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Effect of Broken Kernels, Weevils (Sitophilus Zeamais), and Storage Period on Deterioration Indicators of Maize (Zea Mays) Under Storage Condition

Ferede, Gedifew

BAHIR DAR UNIVERSITY

COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCES

DEPARTMENT OF PLANT SCIENCES

GRADUATE PROGRAM IN PLANT PROTECTION

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MSc Thesis

By:

Gedifew Ferede Tilahun

Main Advisor: Tesfaye Melak (PhD)

Co-Advisor: Admasu Fanta (PhD)

June, 2025

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A Thesis Submitted In Partial Fulfillment of the Requirements for the Degree of Masters of Science in Plant Protection

Major Advisor: Tesfaye Melak (PhD)

Co-Advisor: Admasu Fanta (PhD)

June, 2025

Bahir Dar, Ethiopia

DECLARATION

This is to certify that the thesis entitled "Effect of broken kernels, weevils (*Sitophilus zeamais)*, and storage duration on deterioration indicators of maize (*Zea mays*) under storage condition", submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Protection of Department of Plant Science, Bahir Dar University, is a record of original work carried out by me and has never been submitted to this or any other institutions to get any other degrees or certificates. The assistance and help I received during the course of this investigation have been duly acknowledged.

Gedifew Ferede Tilahun		July 21, 2025
Name of the candidate	Signature	Date

College of Agricultural & Environmental Sciences
Department of Plant Sciences

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DEPARTMENT OF PLANT SCIENCE

APPROVAL OF THESIS FOR DEFENSE

I hear by certify that I have supervised, read, and evaluated this thesis titled "Effect of Broken kernels, Weevils (*Sitophilus zeamais*), and Storage Period on Deterioration Indicators of Maize (*Zea mays*) Under Storage Condition" by Gedifew Ferede Tilahun prepared under my guidance. I recommend the thesis be submitted for oral defense.

Tesfaye Melak Advisor's name	Signature	13/01/2018 Date
Admasu Fanta Co-Advisor's name	Signature	13/01/2018 Date
Yareh Bitew Abaynew Jemal Department Head	Signature	13/01/2018 Date

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Department of Plant Sciences

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THESIS APPROVAL SHEET

As members of the board of examiners, we examined this thesis entitled "Effect of Broken Kernels, Weevils (*Sitophilus zeamais*), and Storage Period on Deterioration Indicators of Maize (*Zea mays*) Under Storage Condition" by Gedifew Ferede Tilahun. We hereby certify that the thesis/dissertation is accepted for fulfilling the requirements for the award of the degree of "Masters of Science (MSc) in Plant Protection."

Board of Examiners

Dawit Tsegaye		13/01/2018
External examiner name	Signature	Date
Abaynew Jemal		13/01/2018
Internal examiner name	Signature	Date
Merkuz Abera		25th Sept, 2025
Chairperson's name	Signature	Date

College of Agricultural & Environmental Sciences
Department of Plant Sciences

DEDICATION

Devotion is to my family, whose unwavering encouragement and support have served as the cornerstone of my journey. Your inspiration in me has motivated me at every turn. To my friends and consultants who have shared their knowledge and passion, helping me overcome obstacles and acknowledging each success. To teachers, their teaching methodology has helped me to successful my academic requirement. And this work is for people who think that knowledge and tenacity are powerful for problem solving, civilization and generation continuity. Great dedication is to those who decided the positive response for any situations.

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ABBREVIATIONS AND ACRONYMS

AF	Aflatoxin
AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
AFIA	Lateral flow immunoassay
AFL	Aflatoxicol
Afs	Aflatoxins
AFT	Total aflatoxins
ANOVA	Analysis of Variance
ASE	Amhara Seed Enterprise
Aw	Water activity
BDU	Bahir Dar University
cal BP	Calibrated Years Before the Present
CGC	Canadian grain commission
CIE	Commission International de L'Eclairage
CRD	Completely randomized design
CSA	Central statistical agency
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization
FB1	Fumonisin B1
FB2	Fumonisin B2
FB3	Fumonisin B3
FBs	Fumonisins
FUM	Total fumonisins
HACCP	Hazard analysis and critical control points

HSCAS	Hydrated sodium calcium alumino silicates
IDK	Insect-damaged kernel
IDE	Integrated Development Environment
Inc.	Incorporated
JECFA	Joint FAO/WHO Expert Committee on Food Additives
Nd	Number of damaged kernel
Nu	Number of undamaged kernel
OGTR	Office of the Gene Technology Regulator
OTA	Ochratoxin A
PPB	Polypropylene bag
ppb	Part per billion
REGWQ	Ryan, einot, gabriel, welsh studentized range q
SSA	Sub-saharan Africa
Tb1	Teosinte branched one
TCTs	Trichothecenes
Tga1	Teosinte glume architecture one
UK	United kingdom
US$	United states dollar
USA	United States of America
UV	Ultra violet
Wd	Weight of damaged kernel
WHO	World health organization
Wu	Weight of undamaged kernel
ZEA	Zearalenone
Zea	Zearalenone
µl	Microliter

Effect of Broken kernels, Weevils (*Sitophilus zeamais*), and Storage Period on Deterioration Indicators of Maize (*Zea mays*) Under Storage Condition

Gedifew Ferede[1], Tesfaye Melak[1], Admasu Fanta[2]

ABSTRACT

*Maize is a highly important crop in Ethiopia for its food purpose and source of income especially for the smallholders; there are a lot of factors that deteriorate it under storage condition. Weevils (Sitophilus zeamais), broken kernel percentage and storage duration are among the factors that deteriorate maize under storage condition. Although maize is essential crop in most areas of country, the deterioration indicators like weevil growth development, moisture change, maize physical damage (maize damaged kernel), weight loss and mycotoxin development are serious problems under storage condition. 60kg of maize was bought from Amhara Seed Site. An experiment was designed to determine the effect of maize broken kernels, weevils and storage duration on deterioration indicators of maize under storage condition. A 0%, 2%, 4% 6% and 8% broken kernels were used in the experiment. Twenty adult maize weevils were added to each 1kg maize sample contained in a woven polypropylene bag. A two time storage period, three months and six months were used to determine weevil infestation level, insect damage kernel, weight loss, mycotoxin (aflatoxin and ochratoxin) occurrence and moisture change of maize under storage condition. The experiment was laid out in a Completely Randomized Design (CRD) with three replications in the grain and seed quality laboratory at the college of Agriculture and environmental Sciences, Bahir Dar University (BDU). Woven polypropylene bag was used for storing the samples for the experimental period. Data logger was used to monitor the external temperature and relative humidity of the laboratory. Moisture content of maize was measured by Agratronix MT-PRO Grain Moisture Tester. Numbers of weevils counted at 3rd and 6th storage period. Damaged and undamaged numbers of thousand maize grains counted and weighed by sensitive balance at end of 3rd and 6th storage period. Coffee grinder was used to grind sample for mycotoxin analysis. The highest results determined from effect of broken kernels, weevils and storage period on weevil development, physical damage, weight loss, aflatoxin and ochratoxin are 1635***, 83.8***, 48.1014***, 19.74*** and 3.99*** respectively for interaction effect and moisture change is 14.4% of main effect.*

Keywords: *Kernel damage, maize grain, mycotoxin development, maize weevil, weight Loss*

CHAPTER ONE

1. INTRODUCTION

1.1. Background

Maize (*Zea mays* L.) is one of the most important crops cultivated around the world and can be easily grown in various climate conditions, under conventional and organic systems (Vili *et al*., 2022). Globally, it is one of the three big global staple cereals namely wheat, rice, and maize. They comprise a major component of the human diet, accounting for an estimated 42% of the world's food calories and 37% of protein intake. The global maize area (for dry grain) amounts to 197 million hectare (ha), including substantive areas in sub-Saharan Africa (SSA), Asia and Latin America (Olaf *et al.,* 2022). Global cereal production for 2024 is estimated at 2,853 million tonnes, a slight decrease of 0.1% compared to 2023 (FAO, 2024).

The average yield of maize is 5.2 tone ha^{-1} (FAO, 2010). However, maize production decreased by 4% from 2021 to 2022 due to a drop in production due to widespread drought (FAO, 2023). The USDA forecasts world corn production to be 1,236 million tones for the 2023/24 season, an increase of 80 million tones (Yildiz, n.d.). Global maize production reached 1.2 billion tones in 2023 (Brief, 2022).

Food production and its security is declining from time to time in developing countries. Among the key constraints to improving food security in Africa including Ethiopia are losses resulting from poor post-harvest management of grains, estimated at 20–30%, amounting to more than US$4 billion annually (FAO, 2010). Some of these losses are caused by insects including weevils and fungi, with the speed at which these multiply being influenced by prevailing environmental conditions (Nukenine *et al*., 2010).

Sitophilus zeamais, the maize weevil, is a serious pest 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 (Suleiman, 2015). The maize weevil (*Sitophilus zeamais*) is a major post-harvest pest of maize. It can be found in numerous tropical areas around the world which infests not only maize but also other types of stored, processed cereal products such as pasta, cassava, and various coarse, milled grains (Nwosu, 2018).

Mycotoxin-causing fungi such as Aspergillus, Fusarium, Alternaria, Penicillium, and other microbes contaminate maize with their different levels and types of mycotoxins. These mycotoxins include aflatoxins (AFs), aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), aflatoxicol (AFL), fumonisin B1 (FB1), fumonisin B2 (FB2), fumonisin B3 (FB3), zearalenone (Zea), deoxynivalenol (DON) and fusarin C, a possible human carcinogen produced by Fusarium species. Some maize samples have been shown to contain up to 51 different types of toxins (Oliveira *et al*., 2013).

Deterioration of stored maize grain and its mycotoxin development by several factors is a very challenging issue in the storage condition. Period of storage, the presence of broken maize grains (broken kernels) that occurred during threshing, and the presence of weevils are the predominant factors of maize grain deterioration in the storage condition that stimulates mould (mycotoxin) development. Those factors need a priority of work for doing possible findings.

1.2. Statement of the Problem

Maize can give a better yield and maintain its quality during pre-storage under a favourable environment. However, there are several factors responsible for maize deterioration and mycotoxin development in storage conditions. Methods of storage, type of storage, storage duration or time length of storage, presence of contaminant insects or insect infestation in storage like weevils (*Sitophilus zeamais*), and physical breakage of grains during threshing (presence of broken kernels) are major factors for deterioration (Rashid A. Suleiman *et al*., 2013).

Threshing (shelling) creates broken kernels which is major post-harvest loss-causing factor of maize in the storage condition (Amare, 2017). Mechanically threshed maize containing 24% moisture content with higher kernel breakage (45%) (GU *et al*., 2019). Besides, traditional storage methods used by Ethiopian farmers are inadequate in terms of storage design and maintenance, and they cause crop storage losses (Abraham, 2008).

Quantity and quality (nutritional, safety, colour,) deterioration of maize occurs in the storage condition due to the interactions among physical, chemical, and biological factors. Among biological factors, weevil insects and fungi (mould) are the major and important factors of post-harvest loss in the storage conditions. Maize kept in quite inadequate storage is vulnerable to weevils, which can cause severe maize damage where weevils and fungi (moulds) highly facilitated mycotoxin (a toxic secondary metabolite produced by fungi (moulds) that can cause disease or death in humans, animals, microorganisms and the deterioration of crops) development in the storage conditions (Mulungu *et al*.,2007).

The fungal species (moulds) and their mycotoxins associated with maize are Aspergillus flavus and aflatoxins, Fusarium verticillioides and F. proliferatum and fumonisins, F. graminearum and trichothecenes and zearalenone. A. flavus can infect maize pre- and post-harvest and an increase in aflatoxin content can occur if the phases of maize drying and storage are poorly managed ood (Marin *et al.,* 2004)

Maize weevil (*S. zeamais*) were one of the major insect pests infesting storage structures (Jemaal Barre & Abaynew Jemal Jenber, 2023). So, determining the effect of those factors on deterioration indicators of maize under storage condition is critically needed in this study. Hence, the absence of well-enough studied management opportunities after maize deterioration is big problem and this study was conducted to determine level of this gap.

1.3. Objectives

1.3.1. General Objective

The main objective of the study was to determine the effect of maize broken kernels, weevils *(Sitophilus zeamais)*, storage duration and their interaction effects on deterioration indicators of maize (*Zea mays*) under storage condition.

1.3.2. Specific Objectives

- To determine the main effect of broken kernels, storage period, and weevils *(Sitophilus zeamais)* on moisture change, insect damaged kernel, weight loss, weevil development and mycotoxin contamination of maize under storage condition.

- To determine the interaction effects of broken kernels, storage period, and weevils on moisture change, insect damaged kernel, weight loss, weevil development and mycotoxin contamination of maize under storage condition.

1.2. Research Questions

1. What are the deteriorating factors of maize grain under storage condition?
2. What are deterioration indicators of maize under storage condition?
3. How much the broken kernels, storage duration, and weevils affect the maize under storage condition?

1.3. Significance of the Study

The quality and quantity of the maize under the storage is bellow-expected due to the development of deterioration indicators through several factors. This study is important to determine effects of broken kernels, weevils, and storage duration on deterioration indicators of maize (*Zea mays*) under storage conditions. So, the study is vital in determining the effects of those factors on development of maize deterioration indicators under storage condition.

This study on the determination of maize deterioration indicators is a big issue because the development of maize deterioration indicators under the storage is a significant and common problem tempo specially. Although maize deterioration indicators can occur every time at everywhere, its level occurrence may vary from time to time and place to place due to variations in several factors. The study was needed to determine the development level of maize deterioration indicators under storage. Its determination is a prerequisite for further study of other possible management options of maize under storage condition.

1.4. Scope of the Study

The study had been limited and prepared to determine the effect of specified critical factors such as the level of maize broken kernel, weevils, and storage period on development of deterioration indicators of maize under storage condition. The study contained three factors such as broken kernel, weevils and storage duration. 1st factor contains 5 levels, 2^{nd} factor contains 2 levels and 3^{rd} factor contains 2 levels, combined to get 20 treatments replicated three times to get 60 sample sizes.

Parameters of the study are temperature, relative humidity, moisture content, number of weevil development, insect damaged kernel in percentage, weight loss in percentage, and mycotoxin development in ppb. The study determined effects of each factor and their interaction effects. The sampling time intervals for the study were three months and six months for the determination of deterioration indicators development of maize under storage condition. The study was also focused on effects of the broken kernel of maize grain on development of deterioration indicators under storage condition.

1.5. Definitions of Terms

Adult progeny: Depending on the period of life being discussed, the word adult progeny might mean several things. Depending on the situation, it could allude to the first generation of adults or later generations. The term adult progeny may also refer to variations in size, ability to reproduce, or behavior depending on the surroundings or the parents, which would make it more difficult to define.

Aflatoxin: Depending on its kind (e.g., B1, B2, G1, G2), which have varying toxicity levels and regulatory limits, the phrase aflatoxin may raise different concerns. It is frequently linked to Aspergillus flavus and Aspergillus parasiticus. Due to cultural agricultural practices and regulatory frameworks, aflatoxin may have distinct connotations in some areas with reference to food safety and economic impact.

Broken Kernels: based on severity levels, the term "broken kernels" may indicate several levels of damage, ranging from little fractures to completely shattered grains, which could affect the context of evaluating quality. According to type of breakage, the word might not make it clear if it refers to just physical damage or if it also includes discoloured or biochemically changed kernels.

Insect Rearing: can refer to a variety of activities, ranging from large-scale breeding for commercial or pest management purposes to laboratory settings for study, which can result in variations in approaches and objectives. In certain cultures, the term "insect rearing" may not apply to scientific or agricultural contexts, but rather to customary methods for producing food or medicine.

Maize (*Zea mays*): In addition to its scientific meaning, "maize" may refer to a variety of processed forms, such as cornmeal or corn syrup, which can have distinct degradation and storage characteristics. Although maize's agronomic and nutritional qualities may not be directly related, in certain societies it might represent subsistence or cultural heritage.

Maize Deterioration Indicators: Depending on the context, indicators may differ greatly; for example, a researcher may focus on fungal growth or nutrient loss while another may consider insect presence to be a vital indicator. Depending on cultural or economic conditions, the significance of some deterioration indicators may vary. For example, the emphasis on nutritional value versus visual quality may differ.

Maize Weevils (*Sitophilus zeamais*): Although weevils are typically thought of as pests, the term may also allude to their role in ecosystems, such as their part in the cycling of nutrients or as food for other animals. Behavioral features for the term weevils may also refer to traits unrelated to their status as pests, such as food preferences or mating habits.

Mycotoxin: is typically thought of as a term used to describe harmful compounds made by fungus, it can also apply to a variety of substances with different toxicological profiles, not just those that harm people or animals. Rather of only being seen as pollutants, mycotoxins may also be determined in relation to their ecological functions, such as the way fungus employ them to compete with other organisms.

Ochratoxin: Although ochratoxin is frequently linked to Aspergillus and Penicillium species, it can also allude to its presence in a variety of animal products, making it difficult to define as a contaminant that is solely produced from plants. Depending on the context, the word may refer to more general health topics, such as chronic exposure hazards that are not as frequently discussed, like possible connections to cancer or renal illness.

R Software: is mostly used by statisticians and data scientists as a programming language and environment for statistical computing and visualization. Because R is an open-source program, users are free to alter and share the code, encouraging a cooperative community to create resources and packages.

R Studio: is a well-known R Integrated Development Environment (IDE) with an intuitive user interface that makes scripting, debugging, and visualization easier. It improves the R programming user experience with features like code completion, syntax highlighting, and integrated version management.

Storage Condition: According to concept Environmental Variability the term storage condition can refer to a broad variety of variables, including temperature, humidity, and light exposure. Based on infrastructure influence the phrase may also refer to the actual storage equipment, such as bins, silos, or bags, which have a big impact on the storage space.

Storage Duration: It is perception of time length or period for storage condition. In short-term context (days) or long-term (months or years) may influence how storage duration is interpreted, which may have an impact on how deterioration processes are seen. Depending on subjective experience of different stakeholders (farmers and distributors) define long or short storage; the phrase may also suggest distinct experiences or outcomes.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Maize Crop and its Origin

Maize is one of the oldest cultivated crops for human consumption. The center of origin for maize 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 European discovery of the Americas in the 15th century. Its cultivation today has spread to different parts of the world (OGTR, 2008 cited by Falayi *et al* 2019). 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).

2.2. History and Taxonomy of Maize

In English-speaking countries, maize (*Zea mays* L.), is also called "corn" or "sweet corn" which is one of the most important food crops cultivated globally. From initial, potential domestication in the Balsas River Valley of tropical south-western Mexico around 9000–8600 years ago (Piperno *et al*., 2009), maize spread across much of Central and South America before 4000–3000 years ago (Iriarte, 2007), to North America around 2000 years ago (Fritz, 2007), and has subsequently dispersed across the globe in the last 500 years or so.

The multidisciplinary history of maize domestication and dispersal is comparatively well-defined through archaeobotanical (macro-fossils and microfossils) and genetics (DNA and aDNA). Multidisciplinary research has enabled the processes of human selection and resultant morphogenetic transformation in the generation of maize to be tracked, as well as domestication traits that are not visible in the archaeobotanical record (Jaenicke-Després *et al*., 2003; Doebley *et al*., 2006; Zeder *et al*., 2006).

Genetic research has shown that maize is descended from a wild grass teosinte (Zea mays ssp. parviglumis or Balsas teosinte) that is native to the Balsas River Valley in tropical southwestern Mexico (Matsuoka *et al*., 2002). Domestication resulted in the transformation of multiple traits in teosinte to create maize. These include archetypical traits of the domestication syndrome in grasses, such as size of edible glume (much larger in maize) and non-shattering rachis (in maize), as well as other traits, such as apical dominance (replacement of multiple stalks with single stalk) and kernel case formation (enclosed casing around kernel in teosinte). Genes have been identified that control specific morphological changes, or domestication traits in maize, including Teosinte branched 1 (tb1, apical dominance) and Teosinte glume architecture 1 (tga1, kernel casing), as well as genes that control varietal differentiation based on kernel color, composition, and sweetness (Doebley *et al*., 2006; Zeder *et al*., 2006).

In 2001, Piperno and Flannery (2001) reported two archaeological cobs of maize dated to c. 6300–6000 cal BP from Guilà Naquitz Cave in the Oaxaca Valley of Mexico. More recent archaeological research at Xihuatoxtla Shelter in the Central Balsas River Valley has identified starch grains and phytoliths of maize on stone artifacts and in associated sediments dating back to c. 9000–8600 cal BP (Piperno *et al*., 2009; Ranere *et al*., 2009).

The degree of multidisciplinary consilience that exists for understanding maize domestication is unusual and shared by only a few other domesticated plants globally (Smith, 2001). There is also relatively good multidisciplinary information for the dispersal of maize in the Americas and the subsequent generation of varieties (Smith, 1998).

Maize was widely cultivated in diverse climate-biological regions across South America by approximately 4,000–3,000 years ago (Perry *et al*., 2006; reviewed in Iriarte, 2007). It was also introduced to the southern United States but remained a relatively minor crop, until c. 1,000 years ago (Fritz, 2007). The maize-bean-squash combination was a significant traditional diet in large parts of the Americas at the time of European conquest and colonization. This nutritionally balanced diet extended from "Argentina to Ecuador, up through Central America and Mexico, and as far north as southern Ontario" (Smith, 1998). Maize was first introduced to Europe in the late fifteenth century, was cultivated for food in Mediterranean regions in the centuries that followed its introduction and has subsequently been dispersed to regions around the globe.

2.3. Maize Crop Production

Maize is one of the crops grown on almost every continent. According to (FAO, 2006), global maize production has increased by nearly 50% over the past ten years. The total global production for 2011/12 fell due to severe drought in some parts of the US, which is the biggest producer of maize (Hoff and O'Kray, 2012 cited by Suleiman *et al*., 2013).

In 2021, the Americas were the top-producing region for maize, with the United States of America and Brazil accounting together for 39% of the world production. China was the second largest producer, with a share of almost 23% (FAO, 2022). Brazil was the first ranked in maize production in 2022, second is China and the third one is United States of America and they account for high production status in the World (FAO, 2023).

2.4. Importance of Maize Crop

Maize grain is one of the major diets of millions of people in the world (Lyon, 2000; Nwosu, 2016). It holds strong promise against poverty and global food crises. In sub-Sahara Africa, for instance, about 50 percent of the population depends on maize for food (FAO, 2002). Maize is a cash crop as well as an industrial raw material (Makate, 2010). It is used in breweries (to produce alcohol) and pharmaceutical companies (to produce oil and as a carrier for vitamins and medicines).

Starch, pulp abrasive, baby cereals, livestock feeds, high fructose syrup, dextrose, maize oil, cosmetics, beverages, crayons, soaps, biodegradable plastics, absorbent material for nappy/diapers, food additives, and food supplements are all industrial products of maize (Tongjura *et al*., 2010). Maize is also used industrially to produce fuel (Young, 2003; Tom, 2008). The fuel is green, in the sense that its use reduces air pollution (Young, 2003).

2.5. Maize Weevils (*Sitophilus zeamais)*

Sitophilus zeamais, maize weevil, the primary storage insect pest of maize, poses a significant difficulty during the post-harvest phase of maize (Barre and Jenber, 2022). Muzemu *et al*., 2013 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 *Sitophilus zeamais* 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* reduced germination percentage and maize production as most farmers in developing countries store grain and seed together (Pingali and Pandey, 2001). Most of smallholder farmers (83%) in SSA sell their maize within two months of harvest to avoid loss from weevil infestation and thus miss opportunities of getting the highest price during the lean season (Suleiman *et al*., 2015).

2.6. Maize Threshing

Maize threshing is the process of separating the edible grain parts from the maize cobs. The threshing devices consist of the cylinder and the concave bumper, which impact and rub the maize. The grains are then threshed from the maize cobs and sieved through the gaps in the concave bumper. Threshing can be done by machine, usually by a combine harvester, which harvests, threshes, and winnows the grain while it is still in the field (Feng *et al*., 2023).

2.7. Maize Storage Condition

Gotera, *gotta*, polypropylene, and jute bags are the common traditional grain storage methods in Ethiopia. *Gotera* is an outdoor storage construction made from mud or cow dung plastered basket work covered with thatched roofing and raised off the ground with stones or wooden platform; Gotta is an indoor grain storage bin made of mud plaster mixed with teff straw (Hengsdijk and De Boer, 2017). Maize grain is known to be susceptible to toxigenic mold infection and associated mycotoxin contaminations (Wagacha and Muthomi, 2008).

2.8. Mycotoxins

Mycotoxins are now attracting worldwide attention because of their implications for food safety, food availability, and international trading. Mycotoxins are common contaminants of stored maize in Sub-Saharan Africa (Kimanya *et al*., 2008; Probst *et al*., 2014). In

Africa however, many factors including environmental conditions, pest infestation, pre and postharvest handling, influence infection of stored products by toxigenic fungi and contamination of maize by mycotoxins (Gnonlonfin *et al*., 2013).

The risk of maize grain contamination is expected to be high in Ethiopia, because of the traditional post-harvest activities and the prevailing environmental conditions. Information availability regarding the happening of mycotoxins in maize produced in Ethiopia is still not the same: they vary in age of grain (duration from harvest to sampling), targeted stage of production and supply chain for sampling, coverage of high-producing areas, or number and type of mycotoxins investigated (Chauhan *et al*., 2016; Getachew *et al*., 2017; Probst *et al*., 2014; Tsehaye *et al*., 2017).

A study conducted by Worku *et al*., in 2019 determines mycotoxin occurrences in stored maize in 150 samples collected from five major maize-growing areas of Ethiopia. Total aflatoxins (AFT), total fumonisins (FUM), and deoxynivalenol (DON) were measured using lateral flow immunoassay (AFIA) whereas the Enzyme-Linked Immunosorbent Assay was used for ochratoxin A (OTA). Results of their studies indicated that incidences of AFT, FUM, OTA, and DON were 100, 32.7, 24, and 7%, respectively.

Among various studies of mycotoxins, most reported studies focus on the occurrence and toxicology of a single mycotoxin (Smith *et al*., 2006), which the majority concerning regulated mycotoxins such as aflatoxins (AFs), ochratoxin A (OTA), zearalenone (ZEA), fumonisins (FBs) and trichothecenes (TCTs) (Escrivá *et al*., 2017). Currently, biologically modified mycotoxins, introduced due to plant metabolism and known as "masked mycotoxins".

2.8.1. Aflatoxin

These are poisonous classes of mycotoxins formed by poisonous molds, particularly Aspergillus parasiticus and Aspergillus flavus, which may contaminate food and feed badly. Aflatoxins are liver killers (Goto *et al*., 1996) and may greatly affect the immune system by suppressing system activity (Peterson *et al*., 2001). Aflatoxin B1, G1, B2, and G2 are the four main types while aflatoxin M1 is a hydroxylated form of B1 which is found in milk and dairy products. B and G stand for the blue and green fluorescence character of aflatoxin under UV light respectively while 1 and 2 refer to the position of these aflatoxins on thin-layer chromatography. Aflatoxin B2 and G2 are less toxic as compared to B1 and G1 and aflatoxin B2 is less toxic than G1 (Sanchis *et al*., 1994).

The fungi species: (Aspergillus flavus and Aspergillus parasiticus) show suitable growth in warm conditions (Chase and Overton, 2012). The optimum environment for aflatoxin growth is 33 °C and 0.99 aw (water activity). Aflatoxin growth in food and feed was observed in stressful growing stages, e.g., drought growth conditions. Under harsh situations, fungi (molds) and spores survive and start decaying vegetation under microbiological actions. Under a suitable environment, attacks on hay and grain especially when moisture content is higher in humid soil (Ominski *et al*., 1994).

Aflatoxins have adverse carcinogenic, toxic, teratogenic, and mutagenic effects and also may cause liver and organ cancer in humans. Direct exposure to aflatoxin may occur frequently by ingestion of food and also by other means like dermal contact and inhalation (Mizrak *et al*., 2009). Aflatoxins impart their noxious effects on humans and animals' health and the severity level of their lethal effects is directly related to the nature of aflatoxin, the age of the consuming body, exposure time, and the food status of a person. The plants, grains, cereals, spices, nuts, and oil seeds that commonly favor the growth of aflatoxin and consequent fungal toxin production are the vital causes of exposure to aflatoxin. The examples include maize, wheat, rice, oilseeds, sunflower, peanut, cotton, almond, pistachio, walnut, coconut, and spices like black pepper, red chilli, turmeric, and coriander (Van Egmond, 2002).

The other commodities which may have aflatoxin contamination are plants parts like vegetables and fruits and also in meat, animal tissues and animal products (Milicevic *et al*., 2010). Different countries have adapted different permissible limits of aflatoxins in feed and food entities. The allowable level of aflatoxin in various food entities is different in different countries (Tritscher *et al*., 2013)

2.8.2. Ochratoxin

The fungal metabolites formed by the species Aspergillus ochraceous and Aspergillus penicillium which are termed as Ochratoxins (Zahra *et al*., 2016; Bayman *et al*., 2002).

Ochratoxin A is the common and pertinent toxin of this group. Ochratoxins A, B and C are structurally related and are equally harmful. Ochratoxin A pollutes agricultural products and due to its growth in food, represents a danger equally to human and animal health globally (Jordan and Pattison, 1996).

Optimum conditions for ochratoxin growth are at 25-30 °C and0.98 aw (water activity). Among three Ochratoxins A is the most common toxic compound found in cereals, barley, nuts, dried fruits, porcine kidney, beer, coffee beans, wines, and moldy bread (JECFA, 2001). Ochratoxins are alleged to be involved in the tumors of urinary organs and the etiology of human nephropathies (Kuiper-Goodman *et al*., 2010).

According to the European Commission 2002, ochratoxin infectivity was set at 3µg/kg in cereals consequent products. In dry grapes the contamination limit fixes to a limit of 10 ppb (ElKhoury and Atoui, 2010).

2.9. Impact of Mycotoxins

Mycotoxicosis may cause severe and deadly diseases in human health. The presence of diverse mycotoxins in food and feed samples may harm human and animal health and also can damage countries' economies to a great extent. The stringent control of foodstuff quality, in both developing and industrialized regions is very highly necessary to avoid cruel and painful circumstances. Sometimes mycotoxin contamination is inevitable and causes huge economic losses.

Generally, mycotoxins cause severe circumstances by intake of unhygienic foods, inhalation of spore-borne toxins and by dermal contact with mold-infected substrates. The effects of mycotoxins are carcinogenic, cytotoxic, teratogenic, estrogenic, immunosuppressant, and neurotoxic (Benkerroum, 2016). This review paper briefly entails mycotoxins information including their structures, major types of mycotoxins, health hazardous effects, their analysis, and different ways to control mycotoxin contamination. Most of the diseases related to mycotoxins are because by contaminated food (Alrabadi *et al*., 2018).

The presence of mycotoxins poses major risks to human health as they may cause many different adverse effects such as hepatotoxicity, genotoxicity, immunosuppression, nephrotoxicity, teratogenicity, and/or carcinogenicity (Claeys, 2020).

2.10. Mycotoxin Control

Mycotoxin contamination may harm humans and animals' health so its control is obligatory. Preventive measures should be adapted, which include preharvest control and post-harvest control while corrective measures include removing mycotoxins from food and feed stuffs. Proper initial drying and subsequent moisture-proof storage are crucial to minimize the growth of toxigenic fungi and toxins development (Bradford *et al*., 2018). Using weevil-resistant varieties like flint corn is also a strategy mentioned by many researchers to reduce mycotoxin infestation (Suleiman *et al*., 2015).

Preventive and corrective measures have great importance regarding the safety of food and feed. There should be strong applicability of rules and regulations in every country to allow the occurrence of permitted limits of these harmful mycotoxins in food and feed stuffs to make food and feed safe. Useful systems like HACCP (Hazard analysis and critical control points) may control the mycotoxins in entire agricultural food chain effectively.

2.10.1. Pre-harvest Control

Suitable cultivation techniques can reduce mycotoxin from the maize grain. For instance, the exclusion of farming waste helps mitigate the contagion of plants and crops by fungal attacks. Fungicides used in field crops may reduce and lower the mycotoxin contamination. Software appliances are accessible to help farmers that predict mycotoxin threats throughout the year which works according to climatic parameters. Various microorganisms as bio-control agents are proposed to reduce aflatoxin infectivity in preharvest; for instance, non-aflatoxigenic strains of the same varieties might be bio-competitive agents (Jard *et al*., 2011).

Resistance propagation is conventionally applied to improve the confrontation of the host plants to fungal attack and infectivity (Anthony *et al*., 2012). To lowering fumonisin in maize and deoxynivalenol in wheat, bio-competitive exclusion shows capable results

(Cleveland *et al*., 2003). By using genetic engineering, the plant genes become less at the risk level to fungal infection.

2.10.2. Post-harvest Control

The contamination of mycotoxin by Aspergillus, Alternaria, Fusarium, and Penicillium species is inevitable in different environmental circumstances. Storage and sorting are the main post-harvest control parameters. Best storage conditions are necessary to lessen the mycotoxin in food and feedstuffs. Water activity, the presence of chemical preservatives, gas composition, temperature, and microbial contacts of the stored products are factors that may affect mycotoxin growth. An integrated control of these factors might give great effective control. No single method is used against a wide range of mycotoxins which may present with co-contamination. Many strategies are introduced for decontamination of mycotoxins. Infected grains do not have the same color or density as protected grains. Thus, the contaminated grains must be separated according to density or appearance (Kabak, 2009; Afolabi *et al*., 2006).

2.10.3. Detoxification

The main purpose of detoxification is to inactivate or remove mycotoxins. The nutritional value and deliciousness of foodstuff should be maintained. Several ways and methods have been investigated for lessening mycotoxin infectivity in feed and foodstuffs. These methods are categorized as physical, chemical and biological methods (Varga and Tóth, 2005).

2.10.4. Physical Methods

The effective control of mycotoxin contamination from the stored maize can be reduced by various physical methods like color-wise sorting, mechanical separation, removal of the fines and density segregation. Washing of grains by simply water or sodium carbonate solution is used to reduce the concentrations of Zearalenone, Deoxynivalenol, and Fumonisins in maize and cereal grains. Gamma radiations were used to control ochratoxin in feeds (Stepanik *et al*., 2007).

2.10.5. Chemical Methods

Aflatoxin contamination is inevitable and capricious which makes it distinctive challenge to both food and feed safety as it directly or indirectly agonies animals and human beings. Although there are many chemicals but it is found that 0.5% hydrochloric acid is the pre-eminent chemical for decontamination of aflatoxin B1 in fish feed and poultry samples (Nisa *et al*., 2012a; 2012b).

The 0.3 and 0.4% NaOCl, 0.5% HCl,0.3% HCl, 0.1% HCl, 10% citric acid, 30% citric acid,50% calcium hydroxide, 96% ethanol and 99% acetone were used for detoxification. The aflatoxins were reduced to 55.1% in super sella rice, 90.9% in super basmati rice, 28.08% in brown rice, and 80.0% in white rice samples. Aflatoxins were detoxified in dal chana, dal mash, dal masoor, haldi and kalongi upto 70.5, 83.0,46.2, 82.09 and 36.9%, respectively. Reduction of aflatoxins was carried out 39.7, 7.1, 39.5, 82.0 and 62.0% in red chilli, makhana, corn flakes, kheer mix and pistachio. The aflatoxins level was reduced in maize grain, damaged wheat, peanut, figs and dates up to 31.3, 64.3, 63.6, 42.7 and 19.8%, respectively (Nisa *et al*., 2013).

Ammoniation is an efficient method used for several years and is mainly used to decrease the level of aflatoxins in feed but it may affect food quality. Similarly, alkalization, acidification and thermal treatment may reduce mycotoxin levels in food and feed samples (Bretz *et al*., 2006). Deamination i.e., the addition of NaNO2 reduces aflatoxin B1 level by deaminating and so, decreases its toxicity (Lemke *et al*., 2001).

2.10.6. Biological Methods

Bio-transformation by adsorbing, binding or detoxifying can reduce mycotoxins. Hydrated sodium calcium alumino silicates (HSCAS) may reduce aflatoxin B1. HSCAS is very important to prevent aflatoxicosis in a diversity of animals. Activated carbon can adsorb Fumonisins B1 and Ochratoxin A in aqueous solutions. Yeast-dried cell mass and wall substance of lactobacillus can merge with mycotoxins. Fungal conidia may bind mycotoxins effectively, especially Zearalenone and Ochratoxins (Jard *et al*., 2009).

Probiotic microorganisms (Saccharomyces cerevisiae and Lactobacillus delbrueckii) were investigated to reduce mycotoxins as biological control agents and found the use of probiotics as an alternative treatment to prevent aflatoxin production in food entities (Silva *et al*., 2015).

2.10.7. Integrated Methods

A variety of methods are used in integrated mycotoxin control in maize stored in storage to stop fungal growth and mycotoxin contamination. These tactics cover combination of every method that used from the pre-harvest to the post-harvest phases including transportation and storage (Fumagalli *et al*., 2021). Reducing mycotoxin levels is intended to protect food and feed safety.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Experimental Materials

The experimental material required for the study was maize (variety BH546). It was collected from Amhara Seed Enterprise (ASE) which is located at Bahir Dar. The maize was cleaned and stored in a deep freezer until it is ready for experimentation. Other experimental material, maize weevils were collected from the entomology laboratory, the College of Agriculture and Environmental Sciences.

Mycotoxin Kits were purchased from Romer Labs Diagnostic GmbH. Hobo data logger digital instrument was also used in the experiment, Coffee grinder machine was used to grind samples at the end of storage periods. Agratronix MT-PRO Grain Moisture Tester was used to measure the moisture content of the maize grain, and woven polypropylene bag was used for storing the samples for the experimental period.

3.2. Insect Rearing

Insect rearing of maize weevils under storage conditions is the practice of maintaining weevil populations, specifically those that infest stored maize or corn (Maceljski & Korunić, 1973). This is often done in a controlled environment for research purposes (Ojo & Omoloye, 2012). The maize weevil (*Sitophilus zeamais*) is a major pest of stored grains, causing significant post-harvest losses. Rearing these insects allows to develop better control methods and storage solutions (Saeed & Laing, 2023).

The maize grain was cleaned and frozen at 13ºC using a deep freezer for a week before the experiment was set to kill any potential predisposed infection (Tadesse & Subramanyam, 2019) (Tesfaye Melak Tadesse and Bhadriraju Subramanyam, 2019). Adult weevils were obtained from entomology laboratory at the College of Agricultural and Environmental Sciences, Bahir Dar University. These weevils were cultured in one litter of transparent plastic jars which contain 500g of disinfected maize grain in an environmental chamber adjusted from 25-30 ºC and 65% humidity. The plastic jars were covered with muslin cloth and held in place by rubber bands to allow aeration and to prevent the escape of weevils.

Adult maize weevils were allowed to oviposit on clean, uninfected maize grain for two weeks after which adults were separated using standard sieves and removed. The maize grains were allowed in the growth chamber for 42 days to get enough newly emerged adults of uniform age to be used in the experiment. All the maize grains were measured the moisture content and grain weight before applying the treatments by grain moisture method in grain and seed quality laboratory, College of Agriculture and Environmental Sciences, Bahir Dar University.

3.3. Sample Preparation

The sixty number of samples are prepared, half for three month storage duration and half for six month storage duration. The storage material used to store maize was a woven polypropylene bag (PPB), which was prepared with a 1kg holding capacity. This storage material is made from woven synthetic fibre and is a low-cost indoor storage material readily available. One kg sample of maize was measured and packed in the woven polypropylene bag which was used for the entire experiment. A small portion of the maize was crushed in order to be used for maintaining the required percentage of broken kernels in the sample. Half of the samples are with 20 weevils and half is with out weevils.

3.4. Experimental Design

The experiment was laid out using a completely randomized design (CRD) in the grain and seed quality laboratory, College of Agriculture and Environmental Sciences under room temperature condition at Bahir Dar University. An experiment consists of combination of three factors; those factors are broken kernel percentage, weevil insect, and storage duration with three replications which were arranged in a factorial design (5*2*2*3 respectively). The proportions of broken kernels in the samples were 0%, 2%, 4%, 6% and 8%. For each proportion, two sets of experiments, one with insects and the other without insects were set for three-month and six-month storage period. Mycotoxin was investigated at storage duration of 3^{rd} and 6^{th} months from both with and without weevil insects for each of broken kernel percentages. The total sample size of the research study was 60 observations.

Table 3.1 Table of treatment combination

Treatment	Insect	%broken kernel	Month
1	Absent	0%	3 months
2	Absent	0%	6 months
3	Absent	2%	3 months
4	Absent	2%	6 months
5	Absent	4%	3 months
6	Absent	4%	6 months
7	Absent	6%	3 months
8	Absent	6%	6 months
9	Absent	8%	3 months
10	Absent	8%	6 months
11	Present	0%	3 months
12	Present	0%	6 months
13	Present	2%	3 months
14	Present	2%	6 months
15	Present	4%	3 months
16	Present	4%	6 months
17	Present	6%	3 months
18	Present	6%	6 months
19	Present	8%	3 months

20	Present	8%	6 months

3.5. Experimental Procedures

The laboratory room was thoroughly cleaned and arranged. Each kilogram of the sample including broken kernel proportions was put in the ready-made woven polypropylene bags. The samples were put in the deep freezer for 72 hours at 13 ℃ to kill any live matter except the sample if it was predisposed before. After 72 hours, all the samples were taken out from the deep freezer and l*et al*l of them stay for 8 hours in order to reach room temperature. Twenty unsexed maize weevils from the newly emerged adults were added to 30 of the samples. The rest, 30 samples were put without insects.

All the samples were arranged on the table in order to reduce other storage pest distractions. A data logger was set in the room to record the temperature and relative humidity of the laboratory room in 30-minute intervals. After 3 months storage period, 3 samples were taken randomly from each treatment. Each sample was sifted using standard sieves to separate insects from the rest of the maize sample. Insects were counted and discarded using deep freezers/alcohol. After removing the insect, the whole samples were ground using a coffee grinder for mycotoxin analysis.

3.6. Data collected

3.6.1 Temperature and Relative Humidity

Insect development like weevils under storage condition may still occur at relative humidity around 35% and temperatures around 15°C (The Storage of Tropical Agricultural Products, n.d.).

3.6.2. Moisture Change

Moisture change of maize grain under the storage condition has measured using moisture meter. The research indicates that moisture content tends to increase during storage, particularly in systems lacking proper ventilation. Maize stored in woven polypropylene bags that had measured by moisture method showed a moisture increase of approximately 12.34% over six months (Geremew Kassa *et al*., 2025). Critical factor for ensuring maize is dried to safe moisture content before storage. A moisture content of 12-14% is generally recommended to prevent mold growth and insect infestation under storage condition (Maize in Human Nutrition - Post-Harvest Technology: Pre-Processing, Post-Harvest Technology: Processing, n.d.).

3.6.3. Adult Progeny

Adult progeny in the context of weevils development under maize storage conditions refers to the offspring of the original (parental) weevil population that have reached adulthood within the stored maize environment (Kasozi *et al*., 2017). Maize weevils (*S. zeamais*) were collected and identified by using a stereomicroscope (Zeiss stemi 2000C with an ocular magnification of 10X and objective magnification of 6X), using identification keys given by (Geremew *et al*., 2023). The separated maize weevils from the maize grain were collected on a tray and both live dead maize weevils were counted for analysis.

3.6.4. Insect-Damaged Kernel (IDK)

Insect damage kernel was recorded by the count and weighing method. Each 1 thousand (1000) of maize grains was taken and weighed using a sensitive balance. The percentage of insect-damaged grains was calculated according to the methods used by (CGC (Canadian Grain Commission), 2014)) as follows:

$$\text{Insect damaged kernel (IDK)}(\%) = \frac{(\text{Number of insect dameged kernel})}{\text{Total number of kernel}} \times 100$$

3.6.5. Weight Loss

The weight loss of maize grain was determined by subtracting the weight of samples at each of the given time storage from the initial weight for each of the 0th-month to 3rd-month, and 3rd-month to 6th-month intervals of data taken based on the methods used by (Wambugu PW *et al*., 2009). Then, the percentage weight loss was calculated as follows and the calculated result was recorded by using of following formula:

$$\text{Weight loss }(\%) = \frac{(\text{Wu}*\text{Nd})-(\text{Wd}*\text{Nu}))}{\text{Wu}(\text{Nd}+\text{Nu})} \times 100$$

Whereas, Wu: indicates weight of undamaged kernel, Wd: indicates weight of damaged kernel, Nu: indicates number of undamaged kernel and Nd: indicates number of damaged kernel

3.6.6. Mycotoxin Analysis

Mycotoxins are toxic secondary metabolites produced by fungi that can contaminate maize under storage condition, which is posing a significant problem to human and animal health (Can Poor Grain Storage Conditions Encourage Mycotoxin Growth? – Randox Food, n.d.). Improper storage facilities can lead to mycotoxin contamination (Zheng *et al*., 2024). Lateral flow immunoassays (LFIA) was to measure total aflatoxins (AFT) in stored maize and wheat samples. Enzyme-Linked Immunosorbent Assay (ELISA) was used to determine ochratoxin A (OTA) levels (Admasu Fanta Worku *et al*., 2019)

3.7. Data Analysis

Some response variable data was transformed to log10(x+1) scale (Bartlett, 1947) to normalize (optimize) heteroscedastic treatment variances. However, untransformed raw data of response variables was presented in tables. Those data that showed significant interaction effects was subjected to a two-way and three-way analysis of variance (ANOVA) to determine significant effects ($P < 0.01$) of interactive effects in R software version 4.4.0 (R 4.4.0 (April, 2024) - SciSpace Literature Review, n.d.). The data that showed significant main effects but non-significant interaction was subjected to one-way ANOVA to determine the significance of treatments. Mean separation of those response data that showed significant effects were done by Tukay HSD multiple range tests in statistical R software.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1. Temperature and Relative Humidity of Maize Storage Condition

The monthly average temperature from August to January of the experimental study ranged from 19.95 to 15.69 °C and average 3rd month to 6th month temperature varies decreasingly from 18.75 to 17.5 °C as shown figure 4.1. According to the study of Geremew *et al*., 2023, the monthly average temperature during January to June of the experimental storage room ranged from 26.26 to 30.44 °C. The temperature of the storage room in January and February was near to the same. Temperature of the storage increases starting from March to April with an average value of 30.25 °C and 30.44°C respectively. Temperature of the storage room decreases slowly from 30.44 °C-29.89 °C and 29.89 °C-28.55 °C in April to May and May to June 2021, respectively.

Monthly average relative humidity from August to January of experimental study ranged from 75.46 to 53.61 % and average 3rd month to 6th month relative humidity varies from 72.46 to 65.55% as shown figure 4.2. Average ambient air temperature and relative humidity of storage varies starting from January (1/1/2021) to June (5/30/2021). The relative humidity of the storage room ranges from 32.89 to 51.82%. The relative humidity of the storage room decreased from 38.63% in January 2021 to 32.19% in March 2021, and then it increased from 35.60% in April before increasing to 51.82% at the end of the trial in June 2021(Geremew *et al*., 2023).

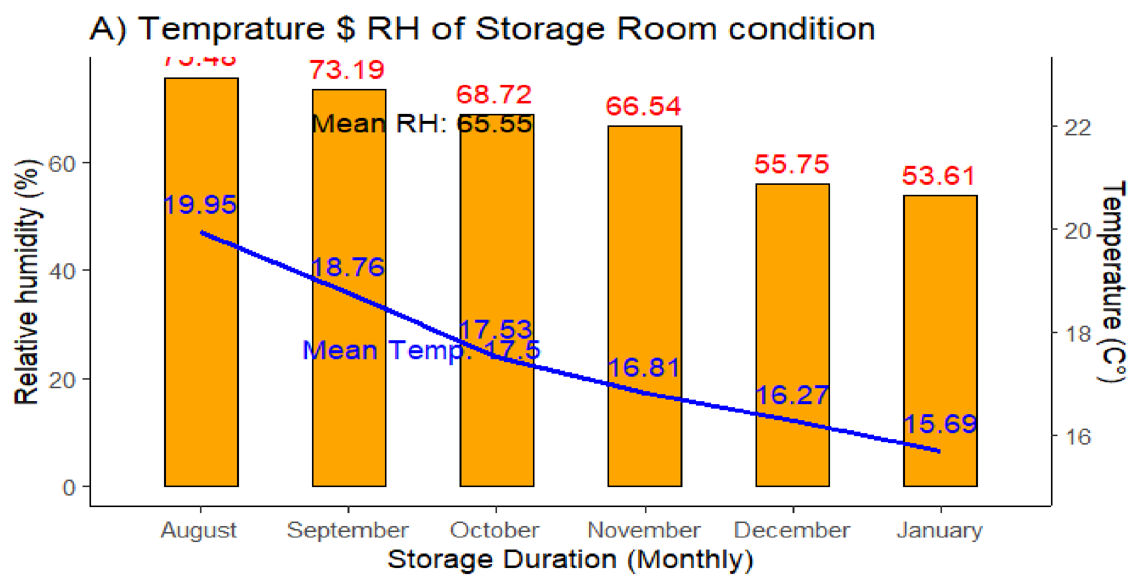

Figure 4.1 Monthly Temperature and Relative Humidity of Maize Storage Condition

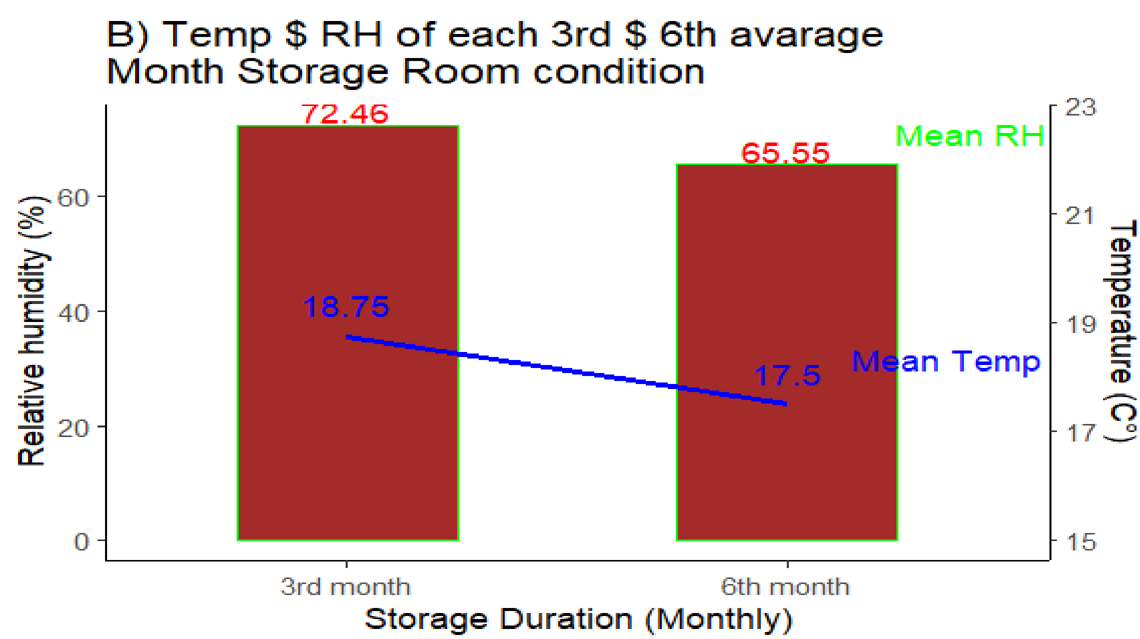

Figure 4.2 Average 3rd month and 6th month Temperature and Relative Humidity of Maize Storage Condition

Whereas RH is relative humidity, Temp is temperature, % is percentage, $ is and sign, °C is degree Celsius and

4.2. Effect of Broken kernels, Weevils (*Sitophilus zeamais*) and Storage Period on Moisture Variation of Maize under Storage Condition

In the Figure 4.3 interaction of all three factors of maize storage insect, maize broken kernel and storage period for the maize moisture content under storage condition stated a p-value of (2.3e-16 ***). This is less than alpha (0.001). So this is an output to reject the null hypothesis. With 99% statistical confidence, there exists sufficient statistical evidence to reject the statement that the three factor interaction of maize storage insect, maize broken kernels and storage period has similar effects across all sets of combinations. Post hoc analyses showed that the interaction of maize containing 20 insects with maize containing 8% broken kernel with 6th month stored maize exhibited the highest effect with mean (14.4%) while the interaction of 0 insects with 0% maize broken kernel with 3rd month exhibited the lowest effect with mean (10.8 %).

The moisture content of maize is critical for its storage life duration. Maize broken kernels tend to retain moisture differently compared to whole kernels, potentially leading to increased moisture levels in stored maize (GuiXiang *et al*., 2023).

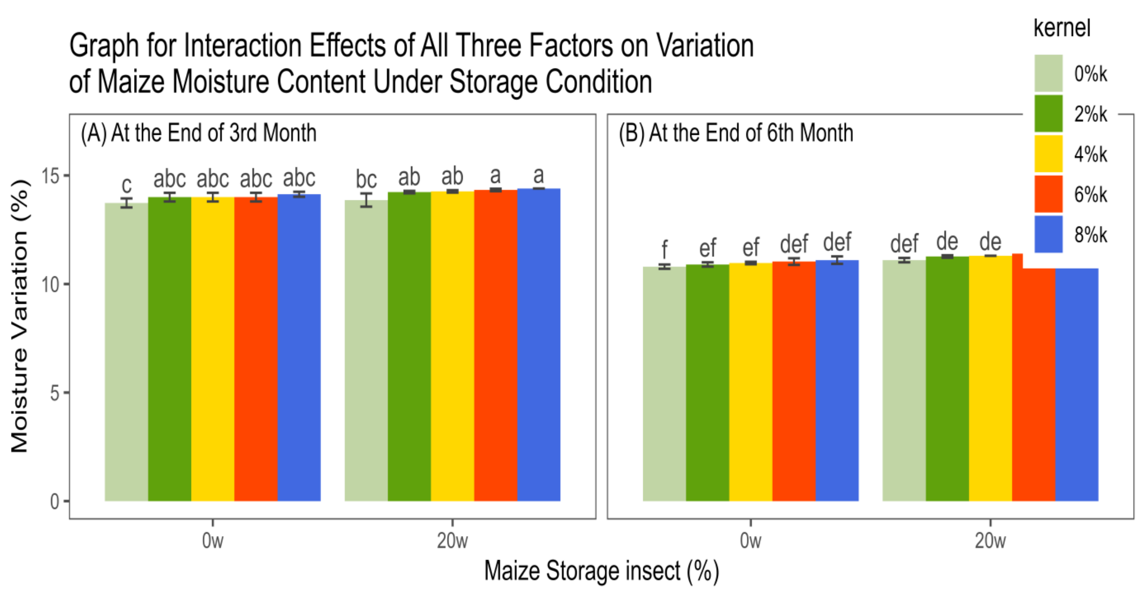

Figure 4.3 Interaction Effect of Broken kernels, Weevils (*Sitophilus zeamais*) and Storage duration on Moisture Variation of Maize under the Storage Condition

Table 4.1 ANOVA outputs for Interaction Effects of Broken kernels, Weevils and Storage Period on Moisture Change of Maize under the Storage Condition

MSE	Df	Mean	CV	MSD	alpha
0.0186667	40	12.61333	1.083187	0.4226062	0.05

Whereas MSE is mean square error, DE is degree of freedom, CV is coefficient of variation, and MSD is mean squared deviation

As the result described from the table 4.2 there is less positive correlation between effects of weevils and maize broken kernel with moisture content of maize under storage condition (0.09769483) and (0.08242581) respectively. Effects of storage period showed negative correlation with moisture content of maize under storage condition (-0.98805001).

Table 4.2 Correlation Matrix of insects, broken kernels and Storage duration with maize moisture content

Variables	**Correlation Matrix Values For Moisture Content**			
Moisture Content	**1.00000000**	**0.09769483**	**0.08242581**	**-0.98805**
With Insects	**0.09769483**	**1.00000000**	**0.00000000**	**0.00000**
Broken Kernel	**0.08242581**	**0.00000000**	**1.00000000**	**0.00000**
Storage Period	**-0.98805001**	**0.00000000**	**0.00000000**	**1.00000**

Whereas MSE is mean square error, DE is degree of freedom, CV is coefficient of variation, and MSD is mean squared deviation

4.3. Effect of Broken kernels, Weevils (*Sitophilus zeamais*) and Storage Period on Weevil Infestation of Maize under Storage Condition

4.3.1 Main Effect of Weevils on Its Infestation under Maize Storage Condition

In the Figure 1.4 the effect of storage insect showed a p-value of (2.2e-16 ***) for the weevil infestation under Maize Storage Condition. This is less than alpha (0.001). The result forces to reject the null hypothesis. With 99% confidence, there is statistically high significant evidence to control the presence of storage insects from maize storage condition. The post hoc analyses showed that the presence of 20 insects had the higher effect than maize without insects under storage condition as mean infestation of (629 weevils infestation) and (6 weevils infestation) respectively.

The initial density of weevils significantly influences their population growth during storage. Studies have shown that higher initial infestations lead to a more rapid increase in the total number of weevils over time. For instance, when maize seeds were infested with varying densities of *Sitophilus zeamais* (0, 36, 72, 108, and 144 individuals per 240 g of seeds), the population growth was directly increasing with the initial number of weevils present (Fufa *et al*., 2021).

The population of *S. zeamais* increased more rapidly in treatments with higher initial infestations, demonstrating a clear relationship between initial density and subsequent population dynamics (Costa *et al*., 2021).

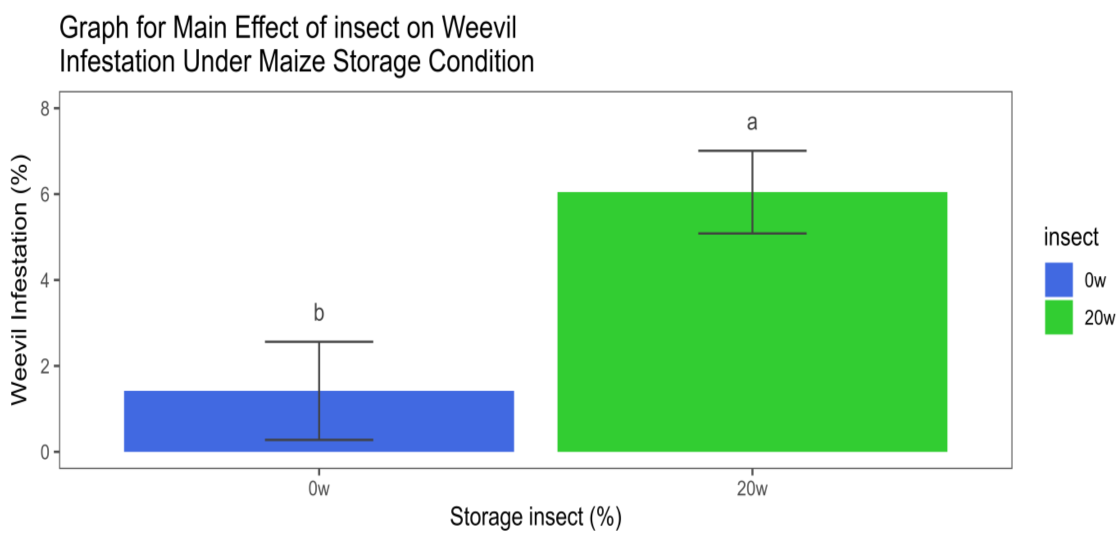

Figure 4.4 Main Effect of Weevils on Weevil Infestation under Maize Storage Condition

4.3.2 Main Effect of Broken kernels on Weevil Infestation of Maize under Storage Condition

In the Figure 4.5 the main effect of maize broken kernels reported a p-value of (2.673e-08 ***) for the effect of weevil infestation of Maize under Storage Condition. This is less than alpha (0.001). Reject the null hypothesis. With 99% confidence, there is statistically high significant evidence to deny the threshing levels (broken kernels) in maize storage condition. The post hoc analyses showed that 8% of broken kernels had the highest effect and 0% of broken kernels had the lowest effect with a mean infestation of (477 weevils infestation) and (207 weevils infestation) respectively. Levels having similar group letters have no significance effect even if they are different in their number of weevil infestation (non-significant effect).

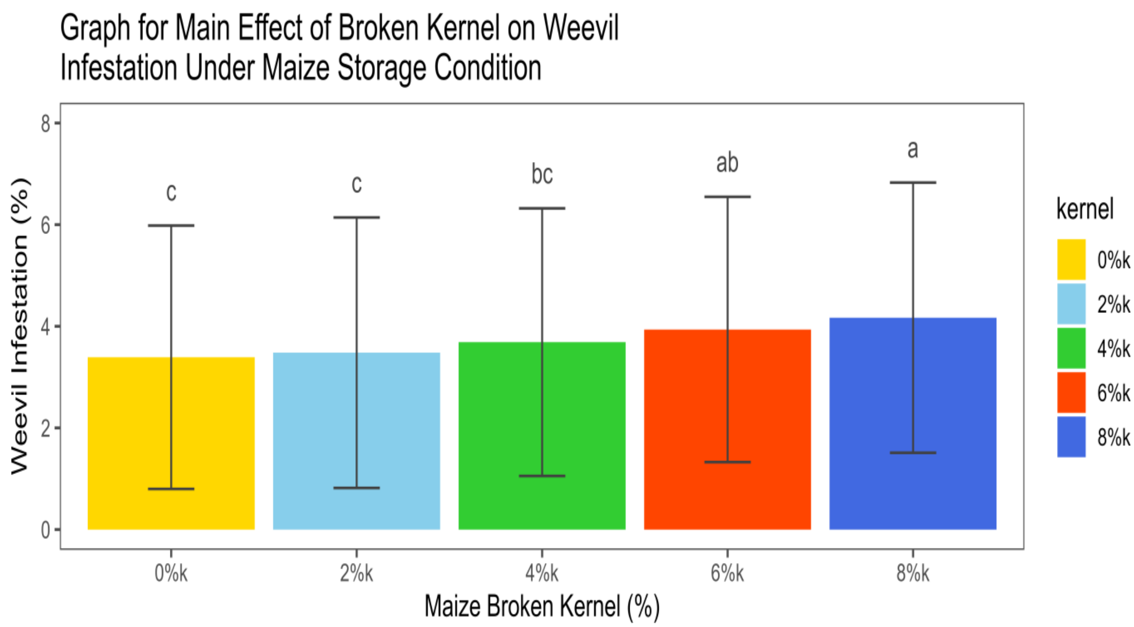

Figure 4.5 Main Effect of Broken kernels on Weevil Infestation of Maize under Storage Condition

4.3.3 Main Effect of Storage Duration on Weevil Infestation of Maize under Storage Condition

In the Figure 4.6, the main effect of storage duration showed a p-value of (2.2e-16 ***) for the weevil infestation of maize under storage condition. This is less than alpha (0.001). Reject the null hypothesis. With 99% confidence, there is statistically high significant evidence of maize Storage duration in storage condition. The post hoc analyses showed that 6th month Storage period had the higher effect than 3rd month Storage period with a mean infestation of (544 weevils infestation) and (91 weevils infestation) respectively. Weevil infestation increases for maize stored for longer time. Different studies indicate that significant damage can occur within just a few months of storage, with losses ranging from 20% to 50% after 3 to 6 months under poor storage conditions (Williams *et al*., 2017).

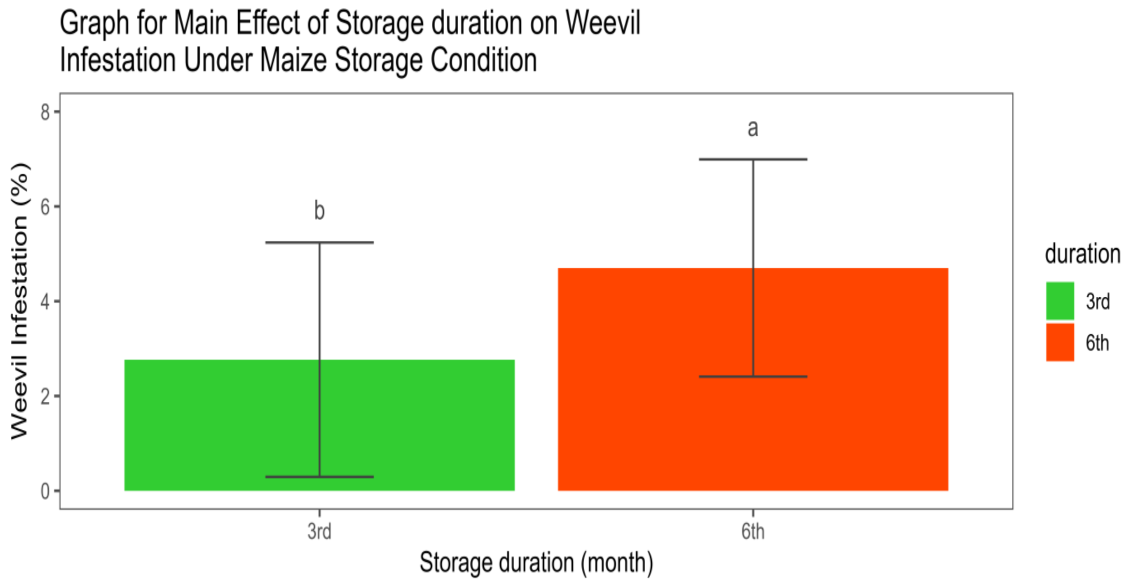

Figure 4.6 Main Effect of Storage Duration on Weevil Infestation of Maize under Storage Condition

4.3.4 Interaction Effects of Weevils and Storage Period on Weevil Infestation under Maize Storage Condition

From the Figure 4.7 Two factors interaction of maize storage insect and storage period for the weevil infestation under Maize Storage Condition reported a p-value of (2.1e-16 ***). This is less than alpha (0.001). Reject the null hypothesis. With 99% statistical confidence, there exists sufficient statistical evidence to reject the claim that the two factor interaction of maize storage insect and storage period has similar effects across all sets of combinations. Post hoc analyses showed that the interaction of 20 insects with 6th month exhibited the highest effect with mean (1076 weevils' infestation) while the interaction of 0 insects with 3rd month exhibited the lowest effect with mean (1 weevil's infestation). This research work is in line with studies that have shown that the presence of multiple pest species can lead to increased competition, which may regulate their population dynamics (Costa *et al*., 2021).

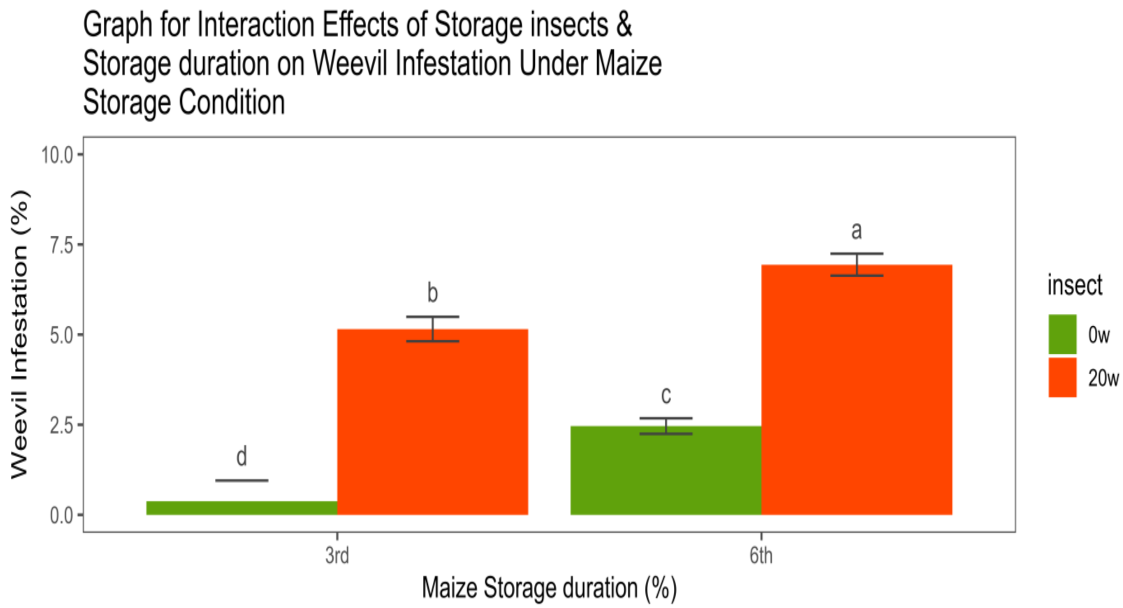

Figure 4.7 Interaction Effects of Weevils and Storage Period on Weevil Infestation under Maize Storage Condition

4.3.5 Interaction Effects of Broken kernels and Storage Period on Weevil Infestation under Maize Storage Condition

In the figure 4.8 two factors interaction of maize broken kernels and storage period for the weevil infestation under maize storage condition reported a p-value of (2.572e-08 ***). This is less than alpha (0.001). The result helps to reject the null hypothesis. With 99% statistical confidence, there exists high statistical evidence to reject null hypothesis that indicates the two factor interaction of maize broken kernels and storage period has similar effects across all sets of combinations.

Post hoc analyses showed that the interaction of 8% broken kernel with 6th month exhibited the highest effect with mean (811 weevils infestation) while the interaction of 0% broken kernel with 3rd month exhibited the lowest effect with mean (56 weevils' infestation). The interaction between the level of broken kernels and the storage period is critical. Maize with a higher percentage of broken kernels stored for extended periods is more likely to experience severe infestations. The other researchers study indicates that maize threshed mechanically containing high breakage at 23% moisture content and stored in traditional bags showed the highest levels of weevil infestation after six months (Geremew Kassa *et al*., 2025).

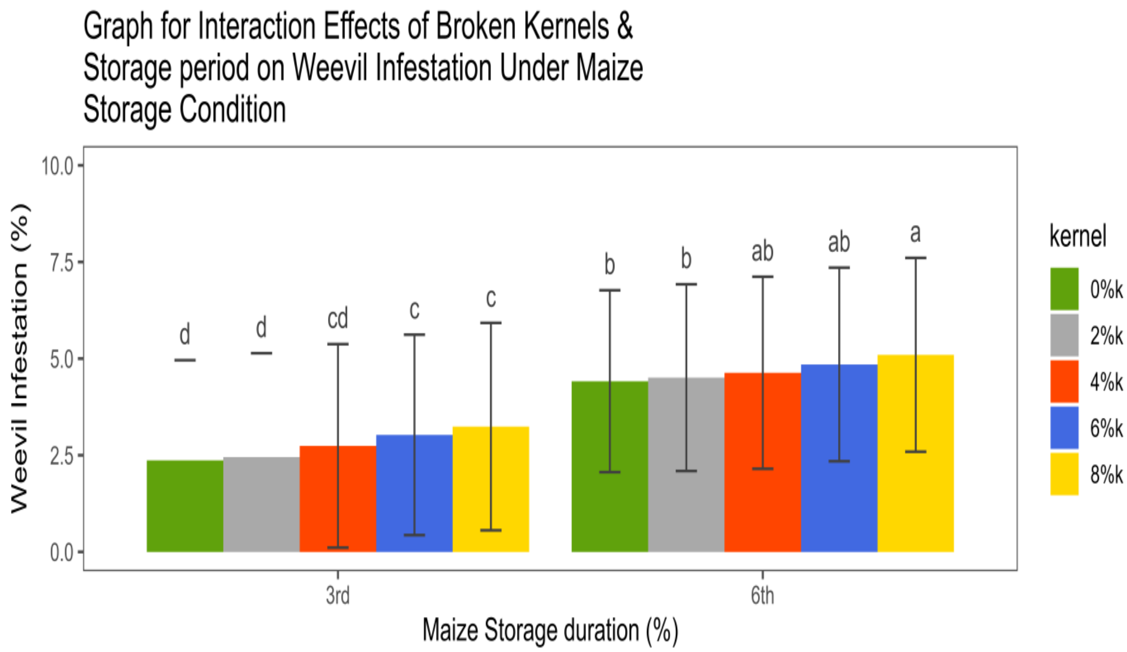

Figure 4.8 Interaction Effects of Broken kernels and Storage Period on Weevil Infestation under Maize Storage Condition

4.3.6. Interaction Effects of Broken kernels and Weevils on Weevil Infestation under Maize Storage Condition

In the figure 4.10 two factors interaction of maize broken kernels and storage insects for the weevil infestation under maize storage condition reported a p-value of (2.5e-16 ***)). This is less than alpha (0.001). The result helps to reject the null hypothesis. Result with 99% statistical confidence, there exist high statistical evidence to reject null hypothesis that indicates the two factor interaction of maize broken kernels and storage insects has similar effects across all sets of combinations. Post hoc analyses showed that the interaction of 8% broken kernel with 20 insects exhibited the highest effect with mean (945 weevils infestation) while the interaction of 0% broken kernel with no insects exhibited the lowest effect with mean (4 weevils infestation).

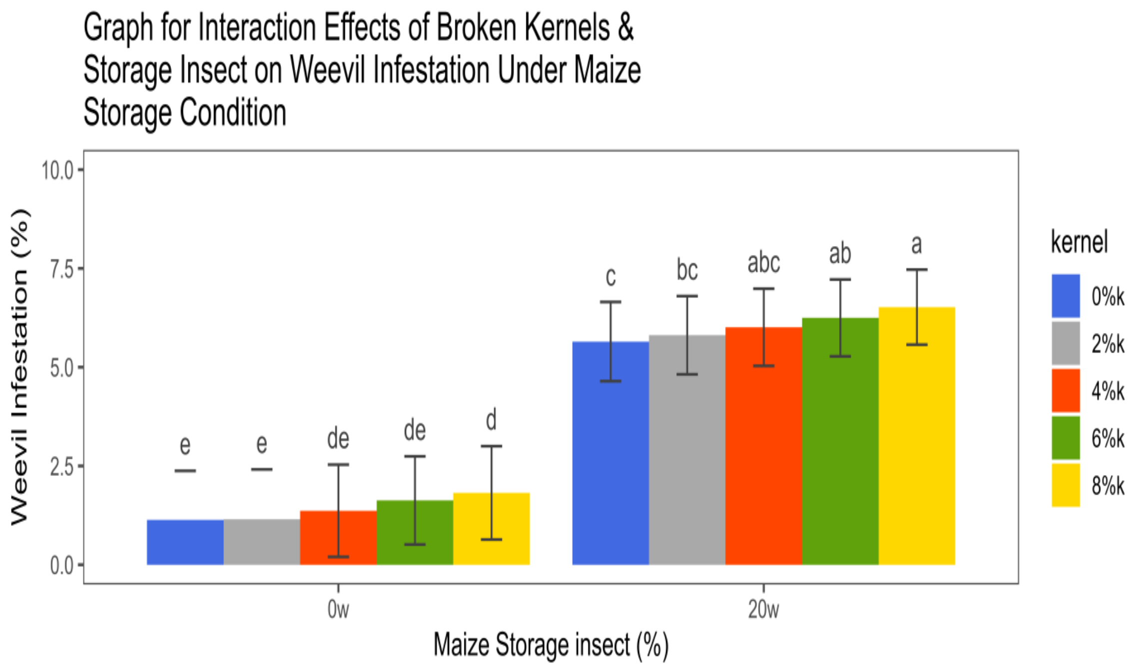

Figure 4.10 Interaction Effects of Broken kernels and Weevils on Weevil Infestation under Maize Storage Condition

4.3.7. Interaction Effects of Broken kernels, Weevils and Storage Period on Weevil Infestation under Maize Storage Condition

From the figure 4.11, all of three factors (broken kernels, weevils and storage period) shown the interaction effect on the weevil infestation of maize under storage condition as reported a p-value of (2.736e-08 ***). This is less than alpha (0.001). So reject the null hypothesis. With 99% statistical confidence, there exists sufficient statistical evidence to reject the claim that the three factor interaction of maize storage insect, maize broken kernels and storage period has similar effects across all sets of combinations. Post hoc analyses had also showed that the interaction of 20 insects with 8% maize broken kernel with 6th month exhibited the highest effect with mean (1606 weevils infestation) while the interaction of 0 insects with 0% maize broken kernel with 3rd month exhibited the lowest effect of mean 0 weevil (no weevil infestation).

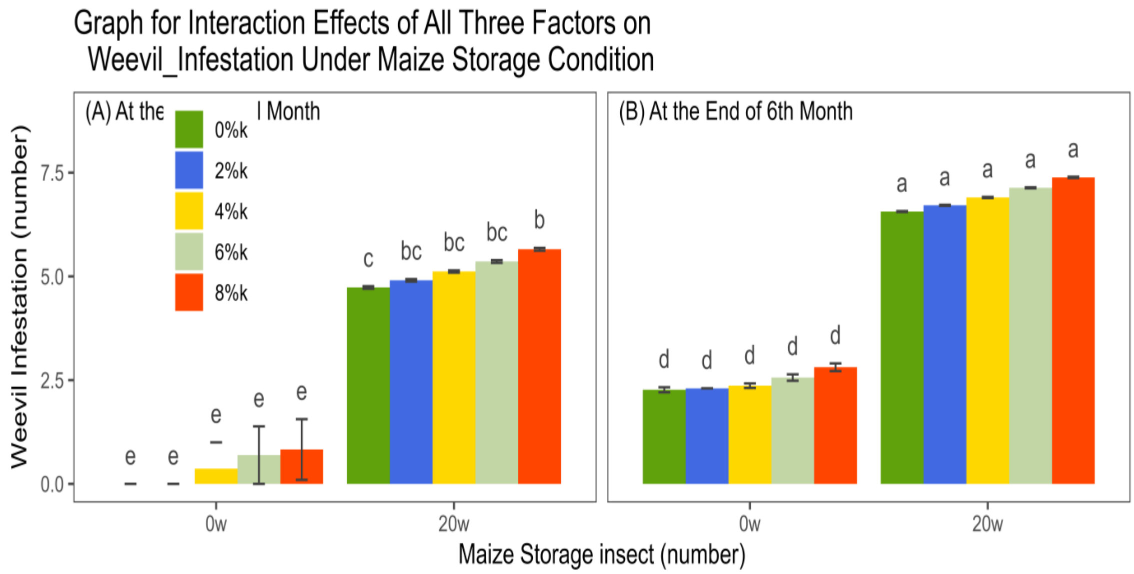

Figure 4.11 Interaction Effects of Broken kernels, Weevils and Storage Period on Weevil Infestation under Maize Storage Condition

Table 4.3. ANOVA outputs for Interaction Effects of Threshing Levels (Broken kernels), Storage Insects and Storage Period on weevil infestation of Maize under the Storage Condition

MSE	Df	Mean	CV	MSD	alpha
0.07225649	40	3.733186	7.200437	0.8314592	0.05

Whereas MSE is mean square error, DE is degree of freedom, CV is coefficient of variation, and MSD is mean squared deviation

As the result described from the table 4.4 there is high positive correlation between effects of weevils with its infestation under maize storage condition (0.9123463). Effects of maize broken kernel and storage period showed less positive correlation with insect infestation under maize storage condition (0.1123842) and (0.3815840) respectively.

Table 4.4 Correlation Matrix of insects, broken kernels and Storage duration with weevil infestation

Variables	**Correlation Matrix Values For Weevil Infestation**			
Weevil Infestation	**1.0000000**	**0.9123463**	**0.1123842**	**0.381584**
With Insects	**0.9123463**	**1.0000000**	**0.0000000**	**0.000000**
Broken Kernel	**0.1123842**	**0.0000000**	**1.0000000**	**0.000000**
Storage Period	**0.3815840**	**0.0000000**	**0.0000000**	**1.000000**

4.4. Effect of Broken kernels, Weevils (*Sitophilus zeamais*) and Storage Period on Dead weevils Infestation of Maize under Storage Condition

In the Figure 4.12 All of three factors interaction of maize storage insect, maize broken kernel and storage period for the Dead Weevils under Maize Storage Condition reported a p-value of (2.673e-08 ***). This is less than alpha (0.001). So this is a result to reject the null hypothesis. With 99% statistical confidence, there exists sufficient statistical evidence to reject the claim that the three factor interaction of maize storage insect, maize broken kernels and storage period has similar effects across all sets of combinations. Post hoc analyses showed that the interaction of 20 insects with 8% maize broken kernel with 6th month exhibited the highest effect with mean (236 dead weevils) while the interaction of 0 insects with 0% maize broken kernel with 3rd month exhibited the lowest effect with mean (0 dead weevils).

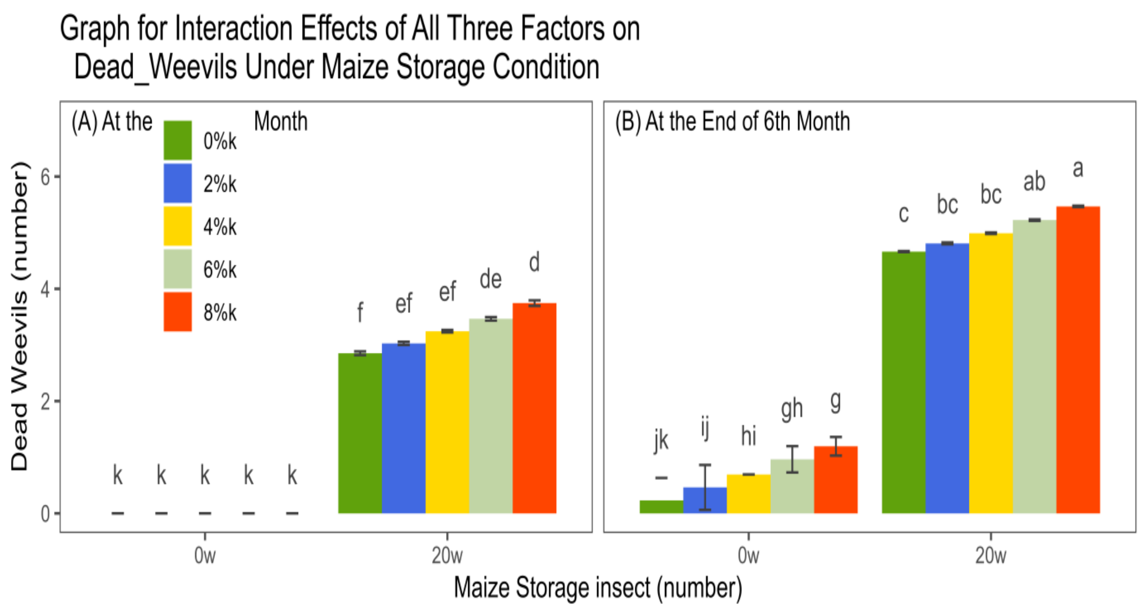

Figure 4.12 Interaction Effects of Broken kernels, Weevils and Storage Period on Dead Weevils under Maize Storage Condition

Table 4.5 ANOVA outputs for Interaction Effects of Threshing Levels (Broken kernels), Storage Insects and Storage Period on dead weevils of Maize under the Storage Condition

MSE	**Df**	**Mean**	**CV**	**MSD**	**alpha**
0.02047203	40	2.251751	6.354187	0.442571	0.05

Whereas MSE is mean square error, CV is coefficient of variation, and MSD is mean squared deviation

As the result described from the table 4.6 there is high positive correlation between effects of weevils with number of dead weevils under maize storage condition (0.9324543). Effects of maize broken kernel and storage period showed less positive correlation with number of dead weevils under maize storage condition which is (0.1159670) and (0. 3038089) respectively.

Table 4.6 Correlation Matrix of insects, broken kernels and Storage duration with Dead weevils

Variables	**Correlation Matrix Values For Dead Insects**			
Dead Weevils	**1.0000000**	**0.9324543**	**0.115967**	**0.3038089**
With Insects	**0.9324543**	**1.0000000**	**0.000000**	**0.0000000**
Broken Kernel	**0.1159670**	**0.0000000**	**1.000000**	**0.0000000**
Storage Period	**0.3038089**	**0.0000000**	**0.000000**	**1.0000000**

4.5. Effect of Broken kernels, Weevils (*Sitophilus zeamais*), and Storage Period on Insect Damaged Kernel of Maize under Storage Condition

In the Figure 4.13 All of three factors interaction of maize storage insect, maize broken kernel and storage period for the maize Insect Damaged Kernel under Storage Condition reported a p-value of (2.673e-08 ***). This is less than alpha (0.001). So this is a reason to reject the null hypothesis. With 99% statistical confidence, there exists sufficient statistical evidence to reject the statement that the three factor interaction of maize storage insect, maize broken kernels and storage period has similar effects across all sets of combinations. Post hoc analyses showed that the interaction of 20 insects with 8% maize broken kernel with 6th month exhibited the highest effect with mean (83 % of IDK) while the interaction of 0 insects with 0% maize broken kernel with 3rd month exhibited the lowest effect with mean (0 % of IDK).

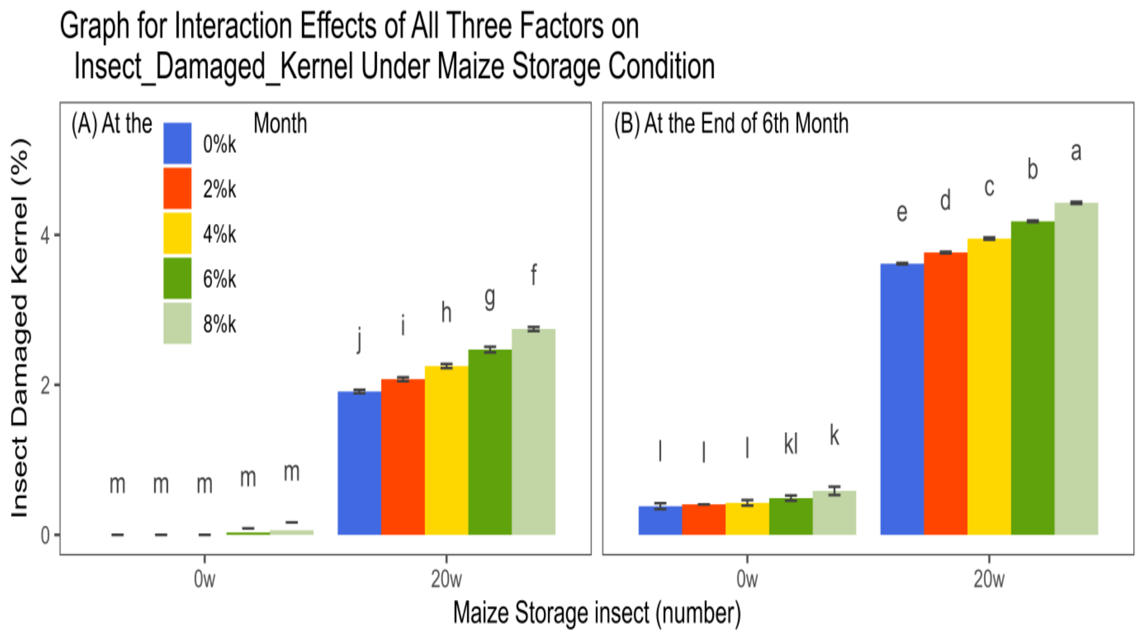

Figure 4.13 Interaction Effects of Broken kernels, Weevils and Storage Period on Insect Damaged Kernel (IDK) of Maize under Storage Condition

Table 4.7 ANOVA for Interaction Effects of Broken kernels, Weevils and Storage Period on Insect Damaged Kernel (IDK) of Maize under Storage Condition

MSE	**Df**	**Mean**	**CV**	**MSD**	**alpha**
0.00131605	40	1.68894	2.147947	0.1122119	0.05

Whereas MSE is mean square error, CV is coefficient of variation, and MSD is mean squared deviation

As the result described from the table 4.8 there is high positive correlation between effects of insects with percent of insect damaged kernel under maize storage condition (0.9113401). Effects of maize broken kernel and storage period showed less positive correlation with percentage of insect damaged kernel under maize storage condition (0.1054415) and (0.3357346) respectively.

Table 4.8 Correlation Matrix of insects, broken kernels and Storage duration with insect broken kernel

Variables	**Correlation Matrix Values For Insect Damaged Kernel**			
Insect Damaged Kernel	**1.0000000**	**0.9113401**	**0.1054415**	**0.3357346**
With Insects	**0.9113401**	**1.0000000**	**0.0000000**	**0.0000000**

Broken Kernels	**0.1054415**	**0.0000000**	**1.0000000**	**0.0000000**
Storage Duration	**0.3357346**	**0.0000000**	**0.0000000**	**1.0000000**

4.6. Effect of Broken kernels, Weevils (*Sitophilus zeamais*) and Storage Period on Maize Weight Loss in the Storage Condition

In the Figure 4.14 All of three factors interaction of maize storage insect, maize broken kernel and storage period for the maize Weight Loss (WtL) under Storage Condition reported a p-value of (2.673e-08 ***). This value is less than alpha (0.001). So reject the null hypothesis. With 99% statistical confidence, there exists sufficient statistical evidence to reject the statement that the three factor interaction of maize storage insect, maize broken kernels and storage period has similar effects across all sets of combinations. Post hoc analyses showed that the interaction of 20 insects with 8% maize broken kernel with 6th month exhibited the highest effect with mean (47.52 % of weight loss) while the interaction of 0 insects with 0% maize broken kernel with 3rd month exhibited the lowest effect with mean (0 % of weight loss).

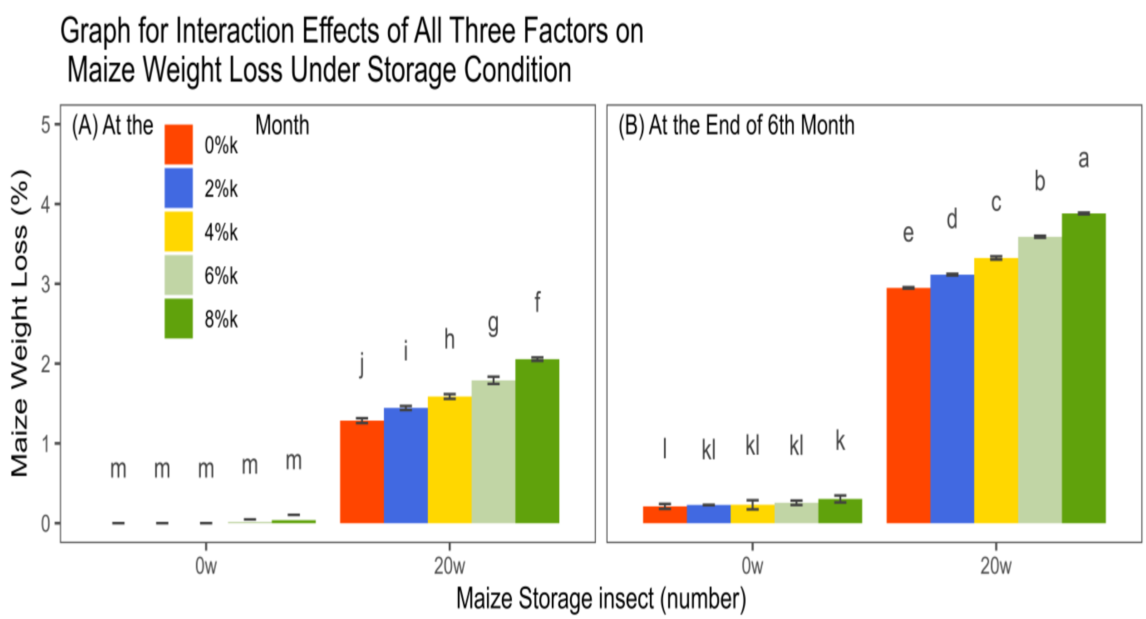

Figure 4.14 Interaction Effects of Broken kernels, Weevils and Storage Period on Weight Loss under Maize Storage Condition

Table 4.9 ANOVA outputs for Interaction Effects of Broken kernels, Weevils and Storage Period on Weight Loss of Maize under Storage Condition

MSE	Df	Mean	CV	MSD	alpha
0.00090083	40	1.315293	2.281823	0.092834	0.05

Whereas MSE is mean square error, CV is coefficient of variation, and MSD is mean squared deviation

As the result described from the table 4.10 there is high positive correlation between effects of insects with percent of maize weight loss under storage condition (0.8748694). Effects of maize broken kernel and storage period showed less positive correlation with percent of maize weight loss under storage condition (0.1182507) and (0.3637055) respectively.

Table 4.10 correlation matrix of insects, broken kernels and storage duration with maize weight loss

Variables	**Correlation Matrix Values For Insect Damaged Kernel**			
Weight Loss	**1.0000000**	**0.8748694**	**0.1182507**	**0.3637055**
With insects	**0.8748694**	**1.0000000**	**0.0000000**	**0.0000000**
Broken Kernels	**0.1182507**	**0.0000000**	**1.0000000**	**0.0000000**
Storage Duration	**0.3637055**	**0.0000000**	**0.0000000**	**1.0000000**

4.7. Effect of Broken kernels, Weevils (*Sitophilus zeamais*) and Storage Period on Mycotoxin (Aflatoxin and Ochratoxin) Contamination of Maize under Storage Condition

4.7.1. Effect of Broken kernels, Weevils (*Sitophilus zeamais*) and Storage Period on Aflatoxin Contamination of Maize under Storage Condition

In the Figure 4.15 All of three factors of maize storage insect, maize broken kernel and storage period on aflatoxin of maize under Storage Condition reported interaction effect with p-value of (4.73e-16 ***). This is less than alpha (0.001). So this is a reason to reject the null hypothesis. With 99% statistical confidence, there exists sufficient statistical evidence to reject the statement that the three factor interaction of maize storage insect, maize broken kernels and storage period has also effects across all sets of combinations. Post hoc analyses showed that the interaction of 20 insects with 8% maize broken kernel with 6th month exhibited the highest effect with mean (19.56 ppb) while the interaction of 0 insects with 0% maize broken kernel with 3rd month exhibited the lowest effect with mean (5.72 ppb).

Presence of higher levels of broken kernels during threshing can lead to increased susceptibility to insect infestation and fungal mold growth. Broken kernels are more prone to damage and provide a greater surface area for fungal infections, including aflatoxin-producing molds. The moisture content at which maize is threshed significantly affects the quality of the grain. Threshing at higher moisture levels can increase the likelihood of kernel breakage, which in turn can enhance the risk of aflatoxin contamination during storage (Geremew Kassa *et al*., 2025).

Sitophilus zeamais is a common pest in stored maize, and its presence can exacerbate the problem of aflatoxin contamination. The weevil damages the kernels, making them more vulnerable to fungal infections (mould development). So the activity of mould that developed by weevils can lead to increased levels of aflatoxin in stored maize. Studies have shown that maize infested with weevils has higher aflatoxin levels compared to non-infested maize, particularly under conditions of high humidity and temperature (Danso *et al*., 2019).

Aflatoxin B1 infestation levels can range from 10 ppb to 50 ppb in maize stored under the typical storage conditions. In some of the studies, levels of aflatoxin development have been reported as high as 100 ppb when moisture content is not adequately controlled (Nazareth *et al*., 2024). At and after six months of storage duration, aflatoxin levels can increase significantly, with values ranging from 20 ppb to 150 ppb. In poorly managed storage conditions, levels may exceed 200 ppb, indicating a higher risk of contamination (Kortei *et al*., 2021).

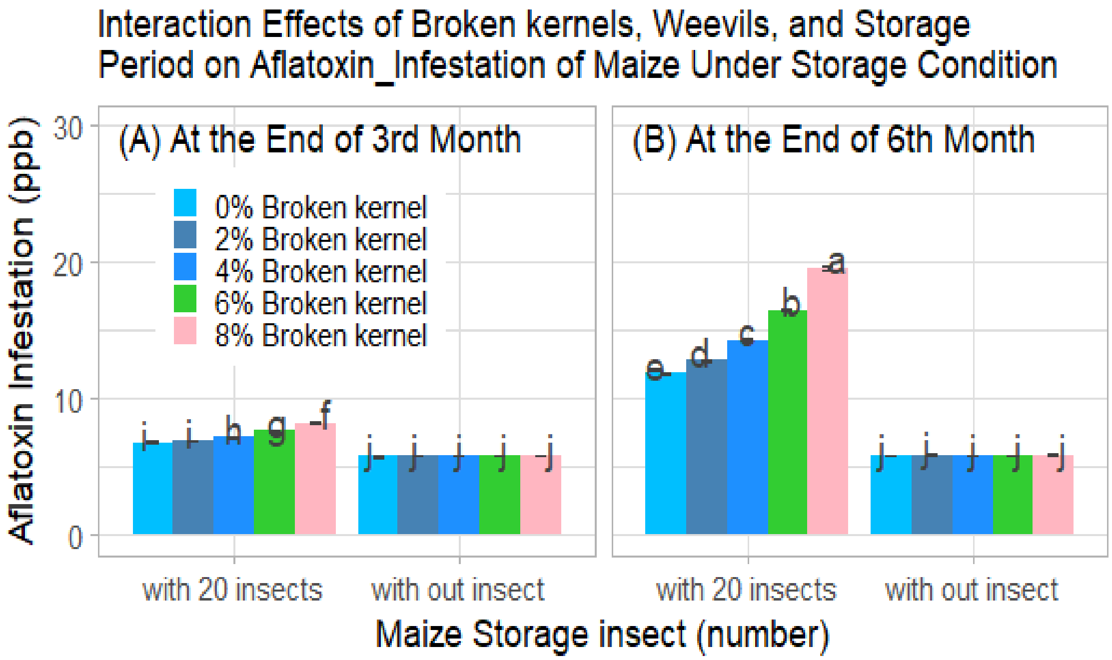

Figure 4.15 Effect of Broken kernels, Weevils (*Sitophilus zeamais*) and Storage Period on Aflatoxin Development of Maize under Storage Condition

Table 4.11 ANOVA outputs for Interaction Effect of Broken kernels, Weevils (*Sitophilus zeamais*) and Storage Period on Aflatoxin Development of Maize under Storage Condition

MSE	**Df**	**Mean**	**CV**	**MSD**	**Alpha**
0.00090759	40	1.321393	2.322818	0.0892839	0.05

Whereas MSE is mean square error, CV is coefficient of variation, and MSD is mean squared deviation

4.7.2. Effect of Broken kernels, Weevils (*Sitophilus zeamais*) and Storage Period on Ochratoxin Development of Maize under Storage Condition

In the Figure 4.16 All of three factors of maize storage insect, maize broken kernel and storage period on ochratoxin of maize under storage condition reported interaction effect with p-value of (2.53e-16 ***). This is less than alpha (0.001). So this is a reason to reject the null hypothesis. With 99% statistical confidence, there exists sufficient statistical evidence to reject the statement that the three factor interaction of maize storage insect, maize broken kernels and storage period has also effects across all sets of combinations. Post hoc analyses showed that the interaction of 20 insects with 8% maize broken kernel with 6th month exhibited the highest effect with mean (3.93 ppb) while the interaction of 0 insects with 0% maize broken kernel with 3rd month exhibited the lowest effect with mean (1.15 ppb).

Higher levels of broken kernels during threshing can increase the susceptibility of maize to fungal infections, including those that produce ochratoxin. Broken kernels are vulnerable to contamination of maize grain due to their damaged structure, which provides an entry point for fungi mould (Geremew Kassa *et al*., 2025). *Sitophilus zeamais* is a major pest in stored maize, and its presence can aggravate the problem of ochratoxin contamination. The weevil damages the kernels, making them more susceptible to fungal infections that produce ochratoxin. The activity of weevils can lead to increased levels of ochratoxin in stored maize. Infested maize is more likely to experience higher fungal growth, particularly under conditions of high humidity and temperature, which are conducive to ochratoxin production (Broadhead *et al*., 2025). Levels of ochratoxin A in maize can range from 5 ppb to 30 ppb at three months of storage period. In humid conditions, its levels may reach up to 50 ppb (Nazareth *et al*., 2024). At six month storage conditions or duration, ochratoxin A levels can increase to between 10 ppb and 60 ppb. In some cases, particularly under high humidity and poor areas, its development levels can reach up to 100 ppb (Kortei *et al*., 2021).

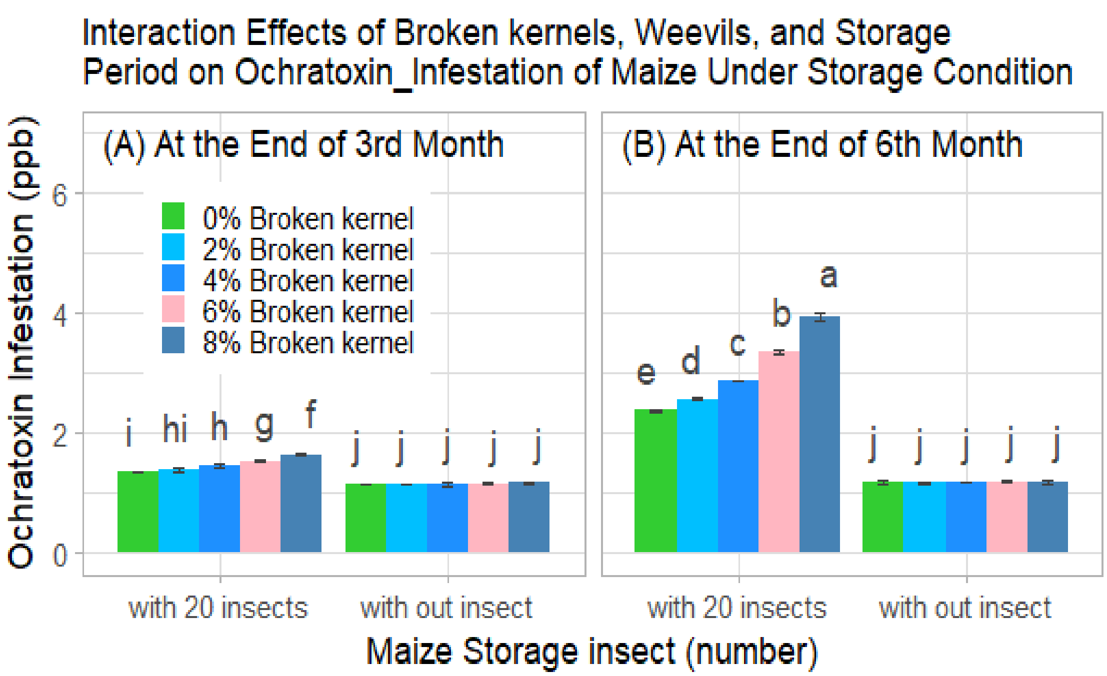

Figure 4.16 Effect of Broken kernels, Weevils (*Sitophilus zeamais*) and Storage Period on Ochratoxin Development of Maize under Storage Condition

Table 4.12 ANOVA outputs for Interaction Effect of Broken kernels, Weevils (*Sitophilus zeamais*) and Storage Period on Ochratoxin Development of Maize under Storage Condition

MSE	**Df**	**Mean**	**CV**	**MSD**	**Alpha**
0.00090134	40	1.356393	2.572818	0.07268339	0.05

Whereas MSE is mean square error, CV is coefficient of variation, and MSD is mean squared deviation

4.8. Correlation Analysis of Dependent and Independent Variables

A statistical technique for determining the direction and strength of a relationship between two or more variables is correlation analysis. It facilitates the discovery of connections and patterns in a data set (What Is Correlation Analysis Definition, Examples Appinio Blog, n.d.). The degree of relationship between the variables is measured by the correlation coefficient, which typically ranges from -1 to +1 (What Is Correlation Analysis: Comprehensive Guide, n.d.).

The colour changing from yellow to green to blue from the graph 4.17 shows increasing of the correlation between/within dependent and independent variables. The increasing value of the correlation indicates that high positive relationship between the variables. This indicates direct proportionality of the variables. The colour changing from blue to green to yellow from the graph 4.17 shows decreasing of the correlation between/within dependent and independent variables. The decreasing value of the correlation indicates that high negative relationship between and within the variables. This indicates indirect proportionality of the variables.

Th green colour shows that the correlation of the variables is zero or there is no correlation between or within the variables. Where the color is near to blue (+1), the correlation is going strongly positive relationship. It indicates the directly relationship of the variables. Where the color is near to yellow (-1), the correlation is going strongly negative relationship. It indicates the inversely relationship of the variables.

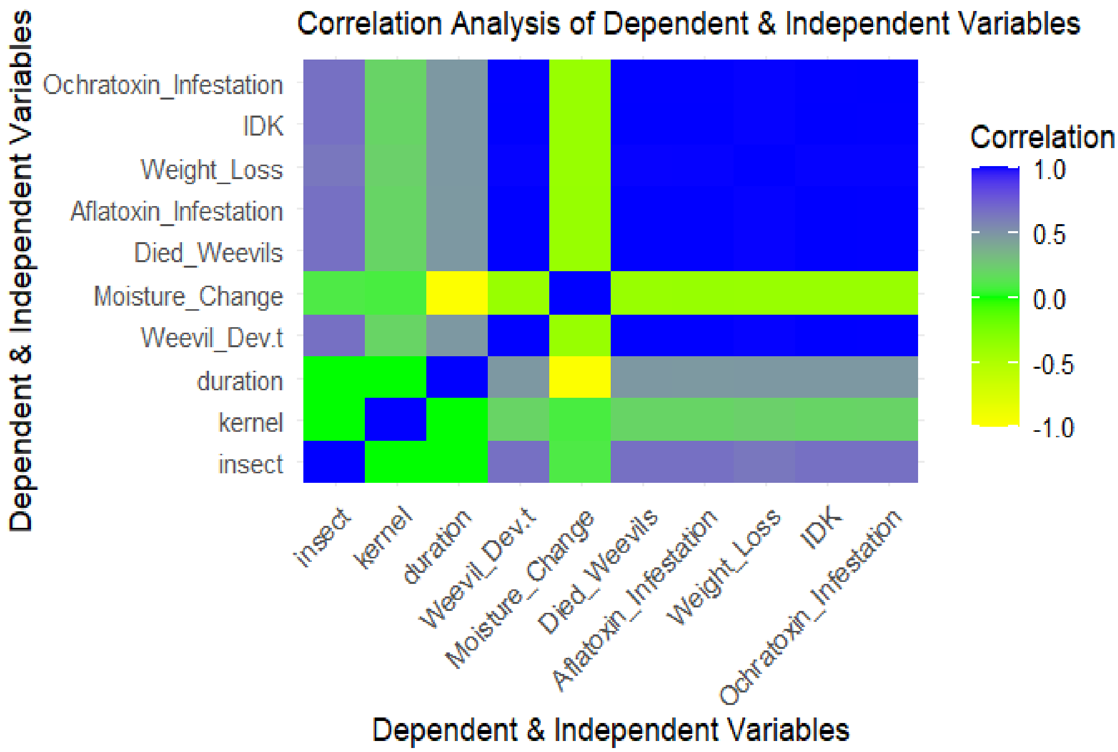

Figure 4.17 Graph for correlation analysis of dependent and independent variables

CHAPTER FIVE

5. CONCLUSION, RECOMMENDATIONS AND FUTURE WORKS

5.1. CONCLUSION

Temperature and relative humidity of study was vary from month to month, the first three months August, September, and October was greater temperature and relative humidity than next 4^{th}, 5^{th} and 6^{th} months; December, November, and January during the experimental study. The maize broken kernels, storage weevils and storage period have significant effect on the maize grain deterioration, quality and quantity loss due to causing weevil infestation, variation on moisture content of maize grain, weight loss, kernel damage or breakage of maize grain and mycotoxin contamination. The maize (variety of BH-546) stored with 20 weevil insects and 8% of broken kernels for 6^{th} month storage condition had caused the highest significant main and interaction effect on maize deterioration indicators like quality and quantity loss, weevil infestation, maize grain kernel damage, weight loss, and mycotoxin occurrence or development.

The reverse is true for the maize stored without weevil insects and 0% of broken kernels for 3^{rd} month storage condition which had caused the lowest main and interaction effect on maize deterioration indicators like quality and quantity loss, weevil infestation, maize grain kernel damage, weight loss and mycotoxin occurrence or development. The Aflatoxin and Ochratoxin are basic mycotoxins occurred and determined in this study.

The maize stored with 20 weevil insects and 8% of broken kernels at the end of 3rd month storage condition which had caused the highest main and interaction effect of maize moisture content. The reverse is true for the maize stored without weevil insects and 0% of broken kernels at the end of 6th month storage condition which had caused the lowest main and interaction effect on maize moisture content. So the moisture content of the maize grain decreased from 3^{rd} month to 6^{th} in the storage condition.

5.2. RECOMMENDATIONS

Careful monitoring and management of both temperature and humidity are essential for maintaining optimal storage conditions for maize grain. The storage condition should have optimum temperature and relative humidity as much as possible. Researcher recommends that the maize grain storage condition should be free of weevil insects and stored without maize broken kernels to boost up storage duration of maize. The maize grain should be free of weevil insects and stored without maize broken kernels to stabilize moisture change and reduce insect growth, weight loss, maize physical damage and mycotoxin development under storage condition.

This research study had determined the effect of broken kernels, weevil insects and storage time interval on moisture change of maize grain, insect development, weight loss, maize physical damage (maize damaged kernel), and mycotoxin (aflatoxin and ochratoxin) contamination of maize (*Zea mays*) under storage condition.

Even if degree of effect is different from treatment to treatment, different levels of weevil insects, broken kernels and storage duration showed significant difference in both main and interaction effect on maize moisture change, insect infestation, weight loss, maize physical damage and mycotoxin (aflatoxin and ochratoxin) development under maize storage condition.

The study determined that, there is increasing significant main and interaction effect of maize stored from combination of broken kernels, weevils and storage period on weevil development, physical damage, weight loss, aflatoxin and ochratoxin with highest mean values 1635***, 83.8***, 48.1014***, 19.74*** and 3.99*** respectively for interaction effect and moisture change 14.4% of main effect.

5.3. FUTURE WORKS

The research of this study can be repeated at different location at different times using methodology amendments to get more accurate and precise result if it will be possible. The effect of other factors on other maize deteriorating indicators under storage condition should be well-studied and determined intensively. The other storage methods should be also determined and identified by considering the different factors primarily, the place where it will be stored, agro-ecological or climatic conditions of the storage location, storage duration, and storage type. Further study on possible prevention and management options of maize deteriorating indicators like mycotoxin contamination under storage condition should be studied by future researchers.

5.4. Conflicts of Interest

The authors announce no conflict of interest to results of the work. The others had no role in the design of the study, in the collection, analysis, or interpretation of data, in writing of the paper, and in the result and decision to publish the findings of the study.

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7. Appendices

7.1. Appendix Table for ANOVA Outputs Using R and RStudio Software

7.1.1. ANOVA Outputs for Effect of Broken kernels, Weevils (Sitophilus zeamais) and Storage on Moisture Variation of Maize under Storage Condition

```
Analysis of Variance Table

 Independent.var      Response of dependent.var
                  Df  Sum Sq Mean Sq   F value    Pr(>F)
fact.A             1   1.291   1.291   69.1429 2.970e-10 ***
fact.B             4   1.044   0.261   13.9866 3.185e-07 ***
fact.C             1 132.017 132.017 7072.3214 < 2.2e-16 ***
fact.A:fact.B      4   0.028   0.007    0.3705    0.8282
fact.A:fact.C      1   0.033   0.033    1.7500    0.1934
fact.B:fact.C      4   0.062   0.015    0.8259    0.5166
fact.A:fact.B:fact.C  4   0.009   0.002    0.1205    0.9744
Residuals         40   0.747   0.019
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

 "R Square 0.994"

$ma[[3]]
[1] "SEm of A: 0.025 , SEd of A: 0.035 , SEm of B: 0.039 , SEd of B 0.056 , SEm of C: 0
.025 , SEd of C: 0.035 , SEm of AB: 0.056 , SEd of AB: 0.079 , SEm of AC: 0.035 , SEd
of AC: 0.05 , SEm of BC: 0.056 , SEd of BC: 0.079 , SEm of ABC: 0.079 , SEd of ABC:
0.112"

$ma[[4]]

        Shapiro-Wilk normality test

data:  model$residuals
W = 0.96214, p-value = 0.05989


$ma[[5]]
[1] "Normality assumption is not violated"

$ma[[6]]
[1] "The means of one or more levels of factor A are not same, so go for multiple compari
son test"

$ma[[7]]
$ma[[7]][[1]]
```

```
    MSerror Df     Mean       CV        MSD
  0.01866667 40 12.61333 1.083187 0.07129684

$ma[[7]][[2]]
   test name.t ntr StudentizedRange alpha
  Tukey fact.A   2         2.858232  0.05

$ma[[7]][[3]]
    dependent.var groups
20w      12.76000      a
0w       12.46667      b


$ma[[8]]
[1] "The means of levels of factor B are not same, so go for multiple comparison test"

$ma[[9]]
$ma[[9]][[1]]
    MSerror Df     Mean       CV       MSD
  0.01866667 40 12.61333 1.083187 0.1593052

$ma[[9]][[2]]
   test name.t ntr StudentizedRange alpha
  Tukey fact.B   5         4.039123  0.05

$ma[[9]][[3]]
    dependent.var groups
8%k      12.76667      a
6%k      12.69167     ab
4%k      12.63333     ab
2%k      12.60000      b
0%k      12.37500      c


$ma[[10]]
[1] "The means of one or more levels of factor C are not same, so go for multiple compari
son test"

$ma[[11]]
$ma[[11]][[1]]
    MSerror Df     Mean       CV        MSD
  0.01866667 40 12.61333 1.083187 0.07129684

$ma[[11]][[2]]
   test name.t ntr StudentizedRange alpha
  Tukey fact.C   2         2.858232  0.05
```

```
$ma[[11]][[3]]
    dependent.var groups
3rd      14.09667      a
6th      11.13000      b


$ma[[12]]
[1] "The means of levels of interaction between A and B factors are same so dont go for a
ny multiple comparison test"

$ma[[13]]
$ma[[13]][[1]]
     MSerror Df     Mean       CV       MSD
  0.01866667 40 12.61333 1.083187 0.2640785

$ma[[13]][[2]]
   test        name.t ntr StudentizedRange alpha
  Tukey fact.A:fact.B  10         4.734513  0.05

$ma[[13]][[3]]
        dependent.var groups
20w:8%k      12.91667      a
20w:6%k      12.86667     ab
20w:4%k      12.78333     ab
20w:2%k      12.75000    abc
0w:8%k       12.61667    bcd
0w:6%k       12.51667    cde
0w:4%k       12.48333     de
20w:0%k      12.48333     de
0w:2%k       12.45000     de
0w:0%k       12.26667      e


$ma[[14]]
[1] "The means of levels of interaction between B and C factors are same so dont go for a
ny multiple comparison test"

$ma[[15]]
$ma[[15]][[1]]
     MSerror Df     Mean       CV       MSD
  0.01866667 40 12.61333 1.083187 0.2640785

$ma[[15]][[2]]
   test        name.t ntr StudentizedRange alpha
  Tukey fact.B:fact.C  10         4.734513  0.05
```

```
$ma[[15]][[3]]
       dependent.var groups
8%k:3rd      14.26667      a
6%k:3rd      14.16667      a
4%k:3rd      14.13333      a
2%k:3rd      14.11667      a
0%k:3rd      13.80000      b
8%k:6th      11.26667      c
6%k:6th      11.21667      c
4%k:6th      11.13333     cd
2%k:6th      11.08333     cd
0%k:6th      10.95000      d


$ma[[16]]
[1] "The means of levels of interaction between A and C factors are same so dont go for a
ny multiple comparison test"

$ma[[17]]
$ma[[17]][[1]]
    MSerror Df     Mean       CV       MSD
  0.01866667 40 12.61333 1.083187 0.1337228

$ma[[17]][[2]]
   test        name.t ntr StudentizedRange alpha
  Tukey fact.A:fact.C   4         3.790685  0.05

$ma[[17]][[3]]
       dependent.var groups
20w:3rd      14.22000      a
0w:3rd       13.97333      b
20w:6th      11.30000      c
0w:6th       10.96000      d


$ma[[18]]
[1] "The means of levels of interaction between all the three factors ABC are same so don
t go for any multiple comparison test"

$ma[[19]]
$ma[[19]][[1]]
    MSerror Df     Mean       CV       MSD
  0.01866667 40 12.61333 1.083187 0.4226062
```

MSE	**Df**	**Mean**	**CV**	**MSD**	**alpha**
0. 01866667	40	12.61333	1.083187	0.4226062	0.05

```
$ma[[19]][[2]]
   test              name.t ntr StudentizedRange alpha
  Tukey fact.A:fact.B:fact.C  20         5.357512  0.05

$ma[[19]][[3]]
           dependent.var groups
20w:8%k:3rd      14.40000      a
20w:6%k:3rd      14.33333      a
20w:4%k:3rd      14.26667     ab
20w:2%k:3rd      14.23333     ab
0w:8%k:3rd       14.13333    abc
0w:2%k:3rd       14.00000    abc
0w:4%k:3rd       14.00000    abc
0w:6%k:3rd       14.00000    abc
20w:0%k:3rd      13.86667     bc
0w:0%k:3rd       13.73333      c
20w:8%k:6th      11.43333      d
20w:6%k:6th      11.40000      d
20w:4%k:6th      11.30000     de
20w:2%k:6th      11.26667     de
0w:8%k:6th       11.10000    def
20w:0%k:6th      11.10000    def
0w:6%k:6th       11.03333    def
0w:4%k:6th       10.96667     ef
0w:2%k:6th       10.90000     ef
0w:0%k:6th       10.80000      f
```

7.1.2. ANOVA Outputs for Effect of Broken kernels, Weevils (Sitophilus zeamais) and Storage on Weevil Infestation of Maize under Storage Condition

```
Response: dependent.var
                   Df Sum Sq Mean Sq   F value    Pr(>F)
fact.A              1 321.12  321.12 4444.2152 < 2.2e-16 ***
fact.B              4   4.99    1.25   17.2539 2.673e-08 ***
fact.C              1  56.17   56.17  777.4207 < 2.2e-16 ***
fact.A:fact.B       4   0.06    0.01    0.2010   0.93633
fact.A:fact.C       1   0.33    0.33    4.6230   0.03765 *
fact.B:fact.C       4   0.14    0.04    0.4963   0.73851
fact.A:fact.B:fact.C  4   0.08    0.02    0.2825   0.88757
Residuals          40   2.89    0.07
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

$rspi[[2]]
[1] "R Square 0.993"
```

```
$rspi[[3]]
[1] "SEm of A: 0.049 , SEd of A: 0.069 , SEm of B: 0.078 , SEd of B 0.11 , SEm of C: 0.
049 , SEd of C: 0.069 , SEm of AB: 0.11 , SEd of AB: 0.155 , SEm of AC: 0.069 , SEd of
AC: 0.098 , SEm of BC: 0.11 , SEd of BC: 0.155 , SEm of ABC: 0.155 , SEd of ABC: 0.2
19"

$rspi[[4]]

	Shapiro-Wilk normality test

data:  model$residuals
W = 0.62671, p-value = 4.339e-11


$rspi[[5]]
[1] "Normality assumption is violated"

$rspi[[6]]
[1] "The means of one or more levels of factor A are not same, so go for multiple compari
son test"

$rspi[[7]]
$rspi[[7]][[1]]
    MSerror Df     Mean       CV       MSD
  0.07225649 40 3.733186 7.200437 0.1402734

$rspi[[7]][[2]]
   test name.t ntr StudentizedRange alpha
  Tukey fact.A   2         2.858232  0.05

$rspi[[7]][[3]]
    dependent.var groups
20w      6.046637      a
0w       1.419734      b


$rspi[[8]]
[1] "The means of levels of factor B are not same, so go for multiple comparison test"

$rspi[[9]]
$rspi[[9]][[1]]
    MSerror Df     Mean       CV       MSD
  0.07225649 40 3.733186 7.200437 0.3134259

$rspi[[9]][[2]]
```

```
  test name.t ntr StudentizedRange alpha
  Tukey fact.B   5         4.039123  0.05

$rspi[[9]][[3]]
    dependent.var groups
8%k      4.169577      a
6%k      3.937579     ab
4%k      3.687935     bc
2%k      3.480016      c
0%k      3.390822      c


$rspi[[10]]
[1] "The means of one or more levels of factor C are not same, so go for multiple compari
son test"

$rspi[[11]]
$rspi[[11]][[1]]
     MSerror Df     Mean       CV       MSD
  0.07225649 40 3.733186 7.200437 0.1402734

$rspi[[11]][[2]]
   test name.t ntr StudentizedRange alpha
  Tukey fact.C   2         2.858232  0.05

$rspi[[11]][[3]]
    dependent.var groups
6th      4.700775      a
3rd      2.765597      b


$rspi[[12]]
[1] "The means of levels of interaction between A and B factors are same so dont go for a
ny multiple comparison test"

$rspi[[13]]
$rspi[[13]][[1]]
     MSerror Df     Mean       CV       MSD
  0.07225649 40 3.733186 7.200437 0.5195629

$rspi[[13]][[2]]
   test        name.t ntr StudentizedRange alpha
  Tukey fact.A:fact.B  10         4.734513  0.05

$rspi[[13]][[3]]
        dependent.var groups
```

```
20w:8%k      6.519729      a
20w:6%k      6.247100     ab
20w:4%k      6.009706    abc
20w:2%k      5.808739     bc
20w:0%k      5.647911      c
0w:8%k       1.819424      d
0w:6%k       1.628059     de
0w:4%k       1.366165     de
0w:2%k       1.151293      e
0w:0%k       1.133732      e


$rspi[[14]]
[1] "The means of levels of interaction between B and C factors are same so dont go for a
ny multiple comparison test"

$rspi[[15]]
$rspi[[15]][[1]]
    MSerror Df     Mean       CV       MSD
  0.07225649 40 3.733186 7.200437 0.5195629

$rspi[[15]][[2]]
   test        name.t ntr StudentizedRange alpha
  Tukey fact.B:fact.C  10         4.734513  0.05

$rspi[[15]][[3]]
       dependent.var groups
8%k:6th      5.098363      a
6%k:6th      4.848904     ab
4%k:6th      4.633898     ab
2%k:6th      4.507540      b
0%k:6th      4.415168      b
8%k:3rd      3.240790      c
6%k:3rd      3.026255      c
4%k:3rd      2.741973     cd
2%k:3rd      2.452491      d
0%k:3rd      2.366475      d


$rspi[[16]]
[1] "The means of levels of interaction between A and C factors are not same, so go for m
ultiple comparison test"

$rspi[[17]]
$rspi[[17]][[1]]
    MSerror Df     Mean       CV       MSD
```

```
 0.07225649 40 3.733186 7.200437 0.2630938

$rspi[[17]][[2]]
  test       name.t ntr StudentizedRange alpha
 Tukey fact.A:fact.C   4        3.790685  0.05

$rspi[[17]][[3]]
      dependent.var groups
20w:6th     6.9396110      a
20w:3rd     5.1536630      b
0w:6th      2.4619383      c
0w:3rd      0.3775307      d



$rspi[[18]]
[1] "The means of levels of interaction between all the three factors ABC are same so don
t go for any multiple comparison test"

$rspi[[19]]
$rspi[[19]][[1]]
   MSerror Df    Mean      CV      MSD
 0.07225649 40 3.733186 7.200437 0.8314592
```

MSE	Df	Mean	CV	MSD	alpha
0.07225649	40	3.733186	7.200437	0.8314592	0.05

```
$rspi[[19]][[2]]
  test              name.t ntr StudentizedRange alpha
 Tukey fact.A:fact.B:fact.C  20        5.357512  0.05

$rspi[[19]][[3]]
          dependent.var groups
20w:8%k:6th     7.3861813      a
20w:6%k:6th     7.1348359      a
20w:4%k:6th     6.9016699      a
20w:2%k:6th     6.7124959      a
20w:0%k:6th     6.5628720      a
20w:8%k:3rd     5.6532777      b
20w:6%k:3rd     5.3593633     bc
20w:4%k:3rd     5.1177413     bc
20w:2%k:3rd     4.9049820     bc
20w:0%k:3rd     4.7329509      c
0w:8%k:6th      2.8105451     d
0w:6%k:6th      2.5629711     d
0w:4%k:6th      2.3661252     d
0w:2%k:6th      2.3025851     d
```

```
0w:0%k:6th     2.2674649      d
0w:8%k:3rd     0.8283022      e
0w:6%k:3rd     0.6931472      e
0w:4%k:3rd     0.3662041      e
0w:0%k:3rd     0.0000000      e
0w:2%k:3rd     0.0000000      e
```

7.1.3. ANOVA Results for Effect of Threshing Levels (Broken kernels) and Weevil Insects (*Sitophilus zeamais*) on weevil death percent in the Maize Storage Condition

```
Analysis of Variance Table

                 Response of dependent.var
                  Df  Sum Sq Mean Sq    F value    Pr(>F)
fact.A             1 215.991 215.991 10550.5250 < 2.2e-16 ***
fact.B             4   3.354   0.839    40.9623 1.223e-13 ***
fact.C             1  22.929  22.929  1120.0052 < 2.2e-16 ***
fact.A:fact.B      4   0.258   0.064     3.1450 0.0243839 *
fact.A:fact.C      1   4.174   4.174   203.8866 < 2.2e-16 ***
fact.B:fact.C      4   0.375   0.094     4.5736 0.0038957 **
fact.A:fact.B:fact.C  4   0.517   0.129     6.3193 0.0004872 ***
Residuals         40   0.819   0.020
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

 "R Square 0.997"

$rspdi[[3]]
[1] "SEm of A: 0.026 , SEd of A: 0.037 , SEm of B: 0.041 , SEd of B 0.058 , SEm of C: 0
.026 , SEd of C: 0.037 , SEm of AB: 0.058 , SEd of AB: 0.083 , SEm of AC: 0.037 , SEd
of AC: 0.052 , SEm of BC: 0.058 , SEd of BC: 0.083 , SEm of ABC: 0.083 , SEd of ABC
: 0.117"

$rspdi[[4]]

        Shapiro-Wilk normality test

data:  model$residuals
W = 0.71148, p-value = 1.486e-09


$rspdi[[5]]
[1] "Normality assumption is violated"

$rspdi[[6]]
```

```
[1] "The means of one or more levels of factor A are not same, so go for multiple compari
son test"

$rspdi[[7]]
$rspdi[[7]][[1]]
     MSerror Df     Mean       CV        MSD
  0.02047203 40 2.251751 6.354187 0.07466504

$rspdi[[7]][[2]]
   test name.t ntr StudentizedRange alpha
  Tukey fact.A   2         2.858232  0.05

$rspdi[[7]][[3]]
    dependent.var groups
20w     4.1490769      a
0w      0.3544258      b


$rspdi[[8]]
[1] "The means of levels of factor B are not same, so go for multiple comparison test"

$rspdi[[9]]
$rspdi[[9]][[1]]
     MSerror Df     Mean       CV       MSD
  0.02047203 40 2.251751 6.354187 0.1668311

$rspdi[[9]][[2]]
   test name.t ntr StudentizedRange alpha
  Tukey fact.B   5         4.039123  0.05

$rspdi[[9]][[3]]
    dependent.var groups
8%k      2.601817      a
6%k      2.413190      b
4%k      2.232135      c
2%k      2.074933     cd
0%k      1.936681      d


$rspdi[[10]]
[1] "The means of one or more levels of factor C are not same, so go for multiple compari
son test"

$rspdi[[11]]
$rspdi[[11]][[1]]
     MSerror Df     Mean       CV        MSD
```

```
  0.02047203 40 2.251751 6.354187 0.07466504

$rspdi[[11]][[2]]
   test name.t ntr StudentizedRange alpha
  Tukey fact.C   2         2.858232  0.05

$rspdi[[11]][[3]]
    dependent.var groups
6th      2.869931      a
3rd      1.633571      b


$rspdi[[12]]
[1] "The means of levels of interaction between A and B factors are not same, so go for m
ultiple comparison test"

$rspdi[[13]]
$rspdi[[13]][[1]]
     MSerror Df     Mean       CV       MSD
  0.02047203 40 2.251751 6.354187 0.2765541

$rspdi[[13]][[2]]
   test        name.t ntr StudentizedRange alpha
  Tukey fact.A:fact.B  10         4.734513  0.05

$rspdi[[13]][[3]]
        dependent.var groups
20w:8%k     4.6063816      a
20w:6%k     4.3446509     ab
20w:4%k     4.1176969     bc
20w:2%k     3.9188171     cd
20w:0%k     3.7578378      d
0w:8%k      0.5972532      e
0w:6%k      0.4817286     ef
0w:4%k      0.3465736    efg
0w:2%k      0.2310491     fg
0w:0%k      0.1155245      g


$rspdi[[14]]
[1] "The means of levels of interaction between B and C factors are not same, so go for m
ultiple comparison test"

$rspdi[[15]]
$rspdi[[15]][[1]]
     MSerror Df     Mean       CV       MSD
```

```
  0.02047203 40 2.251751 6.354187 0.2765541

$rspdi[[15]][[2]]
   test        name.t ntr StudentizedRange alpha
  Tukey fact.B:fact.C  10         4.734513  0.05

$rspdi[[15]][[3]]
       dependent.var groups
8%k:6th      3.331257      a
6%k:6th      3.093674     ab
4%k:6th      2.841759     bc
2%k:6th      2.635737     cd
0%k:6th      2.447229      d
8%k:3rd      1.872378      e
6%k:3rd      1.732705     ef
4%k:3rd      1.622511    efg
2%k:3rd      1.514130     fg
0%k:3rd      1.426133      g


$rspdi[[16]]
[1] "The means of levels of interaction between A and C factors are not same, so go for m
ultiple comparison test"

$rspdi[[17]]
$rspdi[[17]][[1]]
    MSerror Df     Mean       CV       MSD
  0.02047203 40 2.251751 6.354187 0.1400401

$rspdi[[17]][[2]]
   test        name.t ntr StudentizedRange alpha
  Tukey fact.A:fact.C   4         3.790685  0.05

$rspdi[[17]][[3]]
       dependent.var groups
20w:6th     5.0310107      a
20w:3rd     3.2671430      b
0w:6th      0.7088516      c
0w:3rd      0.0000000      d


$rspdi[[18]]
[1] "The means of levels of interaction between all the three factors ABC are not same, so
go for multiple comparison test"

$rspdi[[19]]
```

```
$rspdi[[19]][[1]]
    MSerror Df     Mean       CV      MSD
  0.02047203 40 2.251751 6.354187 0.442571
```

MSE	Df	Mean	CV	MSD	alpha
0.02047203	40	2.251751	6.354187	0.442571	0.05

```
$rspdi[[19]][[2]]
   test              name.t ntr StudentizedRange alpha
  Tukey fact.A:fact.B:fact.C  20         5.357512  0.05

$rspdi[[19]][[3]]
            dependent.var groups
20w:8%k:6th     5.4680067      a
20w:6%k:6th     5.2238916     ab
20w:4%k:6th     4.9903709     bc
20w:2%k:6th     4.8093751     bc
20w:0%k:6th     4.6634094      c
20w:8%k:3rd     3.7447564      d
20w:6%k:3rd     3.4654102     de
20w:4%k:3rd     3.2450230     ef
20w:2%k:3rd     3.0282590     ef
20w:0%k:3rd     2.8522661      f
0w:8%k:6th      1.1945063      g
0w:6%k:6th      0.9634573     gh
0w:4%k:6th      0.6931472     hi
0w:2%k:6th      0.4620981     ij
0w:0%k:6th      0.2310491     jk
0w:0%k:3rd      0.0000000      k
0w:2%k:3rd      0.0000000      k
0w:4%k:3rd      0.0000000      k
0w:6%k:3rd      0.0000000      k
0w:8%k:3rd      0.0000000      k
```

7.1.4. ANOVA Results for Effect of Threshing Levels (Broken kernels) and Weevil Insects (*Sitophilus zeamais*) on Insect Damaged Kernel of Maize in the Storage Condition

```
$idk
$idk[[1]]
Analysis of Variance Table

Response: dependent.var
                  Df  Sum Sq Mean Sq     F value  Pr(>F)
fact.A             1 126.236 126.236 95920.0090 < 2e-16 ***
fact.B             4   1.723   0.431   327.3576 < 2e-16 ***
```

```
fact.C              1  17.132  17.132 13017.8861 < 2e-16 ***
fact.A:fact.B       4   0.897   0.224   170.4458 < 2e-16 ***
fact.A:fact.C       1   5.932   5.932  4507.2013 < 2e-16 ***
fact.B:fact.C       4   0.008   0.002     1.4947 0.22203
fact.A:fact.B:fact.C  4   0.011   0.003     2.1829 0.08835 .
Residuals          40   0.053   0.001
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

$idk[[2]]
[1] "R Square 0.998"

$idk[[3]]
[1] "SEm of A: 0.007 , SEd of A: 0.009 , SEm of B: 0.01 , SEd of B 0.015 , SEm of C: 0.
007 , SEd of C: 0.009 , SEm of AB: 0.015 , SEd of AB: 0.021 , SEm of AC: 0.009 , SEd
of AC: 0.013 , SEm of BC: 0.015 , SEd of BC: 0.021 , SEm of ABC: 0.021 , SEd of ABC
: 0.03"

$idk[[4]]

        Shapiro-Wilk normality test

data:  model$residuals
W = 0.9197, p-value = 0.000746


$idk[[5]]
[1] "Normality assumption is violated"

$idk[[6]]
[1] "The means of one or more levels of factor A are not same, so go for multiple compari
son test"

$idk[[7]]
$idk[[7]][[1]]
     MSerror Df     Mean       CV        MSD
  0.001316052 40 1.688935 2.147947 0.01893099

$idk[[7]][[2]]
   test name.t ntr StudentizedRange alpha
  Tukey fact.A   2         2.858232  0.05

$idk[[7]][[3]]
    dependent.var groups
20w     3.1394271      a
0w      0.2384423      b
```

```
$idk[[8]]
[1] "The means of levels of factor B are not same, so go for multiple comparison test"

$idk[[9]]
$idk[[9]][[1]]
   MSerror Df    Mean       CV        MSD
 0.001316052 40 1.688935 2.147947 0.04229928

$idk[[9]][[2]]
  test name.t ntr StudentizedRange alpha
 Tukey fact.B   5         4.039123  0.05

$idk[[9]][[3]]
   dependent.var groups
8%k      1.954999      a
6%k      1.793352      b
4%k      1.657294      c
2%k      1.561384      d
0%k      1.477646      e


$idk[[10]]
[1] "The means of one or more levels of factor C are not same, so go for multiple compari
son test"

$idk[[11]]
$idk[[11]][[1]]
   MSerror Df    Mean       CV        MSD
 0.001316052 40 1.688935 2.147947 0.01893099

$idk[[11]][[2]]
  test name.t ntr StudentizedRange alpha
 Tukey fact.C   2         2.858232  0.05

$idk[[11]][[3]]
   dependent.var groups
6th      2.223291      a
3rd      1.154578      b


$idk[[12]]
[1] "The means of levels of interaction between A and B factors are not same, so go for m
ultiple comparison test"
```

```
$idk[[13]]
$idk[[13]][[1]]
   MSerror Df     Mean       CV        MSD
 0.001316052 40 1.688935 2.147947 0.07011907

$idk[[13]][[2]]
  test        name.t ntr StudentizedRange alpha
 Tukey fact.A:fact.B  10         4.734513  0.05

$idk[[13]][[3]]
        dependent.var groups
20w:8%k     3.5862323      a
20w:6%k     3.3257127      b
20w:4%k     3.1010982      c
20w:2%k     2.9200345      d
20w:0%k     2.7640576      e
0w:8%k      0.3237651      f
0w:6%k      0.2609909     fg
0w:4%k      0.2134890      g
0w:2%k      0.2027326      g
0w:0%k      0.1912337      g


$idk[[14]]
[1] "The means of levels of interaction between B and C factors are same so dont go for a
ny multiple comparison test"

$idk[[15]]
$idk[[15]][[1]]
   MSerror Df     Mean       CV        MSD
 0.001316052 40 1.688935 2.147947 0.07011907

$idk[[15]][[2]]
  test        name.t ntr StudentizedRange alpha
 Tukey fact.B:fact.C  10         4.734513  0.05

$idk[[15]][[3]]
        dependent.var groups
8%k:6th     2.5071647      a
6%k:6th     2.3355999      b
4%k:6th     2.1890718      c
2%k:6th     2.0852469      d
0%k:6th     1.9993724      e
8%k:3rd     1.4028327      f
6%k:3rd     1.2511037      g
4%k:3rd     1.1255154      h
```

```
2%k:3rd     1.0375201      i
0%k:3rd     0.9559189      j


$idk[[16]]
[1] "The means of levels of interaction between A and C factors are not same, so go for m
ultiple comparison test"

$idk[[17]]
$idk[[17]][[1]]
     MSerror Df     Mean       CV        MSD
  0.001316052 40 1.688935 2.147947 0.03550656

$idk[[17]][[2]]
   test        name.t ntr StudentizedRange alpha
  Tukey fact.A:fact.C   4         3.790685  0.05

$idk[[17]][[3]]
       dependent.var groups
20w:6th    3.98820659      a
20w:3rd    2.29064757      b
0w:6th     0.45837573      c
0w:3rd     0.01850878      d


$idk[[18]]
[1] "The means of levels of interaction between all the three factors ABC are same so don
t go for any multiple comparison test"

$idk[[19]]
$idk[[19]][[1]]
     MSerror Df     Mean       CV       MSD
  0.001316052 40 1.688935 2.147947 0.1122119
```

MSE	Df	Mean	CV	MSD	alpha
0.001316052	40	1.688935	2.147947	0.1122119	0.05

```
$idk[[19]][[2]]
   test               name.t ntr StudentizedRange alpha
  Tukey fact.A:fact.B:fact.C  20         5.357512  0.05

$idk[[19]][[3]]
          dependent.var groups
20w:8%k:6th    4.42757315      a
20w:6%k:6th    4.18098797      b
20w:4%k:6th    3.95116566      c
```

```
20w:2%k:6th   3.76502877    d
20w:0%k:6th   3.61627737    e
20w:8%k:3rd   2.74489152    f
20w:6%k:3rd   2.47043738    g
20w:4%k:3rd   2.25103082    h
20w:2%k:3rd   2.07504022    i
20w:0%k:3rd   1.91183789    j
0w:8%k:6th    0.58675627    k
0w:6%k:6th    0.49021184   kl
0w:4%k:6th    0.42697795    l
0w:2%k:6th    0.40546511    l
0w:0%k:6th    0.38246748    l
0w:8%k:3rd    0.06077385    m
0w:6%k:3rd    0.03177006    m
0w:0%k:3rd    0.00000000    m
0w:2%k:3rd    0.00000000    m
0w:4%k:3rd    0.00000000    m
```

7.1.5. ANOVA Results for Effect of Threshing Levels (Broken kernels) and Weevil Insects (*Sitophilus zeamais*) on Maize Weight Loss in the Storage Condition

```
$wtl
$wtl[[1]]
Analysis of Variance Table

Response: dependent.var
                  Df Sum Sq Mean Sq    F value    Pr(>F)
fact.A             1 84.538  84.538 93852.4371 < 2.2e-16 ***
fact.B             4  1.573   0.393   436.7126 < 2.2e-16 ***
fact.C             1 14.611  14.611 16220.2975 < 2.2e-16 ***
fact.A:fact.B      4  1.163   0.291   322.8959 < 2.2e-16 ***
fact.A:fact.C      1  8.494   8.494  9429.8079 < 2.2e-16 ***
fact.B:fact.C      4  0.025   0.006     6.9861 0.0002307 ***
fact.A:fact.B:fact.C  4  0.009   0.002     2.6153 0.0493775 *
Residuals         40  0.036   0.001
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

$wtl[[2]]
[1] "R Square 0.999"

$wtl[[3]]
[1] "SEm of A: 0.005 , SEd of A: 0.008 , SEm of B: 0.009 , SEd of B 0.012 , SEm of C: 0
.005 , SEd of C: 0.008 , SEm of AB: 0.012 , SEd of AB: 0.017 , SEm of AC: 0.008 , SEd
of AC: 0.011 , SEm of BC: 0.012 , SEd of BC: 0.017 , SEm of ABC: 0.017 , SEd of ABC:
0.025"
```

```
$wtl[[4]]

        Shapiro-Wilk normality test

data:  model$residuals
W = 0.95334, p-value = 0.02241


$wtl[[5]]
[1] "Normality assumption is violated"

$wtl[[6]]
[1] "The means of one or more levels of factor A are not same, so go for multiple compari
son test"

$wtl[[7]]
$wtl[[7]][[1]]
      MSerror Df     Mean       CV        MSD
  0.0009007593 40 1.315293 2.281823 0.01566179

$wtl[[7]][[2]]
   test name.t ntr StudentizedRange alpha
  Tukey fact.A   2         2.858232  0.05

$wtl[[7]][[3]]
    dependent.var groups
20w     2.5022949      a
0w      0.1282905      b


$wtl[[8]]
[1] "The means of levels of factor B are not same, so go for multiple comparison test"

$wtl[[9]]
$wtl[[9]][[1]]
      MSerror Df     Mean       CV        MSD
  0.0009007593 40 1.315293 2.281823 0.03499458

$wtl[[9]][[2]]
   test name.t ntr StudentizedRange alpha
  Tukey fact.B   5         4.039123  0.05

$wtl[[9]][[3]]
    dependent.var groups
8%k      1.569799      a
```

```
6%k      1.413525      b
4%k      1.285530      c
2%k      1.196580      d
0%k      1.111031      e


$wtl[[10]]
[1] "The means of one or more levels of factor C are not same, so go for multiple compari
son test"

$wtl[[11]]
$wtl[[11]][[1]]
      MSerror Df     Mean       CV        MSD
  0.0009007593 40 1.315293 2.281823 0.01566179

$wtl[[11]][[2]]
   test name.t ntr StudentizedRange alpha
  Tukey fact.C   2         2.858232  0.05

$wtl[[11]][[3]]
    dependent.var groups
6th     1.8087597      a
3rd     0.8218256      b


$wtl[[12]]
[1] "The means of levels of interaction between A and B factors are not same, so go for m
ultiple comparison test"

$wtl[[13]]
$wtl[[13]][[1]]
      MSerror Df     Mean       CV        MSD
  0.0009007593 40 1.315293 2.281823 0.05801016

$wtl[[13]][[2]]
   test        name.t ntr StudentizedRange alpha
  Tukey fact.A:fact.B  10         4.734513  0.05

$wtl[[13]][[3]]
        dependent.var groups
20w:8%k     2.9687791      a
20w:6%k     2.6904532      b
20w:4%k     2.4559540      c
20w:2%k     2.2793146      d
20w:0%k     2.1169737      e
0w:8%k      0.1708181      f
```

```
0w:6%k     0.1365962     fg
0w:4%k     0.1151054     fg
0w:2%k     0.1138452     fg
0w:0%k     0.1050876      g


$wtl[[14]]
[1] "The means of levels of interaction between B and C factors are not same, so go for m
ultiple comparison test"

$wtl[[15]]
$wtl[[15]][[1]]
      MSerror Df     Mean       CV        MSD
  0.0009007593 40 1.315293 2.281823 0.05801016

$wtl[[15]][[2]]
   test        name.t ntr StudentizedRange alpha
  Tukey fact.B:fact.C  10         4.734513  0.05

$wtl[[15]][[3]]
       dependent.var groups
8%k:6th     2.0927538      a
6%k:6th     1.9229749      b
4%k:6th     1.7770899      c
2%k:6th     1.6714280      d
0%k:6th     1.5795522      e
8%k:3rd     1.0468434      f
6%k:3rd     0.9040745      g
4%k:3rd     0.7939694      h
2%k:3rd     0.7217319      i
0%k:3rd     0.6425091      j


$wtl[[16]]
[1] "The means of levels of interaction between A and C factors are not same, so go for m
ultiple comparison test"

$wtl[[17]]
$wtl[[17]][[1]]
      MSerror Df     Mean       CV        MSD
  0.0009007593 40 1.315293 2.281823 0.02937491

$wtl[[17]][[2]]
   test        name.t ntr StudentizedRange alpha
  Tukey fact.A:fact.C   4         3.790685  0.05
```

```
$wtl[[17]][[3]]
       dependent.var groups
20w:6th    3.37201513      a
20w:3rd    1.63257468      b
0w:6th     0.24550436      c
0w:3rd     0.01107662      d


$wtl[[18]]
[1] "The means of levels of interaction between all the three factors ABC are not same, so go for multiple comparison test"

$wtl[[19]]
$wtl[[19]][[1]]
      MSerror Df     Mean       CV        MSD
  0.0009007593 40 1.315293 2.281823 0.09283396
```

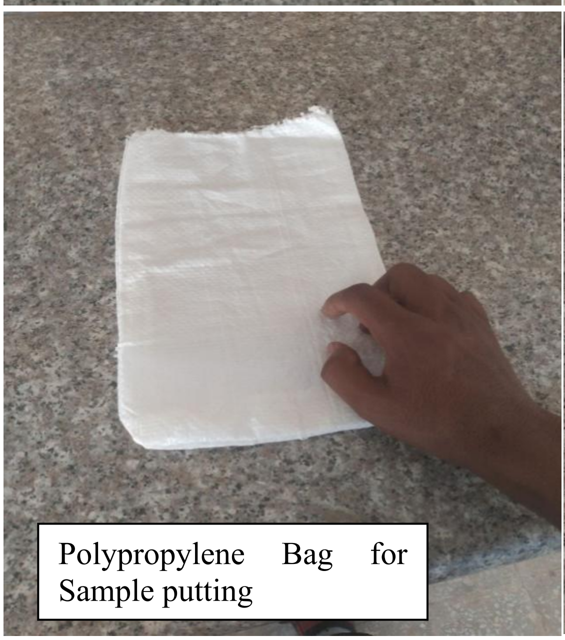
MSE	Df	Mean	CV	MSD	alpha
0.0009007593	40	1.315293	2.281823	0.09283396	0.05

```
$wtl[[19]][[2]]
   test              name.t ntr StudentizedRange alpha
  Tukey fact.A:fact.B:fact.C  20         5.357512  0.05

$wtl[[19]][[3]]
           dependent.var groups
20w:8%k:6th    3.88194507      a
20w:6%k:6th    3.59006678      b
20w:4%k:6th    3.32396908      c
20w:2%k:6th    3.11516553      d
20w:0%k:6th    2.94892918      e
20w:8%k:3rd    2.05561304      f
20w:6%k:3rd    1.79083954      g
20w:4%k:3rd    1.58793885      h
20w:2%k:3rd    1.44346375      i
20w:0%k:3rd    1.28501823      j
0w:8%k:6th     0.30356244      k
0w:6%k:6th     0.25588299     kl
0w:4%k:6th     0.23021081     kl
0w:2%k:6th     0.22769045     kl
0w:0%k:6th     0.21017514      l
0w:8%k:3rd     0.03807371      m
0w:6%k:3rd     0.01730936      m
0w:0%k:3rd     0.00000000      m
0w:2%k:3rd     0.00000000      m
0w:4%k:3rd     0.00000000      m
```

7.2. Appendix Figures of Materials Used

7.2.1. List of equipment and materials that used during the study

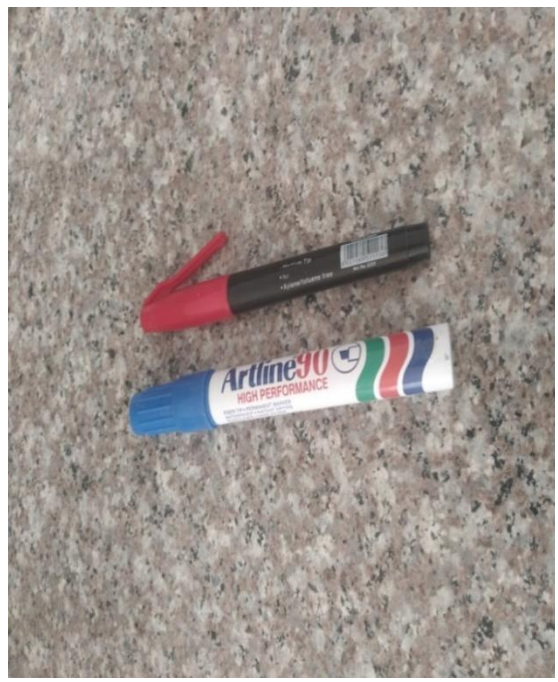


Polypropylene Bag for Sample putting

Plastic Bag for Catching Grinded Maize Sample

Sample from the storage condition

Single sample maize


Artline90
HIGH PERFORMANCE




7.3. Appendix Figures for Activities of the Study

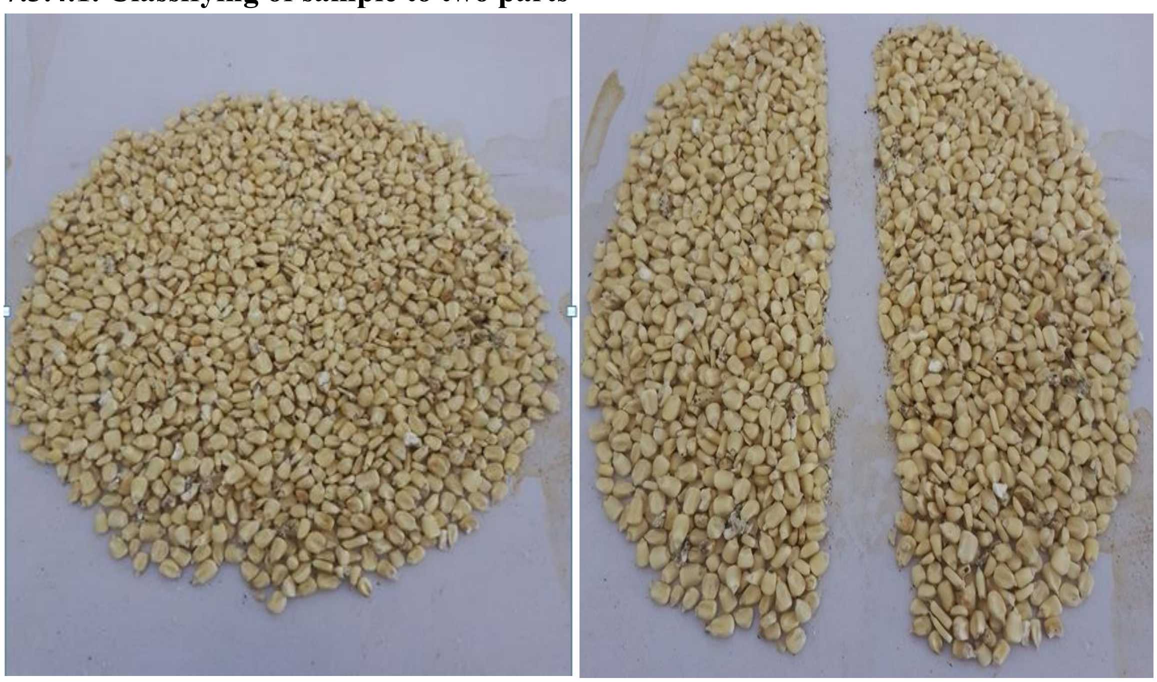
7.3.1. Measuring the moisture content of the sample

7.3.2. Counting of weevil Insects from Maize Storage Duration

7.3.3. Putting counted insects in killing Jarr

7.3.4. Activities to Prepare the Sample for Weight Measurement

7.3.4.1. Classifying of sample to two parts



7.3.4.2. Classifying of sample to four parts

7.3.4.3. Counting of damaged and undamaged kernels from 1000 maize taking single part of the sample

7.3.4.4. Measuring the weight of the damaged and undamaged kernel of the sample

Biography

Gedifew Ferede was born in 1989 E.C from his father Ferede Tilahun and mother Nibret Tefere in Fagita Lakoma, Ethiopa. He completed his undergraduate degree in Plant Science at Injibara University in 2013 E.C. Following this, he pursued a Master's degree in Plant Protection at Bahir Dar University, where his research focused on the impact of broken kernels, weevil infestations, and storage duration on the deterioration indicators of maize (*Zea mays*) under controlled storage conditions. It aims to assess how these factors influence the quality, safety and quantity of stored maize, focusing on parameters (maize deterioration indicators) such as moisture content, weevil development, insect damaged kernel, weight loss, aflatoxin levels, and ochratoxin levels. Determining the interaction effects by analyzing of broken kernels, weevils, and storage duration on maize deterioration indicators, this research seeks to determine insights into effective storage practices that can minimize loss and maintain the nutritional quality of maize.

He has skill on proposal, project, research and review writing. He has knowledge on Mendeley and Endnote software for citation and reference arrangement. He has advanced knowledge and skill on R software and RStudio that R software is latest and very important software which is used for data analysis and documentation by Researchers. He has advanced skill on MS Word, MS Excel, PDF, PPt and basic skill on ArcGIS & remote sensing for study area, location view from the Google Earth. He has basic skill on python, SAS, SPSS, Minitab, and Stata softwares. He has skill on analysis of mycotoxin in the laboratory using kits. He has skill on bacterial, fungal and nematode isolation in the laboratory. He has basic lab work experience on plant diseases analysis.

Currently, Gedifew Ferede is lecturer in department of Plant Science at Injibara University. In addition to his academic work, Gedifew Ferede enjoys Athletics, Taekwondo, Helping others, Reading Research Papers, Journals, Text books, Fiction and Magazines, watching Television news and Movies, online learning on you tube, experience sharing and discussion with colleagues. He plans to continue his research in pursuing a PhD in Plant Pathology/Plant Health/Plant Biotechnology/ Plant Tissue Culture/Integrated Pest Management (IPM)/Modern Agriculture or Crop Management System.