ASSESSMENT OF EFFICACY OF UNSTABILIZED PYRETHRINS AND DIATOMACEOUS EARTH ADMIXTURE ON *SITOPHILUS ZEAMAIS* IN MAIZE GRAINS

BY

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DECLARATION

Declaration by Student

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DEDICATION

To my wife Margaret and children Brent, Bradley and Brinne for their patience and understanding

ABSTRACT

Maize crop provides source of livelihood to all groups of people that depend on it. Its adaptability to different agro-ecological zones has led to its increased production and consumption worldwide. In Kenya maize is regarded as the country's staple food crop. A large proportion of the estimated 30% post-harvest losses are attributed to storage insect pests including *Sitophilus zeamais* (maize weevil) which thrive in tropical climates. Chemical insecticides used have raised concerns on undesirable environmental and human health effects as well as effects on other non-target organisms. In this study, a laboratory investigation was carried out to explore the possibility of controlling *S. zeamais* with a admixture of unstabilized pyrethrins and diatomaceous earth. Adult *S. zeamais* were seeded into glass jars containing maize grains with different ratios of the admixture. Maize grains in separate jars were treated with *pyrethrins* alone and diatomaceous earth alone and also seeded with *S. zeamais*. Untreated maize grains in other jars were seeded with adult insects as the control. Actellic Super Dust – a commercial grain protectant containing admixture of organophosphorous (1.6% *pirimiphos-methyl*) and synthetic pyrethroid (0.3% *permethrin*) was included for comparison purposes. Mortality was monitored in all the treatments at intervals of 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively. Deposition of *pyrethrins*, *pirimiphos-methyl* and *permethrin* in grain were determined on treatment day (24hrs). Residues of these compounds were determined at 90^{th} and 180^{th} day after treatment to establish degradation levels. Comparison of insect pest mortalities was done using one-way analysis of variance (ANOVA) and significant differences separated using Tukey's HSD test. Deposition quantities were analyzed using chi square (χ^2). Results showed that admixtures containing increased quantity of *pyrethrins* resulted in higher mortality of *S. zeamais* on 7^{th}, 14^{th} and 21^{st} days respectively. However, by end of 28^{th} day, all the different ratios had showed 100% mortality. Initial deposited pyrethrins onto the maize grains showed no significant difference with the calculated *pyrethrins* content before application. Residue analysis showed that *pyrethrins* degraded in large proportion over the 180^{th} day period. Milled grains also showed low residue levels indicating high degradation resulting from milling. Based on this study it is recommended that combinations of unstabilized *pyrethrins* and diatomaceous earth be utilized as grain storage protectant.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

ACP	-	Advisory Committee on Pesticides
ADIL	-	African Diatomite Industries Limited
ANOVA	-	Analysis of Variance
ASD	-	Actellic Super Dust
CGC	-	Canadian Grain Commission
CIPS	-	Center for Integrated Plant Systems.
EPAT	-	Environmental and Natural Resources Policy and Training
EPZA	-	Export Promotion Zones Authority
FAO	-	Food and Agricultural Organization
GASGA	-	Group for Assistance on Systems relating to Grain After-harvest.
GIFAP	-	International Group of National Associations of Manufacturers of Agrochemical Products.
GRDC	-	Grains Research and Development Corporation
IGC	-	International Grain Council
IPCS	-	International Programme on Chemical Safety
IRAC	-	Insecticide Resistance Action Committee
KARI	-	Kenya Agricultural Research Institute
LC_{50}	-	Lethal Concentration for 50% mortality
LOQ	-	Limit of Quantification
NARL	-	National Agricultural Research Laboratories
NCPB	-	National Cereals and Produce Board
OPs	-	Organophosphate pesticides
PBK	-	Pyrethrum Board of Kenya
P_f	-	Processing factor
SGR	-	Strategic Grain Reserve
SSA	-	Sub-Saharan Africa
WRCC	-	Western Regional Coordinating Committee

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CHAPTER ONE

INTRODUCTION

1.1 Background to the study

The Maize grains provide a source of livelihood to all groups that depend on it as a staple food and an industrial raw material (Mejia, 2003). Adaptability to different agro-ecological zones has led to increased production and consumption of maize crop worldwide (Mejia, 2003). At the threshold of 21st Century, about 600 million tons of maize grain was produced in the world on 139 million hectares, with 70% of this area being in developing countries such as Latin-America and Africa, where maize crop ranks first (Mejia, 2003). Maize grain in Kenya is regarded as the country's staple food, with the area under maize crop cultivation in 2009 estimated at 1.9 million hectares (Songa & Irungu, 2010) with about 75% production by small-scale farmers. The importance of maize production is evidenced by government policy of subsidizing fertilizer, availing financial resources to the National Cereals and Produce Board (NCPB), maintaining strategic grain reserve (SGR) and assessing and disseminating information on soil suitability for maize growing in various agro-ecological zones (NAAIAP, 2014).

Upon harvesting, most small-scale farmers store sufficient maize grain in own storage facilities for consumption while selling the rest. Likewise, large-scale farmers sell significant quantity of grain to NCPB or grain millers, but retain enough quantity for own consumption (FAO, 2013). Other products derived from maize grain include maize meal flour, corn oil, flour for confectionery, corn flakes, snacks and conversion into glucose syrup and dextrose (EPZA, 2005).

Maize grains whether stored in own or commercial storage facility is susceptible to insect pest infestation leading to quantitative, qualitative, germinative, nutritive and economic losses. Insect pests contribute significant losses to maize grain with damage upto 30% reported in Kenya (Weaver & Petroff, 2004; Hassan and Amupitan 2006; Songa & Irungu 2010; M'mboyi *et al.* 2010; Nukenine, 2010).

Among the major stored grain insect pests is the maize weevil (*Sitophilus zeamais)* which can attack stored maize grain and maize cob in the field and storage facility. Under optimum weather conditions, *S. zeamais* life cycle takes a minimum of 30 days with all developmental stages taking place inside the maize grain. The larva is the most active stage of the insect as it feeds within the grain. Heddi (2011) contends that *Sitophilus spp.* is among the most cereal devastators causing huge agricultural and economic damages, particularly in developing countries. This corroborates other observations that the weevils are the most serious stored grain pests and have spread worldwide through trade (Tabassum *et al*. 1992; Vassanacharoen *et al*. 2007; Koehler 2008; Islam *et al*. 2009; Vasquez-Castro *et al*. 2009). Macharia *et al*. (2006) also indicate that S*itophilus spp.* is a major pest of economic importance throughout Kenya and causes loss of food value in stored cereal grains. Other important grain damaging insect pests include *Prostephanus truncatus* (Larger Grain Borer), *Tribolium castaneum* and *Rhizopertha dominica.*

Mitigation measures used over the years have mainly been chemical insecticides like carbamates, organophosphates and synthetic pyrethroids (ACP, 1991). It is important for users to have knowledge of mode of action, properties, metabolism, residuality and environmental fate of these chemical insecticides in order to make proper appraisal of the benefits and potential hazards of these insecticides (Lorenz, 2009; Morallo-Rejesus and Rejesus, 1988). Gadzirayi *et al*. (2006) argued that over the years, concerns have been raised over the use of chemicals and their effects on human health and ecosystem balance since over-use and abuse of agro-chemicals impose serious costs on a nation's economy while eroding the ecological foundations and thriving agro-ecosystems. There is a global drive towards reduction in pesticide use with the ultimate goal of eventually phasing out deleterious ones such as organophosphates used in grain protection (EPAT, 1994; Belmain, 1999; Mvumi and Stathers, 2003). This has necessitated the need to identify other viable control methods (Cook *et al*. 2002).

Such control methods should offer effective grain protection in addition to minimal risks to biota. Some of these include use of plant extracts and powders. In the current study unstabilized *pyrethrins* contained in the flower heads of *Chrysanthemum cinerariefolium* plant were investigated upon mixing with diatomaceous earth mined in Kariandusi, Kenya. Usage of *pyrethrins* alone are noted to have strong insect flushing effect, agitation, rapid knockdown with appreciable kill albeit quick degradation when exposed to light and high temperatures. Literature on commercial use of *pyrethrins* in maize grain storage is based on stabilized powder or extracts formulations. Unstabilized *pyrethrins* are rarely used in formulations owing to rapid degradation.

Diatomaceous earth dust once mined and processed when used alone or in formulations with chemicals have been utilized and commercialized in grain storage protection and on other crawling insects. Sufficient and prolonged contact of diatomaceous earth with crawling insect pests result in exoskeleton abrasion, piercing, desiccation and eventual insect death. Diatomaceous earth dust is chemically inert, stable and offer grain storage protection.

1.2 Problem Statement

Maize grain represents a source of livelihood as a staple food and an industrial raw material. It is ranked first in developing countries including Sub-Saharan Africa. Maize grain is susceptible to postharvest losses. Under storage conditions, much of the loss is largely attributed to insect pest infestation including *Sitophilus zeamais.* Subsequently, this leads to substantial quantitative, qualitative, nutritive and economic losses. The maize grains must be protected from the insect pest attack to maintain its integrity.

Chemical insecticides have been used extensively in grain storage facilities to control maize grain insect pests. Despite efforts to establish safe residue limits and tolerances of insecticides, there is still increasing consumer concern that continued use of persistent chemical insecticides cumulatively elevates levels in the environment, upsetting ecological balance and affecting non-target organisms. There is also perception that residual insecticides in grain and grain products constitute dietary intake through food consumption posing serious human health risks. The purpose of the current study is to evaluate efficacy of admixture of unstabilized *pyrethrins* and diatomaceous earth mined in Kariandusi, Kenya as part of insect pest management approach and also determine residue levels of *pyrethrins* as measure of food safety.

1.3 Justification of the Study

Post harvest losses attributed to insect infestation on maize grain is quite high, estimated at 30% thereby depleting maize grain stocks available for consumption and industrial raw material predisposing populations to insufficient raw material and food insecurity. Additional insect pest control measures contribute to insect pest management.

Chemical insecticides like organophosphorous and synthetic pyrethroids used in grain protection owing to their persistence contribute to environmental pollution at the site of application and farther away by way of drift and underground seepage. These insecticides become bio-accessible through constant interaction of different types of organisms at various trophic levels in both terrestrial and aquatic ecosystems posing uncertain effects on non-target organisms. Unstabilized pyrethrins with rapid degradation admixed with diatomaceous earth noted as being inert and non-toxic would be a viable option in maize grain protection compared to other chemical insecticides that are more persistent in the environment.

Maize grains treated with chemical insecticides are fed to livestock and chicken as raw grains. The grains may also be boiled or roasted for human consumption or milled into flour for maize meal. These constitute possible dietary intake heightening consumer concerns on health effects of residual chemical insecticides and their metabolites in grain and grain products. A proactive approach to seeking not only for effective control alternatives but also those considered as safe and therefore acceptable to consumers with minimal risks to their health have to be explored.

1.4 Scope of the Study

The study was carried out at the entomological laboratory of Pyrethrum Board of Kenya under controlled conditions. The study evaluated different ratios of unstabilized pyrethrins when mixed with diatomaceous earth mined in Kenya against maize weevil (*S. zeamais)* insect pest. Unstabilized pyrethrins and diatomaceous earth were also evaluated without mixing. A commercial maize grain protectant (Actellic Super Dust) containing *permethrin* and *pirimiphos-methyl* was evaluated alongside the admixture for comparison.

Besides evaluation of the admixture efficacy against the insect pest, *pyrethrins* were also monitored for deposit and residue levels over time. Residue determination on whole maize grain and flour was carried out at 90^{th} and 180^{th} day after application to determine the effect of storage period and grinding on residue levels. *Permethrin* and *Pirimiphos-methyl* residues were also determined for deposition and residuality and compared with recommendations by FAO/WHO standards.

The literature reviewed included use of diatomaceous earth in stored grain protection, pyrethrins in stored grain against insect pests, overview of chemical insecticides in grain protection, environmental and health effects of applying chemical insecticides on stored grain.

1.5 Objectives of the Study

Overall objective

The overall objective of this study was to explore the potential insecticidal activity of a admixture of unstabilized pyrethrins and diatomaceous earth against *S. zeamais* in stored maize grains as part of insect pest management for reducing grain losses.

Specific objectives

1) To determine mortality of *Sitophilus zeamais* by different combinations of unstabilized pyrethrins and diatomaceous earth admixture in maize grains.
2) To determine the initial pyrethrins portion of the admixture deposited onto the maize grains.
3) To determine the residue level of pyrethrins in the different ratios of the admixture at treatment day (24hr), 90^{th} day and 180^{th} day after application in both grain and flour.

1.6 Hypotheses

i. There is no significant difference in level of mortality of *S. zeamais* observed from different ratios of the admixture at different time intervals after application (7^{th}, 14^{th}, 21^{st} and 28^{th} day).
ii. There is no significant difference between the *pyrethrins* in the admixture before treatment and the initial *pyrethrins* deposited onto the maize grains.
iii. No significant proportion of *pyrethrins* in the different ratios of the admixture in grains is degraded between application day and the 180^{th} day of storage.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diatomaceous earth in Stored grain Protection

Diatomaceous Earth has been used as insecticide since antiquity with China indicating its use for over 4,000 years ago (Quarles, 2007). Rojht *et al.* (2010) evaluated the effect of diatomaceous earth of different origin, and at different temperature and relative humidity against adults of *Sitophilus oryzae* in stored wheat. They found out that the mortality of adults increased with increasing dose rates and days of exposure. In all the samples the mortality of *Sitophilus oryzae* adults at the dose level of 900ppm at 21 days of exposure was above 90%. In another study, standardized testing for diatomaceous earth was evaluated by four laboratories against laboratory reared cultures of 7 to 21-day old unsexed adult *Sitophilus oryzae* and *Tribolium castaneum* and the results from the four laboratories were generally in concurrence (Fields *et al.*, 2002).

Shayesteh and Ziaee (2007) investigated the insecticidal efficacy of diatomaceous earth against 7 – 14 day adults of *Tribolium castaneum* in wheat. They found that mortality increased with increase in days of exposure with further observation that adults in treated wheat had their reproductive potential suppressed when compared with those in untreated wheat. Similar findings were reported by Wakil *et al.* (2005) when they evaluated the insecticidal efficacy of the diatomaceous earth formulation SilicoSec® against the adults of *Tribolium castaneum* at laboratory scale. The experiment was conducted on wheat grains, by treating them at dose rates of 75, 100 and 125ppm at 30^{o}C and 60% R.H. The insect mortality was recorded after 14 and 21 days exposure interval, and emergence of

progeny examined after 56 days. Biological and environmental parameters also impact on product efficacy (Arthur, 2002; Kljajic *et al.* 2006). Contrary to this, Vardeman *et al.* (2007) found that exposure interval and temperature had no effect on adult survival or progeny production, although the study was based on surface applications of diatomaceous earth and not homogenized to the entire grain.

Diatomaceous earth at high dosages were found effective against *Tribolium castaneum* based on findings by Marsaro *et al.* (2006) when they evaluated the effectiveness of different dosages of diatomaceous earth to control this insect pest in stored corn in the state of Roraima. Likewise Chanbang *et al.* (2007) reported increased mortality with increase in exposure period and that the two diatomaceous earth commercial products did not completely suppress *Rhyzopertha dominica* on rough rice. They recommended combination treatments with another insecticide to give complete control.

In a study by Baldassari *et al.* (2008) where they evaluated the insecticidal efficacy of a diatomaceous earth formulation against a mixed age population of adults of *Rhyzopertha dominica* and *Tribolium castaneum* as function of different temperature and exposure time, they found that although the insecticide Protector® based on diatomaceous earth was effective it did not attain complete mortality against the age admixture of the populations of the two species of grain pests. Mvumi *et al.* (2004) carried out field assessments of the efficacy and persistence of diatomaceous earth dust admixed with sorghum, maize and cowpeas to protect the grain against insect pests in three agro-ecological zones of Zimbabwe for two consecutive storage seasons. Their findings

indicated that diatomaceous earth formulations were effective and persistent grain protectants against major storage pests attacking sorghum, maize and cowpeas for storage periods of 40 weeks in the climatic conditions found in Zimbabwe, although this was also closely linked to the application concentration and commodities under protection.

Under the most ideal situations where best practices are followed, the least tolerant species (saw-toothed grain beetle) can be killed within 2 weeks and the most tolerant (grain weevil) within 5 weeks when using diatomaceous earth making it an ideal insecticide for treating empty stores when used as part of an integrated strategy as revealed by an efficacy study of diatomaceous earth, applied as structural treatments, against stored product insects and mites (Cook *et al.* 2004; Cao *et al.* 2006). Suppression of progeny emergence and infestation by rice and maize weevils can be achieved using diatomaceous earth (Arthur and Throne, 2003; Arnaud *et al.* 2005; Wakil *et al.* 2006; Lorini and Beckel, 2006).

The abrasive action of diatomaceous earth enhances entomopathogenesis as exhibited by *Beauveria bassiana* and *Metarhizium anisopliae against Tribolium spp. Rhyzopertha dominica* and *Sitophilus oryzae* after the insect cuticle had been damaged and further increasing conidial attachment (Akbar *et al*. 2004; Athanassiou, 2005; Kavallieratos *et al.,* 2006). Athanassiouet al. (2006) investigated diatomaceous earth formulations enhanced with soil bacteria metabolite Abamectin and plant extract Bitterbarkomycin against *Prostephanus truncatus, Rhyzopertha dominica, Sitophilus oryzae* and *Tribolium castaneum* on maize and wheat and found that complete adult mortality and progeny

suppression was achieved with low concentrations of these formulations and therefore could be used with success against stored-grain beetle species, at very low application rates ranging from 75ppm to 125ppm.

Similar results of potential combination treatments were also reported by Chintzoglou *et al.* (2008) when they studied the insecticidal effect of spinosad dust, in combination with diatomaceous earth, against two stored-grain beetle species – *Sitophilus oryzae* and *Tribolium confusum* in maize and wheat. The mix of two or three diatomaceous earth formulations is generally more effective at low dose rates than the application of one diatomaceous earth formulation against major stored-grain beetle species - *Rhyzopertha dominica*, *Sitophilus oryzae*, and *Tribolium confusum* as found out by Athanassiou *et al*. (2007) when they investigated insecticidal effect of three diatomaceous earth formulations - Insecto®, PyriSec®, and Protect-It® when applied alone or in combination, against three stored-product beetle species on wheat and maize. Korunic *et al.* (2010) investigated the long term effectiveness of DE and deltamethrin admixture against *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium castaneum* and found that the admixture provided 100% population reduction of all the three species for up to 12 months with little or no progeny produced.

The foregoing studies indicate that the efficacy of diatomaceous earth against insect pests when used alone is hinged on large quantity applied and longer time of exposure. The limitation associated with high dose is reduced grain flowability and bulk density leading to lower grain grading. The prolonged exposure period required for appreciable efficacy

of diatomaceous earth against *S. zeamais* provides sufficient time to the rapidly multiplying insect pest to inflict more damage to the grain as the larval stage occurs inside the grain which is not easily accessible to diatomaceous earth dust. Application of diatomaceous earth in combination with other grain protectants reduce the limitations noted above in addition to increased efficacy against the target insect pests.

2.2 Pyrethrins in Stored Grain Protection

Pyrethrins found on the flower heads of *Chrysanthemum cinerariefolium* plant are a naturally-occurring group of six chemically-related esters, each of which has insecticidal properties. Three are esters of chrysanthemic acid, and the other three are of pyrethric acid. Once harvested, dried and ground, the natural *pyrethrins* in the powder are relatively unstable at normal temperatures and storage conditions and are light-sensitive compared to the antioxidant-stabilized *pyrethrins*. *Pyrethrins* are normally stabilized prior to its use in formulation of commercial products. The unstabilized powder are rarely used in commercial applications and as such the literature reviewed in this study relate to stabilized *pyrethrins.*

Mulungu *et al.* (2010) investigated efficacy of various grain protectants against *Sitophilus zeamais* and *Prostephanus truncatus* for stored maize and found that pyrethrum flower powder offered effective protection against the two insect pests. Todd *et al.* (2003) contends that, *pyrethrins* degrade rapidly when exposed to natural sunlight and do not persist in the environment beyond a few weeks. Exposure to light accelerates *pyrethrins* degradation from 100% to less than 1% within 5 hours (Gunasekara, 2004). Temperature is a critical factor in the rate of degradation of natural *pyrethrins* based on a study in

Tasmania which established that 26, 65, and 68% of the pyrethrins were lost at 20, 60, and 100^0C, respectively after flower harvest (Atkinson *et al.*, 2004).

Greening (1983), investigated insecticide treatments for farm-stored grain and found out that wheat treated with 1.5 mg/kg of *pyrethrins* remained free of infestations for about 2 months but a low-level infestation was noted on the warmer side of the storage bin which spread peripherally to the cooler side. This was attributed to thermal degradation of *pyrethrins*. Warui et al. (1985), reported satisfactory efficacy of pyrethrum formulation when they evaluated improved pyrethrum formulations to control maize pests mainly *S. zeamais, T. castaneum* and *S. cerealella* in three sites in Kenya.

Kimani and Sum (1999), evaluated essential oils extracted from pyrethrum flowers against adult grain pests *Sitophilus oryzae* and *Tribolium castaneum* and found out that these oils were efficacious against the insect pests and recommended their utilization in pest management. The pyrethrins properties of insect flushing and excitation, quick insect knockdown, rapid degradation and low mammalian toxicity are desired in short-term grain protection, but where long-term storage period is required, there may be need for reapplication or stabilization. Grain protection using *pyrethrins* is tenable only for short periods, but prolonged storage leads to degradation of *pyrethrins* which may lead to insect pest re-infestation.

2.3 Overview of Chemical Insecticides in Grain Protection

The use of chemical insecticides in grain protection spans over many years. Residual chemical grain protectants, chiefly organophosphate, pyrethroid and carbamate insecticides have been used on a world-wide scale in management programs of insect pests in stored raw agricultural commodities (Arthur, 1996). Halliday (1989) indicated that potential hazards to consumers from contamination of food with pesticide residues are currently a major public concern in many countries. This has led to pressure on governments to tighten legislation covering use of chemical insecticides and increase the requirements for research data before uses for individual compounds can be allowed. Most pesticide residues detected in food grains arise from contact chemical insecticides or fumigants, deliberately applied to protect the grain from postharvest insect attack. The ranges of compounds used for this purpose are mostly organophosphates such as malathion, pirimiphos-methyl, fenitrothion and chlorpyrifos methyl, or pyrethroids such as permethrin, deltamethrin and bioresmethrin.

Although assessments of potential hazards to consumers have previously centred on single insecticide, concern has also been expressed about possible additive or interactive effects of pesticide combinations and of particular importance that relate to grain protectants, where combinations of pyrethroids and organophosphates are being used increasingly to provide protection for food grains (GIFAP, 1988). There is need for environmental protection and remediation to reduce possible impact of pesticide pollution by minimizing their use among other measures (Kennedy, 1998). Avino *et al.* (2011) noted that although pesticides are widely used in agriculture, they and in particular the

relative residues in foodstuffs, water and atmosphere, may cause remarkable sanitary problems due to the harmful effects and their spread in waters and atmosphere can produce undesired effects on various organisms and/or water contamination.

2.3.1 Environmental and Health Effects of Chemical Insecticides in Grain

Grains may be stored over long period after insecticide treatment at ambient temperatures in commercial bulk silos or own-farm structures. Most commonly used insecticides for protecting grains against insect pests attack during storage include Organochlorines, Organophosphates and synthetic pyrethroids (Pal and Shah, 2008). The insecticides are applied at post-harvest stage to reduce losses from storage pests, but there is the potential of the residual insecticides being a major source of harm through dietary intake.

Organophosphate and carbamate insecticides are known to inhibit the activity of cholinesterase resulting in build-up of acetylcholine in the body leading to uncontrolled flow of nerve transmissions between nerve cells thereby poisoning the organism's nervous system (Lorenz, 2009). This is attributed to the fact that most insects, fish, humans and other mammals possess acetylcholine – the primary chemical responsible for transmission of nerve impulses across synapse of two neurons. Once transmission is complete, acetylcholine has to be broken down by cholinesterase enzyme in readiness for new transmission (Lorenz, 2009). The effects of organophosphate and carbamate poisoning can result in both systemic and topical symptoms which may include constriction of the eye pupils, blurry vision, eyebrow headache, and severe irritation and reddening of the eyes. Other symptoms may include stomach cramps, nausea, vomiting, diarrhoea, salivation, headache, dizziness, and excessive secretions that cause breathing

difficulties and respiratory failure. In advanced poisonings, the victim will appear pale, sweating and frothing at the mouth leading to death if not treated (Lorenz, 2009).

Pyrethroids have increased stability in sunlight compared to *pyrethrins*, resulting in longer residence times especially in sunlight limited areas such as grain stores and subway tunnels where they can persist for months. Pyrethroids have also been shown to inhibit ATPase enzyme production and utilize a number of different pathways to cause nervous system damage in invertebrates (Clark and Matsumura, 1982). The pyrethroids interfere with sodium channel gating in the nerve cell endings which effectively paralyze organisms by severely limiting neuro-transmission. This paralysis is often preceded by spastic activity of the organism due to the hyper-activity of nerve endings. The spastic activity is caused by sodium channels repeatedly polarizing and depolarizing, mimicking neuro-transmission where none is actually taking place. The inhibition of ATPase enzyme production is of primary importance in understanding effect of pyrethroids especially to aquatic organisms which are much more susceptible to pyrethroid insecticides than terrestrial organisms. Freshwater aquatic organisms must maintain ionic balances and osmoregulation in an extremely dilute environment. Active transport at cellular walls is needed to maintain critical cellular ion levels against a concentration gradient. ATPase enzymes provide the energy needed by cells to maintain this gradient. By inhibiting ATPase enzymes, pyrethroids, breakdown the critical concentration gradient, leading to death of the organism (Clark and Matsumura, 1982).

Pyrethroids have the most serious effects on fish and gill breathing aquatic insects because of the large surface area available to de-ionize after ATPase inhibition (Siegfried, 1993). They have irritant and/or sensitizing properties and are absorbed through the gut and pulmonary membrane. The World Health Organization explains that pyrethroids are neuro-poisons acting on the axons in the peripheral and central nervous systems by interacting with sodium channels in mammals and/or insects (WHO, 1999).

Pyrethroids may act as dermal and respiratory allergens and can result into dermatitis and asthma-like reactions. Other symptoms of acute toxicity due to inhalation include sneezing, nasal stuffiness, headache, nausea, uncoordinated motion, tremors, convulsions, facial flushing and swelling, burning and itching sensations in addition to effects on reproduction, sexual development and interference with immune system and increase in chances of breast cancer (Go *et al.*, 1999). Mueller-Beilschmidt (1990), indicate that lobster, shrimp, mayfly, nymphs, zooplanktons, bluegill and lake trout are some of the most affected non target organisms of pyrethroids with LC_{50} values as low as 1.0µg/L. Pyrethroids are toxic to birds, and may also be indirectly affected because of the threat to their food supply especially the waterfowl and other small insectivorous birds. Mueller-Beilschmidt (1990) further indicates that both beneficial insects and pests are susceptible to pyrethroids thereby disrupting the predator-prey relationship.

The foregoing health effects of organophosphate, carbamate and pyrethroid insecticides cannot be ignored and therefore the use of these insecticides in grain protection exposes pest control operators and other non-target organisms to such effects. Ragnarsdottir (2000) indicates that organophosphate pesticides degradation is a function of microbial composition, pH, temperature, and availability of sunlight. Most organophosphate insecticides are more stable in the pH range (pH: 3 – 6) that may be encountered in the environment (IPCS, 1986). Relative solubility of Organophosphate pesticides facilitates their entry into surface and groundwater where chemical hydrolysis occurs and is also pH dependent. Organophosphate compounds can persist in the environment for long periods of time due to their sorption onto soil particles, making them unavailable for microbial metabolism. The underlying important aspect in evaluating the environmental fate and toxicology of organophosphorous, pyrethroid and carbamates among other pesticides is that they can be transferred to humans through food. This occurs under favourable conditions that preserve these insecticides in the soil. - Evidence suggests that Organophosphates are mutagenic and teratogenic and that a large number of modern-day diseases of the nervous and immune system of mammals can be linked to these pesticides (Ragnarsdottir, 2000).

This therefore calls for a thorough examination in the use of Organophosphates in the context of environmental fate and toxicology. Persistence of OPs in the environment was also reported by Avino and colleagues who noted that they cause various health and safety problems (Avino *et al.,* 2011). Omoyakhi *et al.* (2007), found that weaner rabbits could not tolerate the presence of Actellic dust in feed at concentration of 0.04% as it

could result in adverse effects on hematological and biochemical parameters. Mitra *et al.* (2011) indicate that organophosphate insecticides like chlorpyrifos-methyl and carbamates like aldicarb and carbaryl severely affects birds with worldwide numerous reports of incidences of carbamates induced bird poisoning alongside organophosphates. Sub lethal effects of these pesticides are endocrine disruption, alterations in feeding behaviour and compromised immune systems which affect avian reproduction. Critical bird habitat is affected by pesticide use and can cause bird extinction, behavioural changes and population decline.

Based on surface and ground water studies in the Southern Coast Watershed of Caspian Sea, Iran, Rahmanikhah *et al.* (2010) concluded that the residues of pesticides are major threat to aquatic life of the regional ecosystems. Zidan (2009) evaluated the toxic effects of organophosphate pesticides – chlorpyrifos-methyl, diazinon and profenofos on male reproductive system of rats and results showed that the effect of all tested pesticides on testes and seminal vesicles weights was dose-dependent since all tested pesticides at 50ppm significantly decreased their weights. This evaluation, illustrates the effects of Organophosphates on non target organisms' reproductive organs when exposed to food admixed with organophosphorous.

Mohammed *et al.* (2006) determined residues and decay of Organophosphates insecticides – pirimiphos-methyl, chlorpyrifos-ethyl and synthetic pyrethroids – cypermethrin and fenvalerate in three types of water; distilled water (pH 6.4), well water

(pH 7.6) and standard hard water (SHW, pH 7.8). Water samples were extracted and cleaned-up then analyzed. The data showed that residues could still be detected at 7 days.

El-Sherif *et al.* (2009) investigated the effects of pollutants on some aquatic organisms in Temsah Lake in Egypt which is considered one of the wild life features in Egypt in general and in the Suez Canal region in particular. Concentrations of some pesticides which are used around the area were monitored in the tissues of some birds of prey (wild birds), some species of algae, fish and crustaceans. Among other pesticides the results revealed high residues of organophosphate pesticides represented in malathion and diazinon in most of the tested birds although not high in fish, crustaceans or algae. They deduced that pollutants could be transferred through the food chain leading to biomagnification of the pesticides in the bodies of the higher organisms in the food chain. They recommended that implementation of the environmental management practices need to be undertaken in such ecosystems to prevent more pollution which could affect human health and environment.

Shayeghi *et al.* (2009) determined the whole blood cholinesterase activities of the agriculture and hygiene spray workers exposed to organophosphate and carbamate compounds from different parts of Tehran Province in Iran. Results showed that in 32.4% of the workers, cholinesterase activity had decreased up to extensive poisoning while in 17.6% cholinesterase activity was much decreased at the end of an acute or severe poisoning while no any changes were observed in the remaining 50% of the spray worker blood cholinesterase activity after working. This indicates that there is disruption of cholinesterase activity when human beings are exposed to organophosphorous insecticides. In another study Shayeghi *et al.* (2007) determined organophosphate

insecticides (malathion and diazinon) residue in the drinking water from Karaj river and Amir-Kabir dam, they found that residue of malathion and diazinon in water decreased with increase in distance and time of spraying. The residues of malathion and diazinon insecticides were still more than allowed limits 1-2 months after spraying especially at stations close to spraying places. They concluded that not only the environment, but also the people in area of Karaj River are at risk of chronic toxicity with organophosphorous pesticides through consuming polluted water and agriculture products.

Al-Wabel *et al.* (2011), monitored residues of organophosphorous, Carbamates and Pyrethroids among other pesticides in agricultural soil samples in some regions of Saudi Arabia. They detected twelve pesticide residues in soil samples collected from different regions. They concluded that these pesticides can contaminate the soils. Olabanji *et al.* (2011) investigated the possible link between the mental disorder and the poisoning due to some organophosphorus agrochemicals and found values above the FAO/WHO threshold limits which were an indication that there was a possible links to the mental disorder problems. FMC undertook a study of bifenthrin photo stability and found that the ^{14}C phenyl labelled and ^{14}C cyclopropyl labelled bifenthrin had 106 and 147 days half-life on soils exposed to natural sunlight respectively (FMC, 1983). They also found that it depended on the soil type and conditions of aerobic or anaerobic soil metabolism although it tended to be more stable in anaerobic soil metabolism. This indicated that pyrethroids can persist in soils for a long period of time which may be available to organisms in the higher tropic levels.

Anwar (2003) investigated the toxic effects of *permethrin* on the kidney of newly hatched chicks developed from the eggs injected with a single sub-lethal doses of three different concentrations of *permethrin* insecticide (50; 100 and 200 ppm) on day '0' of incubation. They concluded that although the study was a deliberate introduction of *permethrin* into the chicks, it simulates dietary toxic effects when chicks are fed grain preserved with permethrin insecticides.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Test insects

Two week old *Sitophilus zeamais* used in the test were obtained from National Agricultural Research Laboratories (NARL) – the postharvest research unit of Kenya Agricultural and Livestock Research Organization (KALRO) headquarters in Nairobi, Kenya.

3.1.2 Pyrethrum Powder and *pyrethrins* Standards

Dried pyrethrum flowers were obtained from Pyrethrum Board of Kenya (PBK) factory in Nakuru. They were ground into fine powder using bench mounted Culatti micro mill. The resultant powder was sieved to pass through 106µm aperture sieve and standardized to 1%w/w *pyrethrins* using low *pyrethrins* content powder.

3.1.3 Diatomaceous Earth

Finely ground diatomaceous earth powder of over 85% Si_2O was obtained from African Diatomite Industries Limited (ADIL) factory in Kariandusi, Gilgil, Kenya. The powder was sieved to pass through 106µm aperture sieve.

3.1.4 Actellic Super Dust and *Permethrin and Pirimiphos-methyl* Standards

Actellic Super Dust – a commercial grain protectant containing *permethrin* and *pirimiphos-methyl* active ingredients was bought from Mea Limited – an agrovet shop in Nakuru town while their standards were sourced from Twiga Chemicals Industries Limited as the agent for the manufacture of the technical grades.

3.1.5 Maize Grains

Clean, dry and untreated maize grains were obtained from a farmer in Njoro, Nakuru County then sterilized by subjecting to 60^0C for 4 hours in an oven in the laboratory.

3.2 Methods

3.2.1 Weighing and admixture preparation

Five combinations of pyrethrum powder (containing 1% *pyrethrins)* and diatomaceous earth admixture (See table 1) were weighed using KERN 870 analytical balance (with an accuracy of 0.0001g) and transferred into clean test tubes. The combined admixtures in each test tube were thoroughly shaken to homogenize the admixture. Unmixed pyrethrum powder and diatomaceous earth were also weighed. In each treatment level 4 portions were weighed.

Table 1: Different combinations of admixture

Treatments	Pyrethrum Powder (mg)	Diatomaceous Earth (mg)	Total admixture (mg)	Equivalent Pyrethrins (mg/kg)
D	0	300	300	0
T_1	50	250	300	5
T_2	100	200	300	10
T_3	150	150	300	15
T_4	200	100	300	20
T_5	250	50	300	25
P_y	300	0	300	30

P_y = pyrethrum Powder; D = diatomaceous earth; T_1; T_2; T_3; T_4; T_5 = admixtures

3.2.2 Laboratory Experimental set up

The experiment was carried out at the entomology laboratory of Pyrethrum Board of Kenya (PBK) Nakuru, Kenya and set according to protocol described by Mbugua *et al.* (2011). Experimental design applied was of completely randomized design. The treatments were denoted as T_1, T_2, T_3, T_4 and T_5 for mixed ratios of unstabilized *pyrethrins* and diatomaceous earth; P_y and D for unmixed *pyrethrins* and diatomaceous earth respectively; ASD for Actellic Super Dust and C for the control. Four replicates per treatment were prepared denoted as T_{1a}, T_{1b}, T_{1c} and T_{1d} for T_1 which was done similarly for the other treatments. A weight of 100g maize grains were placed in each of the 36 tagged glass jars and treatments introduced into each jar with thorough mixing. The control was left untreated. All the 36 jars were seeded with 20 adult *S. zeamais* and the jars were laid out in a completely randomized design.

Table 2: Completely Randomized Design

T_{1b}	T_{2a}	T_{4a}	T_{3b}	ASD_2	T_{5a}	ASD_3	D_2	D_4
C_2	T_{1c}	P_{y3}	T_{3a}	T_{2b}	T_{2d}	C_4	T_{4d}	T_{5d}
T_{5b}	P_{y2}	T_{4b}	T_{2c}	D_3	ASD_1	C_1	T_{5c}	P_{y1}
T_{3c}	P_{y4}	T_{1d}	T_{1a}	D_1	C_3	ASD_4	T_{4c}	T_{3d}

The jars were held in a room maintained at temperature and relative humidity conditions of 28 ± 2ºC and 65 ± 5%, respectively.

3.3 Initial *Pyrethrins* Deposition and Residue Determination

Insecticide deposition levels was determined within 24hrs of treatment and compared to the expected calculated values. After mortality monitoring, the maize grains were retained for *pyrethrins* residue determination at 90^{th} and 180^{th} day. This was to investigate level of degradation of pyrethrins in the maize grains. The grains were milled into flour and pyrethrins residue determined. Besides, residues of *permethrin* and *pirimiphos-methyl* in ASD were also determined in grain and flour.

3.3.1 Sample Preparation

From the remaining treated maize grains, 25g sample was extracted for residue determination as described under section 3.3.3. This procedure was repeated on 90^{th} and 180^{th} day. Milling of grain was done using bench mounted grinding mill (Culatti Micro Hammer Mill).

3.3.2 Insecticide Standards Preparation

A series of standard solutions of pyrethrins were prepared using analytical grade. The Standard Pyrethrum Extract for the pyrethrins was obtained from Pyrethrum Board of Kenya. The standards for pyrethrins were prepared in two sets (5.0; 10.0; 15.0; 20.0 and 25.0ppm) and (0.2; 0.4; 0.6; 0.8 and 1.0ppm). Likewise, *Permethrin* (94.7%) and *Pirimiphos-methyl* (91.3%) standards obtained from Twiga Chemicals Industries Limited, Nairobi, working standard solutions were prepared for *permethrin* (0.2; 0.6; 1.0; 1.4 and 1.8ppm) and *pirimiphos-methyl* (1.0; 3.0; 5.0; 7.0 and 9.0ppm).

3.3.3 Extraction of *pyrethrins*

A sample of 25g was placed in a thimble then placed into the main chamber of soxhlet extraction apparatus. A portion of 150 ml of n-hexane was placed in the distillation flask and connected to the main soxhlet chamber. The soxhlet was further equipped with a condenser above it. Refluxing at about 70^0C and condensing the vapours back into liquid flooding the chamber housing thimble containing the sample. The interaction between the maize grain/flour slowly extracted the pyrethrins leaving the insoluble component of the sample. When the Soxhlet chamber was almost full, the chamber automatically emptied by a siphon side arm, with the solvent pouring back to the distillation flask. The extraction process went on for 4 hours. After extraction the solution in the distillation flask was concentrated in a rotary evaporator at 40^0C to near dryness then recovered to 2 ml with n-hexane.

3.3.4 Extract Cleaning

The extract from the flour was further cleaned, since corn oil was also extracted. A portion of 100 ml of extract was shaken vigorously with acetonitrile (2 x 100ml) for 2 mins. The combined acetonitrile phase was shaken for 2 mins with 50 ml of n-hexane. Another 30 ml of acetonitrile was added to upper n-hexane layer then shaken for 2 mins. The combined acetonitrile phases then concentrated up to about 60 ml in a rotary evaporator. This was then transferred to a 1L separatory funnel and the flask washed with 10 ml acetonitrile. A portion of 300ml of sodium chloride solution was added and shaken twice for 2 mins. Another 100 ml n-hexane was added and shaken. The combined n-hexane phases (upper layer) were then dried by filtering through a filter paper with 20g anhydrous sodium sulphate and further concentrated in a rotary evaporator at 40^0C to near dryness then recovered to 2ml with n-hexane. Both the whole grain and milled extracts were stored in -20^0C before the HPLC analysis to prevent *pyrethrins* residue degradation.

3.3.5 Extraction of *Permethrin* and *Pirimiphos-Methyl*

The 25g sample with 5g filter aid (Celite 545) was placed in an Erlenmeyer flask. A 200 ml acetone and n-hexane in the ratio of (2:8) was added and shaken for 30 mins. This was filtered under suction. The filter cake was further washed with 50 ml acetone-hexane admixture. The filtrate transferred to 500 ml graduated cylinder. Addition of n-hexane with careful mixing until the organic phase had a volume of 250 ml. From this, 100 ml was decanted then dried by filtering through a filter paper with 20g of anhydrous sodium sulphate. This was then concentrated in a rotary evaporator at 40^0C to near dryness then recovered to 2 ml with n-hexane.

The cleaning of the extract from the flour was performed as described under section 3.3.4 above. Both the whole grain and milled extracts were stored under freezing temperature conditions (-20^0C) before the HPLC analysis for *permethrin* and GC analysis for *pirimiphos-methyl*.

3.4 Mortality Monitoring

Mortality of *S. zeamais* was monitored at intervals of 7^{th}, 14^{th}, 21^{st} and 28^{th} day post treatment. The monitoring process involved grain sieving, counting the live insects and returning them to the respective glass jars. Isolation and confirmation of the dead insects was by prodding and counting. The mortality data was subjected to one-way analysis of variance (ANOVA) by the F test and significant differences in the treatment means were separated using Tukey's HSD test.

3.5 Residue Analysis

Extracted *pyrethrins* and *permethrin* residues were analyzed using High Performance Liquid Chromatography (Varian 5000) while *pirimiphos-methyl* residues were analyzed using Gas Chromatography (Shimadzu 2010).

3.6 HPLC and GC operating parameters

Table 3: HPLC (Varian 5000) operating conditions

Parameter	Conditions
Injected Volume	10µl
Mobile Phase	Acetonitrile-water: (98:2) v/v
Detector	UV
Column Temperature	25^0C
Flow Rate	0.8ml/min
Detection λ	215nm (*Permethrin*), 225nm (*Pyrethrins*)
Limit of Quantification (LOQ)	0.01mg

Table 4: GC (Shimadzu GC 2010) operating conditions

Parameter	Conditions
Injected Volume	10µl
Detector	Nitrogen-Phosphorous Detector (NPD)
Column	30m Zebron ZB-5 with i.d. 0.32mm and film thickness of 0.25mm; 5% Phenyl and 95% Dimethylpolysiloxane
Injector Temperature	275^0C
Detector Temperature	300^0C
Column Temperature	225^0C
Flow Rate	2 ml/min

3.7 Residue Quantification

The pesticide residue present in both grains and flour were quantified according to formula by Food Safety and Standards Authority of India (FSSAI, 2012). The calculation of the amount of the pesticide residue present was carried out by comparing the peak heights for the samples with the corresponding peak heights for the standards:

$$\textbf{Insecticide residue, mg/kg} = \frac{\textbf{ph smpl.}}{\textbf{ph std.}} \times \frac{\textbf{Vstd.}}{\textbf{Vsmpl.}} \times \frac{\textbf{Vf.}}{\textbf{G smpl.}} \times \textbf{C} \quad \textbf{..... (Eq. 1)}$$

Where:

$ph_{smpl.}$ = Peak height of Sample

ph_{std} = Peak height of Standard

V_{std} = Volume of standard injected (in µL)

V_{smpl} = Volume of Sample injected (in µL)

V_f = Final Volume of the sample concentration (in mL)

G_{smpl} = Mass in grams of sample

C = Concentration in ppm of reference Standard

Residue change by conversion of maize grains to flour was determined by calculating the processing factor (FAO, 2009) to indicate the ratio of the residue found in the flour to the residue in the grains.

$$P_f = \frac{\text{Residue level [mg/Kg] in flour}}{\text{Residue level [mg/Kg] in grains}} \quad \textbf{..... (Eq.2)}$$

CHAPTER FOUR

RESULTS

4.1 Mortality due to different ratios of admixture

The mean percentage mortality of *S. zeamais* caused by different ratios of admixture, pyrethrins alone, diatomaceous earth alone, ASD and Control are represented graphically below.

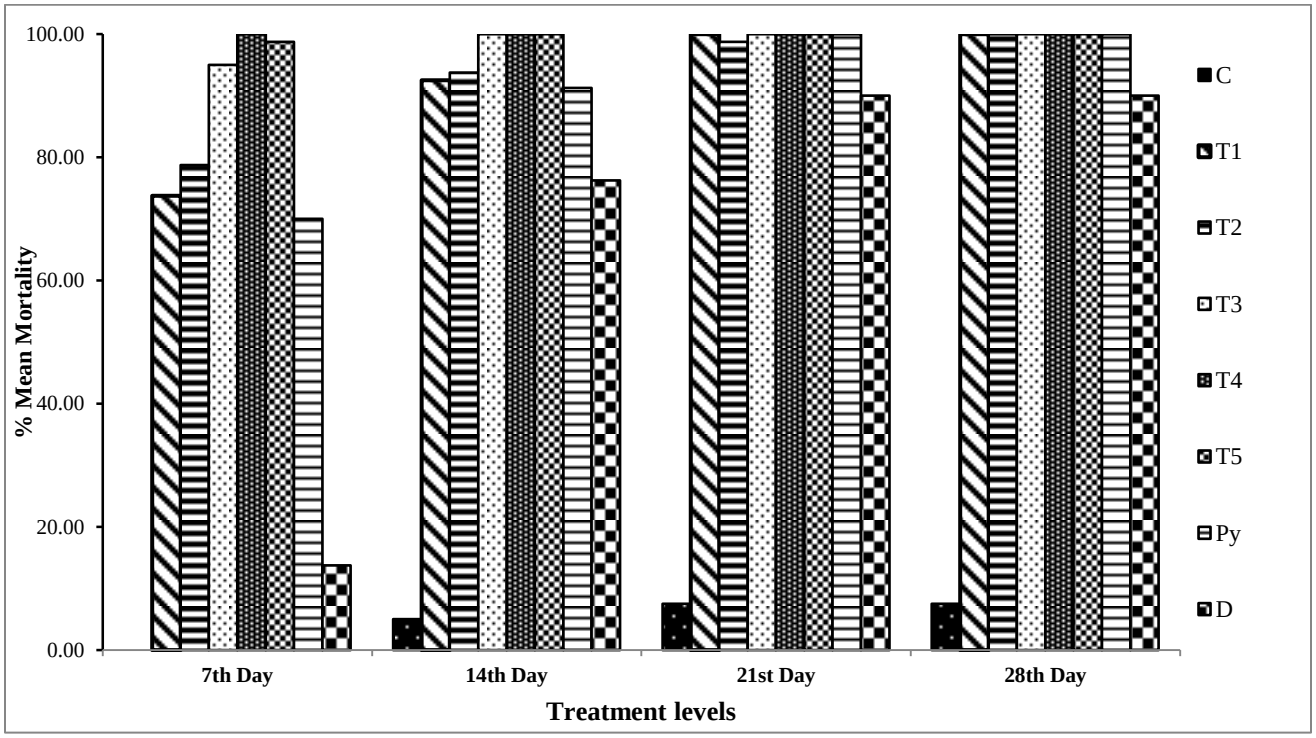

Fig 1: Percent mean mortality against treatment levels

The observations of mortalities at 7^{th}, 14^{th}, 21^{st} and 28^{th} day were based on average of four replications in each treatment level. On the 7^{th} day, the percentage mortalities showed direct proportion to increase in *pyrethrins* in the admixture (73.75%; 78.75%; 95.00%; 100% and 98.75% in T_1; T_2; T_3; T_4; and T_5) respectively. There was no mortality observed within the first 7 days in the untreated maize grain (control). The T_4 ratio

resulted in 100% mortality within the 7 days. However, there was a deviation in T_5 where there was slight drop in mortality compared to T_4 although the proportion of *pyrethrins* in the admixture was higher in the treatment. At 14th day, mortality increased to 92.50% and 93.75% for T_1 and T_2 respectively, while there was 100% mortality observed in T_3, T_4 and T_5. There was mortality of 5% in the control. By 21st day, there was 100% mortality in all admixture treatments except in T_2 which was 98.75%. Mortality in control had also increased to 7.5%. By the 28th day, there was 100% mortality in all the different ratios of the admixture. There was 100% mortality in pyrethrins alone by the 21st day, unlike in diatomaceous earth alone where 90% mortality was noted at the end of the bio-assay experiment. Insect pest mortality data was subjected to one-way analysis of variance (ANOVA) to establish any significant difference in the admixture treatment levels (T_1, T_2, T_3, T_4 and T_5) using the F-test. The results for the 7th day interval assessment period were as indicated on Table 5 below.

Table 5: Results of One-Way ANOVA after 7 days of treatment

	Sum of Squares	**df**	**Mean Square**	**F**	**Sig. level**
Between Groups	94.30	4	23.575	4.519	0.014
Within Groups	78.25	15	5.217		
Total	172.55	19			

The results indicated that on the 7th day after treatment there was significant difference among the different ratios of treatments as F-test statistic value ($F_{(4,15)} = 4.519$, $p<0.05$) was larger than the $F_{critical}$ ($F_{critical} = 3.056$).

By the 14th day post treatment, all the different ratios of the admixture treatments were noted to have no significant difference in insect mortality as F-test statistic $F_{(4,15)} = 2.937$, $p > 0.05$ was slightly lower than the $F_{critical}$ value ($F_{critical} = 3.056$) as noted on Table 6 below.

Table 6: Results of One-Way ANOVA after 14 days of treatment

	Sum of Squares	df	Mean Square	F	Sig. level
Between Groups	9.20	4	2.30	2.937	0.056
Within Groups	11.75	15	0.783		
Total	20.95	19			

Treatments, T_1 and T_2 which posted mortality of 92.50% and 93.75% respectively were found not to be significantly different from the higher dosage of *pyrethrins* and diatomaceous earth admixture based on the F-test statistic value of 2.937 lower than the $F_{critical}$ value of 3.056.

Based on the 14th day ANOVA analysis with no significant difference noted, it was inferred that there was no significant difference expected with additional exposure period (21st and 28th day) as F-test statistic values were expected to be lower than the $F_{critical}$ value of 3.056 since at 14th day post-treatment the F-test statistic was already below the $F_{critical}$ value. The mean percentage mortality was subjected to Tukey's HSD test to separate the homogeneous and heterogeneous means as noted from the one-way ANOVA analysis.

Table 7: Percentage Mean mortality of *S. zeamais*

Treatment Combinations	% mean ± SE mortality of four replications per treatment			
	7th Day	14th Day	21st Day	28th Day
T_1	73.75±7.74[a]	92.50±3.23[a]	100.00±0.00[a]	100.00±0.00[a]
T_2	78.75±9.44[b]	93.75±3.75[a]	98.75±1.25[a]	100.00±0.00[a]
T_3	95.00±3.54[c]	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]
T_4	100.00±0.00[d]	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]
T_5	98.75±1.25[cd]	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]
ASD	100.00±0.00[d]	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]
Control	0.00±0.00[e]	5.00±2.04[b]	7.50±3.82[b]	7.50±3.82[b]

Means within the same column followed by same letter do not significantly differ, Tukey's HSD test ($\alpha = 0.05$)

4.2 Initial Deposition of *Pyrethrins* onto maize Grain

Determination of pyrethrins initially deposited onto the maize grains was quantified to establish effective dosage. The *pyrethrins* content based on calculation before application onto the maize grains are indicated on Table 9. The actual initial deposited *pyrethrins* on the maize grains are also indicated on the table. The quantities were noted to be lower than the expected calculated values. The initial pyrethrins deposited were determined using HPLC.

Table 8: Percentage deposition of *pyrethrins* on maize grains

Treatment	Calculated (mg/kg)	Deposited in Grain (mg/kg)	% Deposition
T_1	5.00	4.24	84.80
T_2	10.00	8.61	86.10
T_3	15.00	12.79	85.27
T_4	20.00	17.78	88.90
T_5	25.00	21.96	87.84
$ASD_{(Permethrin)}$	1.67	1.58	94.61
$ASD_{(pirimiphos\text{-}methyl)}$	8.89	8.19	92.13

The figure below indicates initial percentage deposition of the *pyrethrins* in the different ratios and that of *permethrin* and *pirimiphos-methyl* active ingredients in ASD.

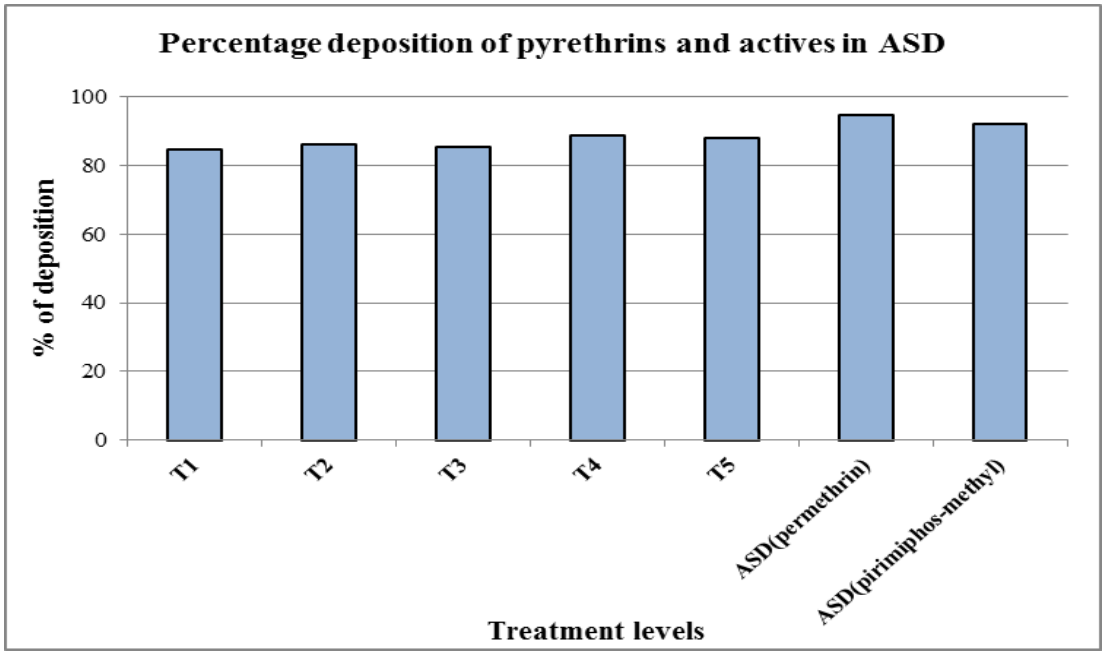

Figure 2: Initial percent deposited *pyrethrins* and actives in ASD

The initial deposition of pyrethrins data was subjected to Chi-Square (χ^2) analysis to establish if there is any significant difference between the calculated pyrethrins (E) before treatment and the initial deposited pyrethrins (O) onto the maize grains as analyzed (see table below).

Table 9: Chi-Square (χ^2) for pyrethrins deposition

Treatments	**Determined pyrethrins (O)**	**Expected pyrethrins (E)**	**(O-E)**	**$(O-E)^2$**	**$\frac{(O-E)^2}{E}$**
T_1	4.24	5.00	-0.76	0.58	0.116
T_2	8.61	10.00	-1.39	1.93	0.193
T_3	12.79	15.00	-2.21	4.88	0.326
T_4	17.78	20.00	-2.22	4.93	0.246
T_5	21.96	25.00	-3.04	9.24	0.370

Determined Chi-Square χ^2= 1.251; Chi Square (χ^2) Table Value = 9.488 (df = 4; ($\alpha = 0.05$)

The results indicated that the Chi-Square calculated (χ^2 = 1.251) was less than the Chi-square table value (9.488) implying that the pyrethrins content deposited onto the maize grains was not significantly different from the pyrethrins content initially before the treatment.

4.3 Residue Determination

Over the 90-day and 180-day storage period, the proportion of degradation of all insecticides (Table 10) from the initial deposition in the maize grain revealed that pyrethrins, permethrin and pirimiphos-methyl degrade in varying proportions with increased storage period with high proportion of degradation noted in pyrethrins compared to *permethrin* and *pirimiphos-methyl* in ASD.

Table 10: Degradation of insecticides in maize grains during storage period

Treatments	Time after Treatment (Days)				
	(24hrs)	90		180	
	Grain (mg/kg)	Grain (mg/kg)		Grain (mg/kg)	
T_1	4.24*	3.07	(27.59)	0.82	(80.66)
T_2	8.61*	5.88	(31.71)	0.96	(88.85)
T_3	12.79*	6.83	(46.60)	1.20	(90.62)
T_4	17.78*	7.98	(55.12)	2.41	(86.45)
T_5	21.96*	9.08	(58.65)	3.97	(81.92)
$ASD_{(Permethrin)}$	1.58*	1.23	(22.15)	0.71	(55.06)
$ASD_{(Pirimiphos\text{-}methyl)}$	8.19*	5.12	(37.48)	3.03	(63.00)

*Initial deposition; Figures in parentheses indicate % reduction based on initial deposition

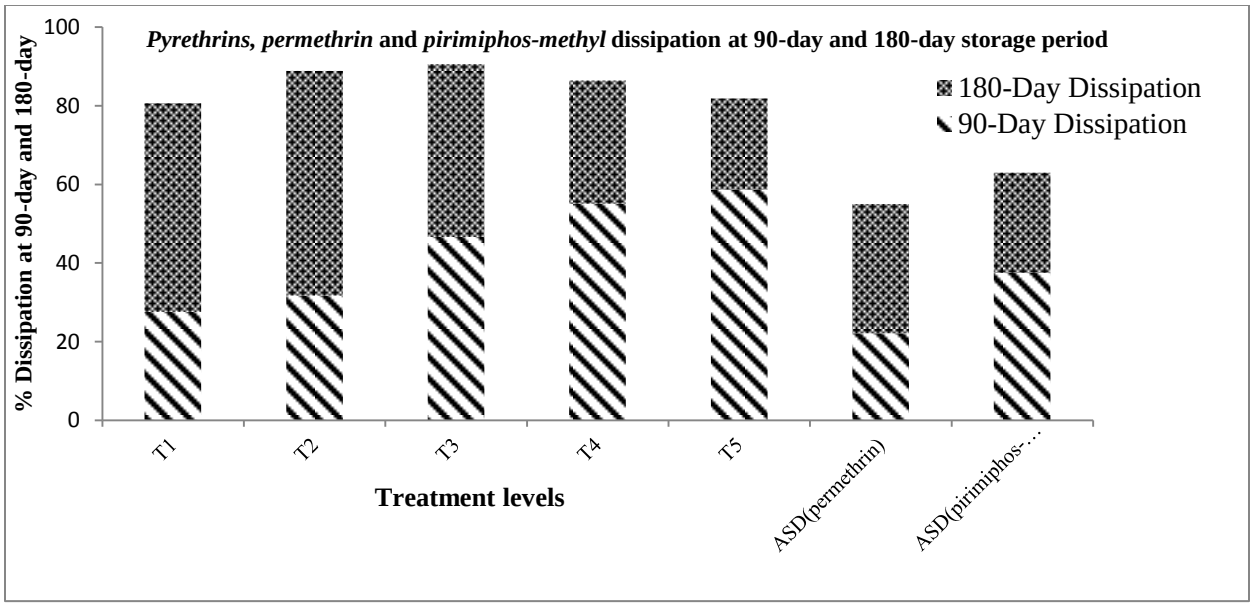

Figure 3: Proportion of degraded insecticides at 90-day and 180-day

Processing factors greater than one ($P_f > 1$) indicate that a particular process concentrates the insecticide while factors below one ($P_f < 1$) indicate that a process reduces the insecticides. Processing factors were calculated using equation 2 in section 3.7 above after the milling process at 90^{th} day and also at 180^{th} day. It was observed from the values obtained that by milling the treated grain into flour, there was further reduction of insecticide residues (Table 11) which is attributed to the heat generated during the grinding process. *Pyrethrins* are thermally sensitive and degrade when subjected to heat conditions. The grinding process led to the thermal degradation of the *pyrethrins*.

Table 11: Degradation of insecticides on milling process

Treatments	24hr Grain (mg/kg)	90 Flour (mg/kg)	180 Flour (mg/kg)	Processing factors 90th Day	Processing factors 180th Day
T_1	4.24*	1.05 (75.24)	0.51 (87.97)	0.34	0.63
T_2	8.61*	2.81 (67.36)	0.58 (93.26)	0.48	0.60
T_3	12.79*	3.37 (73.65)	1.02 (92.03)	0.49	0.85
T_4	17.78*	5.11 (71.26)	1.27 (92.86)	0.64	0.53
T_5	21.96*	5.07 (76.91)	2.15 (90.21)	0.56	0.54
$ASD_{(Permethrin)}$	1.58*	1.02 (35.44)	0.63 (60.13)	0.83	0.89
$ASD_{(Pirimiphos\text{-}methyl)}$	8.19*	3.49 (57.39)	1.74 (78.75)	0.68	0.57

*Initial deposition; Figures in Parentheses indicate % reduction based on initial deposition in grain

CHAPTER FIVE

DISCUSSION

5.1 Mortality due to different ratios of admixture on *S. zeamais*

The study results showed that the level of mortality of the *Sitophilus zeamais* insect pest is dependent on the quantity of *pyrethrins* portion in the admixture. It indicated that the more the quantity of the *pyrethrins* portion in the admixture, the more mortality of insect pests is achieved. This was evident in T_4 where *pyrethrins* portion in the admixture was more than diatomaceous earth resulting in 100% *S. zeamais* mortality. This observation apparently masked the role of diatomaceous earth in the admixture as a grain protectant. Using unstabilized *pyrethrins* alone insect mortality was 70% within the first 7-day interval unlike diatomaceous earth alone where 13.25% insect mortality was observed during the first 7 days period of exposure. This indicates that pyrethrins' effectiveness is superior to that of diatomaceous earth alone within a few days of application. However, on mixing pyrethrins with diatomaceous earth, it was observed that mortality within the first seven days for all the treatments were above the mortality for individual treatments. This corroborates a study by Ceruti and Lazzari (2005), using admixture of *deltamethrin* and diatomaceous earth found out that combined treatments against *S. zeamais* in stored corn, resulted in faster mortality than in treatments using diatomaceous earth alone. They concluded that combining *deltamethrin* with diatomaceous earth, reduces the quantity requirement for diatomaceous earth in high dosage for effectiveness.

In the study, it was noted that *S. zeamais* mortality increased with increase of pyrethrins portion and that significant differences existed within the first 7 days of the post treatment except mortality of T_4 and T_5 which showed results with no significant difference after 7 days of application. Apparently, insect mortality was also dependent on time period of exposure. It was observed that where diatomaceous earth was used alone, mortality had increased from 13.25% at 7^{th} day interval to 76.25% at 14^{th} day interval, an indication that there was sufficient exposure time period by the 14^{th} day for the diatomaceous earth to get in contact with the insect pests to produce the increase in mortality. The mode of action for diatomaceous earth is through abrasion, piercing of insect cuticle and absorbing the fluid thereby desiccating the insect pest. This action requires sufficient contact time between the insect and diatomaceous earth. A study by Mvumi *et al.* (2006) found out that efficacy increased with increasing concentration of diatomaceous earth and exposure period although the lower dose of 1000ppm did not show satisfactory mortality based on a study when they treated stored corn with 1000ppm, 2500ppm and 5000ppm. They suggested that with sufficient supplies of diatomaceous earth, small-scale farmers in Sub-Saharan Africa would have alternatives to the organophosphate pesticides currently used on stored grain.

It was observed that between 7^{th} and 14^{th} day interval, the incremental *S. zeamais* mortality was lower in the treatment where unstabilized *pyrethrins* alone (70% at 7^{th} day to 91.25% at 14^{th} day) was used compared with the increase observed when in diatomaceous earth alone (13.25% at 7^{th} day to 76.25% at 14^{th} day) was used. This implied that for the first 7 days, unstabilized *pyrethrins* application produced more effect

compared to subsequent exposure period while diatomaceous earth effects become pronounced with increased storage period. When mortality for the treatments T_1, T_2, T_3, T_4 and T_5 were compared at 14 days post treatment, there were no significant differences in mortality of *S. zeamais* in all the treatments. Mortality at 21st and 28th day was affected mainly by the exposure period since all the admixture treatments showed 100% insect mortality except for the T_2 that had 98.75% mortality at 21st day interval which was not significantly different from the other admixture treatments. Unmixed diatomaceous earth treatment peaked at 90% mortality within the 21st and 28th day.

It was also observed that the live insects appeared weak and shrivelled indicating continued mechanical effects of diatomaceous earth. Marsaro Jnr *et al.* (2006) found out that dosages of 1,000 and 800 g/ton reached 95% of mortality 5 and 6 days after treatment, respectively, while 600 and 400 g/ton took 9 and 12 days, respectively, to reach the same level of mortality. In the dosage of 200 g/ton the maximum mortality was 91% after 28 days of exposure. They concluded that mortality of adults was influenced by the dosage and the exposure time of insects to diatomaceous earth. Similar findings were also obtained by Kabir *et al.* (2012) and Beriş *et al.* (2011). It was observed that there was no insect pest mortality within the first 7 days in the control, but at 14th day, 5% mortality was recorded which increased to 7.5% by 21st day remaining the same through upto 28th day. The combination ratio for T_4 treatment resulted in *S. zeamais* mortality that was comparable to that of ASD as there was 100% mortality within the first 7 days. It was observed that the mortality for treatment T_5 within the first 7 days was at 98.5% although it had more portion of the *pyrethrins* content in the admixture.

The *pyrethrins* component of the admixture have the property of insect repellency, agitation, insect flashing and fast knock down. As defensive mechanism, the insect pests move restlessly between the spaces in the grains in an effort to escape the effect of *pyrethrins*. The agitated insects, establish sufficient contact with diatomaceous earth portion of the admixture which pierces the insect's cuticle and absorbs the fluid that oozes out. The insect eventually dies as a result of dehydration.

5.2 Initial *Pyrethrins* Deposition

The results from the study demonstrated that in all the admixture treatments, the initial *pyrethrins* deposited and determined within one day after application was lower than *pyrethrins* content values before application. Subjecting the initial pyrethrins deposition data to chi-square analysis indicated that this drop in *pyrethrins* content was not significantly different from the pyrethrins before application. This corroborates Caboni et al. (2007) findings when they studied the degradation of *pyrethrins* residues on stored durum wheat after post-harvest treatment and found that in all trials, the initial deposition of *pyrethrins* levels, were below the maximum residue level of 3mg/kg. Earlier findings by Ong et al. (1994) when they studied persistence of grain protectants in maize, indicated that *pirimiphos-methyl* initial deposition was 4.2mg/kg from a target of 6.0mg/kg and that of *permethrin* was 1.1mg/kg from a target of 2.0mg/kg. Initial deposition of all the different admixture ratios were lower compared to *permethrin* and *pirimiphos-methyl* in ASD. This observation could be attributed to possibility of uneven mixing of the *pyrethrins* and diatomaceous earth at the time of blending the ratios. Other contributory factors leading to lower deposition in all treatments, is the handling and

possible uneven distribution of applied insecticides in the maize grains, translocation of the powder admixtures over the storage period to the bottom of the test jars and incomplete extraction of the insecticide.

5.3 Residue degradation

There was a general reduction in residues in all the insecticides in maize grain with increase in storage period. The pyrethrins in the admixture treatments degraded in grains from initial deposition of 4.24, 8.61, 12.79, 17.78 and 21.96mg/kg to 3.07, 5.88, 6.83, 7.98 and 9.08mg/kg respectively by end of three months storage period (90 days). By 180th day, the *pyrethrins* residue levels had degraded further in grains to 0.82, 0.96, 1.20, 2.41 and 3.97mg/kg respectively. The figure below (Figure 4) indicates the degradation trend of *pyrethrins* residue over the 180-day storage period.

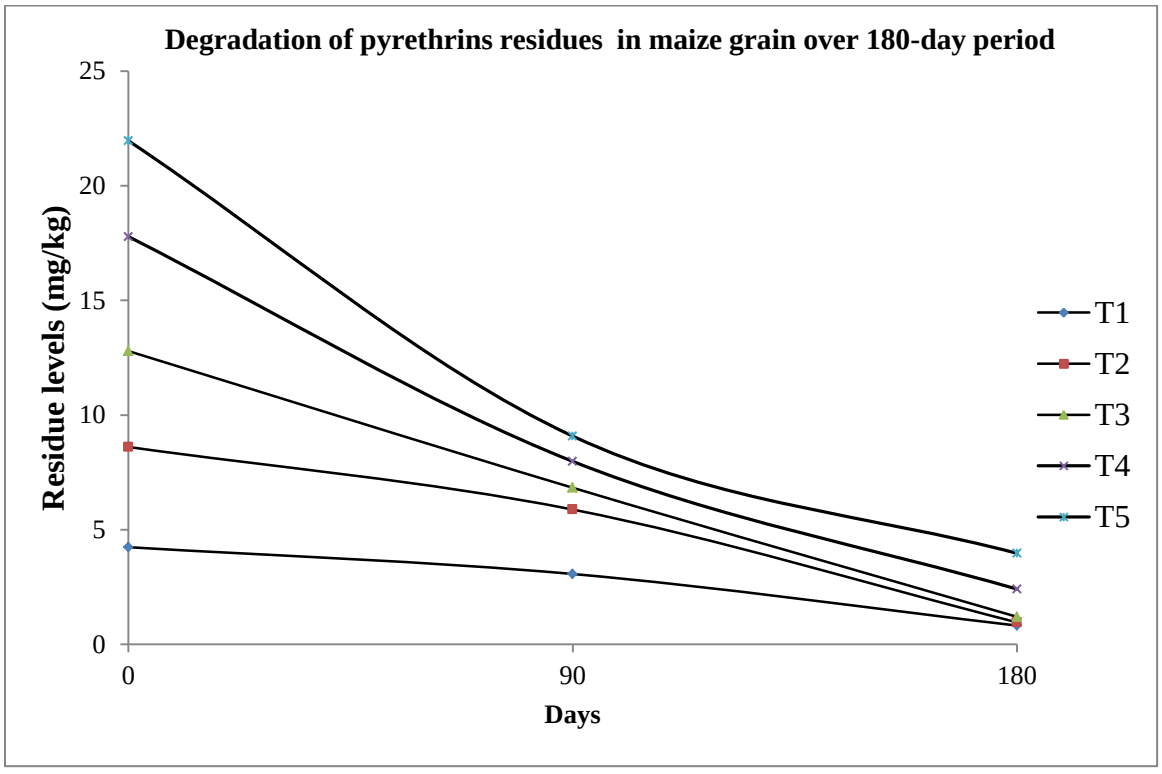

Fig 4: Degradation trend of *pyrethrins* residues in grain over the 180-day storage period

Processing the maize grains by grinding into flour at 90th day resulted in further reduction of *pyrethrins* to 1.05, 2.81, 3.37, 5.11 and 5.07 mg/kg respectively. This implied that processing grains lead to further *pyrethrins* degradation. Additional reduction in *pyrethrins* residues in flour at 180th day were 0.51, 0.58, 1.02, 1.27 and 2.15 mg/kg respectively. The grinding process further reduces *pyrethrins* residue reducing process. The fugure below (figure 5) indicates the degradation trend of pyrethrins residue in flour.

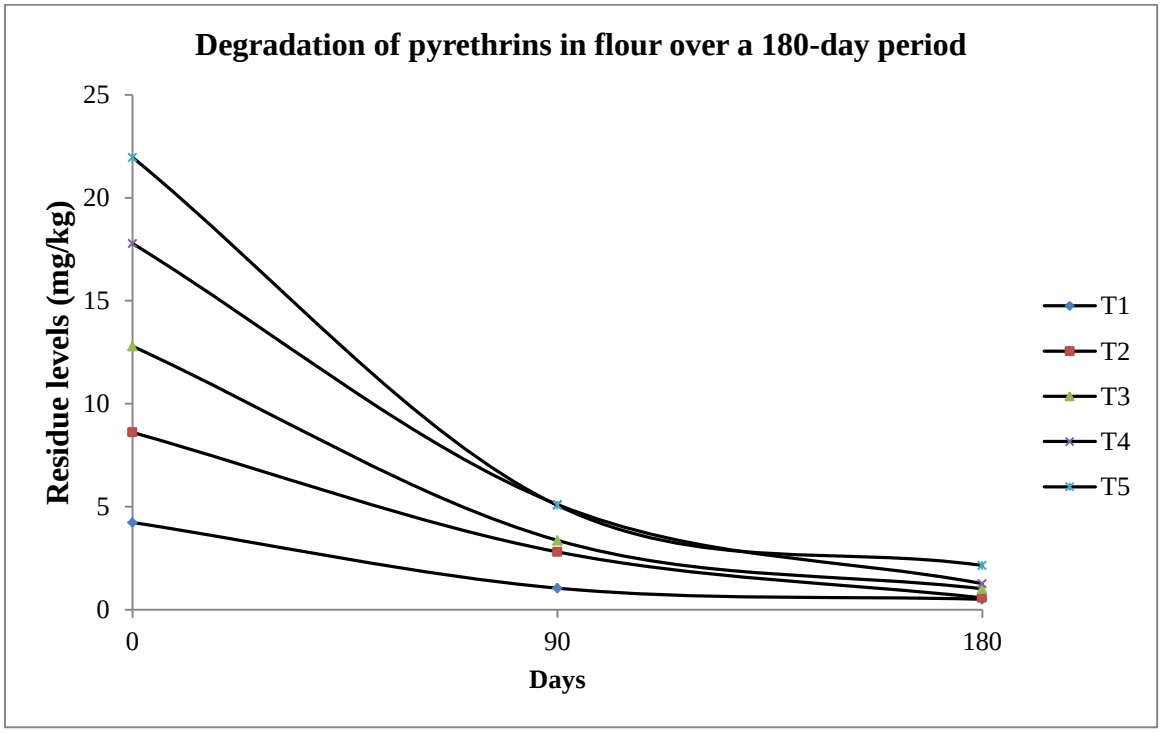

Figure 5 : Degradation trend of *pyrethrins* residues in flour over the 180-day storage period

An earlier study by Caboni et al. (2007), found that the fate of *pyrethrins* in two experiments were similar. They had used single and double dose as recommended by the manufacturer and noted that the total content of *pyrethrins* remained unchanged for 22 days and complete degradation had occurred in 8 months. Similar results were obtained by Extension Toxicological Network (1994) who established that in stored grain, 50% or

more of applied *pyrethrins* disappeared during the first 3 to 4 months of storage and that at least 80% of the remainder is removed by handling, processing, and cooking.

Permethrin and *pirimiphos-methyl* in ASD had degraded from 1.58 and 8.19 mg/kg initial deposition to 1.23 and 5.12 mg/kg in grain respectively at 90^{th} day. In the study by Ong *et al.* (1994) they found out that after 12 weeks (3 months) of storage, *pirimiphos-methyl* had degraded to 2.8 mg/kg while *permethrin* was at 0.9 mg/kg. In this study, a further reduction to 1.02 and 3.49 mg/kg respectively was noted to occur grinding the grain into flour at 90^{th} day. Increased storage period resulted to additional degradation of the *permethrin* and *pirimiphos-methyl* just like for the *pyrethrins*. Degradation of *permethrin* and *pirimiphos-methyl* at 180^{th} day was 0.71 and 3.03 mg/kg respectively while that for flour were 0.63 and 1.74 mg/kg respectively. Ong and colleagues also noted that *pirimiphos-methyl* and *permethrin* had degraded by 24 weeks (6 months) to 1.1 and 0.7 mg/kg respectively.

The percentage degradation during the 90-day of storage period were low for *permethrin* (22.15%) in ASD while that of *pyrethrins* was at 58.65% in T_5 in the admixture. The degradation of *pyrethrins* was generally observed to be of a larger range compared to that of *permethrin* and *pirimiphos-methyl* in ASD. A study by Afridi *et al.* (2001) established that *pirimiphos-methyl* and *permethrin* had degraded by 44.5% and 26.4% respectively in 13 weeks of wheat storage at a temperature of 25^0C and 13% grain moisture. In this study, by 180^{th} day storage period the degradation of *pyrethrins* in the admixture had substantially increased with the highest being 90.62%, compared to that of *permethrin* and *pirimiphos-methyl* in ASD which was at 55.06% and 63.00% respectively.

Findings by Afridi and colleagues also found that after 26 weeks, *pirimiphos-methyl* and *permethrin* had degraded by 63.2% and 35.9% respectively. The percentage decrease of *pyrethrins* from the initial deposition on maize grains is shown on table 12 below.

Table 12: Percentage *pyrethrins* Degraded by 3months and 6 months

Treatment	**% Degraded by 3 months (90 days)**	**% Degraded by 6 months (180 days)**
T_1	27.59	80.66
T_2	31.71	88.85
T_3	46.60	90.62
T_4	55.12	86.45
T_5	58.65	81.92
$ASD_{Permethrin}$	22.15	55.06
$ASD_{Pirimiphos\text{-}methyl}$	37.48	63.00

It was observed that the first three months (90 days) of storage period indicated increase of *pyrethrins* degradation with more pyrethrins content. The proportion of degradation in all the admixture treatments was over 80%. This observation was contrary to the hypothesis that there was no significant portion of *pyrethrins* degraded in the different ratios in grains by the 180^{th} day of storage.

These findings showed that degradation of *pyrethrins* was much higher than for pyrethroid and organophosphate insecticides in storage conditions. This supports a study by Atkinson *et al*. (2004) who found out that *pyrethrins* degraded during storage period by identifying the possible causes as generation of heat, presence of moisture, oxygen and microbial activity. The pesticide residue levels in all the admixture treatments after the 180th day of storage were noted to be generally within the maximum residue limits (MRLs) set by the Food Agricultural Organization (FAO) and World Health Organization (WHO) for pyrethrins (3 mg/kg) for cereal grains including maize grains (FAO & WHO, 2013). Physical handling and milling operation result in translocation of most insecticide residues as they are on the surface of the grains. Processing the grains by milling into flour was to further establish the effect of the milling process on residues of the insecticides and therefore indicate dietary intake risk to consumers. Most raw agricultural commodities undergo household preparation or industrial processing prior to final consumption. The processing may alter the residues levels which might lead to concentration or reduction. This is denoted by processing factor (P_f). Federal Institute for Risk Assessment (2011) indicates that processing factor greater than 1 ($P_f > 1$) implies an increase in residue levels during processing and less than 1 ($P_f < 1$) indicate decreased residue levels.

At the 90th day and 180th day period the grains were milled and the residue levels in the flour were determined and the processing factors were calculated (Table 11) in Chapter Four above). Milling the grain at the 90th and 180th day reduced the residue levels in all the grains. However, it was noted that the processing factors decreased variedly in the

different ratios. The difference in ratio of residues in the grain to that in the flour after the 90 day period was noted to be a significant proportion in the first three cocktail treatments (T_1, T_2 and T_3) with processing factors of 0.34, 0.48 and 0.49 indicating high residue reduction as compared to those of T_4, T_5 and also *permethrin* and *pirimiphos-methyl* in ASD. At the 180th day period, the residue reduction (P_{fs}: 0.65, 0.60 and 0.85) was lower. It was also noted that *permethrin* residue reduction on milling process on both periods (90th day and 180th day) was minimal (P_{fs}: 0.83 and 0.89) compared to the counterpart *pirimiphos-methyl* in the same period interval (P_{fs}: 0.68 and 0.57) and those of the admixture treatments.

5.4 Environmental fate of pyrethrins

Compared to many other pesticides, most notably the synthetic pyrethroids, *pyrethrins* have shorter persistence. The six chemical components pyrethrins (pyrethrin I, Pyrethrin II, Jasmolin I, Jasmolin II Cinerin I and Cinerin II) are rapidly degraded in the environment through photolysis, hydrolysis, thermal degradation and biodegradation into essentially non-toxic breakdown products. The primary route of elimination in the environment is photolysis (UV light mediated). Photodegradation of *pyrethrins* in sunlight is rapid and results in the isomerization of the side-chains, photooxidation to a variety of carboxylic acids, and isomerization of the cyclopropane acids.

In the ambient atmosphere, *pyrethrins* are rapidly degraded through reaction with atmospheric oxidants or by direct photolysis. The aqueous hydrolysis data indicate that *pyrethrins* are slow to degrade in deep waters unlike in surface water. However, in the

presence of microbial communities, the degradation is expected to be faster via oxidative metabolism (Gunesekara 2004). The properties and environmental fate along with the ready metabolism of *pyrethrins* in various species in the food chain from microbes through fish indicate that *pyrethrins* are short lived in the environment and are unlikely to bioaccumulate to appreciable levels.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The results from the study have demonstrated that unstabilized *pyrethrins* in combination with diatomaceous earth mined in Kariandusi, Kenya offer grain protection comparable to the commercial chemical grain protectants.

There was increased efficacy with increased portion of unstabilized *pyrethrins* in the admixture masking the role of diatomaceous earth in the admixture which also has grain protection property.

The results of the combination of unstabilized *pyrethrins* and diatomaceous earth did not clearly show whether the relationship was synergistic, additive, antagonistic or potentiation.

The study also showed that actual initial deposition of pyrethrins was lower than the pyrethrins content before the application but not significantly different.

Increased storage period leads to further degradation of unstabilized *pyrethrins* as evident from the determination of residue levels at 90^{th} day and 180^{th} day.

Processing the grains into flour by milling reduce residue levels of *pyrethrins* further.

6.2 Recommendations

The combination of unstabilized *pyrethrins* and diatomaceous earth mined in Kariandusi, Kenya should be utilized as part of integrated pest management (IPM) strategy.

Processing of grain is encouraged before consumption as it reduces levels of dietary intake and health risks posed especially by chemical insecticides.

Further study is recommended to establish the actual relationship between *pyrethrins* and diatomaceous earth admixture whether such an interaction would be synergistic, potentiation, additive or antagonistic.

Susceptibility of other potentially grain damaging insect pest species like *P. truncatus*, *S. oryzae* and *T. castaneum* should be investigated using unstabilized *pyrethrins* and DE admixture.

The study was conducted under laboratory controlled conditions; field conditions that simulate actual farmer storage conditions especially on temperature and humidity would provide valuable data for admixture performance.

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APPENDIX

Efficacy Data based on 4 replications per treatment of S. zeamais (n = 20)

Efficacy of different ratios of the admixture					
		% Mean mortalities at different interval days			
Treatment levels	**Insects seeded**	**7th Day**	**14th Day**	**21st Day**	**28th Day**
C	20	0.00	5.00	7.50	7.50
T_1	20	73.75	92.00	100.00	100.00
T_2	20	78.75	93.75	98.25	100.00
T_3	20	95.00	100.00	100.00	100.00
T_4	20	100.00	100.00	100.00	100.00
T_5	20	98.75	100.00	100.00	100.00
P_y	20	70.00	91.25	100.00	100.00
D	20	13.75	76.25	90.00	90.00
ASD	20	100.00	100.00	100.00	100.00

C = Control; T_1, T_2, T_3, T_4 and T_5 are admixtures
Py = Pyrethrins
D = Diatomaceous Earth