringe (BD Luer-Lok™, 1 Becton Drive, Franklin Lakes, NJ 07417, USA) passed through a sterile syringe filter of 0.45 microns (Corning Incorporated, Corning, NY 14831, Germany) and collected in a clean test tube, and labeled appropriately. Five hundred μL of sample diluent was added to the red dilution cup (provided in the kits) and 100 μL of the filtrate was added to the red dilution cup and mixed up and down five times. Then, 100 μL of the filtered dilute extract

solution was pipetted and transferred onto the white sample cup (provided in the kits), and the Reveal Q⁺ strips were inserted for either aflatoxin, fumonisin or zearalenone, and then incubated for six minutes. After the incubation, the developed strips were removed and inserted into a Reveal AccuScan Pro 2.0 Reader System (620 Lesher Place, Neogen® Corporation, Lansing, MI 48912 USA) to determine aflatoxin, fumonisin or zearalenone content of the sample. The Reveal Q⁺ assay is quantitative for total aflatoxins, fumonisin, and zearalenone with a range of detection of 2–150 ppb, 0.3-6 ppm and 50-1200 ppb for aflatoxin, fumonisin, and zearalenone, respectively. All maize samples were analyzed in duplicate.

11.2.4. Statistical Analysis

Collected data were coded and entered into Microsoft Excel 2016 and analyzed using SPSS version 16.0 (SPSS, Chicago, IL, USA). Descriptive statistics were performed to compute relevant variables. The mean and standard deviation of aflatoxin, fumonisin, and zearalenone for each district was calculated using Microsoft Excel 2016 and expressed as a mean \pm standard deviation.

11.3. Results

11.3.1 Assessment of Postharvest Practices and Awareness of Mycotoxins Contamination

The assessment of postharvest practices and awareness of mycotoxins contamination in three agro-ecological zones of Tanzania were divided into three main categories: farmers, traders, and consumers.

11.3.2. Farmers

The farmer responses to the survey from the three agro-ecological zones are presented in Table 42. The results show women constituted 80% of the farmers interviewed in Kilosa and Chamwino districts and 40% in Babati. Seventy percent of the farmers have at least a primary education. The mean age of all farmers was 25 ± 6.4 years (Table 42). The survey found 70, 70, and 40% of the farmers in Kilosa, Chamwino, and Babati respectively cultivated an average of five to ten ha for maize production. All of the respondents (farmers) across all zones experience postharvest losses of maize, mainly due to weather conditions and insect infestation. The result shows most of the farmers sort their maize prior to storage. Also, the study found damaged maize was used as feed and discarded when totally moldy. In addition, the result shows that postharvest losses were mainly (over 60%) occurring during storage as shown in Table 42. The majority of farmers (over 80%) said they do not have any knowledge or they never heard about mycotoxin contamination before (Figure 80).

Table 42. Farmer's Responses on Postharvest Practice and Mycotoxins Awareness in three Agro-Ecological Zones (%) (N = 30 for each District).

Post-harvest practice and mycotoxins awareness	Parameter	Percent respondents (%)		
		Kilosa	Chamwino	Babati
Biodata	Male	20	20	60
Gender	Female	80	80	40
Education level	None	10	10	0
	Primary	60	50	100
	Secondary	30	40	0
Age group	18-25 years	30	25	0
	25-40 years	20	35	40
	Over 40 years	50	40	60
Total production area	Below 5ha	30	30	60
	5-10ha	70	70	40
Total yield in bags (1bag = 100 kg)	Less than 5 bags	20	20	60
	5-10 bags	70	70	20
	Above 10 bag	10	10	20
Main cause (s) of maize losses	Pest infestation	60	60	33.3
	Poor storage	0	0	6.7
	Weather conditions	40	40	60
How long do you store your maize	Less than 3 months	0	0	3.3
	Three months	100	100	90
	Six months	0	0	6.7
	Over six months	0	0	0
Sorting practices (criteria)	Color	30	30	0
	damage	70	70	100
Handling practices- with damage maize & level of discard	Used as food	0	0	10
	Used as feed	100	100	100
	When totally mold	100	100	86.7
	Not discarded	0	0	13.3
Knowledge of mycotoxins contamination	Yes	50	40	20
	No	50	60	80
Major causes of PHL in the value chain	Transport	30	30	20
	Drying	0	0	20
	Storage	70	70	60

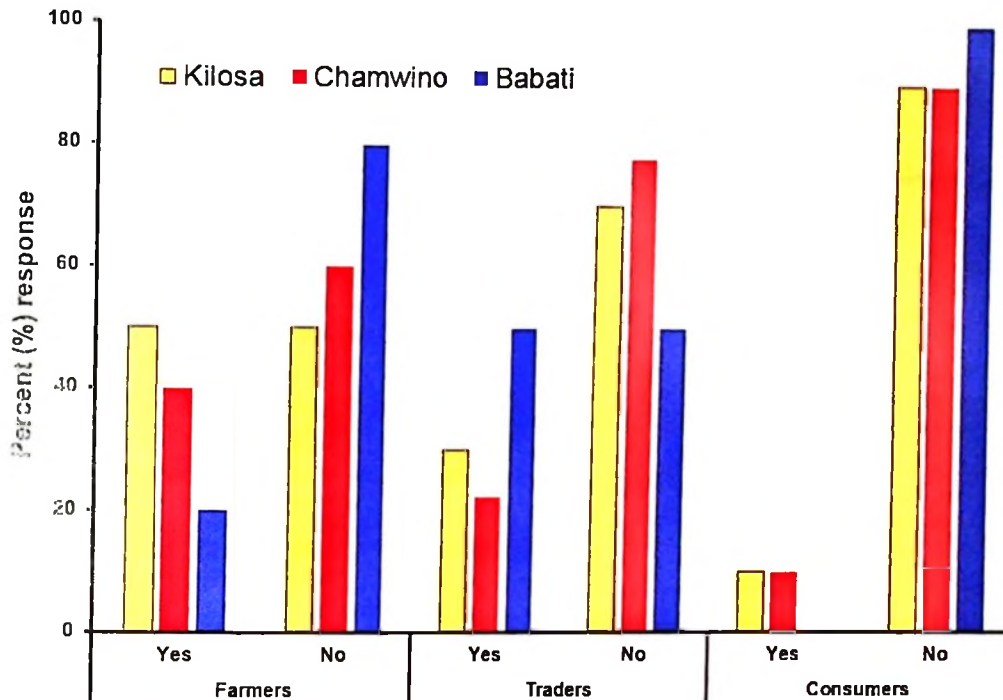


Figure 80. Mycotoxins Awareness of Farmers, Traders and Consumers in three Districts.

11.3.3. Traders

A descriptive summary of Trader's is shown in Table 43. As expected, most of the traders were male: 100% in Kilosa, 88.9% in Chamwino, and 100% in Babati. The majority of the traders have at least a primary education: 70, 77.8, and 60% for Kilosa, Chamwino, and Babati respectively. The mean age of traders was 27 ± 4.6 years. The study also found most of the traders store their maize in the living house without proper storage structures (Table 43). Also, the result shows insect infestation is the main cause of maize losses in storage: 100, 88.9, and 90% for Kilosa, Chamwino, and Babati respectively. Chemical insecticides were used by over 75% of traders to control insects in storage. Mixed results were obtained when traders asked when they discard their maize, 70 and 66.7% in Kilosa and Chamwino discard their maize only it when it shows signs of mold contamination, but 70%

of the traders in Babati discard maize when is totally moldy. Furthermore, over 50% of the traders surveyed used damage maize for animal feed. Also, the result shows over 87% of the losses occur in the storage. In addition, a nearly two-thirds of the participants has no knowledge of mycotoxins contamination (Figure 80).

Table 43. Traders' Responses on Postharvest Practice and Mycotoxins Awareness in three Agro-Ecological Zones (%) (N= 30 for each District).

Post-harvest practice and mycotoxins awareness	Parameter	Percent respondents (%)			
		Kilosa	Chamwino	Babati	
Biodata	Male	100	88.9	100	
	Female	0	11.1	0	
Education level	Primary	70	77.8	50	
	Secondary	30	22.2	40	
Age group	Under 18 years	10	0	0	
	18-25 years	0	22.2	10	
	25-40 years	80	66.7	20	
	Over 40 years	10	37.5	70	
	Maize storage	Traditional granary	0	22.2	10
		Living house without improved structure	100	77.8	30
		Living house with improved structure	0	0	40
Storage duration	Less than three months	0	11.1	20	
	Three months	40	66.7	10	
	Six months	60	22.2	30	
	Over six months	0	0	40	
Insecticide application	Yes	100	77.8	50	
	No	0	22.2	50	
Main pest	Insects	100	88.9	90	
	Rodent	0	11.1	10	
Do you sell maize when damaged	Yes	70	100	90	
	No	30	0	10	
When do you discard your maize	Show sign mold contamination	20	0	10	
	Totally moldy	70	66.7	20	
	Not discarded	10	33.3	70	
What do you do with damage maize	Give away	0	0	20	
	Used as food	30	0	20	
	Used as feed	50	55.6	60	
	Mix with others and sell	20	44.4	0	
Major causes of PHL	Transport	0	11.1	3.4	
	Drying	0	0	24.1	
	Storage	100	88.9	72.4	

11.3.4. Consumers

Table 44 shows a descriptive summary of the responses of consumers. The results show that most of the consumer of maize are female: Chamwino (60%), and Babati (90%). However, males were the majority in Kilosa with 70 percent. The average age of the consumers in all districts was 25 ± 4.2 years. It was observed that the majority of consumers have primary educations, except in Chamwino (Table 44). The main quality criteria used by consumers across all regions were maize to be free from insects and mold contamination (60, 80, and 60% for Kilosa, Chamwino, and Babati respectively). Price seemed to not be an important factor to consumers in Chamwino and Babati districts, but was very important in Kilosa (70%). Also, the results show that insect infestation is the major cause of postharvest losses. Like in the other two categories (farmers and traders) most of the consumers interviewed believe major losses of maize occurred during storage. Finally, most of the consumers interviewed have no knowledge of mycotoxin contamination (Figure 80).

11.3.5. Mycotoxin Contamination of Maize in Three Agro-Ecological Zones

The overall mean concentration of mycotoxin contamination (aflatoxin, fumonisin, and Zearalenone) is presented in Table 45. All maize samples collected contained detectable levels of mycotoxins. The maximum concentration of aflatoxins, fumonisin, and Zearalenone in maize samples was 19.20ppb, 7.60ppm, and 189.90ppb respectively. The highest aflatoxin concentration was observed in the Kilosa district with concentrations of 19.2 and 17.3ppb, and lowest concentration was detected in Babati district with concentration of 2.0ppb (Figure 81). In addition, the highest concentration of fumonisin and Zearalenone was detected in Babati district: 7.6ppm and 189.9ppb respectively.

Table 44. Consumer Responses on Postharvest Practice and Mycotoxin Awareness in three Agro-Ecological Zones (%) (N = 30 for each District).

Post-harvest practice and mycotoxins awareness	Parameter	Percent respondents (%)		
		Kilosa	Chamwino	Babati
Biodata	Male	70	40	10
Gender	Female	30	60	90
Education level	None	0	10	20
Age group	Primary	90	20	60
	Secondary	10	30	20
	College	0	40	0
	18-25 years	10	20	10
	25-40 years	40	70	40
	Over 40 years	50	10	50
Main quality criteria to buy maize	Free from insects and mold contamination	60	80	60
	Quality of maize	40	20	40
Most important parameter	Quality	70	60	70
	Price	30	40	30
Most parameter do you check before buy maize	Moisture of maize	10	10	20
	Insects contamination	60	50	40
	Mold contamination	30	40	40
Could you buy mold maize under reduced price	Yes	70	10	40
	No	30	90	60
Major causes of PHL	Insects	60	40	60
	Spillage	0	10	0
	Rodents	10	10	0
	Poor storage structure	30	40	40
Major PHL in the supply chain	Transport	30	30	0
	Drying	0	15	40
	Shelling	20	10	20
	Storage	50	45	40

In general, 33% of all samples collected exceeded the maximum limit set by Tanzania Bureau of Standard (TBS) for total aflatoxin (10ppb).

Table 45. Mycotoxin Contamination in Maize Grain.

Parameter	Aflatoxin (ppb)	Fumonisin (ppm)	Zearalenone (ppb)
Overall mean \pm S.D	4.2 \pm 2.9	1.4 \pm 1.3	57.8 \pm 13.5
Range, all samples	2.0– 19.2	0.3 – 7.6	50.0 - 189.9
Number of districts	3		
	30		

11.4. Discussion

The results of this study are consistent with previous authors (Hell et al., 2000; Kimanya et al., 2008; 2010; 2014; Mboya et al., 2012; TFDA, 2012; Shirima et al., 2014; Kamala et al., 2015; 2016) who show that postharvest practices and lacks of public awareness on mycotoxins have an influence on contamination of maize with mycotoxins. In general, the study found most of the participants in agriculture were women rather than male. This result are comparable to the findings of Ellis et al. (2007) who reported women in Tanzania were more active in agricultural activities and account for about 52%. Likewise, a study conducted by the United Nations Development Programme (UNDP) found women make up about 60-80% of the agricultural labor force in Nigeria (Ogunlela and Mukhtar, 2009). In contrast, Jolly et al. (2009) found a high proportion of farmers in Ghana are male rather than women.

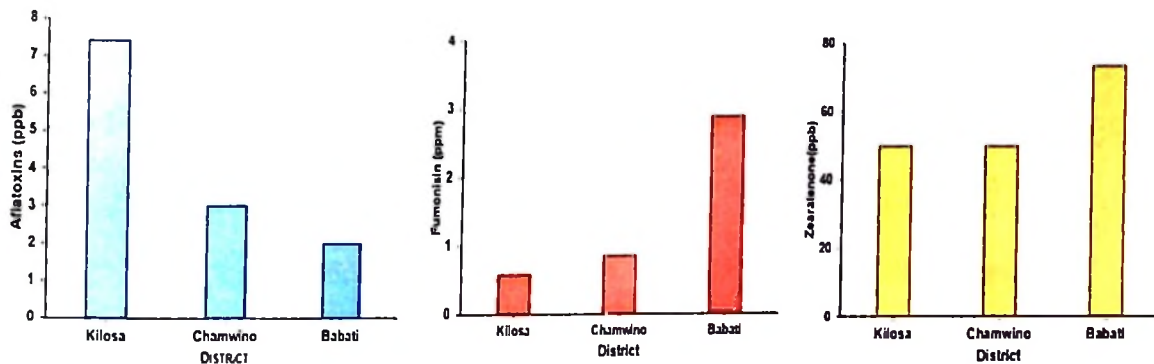


Figure 81. Mycotoxins Concentration Levels (Aflatoxins, Fumonisin, & Zearalenone) in Three Districts.

In addition, Ellis and others found women in Tanzania were more engaged in trade than male (Ellis et al., 2007). However, this contrasts with our finding where over 90% of the traders surveyed were male. Most of the participants had a primary education over 50%

across three categories (farmer, trader and consumer) in all agro-ecological zones. A similar finding was reported by the National Bureau of Statistics (NBS) that over 80% of the population in Tanzania mainland attained primary education (NBS, 2013). Education level seems to be directly related to mycotoxins awareness. As shown in Figure 79, overall mycotoxin contamination (aflatoxin and fumonisins) in Chamwino district was significantly lower compared to Kilosa and Babati. In addition, the surveyed conducted by Dosman et al. (2001) found that people who are more educated are more aware of the risks associated with food safety, such as aflatoxin contamination, compared to less educated people. Also, Baker (2003) found a high correlation between education and income and food safety. Another study conducted by Jolly et al. (2006) on awareness and perceptions of groundnut aflatoxin among Ghanaians found education level had a positive effect on the awareness of aflatoxin contamination and concluded that more highly educated participants to have a better knowledge of aflatoxin and are more aware of groundnut contamination compared to less educated participants. However, a survey conducted by Leong et al. (2012) in Malaysia found no significant association between aflatoxin levels with gender and education level.

Moreover, the study found a high percentage of postharvest losses of maize. One hundred percent of all participants surveyed experience PHL of maize mainly by insect infestation. The study also found main losses occurred during storage; this result concurred with previous reports (Rugumanu, 2004; Demissie et al., 2008; FAO, 2011; Abass et al., 2014; Kaminski and Christiaensen, 2014; Affognon et al., 2015) that significant loss of maize grain in developing countries occurs during storage (15-25%). Furthermore, the results showed a noteworthy portion of the population has little or no knowledge of mycotoxin contamination. This could be the reason of high mycotoxin contamination in some regions like Kilosa and

Babati. According to Gong et al. (2002), increasing awareness and knowledge about aflatoxins may reduce aflatoxin as well as other mycotoxins contamination of cereal grain. Nandi and Häggblom (1984) reported that the problem of mycotoxin contamination in agricultural commodities in developing countries is made worse by lack of public awareness of mycotoxin contamination.

In addition, the occurrences of aflatoxin and fumonisin in this study are significantly lower compared to other studies conducted by TFDA, 2012; Kamala et al., 2015; 2016). A greater variation in types and levels of mycotoxin contamination was observed across agro-ecological zones and this aligned with the results of previous studies (Kamala et al., 2015; 2016). This could be explained by postharvest practices and climatic conditions. For instance, the average mean temperature and relative humidity during the time of data collection (December 2015) were 30 °C and 69% R.H in Kilosa (Morogoro), 28°C and 66% in Chamwino (Dodoma), 26°C and 64% R.H in Babati (Manyara). These conditions are favorable for the growth and development of mold growth and subsequent toxin production (Kaaya and Kyamuhangire, 2006). It has been noted by Paterson and Lima (2010) and Tran-Dihn (2013) that environmental factors and irregular weather conditions contribute to mycotoxins contamination in tropical countries. In addition, the study also determined the concentration of Zearalenone in several maize samples. The overall result is shown in Table 5. Results of this study were within the range of the results obtained by Doko et al. (1996). However, they were significantly low compared to those reported by Degraeve et al. (2016).

11.5. Conclusions

This study assessed the postharvest practices and awareness of mycotoxins. The results show postharvest losses of maize are quite high and a significant portion of the population are unaware and have no knowledge of mycotoxin contamination. Mycotoxins (aflatoxins, fumonisins, and zearalenone) was detected in all samples collected. This information shows a necessity of creating a monitoring, surveillance, and intervention program on mycotoxins. Also, the necessary effort is needed to educate the general public about the risks of mycotoxin contamination and affordable techniques should be provided to reduce postharvest losses of maize in Tanzania.

11.6. References

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CHAPTER 12. GENERAL CONCLUSIONS

Agriculture is the backbone of the Tanzanian national economy. It accounts for about one-third of the gross domestic product, provides 85 percent of all exports and serves as a livelihood to over 80 percent of the total population. Maize is a primary staple crop grown in nearly all agro-ecological zones in the country. Tanzania is a major maize producer in Sub-Saharan Africa. However, despite being the highest producer of maize in the EA region, post-harvest losses of maize remained significantly higher. Such loss often aggravated by inappropriate handling, poor storage facilities, insects, and other pests, and contamination by spoilage fungi. The major effects of fungi on maize are discoloration, reduce quality and contaminate maize with mycotoxins. Mycotoxins are toxic secondary metabolites of fungi that frequently contaminate the maize in the field and/or during storage. Mycotoxins contamination of maize poses a health risk to humans and domesticated animals if not properly managed because of their acute and chronic effects. The most important mycotoxins in maize are the aflatoxins, fumonisins, deoxynivalenol, and ochratoxin. The strategies to reduce mycotoxins in maize include pre-harvest and post-harvest strategies. The pre-harvest strategies include the application of atoxigenic fungal strains and antagonistic micro-organisms, crop rotation, tillage practices, appropriate application of fertilizers, weed control, irrigation, insect control, genotypes of seed planted. The post-harvest strategies include proper storage (hermetic storage), improve drying conditions and grain milling. Also, minimizes times between harvesting and drying, sanitation, efficiently drying to below 14% moisture content and physical separation of damaged grains.

The postharvest losses are a major factor negatively affecting smallholder farmers in Tanzania. The major constraints to maize production include pests (*S. zeamais* and *P. truncatus*), diseases, weeds, pathogens, and viruses. Reducing PHL has positive consequences for a society like poverty alleviation, increase food security, improving nutrition status, and increases household income. The main strategies to reduce PHL include like using improved maize varieties, harvest at the right time, and improve storage structures like hermetic storage containers such as PICS bags and metal silos. As well as improving drying efficiency, uses of moisture and temperature meters, proper hygiene and sanitation and access to market information. Moreover, the study found dent corn is more susceptible to *S. zeamais* than flint corn. Other factors, such as time and temperature, play large roles in corn infestation. Most damages occurred at 27°C and 30 days storage time. Hence, flint corn, or a hybrid of flint and dent, could be a viable approach to reducing the problem of infestation and damage in developing countries. Likewise, the study on evaluation of maize weevils *S. zeamais* infestation on seven varieties of maize found three maize varieties (orange flint corn, yellow popcorn, and white popcorn) were resistant to *S. zeamais* infestation based on the number of live *S. zeamais*, seed weight damaged, percentage seed weight losses and weight of powder produced. Thus, orange flint corn and popcorn may be potential maize varieties to be used to reduce the postharvest loss of maize in tropical countries due to *S. zeamais*. The study also found some similarity and difference between techno-economic analysis and life cycle assessment of maize storage for middle scale farmers. As the storage capacity increased the total storage cost per kg decreased. For the LCA, as storage capacity increased more energy is needed to operate the equipment. Consequently, more GHS's emissions were emitted.

Further, in the determination of measured and predicted temperature of maize under hermetic conditions, we found the predicted temperatures were closely matched with measured values throughout the experiments. In general, maize grain temperature oscillated greatly on the boundary of the bin and slightly at the center. The temperature in the hermetically sealed cylindrical bins varied, mostly in the radial direction and very little in the axial vertical directions. Conversely, no noticeable change was observed in room condition. The lag time between the center temperature and the side (right, left, top and bottom) was greater in the radial direction compared to vertical temperature. Also, the temperature in the grain changed more rapidly in the freezing conditions than in the room and cooling conditions. In addition, the study was conducted to determine the impact of moisture content and *S. zeamais* on maize quality during hermetic and non-hermetic storage conditions. The results show that *S. zeamais* and moisture content play significant roles in maize quality during storage. A positive and significant relationship between fungal growth and aflatoxin accumulation was observed in maize grain stored at high moisture content with weevil treatments. However, no aflatoxin accumulation was detected in low moisture maize and under hermetic conditions. Total mortality (100%) of *S. zeamais* was obtained at the end 60 days storage of hermetic storage, but the number of insects increased as moisture content and storage time increased for NHW treatments. The moisture content of maize grain stored in hermetic conditions was relatively constant, but a linear relationship between storage time and moisture content was in a non-hermetic with weevil treatment.

In another study, the low synergistic interaction between *P. truncatus* and *S. zeamais* in terms of number live populations and the weight of powder was observed. A higher number of *S. zeamais* was observed at the end of the study but was outnumbered by *P. truncatus* after

60 days of storage. The percentage grain damage, and the weight of powder produced was higher in *P. truncatus* and mixed treatments than *S. zeamais* treatments. Percentage seed weight loss was decreased after 60 days for *P. truncatus* and mixed treatments, but was sharply increased for *S. zeamais*. Both insects cause significant damage when coexisting during storage. However, *P. truncatus* was the major contributor of grain damage both in the individual treatments as well as in the mixed treatments in the hermetic and non-hermetic storage. In addition, the study found the periodic physical disturbance was a very effective and may be a feasible method to protect maize grain from *S. zeamais* infestation. The result has shown that disturbed containers twice a day could reduce *S. zeamais* infestation significantly with minimum mechanical damage of the maize grains. After three months of storage percentage the mortality rate of *S. zeamais* were 98%. After three months of storage of maize, there was no live *S. zeamais* in any container in any of the three regions. Hence, this study demonstrates the potential of a simple, affordable, feasible, safe, and effective method of protecting maize grain for small-holder farmers in developing countries who cannot afford modern and costly methods to control maize grain from insect infestation. Finally, in the assessment of postharvest practices and awareness and determination of mycotoxins in maize in three agro-ecological zones in Tanzania, on farmers, traders, and consumers of maize. The study found over 80% of the farmers, traders, and consumers of maize were unaware of mycotoxins contamination. Also, all maize samples collected contained detected levels of mycotoxins concentration. The maximum concentration of aflatoxins, fumonisin, and Zearalenone in maize samples was 19.20 ppb, 7.60 ppm, and 189.90 ppb respectively. Therefore, the necessary effort is needed to educate the general public about the risks of mycotoxins contamination and affordable techniques should be provided to reduce postharvest losses of maize in Tanzania.

APPENDIX 1. QUESTIONNAIRE FOR POSTHARVEST LOSSES PRACTICES AND MYCOTOXINS

A: General information

1. Name of street/ village
2. Name of District.....
3. Name of interviewee.....
4. Gender (a) male [] (b) Female []
5. Age (a) under 18 [] (b) 18-25 [] (c) 25-40 (d) over 40
6. Education level
.....
(0= none, 1 = Primary, 2 = Secondary, 3 = College, 4 = none, 5= other (specify).....)
7. What type of activity are you involved in.....
(1 = farming, 2= trader, 3= consumer, 4 = both 1, 2, & 3, 5 = other (specific).....)

Instructions

- If Qn 8=1 go to Section B Information on Farming
 If Qn 8=2 go to Section C Information on Trader
 If Qn 8=3 go to Section D Information on Consumer

B: Information on Farmers

8. Total area cultivated (Ha) (a) below 5 [] (b) 5-10 [] (c) 10-50 [] (d) above 50 []
9. During the last season how many bags or kg of maize did you harvest
10. Out of the bags you harvested were there any loses.....
(1 = Yes, 2 = No)
11. What are the main reasons for post-harvest loses.....
(1=lack of storage, 2 = Pest infestation, 3 = Poor storage, 4= Poor weather, 5= other (Specify).....)

If answer is 2 go to Question 13

12. What types of pest infestation.....?
(1= insects, 2 = mold, 3= rodent/mice, 4= birds, 5 = other (specify).....)

If answer is 1 go to Question 14

13. What types of insect.....?

(1= maize weevils, 2 = larger grain borer, 3= other (specify).....)

14. Average number months maize will be held

(1 = less than 1 month, 2 = three months, 3 = six months, 4 = one year, 5 = other (specify).....)

15. How do you dry your maize grain.....?

(1= No drying, 2= sun drying, 3= solar drying, 4= mechanical drying, 5= other (specify).....)

16. After harvesting do you sort out certain cobs.....

(1= Yes, 2=No), If yes go to question 21

17. What the criteria for sorting.....

(1 = color, 2= cob size, 3=grain size, 4= damage, 5 = other (specify).....)

18. What do you with the damage maize cobs.....?

(1=throw them away, 2= domestic consumption, 3= animal feeds, 4=sell them. 5= other (specify)....)

19. At what level do you discard maize grain.....?

(Picture: 1= when show sign of mold growth, 2 = when show clear sign of mold growth. 3 = when is total moldy, 4 = not discard, 5 = other (specify).....).

20. What methods of discard

(1 = used as animal feeds, 2 = burning, 3 = burial, 4= left in the field. 5 = other (specify)....).

21. Do you have any knowledge about effect of moldy maize.....

(1 = Yes, 2 = No)

22. Have you heard of the word mycotoxins (aflatoxins) before

(1 = Yes, 2 = No)

23. Are you aware of mycotoxins contamination in crop (maize).....

(1 = Yes, 2 = No)

24. Are you aware of effects of mycotoxins on human and animals.....

(1 = Yes, 2 = No)

25. In your view, where in the post-harvest maize value chain do the major losses occur?

(1= transport from field to home, 2= drying, 3= shelling, 4= storage, 5 = other (specify).....)

C: Information on Trader

26. Where do you store your maize.....

(1= in traditional granary, 2= in living house without improved structure, 3= in living house with improved structure, 4 = rented facility, 5 = other (specify).....).

27. How long you store your maize.....

(1 = less than 1 month, 2 = three months, 3 = six months, 4 = one year, 5 = other (specify).....).

28. Are there any challenges/problems/constraints for storing maize.....?
(1= lack of finances to build or to rent storage facilities, 2 = lack or inefficacy of pesticides, 3 = uncertainty in profitability and market prices, 4 = theft, 5 = other (specify)?)
29. Do you use chemical to treat your maize in storage.....
(1= Yes, 2=No)
30. What is the main pests in your storage.....
(1= insects, 2 = molds, 3 = rodent/mice, 4 = other (specify).....)
31. Do sell maize when damaged by insect or molds.....
32. At what level do you discard maize grain.....?
(Picture: 1= when show sign of mold growth, 2 = when show clear sign of mold growth, 3 = when is total moldy, 4 = not discard, 5 = other (specify).....).
33. What do you with the damage maize cobs.....?
(1=throw them away, 2= domestic consumption, 3= used as animal feeds, 4=sell them, 5= other (specify).....).
34. Any food inspector or health officer inspect your product (maize).....
(1 = Yes, 2 = No), If yes go to question 33, no go to question 34.
35. How often food inspector visit your shop or site.....
(1= none, 2 = once per year, 2= twice per year, 3=randomly 4 = other (specify).....).
36. Do you have any knowledge about effect of moldy maize.....
(1 = Yes, 2 = No)
37. Have you heard of the word mycotoxins (aflatoxins) before
(1 = Yes, 2 = No)
38. Are you aware of mycotoxins contamination in crop (maize).....
(1 = Yes, 2 = No)
39. Are you aware of effects of mycotoxins on human and animals.....
(1 = Yes, 2 = No)
40. Do you think the people that handle the maize after harvest have the requisite knowledge on proper handling.....?
(1= Yes, 2 =No)
41. In your view, where in the post-harvest maize value chain do the major losses occur?
(1= transport from field to home, 2= drying, 3= shelling, 4= storage, 5 = other (specify).....)

D: Information on Consumer

42. What your quality criteria when you buy maize.....

(1= free from insects & molds, 2= quality of maize, 3= other (specify).....).

43. Which of the following is most important to you as consumer.....

(1= quality, 2= price, 3= other (specify).....).

44. What do you check when you buy maize.....?

(1= moisture of maize, 2 = rodents & insects contamination, 3= mold/discoloration, 4=other (specify)... ..).

45. Do you buy mold or damaged grain if sell under reduced price.....

(1= Yes, 2=No)

46. In your view, where in the post-harvest maize value chain do the major losses occur?

(1= transport from field to home, 2= drying, 3= shelling, 4= storage, 5 = others (specify).....)

47. In your view, what is the main cause of post-harvest maize losses?

(1= insects/molds, 2= spillage, 3=rodents, 4= poor storage structures, 5 = poor handling, 6 = others (specify)...

**APPENDIX 2. MORE DATA FOR TECHNO-ECONOMIC ANALYSIS AND LIFE
CYCLE ANALYSIS**

Unit Conversions Table		
3.11 Kg of CO ₂	=	1L of diesel fuel
CO ₂ moleccule	=	27.3% C by mass
1 tonne	=	1.10231 tons
39.6 MJ per Liter of liquid diesel fuel		
1 ha		10,000m ²
6.9 g/bhp-hr of Nox		
1 mole of CO ₂	=	44.01g
1 mole of N ₂ O	=	44.013g
1 mole of CH ₄	=	16.04 g
1 Kg of N ₂ O	=	298 CO ₂ -eq
1 Kg of CH ₄	=	25 CO ₂ -eq
1 ft	=	12 in
1 kw	=	1000 watts
1 dollar	=	100 cents
1 lb	=	0.0005 tons
1 gal	=	0.134ft ³
BTU's/ bu (dry corn 22 to 14%)	=	16774
1 Liquid propane (LP) BTU/bu	=	92,000
Air flow rate (cfm/bu)	=	100
Air temperature (F)	=	180

Techno-Economic Analysis Calculations

	Scenarios			
	I	II	III	
Daily storage capacity (bu/d)	50	500	5,000	
Yearly storage rate (G)- bu/y	25,000	250,000	2,500,000	
Interest rate (I)	8.00%	8.00%	8.00%	
Life expectancy (L), y	25	25	25	
Operation hours (OH), h/y	1000	1000	1000	
Electricity price to run motor and other equipment (EP), \$/kWh	8.01	8.01	8.01	
Depreciation	4.00%	4.00%	4.00%	
Insurance	0.50%	0.50%	0.50%	
Taxes	1.00%	1.00%	1.00%	
Overhaul	0.50%	0.50%	0.50%	
Shrinkage	2.00%	2.00%	2.00%	
Harvesting	0.05	0.05	0.05	
Repairs	1.00%	1.00%	1.00%	
Total % Cost	15.00%	15.00%	15.00%	Annual % Cost
Grain Bin Cost (\$)	1,800	18,000	180,000	
Cost/kg (S)	0.072	0.072	0.072	Investment cost per bushel
Cost/kg-Year	0.0108	0.0108	0.0108	Annual investment cost per bushel

Assumptions			
Truck travel distance	10 km	6.21 miles	
2-Axle single unit truck	6,820 kg	15,000 lb	33 m ³
Functional unit =	1 kg (maize)		
Electricity (kWh)	0.75 kWh/bu	(21 to 16)% M.C	
Propane needed/ bu	26 gallons	120 bushels of corn	
CO ₂ emissions factor for electricity generation in IOWA		0.875	
Nox emission factor for electricity generation		0.000662	
SO ₂ emission factor for electricity generation		0.000402	
Fuel consumption for combine		2.24 gallons/ acre	
Fuel consumption	4.25 mpg		
Liquid propane	0.02 gallons /bushel/ per % M.C		
Initial M.C (%)	21 %		
Final M.C (%)	16 %		
Corn yields	1 acre	164 Bushels	
Scenario I	152.44	25000 Bushels	
Scenario II	1524.39	250000	
Scenario III	15243.90	2500000	
Emission coefficients (Diesel powered vehicles)	0.15 kg CO ₂ /tonne-km		
	0.00174 kg NO _x /tonne-km		
Energy consumption (grain dryer)	2 kWh/ 1000 bushels		

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