SYNERGISTIC QUALITIES OF SELECTED PLANT EXTRACTS ON POTENCY OF PYRETHRINS AGAINST THE MAIZE WEEVIL, *SITOPHILUS ZEA-MAIS* (MOTSCH.) (COLEOPTERA: CURCULIONIDAE)

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A Thesis Submitted to Graduate School in Fulfilment of the Requirements for the Award of the Degree of Doctor of Philosophy in Zoology (Entomology) of Chuka University.

CHUKA UNIVERSITY
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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented for an award of a diploma or conferement of a degree in any University or Institution.

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Recommendation

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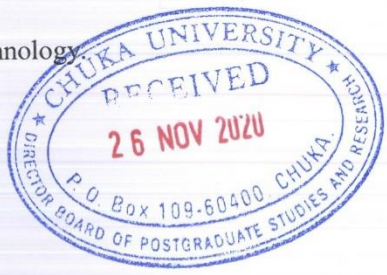

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DEDICATION

This work is dedicated to my late mother Pasculina Kabon Kiptarus, who though did not have formal education encouraged me to pursue education to greater heights.

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The completion of this research work is attributed to many valuable individuals and institutions which in one way or another have contributed to its success. First, I am grateful to God for the good health and sound mind during my entire study period.

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ABSTRACT

Post-harvest losses of grains are a major hindrance to attainment of food security in Kenya due to infestation of coleopteran grain pests such as weevils. Although synthetic chemical insecticides are being used in their control, overuse and misuse of these insecticides have resulted in problems of pesticide resistance, environmental contamination, pest resurgence and even consumer poisoning. Several studies have been done on utilizing plant essential extracts and oils in control of storage pests but their limitations, such as inconsistencies in efficacy, lack of persistence and residual effect have hindered their use as stand-alone products for pest management. Piperonyl butoxide (PBO) is often the synergist used to enhance efficacy of insecticides like pyrethroids and pyrethrum formulations. Synergists enable the use of an active ingredient in very small quantities by preventing detoxification within the insect thus un-synergised formulations are rarely used. This study aimed at evaluating synergistic qualities of selected plant extracts on potency of pyrethins formulations against the maize weevil, *Sitophilus zea-mais* (Motsch.) (Coleoptera: Curculionidae). Plants extracts (Black pepper seeds, Nutmeg seeds, Coriander leaves and roots) "synergists" used were selected on the basis of possessing a methylenedioxyphenyl (MDP) ring structure similar to that of PBO. Full dose response, dose-mortality, synergism experiments were carried out on *S. zea-mais* at four concentrations of synergists, each at four ratios (synergist: pyrethrins) while infestation was carried out on maize treated with plant ectracts-pyrethrins formulations. Topical application of pyrethrins/synergist/formulation on *S. zea-mais* was done in triplicate in a Completely Randomised Design. Experiments were conducted under controlled laboratory conditions of 27 ± 2^{O}C and 60 ± 5% RH with normal day light hours. Probit analysis was used to determine the lethal concentration (LC) of pyrethrins to the *S. zea-mais*. Analysis of Variance was used to obtain the mean mortality differences of *S. zea-mais*at $P \leq 0.05$ while Duncan's Multiple Range Test was used to rank significant concentration means within a synergist. Results obtained found LC_{20} value for pyrethrins to be 2,200 ppm. To obtain LC_{50}, 14227 ppm, 13780 ppm and 8868 ppm of pyrethrins wwere required over a 24 h, 48 h and 72 h exposure time respectively. Black pepper seed hexane extract (BPSHE) and PBO after 48 h exposure were statistically significant ($P \leq 0.05$) with the average percentage mortalities of 10% and 20% at 10,000 ppm and 20,000 ppm respectively, while PBO and Coriander leaves hexane extract (CLHE) were significant $P \leq 0.05$ after 72 h. PBO was the most toxic synergist (36.67 ± 3.33%) followed by CLHE (26.67 ± 3.43%) at 20,000 ppm. In the formulations of synergist: pyrethrins, CLHE, Nutmeg seed hexane extract (NMHE) and Black pepper seed methanol extract (BPSME) at ratio 1:1 were statistically significant ($P \leq 0.05$) 24 h after exposure. PBO registered higher percentage (83.33 ± 12.02%) mortality followed by CLHE (46.67 ± 3.33%), BPSME (43.33 ± 6.67%) and NMHE (26.67 ± 3.33%) at ratio of 3:1 while BPSHE was most effective synergist at ratio 2:1. BPSME and NMHE co-toxicity values were below 20 and -20 respectively while in PBO, BPSHE and CLHE values were above 20. Low concentrations of pyrethrins were required to achieve higher percentage mortality of *S. zea-mais* when exposure time was extended to 72 h. The toxicity of plant extracts tested was low hence qualified as potential synergists to replace the standard, PBO in pyrethrins formulations. BPSHE was a better synergist than PBO at a concentration of 5,000 ppm followed CLHE while BPSME was an additive to the pyrethrins while NMHE was antagonistic to pyrethrins at 1,000 ppm. It is recommended that time of exposure of a synergist and an insecticide play a critical role in high mortality rates of *S. zea-mais* regardless of the ratio of synergist: pyrethrins and should be considered when formulating insecticides.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
BPSME	Black Pepper Seeds Methanol Extract
BPSHE	Black Pepper Seeds Hexane Extract
BRA	Botanical Resources Australia
CLME	Coriander Leaves Methanol Extract
CLHE	Coriander Leaves Hexane Extract
CRHE	Coriander Roots Hexane Extract
CYP	Cytochrome P-450
DDT	Dichlorodiphenyl trichloroethane
DMRT	Duncan's Multiple Range Test
EPA	Environmental Protection Agency
GDP	Gross Domestic Product
h	hours
KALRO	Kenya Agricultural and Livestock Research Organization
LC	Lethal Concentration
LD	Lethal Dose
LGB	Larger Grain Borer
MDP	Methelenedioxyphenol
MFOs	Mixed Function Oxidases
NASS	National Agricultural Statistics Service
NCPB	National Cereals and Produce Board
NMHE	Nutmeg Seeds Hexane Extract
PBO	Piperonyl butoxide
PHL	Postharvest Losses
PPCK	Pyrethrum Processing Company of Kenya
ppm	Parts per million
RH	Relative Humidity
S.E	Standard error
SDGs	Sustainable Development Goals
SSA	Sub-Saharan Africa

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Agriculture is the backbone of Kenya's economy and central to its development strategy. More than 75% of Kenyans make some part of their living in agriculture and it accounts for more than 34.2% of Kenya's Gross Domestic Product (GDP) (Kenya's Facts and Figures, 2018). This sector is critical in realizing targets that are set out in the Sustainable Development Goals (SDGs) especially of food sufficiency through sustainable agriculture, improved food security and better nutrition (Kenya Economic report, 2018), Kenya's vision 2030 and the Big Four Agenda, one main pillar being food security and nutrition. This is envisioned to be achieved by increasing maize production by more than 27 million bags of 90 kgs among other staple foods by the year 2022.

Globally, food demand is emerging as a big challenge to mankind with the population expected to grow to 9.1 billion people by the year 2050 requiring about 70% extra food to feed them (Godfray *et al*., 2010 & Parfit *et al*., 2010). Most of this population rise is attributed to developing countries with several of them already facing issues of hunger and food insecurity. Factors like climate change, land use change, increasing urbanization, declining freshwater resources and land infertility have further aggrevated this problem. These concerns call for intergrated and innovative approaches towards global efforts in ensuring sustainability of food production and consumption (FAO, 2019).

Approximately one-third of food produced (about 1.3 trillion ton) globally, worth about USD one trillion is lost during postharvest operations every year (Gustavsson *et al*., 2011). Postharvest losses (PHL) account for quality losses reducing the economic value of the food and making it unsuitable for human consumption. In addition, physical losses reduce the quantities available. In severe cases losses of upto 80% of the total food production have been recorded (Fox, 2013). In Europe, 32% of all food purchased is not eaten while in USA, 30% is thrown away each year. In Africa, PHL of cereal food has been estimated to range between 20% and 40% of the total crop harvested and as much as 50-60% cereal grains losses recorded in Kenya (Kumar & Kalita, 2017). These losses are highly significant considering the low agricultural productivity in

several regions of Africa (Abass *et al*., 2014; Tadele, 2012) where the number of insecure populations still remains high. One way of strengthening this food security is by reducing post-harvest losses (WFP & FAO, 2012).

Despite significant increase in the area of land under cultivation and the yield per acre of maize over the last two decades in Kenya, food security is still an uphill task. The Kenyan Ministry of Agriculture has reported upto 50% loss of maize due to pest infestation particularly during grain storage. About 80% of cultivated maize is stored by the farmers on their and has been found to sustain losses of about 30% within six months of harvesting when no control measures are taken while about 2.2% loss occur in central storage at the National Cereals and Produce Board (NCPB) (Mahihu, 2013). In monetary terms, total losses translate to over 1.8 million 90 kg bags valued at Kenya shillings 8.1 billion annually yet in most cases these losses are under-estimated (Likhayo *et al*., 2013). PHL impact on the available food volumes and trade in values of the commodities on the life of millions of small holder farmers (Pathak & Gupta, 2015; Zorya *et al*., 2011). Thus, more effort is required in post-harvest management practices to provide safe and quality food which meets dietary needs for an active and healthy life as envisioned in the economic pillar of the Kenya Vision 2030 (Republic of Kenya, 2007).

Grains need to be stored from one harvest to the next in order to maintain their constant supply all year round and to preserve their quality until required for use. To ensure household food supplies, reserves and availability of seed for planting, proper post-harvest management of surplus produce is important (Midega *et al*., 2016). Insect pests cause major damage to stored grain and foodstuffs, reducing the products weight, quality and value. It is estimated that about 10- 40% of the total damage to stored grains world-wide is caused by insect pests (Ojo & Omoloye, 2012), 20% in Africa (Youdeowei & Service, 1986) and 30% in Kenya (Richter *et al*., 2007; Mahihu, 2013).

The most damaging post-harvest insect pests are the weevils, the grain borers, lepidopteran stem and cob borers (Kumar & Kalita, 2017). In Mexico, high losses of grains in storage (76%) and grain damage (100%) have been recorded after a one-year storage (Garcia-Lara *et al*., 2019). Similarly, in African countries such as Togo, 80% - 90% of storage losses in grains have been attributed to insect pests. A common pulse

weevil, *Callosobruchus maculatus* alone, was found to be responsible for upto 24% losses in stored pulses in Nigeria while in Ghana, Cameroon and Benin, about 50%, 44%, and 23% maize losses respectively have been attributed to weevils and borers (Kimenju & de Groote, 2010).

In Kenya, stored maize infestations with Larger Grain Borer (LGB), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) (Lamboni and Hell, 2009) have recorded losses as high as 90% and upto 20% with maize weevil (MW), *Sitophilus zea-mais* (Motsch) (Coleoptera: Curculionidae) (Likhayo *et al*., 2013). The maize weevil causes severe qualitative and quantitative losses. Larvae and adults feed internally in seeds, causing losses in weight and quality and increasing infection by pathogens, which are harmful for human health (Fontes *et al.,* 2003; Hell *et al*., 2000).

Synthetic insecticides are the main control methods used in various countries to control grain pests and reduce loses during storage (Guedes *et al*., 2012). This practice has been widely adoped by farmers in African countries like Kenya, where more than 93% of farmers have been reported to use insecticides for their control (Kimenju & de Groote, 2010). Insecticides used include fumigants like aluminium and magnesium phosphide, contact insecticides like fenithrothion and pirimiphos-methyl (organophosphate), bifenthrin and deltamethrin (pyrethroids) and esfenvalerate (pyrethroid) plus fenithrothion (organophosphate) (Brasil, 2016; U.S. EPA, 2003; Fang *et al*., 2002; NASS, 1999). Because of their non-specific mode of action acting on the insect's central nervous system and on energetic metabolism of insects (Brazillian Committee of Action to Prevent Resistance to Insecticides – IRAC-BR, 2016) these products can be harmful to non-target organisms.

Furthermore, insecticides have had limitations such as high costs, development of resistance in treated pests, health hazards due to toxic residues and environmental contamination (Shaaya *et al*., 2016). Many stored grain insects, including *S. zea-mais*have exhibited some resistance (Ribeiro *et al*., 2003; Lorini and Galley, 1999; Collins *et al*., 1993;). The main groups of compounds from which resistance has developed are organophosphates, pyrethroids and juvenile hormone analogues.

In recent years, research has focussed on the use of botanical insecticides as alternatives to conventional insecticides. In other studies, it was found that, several plant species and their extracts with natural pesticide ability are commonly used as a traditional practice for short term protection of grains from insects especially in Asian and several African countries (Kumar & Kalita, 2017). Generally, plant based insecticides are known to be biodegradable, environmentally friendly and relatively safe for human health. Natural plant compounds containing pyrethrins, azardirachtin, rotenoids and alkaloids have been used widely in household and horticultural pest control but less in storage pest control. This is due to the fact that these compounds are relatively unstable, not available in sufficient quantity and purity for their bioactive evaluation and high costs associated with production (Joffe *et al*., 2012).

Nevertheless, when formulated with effective synergists and antioxidants or stabilizers, they may be economically viable insecticides. Synergism has the role of increasing the potency of insecticides and speeding their reaction time by preventing detoxification within the insect. Examples of some synergisms which have been documented are those of malathion toxicity by other organo-phosphorus compounds, the mode of action of the herbicide synergist tridiphane, pyrethrins and pyrethroids by the synergists piperonyl butoxide and MGK-264 (Lang'at *et al*., 2008; Hodgson, 1999).

Studies done by Liu *et al*. (2015) on synergistic effects of various compounds to pyrethrins, documented an optimal biological ratio for different pest species and each individual synergist. Piperonyl butoxide (PBO) is the main synergist used to boost efficacy of low levels of pyrethrins by binding onto the cytochrome P-450 dependent microsomal oxidase, the defence mechanisms employed by the insects in counteracting pyrethrins (Hamilton, 1995). Synergists enable the use of an active ingredient in very small quantities by preventing its detoxicification within the insect thus unsynergised formulations are rarely applied for the control of insect pests (Formulating Pyrethrum, 1987). Bioactive compounds in plants are usually found in small quantities and therefore synergism maybe a viable way of ensuring that these compounds are available for use in insect pest management.

Currently, focus has shifted to the demand of organic products, decrease in environmental contamination and safe use of pesticides thus a natural compound for

use as a synergist would be ideal. Although PBO is still an effective synergist, its classification as organic product has changed in many countries; it is also expensive, toxic and in short supply (Lang'at *et al*., 2008). Several plant extracts/oils have been tested for synergistic activity with pyrethrum such as sesamin, sesamolin, safrole, myristicin, dillapiole, haplophyllidine, karanjin, elemicin, sesanglolin and piperine (Scott *et al*., 2019; Lang'at *et al*., 2008). Though dillapiole and piperine have been found to be relatively effective as pyrethrum synergists on the housefly, *Musca domestica* (Lang'at *et al*., 2008; Saxena *et al*., 1977; Sigh *et al*., 1976), a wide range of insect pests have not been subjected with insecticides formulated with these synergists in order to verify their synergistic activity. Currently, most pyrethroid and pyrethrins insecticides are still synergised with the semi-synthetic PBO which renders the product "not safe". Also, searches for effective synergists with a viability equivalent to that of PBO are still required in order provide variety of organic pesticides.

Though several studies have been done on the possibility of utilizing plant essential extracts and oils in control of storage pests, their limitations have majorly restricted their use as stand alone products for pest management and thus alternatine strategies have to be sought. This study focussed on possible combinations of selected plant extracts as synergists with pyrethrins in order to make them viable insecticides for possible use in protection of stored grains against coleopteran pests.

1.2 Statement of the Problem

Post-harvest losses of important grain crops such as maize are a major hindrance to attainment of food security in developing countries such as Kenya. Stored maize coleopteran pests such as larger grain borer and maize weevil infest the grains before shelling, lay eggs which hatch and mature before boring their way out to re-infest other grains. Although synthetic chemical insecticides have been instrumental in their control, overuse and misuse of these pesticides have resulted in problems of pesticide resistance, environmental contamination, pest resurgence and consumer poisoning. Chronic health effects may occur years after even minimal exposure to pesticides in the environment, or result from pesticide residues ingested through food and water. Precautionary measures such as rinsing off chemical residues or allowing sufficient duration for degradation of the chemicals are rarely taken into consideration by the resource poor populace. In order to minimize resistance, ensure food security, save the

population from health hazards, reduced-risk tactics for storage pests' management are required. Although plant essential oils have been tried for use as alternative to synthetic insecticides, their levels of control are still wanting. This is because plant ingredients have been found to be in small quantities, lack residual activity and expensive to produce. An alternative strategy needs to be sought such as use of plant extracts as synergists of biochemical active compounds like pyrethrum to obtain viable products for grain protection which would minimize the amount of synthetic insecticides applied on stored maize grains against *S. zea-mais*.

1.3 Objectives of the Study

1.3.1 Broad Objective

To evaluate the synergistic qualities of selected plant extracts on potency of pyrethrins formulations against the maize weevil, *Sitophilus zea-mais* (Motsch.) (Coleoptera: Curculionidae)

1.3.2 Specific Objectives

i. To determine *in vivo* the lethal concentration (LC) values for pyrethrins on the maize weevil, *S. zea-mais*.
ii. To determine *in vivo* the effect of the selected plant extracts on the maize weevil, *S. zea-mais*.
iii. To determine the potency of plant extracts-synergised pyrethrins formulations at different rates and concentrations on stored maize against maize weevil, *S. zea-mais*.
iv. To evaluate the efficacy of plant extracts-synergised pyrethrins formulations treated maize on infestation by the maize weevil, *S. zea-mais*.

1.4 Research Hypotheses

$H0_1$: Pyrethrins have no statistically significant effect on *S. zea-mais* mortality at different concentrations.

$H0_2$: Plant extracts have no statistically significant effect on *S. zea-mais*.

$H0_3$: Plant extracts-synergised pyrethrins formulations do not cause statistically significant mortality of *S. zea-mais* on stored maize at different rates and concentrations.

$H0_4$: Plant extracts-synergists pyrethrins formulations do not protect maize from infestation by *S. zea-mais*.

1.5 Significance of the Study

The study was important in providing information that would add to the existing scholary literature on control of pests of stored products hence enhance academic research.

The research was important in providing more options of safe and affordable insecticides for use on stored grains. This would enable reduction of post-harvest losses due to storage pests and increase food availability without requiring additional production resources and hence contribute to food security. Thus contributing partly to Kenya's vision 2030 and the "Big4 Agenda" whose goal is food sufficiency, universal health and increase industrialization by year 2030.

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The study was also important in providing insight for consideration by farmers to use safe formulations for protecting and prolonging their farm grains from storage pests. The use of these botanical insectide formulations has the potential to improve their health and the food surplus would increase their income which could inturn improve the living standards of the population. Traders also improve their returns through minimizing losses associated with grain pests.

The addition of an inexpensive compound functioning as an effective synergist would benefit both the pyrethrum industry and farmers alike, as it will expand the production of pyrethrum in Kenya while making it more viable and affordable method of control of storage pests. Insecticide manufacturers will benefit by adding safe products to the market hence increasing their income. In addition, the industries will create more jobs to the members of the society as stipulated in the Kenya's Big4 Agenda.

1.8 Operational Definition of Terms

The following are operational definitions of key terma as they are used in this study.

Active Ingredient: The essential component of the technical grade that exerts toxic actions on an organism

Additive Effect: The result of synergist-pyrethrins formulations that produced an efficacy more or less equal to the sum of their separate efficacy

Antagonistic Effect: The result of synergist-pyrethrins formulations that produced lower effect than their individual efficacies

Concentration of a Substance: Quantity of solute present in a given quantity of solution. In this study concentrations in parts per million (ppm) were prepared and used for plant extracts and pyrethrins

Efficacy: The maximum effect that a given active ingredient, synergist or formulation will produce regardless of the dose on pests.

Formulation: A mixture of an active ingredient (pyrethrins) and synergists (plant extracts) which effectively control a pest (maize weevils)

Lethal Concentration: Amount of an active ingredient or compound required to kill individuals of a test population. It is expressed in LC values, for instance, LC20 means the lethal concentration of the compound estimated to kill 20% of individuals of the test population.

Piperonyl Butoxide (PBO): The standard synergist for formulations of pyrethrins that is already commercialized

Plant Extract/Oil: A substance made by extracting a plant part (seeds, roots, leaves, shoots) using a solvent. The extract/oil is an active with desirable properties and is used for a particular purpose.

Post Harvest Losses (PHL): The degradation in both quantity and quality of a food production fron harvest to consumption.

Potency: Expression of the activity of a compound/plant extracts ("synergists") or formulations in terms of concentration or amount required to produce a defined effect

Potential Synergists: The term "potential synergists" or "synergists" are used to refer to plant extracts tested for synergism in this study and these terms are used interchangeably even if the plant extracts may not have shown any synergistic activity.

Synergist: A compound that is not toxic or negligibly toxic to insects when it is applied on its own, but when combined with an insecticide, it enhances the efficacy of that insecticide while using the minimum amount of active ingredient making the formulation more cost effective.

Synergistic Effect: The term used used to describe the results of synergist-pyrethrins formulation that produced greater effect than the effect that synergists and pyrethrins produced individually

CHAPTER TWO
LITERATURE REVIEW

2.1 Overview of Pests in Grain Storage

Insects form one of the most important agents of loss in grain storage. There are approximately 100 insect species associated with stored grain but only about 20 are major pests of cosmopolitan distribution (Hall, 1980). The major grain pests belong to two orders: Coleoptera (beetles) and Lepidoptera (moths). Both the larvae and the adults in beetles cause damage to the grains while in moths only the caterpillars are important. Some characteristics of these insect pests include very high rates of multiplication with some stages of growth occuring within the grains making them very difficult to see hence a major cause of grain damage in storage.

Depending on type of infestation and extent of attack on the grains, storage insect pests can be grouped into primary, secondary and tertiary pests, parasites and predators (Wheatley, 1965). Primary pests are the major pests of stored grains and cause significant loss (Mahihu, 2013). Important pests include the beetles, *Sitophilus zea-mais* on maize and other cereals, *Sitophilus oryzae* on wheat, sorghum and rice and *Prostephanus truncatus* on maize. These possess snout-shaped mouthparts and feed on grain by boring thus grouped as "true" weevils. Both the adult and larva damage the cereal grain by chewing leading to the loss of germplasm. Several larvae of *S. zeamais* can develop inside a single grain of maize causing severe infestations and lowering the food value. Studies have recorded emergence of upto 100 adults per kilogram of maize grains per day five weeks after infestation (Pest web, 2017). Contamination of the produce can be serious due to accumulation of uric acid excreted by the weevils, frass and dead weevils (Dennis, 1990) rendering grain unpalatable. Adults fly into the ripening crops in the fields thus many infestations start in the field.

On legume grains, *Acanthoscelides obtectus* and *Callosobruchus maculatus* are the most destructive pests. The larvae feed internally and make holes on the grain but adults do not feed and the snout is not used for drilling holes in grains hence they are called "false" weevils or bruchids.

Secondary pests do not cause serious damage to dry cereal and pulse grains but are capable of multiplying very rapidly to cause damage. They develop on grains that have been damaged by primary pests or on broken grain (Mahihu, 2013). Common beetles include *Tribolium castaneum* and *Oryzaephilus surinamensis* on maize, wheat, rice

and milled cereal products. Among the moths are *Ephestia cautella* and *Plodia interpunctella*. Table 1 summarises some common primary and secondary insect pests found on stored products in Kenya.

Table 1: Common Insect Pests of Grain in Kenya

Scientific Name	Common Name	Main products Attacked
Primary Pests		
Sitophilus zea-mais	Maize weevil	maize, sorghum, cereals
Sitophilus oryzae	Rice weevil	maize, sorghum, rice, wheat
Sitotroga cerealella	Angoumois grain moth	maize, sorghum, wheat, rice
Prostephanus truncatus	Larger grain borer	maize, cassava
Rhizopertha dominica	Lesser grain borer	maize, wheat, rice, sorghum
Acanthoscalides obtectus	Common bean weevil	Beans
Callosobruchus maculatus	Cowpea weevil	cowpea, grams
Secondary Pests		
Tribolium castaneum	Rust red flour beetle	maize, wheat, rice, sorghum, milled cereal products
Ephestia cautella	Tropical warehouse moth	maize, milled grain products, rice, milled grain products
Lasioderma serricorne	Tobacco beetle	cassava, tobacco
Dermestes species	Hides beetle	dried fish, hides and skins
Oryzaephilus surinamensis	Saw-toothed grain beetle	maize, rice, wheat, oil seeds
Corcyra cephalonica	Rice moth	maize, wheat, rice, sorghum, milled grain products

Source: Mahihu 2013

2.2 Post- Harvest Losses of Maize

Maize provides food and income for over 300 million resource poor small holders in Eastern and South of Africa. Due to the lack of effective pest control farmers end up selling their maize soon after harvest, when prices are at their lowest, partly to curb post-harvest losses and to meet other financial needs. The same farmers are forced to buy the grains back at more than twice the price later in the season resulting in a continual poverty trap (EGSP II, 2013). Therefore, post-harvest losses fuel food insecurity and impoverishment.

Maize (*Zea mays* L.) is one of the most important cereal food crops for resource poor and small holders in Sub-Saharan Africa (SSA) providing food and income to millions. It is the main source of carbohydrate, protein, iron, vitamin B and minerals and also a stable food for more than 50% of the population (Gairns *et al*, 2013; Tadele, 2012).

Maize is mostly grown by small scale farmers for subsistence as part of mixed agricultural systems. However, yields are generally low due to moisture stress, poor soil fertility and post-harvest losses despite improved germplasm and on-farm crop management (Farm Link, 2017; Chabi-Olaye *et al*., 2005). According to the World Bank report, Sub-Sahara Africa alone loses food grains worth about 4 USD billion annually (Zorya *et al*., 2011; FAO, 2010). This is equivalent to a decade of food aid for the region, or equivalent to food for about 48 million people (EGSP II, 2013).

Globally, post-harvest losses of about 20 to 90% due to the maize weevil have been reported for when maize is not treated with a protectant (Ojo & Omoloye, 2012; Giga *et al*., 1991). In These losses have been estimated to be between 20% and 40% (Abass *et al*., 2014) and in severe cases losses upto 80% have been reported (Fox, 2013) in Africa. Grain losses for cereals and pulses stands at 20% (Youndeowei & Service, 1986) while that of maize averages at 30% mainly due to the maize weevil (10-20%) and LGB (30-90%) (Likhayo *et al*., 2013). In Benin, losses as high as 36% have been recorded on stored maize (Lamboni & Hell, 2009) and 50% in Cameroon (Nukenine *et al*., 2002) due to infestation with LGB. Pantenius (2008) estimated upto 11.8% weight loss due to insect infestation in maize after six months of storage in traditional granaries in Togo.

It is noted that since the LGB was first found in Zambia in 1993, there has been sporadic outbreaks causing substantial losses in maize ranging from 5 to 74% (Lamboni and Hell, 2009). In Guatemala, due to lack in storage structures along with the regions high humidity, storage loses have been estimated between 40% and 45% (Inter-American Institute for Cooperation on Agriclture, 2013). In Kenya, weight loss of stored maize increased from 4.5 to 30% twenty years after the introduction of LGB in the country (Ritcher *et al*., 2007). Heavy infestations of the LGB can result in complete harvest losses (Likhayo, 2013).

The potential impact of increased maize productivity on poverty reduction and greater livelihood security will not be realised unless complementary and additional insect pest control strategies are developed and used. Despite the development of improved storage technologies such as grain metal bins and super bags, coleopteran grain pests begin

infestations from the fields. The infested maize need to be protected from re-infestation once in storage thus the need for safe, effective insecticides for their control.

2.3 Control of Stored Grain Insect Pests

Stored grain pests are a big challenge to control in an agricultural system. Different approaches have been used for control, for example, from stockpiling grain and grain products. Methods such as storing grain at cold or in controlled temperatures have been found to prevent infestation (Isman & Grieneisen, 2014). Physical methods such as removal of adult insects by sieving and addition of inert dusts such as ash and clay to grains has been found to reduce insect numbers by causing insects to die by desiccation (Dent, 2000; Gaby, 1988). In laboratory studies to test the toxicity of various plant ashes against F_1 progeny of *S. zea-mais,* Akob & Ewete (2007) found that using leaf ashes of *Cupressus arizonica*, *Eucalyptus grandis*, *Ocimum gratissimum* and root ashes of *Vetiveria zizanioides* significantly reduced the number of emerged weevils.

Hermetic storage (HS) for cereals, pulses, coffee and cocoa beans has also been used widely in developing countries. The method creates an automatic modified atmosphere of high carbon IV oxide concentration using sealed waterproof bags or structures (Global Harvest Iniative, 2014). The low air permeability created by these structures reduces oxygen levels available for the insects and thus the high levels of CO_2 causes death of the insects (Murdock *et al*., 2012). However, this method is slow and may not give high levels of mortality after an infestation even when well managed. Garcia *et al* (2020) also found that when chickpeas are packed wth high CO_2 in modified atmospheres, a decrease in the mortality of eggs and adults of *Ryzopertha dominica* occurred due to pulse resorption.

The interest in utilization of botanical insecticides and plant essential oils have become increasingly relevant in the control of insect pests (Isman & Grieneisen, 2014; Regnault-Roger *et al*., 2012). Many plant products and their bioactive compounds with repellent, antifeedant or insecticidal activity against stored product insect pests have been reported (Akhtar *et al*., 2008; Rajendran & Sriranjini, 2008). Essential oil extracts from leaves of wormseed, *Chenopodium ambrosioides* Linn. (Chenopodiaceae) has been found to be effective aganst six common species of grain beetles in western highlands of Cameroon. Filter paper discs were treated with the oil diluted in acateone

at different concentrations (0 to 6µl/cm^3) to check its effectiveness. Mortality was found to be 100% for the bruchid, *Callosobruchus sinensis* in 48 h compared to that in weevils and borers. Also the ground leaves were effective in inhibiting the F1 progeny production of the insects and adult emergence of the insects (Kumar & Khalita, 2017). In laboratory experiments, Carvacrol, a compound from *Thujapois dolabrata*; linalool, a bioactive molecule from *Ocimum canum*; estragole and fenchone from *Foeniculum vulgare* have been found to be toxic against adults of *S. oryzae* and *R. dominica* (Akhtar *et al*., 2008; Kim et al., 2003).

Bioactive compounds isolated from roots of *Decalepis hamiltonii* have shown protection of grains by suppressing the emergence of F_1 progeny of *S. oryzae, R. Dominica, T. casteneum* and *C. cinensis* in treated grain by contact bioassays for a period of upto three months (Rajashekar *et al*., 2010). Shaaya *et al* 2016) tested four edible oil of pure soyabean pure, crude cotton seed oil, crude rice bran oil and crude palm kernel oil as fumigants against insects in beans and wheat which were found to be effective during the initial four months storage period on average. The major issue with these plant materials is that the oil yields are low and expensive to use on a commercial scale (Kumar and Kalita, 2017).

Azadirachtin, derived from the seeds of neem tree, *Azadirachta indica*, belonging to the meliaceae (mahogany) family have shown insecticidal activity against a variety of insect pest species (Schmutterer, 1990). It is a complex compound bearing a mixture of related substances extracted from the neem seed kernels. Azadirachtin, which makes up the active ingredient (a.i) is the most active and abundant phytochemical in neem (Gahukar, 2014). Neem extracts act on the physiological process of insects related to metamorphosis and ecdysis (Mordue & Nisbet, 2000), antifeedant, repellent, sterillant and oviposition inhibitor (Raymond, 2014). Also, Azadiractin is largely responsible for both repellents (behavioural) and toxic (physiological) actions on stored products beetles. However, its bitter taste and lack of contact toxicity restricts its use on stored products meant for consumption (Mahihu, 2013).

Spinosad, a bio-pesticide produced from bacterial fermentation has been used against insects of stored grains. Its efficacy has been found to vary greatly with insect species though high potency has been reported against lesser grain borer, *Rhizopertha dominica* (F.) (Athanassiou *et al*., 2008; Huang & Subramanyam, 2007; Fang *et al*., 2002).

Although spinosad has low mammalian toxicity and degrades quickly when exposed to sunlight, it needs to be applied in binary combination with other protectants such as chlorpyrifos, methomyl, avermectin and fenvalerate if high levels of control of a range of insect species are to be achieved since cases of resistance have been reported in some cases (Roditakis *et al*., 2018).

However, spinosad resistance is emerging in various pest species which have been associated with alterations with the target-site receptors (Hojland *et al*., 2014). Markussen & Kristensen (2012) found that spinosad resistance involved alterations of cytochrome P450 gene expression in female *Musca domestica* L. from field populations and that polymorphic sites were found in promotor regions of P450 genes in spinosad resistant strains collected in Denmark (Mahmood *et al*., 2016). This gene amplication effect has also been observed in many other populations such as in the vector mosquitoes *A. aegypti* (Poupardin *et al*., 2014) and *A. albopictus* (Grigoraki *et al*., 2017). Other reports have indicated that resistance to spinosad in the cotton bollworm, *Helicoverpa armigera* was associated with an increase in cytochrome P450 monooxygenase (Wang *et al*., 2009).

Biological control of *P. truncatus* using its natural enemy, *Teretrius nigrescens* (Coleoptera), has been tried as an alternative control method (Golob, 2002). However, various research groups have had varied conclusions on how to measure the effectiveness of this biocontrol agent on *P. truncatus* since part of its life cycle is within the grains (Meikle *et al*., 2002).

The possibility of controlling grain pests by means of their parasites have been studied both in Europe and America (Raymond, 2014) but the general conclusion reached is that though the parasites are valuable allies, they are not likely by themselves to keep pests within reasonable limits because they have been found to work on the principle of one parasite to one pest. Various parasitoids (*Cephalonomia tarsalis, Laryophagus distinguendus* and *Theocolax elegans*) have been thought to be effective if introduced early in the storage period (Dent, 2000; Youndeowei, 1993). The fungus, *Beauveria bassiana* and bacterium, *Bacillus thuringiensis* have also been tested but none has achieved significant control since most of the life cycle of *S. zea-mais*is within the grains and storage-controlled conditions do not often favour the growth of these

microorganisms. Several nano-materials loaded with natural products have also been evaluated against stored grain pests (Dimetry & Hussein, 2016). Though still a new technology on development phase, products based on alumina, silica, silicon dioxide, zinc and silver nano-particles have been shown to be active against such storage pests (Stadler *et al*., 2018; Routray *et al*., 2016; Rouhani *et al*, 2013).

In practice fumigation with methyl bromide is often used for disinfestation of storage rooms or with hydrogen phosphide for direct uses on the grains. Fumigation with phosphine and methyl bromide are effective in large scale stores (Dent, 2000). Methyl bromide though a rapidly acting fumigant that has been reported to give complete disinfestations in 12-48 h however, its use been prohibited by European Union since the year 2010 for use in storage protection due to damaging effects on the ozone layer (Anon, 2017). Studies on use of ethyl formate for control of stored grain pests have shown that varied dosages in an exposure period of 48-72 h control all stages of insects in stored grains and their products (Muthu *et al*., 2012).

2.3.1 Chemical Control of Insect Pests

Chemical treatment of stored grains has profound effect in maintaining cereal stocks by reducing losses caused by insect pests. In many climatic zones, long-term storage of grains without loss of quantity or quality would be not be possible if some form of post harvest chemical treatment is not used (Annis, 2016). Despite this, chemicals used have had negative effects which warrant their reduction in grains in storage (Holztman, 2010). Treated grains have many entry points for residues into food used for human consumption, either directly or indirectly via animal products such as meat or milk which complicates the pathway of registration of new active compounds for use on stored grains (Annis, 2016).

The use of hemical insecticides to control stored product insect pests' dates back to the 1950s. These insecticides are commonly used because of their reliability, efficacious speed, ease of use and the fact that short term pest level reduction are almost assured (Cherry *et al*., 2005). Their use still remains the most effective option in controlling pests and reducing losses during storage of grains and that losses of upto 100% can occur if maize is not protected with insecticides before storage (The MDG Centre, East and Southern Africa, 2008). Current recommended chemical insecticides include

organophosphates, pyrethroids, diamides and juvenile hormone analogues (Silva *et al*., 2019).

However, the widespread use of these insecticides is of global concern with respect to environmental and mammalian hazards, insecticide resistance development, chemical residues in food, side effects on non-target organisms and the associated high costs (Cherry *et al*., 2005). Further, review process of active substances by the regulation of European Parliament and Council of 23rd Frebruary, 2005 on maximum residue levels of pesticides in and on food found that the number of registered insecticides for use in stores were few and that new active substances had not been added to the list (Holztman, 2010). Consequently, the few available actives have been overused increasing resistance problems in pests of stored products

2.3.1.1 Use of Organophosphates in Control of Stored Grain Pests

Pirimiphos-methyl (Actellic^{R}) is registered in the U.S at the rate 8mg/kg for use on stored corn and sorghum (U.S. EPA, 1999; 2003) while in Kenya it is recommended at very high dosage of 555mg/kg on shelled maize. Furthermore, this insecticide breaks down rapidly and many stored grain insects have developed high levels of resistance (Subramanyam & Hagstrum, 1995). Fang *et al*. (2002) suggested that alternative pest management strategies were needed in post-harvest commodities to replace or complement the existing organophosphate grain protectants.

Malathion 2% applied at rate of 50gm/90kg shelled maize is also registered for use on coarse and small grains but it breaks down rapidly and many stored grain insects have developed resistance (Guedes *et al*., 1996; Subramanyam & Harein, 1990; Zettler & Cuperus, 1990). Malathion has been found to be ineffective against pests such as *T. casteneum* and *R. dominica* in many countries (Mahihu, 2013; Subramanyam & Hagstrum, 1995) due to pest resistance. Thus, it is no longer recommended as a grain protectant by the cooperative extension service specialists and post-harvest researchers (Mahihu, 2013).

2.3.1.2 Use of Synthetic Pyrethroids in Control of Sored Grain Pests

These are modern synthetic insecticides chemically similar to natural pyrethrins but modified to increase stability in the natural environment. They have a residual effect

that provides for longer control periods, more specific targets on insect species, cheaper to produce and have low toxicity towards mammals (Brown, 2006; Klaassen, 2001). The development of synthetic pyrethroids was based on the pyrethrins structure (either pyrethrins I or II) (Staudinger & Ruzicka (1924). This development aimed at obtaining a stable molecule that is not easily degraded by sunlight and with higher efficacy (Davies *et al*., 2007; Litchfield, 1985). As a result, the first pyrethroid molecule developed was permethrin that was similar to pyrethrins I which was later followed by deltamethrin and cypermethrin (Davie *et al*., 2007; Davies, 1985). Molecules making up pyrethroid mixtures stem from mixtures of isomers which contribute to their biological activity (Litchfield, 1985).

The advantage that synthetic pyrethroids have include persistence due to their stability in sunlight and hence greater insecticidal effect unlike natural pyrethrins (Thacker, 2002). Because of this, they have been used widely to control insect pests as plant sprays, household sprays, pet sprays and in crop protection (Klaassen 2001; Mrak, 1973). In protecting stored grains, synthetic pyrethroids insecticides such as permethrin and deltamethrin are not very effective against maize weevil which is more susceptible to organophosphate insecticides such as fenithrothion and pirimophos-methyl (CABI, 2010). However, better control is achieved when formulated with additional active ingredients from other group of compounds to increase their efficacy. Some of these misures include Actellic Super dust, a combination of pirimiphos methyl 1.6% + permethrin 0.3%, Skana super grain dust and sumicombi (fenithrothion 1.5% + fenvalerate 0.3%). The intensive use of these synthetic pyrethroids have led to the development of resistance in stored product pests (Joffe, 2012).

Focus has now shifted to the use of botanical actives such as pyrethrum due to the complexity of its molecule having six active esters and its non persistence in the environment. The demand for safe pest control products particularly, on human health and the and the environment have contributed to the interest in using natural pyrethrum for the control of stored prduct pests. Advantages of pyrethrum such as quick knockdown of arthropod pests and lack of persistence in the environment is an ideal product for use on grains that are directly consumed (Narahashi, 1982). Pyrethrum in combination with other molecules such as natural synergists can reduce its cost and play a critical role in control of storage pests.

2.3.2 Use of Botanical insecticides in Storge Pest Managment

Pyrethrins are insecticidal compounds obtained from white daisy like flowers of the plant pyrethrum, *Chrysanthemum cinerariefolium*. They are extracts from the flowers that contain six active pyrethrin compounds (Kenya Pyrethrum, 2001; Ray, 1991; Casida, 1973) which are extracted as an oil or dry powder shortly after the flower blooms. Flowers contain about 1-2% pyrethrins, relative to its dry weight, but approximately 94% of the total yield is concentrated in the seeds of the flower (Casida and Quistad, 1995). The use of pyrethins dates back to Persia and Yugoslavia about 1800's (Grosby, 1996). The flowers have historically been grown in commercial quantities in Ecuador, Japan, Yugoslavia, Kenya, Tanzania and Tasmania. However, after World War II, increased production was observed in Kenya. By the 1960's, Kenya supplied more than 90% of the world's pyrethrum (Grosby, 1996.

There are six biologically active compounds in pyrethrins that are responsible for knockdown properties of the insecticide (Kumar *et al*., 2005, Head,1973) They are divided into two groups namely Pyrethrins I (Pyrethrins I, cinerin I and JasmolineI) and Pyrethrins II (Pyrethrins II, cinerin II and JasmolineII). Pyrethrins I are esters of chrysanthemic acid and Pyrethrins II are esters of pyrethric acids. These combine with one of three alcohols (pyrethrolone, cinerolone and jasmololone) to form the respective six active ingredients (Head, 1973). Group I pyrethins are insoluble in water but soluble in hydrocarbons and organic solvents such as alcohol (WHO, 1975).

Pyrethrins have a unigue mode of action that include flushing action which cause insects to move out of their hiding thereby getting in contact with the insecticide (BRA, 2010). They are also strong repellants at low concentrations which can be good in repelling stored grain pests. Pyrethrins are contact insectides that affect the nervous system of insects causing paralysis and "knockdown" effect. They bind to sodium channels of nerve cells causing repetitive action potentials, prolonging their opening and thereby causing death (Brown 2006; Tomlin, 2000).

Pyrethrins are easily degraded by sunlight, alkaline conditions, high temperatures and UV light thus they do not leave residues in environment and even on food stuffs (Ashamo *et al*., 2013). Pyrethrins are easily metabolized by warm blooded animals thus

have low toxicity to mammals. These properties make pyrethrins universally accepted biopesticides and it is included in the list of approved organic insecticides in the world.

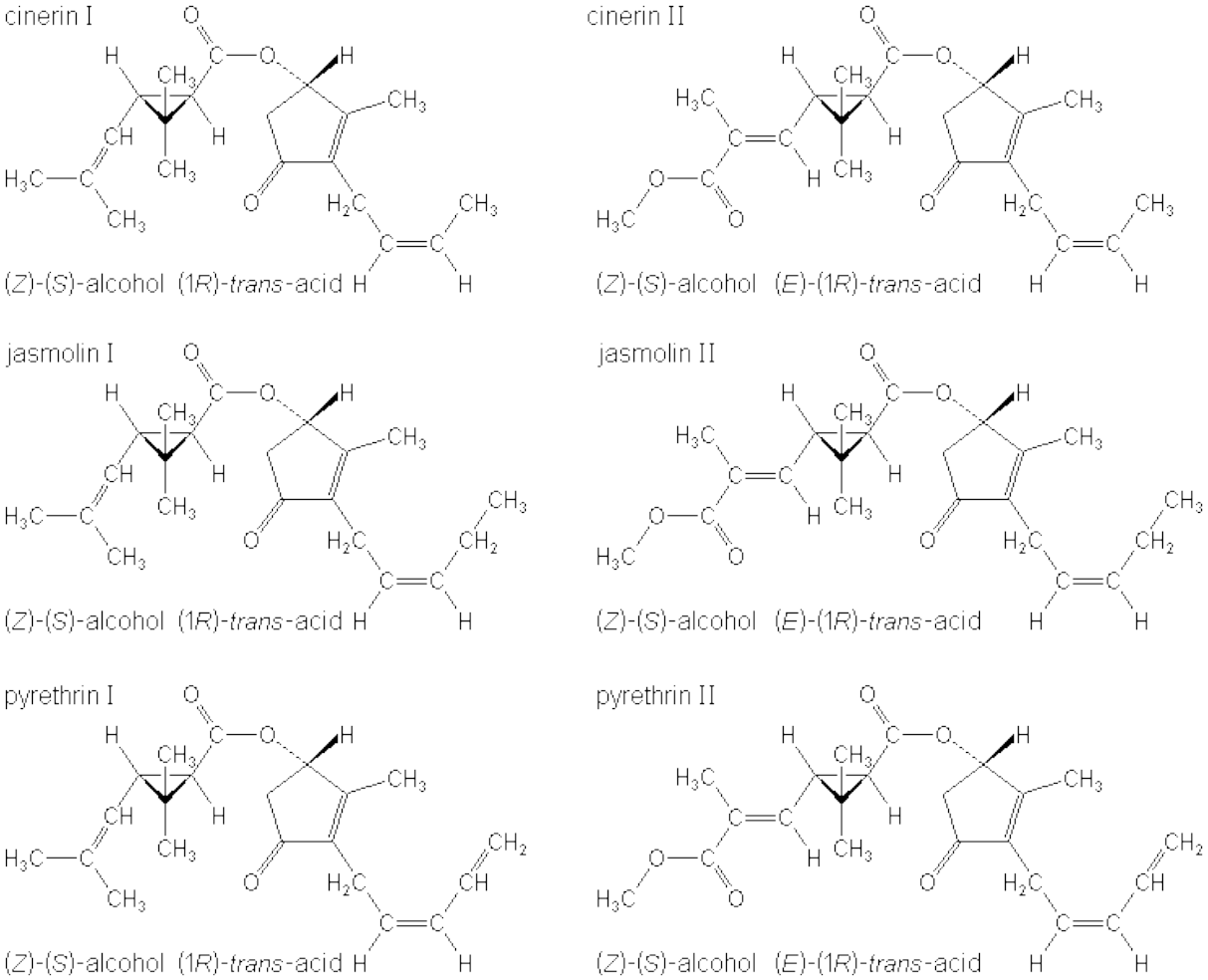

Figure 1: Structure of Pyrethrins (Formulating Pyrethrum, 1987)

Natural pyrethrum is generally used in combination with the semi-synthetic synergist PBO to increase its toxicity. Pyrethrins alone or in combination with this synergist have been used for treating empty storage facilities and direct on grain treatment to manage stored product insects. However, Desmachelier (1977) reported that pyrethrins at 1mm/kg synergist with PBO (in 1:10 ratio) were ineffective against *R. dominica*. Similar findings were found where synergist pyrethrins with PBO applied at a rate 1.5mg/kg were ineffective against five insect pests of stored products (Ashamo *et al*., 2013) and with increased rates (4mg/kg), significant control of *R. dominica* could be achieved for more than 140 days at 20 - 30^{o}C but the costs are prohibitive.

However, there are emerging issues on the quest for use of natural products in the control of pests. This quest driven by organic foods and healthy products is greatly increasing. The need to use eco-friendly and cheap biopesticides in pest management to reduce crop loss due to infestations is greatly desired (Ashamo *et al*., 2013). This will help reduce food shortages in developing economies and ensure sustainable food security. Thus, continued use of synthetic insecticides is not sustainable but high costs of producing botanical formulations and their efficacy necessitate further investigations. Synergism therefore, is one viable way to reduce costs and enhance efficacy.

New chemicals extracted from plant oils have shown promise for potential use in the development of new pesticides (Gross *et al*., 2017) though limitations such as inconsistencies in efficacy and composition, lower potency against target pests compared to synthetic insecticides, lower persistence and residual activity may restrict their use as stand-alone products for crop protection in many situations (Isman, 2012, 2006).

2.4 Development of Insecticide Resistance

The overuse of pesticides has resulted in many insect species developing resistance to about 400 different insecticide molecules. Out of about 870 insect species, about 489 have become resistant (Whalon *et al*., 2010). Almost all classes of insecticides have reported cases of resistance including pyrethroids, diamide, carbamates, cyclodienes, DDT and organophosphates (Roditakis *et al*., 2018; Brattsen *et al*., 1986). Diamides, one of the few effective and recently introduced classes of insecticides against the tomato pin worm, *Tuta absoluta*, has been reported in Brazil, Europe and Italy to have very high cases of resistances in field poplations (Silva *et al*., 2019; Roditakis *et al*., 2018).

According to the World Health Organization (WHO) resistance is defined as "the development of an ability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species" (WHO, 1975). Resistance in most cases develops as result of over exposure of the same class of insectide to the same population of insects. Application of an insectide does not usually eradicate the whole population

The development of new compounds have been found to be slow and expensive and thus limited control options readily become available to farmers. Increased application rates result in higher risk of exposure to pesticide and more environmental contamination. The cost of controlling insects also increases drastically (Bett et al., 2016) and ecologically sound pest control strategies are disrupted. Diseases in humans, animals and plants increase where transmission is by insect vectors. Insecticide resistance can also lead to complete destruction of agricultural production (Soderlund & Bloomquist, 1990), such as the loss of cotton production in the Ord valley, Australia, due to DDT resistance in the cotton bollworm, *Heliothis armigera* (Lepidoptera: Noctuidae) (Castle 2002).

The development of resistance in insects depends on a variety of biochemical, genetic and ecological factors, which vary between species and populations (Brattsen *et al*. 1986; Nwana & Akibo, 1982). Factors such as short generation time, high fecundity rate, dispersal ability of the insect, mode of inheritance and fitness costs associated with resistance all play a role in the development of resistance, together with the frequency of insecticide applications, dosage rates applied and persistence of insecticide residues (Tabashnik, 1990).

2.4.1 Insecticide Resistance Mechanisms in Insects

It has been argued that, the development of resistance in insects has been attributed to co-existence of insects and plants together over a long period of time which has led to insects developing a number of mechanisms to cope with toxic plant allelochemicals, such as alkaloids, terpenes and phenols. These mechanisms are developed by plants as defence mechanisms against herbivorous action of insects. As a result, insects have also developed some coping mechanisms which include: - behavioural adaptations, physiological processes, target site insensitivity and metabolic mechanisms. In this defensive adaptive behaviour, one resistance mechanism is capable of conferring cross-resistance to other insecticides and a combination of these mechanisms can occur within one insect population (Brattsen *et al*., 1986; Scott, 1990; Soderlund & Bloomquist, 1990).

It has been proposed that, the reason why insects develop rapid resistance is because of intensive use of insecticides in areas favouring rapid pest reproduction, such as crop monocultures and the insecticide is the only selecting agent for insecticide resistant genome (Brattsen *et al*., 1986). In most cases, for the insecticide to cause an effect it must reach the target site by first penetrating the cuticle of the insect and/or other barrier tissues. When an insect cuticle is altered it will slow down the penetration of the insecticide hence less insecticide passes through to the insect's body. This confers low levels of resistance mechanism on its own, but is usually found in combination with other resistance mechanisms, such as detoxification enhancing the effect of the mechanism (Soderland & Bloomquist, 1990).

If penetration of insecticide is delayed from reaching the tissues, there is will be more time for detoxification. (Brooks, 1976). In another study it was observed that, diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), resistance to fenvalerate was due to reduced cuticular penetration (Noppun *et al*., 1989), reduced target and higher metabolic detoxification rate (Yao *et al*., 1988). In resistant *H. armigera* from Thailand, Ahmad & McCaffery (1999) found that, delayed penetration of cypermethrin through the cuticle to be a minor mechanism of pyrethroid resistance, functioning together with nerve insensitivity. The study has shown that, decreased cuticular penetration also plays a role in pyrethroid resistance in *H. armigera* (Gunning *et al*., 1991).

Studies have further shown that insensitivity of the nervous system and modified sodium channels are the most common mechanism of resistance towards pyrethroids, DDT and DDT analogues. Decreased nerve sensitivity was first noted in adult house flies *Musca domestica* (L.) (Diptera: Muscidae) (Busvine, 1951). Insensitivity of the nervous system confers resistance not only to the lethality of pyrethrins and DDT, but also to their rapid paralytic effect (knockdown), and has therefore been termed "knockdown resistance" or kdr. Insects that show decreased sensitivity to insecticides have the ability to afford cross resistance to all the pyrethroids and DDT, and the failure of synergists to increase toxicity to the insecticides. Kdr arise from alterations in the binding site for DDT and pyrethroids on the insect nerve cell membranes (Soderlund & Bloomquist, 1990)

Cases of insecticide resistance have been found in most classes of synthetic insecticides including carbamates, organophosphates, pyrethroids and DDT (Brattsen *et al*., 1986). Resistance to deltamethrin in *H. armigera* in West Africa has been associated with an increase in Mixed Function Oxidases (MFO's) activity (Mansour & Aly, 2015). Chen *et al*., (2005) and Yang *et al*., (2004) found elevated cytochrome P450 monooxygenases to be important in pyrethroid resistance in *H. armigera* larvae. The study found correlation of *M. domestica* pyrethroid resistance and increase in monooxygenase activity (Lee and Scott, 2015). Darbon *et al*., (2002) found that the over transcription of a single P450 gene, *Cyp6g1*, was responsible for resistance to DDT in the fruitfly, *Drosophila melanogaster* (Diptera: Drosophilidae). The presence of PBO in a compound makes it bind with the MFOs causing the availability of insecticide to block the sodium-potassium channels in axonal membranes, resulting in repeated nerve excitation due to release of neurosecretory hormones such as diuretic hormones which eventually causes death of the insect (Hamilton, 1995).

Once inside the body, a percentage of the insecticide gets stored in the tissues, such as adipose tissue, and the remainder is distributed within the body of the insect. A portion of the transported insecticide is detoxificated by enzymes and a portion excreted. The remainder is then available to act on the target site (Brooks, 1976). Mutations affecting any of these processes may result in a reduction in the affinity of insecticide towards its target site, conferring some level of resistance to the insect (Soderland and Bloomquist, 1990)

When cross-resistance and multiple resistance occur together it will be of particular importance in the control of pests, due to addictive effect caused by their presence. There is likely to be an evidence indicating that, cross-resistance may lead to control failures with a much broader range of insecticides than those initially used for their control thus, eliminating these additional insecticides from possible use in the future. Pest populations with extreme resistance to pesticides and presence of multiple resistance mechanisms can be extremely difficult to control cotrol (Soderlund & Bloomquist, 1990). Multiple resistance and its involvement in reduced cuticular penetration and an insensitive target site, combined with metabolic resistance factor, leads to confering cross-resistance to other classes of insecticides that have similar mode of action (Brattsen *et al*., 1986).

An example of multiple resistances is demeton-S-methyl-resistant melon ahid, *Aphis gossypii* (Hemiptera: Aphidae), where resistance is as aresult of insensitive AChE together with enhanced esterase activity (Han *et al*. 1998). Cross-resistance between pyrethroids has been established in pyrethroid-resistant tobacco budworm *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) (Leonard *et al*., 1988) and in pyrethroid-resistant *H. irritans* (Byford *et al*., 1985). In spotted bollworm *Erias vittella* (F.) (Lepidoptera: Noctuidae), cross-resistance was sited in a fenvalerate-resistant population towards endosulfan and carbaryl, whereas a cypermethrin-resistant population developed cross-resistance to endosulfan, fenvalerate and carbaryl (Saini *et al*., 1989).

2.4.2 Management of Insecticide Resistance

Resistance mechanism is a dynamic phenomenon which is known to cause resistance and may change over time. It is recommended that, continued monitoring is important to determine whether management strategies remain valid or need to be revised in light of changing circumstances or new knowledge acqusition (Denholm & devine, 2013). In the past practices, resistance was managed by either increasing pesticide dosages, applying new compounds as alternative methods for control and on the concept of insecticide rotation. The outbreaks of resistant *H. armigera* in Australia was based primarily on insecticide rotations (Joffe, 2012). This approach, has not yielded good results, and is no longer viable due to the increasing concerns about environmental contamination by chemical pesticides. The high cost of developing new pesticides has resulted in a decline in their rate of development thus there will be often a very limited number of insecticides available for use in management strategies. The availablabilty of pesticide chemistries are limited and need to be conserved for their extended use. It can be conluded that, it is essential to prevent development of resistance, or to slow it down and reduce its impact as much as possible (Castle, 2002; Soderlund & Bloomquist, 1990).

Rational and informed strategies in resistance management to delay, prevent or reverse the development of resistance in pests has been recommended. Resistance management can also include an increase in the development of resistance of beneficial species, such as natural enemies, which would contribute to controlling pests. An understanding of

the resistance mechanisms in insects is essential for development of successful resistance management program (Soderlund & Bloomquist, 1990; Tabashnik, 1990).

Ways of overcoming resistance mechanisms can be devised when the mechanism is known, for example, by using insecticide synergists (Scott, 1990). PBO is recommended for control of severely pyrethroid-resistant, *H. armigera* in Australia (Forrester, 1988). An increasing understanding of the physiology and biochemistry of resistant insects is essential in the search for effective insecticides (Brattsen *et al*., 1986). Synergists may also play a role in delaying the development of resistance, since insects with resistant genes would be killed in equal proportion to the susceptible ones, removing the selective advantage conferring the resistance (Ranasinghe & Georghiou, 1979). Some specific synergists could be selected that which specifically targets a mechanism of resistance present in the pest species but not in the natural enemies. The efficacy of an insecticide could be increased against the target pest and still keep natural enemy populations high (Ishaaya & Casida, 1981; Plapp & Vinson, 2017).

2.5 Use of Synergists in Insecticide Formulations

The researchers have discovered synergists as copmpounds that are either negligibly toxic or non-toxic to insects when applied on their own. When synergy is used in combination with insecticide, enhance the efficacy of that insecticide. Synergists can be used in combination with pesticides against insects possessing metabolic resistant mechanisms and in susceptible insect strains since synergists basically act by inhibiting the metabolic pathway involved in detoxification of an insecticide (Casida, 1970; Metcalf, 1967). The use of a synergist to enhance efficacy of formulations in both resistant and susceptible insect strains and to allow more cost-effective formulations. Processes other than enzyme inhibition, such as increasing the penetration of an insecticide through the cuticle or preventing the deterioration of insecticide are not considered as classical synergism (Metcalf, 1967).

To protect themselves from naturally occurring toxins, such as plant allelochemicals, all living organisms' posses an array of defence mechanisms. Enzymes systems are also utilized in the detoxification of insecticides in resistant insects. The action of these enymes is such that they mobilize toxins or insecticides by changing their molecular structure in such a way that the product is rendered less toxic than before, or it can be

eliminated rapidly from the body, or both (Brattsen *et al*., 1986). Many of the enzymes are temporarily induced by the toxin and persist as long as the toxin is present in sufficient quantities under natural circumstances. In metabolic resistance, individuals with permanently expressed high enzyme activities survive and reproduce in the presence of insecticides resulting in resistant populations (Brattsen *et al*., 1986; Puinean *et al*., 2010). It can be authenticated that, Synergism therefore, has the role of increasing potency of insecticides and increasing the reaction time of insecticide by preventing detoxification within the insect. At the physiological level, the effect of a synergist is often related to an interaction with transport and/or metabolism mechanisms or to a complementary physiological effect (Berenbaum, 1989).

Quite a number of synergists are availablein the market, commercially manufactured and their activities vary depending on the type of insecticide they are combined with. Some of the wel known synergies are PBO and MGK-264 a which are the most commonly used synergists with unique mode of action, others are triphenyl phosphate and diethyl maleate (DEM) (Wang *et al*., 2013). The other studies have found that, insects have in-built complex systems that will counteract an insecticide once it enters its body. Some of the well- known oxidases such as MFOs or microsomal oxidases are some of these types of defence mechanisms, which work by binding with the insecticide rendering it ineffective. MFOs play important ole in the detoxification of xenobiotics (substances foreign to an organism, such as insecticides or allelochemicals), as well as in metabolism of endogenous substances such as hormones, pheromones and fatty acids (Feyereisin, 2012; Mansuy, 1998). The main function of these enzymes is to convert lipophilic substances into more polar substances which are more readily eliminated from the body. The respiratory apparatus, the integument and the digestive tract, especially the insect mid-gut, contain the highest concentration of MFOs since these are the first tissues to come in contact with xenobiotics (Nakatsugawa & morelli, 1976).

Synergism has particularly been associated with the use of pyrethroids and pyrethrins formulations because though potent knockdown agents, they are only moderately toxic to most insects without the addition of a synergist (Norries *et al*., 2019). Some studies have found that, many insects are able to detoxify pyrethrins and survive its application (Casida & Quistad, 1995; Scott, 1990). Pyrethrum products are likey more expensive and therefore a search was made for compounds to be used in combination with

pyrethrins in order to enhance their efficacy. Sesame oil being among the first compounds and was found to synergise pyrethrum, having no insecticidal activity of its own. The active compounds were shown to be sesamin and the more potent sesamolin (Beroza, 1954; Haller *et al*., 1942a, 1942b). The synergistic effects were attributed to its methylenedioxyphenyl (MDP) ring (Figure 2), and the constituents on the benzene ring (Haller *et al*., 1942a).

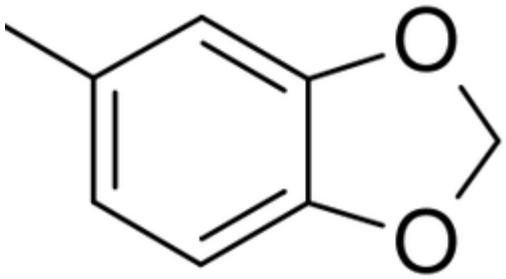

Figure 2: Methylenedioxyphenyl (MDP) ring (Kenya Pyrethrum, 2001)

The MDP substituent is a structural feature which is present in many plant chemicals that prevent foraging by predatory insects and herbivores. Exposure of insects to MDP containing synergists in the environment, in the absence of co-administered pesticides may also enhance xenobiotic detoxification (Murray, 2012). Early studies found that most MDP agents themselves posses relatively low intrinsic toxicity, but strongly influence the actions of other xenobiotics in mammals and insects by modulating cytochrome P-450 (CYP)-dependent biotransformation (Murray, 2012). Such methylenedioxyphenyl containing natural products are frequently associated with prolonged inhibition of CYP450 activities. They undergo biotransformation to reactive intermediates generating tight-binding complexes with the cytochrome. After exposure to MDP chemicals, an initial phase of CYP inhibition is followed by sustained phase of CYP induction. In insects CYP inhibition by MDP agents underlies their use as pesticide synergists (Murray, 2012)

Severall studies were contacted and it was established that, Sesame oil became one of the first commercially available synergists but its use was limited because of the difficulty in preparing it in suitable quantities. It has low solubility in freon and petroleum hydrocarbons (used in insecticidal sprays). Piperonyl cyclohexenone was

subsequently commercialized as a synergist, but an even more active. compound was PBO which was found to be completely soluble in freon, and petroleum hydrocarbons and relatively non-toxic to mammals (Wachs, 1947). Of the synergists commercialized, quite a number with the MDP ring have since been commercialised, for example, sulfoxide, propyl isome and tropital but none have found as much practical use as PBO. PBO is synthesized from the natural product safrole (Casida, 1970). A rich source of safrole originates from tree species in Brazil of the genus *Ocotea*, however safrole is also produced synthetically (Casida and Quistad, 1995). Safrole has also been found occuring naturally in a number of other plant species including black pepper (Russel & Jennings, 1969) and nutmeg (Power & Salway, 1970).

It has been established that, the efficacy of MDP compounds as synergists are influenced by the length of side chains, the number of methoxyl groups present and the position of the double bond in the side chain attached to the benzene ring (Wen *et al*., 2006). A long polyether or oxygen-containing side chain seems likely to be involved in synergism (Casida, 1970). Moore & Hewlett (1958) found that a side chain length of six to ten carbon atoms showed significant synergistic activity whereas fewer or more carbon atoms had less activity. Lichtenstein & Casida (1963) in another study, found that synergistic activity of MDP ring is directly related to the number of methoxyl groups present. Further examination it was found that, in using the compound eugenol (one methoxy group), methyl eugenol (two methoxy groups) and elemecin (three methoxy groups), it was found that the property of these compounds increased toxicity of pyrethrins to houseflies, *M. domestica* with increased number of methoxy groups present (Lichtenstein *et al*., 1974). Several tests with myristicin (one methoxy groups) and apiol (two methoxygroups) seemed to confirm this principle with fruit fly, *D. Melanogaster*. The study asserted that, Apiol was more toxic that myristicin and also more pronounced synergistic activity of parathion than with mysisticin ((Lichtenstein *et al*., 1963).

Other studies revealed that, the action of synergists may not necessarily be restricted to one specific metabolic pathway but can be involved in the inhibition of several enzymes. It is alluded that, relatively non-toxic organophosphate esters, such as DEF (S,S,S-tributyl phosphorothioate) acts on both P450s and esterases (Scott, 1990). PBO acts by inhibiting the Mxed Function Oxidases (MFOs) that have been reported to be

involved in detoxification of various insecticides (Oppert *et al*., 2015). PBO has long been known to inhibit P450s (Wilkinson, 1976), esterases (Gunning *et al*., 1999; Young *et al*., 2005, 2006) and acetylcholinesterase (AChE) (Gunning, 2006; Kang *et al*., 2006). Studies conducted by Phillipio *et, al*. (2013) alluded in their report, that PBO can inhibit both P450 and esterase activity by binding with E4 (a resistance-associated esterase) to acceletate small substrates to the active-site triad, while acting to block larger insecticidal molecules. Derivatives of PBO with alkynyl side chains have also indicated effective inhibitors of P450 activity in vitro than PBO and subsequently demonstrating high levels of synergism *in vivo* with upto 290-fold synergism of imidacloprid against imidacloprid-resistant *Myzus persicae* (Hemiptera: Aphididae) (Phillipou *et al*., 2016). It is alluded from this study that, the effects of PBO seem to be multiple, which could explain its high efficacy as a synergist.

The efficacy of a synergist is commonly expressed as the synergism factor (SF), which is the ratio of the LC_{50} of insecticide alone to the LC_{50} of insecticide with the synergist (Yammamoto, 1973). Other studies have established that, PBO has been shown to increase the toxicity of many insecticides in resistant insects, resulting in high SF values. It has also been asserted that, in sweetpotato, white fly *Bemisia tabaci* (Hemiptera: Aleyrodidae), PBO significantly synergised methamidophos, chlorpyrifos, fenvalerate, avermectin, amamectin benzoate, spinosads, fibronil and imidacloprid (Kang *et al*., 2006). In pyrethroid-resistant *H. virescens*, PBO mixed with amitraz increased the toxicity of cypermethrin (Bagwell & Plapp, 1992). The study akso affirmed that, significant synergistic effects were found using PBO with methamidophos, fenvalerate, fipronyl and avermectin in *Plutella xylostella, Phyllotreta striolata* (F.) (Coleoptera: Chrysomellidae), *Liriomyza sativae* Blanchard (Diptera: Agromyzidae), *Propylea japonica* Thunberg (Coleoptera: Coccinellidae) and *Cotesia plutellae* Hurdjumov (Hymenoptera: Braconidae) (Wu *et al*., 2007).

Various plant essential oils have been shown to enhance pyrethroids to differing degrees although attributable to their intrinsic toxicity (Norries *et al*., 2019). Deltametrin efficacy has been found to increase when exposured to cinnamon (*Cinnamomum cassia*), tagetes (*Tagetes bipinnata*) and sage (*Salvia officinnalis*) oils while efficacy was decreased when exposed to amyris (*Amyris balsamifera*) oil, effects being mediated by changes in cytochrome P450 activity. Also, some plant-derived

essential oils have been found to increase efficacy of deltamethrin similar to standard synergists such as PBO (Scott *et al*., 2019). However, Jun-Hyung *et al* (2020) on evaluating 17 plant essential oils against two strains of mosquito found that when the essential oils were mixed with permethrin, they failed to show synergism of insecticidal activity. Other reports on some plant constituents like linalool from lavender (*Lavendula angustifolia*) and thymol from thyme (*Thymus vulgaris*) have shown antagonistic action in imidacloprid against green peach aphid, *Myzus persicae* (Faraone *et al*, 2015).

Several studies conducted on different plant species indicate that, there is no one particular general combination or ratio at which to administer a synergist and an insecticide. That the the synergistic effect is siginicantly influenced by the synergist itself, the insecticide used and the insect species involved. It is claimed that, for it to be effective, a synergist should penetrate the insect and be transported to the target enzyme preferably faster than the insecticide. It means therefore that; the synergist should have a high affinity for the target enzyme and a lower metabolism rate than the insecticide. The studies alluded that, the specificity of a synergist can be greatly influenced by any of these processes (Casida, 1970; Yamamoto, 1973).

Other similar studies have established that, temporal synergism has been found to be significant when administering synergists in combination with an insecticide. This refers to the amount of time between the application of the synergist and the insecticide (Gunning *et al*., 1999, Moore *et al*., 2005; Scott, 1990). Pre-treatment with a synergist can increase the amount of synergism found due to the considerable time it can take for synergist to maximally inhibit the specific metabolic enzymes involved in resistance (Bingham *et al*., 2007; Young *et al*., 2005, 2006). The concept of temporal synergism has led to the development of microencapsulated insecticides (Bingham *et al*., 2007).

It has been found that, a synergist added to a small quantity of an active ingredient has the capacity to enhance efficacy without taking part in the process. This indicates that, obtaining a natural synergist is expected to stabilize botanical insecticides and increase their efficacy for uses in grain storage hence obtaining a safe efficaceous product which is affordable less or no resistance on pest control and management (Bingham et al., 2007).

2.6 Plants Possessing MDP ring and Synergism

Black pepper (*Piper nigrum* L.) (family Piperaceae), also known as "King of spices" and "Black gold" is a spice vine crop cultivated for its fruit which is usually dried and used as a spice and seasoning widely and extensively in the world. It has multiple uses in the food industry, kitchens, permfumery and traditional medicines (Abou-Zeid, 2014). It is a perennial climber that uses its ivy-like roots for support. Flowers are sessile, borne on pendulous, dense, slender spikes. The berry-like fruits, also called peppercorns are roundabout 0.5-1cm in diameter and contain a single seed. Young berries are green or light purple that change to red on ripening (Ravindran, 2000). Black pepper is mainly grown in south west India, particularly western coastal regions and the first oriental spice to be introduced in the western world.

Black pepper is valued for its pungency and flavour which is attributed by the alkaloid piperine and volatile oils (alkamides, piptigrine, wisanine, dipiperamide D and dipiperamide) (Tripathi *et al*., 2009) which is contained in all the plant parts, high concentrations being on fully differentiated shoots and seeds. Piperine is a trans- isomer of 1-piperoyl piperidine and represents 90-95% of the total pungency of black pepper (Anil *et al*., 1994). Piperine is isolated in good yield from ground black pepper seeds which is made up of 5-9% of alkaloids that also include piperidine, piperettine and piperanine (Ikan, 1991). Pepper's pungency was found in 1821 to be due to piperine and piperanine (Integrated Spectral Data Base System for Organic Compounds, 2001)

The specific compound, piperine is commercially utilized to prepare different insecticides for use against houseflies and other insect pests (Jafri & Mehta, 2014). Piperine has also been documented to enhance the bioavailability of a number of therapeutic drugs as well as phytochemicals through its inhibitory influence on enzymatic drug biotransforming reactions in liver and intestine (Swathy et al., 2018; Islam et al., 2015). In some in vitro studies, an extract of P. nigrum seeds, piperine showed 50-65% inhibitory activity on acetylcholinesterase (Abou-zeid et al, 2014). Constituents isolated from P. nigrum including piperine and dipiperamides D and E potentially inhibited some CYP450 metabolic pathways (Vasavirama & Upender, 2014; Continquiba *et al*., 2011))

In synergistic studies, *P. nigrum* extract used in conjunction with pyrethrum upon gene expression in *D. melanogaster* showed that it maybe an effective a synergist like PBO with a synergistic ratio of 11:6 (Jensen *et al*., 2006). PBO when used in combination with pyrethrum against *M. domestica* resulted in a synergistic ratio of 15:5 (Incho and Greenberg, 1952; Nash, 1954). *P. nigrum* acts by inhibiting polysubstrate mono-oxygenase (PSMO) activity and slowing detoxification (Dalvi and Dalvi, 1991) while PBO binds with MFOs within the insect rendering them ineffective (Hamilton, 1995). The use of *P. nigrum* extract, rather than a pure compound, may have contributed to the synergy. Scott *et al.,* 2002 reported that tertiary mixtures of piperamides showed synergy due to the presence of analogue synergism. The use of *P. nigrum* as a synergist could be useful against insects which have evolved resistance. For example, an extract of *P. tuberculatum* has been reported to be effective against a strain of Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomellidae) with long history of multiple resistance. Also, it has low mammalian toxicity making it a safe product in the control of maize grain pests (Scott *et al*., 2003).

Coriander, *Coriandrum sativum* L. (family apiaceae) is a slender, soft, hairless, glabrous, branched, aromatic annual herb belonging to the family Apiaceae (Duke *et al*., 2002). This plant is commonly known as “Dhaniya” in Hindi or Cilantro and is one of the oldest spices with evidence of its use dating back to more than 5,000 years ago. The plant is an herb about 50cm tall and matures in two to three months after sowing. It is normally pulled out with the roots after maturity. The stem is more or less erect and sympodial; monochasial branched, sometimes with several sides of branching at the basal node. Each branch ends with an inflorescence with pink or white flowers in small loose umbels. The stem is hollow, green and sometimes turns red or violet during the flowering period. The plant is cultivated for its leaves and seeds (Preedy *et al*., 2011). *C. sativum* is grown almost everywhere for the leaves (cilantro), the seeds (coriander) or both (Pandey, 2010). Coriander roots have deeper, more intense flavour than the leaves and is used in a variety of Asian cuisines such as soups or curry pastes. Essential oils from the leaves and seeds contain mixed polyphenols and terpenes including linalool as the major constituent accounting for the aroma and flavour of coriander (Zheljazkov *et al*., 2014; Diederichsen *et al*., 1996).

Coriander oil is among the most used essential oils worldwide (Burdock & Carabin, 2009). The extraction of essential oil from coriander seeds and leaves is carried out through hydro-distillation. The predominant constituent of coriander is linalool which forms approximately two-thirds of the oil (Jensen, 1981; Goock, 1977). The leaf oil contains 44 compounds mostly of aromatic acids of which the major is 2-decenoic acid (30.82%), linalool (coriandrol) (13.97%), E-11-tetradecenoic acid (13.4%), capric acid (12.7%), tridecanoic acid (5.5%) and undecanoic acid (7.1%) (Telsi *et al*., 2006; Simon & Quinn, 1988). *C. sativum* seed oil contains linalool as the major constituent (60-70%) and 20% terpenes (Yang *et al*., 2004, Ghani, 2003; Yusuf *et al*., 1994; Coleman & Lawrence, 1992). Many aromatic plants and spices, especially *C. sativum* seeds and leaves essential oils have been known to support various biological activities such as antimicrobial, antifungal and antioxidant properties (Wangensteen *et al*., 2004). Generally, the antioxidant activities of non-volatile extracts of coriander leaves were found to be higher than those of the seeds which might be due to the presence of high phenolic content (Guerra *et al*., 2005).

Synergistic activity of coriander oil when mixed with conventional antibiotics against *Acinetobacter baumannii* showed that coriander essential oil with ciprofloxacin, gentamicin and tetracycline against *A. Baumannii* was effective indicating a possible synergistic interaction (Duarte *et al*., 2012). Shahwar *et al.* (2012), in their study on antifungal activity of *C. sativum* essential oil against candida species and potential synergism with amphotericin B found that a synergistic effect between coriander oil and amphotericin B was obtained for *Candida albicans* strain while for *C. tropicalis* only an additive effect was observed. Evaluation of synergistic antibacterial and antioxidant efficacy of essential oils have also shown that coriander/cumin combination had synergistic interaction against bacteria (Anwesa & Ranjan, 2015).
In insecticidal activity, linalool has been found to create a synergistic effect with the codling moths' pheromone, codlemone which increases attraction of males (Yang *et al*., 2004). Also, it has been used in some mosquito repellent products (South China Morning Post, 2015) however, EPA has not supported this claim to repel mosquitoes as the sole active ingredient (EPA Linalool Summary Registration Review, 2007).

Nutmeg, *Myristica fragrans* (family Myristicaceae) is a tropical evergreen tree that is native to the islands of Indonesia and cultivated in the West Indies. The seeds produce

a spice that has distinctive pungent fragrance and a slightly sweet taste and is used to flavour many kinds of foods. Nutmeg trees may reach upto a height of 20 metres. The fruit is a pendulous drupe and when fully mature it splits into two exposing a crimson coloured aril surrounding a single brown seed, the nutmeg. The nutmegs are dried gradually in the sun during which the nutmeg shrinks away from its hard seed coat. Dried nutmegs are greyish brown ovals with furrowed surfaces (Jbilou & Sayah, 2006).

The name nutmeg is also applied in different countries to other fruits and seeds: - the Jamaican or calabash nutmeg is derived from *Monodora myristica* (family Annonaceae), Brazillian nutmeg from *Crytocarya moschata* (family Lauraceae), Peruvian nutmeg from Laurelia aromatic (family Atherospermataceae), the Madagascar or clove nutmeg from Ravensara aromatic (family Lauraceae) and Carlifonian or stinking nutmeg from *Torreya carlifornica* (family Taxaceae) (Vieira *et al*., 2017)).

Nutmeg contains 7 to 14% essential oils, the principal components which are volatile terpenes and phenylpropanoids, including d-pinene, limonene, geraniol, safrole and myristicin (Abourashed & El-Alfy, 2016; Piras *et al*., 2012). Nutmeg on extraction yields 24 to 34% fixed oil, nutmeg oil, the principal component being myristicin or methoxysafrole. Myristicin also occurs in essential oil from other seasonings plants like dill or parsnip (Sousa *et al*., 2015) and in parsley (Simon & Quinn, 1988) which has been found to contain insecticidal and synergistic components for carbamates, organophosphates, pyrethroids and pyrethrins (Joffe, 2012). Myristicin isolated from edible parts of parsnips (family Umbelliferae) was found to have insecticidal and synergistic activity on several insect species (Lichtenstein & Casida, 1963). Myriticin was found both in dill greens and roots while apiol and dill-apiol were major insecticidal components of dill roots (Okosum & Adedire, 2010). Tests with myristicin (one methoxy group) and apiol (two methoxy groups) on the fruitfly, *D. melanogaster* found that apiol was more toxic than myristicin and also showed more pronounced synergistic activity of parathion compared to mysisticin (Bett *et al*., 2016; Oppert *et al*, 2015) though in its pure form, Rahman *et al* (2015) also found myristicin as a toxin.

2.7 Insecticide toxicology bioassays and methods

Insecticide bioassay refers to any quantitative procedure used to determine the relationship between the amount (dose or concentration) of an insecticide administered

and the magnitude of response in a living organism. If the toxic effect e.g death indeed results from the action of an insecticide, there must be a positive correlation between the appropriate range of the insecticide dose administered and the magnitude of the effect. Such relationship is known as dose-response relationship and is fundamentally important in insecticide bioassay (Norris *et al*., 2019; Finney, 1971).

Insecticide bioassay with insects or arthropods often is used to estimate the median lethal dose (LD_{50}) or concentration (LC_{50}) and its associated 95% confidence intervals (95% CI) from a dose response model. The LD_{50} or LC_{50} is the lethal dose concentration required to kill 50% of a given population or strain under specified conditions (Finney, 1971).

A synthesized insecticidal chemical prior to formulaions is refered to as technical grade compound often with very high purity. The essential component of the technical grade is the active ingredient (a.i) that exerts toxic actions on an organism. Technical grade insecticides are not often used directly for pest control because their physical and chemical properties are not suitable for commercial use. Therefore, technical grade should be brought into more appropriate forms (formulations) for application e.g sprays, powders, granules, fumigants, baits among others.

Insecticide toxicology has been widely studied extensively, primarily in laboratory experiments designed to measure susceptibility of chemicals or to define the metabolic fate of insecticides applied to the insect (Gao & Zhu, 2000). Laboratory investigations provide an understanding of insect-insecticide interaction and many chemicals can be tested rapidly within a short period of time. Controlled conditions allow definitive interpretation of data (Tarcasio *et al*., 2011).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Experimental Site

The experiments were carried out at Chuka University Chemistry and Plant Sciences laboratory, Kenya. Chuka University is located near Chuka town in Tharaka Nithi County approximately 186 km from Nairobi along Nairobi-Meru highway on the eastern slopes of Mt. Kenya at an altitude of about 2000 m above sea level. Temperatures range from 16-24 ^{0}C and an average annual rainfall of 1,000 mm. All the bioassays were maintained throughout under controlled storage experimental growth chamber conditions of 27 ± 2 ^{0}C and 60 ± 5% RH with normal day light hours.

3.2 Experimental Design

Completely Randomised Design (CRD) was used to set ut the experiments to determine *in vivo* the lethal concentration (LC) values for pyrethrins on *S. zea-mais*. Pyrethrins at ten concentrations and time of exposure at three levels (24 h. 48 h. 72 h) were assessed.

To determine *in vivo* the effect of the selected plant extracts on *S. zea-mais*, a 7x4x3 factorial experiment laid out in a CRD and replicated three times was used. The

treatments were plant extracts at eight (8) levels (6 plant extracts, PBO and acetone), concentrations at four levels (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) and time of exposure at three levels (24 h, 48 h and 72 h). A sample of the layout is outlined in Table 2

To determine the potency of plant extracts-synergised pyrethrins formulations at different rates and concentrations on stored maize against *S. zea-mais,* a 3x4x4x7 factorial experiment was laid out in a CRD and replicated three times. The synergists were seven (6 plant extracts and PBO), concentrations at four levels (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm), formulations at four ratios (1:1, 2:1, 3:1 and 4:1) and time of exposure at three levels (24 h, 48 h and 72 h)

Table 2: Layout for Experiment to Determine *in vivo* the Effect of the Selected Plant Extracts on *S. zea-mais*

Concentration (ppm)		Potential synergists/PBO/solvent											
		Synergist A			Synergist B			PBO			solvent		
		R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
C1	24 h	C_IR_I	C_1R_2	C_1R_3									
	48 h	C_1R_3	C_IR_3	C_1R_1									
	72 h	C_1R_2	C_1R_1	C_1C_2									
C2	24 h	C_2R_3	C_2R_2	C_2R_1									
	48 h	C_2R_1	C_2R_3	C_2R_2									
	72 h	C_2R_2	C_2R_1	C_2R_3									
C3	24 h												
	48 h												
	72 h												
C4	24 h												
	48 h												
	72 h												

*The sample layout using two concentrations of synergist A (representing a plant extract) was replicated for all the other extracts and concentrations, including PBO and acetone

Table 3: Layout for Experiment to Determine the Potency of Plant Extracts-Synergised Pyrethrins Formulations at Different Rates and Concentrations on Stored Maize against Maize Weevil, *S. zea-mais*

Synergist	Conc. ppm	Time	Formulation (synergist: pyrethrins)			
			Ratio 1:1	Ratio 2:1	Ratio 3:1	Ratio 4:1

			R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
A	1,000 ppm	24 h	F1	F2	F3	F2	F1	F3	F1	F2	F3	F1	F2	F3
		48 h	F3	F1	F2	F1	F3	F2	F3	F1	F2	F3	F1	F2
		72 h	F2	F3	F1	F3	F1	F3	F2	F3	F1	F2	F3	F1
	5,000 ppm	24 h												
		48 h												
		72 h												
	10,000 ppm	24 h												
		48 h												
		72 h												
	20,000 ppm	24 h												
		48 h												
		72 h												
B														

*F represents formulation done at a concentration of 1,000 ppm at the four ratios while while the number on the F represent the replicate. The sample layout shown was used in all concentrations and ratios under study.

3.3 Experimental Materials

3.3.1 Experimental maize

Freshly harvested shelled and susceptible maize variety H512 (5x90kg bags) was procured from Kenya Agriculture and Livestock Research Organization (KALRO), Katumani Research Institute, Kenya. Dust and debris of shelled maize grains were removed by sieving using a 6.0 mm sieve. The cleaned maize was then dried in the sun until the moisture content was less than 13% then transferred into a storage chamber free from insecticides and storage pests.

3.3.2 Pyrethrins

Pyrethrins, of technical grade solutions of 50% (w/w) were sourced from Pyrethrum Processing Company of Kenya (PPCK) Ltd, Nakuru, Kenya. Dilutions of the pyrethrins were prepared to obtain different concentrations of pyrethrins, 100 ppm (0.01%), 1,000 ppm (0.1%), 2,500 ppm (0.25%), 5,000 ppm (0.5%), 10,000 ppm (1%), 20,000 ppm (2%), 25,000 ppm (2.5%), 50,000 ppm (5%), 100,000 ppm (10%) and 200,000 ppm (20%). A conversion of 0.0001% = 1 ppm was used for this study. To obtain these concentrations the formula $C_1V_1 = C_2V_2$ was used where C_1 = 50% (500,000 ppm), V_1 = volume to be measured, C_2 = required concentration and V_2 = required volume. These concentrations were then tested on the maize weevils to obtain the lethal concentration values. Acetone was used to make solutions of different concentrations for grain treatment.

3.3.3 Synergists

The plant extracts selected to be tested as synergists for pyrethrins, and the standard synergist, PBO used in this study are summarized in Table 2.

The plants used in the study were chosen on the basis of possessing a methylenedioxyphenyl (MDP) ring structure similar to that of the standard synergist, PBO. The plant extracts and oils were prepared for use in this study at the Chemistry Research Laboratory, Chuka University, Kenya and at the Natural Chemistry Laboratory, Egerton University, Kenya.

Table 4: Codes given to Standard Synergist and Plant Extracts Possessing MDP ring tested for Synergistic Potential

Codes of Plant extracts/oils (synergists)	Description of sample	Extract/oil
PBO	Piperonyl butoxide	standard synergist
BPSHE	Black pepper seeds	Hexane extract
BPSME	Black pepper seeds	Methanol extract
CLHE	Coriander leaves	Hexane extract
CLME	Coriander leaves	Methanol extract
CRHE	Coriander roots	Hexane extract
CRME	Coriander roots	Methanol extract
NMHE	Nutmeg seeds	Hexane extract
NMME	Nutmeg seeds	Methanol extract

3.4 Collection of Plant Parts and Extraction

Seeds of *P. nigrum* and *M. fragrans* originally from Kerala, India were obtained from a commercial spice supplier in Nakuru, Kenya. Fresh leaves and roots of *C. sativum* were obtained from Farming Systems of Kenya, Nakuru branch. The plant materials (roots, leaves or seeds) were authenticated at the Botany Department of Egerton University, Kenya. The plant materials were air dried in a well-ventilated room temperature away from direct sunlight to avoid any decomposition of the compounds present by ultraviolet light. Drying was allowed until a constant weight was obtained so as to enhance maximum extraction of the compounds.

The seeds of *P. nigrum* and *M. fragrans*, dried leaves and roots of *C. sativum* were milled into powder using a Binatone electric blender (BLG-400) fitted with a 2mm sieve. The powders were each, successively extracted using Soxhlet apparatus, initially using analytical grade *n*-hexane (1 L) and followed by extraction using analytical grade methanol (1 L) with each solvent for 24 h. The solvents were evaporated to dryness using a rotary vacuum evaporator (Resona type WB) under reduced pressure. *n*-hexane solvent was used to extract non-polar compounds whereas methanol solvent was used to extract semi-polar and polar compounds. Using these two solvents offers partitioning of compounds to two types of extracts with different polarities. The resulting extracts/oils were air-dried at room temperature to remove excess solvent. The quantities of extracts/oils obtained are given in Table 3. The concentrated extracts/oils were then kept in vials at 4 ^{o}C until ready for use. The plant extracts/oils used as synergists were given codes for easy identification (Table 2). Likewise, the formulation containing either of the plant extracts and pyrethrins was given a code similar to that of the plant extract.

Table 5:Mass of each Extract obtained after Extraction of Plant Parts

Plant part	Mass of powder before extraction (g)	Mass of crude extract (g)
BPSHE	210.62	15.21
BPSME	170.20	13.20.
CLHE	93.49	10.71
CLME	82.89	12.24
CRHE	10.11	3.79
CRME	4.59	0.09
NMHE	72.30	9.78
NMME	57.11	7.31
PBO	N/A	N/A

Dilutions of the plant extracts were prepared to obtain different concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm, 20,000 ppm) of each synergist. 20 mls of each concentration was prepared by weighing the required weight of the extract using a weighing balance and then transferred into 50 mls vials. Approximately 20 mls of acetone was measured using a measuring cylinder to dissolve plant extracts. The weights used were 0.002 gm, 0.01 gm, 0.02 gm and 0.04 gm which yielded concentrations of 1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm respectively.

However, plant extracts CRME and NMME had low solubility in petroleum hydrocarbons and therefore were not included in the bioassays.

3.5 Rearing of Insects

Maize weevil parent stock was supplied by National Agriculture Research Laboratory (NARL), Nairobi, Kenya. Cultures of the maize weevil were established to supply similar aged weevils for the experiments. About 25 kilograms (kg) grain of untreated maize variety H512 were cleaned to remove grains with visible damage. Cleaned grains were then dried in the sun until 13% moisture content was attained.

Maize weevils were cultured on the clean disinfected maize grains in 14 jars, each jar with 1.5L capacity, 500 gms maize grains were put into the jars. 50 Unsexed adult maize weevils were introduced into each of the seven (7) jars of grain. The jars were then covered with muslin cloth and fixed with a rubber band to allow for aeration and prevent escape of insects. After seven days (period allowed for oviposition), all parent insects were removed from each jar by sieving using a 6.0 mm sieve and placed on the other seven jars with grains and kept at the same conditions. Removal of parent insects and placement on a fresh maize medium was repeated until sufficient numbers of laboratory reared weevils were obtained. The jars were kept at the experimental growth chamber maintained at a constant temperature of $27 \pm 2\ ^{0}C$ and $60 \pm 5\%$ RH with normal day light hours.

3.6 Data Collection

Four types of bioassays were used to collect data in this study: full dose response, Dose-mortality biossay, synergism bioassays and infestation on treated maize.

3.6.1 To Determine in Vivo the Lethat Concentration (LC) Values for Pyrethrins on *S. zea-mais*

A method previously described by ffrench-Constant & Roush (1990) and Finney (1971) was used in this bioassay. To determine dose response for pyrethrins, ten pyrethrins dosages (100 ppm, 1,000 ppm, 2,500 ppm, 5,000 ppm, 10,000 ppm, 25,000 ppm, 50,000 ppm, 100,000 ppm and 200,000 ppm) diluted in acetone were each applied on the maize weevils separately. Technical grade (50% w/w) pyrethrins were used to make serial dilutions as described in section 3.3.1 for the bioassays.

Batches of 10 newly hatched unsexed adult maize weevils aged between 7-14 days were selected for the treatments. The adult weevils were obtained by sieving from the maize cultures into a petri-dish, covered with a muslin cloth and placed in a freezer (-20^{o}C) for 10 minutes to immobilize them. The pyrethrin dosages were applied separately on the dorsal part of the thorax of each test insect using a hand operated 10-µL topical applicator to dose each insect with 1µL of pyrethrin dosage (Plate 1). Dosages were done in a geometric progression from the lowest concentration to the highest.

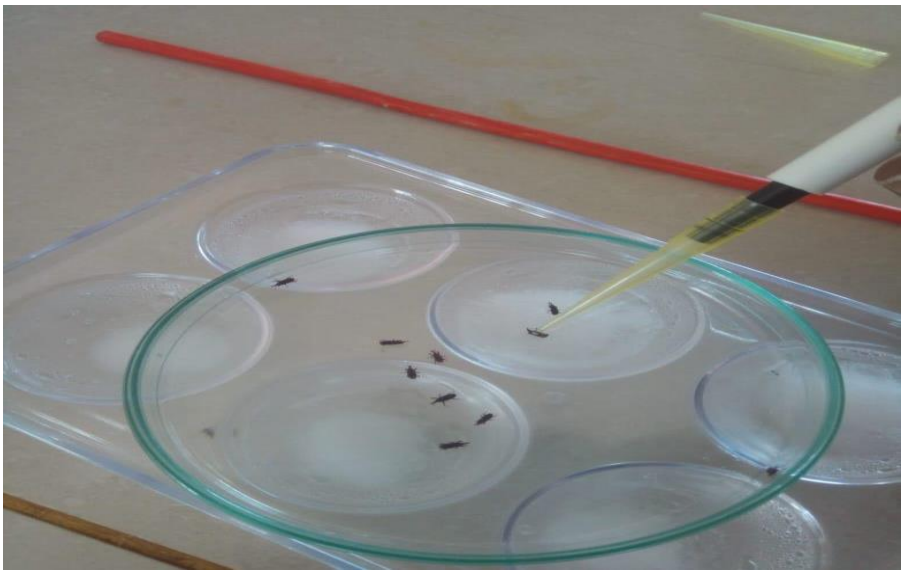

Plate 1: Topical Application of Dosages on Maize Weevils

After each dose, test insects were transferred to 250 ml plastic containers with sufficient quantity of food (fresh maize) and covered with muslin cloth held in place by rubber bands to allow ventilation. The containers were then kept in a recovery growth chamber maintained at a constant temperature of 27 ± 2 ^{0}C and 60 ± 5% RH with normal daylight hours (Plate 2). For pyrethrins treatments, mortality was assessed after 24 h, 48 h and 72 h exposure period.

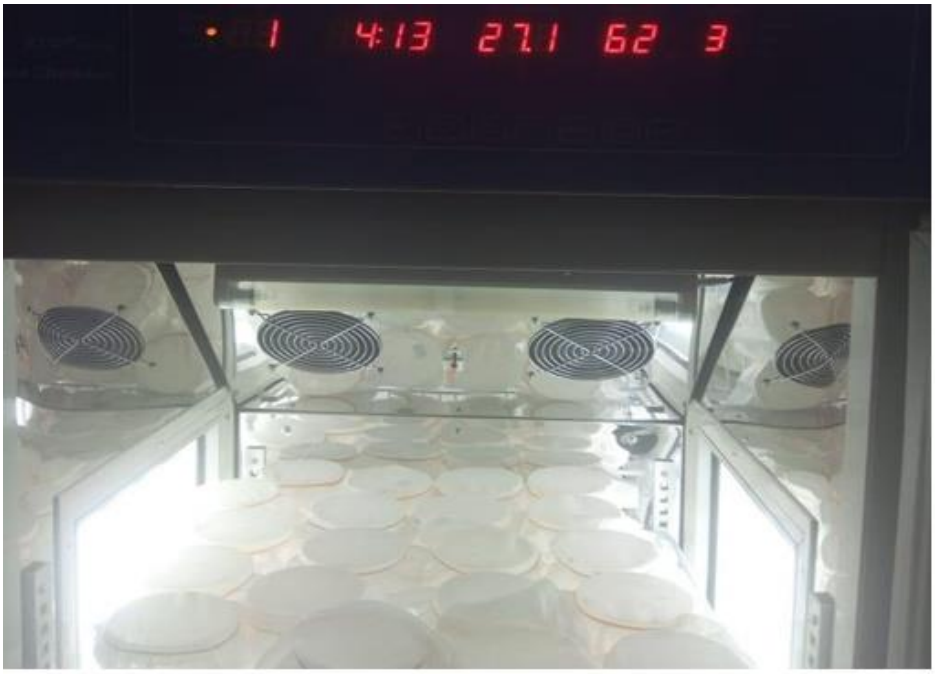

Plate 2: Experimental Growth Chamber Maintained at 27 ± 2 ^{0}C and 60 ± 5% RH

Insects were considered dead if they were incapable of moving when probed with a fine forceps at the abdomen (i.e two subsequent touches in one-minute interval) were counted as dead (Plate 3). Three replications were done for all concentrations.

The data was analysed with probit analysis (Finney, 1971) to determine the lethal concentration (LC) of pyrethrins to the maize weevils. The probits obtained were used to calculate lethal concentration (LC_{10} to LC_{95}) values, giving the concentration required to kill 10% to 95% of the test population respectively. From this data, the LC20 was used as the discriminating dose for synergism bioassays. This was used as a fixed concentration of pyrethrins that was formulated with potential synergists, in order to assess their efficacy on *S. zea-mais.*

Plate 3: Direct Counts of Dead Maize Weevils

3.6.2 To Determine *in vivo* the Effect of the Selected Plant Extracts on *S. zea-mais*

Dose-mortality bioassays were conducted to determine the toxicity of plant extracts to *S. zea-mais*. The accurate quantification of toxicity requires that a known quantity of toxicant is applied onto the test insect (Ribiero *et al*, 2016). *S. zea-mais*was exposed to a four concentrations of each potential synergists to ascertain their toxicity before formulating with pyrethrins.

These bioassays followed procedures previously described by Viteri Jumbo *et al*,(2014). Prepared concentrations of the plant extracts were applied by topical application as described in section 3.6.1. Four concentrations of each plant extract (e.g BPSHE; 1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) were used in the bioassays. As a positive control, the standard synergist, PBO was used also at the concentrations specified. Acetone was included in the bioassay since it was used as the solvent in preparation of the concentrations. Three replications were done concentration and the control treatments.

3.6.3 To Determine the Potency of Plant Extracts-Synergised Pyrethrins Formulations at Different Rates and Concentrations on Stored Maize against *S. zea-mais.*

These were done using discriminatory dose-mortality bioassays of *S. zea-mais*using mixtures of pyrethrins plus synergists. A concentration of 2,200 ppm pyrethrins were chosen as discriminating doses equivalent to approximately LC20 of pyrethrins. Low

LC-values with the active ingredient alone were chosen so that the synergistic abilities of the plant extracts/oils (synergists) could be compared. PBO, the standard synergist was used as the control.

Synergism bioassays followed a previously described method (Ribiero *et al*., 2003). Here, formulations were prepared to contain the synergists and the discriminatory dose of pyrethrins (2,200 ppm) at four ratios of synergist: pyrethrins i.e 1:1, 2:1, 3:1 and 4:1 respectively for each concentration of the synergists (Plate 4). The standard formulation with PBO is commonly at a ratio of 4:1 for commercial insecticides thus lower ratios of synergists were chosen to compare efficacies of the formulations. Formulations with PBO were used as controls. Prepared concentrations of the plant extracts and PBO in their respective ratios were applied by topical application as described in section 3.6.1. Three replications were done for all the formulations.

Plate 4: Formulations at Different Ratios of Synergists: Pyrethrins

3.6.4 To Evaluate the Efficacy of Plant Extracts Synergised –Pyrethrins Formulations treated Maize on Infestation by *S. zea-mais*

The method by Derera *et al*. (2001) was adopted. Briefly, 500 gm of maize were placed in separate 1.5 litre glass jars. The 500gm lot in each jar were treated with formulations as described in 3.6.3. PBO formulation in the ratio 4:1 served as positive control and distilled water as negative control.

The jars holding test maize and controls were closed with metal lids then briefly shaken and tumbled mechanically in a tumbling drum for 10 minutes to mix the maize with treatment. 100gm of maize from each jar were then placed in three separate 250ml plastic containers. Thirty newly emerged unsexed adults of maize weevils were introduced to the 250ml plastic containers with various treatments to infest the 100gm grains. They were then kept for seven days for oviposition to take place. After introduction of weevils the plastic containers were covered with muslin cloth and placed in a recovery growth chamber maintained at a constant temperature of 27 ± 2 ^{0}C and 60 ± 5% RH with normal daylight hours. The treatments were arranged in a CRD with three replications.

Mortality was assessed **7** days after introduction of the test insects. All insects were removed, dead and live ones counted and recorded. Insects that were unable to move when prodded with a fine forceps at the abdomen were considered dead.

3.6.5 Synergism Calculations

Synergy of plant extracts and pyrethrin formulations was obtained using a previously established method (Mansour *et al*., 1966) where the co-toxicity factor was calculated using the following equation:

$$\text{Co-toxicity factor} = \frac{(\text{Observed \% mortality} - (\text{Expected \% mortality}}{(\text{Expected \% mortality})} \times 100$$

Where expected mortality was calculated as the sum of the average percentage mortalities achieved by pyrethrins at LC20 and the plant extracts. The co-toxicity factor was used to assess whether a plant extract could be antagonistic, additive or synergistic within the formulations compared with the individual components. Values lower than -20 suggest an antagonistic relationship, values between -20 and 20 suggest an additive character and values greater than 20 suggest synergism.

3.7 Data Analysis

Both descriptive and inferential statistics were used in the data analysis. The former was done through computation of mean and standard error on response variable. Analysis of Variance (ANOVA) was used to test the significant difference of the mean mortality of maize weevils at 5% significance level. Duncan's Multiple Range Test

(DMRT) was used to rank the mean mortalities of maize weevils between the concentrations where significant $P < 0.05$ results were obtained while Co-toxicity factors were used to determine whether a plant extract was a synergist, antagonist or an additive. Data analysis was done via Statistical Package for Social Scientist (SPSS) and Microsoft Excel and the report was done on Microsoft Word.

3.8 Ethical Considerations

The information contained in this thesis was cited appropriately and represented as it is to avert plagiarism. The research clearance and approval was granted by Chuka University Ethics Review committee (Appendix XXII) and by the National Commission for Science and Technology (Appendix XXIII and XXIV). The results presented in this study are based on the true findings of the research carried out.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Determination *in vivo* the lethal concentration (LC) Values for Pyrethrins on *S. zea-mais.*

The results of exposure of *Sitophilus zea-mais* to unsynergised pyrethrins at a range of concentrations by topical application under standardized conditions are presented in Tables 6, 7 and 8. The number of insects killed was recorded over time (24 h, 48 h and 72 h). The ratio of the number of insect deaths to that of the insects exposed gave the percentage of dead insects at a particular pyrethrin concentration which was subjected to probit analysis to obtain the percentage mortality. From these results, the lethal concentration (LC) values for pyrethrins were obtained (LC_{10} to LC_{95}). These were used to determine the LC20 (concentration that kills 20% of the *S. zea-mais*) which was found to be 2,200 ppm thus this pyrethrins concentration was used for formulations with the plant extracts in the discriminatory dose bioassays.

Table 6: Toxicity of Pyrethrins to *S.zea-mais* 24 h after Exposure

Pyrethrins conc. (ppm) (X)	Log conc. X	Response (Y)	Z-Score	Percentage kill (P)	No. of insects (n)	Observed deaths
100	2.000	1.275	-2.24	1.2	10	0

1,000	3.000	4.437	-1.19	11.7	10	1
2500	3.398	5.695	-0.77	22.1	10	2
5,000	3.699	6.647	-0.45	32.6	10	4
1,0000	4.000	7.599	-0.13	44.8	10	4
20,000	4.301	8.551	0.18	57.1	10	5
25,000	4.398	8.857	0.29	61.4	10	4
5,0000	4.699	9.809	0.60	72.6	10	5
1,00000	5.000	10.761	0.92	82.1	10	6
20,0000	5.301	11.713	1.24	89.3	10	8

Mean=4 and SD=2 ; Y=-5.049+3.162Log(X)

Table 6 indicates the observed deaths and predicted percentage adult mortality of *S. zea-mais*after 24 h exposure to unsynergised pyrethrins. The percentage kill (P) obtained show that an increase in pyrethrins concentration corresponded to an increase in mortality of *S. zea-mais*. For instance, at 2500 ppm a response adult mortality of 5.7 which is approximately 6 adult weevils obtained which translates to mortality of 22.1% in a natural population. This percentage is slightly higher than that of observed deaths (20%) because in a predicted model, it assumes deaths in a natural population whereas in observed deaths, it was based on a sample of ten (10) weevils. Similarly, a case of higher pyrethrins concentration of 20,000 ppm corresponded to a predicted adult mortality of approximately twelve (12) adult weevils which translated to 89.3% deaths in a natural population. This value is slightly higher compared to 80% of observed deaths in an empirical experiment of a sample size of 10 weevils.

The observed and predicted adult mortality of *S. zea-mais adults* after 48 h exposure to unsynergised pyrethrins are shown in Table 7.

Table 7: Toxicity of Pyrethrins to *S.zea-mais* 48 h after Exposure

Pyrethrins conc. (ppm) (X)	Log conc. X	Response (Y)	Z-Score	Percentage kill (P)	No. of insects (n)	Observed deaths
100	2.000	0.135	-2.29	1.1	10	1
1,000	3.000	3.383	-1.21	11.3	10	2
2500	3.398	4.675	-0.77	21.8	10	4
5,000	3.699	5.653	-0.45	32.6	10	7
1,0000	4.000	6.631	-0.12	45.2	10	7
20,000	4.301	7.609	0.20	57.9	10	5
25,000	4.398	7.923	0.31	62.2	10	10
5,0000	4.699	8.901	0.63	73.6	10	10
1,00000	5.000	9.879	0.96	83.1	10	10
20,0000	5.301	10.857	1.29	90.1	10	10

Mean=7 and SD=3; Y=-6.361+3.248Log(X)

The study showed that increasing exposure time translated to an increase in percentage mortality of insects tested in both the observed and probit analysis but upto a pyrethrins concentration of 25,000 ppm for the observed deaths. In this case it was found that the percentage kill from probit model continued to increase with an increase in the concentration of pyrethrins. For instance, 25,000 ppm pyrethrins concentration gave a percentage kill of 62.2% which represents a natural population of insects while in the experiment (observed deaths) it accounted for 100% mortality. This variance is due to the large population of insects in nature where it is not possible to individually dose all the insects unlike in the empirical experiment (Finney, 1971).).

The observed deaths and predicted percentage adult mortality of *S. zea-mais*after 72 h exposure time (Table 8. The results showed that longer exposure time increased percentage mortality of the maize weevils. This was realized across all the concentrations tested except for the experiments where the number of observed deaths stood at 100% mortality after 25,000 ppm pyrethrins concentration.

Table 8: Toxicity of Pyrethrins to *S. zea-mais*72 h after Exposure

Pyrethrins conc. (ppm) (X)	Log conc. X	Response (Y)	Z-Score	Percentage kill (P)	No. of insects (n)	Observed deaths
100	2.000	-0.436	-2.22	1.30	10	1
1,000	3.000	1.755	-1.12	13.10	10	2
2500	3.398	2.627	-0.69	24.50	10	6
5,000	3.699	3.287	-0.36	35.90	10	8
1,0000	4.000	3.946	-0.03	51.20	10	9
20,000	4.301	4.605	0.30	61.80	10	9
25,000	4.398	4.818	0.41	65.90	10	10
5,0000	4.699	5.478	0.74	77.00	10	10
1,00000	5.000	6.137	1.07	85.80	10	10
20,0000	5.301	6.796	1.40	92.50	10	10

Mean=8 and SD=3 ; Y=-4.818+2.19Log(X)

The results suggest that exposure duration is critical in determining mortality rates of the maize weevils. In this case, for instance, at a concentration of 10,000 ppm, the mortality rate recorded in the probit model was 44.8%, 45.2% and 51.2% at 24, 48 and 72 h exposure duration respectively. This implies that the mortality of the maize weevils

exposed to unsynergised pyrethrin is time dependent, that is, the longer the time (more than 24 h) after exposure of insecticide the more the deaths.

Probits plotted against the pyrethrins concentration (ppm) to obtain LC values that kill between 10% and 95% of adult S. zeamais are presented in Figure 3. The estimated LC values are shown in Table 7. LC20 was found to be 2200 ppm (concentration of pyrethrins required to kill 20% of *S. zea-mais*population). The low LC value was used for the discriminatory dose bioassays to ensure low percentage mortality with unsynergised pyrethrins and allow for the observation of synergistic activity by the potential synergists.

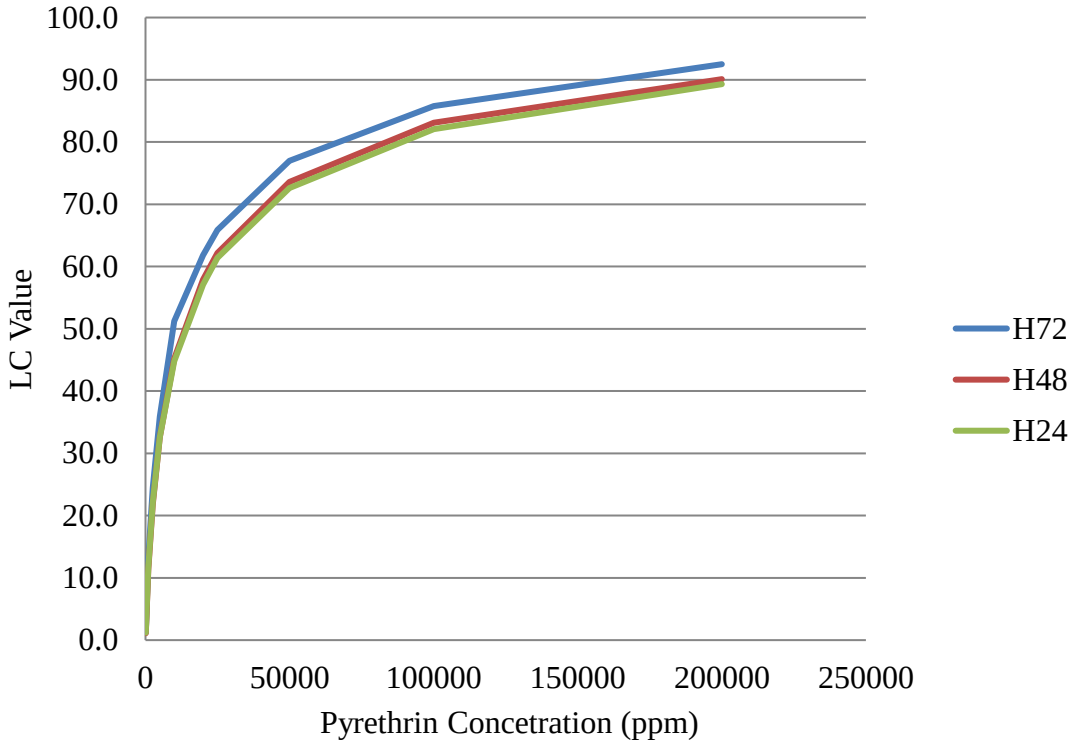

Figure 3: Dose-Response Curve of Pyrethrins on *S. zea-mais* Mortality after Topical Application 24 h, 48 h and 72 h exposure time.

Table 9: The Lethal Concentration Values (LC) for Pyrethrins obtained from Full-dose Response Bioassays Against *S. zea-mais* (adults) 24 h, 48 h and 72 h exposure.

LC value	Pyrethrins dose (ppm)		
	24 h	48 h	72 h
LC_{10}	854	885	764
LC20	2197	2243	1908

LC_{30}	4381	4398	1306
LC_{40}	8032	7537	6340
LC_{50}	14227	13780	8868
LC_{60}	23372	22442	17805
LC_{70}	44196	42105	34234
LC_{80}	88987	83684	67046
LC_{90}	209772	198571	162687
LC_{95}	297166	270000	237313

The LC20 of pyrethrins was successfully determined in this study in order to later evaluate the synergistic potentials of plant extracts/oils and compare with that of the standard synergist PBO. Two aspects were tested: LC values and time of exposure. In general, the LC values obtained followed the same trend from values obtained in literature (Finney, 1971; Joffe, 2012). Ten concentrations of pyrethrins and more than 300 unsexed adult maize weevils were used to calculate the LC values. Probit analysis model used sufficiently described the dose-response data of pyrethrins to the maize weevils. It was found that the number of adult mortality continued to increase with an increase in concentration of pyrethrins. For instance, pyrethrins at 25,000 ppm concentration accounted for 62.2% mortality in a natural population of insects while in the experiment, it accounted for 100% mortality. This variance is due to large populations of insects in nature where it is not possible to individually dose all the insects unlike in an empirical experiment (Ribiero *et al*., 2003; Finney, 1971).

The time of exposure to the serial concentrations was considered in the present study. The pyrethrins concentration required to kill a certain percentage of the maize weevil reduced when exposure time was prolonged to 72 h period. This was necessary to find out if time of exposure affected the efficacy of pyrethrins. It was found that longer exposure time enabled pyrethrins to sufficiently interact with the insect and as a result higher mortalities were observed with time. This could be due to the fact that the maize weevil's body is highly sclerotized and the elytra is rigid thus more time could be needed for penetration of the insect's cuticle and subsequent toxicity. The hardened nature of the cuticle may dictate the rate at which the plant extracts or even an insecticide penetrates the cuticle hence the necessity of exposure duration. As a result, it was found that lower concentrations of pyrethrins were needed to achieve higher percentage mortality with prolonged exposure. For instance, to obtain LC_{50}, 14227

ppm, 13780 ppm and 8868 ppm of pyrethrins was required over 24 h, 48 h and 72 h exposure time respectively.

Srivastava *et al* (2008) also found that larvae of *Spilarctica oblique* fed with single dose of myristicin in diet mix bioassay resulted in low mortality after 24 h but with increased duration of time to 72 h the same single dose gave complete mortality after 72 h. Allowing sufficient time for pyrethrins to interact with the maize weevil can be cost effective and reduce levels of contamination through residues on stored maize since use of lower concentrations and quantity can be used to achieve significant control of this pest. Therefore, when formulating using pyrethrins there is need to clearly indicate exposure time on the pesticide labels in order to reduce or prevent over dose of insecticide that can result to other problems like resistance.

4.2 Determination *in vivo* of the Effect of Plant Extracts/Potential Synergists on the *S. zea-mais*

The study sought to determine the effect of plant extracts on *S. zea-mais*. The mean percentage mortality rate (± S. E) of *S. zea-mais*(adults) treated topically with potential synergists, PBO and acetone 24, 48 and 72 h after treatment are presented in Table 10 and appendices I, II &III

It was found that after 24 h exposure (Table 10 and Appendix I) the mean difference of percentage deaths of BPSME and CLHE were both statistically significant ($P \leq 0.05$) ($P = 0.015$ and $P = 0.017$ of BPSME and CLHE respectively). This implied that as the concentration of the extracts increased, higher percentage mean mortality in both BPSME and CLHE also increased. CLHE had higher average mortality 16.67 ± 3.33% mortality compared with BPSME with an average mortality rate of 13.33 ± 3.33% at synergist concentration of 20,000 ppm. BPSME at 1,000 ppm was shown to be statistically different from 20,000 ppm while 5,000 ppm and 10,000 pm were not statistically different according to DMRT. In CLHE, concentration at 20,000 ppm ranked differently from the lower concentrations tested (1,000 ppm, 5,000 ppm and 10,000 ppm) which were not statistically different.

PBO and the plant extracts BPSHE, CLME, NMHE and CRHE were not statistically significant ($P \leq 0.05$). These extracts showed no difference in mortality means with

increase in their concentration. For instance, toxicity of BPSHE at 20,000 ppm, one could achieve the same results as at 10,000 ppm (average mortality 6.67 ± 0.33%). Similarly, in the case of CLME, the percentage mortality at 5,000 ppm was 10.00 ± 3.77% compared to percentage mortality of 3.33 ± 0.133% at 10,000 ppm and 20,000 ppm making the mean mortality difference statistically insignificant ($P > 0.05$) in terms of concentration levels under study. At 1,000 ppm, PBO, acetone and most plant extracts except CLME and NMHE were not toxic to *S. zea-mais.* And the solvent, acetone was not toxic the entire duration thus toxicity observed were due to the extracts.

Table 10: Mean percentage mortality of *S. zea-mais* Adults Treated Topically with Potential Synergists, PBO and Acetone (control) 24 h, 48 h and 72 h after Treatment

24 h EXPOSURE																
Synergist concentration	Mortality (%)												PBO		Acetone	
	BPSHE		BPSME		CLME		CLHE		NMHE		CRHE					
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
1,000 ppm	0.00[a]	0.000	0.00[a]	0.000	6.67[a]	0.333	0.00[a]	0.000	6.67[a]	0.333	0.00[a]	0.000	0.00[a]	0.000	0.00	0.000
5,000 ppm	3.33 [a]	0.133	6.67[ab]	0.667	10.00[a]	3.774	3.33[a]	0.133	3.33[a]	0.133	6.67[a]	0.333	0.00[a]	0.000	0.00	0.000
1,0000 ppm	6.67 [a]	0.333	10.00[ab]	0.000	3.33[a]	0.133	6.67[a]	0.333	6.67[a]	0.333	0.00[a]	0.000	3.33[a]	0.133	0.00	0.000
20,000 ppm	6.67 [a]	0.333	13.33[b]	3.333	3.33[a]	0.133	16.67[b]	3.333	6.67[a]	0.333	-	-	6.67[a]	3.333	0.00	0.000
$P_{(0.05)}$values	0.363		0.015		0.627		0.017		0.931		0.079		0.219		N/A	
48 h EXPOSURE																
Synergist concentration	Mortality (%)												PBO		Acetone	
	BPSHE		BPSME		CLME		CLHE		NMHE		CRHE					
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
1,000 ppm	13.33[ab]	3.333	26.67[a]	3.333	16.67[a]	3.333	3.33[a]	3.333	16.67[a]	3.333	6.67[a]	0.330	6.67[a]	0.333	6.67[a]	0.330
5,000 ppm	6.67[a]	0.333	16.67[a]	3.667	10.00[a]	5.774	10.00[ab]	5.774	13.33[a]	3.333	13.33[a]	3.333	3.33[a]	0.133	6.67[a]	0.330
1,0000 ppm	10.00[a]	0.000	23.33[a]	4.133	6.67[a]	0.333	16.67[ab]	3.333	16.67[a]	4.819	23.33[a]	4.133	10.00[ab]	0.000	6.67[a]	0.330
20,000 ppm	20.00[b]	0.000	26.67[a]	3.343	13.33[a]	3.333	23.33[b]	4.333	20.00[a]	5.774			20.00[b]	5.774	6.67[a]	0.330
$P_{(0.05)}$values	.021		0.389		0.4		0.041		0.878		0.115		0.04		1.00	
72 h EXPOSURE																
Synergist concentration	Mortality (%)												PBO		Acetone	
	BPSHE		BPSME		CLME		CLHE		NMHE		CRHE					
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
1,000 ppm	13.33[ab]	3.333	30.00[ab]	0.000	16.67a	3.333	6.67[a]	3.333	16.67[a]	3.333	6.67[a]	0.333	6.67[a]	0.333	10.00	0.000
5,000 ppm	6.67[a]	0.333	20.00[a]	5.774	16.67[a]	3.333	10.00[a]	5.774	13.33[a]	1.333	16.67[a]	3.333	6.67[a]	0.333	10.00	0.000
1,0000 ppm	20.00[ab]	5.774	26.67[ab]	3.343	16.67[a]	3.333	16.67[ab]	3.333	16.67[a]	3.333	23.33[a]	4.133	10.00a	0.000	10.00	0.000
20,000 ppm	23.33[b]	4.133	33.33[b]	3.833	23.33[a]	4.133	26.67[b]	3.343	23.33[a]	4.133			36.67[b]	3.333	10.00	0.000
$P_{(0.05)}$values	.080		0.015		0.441		0.036		0.268		0.115		0.0001		N/A	

*Means within the same column having the same symbol do not differ significantly from one another at p=0.05 test level

The synergists toxicities 48 h. exposure duration PBO, BPSHE and CLHE showed that the mean difference of percentage deaths was statistically significant at 5% significance level (P = 0.04, P = 0.021 and P = 0.041 of PBO BPSHE and CLHE respectively) (Table 10 and appendix II) CLHE had higher average mortality at 10,000 ppm and 20,000 ppm of 16.67 ± 3.33% and 23.33 ± 4.33% respectively compared with PBO and BPSHE each with 10 ± 0% and 20 ± 5.77% average mortalities respectively. PBO and BPSHE performed more or less the same at higher synergist concentrations (10,000 ppm and 20,000 ppm) tested but different at 5,000 ppm mortalities of 6.67 ± 0.33% (BPSHE) and 0% (PBO). Also, the mean percentage deaths in CLHE increased consistently with increase in concentrations being higher than PBO and BPSHE at 5,000 ppm, 10,000 ppm and 20,000 ppm (10.00 ± 5.77%, 16.67 ± 3.33% and 23.33 ± 4.33% respectively). PBO was shown to be the least toxic at lower concentrations (1,000 ppm, 5,000 ppm and 10,000 ppm) followed by BPSHE at all concentrations. BPSHE was the most toxic at 1,000 ppm (13.33 ± 3.33%) followed by PBO (6.67% ± 0.33%) while CLHE was the least toxic (3.33 ± 3.33%). This trend is reversed at higher concentrations where CLHE becomes the most toxic followed by BPSHE at 5,000 ppm (6.67 ± 0.33%) (Appendix II).

However, plant extracts namely; BPSME, CLME, NMHE and CRHE were not significant at 5% significance level. This implied that there was no difference in mortality means with increase in concentration of these extracts. For instance, in BPSME at 1,000 ppm and 20,000 ppm, one could achieve the same results of an average of 26.67%. Similarly, in the case of CLME, the percentage mortality at 1,000 ppm is higher (16.67%) compared to percentage mortality of 6.67% at 10,000 ppm. The increase in average percentage mortality of *S. zea-mais* after 48 h of exposure suggest that plant extracts and PBO were slow in acting on the insects at first or their toxicity might have been contributed by the solvent, acetone which recorded an average percentage mortality of 6.67% mortality since it was used in dissolving the synergists.

It was found that after 72 h exposure (Table 10 and Appendix III), the mean difference of percentage deaths of PBO, CLHE and BPSME were statistically significant $P \leq 0.05$ (P = 0.001, P= 0.036 and P = 0.015 of PBO, CLHE and BPSME respectively). PBO had the higher average mortality of 36.67 ± 3.33% and the most toxic synergist followed by BPSME (33.33 ± 3.83%) and CLHE (26.67 ± 3.43%) at a concentration of 20,000

ppm. At 1,000 ppm, both CLHE and PBO had percentage mortality of 6.67 ± 3.33% while BPSME was the most toxic (30.00%). BPSME toxicity was generally slightly higher in all the concentrations under study. PBO at 1,000 ppm and 5,000 ppm registered a percentage mortality of 6.67 ± 3.33% same as that of CLHE at 1,000 ppm. These plant extracts could be utilized at the respective concentrations in pyrethrins formulaions. However, PBO was shown to be more toxic (36.67 ± 3.33%) to the insects than CLHE (26.67 ± 3.43%) when tested at 20,000 ppm concentration.

BPSHE, BPSME, CLME, NMHE and CRHE were not statistically significant at 5% significance level as there was no difference in mortality means when the concentration of the extracts was increased. For instance, in CLME at 1,000 ppm, 5,000 ppm and 10,000 ppm the average percentage mortality was 16.67% meaning the synergist concentration did not affect percentage mortality of the test insects. This consistency shows that CLME could be utilized at any concentration as a synergist since it showed minimal variations in percentage mortality of the test insects. Acetone registered a constant percentage mortality of 10% at 48 h exposure time. This percentage could contribute to the the toxicities of the synergists under study but since it registered a constant value, the overall effect would probably not change the observed result

Generally, at lower concentrations (1,000 ppm and 5,000 ppm), toxicity of all synergists tested was low. These percentage mortalities are important when selecting a suitable synergist for pyrethrins Toxicities of plant extracts are often used to form a basis to determine whether it can be a synergist or an additive in an insecticide formulation (Joffe, 2012). If high mortalities have been recorded, it would imply that the compounds contribute to the overall mortalities whenever they are used in formulations of insecticides. At 24 h exposure time, CLHE and BPSME recorded 16.67% and 13.33% mortalities at 20,000 ppm concentration respectively. With increase of exposure time to 48 h and 72 h, CLHE percentage mortality of the maize weevils increased to 23.33% and 26.67% respectively. This consistency of increase mortality of *S zea-mais* in CLHE could indicate presence of particular chemical component in coriander leaves which gets activated with time and could be responsible for the results obtained. Telci *et al* (2006) found that coriander leaf oil contained 44 compounds mostly of aromatic acids and linalool which could be responsible for the observed results (Yang et al, 2004;

Ghani, 2003). Characterization of these compounds may explain which component would be responsible for these results.

BPSHE and PBO after 48 h exposure were statistically significant at 5% significance level with the average percentage mortalities of 10% and 20% at 10,000 ppm and 20,000 ppm respectively while after 72 h, PBO and CLHE were significant. It did not follow that plant extracts that were significant at 24 h exposure performed similarly at 48 h. and 72 h duration. These results imply that the plant extracts tested and statistically significant ($P \leq 0,05$) at a particular exposure time could be used as potential synergists for pyrethrins. CRHE, CLME and NMHE were not statistically significant at all concentrations tested. Though these plants possess the MDP ring structure as the standard, PBO, their varied toxicities show that the components in these plant extracts do not act in the same way. These results suggest that various components in these plants may be acting differently.

Early studies found that most MDP agents themselves possess relatively low intrinsic toxicity but strongly influence the action of other xenobiotics in mammals and insects by modulating cytochrome P-450 thus in insects CYP inhibition by MDP agents underlies their use as pesticide synergists (Murray, 2012). The low average mortalities of the plant extracts tested and statistically significant in this study agree with this finding however, the concentrations at which these plant extracts operate vary from one plant species to another. The exposure time also tends to increase the toxicity of the extracts though some contribution to this toxicity could by due to the solvent.

4.3 Determination of the Potency of Plant Extracts-Synergised Pyrethrins Formulations at Different Rates and Concentrations on Stored Maize against *S. zea-mais.*

The study determined whether percentage of mortality of the test insects subjected to discriminating dose of the plant extracts increased after synergist treatment using four concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) each applied at four ratios namely 1:1, 2:1, 3:1 and 4:1 (synergist: pyrethrins). Results are recorded under each synergist: pyrethrins ratio, concentration and exposure time.

4.3.1 Percentage Mortality of *S. zea-mais* Exposed to Formulations at Ratio 1:1 after 24 h, 48 h and 72 h exposure.

The results of formulations at the ratio of 1:1 synergist: pyrethrins are presented in Table 11. It was found that after 24 h exposure, formulations with PBO and the plant extracts CLHE, NMHE and BPSME were statistically significant at 5% significance level ($P = 0.0126$, $P=0.033$, $P = 0.013$, and $P = 0.009$ of PBO, CLHE, NMHE and BPSME respectively). As the synergist concentration increased, higher percentage mean mortality resulted in the synergist formulations. However, PBO registered higher percentage mortalities than all the synergists under the concentrations tested. For example, at 20,000 ppm, PBO had 83.33 ± 12.02% mortality of *S. zea-mais,* followed by CLHE (46.67 ± 3.33%), BPSME (43.33 ± 6.67%) and NMHE (26.67 ± 3.33%). Ranking of the means revealed that concentration of 20,000 ppm was different from the other lower concentrations in the synergists that were significant ($P < 0.05$) except for NMHE where means at higher concentrations (5,000 ppm, 10,000 ppm and 20, 000 ppm) were different from that at 1,000 ppm with the highest percentage mortality recorded at 10,000 ppm

In addition, the plant extracts when formulated with pyrethrins at this ratio gave significantly higher mortality than unsynergised pyrethrins (20% mortality at 2200 ppm) except BPSME and NMHE at 1,000 ppm (16.67 ± 3.33% and 6.67 ± 3.33% mortality respectively) (Appendix IV). This could mean that there was an antagonistic effect of BPSME and NMHE with pyrethrins that brought the overall mortality below 20%. From these results, PBO and CLHE were shown to be the most effective synergists with significantly higher mortality when applied with pyrethrins than all the other treatments in all the four concentrations tested. However, formulations with CLME, BPSME and CRHE were not significant ($P \leq .0.05$). These extracts did not show differences in average percentage mortality of *S. zea-mais* with regard to increasing concentrations

Plant extracts when applied alone did not show inherent toxicity towards maize weevils at the concentrations tested including PBO and acetone. When formulated with pyrethrins, BPSME, CLHE and PBO had significantly higher mortalities than unsynergised pyrethrins (20% mortality) treatment ($P \leq 0.05$). The increase in mortality could be ascribed to synergistic or additive effects. BPSME and NMHE with 16.67 ±

3.33% and 6.67 ± 3.33% respectively had mortalities below that of unsynergised pyrethrins of 20% mortality rate hence these plant extracts could have some antagonistic effects at 1,000 ppm level.

At 48 h exposure of synergised pyrethrins, the mean difference of percentage deaths of CLHE, BPSME, and NMHE were statistically significant at ($P \leq 0.05$). ($P = 0.032$, $P = 0.026$, and $P = 0.001$ of CLHE, BPSME, and NMHE respectively) while CLME was marginally significant ($P = 0.059$). However, formulations with BPSHE, CRHE, and PBO were not significant ($P > 0.05$) (Table 11)

The study showed that the plant extracts that were statistically significant at 24 h were also significant ($P \leq 0.05$) at 48 h exposure time except PBO. Also, a higher average percentage mortality was recorded in the formulations (Appendix V). More time allowed the plant extracts to interact with pyrethrins yielding higher percentage mortality compared to 24 h duration. BPSME was the most effective pyrethrins synergist at 1,000 ppm (70.00 ± 5.77% mortality) followed by the CLHE (63.33 ± 3.33% mortality) and NMHE (16.67 ± 3.33%) of which mortalities recorded were higher than that of unsynergised pyrethrins (20% mortality) except for NMHE (16.67 ± 3.33%). BPSME and C±±LHE had 90 ± 5.77% average mortality at a synergist concentration of 20,000 ppm infering that BPSME and CLHE could have synergised pyrethrins at their respective concentrations since their toxicities to *S. zea-mais*were low (toxicity less than 20%).

Ranking the significant means showed that in CLHE concentration of 1,000 ppm was significantly different from 20,000 ppm while 5,000 ppm and 10,000 ppm were the same. In CLME, 10,000 ppm ranked highest and differently from 1,000 ppm while 5,000 ppm and 20,000 ppm were similar. In BPSME, 20,000 ppm was significantly different from the lower concentrations yet the highest percentage mortality was achieved at 10,000 ppm (93.33 ± 3.33%) compared with 20,000 ppm (90 ±5.77%). Also, the efficacies of each plant extract were inconsistent in ranking and percentage mortalities at different concentrations.

Table 11: The Mean Percentage Mortality after 24 h, 48 h and 72 h exposure of *S. zea-mais* Adults with Pyrethrins over a range of Synergist Concentrations in the Ratio of 1:1 (Synergist: Pyrethrins)

24 h EXPOSURE														
Synergist concentration	Mortality (%)												PBO	
	BPSHE		BPSME		CLME		CLHE		NMHE		CRHE			
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
1,000 ppm	43.33^{a}	3.333	16.67^{a}	3.333	30.00^{a}	5.774	23.33^{a}	3.333	6.67^{a}	3.333	23.33^{a}	3.333	56.67^{a}	3.333
5,000 ppm	53.33^{a}	3.333	23.33^{a}	3.333	43.33^{b}	3.333	30.00^{ab}	5.774	26.67^{b}	3.333	30.00^{a}	10.000	70.00^{ab}	5.774
1,0000ppm	46.67^{a}	3.333	30.00^{a}	0.000	26.67^{a}	3.333	40.00^{bc}	5.774	33.33^{b}	6.667	36.67^{a}	8.819	80.00^{ab}	5.774
20,000 ppm	63.33^{a}	12.019	43.33^{b}	6.667	26.67^{a}	3.333	46.67^{c}	3.333	26.67^{b}	3.333	-	-	83.33^{b}	12.019
$P_{(0.05)}$values	0.234		0.009		0.059		0.033		0.013		0.531		0.0126	
48 h EXPOSURE														
Synergist concentration	Mortality (%)												PBO	
	BPSHE		BPSME		CLME		CLHE		NMHE		CRHE			
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
1,000 ppm	96.67^{a}	3.333	70.00^{ab}	5.774	53.33^{a}	3.333	63.33^{a}	3.333	16.67^{a}	3.333	76.67^{a}	8.819	93.33^{a}	3.333
5,000 ppm	70.00^{a}	5.774	66.67^{a}	8.819	66.67^{ab}	3.333	73.33^{ab}	6.667	53.33^{a}	3.333	63.33^{a}	8.819	93.33^{a}	3.333
1,0000 ppm	83.33^{a}	12.019	93.33^{bc}	3.333	70.00^{b}	5.774	73.33^{ab}	3.333	73.33^{a}	8.819	70.00^{a}	11.547	90.00^{a}	0.000
20,000 ppm	90.00^{a}	10.000	90.00^{c}	5.774	56.67^{ab}	3.333	90.00^{b}	5.774	53.33^{a}	6.667			90.00^{a}	10.000
$P_{(0.05)}$values	0.226		0.026		0.059		0.032		0.001		0.651		0.945	
72 h EXPOSURE														
Synergist concentration	Mortality (%)												PBO	
	BPSHE		BPSME		CLME		CLHE		NMHE		CRHE			
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
1,000 ppm	100.00^{a}	0.000	86.67^{a}	3.333	76.67^{a}	3.333	86.67^{a}	3.333	16.67^{a}	3.333	86.67^{a}	3.333	100.00	0.000
5,000 ppm	93.33^{a}	3.333	90.00^{ab}	5.774	86.67^{a}	3.333	90.00^{a}	0.000	76.67^{b}	3.333	96.67^{a}	3.333	100.00	0.000
1,0000 ppm	96.67^{a}	3.333	100.00^{b}	0.000	83.33^{a}	3.333	86.67^{a}	3.333	93.33^{b}	6.667	83.33^{a}	6.667	100.00	0.000
20,000 ppm	100.00^{a}	0.000	96.67^{ab}	3.333	83.33^{a}	3.333	96.67^{a}	3.333	93.33^{b}	6.667	-	-	100.00	0.000
$P_{(0.05)}$values	0.219		0.011		0.268		0.119		0.0001		0.196		0	

*Means within the same column having the same symbol do not differ significantly from one another at p=0.05 test leve

After 48 h duration, results showed that BPSME at 10,000 ppm (93.33 ± 3.33%) was the most efficacious synergist to pyrethrins followed byCLHE and BPSME at 20,000 ppm (90 ± 5.77%), CLHE (5,000 ppm and 10,000 ppm) and NMHE (10,000 ppm) with 73.33 ± 6.67%. There was no specific concentration at which to administer a synergist rather results show that the percentage mortality of *S. zea-mais*depended on the plant extract

The trends of synergist efficacy after 72 h exposure duration (Appendix VI). Only BPSME and NMHE mortalities were statistically significant at $P \leq 0.05$ ($P = 0.012$, and $P = 0.0001$ of BPSME and NMHE respectively). NMHE at 1,000 ppm had 16.67 ± 3.33% mortality which was below that of unsynergised pyrethrins (20% mortality) implying that NMHE could have an antagonistic effect to pyrethrins at 1,000 ppm compared to enhanced efficacy at 5,000 ppm (76.67 ± 3.33%) and 10,000 ppm (93.33± 6.67%) mortalities respectively. When the percentage mortality due to NMHE concentrations were ranked, 1,000 ppm was found to be significantly different from the higher concentrations (5,000 ppm, 10,000 ppm and 20,000 ppm) which in turn did not yield significantly different ($P \leq 0.05$) percentage mortalities (Table 11). This infers that it would be economical to use this plant extract at 5,000 ppm in formulations with pyrethrins as the percentage mortalities at higher concentrations (10,000 ppm and 20,000 ppm) would not yield different results.

BPSME at 10, 000 ppm was the most efficacious concentration with 100% mortality of *S. zea-mais*and it was different when ranked from the rest of the concentrations. This was followed by 20,000 ppm (96.67 ± 3.33%), 5,000 ppm (90 ± 5.77%) and 1,000 ppm (86.67 ± 3.33%). Generally, the BPSME and NMHE recorded increased average percentage mortalities of *S. zea-mais* consistently over the exposure time of 24 h, 48 h and 72 h with higher percentage mortality observed after 72 h exposure for all the extracts.

The results of the joint action between the plant extracts (synergists) at the four concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) and pyrethrins at LC20 in the ratio of 1:1 (synergist: pyrethrins) determined according to the equation of co-toxicity factors (Tables 12, 13 and 14)

After 24 h exposure (Table 12), BPSHE and PBO co-toxicity values obtained showed synergism (> 100 fold) in all the concentrations and ratios tested. BPSME with co-toxicity values of -16.7, -12.5 and 0 at concentration of 1,000 ppm, 5,000 ppm and 10,000 ppm respectively over 24 h period. These values show that BPSME was an additive to the formulation and only a synergist at 20,000 ppm. NMHE is antagonistic to pyrethrins at 1,000 ppm with co-toxicity value of -75 while at 5,000 ppm and 20,000 ppm it was an additive (14.3 and 0 values respectively). CLHE was only an additive at 1,000 ppm and a synergist at higher concentrations.

The co-toxicity values after 48 h exposure (Table 13). NMHE was an antagonist at 1,000 ppm (-54.5) when formulated with pyrethrins and a synergist at 5,000 ppm, 10,000 ppm and 20,000 with co-toxicity values of 90, 100 and 33 respectively. All the other plant extracts showed synergism. BPSHE and PBO had same synergism value (125 fold) followed by CLHE (107.7 fold) at 20,000 ppm while at 5,000 ppm, PBO was a better synergist (300 fold) followed by the plant extracts BPSHE (162.5 fold), CLHE (144.4 fold) and CLME (122.2 fold).

At 72 h exposure (Table 14), most plant exracts had better synergism than the standard, PBO at a concentration of 20,000 ppm. For instance, BPSHE (130.8), NMHE (115.4), CLHE (107.1), CLME (92.3) and BPSME (81.3) while PBO had co-toxicity value of 76.5. These plant extracts could replace PBO when formulated at this ratio and concentration.

Table 12: Co-toxicity Factors Calculated on the basis of LC_{20} Pyrethrins and Synergists Applied on *S. zea-mais* at Ratio of 1:1 (Synergist: Pyrethrins) 24 h Exposure.

Plant extract/ synergist	Synergist - 1,000 ppm			Synergist -5,000 ppm			Synergist 10,000 ppm			Synergist 20,000 ppm		
	% Mortality			% Mortality			% Mortality			% Mortality		
	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor
BPSHE	20.0	43	117	23.3	53	129	26.7	47	75.0	26.7	63	138
BPSME	20.0	17	-16.7	26.7	23	-12.5	30.0	30	0.0	33.3	43	30.0
CLME	26.7	30	12.5	30.0	43	44.4	23.3	27	14.3	23.3	27	14.3
CLHE	20.0	23	16.7	23.3	30	28.6	26.7	40	50.0	36.7	47	27.3
NMHE	26.7	7	-75.0	23.3	27	14.3	26.7	33	25.0	26.7	27	0.0
CRHE	20.0	23	16.7	26.7	30	12.5	20.0	37	83.3			
PBO	20.0	80	300	20.0	57	183	23.3	70	200	26.7	83	213

- Values lower than -20 suggest antagonism, values between -20 and 20 suggest additive character and values greater than 20 suggest synergism
- The expected value is contributed by the constant 20% mortality of *S. zea-mais*due to pyrethrins (LC20) and toxicity of the plant extract/synergist alone while observed mortality is due to the synergist-pyrethrins formulation

Table 13*:* Co-toxicity Factors Calculated on the basis of LC_{20} Pyrethrins and Synergists applied on *S. zea-mais* at ratio 1:1 (Synergist: Pyrethrins) 48 h Exposure.

Plant extract/synergist	Synergist at 1,000 ppm			Synergist at 5,000 ppm			Synergist at 10,000 ppm			Synergist at 20,000 ppm		
	% Mortality			% Mortality			% Mortality			% Mortality		
	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor
BPSHE	33.3	97	190.0	26.7	70	162.5	30.0	83	177.8	40.0	90	125.0
BPSME	46.7	70	50.0	36.7	67	81.8	43.3	93	115.4	46.7	90	92.9
CLME	36.7	53	45.5	30.0	67	122.2	26.7	70	162.5	33.3	57	70.0
CLHE	23.3	63	171.4	30.0	73	144.4	36.7	73	100.0	43.3	90	107.7
NMHE	36.7	17	-54.5	33.3	53	60.0	36.7	73	100.0	40.0	53	33.3
CRHE	26.7	77	187.5	33.3	63	90.0	43.3	70	61.5			
PBO	26.7	93	250.0	23.3	93	300.0	30.0	90	200.0	40.0	90	125.0

- Values lower than -20 suggest antagonism, values between -20 and 20 suggest additive character and values greater than 20 suggest synergism
- The expected value is contributed by the constant 20% mortality of *S. zea-mais*due to pyrethrins (LC_{20}) and toxicity of the plant extract/synergist alone while observed mortality is due to the synergist-pyrethrins formulation

Table 14: Co-toxicity Factors Calculated on the basis of LC_{20} Pyrethrins and Synergists applied on *S. zea-mais* at ratio 1:1 (Synergist: Pyrethrins) 72 h Exposure.

Plant extract/ synergist	Synergist at 1000ppm			Synergist at 5000ppm			Synergist at 10000ppm			Synergist at 20000ppm		
	% Mortality			% Mortality			% Mortality			% Mortality		
	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor
BPSHE	33.3	100	200.0	26.7	93	250.0	40.0	97	141.7	43.3	100	130.8
BPSME	50.0	87	73.3	40.0	90	125.0	46.7	100	114.3	53.3	97	81.3
CLME	36.7	77	109.1	36.7	87	136.4	36.7	83	127.3	43.3	83	92.3
CLHE	26.7	87	225.0	30.0	90	200.0	36.7	87	136.4	46.7	97	107.1
NMHE	36.7	17	-54.5	33.3	77	130.0	36.7	93	154.5	43.3	93	115.4
CRHE	26.7	87	225.0	36.7	97	163.6	43.3	83	92.3			
PBO	26.7	100	275.0	26.7	100	275.0	30.0	100	233.3	56.7	100	76.5

- Values lower than -20 suggest antagonism, values between -20 and 20 suggest additive character and values greater than 20 suggest synergism
- The expected value is contributed by the constant 20% mortality of *S. zea-mais*due to pyrethrins (LC20) and toxicity of the plant extract/synergist alone while observed mortality is due to the synergist-pyrethrins formulation

In the synergism bioassays, each plant extract at four concentrations was mixed with pyrethrins (LC_{20}) at 4 ratios and topically applied to the maize weevil. From the results obtained at ratio 1:1 of synergist: pyrethrins, PBO showed the highest efficacy as a pyrethrins synergist in all concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) tested, followed by CLHE at 24 h after treatment. NMHE, although statistically significant had low percentage mortalities below that of LC20 at 1,000 ppm (6.67%, 16.67% and 16.67%) over 24 h, 48 h and 72 h respectively while BPSME had 16.67% mortality at 24 h suggesting that these plant extracts may have compounds that are either not sufficient enough to detoxify enzymes within the maize weevil or they are slow in penetrating the cuticle giving chance to the insect's enzymes to detoxify pyrethrins and render it ineffective.

At higher concentrations of 5,000 ppm both BPSME and NMHE showed minimal increase in percentage mortalities but more than that of LC20, the difference possibly being due to the toxicity of the plant extracts. The fact that high synergist concentrations reduced synergism of pyrethrins could be due to the mechanism of insecticide metabolism within the insect which is not altered by the synergist. Casida (1970) found that a synergist could influence the reaction rate or shift significant detoxification reactions to non-oxidative mechanisms and that oxidation or hydroxylation reactions form products of either reduced potency (detoxification) or enhanced potency (activation) enabling a synergist to increase or decrease insecticide toxicity. The most efficacious plant extract when compared to PBO at 24 h was CLHE at 20,000 ppm.

Increasing the exposure period of the formulations to 48 h and 72 h also increased the overall percentage mortality of the maize weevils. For instance, NMHE at 10,000 ppm achieved 33.33%, 73.33% and 93.33% while CLHE had 40.00%, 83.33% and 86.67% at 24 h, 48 h, and 72 h respectively. This indicates that when the formulations are allowed time to interact with the maize weevils, higher mortalities could be achieved. Though not tested in the present study, pre-treatment of insects with synergists have been found to increase the amount of synergism due to the time it takes for the synergist to maximally inhibit the enzymes within the insect (Bingham *et al*., 2007). In other studies, using of ethyl formate for control of stored grain pests it was shown that varied dosages in an exposure period of 48-72 h controlled all stages of insects in stored grains

and their products (Muthu *et al*., 2012) and methyl bromide completely eradicated infestations 12-48 h after application (Anon, 2017).

Generally, increasing concentration of a synergist did not increase the percentage mortality of *S. zea-mais*geometrically except for CLHE (24 h and 48 h) and PBO (24 h) where increase in concentration of synergist increased the percentage mortality of the maize weevil. In BPSME for instance, concentrations of 1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm yielded 70.00%, 67.67%, 93.33% and 90.00% percentage mortalities of *S. zeamais* respectively while NMHE yielded 73.33% and 53.33% mortalities at 10,000 ppm and 20,000 ppm respectively. The findings suggest that penetration of the insect's cuticle may depend on the plant extract and not the concentration at which it is applied.

To find out if the plant extracts synergized pyrethrins at ratio 1:1 synergist: pyrethrins, co-toxicity factor was used as a means of assessing the synergistic potential of plant extracts when formulated with pyrethrins at different concentrations. The findings of this study showed that BPSHE and PBO synergized the toxicity of pyrethrins in all the concentrations and ratios tested over the 72 h exposure time with co-toxicity factors above 10, the highest being PBO at 1,000 ppm synergizing pyrethrins 300 fold which reduces as concentration is increased. BPSHE also consistently showed the highest co-toxicity factor of all the plant extracts tested. The results shown by BPSHE, CLHE and PBO are in harmony to a study done by Joffe (2012) who found that an increase in the synergist concentration tended to correspond with a decrease in mortality of pollen beetles, *Meligethes aeneus* having to do with penetration of the cuticle of the insect, with an increasing amount of synergist perhaps blocking the penetration of pyrethrins through the cuticle. However, this finding was contrary with respect to the plant extracts NMHE and BPSME which show antagonism and additive ffect at lower concentarions and only synergists at higher concentarions.

Other studies have also shown that some synergists reduce insecticide penetration through the cuticle, for example, piperonyl cyclonene, which reduced the absorption of topically applied pyrethroids in house flies *M. domestica* (Winteringham *et al*., 1955) and sesamex which reduced the absorption of both labeled C^{14}-pyrethrin 1 and C^{14}-cinerin 1 by approximately half in houseflies, suggesting that sesame viscous oil

possibly compete with the housefly epicuticle for any lipophilic compound (Joffe, 2012)

CLHE was only an additive at 1,000 ppm over 24 h exposure and a synergist in the rest of the concentrations and time. These observations indicate the potential of BPSHE to replace PBO in pyrethrins formulation at a ratio of 1:1 synergist: pyrethrins at all concentrations tested while CLHE can replace PBO when formulated with pyrethrins at 5,000 ppm, 10,000 ppm or 20,000 ppm.

BPSME with co-toxicity values of between -20 and 20 at concentration of 1,000 ppm, 5,000 ppm and 10,000 ppm respectively over 24 h period was an additive to the formulation and only a synergist at 20,000 ppm while NMHE is antagonistic to pyrethrins at 1,000 ppm with co-toxicity values less than -20 over 24 h, 48 h and 72 h exposure period. NMHE showed significant synergism at concentrations of 5,000 ppm, 10,000 ppm and 20,000 ppm after 48 h and 72 h exposure period. This suggests that some components in nutmeg plant extracts may have acted to inhibit oxidative processes important for the activation of pyrethrins when formulated at the 1,000 ppm regardless of the exposure time. Faraone *et al*. (2015) found that some plant constituents like linalool from lavender (*Lavendula angustifolia*) and thymol from thyme (*Thymus vulgaris*) showed antagonistic action in imidacloprid against green peach aphid, *Myzus persicae* with their extracts showing synergism.

A component in NMHE, though not isolated in this sudy may restrict the use of nutmeg oil as synergist at a 1,000 ppm. Studies by Gross *et al.* (2017) suggested that plant essential oils enhance the toxicity of various type II pyrethroids and natural pyrethrins though the mechanism of action had not been fully explored. The plant extracts tested in this study were expected to portray the same results as PBO because of the MDP ring thus the differences observed in their efficacy will have to be further investigated. The data also suggest that some of these plant extracts may have higher levels of compounds that interfere with the insect's enzyme system than others which accounts for the variations in synergism observed. Further work need be done to specifically elucidate the structures of these plant extracts particularly that of CLHE whose efficacy compares similarly with that of PBO.

This study found out that longer exposure time yielded higher mortality rates even with lower concentrations of synergists. This could be an important consideration in formulating insecticides that are cost effective and efficacious.

4.3.2 Percentage Mortality of *S. zea-mais* Exposed to Formulations at Ratio 2:1 (Synergist: Pyrethrins) after 24 h, 48 h and 72 h Exposure.

The study was conducted to establish whether an increase in the amount of synergist at the different concentrations in pyrethrins formulations caused higher percentage mortality of *S. zea-mais*when exposed over time. After 24 h exposure (Table 15 and Appendix VII), the percentage mortalities obtained for synergists BPSHE and CRHE were statistically significant at $P \leq 0.05$ ($P = 0.022$ and P0.045 of BPSHE and CRHE respectively). BPSHE registered higher percentage mortality at 1,000 ppm (43.33 ± 6.67%) and 5,000 ppm (56.67 ± 3.33%) compared with CRHE at the same concentrations with 26.67 ± 6.67% and 36.67 ± 3.33% respectively. However, at 10,000 ppm CRHE registered higher percentage mortality (53.33 ± 6.67%) than BPSHE (46.67 ± 3.33%). Generally, formulations of these extracts with pyrethrins at this ratio gave significantly higher mortality than unsynergised pyrethrins (20% mortality at 2,200 ppm). When significant means were ranked, BPSHE showed that 20,000 ppm was different from 1,000 ppm and that 5,000 ppm and 10,000 ppm were statistically the same. In CLHE ranked means were all different indicating with each concentration having very independent percentage mortalities.

Formulations with PBO, BPSME, CLME and NMHE were not significant ($P > 0.05$) inspite of the increase in concentration of the extracts. PBO, though being the standard synergist, its efficacy could not be compared with the plant extracts at 24 h exposure.

Table 15: The Mean Percentage Mortality after 24 h, 48 h and 72 h Exposure of *S. zea-mais* Adults with Pyrethrins Over A Range of Synergist Concentrations in the Ratio of 2:1 (Synergist: Pyrethrins)

24 h EXPOSURE														
Synergist concentration	Mortality (%)												PBO	
	BPSHE		BPSME		CLME		CLHE		NMHE		CRHE			
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
1,000 ppm	43.33[a]	6.667	16.67[a]	3.333	40.00[a]	5.774	33.33[a]	3.333	46.67[a]	8.819	26.67[a]	6.667	50.00[a]	5.774
5,000 ppm	56.67[ab]	3.333	33.33[ab]	8.819	36.67[a]	3.333	36.67[a]	3.333	46.67[a]	3.333	36.67[ab]	3.333	56.67[a]	3.333
1,0000 ppm	46.67[ab]	3.333	26.67[a]	3.333	36.67[a]	3.333	46.67[a]	6.667	30.00[a]	5.774	53.33[b]	6.667	43.33[a]	6.667
20,000 ppm	66.67[b]	8.819	46.67[b]	3.333	43.33[a]	8.819	46.67[a]	3.333	40.00[a]	5.774	-	-	53.33[a]	3.333
$P_{(0.05)}$values	.022		0.091		0.821		0.140		0.265		0.045		0.34	
48 h EXPOSURE														
Synergist concentration	Mortality (%)												PBO	
	BPSHE		BPSME		CLME		CLHE		NMHE		CRHE			
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
1,000 ppm	90.00[a]	5.774	76.67[a]	3.333	66.67[a]	3.333	70.00[a]	5.774	70.00[b]	10.000	60.00[a]	0.000	80.00[a]	5.774
5,000 ppm	83.33[a]	12.019	80.00[ab]	10.000	73.33[a]	3.333	70.00[a]	5.774	63.33[b]	3.333	66.67[a]	8.819	80.00[a]	5.774
1,0000 ppm	93.33[a]	6.667	90.00[ab]	5.774	83.33[a]	6.667	63.33[a]	3.333	40.00[a]	5.774	66.67[a]	3.333	86.67[a]	8.819
20,000 ppm	90.00[a]	5.774	100.00[b]	0.000	80.00[a]	5.774	96.67[b]	3.333	60.00[ab]	5.774			86.67[a]	3.333
$P_{(0.05)}$values	0.842		0.091		0.168		0.005		0.06		0.630		0.770	
72 h EXPOSURE														
Synergist concentration	Mortality (%)												PBO	
	BPSHE		BPSME		CLME		CLHE		NMHE		CRHE			
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
1,000 ppm	100.00[a]	0.000	96.67[a]	3.333	86.67[a]	3.333	93.33[a]	6.667	80.00[a]	11.547	86.67[a]	6.667	100.00[a]	0.000
5,000 ppm	93.33[a]	6.667	96.67[a]	3.333	90.00[a]	5.774	93.33[a]	6.667	96.67[a]	3.333	93.33[a]	3.333	100.00[a]	0.000
10 000 ppm	100.00[a]	0.000	100.00[a]	0.000	90.00[a]	5.774	86.67[a]	3.333	70.00[a]	10.000	96.67[a]	3.333	96.67[a]	3.333
20 000 ppm	93.33[a]	6.667	100.00[a]	0.000	93.33[a]	3.333	100.00[a]	0.000	93.33[a]	6.667			96.67[a]	3.333
$P_{(0.05)}$values	0.596		0.546		0.802		0.735		0.178		0.375		0.596	

Means within the same column having the same symbol do not differ significantly from one another at p=0.05 test level

When time of exposure was prolonged to 48 h, only CLHE is statistically significant at $P \leq 0.05$ ($P = 0.005$) with NMHE and BPSME showing mortalities that are marginally significant ($P = 0.059$ and $P = 0.091$ of CRHE and BPSME respectively) (Table 15 and Appendix VIII). Percentage mortality increased with increase in concentration of the synergist with CLHE showing a mean significant difference at 20,000 ppm compared with the lower concentrations (1,000 ppm, 5,000 ppm and 10,000 ppm) which were not different in synergizing pyrethrins according to DMRT. BPSME was shown to be the most effective synergist at a concentration of 20,000ppm (100% mortality) followed by CLHE (96.67 ± 3.33%) then NMHE (60 ± 5.77%) mortality at the same concentration. This bioassay further showed that BPSME was a better synergist of pyrethrins at all concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) tested with 76.67 ± 3.33%, 80.00 ± 10.0%, 90.00 ± 5.77%, and 100% mortalities respectively followed by CLHE with 70 ± 5.77%, 70 ± 5.77%, 63.33 ± 3.33% and 96.67 ± 3.33% mortalities respectively.

Results also showed that increasing concentrations did not correspond to geometric increase in percentage mortality of *S. zea-mais*. For instsnce, CLHE at 10,000 ppm yielded lower percentage mortality (63.33 ± 3.33%) compared to 1,000 ppm and 5,000 ppm (10.00 ± 5.77%) while in NMHE at 10,000 ppm showed 40.00 ± 5.77% yet the highes was at 1,000 ppm (70.00 ± 10%). In general, all the plant extracts tested and statistically significant ($P \leq 0.05$) for synergism at the ratio of 2:1 (synergist: pyrethrins) in all the concentrations had mortalities of above 40% which is higher than unsynergised pyrethrins (20%). It was found that after 72 h exposure of synergised - pyrethrins, PBO and all the plant extracts BPSHE, CLHE, CLME, CRHE, NMHE and BPSME were not significant ($P > 0.05$). All the plant extracts and PBO had reached their maximum efficacy levels and there were no significant differences in their concentrations (Table 15 and Appendix IX). The co-toxicity values presented in Table 16 show that at the ratio of 2:1 synergist: pyrethrins, BPSME is the only plant extract that is shown to be an additive at 1,000 ppm (-16.7) and 10,000 ppm (-11.1) after 24 h. At 20,000 ppm, BPSHE (146.9) is a better synergist than PBO (97.53). When exposure time was extended to 48 h (Appendix XVI), BPSHE had higher co-toxicity values at 10,000 ppm (211.1) and 20,000 ppm (188.9) compared to PBO at same concentrations (125 and 116.7) respectively

Table 16: Co-toxicity Factors Calculated on the basis of LC_{20} Pyrethrins and Synergists Applied on *S. zea-mais* at Ratio 2:1 (synergist: pyrethrins) 24 h Exposure.

	Synergist 1,000 ppm			Synergist 5,000 ppm			Synergist 10,000 ppm			Synergist 20,000 ppm		
	% Mortality			% Mortality			% Mortality			% Mortality		
	Expected	Observed	Co-toxicity facfor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor
BPSHE	20	43	116.7	23	57	146.4	27	47	72.84	27	67	146.9
BPSME	20	17	-16.7	27	33	23.46	30	27	-11.1	33	47	41.41
CLME	27	40	48.15	30	37	22.22	23	37	59.42	23	43	88.41
CLHE	20	33	66.67	23	37	59.42	27	47	72.84	37	47	26.13
NMHE	27	47	72.84	23	47	102.9	27	30	11.11	27	40	48.15
CRHE	20	27	33.33	27	37	35.8	20	53	166.7			
PBO	20	50	150	20	57	183.3	23	43	88.41	27	53	97.53

- Values lower than -20 suggest antagonism, values between -20 and 20 suggest additive character and values greater than 20 suggest synergism
- The expected value is contributed by the constant 20% mortality of *S. zea-mais*due to pyrethrins (LC20) and toxicity of the plant extract/synergist alone while observed mortality is due to the synergist-pyrethrins formulation

At 72 h (Appendix XVII), all the plant extracts had higher co-toxicity values than PBO at 20,000 ppm with BPHSE, CLME and NMHE (117.1) followed by CLHE (112.8), BPSME (88.68) and PBO (69.59). The co-toxicity values correspond to the results presented.

The overall effect of the plant extracts synergized- pyrethrins that were tested and statistically significant at 5% significance level showed a similar trend with that of formulations at the ratio of 1:1 (synergist: pyrethrins) though CRHE was not significant at the ratio of 1:1 ($P > 0.05$). The percentage mortalities were slightly higher implying that increase in dosage of synergists effectively enhanced the efficacy of the formulations. This may be attributed to their intrinsic toxicities or increased penetration of the insect's cuticle. The potential synergists could have similar qualities that confer similar effects to synergism. BPSHE, BPSME and CRHE at 24 h were highly significant while CLHE, NMHE and BPSME were statistically significant at 48 h, consistent with percentage mortalities observed at ratio of 1:1 and at 72 h, BPSHE and CLHE were significant. It followed that PBO was not significant at this ratio. Increasing the ratio of PBO: pyrethrins to 2:1 did not show any differences among the concentrations in relation to its efficacy over the 72 h period.

The co-toxicity values obtained indicated that BPSME contributed towards the toxicity of pyrethrins by being an additive rather than a synergist while NMHE synergized pyrethrins except at 1,000 ppm concentration. BPSHE with values above 100 in all concentrations was the most efficacious plant extract with more synergism (146 fold) than the standard PBO (97.53) at 20,000 ppm after 72 h exposure period. The plant extract BPSHE was the most effective potential synergist that could replace PBO in pyrethrins formulations. *P. nigrum* extract containing piperine has been shown to be an effective synergist as PBO with a synergistic ratio of 11:6 (2:1) (Jensen *et al*., 2006) as compared to that of PBO at 15:5 (3:1) (Incho & Greenberg, 1952; Nash, 1954), the difference being attributed to their modes of action. *P. nigrum* is thought to act by inhibiting polysubstrate monooxygenase (PSMO) activity and slowing detoxification (Dalvi & Dalvi, 1991) while PBO binds to MFOs within the insect rendering the insecticide ineffective (Hamilton, 1995). The findings in the current study showed BPSHE to be the most effective synergist at ratio 2:1.

Generally, these results also show that the longer the exposure time to the synergist formulations upto 48 h, increases efficacy of the synergist formulations. At 2:1 synergist: pyrethrins ratio, all the plant extracts tested and statistically significant at 5% significance level could be potential pyrethrins synergists however the most economical concentration could be at 1,000 ppm. Thus, the overall percentage mortalities obtained could be explained as due to synergistic rather that additive effect of the plant extracts. The fact that all the plant extracts and PBO had reached their maximum efficacy at this ratio indicate that more synergist may help prevent detoxicification of pyrethrins within the insect. With exposure time extended to 72 h, plant extracts achieved high mortalities even at low concentrations (1,000 ppm) with PBO and BPSHE achieving 100% mortality.

4.3.3 Percentage Mortality of *S. zea-mais* Exposed to Formulations at Ratio 3:1 (Synergist: Pyrethrins) after 24 h, 48 h and 72 h exposure.

The study was carried out to establish the effect of increasing the synergists by three-fold in the formulations against *S. zea-mais*. It was found that after 24 h exposure (Table 17), formulations with PBO and the plant extracts CLHE, NMHE and BPSHE were statistically significant ($P \leq 0.05$) ($P = 0.0204$, $P = 0.024$, $P = 0.049$ and $P = 0.0421$ of PBO, CLHE, NMHE and BPSHE respectively). PBO registered higher percentage mortalities under all the synergist concentrations tested (Appendix X). For instance, at 20,000 ppm, PBO had 63.33 ± 6.67% mortality of *S. zea-mais*, followed by BPSHE (56.67 ± 56.67%), NMHE (53.33 ± 5.77%) and CLHE (50.00 ± 5.77%). In addition, these compounds when formulated with pyrethrins at this ratio (3:1) gave significantly higher average mortality than unsynergised pyrethrins (20% mortality at 2,200 ppm). The findings of this study showed that, PBO was shown to be the most effective synergist with significantly higher mortality when applied with pyrethrins than all the other synergists in all the four concentrations tested.

In addition, significant means when ranked using DMRT showed that the efficacy of PBO, BPSHE and NMHE and CLHE at 20,000 ppm was significantly different at ($P \leq 0.05$) from the lower concentrations (1,000 ppm, 5,000 ppm and 10,000 ppm) (Appendix XI). Formulations with BPSME., CLME and CRHE were not significant at 5% significance level meaning there was no difference in average percentage mortality

with increase in concentration of the extracts resulting in the mean difference being statistically insignificant in terms of synergizing pyrethrins in this study.

BPSHE at 20,000 ppm and PBO at 5,000ppm showed similar efficacy of 56.67 ± 3.33% while at 10,000 ppm it is equivalent to PBO at 1000ppm and NMHE at 20,000 ppm with average mortality of 53.33 ± 8.819%after 24 h exposure thus BPSHE can replace PBO when formulated at 20.000 ppm with PBO at 5,000 ppm. These comparisons are important when deciding on use of a synergist. The factors that would be needed for consideration using results in the current study would be a combination of concentration, ratio and time of exposure that work best for a synergist.

After 48 h of exposure (Table 17), only PBO, CLHE, BPSHE and BPSME resulted in average mortalities of *S. zeamais* that were statistically significant at ($P \leq 0.05$) ($P = 0.015$, $P = 0.009$, $P = 0.044$ and $P = 0.012$ of PBO, CLHE, BPSHE and BPSME respectively). Percentage mortality increased with increase in concentration of the synergist. These synergists were shown to be effective at all synergist concentrations. The general trend at the ratio of 3:1 (synergist: pyrethrins) showed various synergists had mortalities above 80% in at least one concentrations. This could mean that 3:1 ratio is economically viable for synergism. However, CRHE, CLME and NMHE were not statistically significant ($P > 0.05$).

Further comparisons on the extracts show that BPSHE and BPSME at 20,000ppm recored higher mortalities (93.33 ± 3.33%) than PBO (90 ± 5.77%) followed by CLHE (86.67 ± 3.33%) at the same concentration while BPSHE and PBO at 1,000 ppm both registered 83.33 ± 3.33% after 48 h exposure time

Table 17: The Mean Percentage Mortality after 24, 48 and 72 h Exposure of *S. zea-mais* Adults with Pyrethrins Over a Range of Synergist Concentrations in the Ratio of 3:1 (Synergist: Pyrethrins)

24 h EXPOSURE														
	Mortality (%)													
Synergist concentration	BPSHE extract		BPSME		CLME		CLHE		NMHE		CRHE		PBO	
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
1,000 ppm	43.33^{a}	3.333	36.67^{a}	3.333	43.33^{a}	6.667	33.33^{a}	3.333	26.67^{a}	3.333	23.33^{a}	3.333	53.33^{a}	8.819
5,000 ppm	50.00^{a}	10.000	33.33^{a}	3.333	43.33^{a}	6.667	40.00^{a}	5.774	30.00^{a}	8.819	36.67^{a}	8.819	56.67^{a}	3.333
1,0000 ppm	53.33^{ab}	8.819	30.00^{a}	5.774	30.00^{a}	5.774	45.00^{a}	5.774	30.00^{a}	5.774	43.33^{a}	3.333	58.33^{a}	3.333
20,000 ppm	56.67^{b}	3.333	46.67^{a}	6.667	50.00^{a}	5.774	50.00^{b}	5.774	53.33^{b}	5.774			63.33^{b}	6.667
$P_{(0.05)}$values	.0421		0.182		0.223		0.024		0.049		0.118		0.0204	
48 h EXPOSURE														
Synergist concentration	Mortality (%)													
	BPSHE		BPSME		CLME		CLHE		NMHE		CRHE		PBO	
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
1,000 ppm	83.33^{a}	6.667	70.00^{a}	10.000	76.67^{a}	8.819	66.67^{a}	3.333	50.00^{a}	5.774	56.67^{a}	6.667	83.33^{ab}	3.333
5,000 ppm	93.33^{b}	6.667	83.33^{ab}	3.333	90.00^{a}	5.774	73.33^{a}	3.333	76.67^{a}	8.819	70.00^{a}	5.774	76.67^{a}	3.333
1,0000 ppm	76.67^{a}	3.333	86.67^{ab}	3.333	83.33^{a}	3.333	66.67^{a}	3.333	60.00^{a}	11.547	53.33^{a}	3.333	80.00^{ab}	0.000
20,000 ppm	93.33^{b}	3.333	93.33^{b}	3.333	90.00^{a}	5.774	86.67^{b}	3.333	70.00^{a}	5.774			90.00^{b}	5.774
$P_{(0.05)}$values	.044		0.012		0.423		0.009		0.199		0.152		0.015	
72 h EXPOSURE														
Synergist concentration	Mortality (%)													
	BPSHE		BPSME		CLME		CLHE		NMHE		CRHE		PBO	
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
1,000 ppm	96.67^{a}	3.333	90.00^{a}	0.000	93.33^{a}	3.333	93.33^{a}	3.333	86.67^{ab}	6.667	83.33^{a}	8.819	96.67^{a}	3.333
5,000 ppm	100.00^{a}	0.000	100.00^{b}	0.000	96.67^{a}	3.333	96.67^{a}	3.333	96.67^{b}	3.333	96.67^{a}	3.333	96.67^{a}	3.333
1,0000ppm	90.00^{a}	5.774	96.67^{ab}	3.333	93.33^{a}	3.333	96.67^{a}	3.333	70.00^{a}	5.774	90.00^{a}	5.774	100.00^{a}	0.000
20,000 ppm	93.33^{a}	3.333	93.33^{ab}	3.333	96.67^{a}	3.333	96.67^{a}	3.333	83.33^{ab}	3.333			100.00^{a}	0.000
$P_{(0.05)}$values	.330		0.077		0.802		0.859		0.033		0.394		0.596	

*Means within the same column having the same symbol do not differ significantly from one another at p=0.05 test level

After 72 h exposure duration (Appndix XII). The mean difference in percentage mortality caused by NMHE wase statistically significant at 5% significance level (P = 0.033) The results further revealed that mortality differences due to NMHE concentrations did not differ except at 5,000 ppm and 10,000 ppm when ranked (DMRT). However, PBO, BPSME, BPSHE, CLHE, CLME and CRHE were not significant ($P > 0.05$) implying that increase in synergist concentration did not correspond to an increase in percentage mortality of the *S.zea-mais*. At the ratio of 3:1, the plant extracts tested and statistically significant could be potential synergists to replace PBO in pyrethrins formulations. The most economical concentration could be at 5,000 ppm with NMHE since 96.67 ± 3.33% percentage mortality of *S.zea-mais* was achieved after 72 h. NMHE could have been slow in acting on the test insects and its active components need a prolonged time to show effect compared with the other plant extracts which were significant ($P \leq 0.05$) after 48 h. Generally, there was no consistency of results at $P \leq 0.05$ after 48 h and 72 h exposure time.

The co-toxicity values presented in Table 18 indicate that BPSME is inconsistent with concentration at 24 h exposure. NMHE is an additive and only a synergist at 5,000 ppm. At 48 h (Appendix XVIII), BPSHE at 5,000 ppm has a higher value (245.7) than PBO (233.3) and the other plant extracts. CLME was shown to be a better synergist at 10,000 ppm (208.6) and 20,000 ppm (172.7) than PBO (166.7 and 125 values respectively). When exposure time is extended to 72 h (Appendix XIX), BPSHE at 5,000 ppm had higher value (270.4) than PBO (258) and that all plant extracts were better than PBO at 20,000 ppm.

Table 18: Co-toxicity Factors Calculated on the Basis of LC_{20} Pyrethrins and Synergists Applied on *S. zea-mais* at ratio 3:1 (Synergist: Pyrethrins) 24 h Exposure.

	Synergist 1000ppm % Mortality			Synergist 5000ppm % Mortality			Synergist 10000ppm % Mortality			Synergist 20000ppm % Mortality		
	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor
BPSHE	20	43	116.7	23	50	117.4	27	43	60.49	27	57	109.9
BPSME	20	37	83.33	27	33	23.46	30	30	0	33	47	41.41
CLME	27	43	60.49	30	43	44.44	23	30	30.43	23	50	117.4
CLHE	20	33	66.67	23	40	73.91	27	40	48.15	37	50	35.14
NMHE	27	27	-1.23	23	53	131.9	27	30	11.11	27	30	11.11
CRHE	20	23	16.67	27	37	35.8	20	43	116.7			
PBO	20	53	166.7	20	57	183.3	23	43	88.41	27	63	134.6

- Values lower than -20 suggest antagonism, values between -20 and 20 suggest additive character and values greater than 20 suggest synergism
- The expected value is contributed by the constant 20% mortality of *S. zea-mais*due to pyrethrins (LC20) and toxicity of the plant extract/synergist alone while observed mortality is due to the synergist-pyrethrins formulation

Generally, formulations at ratio 3:1 (synergist: pyrethrins) reveal that the plant extracts BPSHE is similar in efficacy to PBO. For instance, at synergist concentration of 1,000 ppm, percentage mortality of *S. zea-mais*due to PBO and BPSHE is 83.33% after 48 h of treatment. BPSHE was also shown to be a better synergist to PBO when compared at a concentration of 5,000 ppm with percentage mortalities of 93.33% (BPSHE) and 76.67% (PBO) after 48 h exposure respectively followed CLHE at the same concentration with 73.33% mortality of *S. zea-mais*. It was found that after 72 h exposure of synergised pyrethrins, the mean difference of percentage deaths of NMHE was statistically significant at 5% significance level (P =0.033) The results further revealed that the difference in concentrations did not differ significantly except at 5,000 ppm and 10,000 ppm. Data obtained also showed that the longer the exposure time of the statistically significant synergist formulations at 5% significance level, the higher the percentage mortalities of *S. zea-mais*.

The co-toxicity values obtained at this ratio show NMHE being an additive at all concentrations after 24 h of treatment and only a synergist to pyrethrins at 5,000 ppm. As the exposure time is increased to 48 h and 72 h, NMHE synergism increases dismally in the concentrations that were earlier additives. The ability of NMHE to counteract the mechanism of metabolic detoxification and avail pyrethrins to kill *S. zea-mais*was not established in this study contrary to other findings. Nutmeg has been found to contain between 7 to 14% essential oils, the principal components being volatile terpenes and phenylpropanoids, including d-pinene, limonene, geraniol, safrole and myristicin (Abourashed & El-Alfy, 2016; Piras *et al*., 2012).

Nutmeg on extraction yields nutmeg oil, the principal component being myristicin or methoxysafrole. Tests with myristicin (one methoxy group) and apiol (two methoxy groups) on the fruitfly, *D. melanogaster* found that apiol was more toxic than myristicin and also showed more pronounced synergistic activity of parathion compared to mysisticin (Bett *et al*., 2016; Oppert *et al.*, 2015) though in its pure form, Rahman *et al.* (2015) also found myristicin as a toxin. Thus nutmeg, though it has been found to contain safrole, the compound associated with the synergism in PBO and myristicin, its synergistic activity still need to be ascertained. The fact that NMHE was found to be additive 24 h duration at low concentration suggest that if formulated at the right

concentration and allowed enough time to interact with the insects, it would still be a viable synergist

However, formulations at 3:1 synergist: pyrethrins ratio, the BPSHE and CLHE could be potential pyrethrins synergists to replace PBO with the most economical concentrations being dependent on the synergist. Since the natural plant oils and extracts used in this study were not pure compounds, synergism could be attributed to any of the constituents present in the oils or extracts even in small amounts. In sesame oil, Beroza (1954) found sesamolin to be about five times more active as pyrethrum synergist than sesamin, and even though sesamolin was present in smaller amounts, it accounted for most of the synergistic activity of sesame oil.

4.3.4 Percentage Mortality of *S. zea-mais* Exposed to Formulations at Ratio 4:1 (Synergist: Pyrethrins) after 24 h, 48 h and 72 h exposure.

The results obtained at 24 h exposure time are presented in Table 19. Percentage mortalities obtained for synergists PBO, CLHE, CRHE, BPSHE and BPSME were statistically significant at ($P \leq 0.05$) ($P = 0.011 < 0.05$, $P = 0.043$, $P = 0.002$, $P = 0.009$ and $P = 0.013$) of PBO, CLHE, CRHE, BPSHE and BPSME respectively). Further, CRHE and BPSHE showed no significant difference at lower concentrations (1,000 ppm and 5,000 ppm) which were also different from 10,000 ppm and 20,000 ppm (DMRT) ($P < 0.05$) (Appendix XIII). However, PBO had a much higher percentage mortality at 20,000 ppm (8 ± 0%) followed by the plant extracts BPSHE (56 ± 3.33%), CLHE (46 ± 3.33%) and BPSME (40 ± 5.77%). Generally, formulations at this ratio gave significantly higher mortality than unsynergised pyrethrins (20% mortality at 2,200 ppm). However, formulations with CLME and NMHE were not significant at 5% significance level and therefore there was no significant difference in percentage mortality with increase in concentration of the extracts.

Ranking the significant means showed that PBO and CLHE at 1.000 ppm was ranked differently from the higher concentrations (5,000 ppm, 10,000 ppm and 20,000 ppm) which were not different from the other. In BPSHE, lower concentrations (1,000 ppm and 5,000 ppm) ranked the same but different from the higher concentrations (10,000 ppm and 20,000 ppm) which were also the same.

Table 19: The Mean Percentage Mortality after 24 h, 48 h, and 72 h Exposure of *S. zea-mais* Adults with Pyrethrins over a Range of Synergist Concentrations in the Ratio of 4:1 (synergist: pyrethrins)

24 h EXPOSURE														
Synergist concentration	Mortality (%)													
	BPSHE		BPSME		CLME		CLHE		NMHE		CRHE		PBO	
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
1,000 ppm	46.67[a]	3.333	20.00[a]	5.774	50.00[a]	5.774	36.67[a]	3.333	50.00[a]	5.774	26.67[a]	3.333	46.67[a]	3.333
5,000 ppm	40.00[a]	0.000	43.33[b]	8.819	53.33[a]	3.333	43.33[ab]	3.333	40.00[a]	5.774	33.33[a]	3.333	70.00[b]	5.774
1,0000ppm	56.67[b]	3.333	30.00[ab]	5.774	43.33[a]	3.333	53.33[b]	3.333	36.67[a]	8.819	56.67[b]	3.333	70.00[b]	5.774
20,000 ppm	56.67[b]	3.333	40.00[ab]	5.774	53.33[a]	3.333	46.67[ab]	3.333	40.00[a]	0.000			80.00[b]	0.000
$P_{(0.05)}$values	.009		0.013		0.33		0.043		0.472		0.002		0.011	
48 h EXPOSURE														
Synergist concentration	Mortality (%)													
	BPSHE		BPSME		CLME		CLHE		NMHE		CRHE		PBO	
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
1,000 ppm	93.33[a]	3.333	66.67[a]	6.667	80.00[a]	5.774	73.33[a]	3.333	56.67[a]	3.333	66.67[a]	8.819	83.33[a]	3.333
5,000 ppm	86.67[a]	8.819	60.00[a]	25.166	100.00[b]	0.000	73.33[a]	6.667	63.33[a]	8.819	76.67[a]	8.819	93.33[a]	3.333
1,0000ppm	76.67[a]	8.819	80.00[a]	5.774	86.67[a]	3.333	76.67[ab]	3.333	50.00[a]	5.774	70.00[a]	5.774	86.67[a]	3.333
20,000 ppm	90.00[a]	5.774	93.33[a]	6.667	100.00[b]	0.000	83.33[b]	3.333	53.33[a]	8.819			90.00[a]	10.000
$P_{(0.05)}$values	.427		0.384		0.006		0.038		0.606		0.680		0.465	
72 h EXPOSURE														
Synergist concentration	Mortality (%)													
	BPSHE		BPSME		CLME		CLHE		NMHE		CRHE		PBO	
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
1,000 ppm	96.67[a]	3.333	86.67[a]	3.333	96.67[a]	3.333	96.67[a]	3.333	83.33[a]	8.819	93.33[a]	3.333	100.00[a]	0.000
5,000 ppm	96.67[a]	3.333	60.00[a]	25.166	100.00[a]	0.000	96.67[a]	3.333	90.00[a]	5.774	90.00[a]	0.000	100.00[a]	0.000
1,0000ppm	93.33[a]	3.333	96.67[a]	3.333	93.33[a]	3.333	100.00[a]	0.000	73.33[a]	8.819	100.00[b]	0.000	96.67[a]	3.333
20,000 ppm	100.00[a]	0.000	96.67[a]	3.333	100.00[a]	0.000	100.00[a]	0.000	83.33[a]	3.333			100.00[a]	0.000
$P_{(0.05)}$values	.487		0.225		0.219		0.596		0.464		0.027		0.51	

*Means within the same column having the same symbol do not differ significantly from one another at p=0.05 test level

Formulating with concentrations that show the same means would require that the lower concentration can be used since the percentage mortality achieved would not be different. This would be an important factor to consider in formulations in order to obtain cost effective but efficacious formulations

After 48 h of exposure (Appendix XIV and Table 19), it was found that CLHE and CLME mortalities were statistically significant at ($P \leq 0.05$) ($P = 0.038$ and $P = 0.006$ respectively). Percentage mortality increased with increase in concentration of the synergist with CLHE being different at 20,000 ppm from the lower concentrations of 1,000 ppm, 5,000 ppm and 1,0000 ppm (Appendix XV). CLME were shown to perform better at 20,000 ppm (100% mortality) followed by CLHE (83.33 ± 3.33%). PBO was statistically not significant ($P > 0.05$) after 48 h exposure. In general, all the plant extracts tested and significant for synergism at the ratio of 4:1 (synergist: pyrethrins) in all the concentrations had mortalities of above 40% which is higher than unsynergised pyrethrins (20%). The overall effect of the plant extracts and PBO synergised pyrethrins showed a consistent trend with that of formulations at the lower ratios (1:1, 2:1, 3:1) with the average percentage mortalities slightly higher.

Further, the study was extended over a period of 72 h exposure and the results are presented in (Table 19). It was found that the mean difference of percentage deaths in CRHE were statistically significant at $P \leq 0.05$ ($P = 0.027$). The lower concentrations (1,000 ppm and 5,000 ppm) had statistically same efficacy that was different from that of 10,000ppm.

Co-toxicity values obtained at ratio 4:1 synergist: pyrethrins at 24 h exposure are presented in Table 20. All plant extracts were synergists to pyrethrins except BPSME that was shown to be an additive at all concentrations except at 5,000 ppm (60.49) where it was a synergist. At 48 h (Appendix XX), BPSHE and PBO both had co-toxicity vaue of 125 at 20,000 ppm followed by BPSME (98.58), CLHE (93.8) with CLME being the highest at a value of 221. Results at 10,000 ppm also showed CLME (203) followed by PBO (188.9), BPSHE (155.6) and CLHE (107.2). Prolonging the time to 72 h (Appendix XXI) show that all the plant extracts were better syneygists than PBO at 20,000 ppm.

Table 20: Co-toxicity Factors Calculated on The Basis of LC_{20} Pyrethrins and Synergists Applied on *S. zea-mais* at Ratio 4:1 (Synergist: Pyrethrins) 24 h Exposure.

	Synergist at 1000ppm			Synergist at 5000ppm			Synergist at 10000ppm			Synergist at 20000ppm		
	% Mortality			% Mortality			% Mortality			% Mortality		
	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor
BPSHE	20	47	133.3	23	40	73.91	27	57	109.9	27	57	109.9
BPSME	20	20	0	27	43	60.49	30	30	0	33	40	21.21
CLME	27	50	85.19	30	53	77.78	23	43	88.41	23	53	131.9
CLHE	20	37	83.33	23	43	88.41	27	53	97.53	37	47	26.13
NMHE	27	50	85.19	23	40	73.91	27	37	35.8	27	40	48.15
CRHE	20	27	33.33	27	33	23.46	20	57	183.3			
PBO	20	47	133.3	20	70	250	23	70	204.3	27	80	196.3

- Values lower than -20 suggest antagonism, values between -20 and 20 suggest additive character and values greater than 20 suggest synergism
- The expected value is contributed by the constant 20% mortality of *S. zea-mais*due to pyrethrins (LC20) and toxicity of the plant extract/synergist alone while observed mortality is due to the synergist-pyrethrins formulation

In the synergism bioassay with synergists at the ratio of 4:1 synergist: pyrethrins all the plant extracts and PBO were statistically significant at 5% significance level 24 h after treatment except CLME and NMHE. This meant that if formulations were to be done at this ratio then the plant extracts could be synergists for pyrethrins formulations. Also, efficacy of the synergists increased with time over the 72-h exposure for example, at 1,000 ppm, all synergists had more than 90% average mortality of *S. zea-mais*. This implies that formulations at lower concentrations could be economically viable since average percentage mortalities will not be different from that of higher concentrations.

The co-toxicity values all showed that the plant extracts and PBO were synergists to pyrethrins except BPSME at 1,000 ppm which showed additive values. Also, the co-toxicity values of all the plant extracts were higher than that of PBO at 20,000 ppm indicating that all the plant extracts were better synergists than PBO at this concentration. Results from section 4.2 had shown that PBO, when applied on *S. zea-mais* was most toxic synergist compared to the plant extracts. This toxicity could partly explain PBO's reduced synergism at 20,000 ppm or it could be due to high concentration of synergist blocking penetration of pyrethrins on the insect's cuticle. BPSHE and CLHE were shown to be better synergists at 20,000 ppm than PBO.

The MDP ring structure considered important for synergism of pyrethrins (Beroza, 1954; Casida, 1970; Haller *et al*., 1942) present in PBO and the plant extracts tested did not confirm show consistency in the results. PBO though widely used in insecticide synergism is only more efficacious at lower concentrations. PBO synthesized from sassafras oil extracted from ocotea tree contains safrole as the main component (Casida & Quistad, 1995). Safrole has also been found to occur naturally in a number of other plant species including black pepper (Russel & Jennings, 1969) and nutmeg (Power &Salway, 1970) which were also tested in these experiments. In the current study, it was found that black pepper seed hexane extract was similar in efficacy with PBO except at 20,000 ppm where BPSHE was a better synergist while nutmeg seed extract was more of an antagonist or an additive at lower concentrations and only a synergist at concentration beyond 5,000 ppm.

BPSHE contain piperine among other essential oils which has been found to show some synergism when used in conjuction with pyrethrum upon gene expression in *D.*

melanogaster with a synergistic ratio of 11:6 (Jensen *et al*., 2006). Also, an extract of *Piper tuberculatum* has been shown to be effective against a resistant strain of Colorado potato beetle, *L. decemlineata* with long history of multiple resistances to several insecticide classes including carbamates, organophosphates, organochlorines and pyrethroids (Scott *et al*., 2003). The structural similarity of the plant extracts used in this study could account for synergistic activity found with CLHE and NMHE.

Nutmeg seed oil has been found to contain myristicin and apiole both compounds with the MDP ring structure characteristic of piperine and PBO. Both myristicin and apiole have previously shown some synergistic properties on pyrethrins and carbamates against *M. domestica* and *D. melanogaster* (Barenbaum & Neal 1985, Lichtenstein *et al* 1974; Lichtenstein & Casida 1963). Also, myristicin has also been shown to effectively synergise xanthotoxin, a naturally occurring insect toxicant, against corn earworm *Heliothis zea* (Berenbaum & Neal 1985). NMHE, in the current study was found to be more of an antagonist to pyrethrins at lower concentrations and ratios and time of exposure. If nutmeg seed extract/oil is to be used as a synergist for pyrethrins, its concentration and time of exposure has to be considered. It is poposed that some compounds in nutmeg extract get activated with time and higher concentrations work better unlike PBO where more concentration and exposure time tend to slow down its efficacy.

Parsley seed and leave extracts have also been found to possess myristicin and apiole among other components. *Coriandrum sativum* has previously not been tested as synergist for insecticides though the seeds and leaves essential oils have been known to support various biological activities such as antimicrobial, antifungal and antioxidant properties (Wangensteen *et al*., 2004). Synergistic activity of coriander oil and various antibiotics against *Acinetobacter baumannii* showed that a combination of coriander essential oil with ciprofloxacin, gentamicin and tetracycline against *A. Baumannii* showed *in vitro* effectiveness which indicated a possible synergistic interaction (Duarte *et al*., 2012). Shahwar *et al* (2012), in their study on antifungal activity of *C. sativum* essential oil against candida species and potential synergism with amphotericin B found that a synergistic effect between coriander oil and amphotericin B was obtained for *Candida albicans* strain while for *Candida tropicalis*, only an additive effect was observed. Similar findings were shown in the current study where CLHE at 1,000 ppm

and formulated at ratio 1:1 synergist: pyrethrins was shown to be an additive to pyrethrins 42 h exposure and only a synergist at higher concentrations (5,000 ppm, 10,000 ppm and 20,000 ppm) Evaluation of synergistic antibacterial and antioxidant efficacy of coriander essential oils have also shown that coriander/cumin combination had synergistic interaction against bacteria (Anwesa & Ranjan, 2015). Apiol, contained in dill and parsley root and leave extracts have been shown to effectively synergise parathion against *D. melanogaster* (Lichtenstein, 1974). The results of these studies are in harmony with those of the current study.

In synergistic bioassay with *S. zea-mais*at lowered concentration of 1,000 ppm after 24 h exposure, PBO showed the highest efficacy as a pyrethrins synergist. However, at a higher concentration of synergist, coriander leaf extract and black pepper seed extract also enhanced synergism with pyrethrins. Though the mode of action of the synergists was not tested in this study, the high efficacy of PBO as a synergist could possibly be attributed at other factors like its surfactant properties which could facilitate the penetration of pyrethrum through the insect cuticle. PBO has been found to increase the penetration of esfenvalerate (Gunning *et al*., 2006) and permethrin (Kennuagh *et al*. 1993).

The differences observed with n-hexane and methanol extracts of Black pepper seed and coriander leaves extracts could be used to explain the differences observed in their efficacy. Methanol extracts were inconsistent in synergising pyrethrins. BPSME was more of an additive in the formulations while CLHE was a synergist. Since n-hexane dissolves non-polar compounds and methanol dissolves polar compounds, further investigations need to be done to ascertain the actual compounds involved in the synergism/additive observed.

4.3.5 Overall Percentage Mortality of *S. zea-mais* calcutated from the Four Ratios of Synergists: Pyrethrins Formulations 24 h, 48 h and 72 h exposure

The mean percentage mortality of *S. zea-mais adults* per synergist calculated from discriminatory dose bioassay with mixtures of potential synergists each at the four ratios of synergist: pyrethrins (1:1, 2:1, 3:1 and 4:1) after 24 h exposure is shown on Figure 4. PBO had higher average mortality at all ratios of synergist: prethrins but higher at ratio 1:1 with 73% average mortality of weevils followed by the ratio of 4:1

at 67% average mortality. This implies that PBO yield higher mortality rate at the ratio of 1:1 (synergist: pyrethrins) in the current study. In addition, BPSHE plant extract had highest mean mortality rate compared to the other plant extracts under study. In this case, the ratio of 2:1 (synergist: pyrethrins) had higher mortality (56%) than PBO (51%) thus BPSHE was a better synergist to pyrethrins than PBO at ratio 2:1.

The information in Figure 5 shows the mortality rates of PBO and plant extracts after 48 h treatment. It was found that the highest mortality rate was that of PBO (92%) and CLME (92%) at different ratios of 1:1 and 4:1 synergist: pyrethrins respectively. In this case CLME recorded the same mortality rate with PBO after 48 h exposure period but at different ratios of synergist: pyrethrins. Similarly, BPSHE and PBO had mortality rates of 85% at different ratios of 2:1 and 4:1 synergist: pyrethrins respectively. All the plant extracts and PBO recorded significantly higher mortality rates at the ratio of 4:1 with the lowest recording 56% average mortality.

It was also noted that the synergist/acetone recorded a slightly higher mortality rate after 48 h compared to a previous time exposure of 24 h. In this case synergist contributed close to 23% mean mortality rate in BPSME. However, PBO and the plant extracts still had high mortality means at different ratios with an overall achievement of at least 50% average mortality rates.

With 72 h exposure (Figure 6) PBO and most of the plant extracts namely: - BPSHE, BPSME, CLME and CLHE yield mortality rates is almost 100% for different rations of synergist: pyrethrins. It can also be noted that PBO had over 90% mortality rates for all the ratios under the current study. It is important to note that under BPSHE, BPSME, CLME, CLHE and CRHE, the ratio 4:1 yielded over 90% average mortality rates. After 72 h exposure, synergists recorded mortality rates of upto 28% under BPSME in comparison to 24 and 48 h exposure.

From these results, there seems to be no particular ratio at which to administer a synergist in an insecticide. Yamamoto, 1973 showed that a synergistic effect is greatly influenced by several factors including the synergist itself, the insecticide used and insect species involved. Thus in order to be effective, a synergist should penetrate the insect and be transported to the target site more rapidly than the insecticide (Casida,

1970; Yamamoto, 1973). Nash (1954) found that there exists an optimal biological ratio for different pest species and probably each individual synergist. Although not directly tested in this study, pre-treatment with synergists has been found to increase the amount of synergism due to considerable time taken by synergist to maximally inhibit the specific metabolic enzymes within the insect (Bingham *et al*., 2007; Young *et al*., 2005, 2006).

However, this study, longer time of exposure to the formulations greatly increased the average percentage mortality of *S. zea-mais* after 72 h even at lower concentrations (1,000 ppm and 5,000 ppm) of the synergists. Desmachelier (1977) reported that pyrethrins at 1mg/kg synergist with PBO (1:10 ratio) were ineffective against *R. dominica* and synergist pyrethrins with PBO applied at a rate 1.5mg/kg were ineffective against five insect pests of stored products (Subramanyam and Fangeng, 2005). With increased rates (4mg/kg), Ashamo *et al*. (2013) found that significant control of *R. dominica* could be achieved for more than 140 days contrary to the present study.

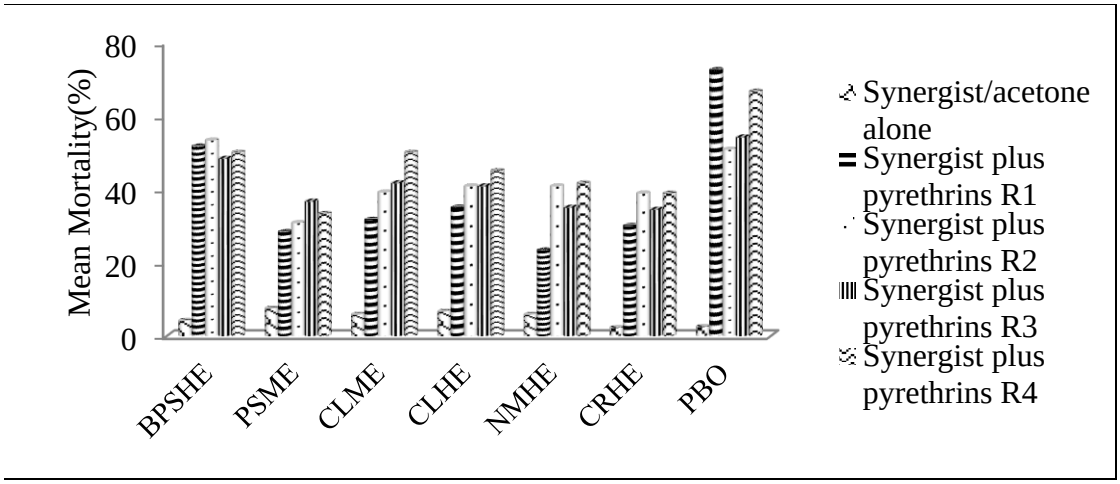

Figure 4: The mean percentage mortality of *S. zea-mais* adults calculated from discriminatory dose bioassays with mixturesof potential synergist each at four ratios of synergist: pyrethrins (1: 1, 2:1, 3:1 and 4:1) designated R1, R2, R3and R4 respectively and with synergist alone 24 h after treatment

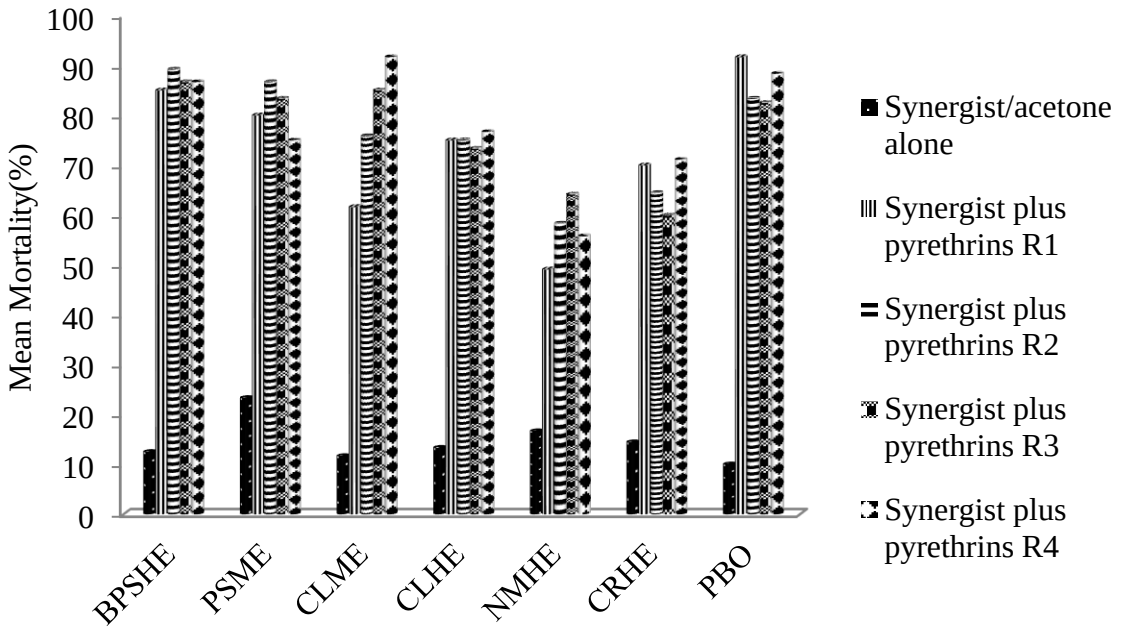

Figure 5: The mean percentage mortality of *S. zea-mais adults* calculated from discriminatory dose bioassays with mixtures of potential synergist each at four ratios of synergist: pyrethrins (1: 1, 2:1, 3:1 and 4:1) designated R1, R2, R3and R4 respectively and with synergist alone 48 h after treatment

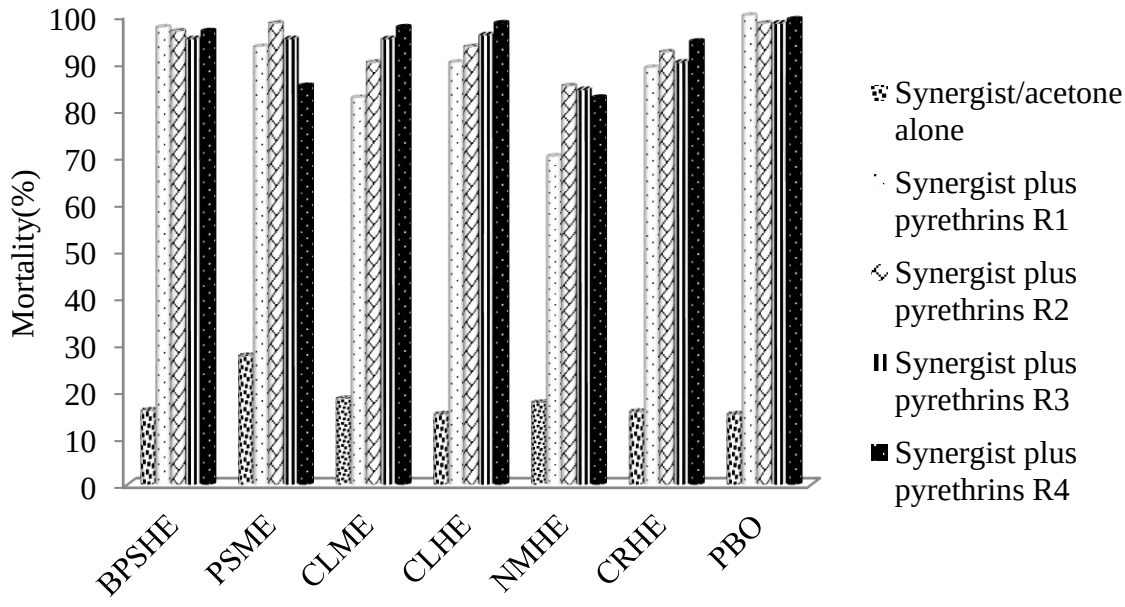

Figure 6: The Mean Percentage Mortality Of *S. zea-mais adults* Calculated From Discriminatory Dose Bioassays With Mixturesof Potential Synergist Each At Four Ratios Of Synergist: Pyrethrins (1: 1. 2:1, 3:1 And 4:1) Designated R1, R2, R3and R4 Respectively And With Synergist Alone 72 H After Treatment

4.4 Evaluation of the Potency of Plant Extracts-synergised Pyrethrins Formulations on *S.zea-mais*

The study sought to evaluate the potency of plant extracts-synergised pyrethrins on *S. zea-mais*. Table 13 shows that after 7 days' exposure, formulations with PBO and the plant extracts namely: -BPSME, CLME CLHE and BPSME were statistically significant at 5% significance level (P = 0.01, P = 0.04, P = 0.000, and P = 0.000 of PBO, CLME, CLHE, and BPSME respectively). More synergist concentrations led to higher the average percentage mortality in these formulations. However, PBO registered higher percentage mortalities in all the synergist concentrations. For instance, at 20 000ppm, PBO had 96.67% mortality of *S. zea-mais,* followed by BPSME (92%), CLHE (89%) and CLME (88.67%). However, formulations with BPSHE, NMHE and CRHE were insignificant (P > 0.05).

The high average mortalities in all the plant extracts showed consistency with results when time of exposure is increased. At 20,000 ppm concentration, BPSHE, BPSME, CLME and CLHE had mean average mortality above 89% which was higher compared to the other concentrations for the same plant extracts. NMHE at a concentration of 5,000 ppm higher average mortality (65%) compared to other concentrations which had lower mortalities. However, CRHE had 71% average mortality at 5,000 ppm compared to the other concentration levels. The results indicated that at a concentration level of 1,000 ppm, 65% and above mortality rates of the maize weevil adults can be achieved on all the plant extracts tested and PBO except NMHE. This implies that low concentration of 1,000 ppm can achieve significant protection of maize grains within a period of seven days' exposure.

Further, it was found that at concentration of 20,000 ppm, NMHE had the lowest mortality rate of 61% as compared to lower concentrations. This could be attributed to the fact NMHE could be containing a mixture of components that may not be acting at the same time therefore delaying its effect. Nutmeg oil has been found to contain the component myristicin which also occurs in essential oil of plants like dill or parsnip and parsley (Simon & Quinn, 1988). This could be explained by the fact that myristicin with its one methoxy group on the MDP ring have less pronounced synergistic activity (Oppert *et al*, 2015), thus the low percentage mortalities exhibited by NMHE.

Table 21: The Mean Percentage Mortality 7 Days after Exposure of *S. zea-mais adults* with Pyrethrins over a Range of Synergist Concentrations in the Ratio of 4:1

Synergist concentration	Mortality (%)													
	BPSHE		BPSME		CLME		CLHE		NMHE		CRHE		PBO	
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
1,000 ppm	92.00[a]	1.000	69.00[a]	1.000	74.67[a]	3.930	67.67[a]	2.906	51.33[a]	2.963	61.33[a]	2.963	84.33[a]	1.333
5,000 ppm	87.67[a]	2.906	85.67[bc]	2.963	72.33[a]	2.906	72.00[a]	1.000	65.33[b]	2.333	71.33[b]	2.963	82.33[a]	3.930
1,0000ppm	84.33[a]	4.667	84.33[b]	2.963	76.67[a]	3.756	67.67[a]	2.906	60.00[ab]	4.041	69.00[ab]	1.000	88.00[a]	1.000
20,000 ppm	90.00[a]	0.000	92.00[c]	1.000	88.67[b]	2.963	89.00[b]	1.000	61.00[ab]	4.933	-	-	96.67[b]	2.028
$P_{(0.05)}$values	0.31		0.00		0.04		0.00		0.13		0.07		0.012	

*Means within the same column having the same symbol do not differ significantly from one another at p=0.05 test level

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary of Findings

The research study aimed at investigating the synergistic qualities of natural plant extracts with pyrethrins against the maize weevil, *S. zea-mais*(Motsch) (Coleoptera: Curculionidae). Plant extracts obtained from black pepper seeds (Piper nigrum), Nutmeg seeds, (Myristica fragrans), coriander leaves and roots, (Coriandrum sativum) were tested for synergism of pyrethrins against *S.zea-mais* and compared to the standard synergist, PBO. Bioassays were maintained under controlled storage experimental growth chamber conditions of 27 ± 2 ^{0}C and 60 ± 5% RH with normal day light hours at Plant Sciences laboratory of Chuka University, Kenya. Three types of bioassays were used in a completely randomized design. Topical application of seven potential synergists were used to dose the insects with a synergist or formulation in triplicate.

The study sought to to determine in vivo the Lethat Concentration (LC) values for Pyrethrins on *S. zea-mais*. Probit analysis model used sufficiently obtain LC20 value for pyrethrins of 2,200. This low LC value was used for the synergistic bioassays to ensure low percentage mortality with unsynergised pyrethrins and allow for the synergistic activity by the porential synergists It was found that percentage mortality of insects increased with an increase in concentration of pyrethrins. The results also showed that longer exposure time increased percentage mortality of the maize weevils. At a concentration of 10,000 ppm, the mortality rate translated to 44.8%, 45.2% and 51.2% at 24, 48 and 72 h exposure duration respectively. Also, lower concentrations of pyrethrins were needed to achieve higher percentage mortality with prolonged exposure. To obtain LC_{50}, 14227 ppm, 13780 ppm and 8868 ppm of pyrethrins is required over 24 h, 48 h and 72 h exposure time respectively. Allowing sufficient time for pyrethrins to interact with the maize weevil can be cost effective since use of lower concentrations and quantity can be used to achieve significant control of this pest.

The study sought to determine *in vivo* the effect of the selected plant extracts on *S. zea-mais* The mean difference of percentage deaths of BPSME and CLHE were both statistically significant ($P \leq 0.05$). CLHE showed higher toxicity 16.67 ± 3.33% than BPSME with an average mortality rate of 13.33 ± 3.33% at synergist concentration of 20,000 ppm 24 h after treatment. CLHE had higher average mortality at 10,000 ppm

and 20,000 ppm of 16.67 ± 3.33% and 23.33 ± 4.33% respectively compared with PBO and BPSHE each with 10 ± 0% and 20 ± 5.77% mortalities respectively. PBO was shown to be the toxic synergist (36.67 ± 3.33%) than CLHE (26.67 ± 3.43%) at 20,000 ppm. Generally, at lower concentrations (1,000 ppm and 5,000 ppm), toxicity of all synergists tested was low. At 24 h exposure time, CLHE and BPSME recorded 16.67% and 13.33% mortalities at 20,000 ppm concentration respectively. With increase of exposure time to 48 h and 72 h, CLHE percentage mortality of the maize weevils increased to 23.33% and 26.67% respectively. BPSHE and PBO after 48 h exposure were statistically significant ($P \leq 0.05$) with the average percentage mortalities of 10% and 20% at 10,000 ppm and 20,000 ppm respectively while after 72 h, PBO and CLHE were significant. The plant extracts tested and statistically significant at a particular exposure time can be used as synergists for pyrethrins since their inherent toxicity is low.

Further the study sought to determine the potency of plant extracts-synergised pyrethrins formulations at different rates and concentrations on stored maize against *S. zea-mais*. At the ratio of 1:1, formulations with PBO and the plant extracts CLHE, NMHE and BPSME were statistically significant ($P \leq 0.05$) 24 h after exposure. PBO registered higher percentage (83.33 ± 12.02%) mortality followed by CLHE (46.67 ± 3.33%), BPSME (43.33 ± 6.67%) and NMHE (26.67 ± 3.33%). In addition, these compounds when formulated with pyrethrins at this ratio gave significantly higher mortality than unsynergised pyrethrins (20% mortality at 2200 ppm) except BPSME and NMHE at 1,000 ppm (16.67 ± 3.33% and 6.67 ± 3.33% mortality respectively). NMHE, though statistically significant ($P \leq 0.05$) had low percentage mortalities below that of LC20 at 1,000 ppm (6.67%, 16.67% and 16.67%) over 24 h, 48 h and 72 h respectively while BPSME had 16.67% mortality at 24 h.

Increasing the exposure period of the formulations to 48 h and 72 h also increased the overall percentage mortality of the maize weevils indicating that when the formulations are allowed time to interact with the maize weevils, higher mortalities could be achieved. Generally, it did not follow that increasing concentration of a synergist increased the percentage mortality of *S. zea-mais*geometrically except for CLHE (24 h and 48 h) and PBO (24 h) where increase in concentration of synergist increased the percentage mortality of the maize weevil

From the results obtained BPSHE and PBO synergized the toxicity of pyrethrins in all the concentrations and ratios tested over the 72 h exposure time with co-toxicity factors above 10, the highest being PBO at 1,000 ppm synergizing pyrethrins 300 fold which reduces as concentration is increased. BPSHE also consistently showed the highest co-toxicity factor of all the plant extracts tested. CLHE was only an additive at 1,000 ppm over 24 h exposure and a synergist in the rest of the concentrations and time. BPSME with co-toxicity values of between -20 and 20 at concentration of 1,000 ppm, 5,000 ppm and 10,000 ppm respectively over 24 h period was an additive to the formulation and only a synergist at 20,000 ppm while NMHE is antagonistic to pyrethrins at 1,000 ppm with co-toxicity values less than -20 over 24 h, 48 h and 72 h exposure period. NMHE showed significant synergism at concentrations of 5,000 ppm, 10,000 ppm and 20,000 ppm after 48 h and 72 h exposure period

BPSHE and CRHE were statistically significant at $P \leq 0.05$ ($P = 0.022$ and $P = 0.045$ of BPSHE and CRHE respectively). BPSHE registered higher percentage mortality at 1,000 ppm (43.33 ± 6.67%) and 5,000 ppm (56.67 ± 3.33%) compared with CRHE at the same concentrations with 26.67 ± 6.67% and 36.67 ± 3.33% respectively. When significant means were ranked, BPSHE showed that 20,000 ppm was different from 1,000 ppm and that 5,000 ppm and 10,000 ppm were statistically the same. In CLHE, ranked means were all different indicating with each concentration having very independent percentage mortalities. After 48 h duration, only CLHE was statistically significant ($P \leq 0.05$) ($P = 0.005$) with NMHE and BPSME showing mortalities that are marginally significant ($P = 0.059$ and $P = 0.091$ of CRHE and BPSME respectively). BPSME was shown to be the most effective synergist at a concentration of 20,000ppm (100% mortality) followed by CLHE (96.67 ± 3.33%) then NMHE (60 ± 5.77%) mortality at the same concentration. Results also showed that increasing concentrations did not correspond to geometric increase in percentage mortality of *S. zea-mais*.

BPSME is the only plant extract that is shown to be an additive at 1,000 ppm (-16.7) and 10,000 ppm (-11.1) after 24 h. At 20,000 ppm, BPSHE (146.9) is a better synergist than PBO (97.53). The co-toxicity values obtained indicated that BPSME contributed towards the toxicity of pyrethrins by being an additive rather than a synergist while NMHE synergized pyrethrins except at 1,000 ppm concentration. BPSHE with values

above 100 in all concentrations was the most efficacious plant extract with more synergism (146 fold) than the standard PBO (97.53) at 20,000 ppm after 72 h exposure period. The plant extract BPSHE was the most effective potential synergist that could replace PBO in pyrethrins formulations. With exposure time extended to 72 h, plant extracts achieved high mortalities even at low concentrations (1,000 ppm) with PBO and BPSHE achieving 100% mortality.

At the ratio of 3:1, formulations with PBO and the plant extracts CLHE, NMHE and BPSHE were statistically significant ($P \leq 0.05$) ($P = 0.0204$, $P = 0.024$, $P = 0.049$ and $P = 0.0421$ of PBO, CLHE, NMHE and BPSHE respectively) after 24 h exposure. PBO registered higher percentage mortalities under all the synergist concentrations tested. Ranking of the means showed that the efficacy of PBO, BPSHE and NMHE and CLHE at 20,000 ppm was significantly different at ($P \leq 0.05$) from the lower concentrations (1,000 ppm, 5,000 ppm and 10,000 ppm) BPSHE at 20,000 ppm and PBO at 5,000ppm showed similar efficacy of 56.67 ± 3.33% while at 10,000 ppm it is equivalent to PBO at 1000ppm and NMHE at 20,000 ppm with average mortality of 53.33 ± 8.819%after 24 h exposure thus BPSHE can replace PBO when formulated at 20.000 ppm with PBO at 5,000 ppm. These comparisons are important when deciding on use of a synergist. The factors that would be needed for consideration using results in the current study would be a combination of concentration, ratio and time of exposure that work best for a synergist.

The most economical concentration could be at 5,000 ppm with NMHE since 96.67 ± 3.33% percentage mortality of *S. zea-mais*was achieved after 72 h. NMHE could have been slow in acting on the test insects and its active components need a prolonged time to show effect compared with the other plant extracts which were significant ($P \leq 0.05$) after 48 h. Generally, there was no consistency of results at $P \leq 0.05$ after 48 h and 72 h exposure time. The co-toxicity values obtained at this ratio show NMHE being an additive at all concentrations after 24 h of treatment and only a synergist to pyrethrins at 5,000 ppm. As the exposure time is increased to 48 h and 72 h, NMHE synergism increases dismally in the concentrations that were earlier additives. However, formulations at 3:1 synergist: pyrethrins ratio, the BPSHE and CLHE could be potential pyrethrins synergists to replace PBO with the most economical concentrations being dependent on the synergist

In general, all the plant extracts tested and significant for synergism at the ratio of 4:1 (synergist: pyrethrins) had mortalities of above 40% which is higher than unsynergised pyrethrins (20%) in all the concentrations. The plant extracts were synergists to pyrethrins except BPSME that was shown to be an additive at all concentrations except at 5,000 ppm (60.49) where it was a synergist. At 48 h, BPSHE and PBO both had co-toxicity vaue of 125 at 20,000 ppm followed by BPSME (98.58), CLHE (93.8) with CLME being the highest at a value of 221. Results at 10,000 ppm also showed CLME (203) followed by PBO (188.9), BPSHE (155.6) and CLHE (107.2). Prolonging the time to 72 h show that all the plant extracts were better syneygists than PBO at 20,000 ppm.

In the synergism bioassay with synergists at this ratio, all the plant extracts and PBO were statistically significant ($P \leq 0.05$) level 24 h after treatment except CLME and NMHE. This meant that if formulations were to be done at this ratio then the plant extracts could be synergists for pyrethrins formulations. Also, efficacy of the synergists increased with time over the 72-h exposure for example, at 1,000 ppm, all synergists had more than 90% average mortality of *S. zea-mais*. This implies that formulations at lower concentrations could be economically viable since average percentage mortalities will not be different from that of higher concentrations. The co-toxicity values all showed that the plant extracts and PBO were synergists to pyrethrins except BPSME at 1,000 ppm which showed additive values

NMHE, in the current study was found to be more of an antagonist to pyrethrins at lower concentrations and ratios and time of exposure. If nutmeg seed extract/oil is to be used as a synergist for pyrethrins, its concentration and time of exposure has to be considered. At a lower concentration of 1,000 ppm after 24 h exposure, PBO showed the highest efficacy as a pyrethrins synergist. However, at a higher concentration of synergist, coriander leaf extract and black pepper seed extract also enhanced synergism with pyrethrins. Methanol extracts were also shown to be inconsistent in synergising pyrethrins. BPSME was more of an additive in the formulations while CLHE was a synergist. Since n-hexane dissolves non-polar compounds and methanol dissolves polar compounds, further investigations need to be done to ascertain the actual compounds involved in the synergism/additive observed. The plant extracts tested in this study were

expected to portray the same results as PBO because of the MDP ring thus the differences observed in their efficacy will have to be further investigated. It was found that longer exposure time yielded higher mortality rates even with lower concentrations of synergists. This could be an important consideration in insecticide formulations that are cost effective and efficacious

The overall mean mortality results comparing efficacy of each synergist at ratio 4:1showed geometric increase in mortality consistenly over the 72 h period. Pyrethrins-synergised with PBO (1,000 ppm, 5,000 ppm and 20,000 ppm), BPSHE (20,000 ppm), CLME (5,000 ppm and 20,000 ppm), CLHE (1,0000 ppm and 20,000 ppm) yield 100% mean mortality on *S. zea-mais*. The statistically significant mortalities ($P \leq 0.05$) by the synergists at 1,000 ppm implied that formulations at lower concentrations could be economically viable than at higher concentrations.

The study sought to evaluate the efficacy of plant extracts synergised pyrethrins formulations treated maize on infestation by *S. zea-mais.* After 7 day-exposure, PBO registered higher percentage mortalities under all the synergist concentrations tested. For instance, at 20,000 ppm, PBO had 96.67% mortality of *S. zea-mais*, followed by BPSME (92%), CLHE (89%) and CLME (88.67%).

5.2 Conclusion

From the findings of the study the following conclusions were drawn: -

LC20 for pyrethrins for pyrethrins was found to be 2,200 ppm which was used for synergism experiments. Percentage mortality of insects increased with an increase in concentration of pyrethrins and that longer exposure time increased percentage mortality of the maize weevils. Allowing sufficient time for pyrethrins to interact with the maize weevil can be cost effective since use of lower concentrations and quantity can be used to achieve significant control of this pest

Toxicity of plant extracts tested was low hence qualified as potential synergists to replace the standard, PBO in pyrethrins formulations. At a higher concentration (20,000 ppm) PBO was more toxic than the plant extracts tested.

Plant extracts BPSHE and CLHE and PBO synergized the toxicity of pyrethrins in all concentrations at the ratio of 1:1 synergist: pyrethrins over 72 h exposure duration with co-toxicity factors above 100. Increasing the exposure period of the formulations increased the overall percentage mortality of the maize weevils indicating that when the formulations are allowed time to interact with the maize weevils, higher mortalities could be achieved. BPSHE consistently showed the highest co-toxicity factor of all the plant extracts tested. CLHE was only an additive at 1,000 ppm over 24 h exposure and a synergist in the rest of the concentrations and time. BPSME was shown to be an additive to the pyrethrins formulation and only a synergist at 20,000 ppm while NMHE is antagonistic to pyrethrins at 1,000 ppm and a synergist at concentrations of 5,000 ppm, 10,000 ppm and 20,000 ppm after 48 h and 72 h exposure period

Increasing the concentration of a plant extract does not correspond to a geometric increase in percentage mortality of *S. zea-mais*. The plant extract BPSHE was the most effective potential synergist that could replace PBO in pyrethrins formulations. With exposure time extended to 72 h, plant extracts achieved high mortalities even at low concentrations (1,000 ppm) with PBO and BPSHE achieving 100% mortality

BPSHE was a better synergist than PBO at a concentration of 5,000 ppm followed CLHE. NMHE was an additive at all concentrations after 24 h of treatment and only a synergist to pyrethrins at 5,000 ppm. BPSHE and CLHE are potential synergist to replace PBO while NMHE is an additive when formulated at the ratio of 3:1 synergist: pyrethrins with the most economical concentrations being dependent on the synergist

NMHE, in the current study was an antagonist to pyrethrins at lower concentrations. If nutmeg seed extract/oil is to be used as a synergist for pyrethrins, its concentration and time of exposure has to be considered Methanol extracts were also shown to be inconsistent in synergising pyrethrins. BPSME was more of an additive in the formulations while CLHE was a synergist. Since n-hexane dissolves non-polar compounds and methanol dissolves polar compounds, further investigations need to be done to ascertain the actual compounds involved in the synergism/additive observed. The plant extracts tested in this study were expected to portray the same results as PBO because of the MDP ring thus the differences observed in their efficacy will have to be further investigated. It was found that longer exposure time yielded higher mortality

rates even with lower concentrations of synergists. This could be an important consideration in insecticide formulations that are cost effective and efficacious

Efficacy of the synergist-synergist pyrethrins increased over the 72 h exposure for example, at 1,000 ppm, all synergists had more than 90% average mortality of *S. zea-mais*. Formulations at lower concentrations could be economically viable since average percentage mortalities will not be different from that of higher concentrations.

5.3 Recommendation

The following recommendations were made:

i. Time of exposure of a synergist and an insecticide play a critical role in achieving high mortality rates of *S. zea-mais*regardless of the ratio of synergist: pyrethrins
ii. Hexane extracts of black pepper seed (*P. nigrum*) and coriander leaves (*C. sativum*) can replace piperonyl butoxide (PBO) in pyrethrins formulations against *S. zea-mais*
iii. Increasing the concentration of a plant extract does not correspond to a geometric increase in efficacy when formulated with pyrethrins
iv. It is economical to achieve high mortality rates of *S. zea-mais*at low concentration of pyrethrins or in formulations over a prolonged duration 72 h

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5.4 Suggestions for Further Study

The following were areas suggested for further studies

i. *In vitro* studies of refined constituents of BPSHE and CLHE to determine their mode of action in insects
ii. Chemical structure and composition of *C. sativum* and *P. nigrum* need to be elucidated
iii. Further investigation to acertain the observed differences in efficacy of plants possessing MDP ring structure thought to be involved in synergism
iv. Investigate the ability of NMHE to counteract the efficacy of pyrethrins as an antagonist in pyrethrin formulations.
v. Further investigations need to be done on polar and non-polar compounds of plants to ascertain the actual compounds involved in the synergistic, additive or antagonistic effects of pyrethrins.

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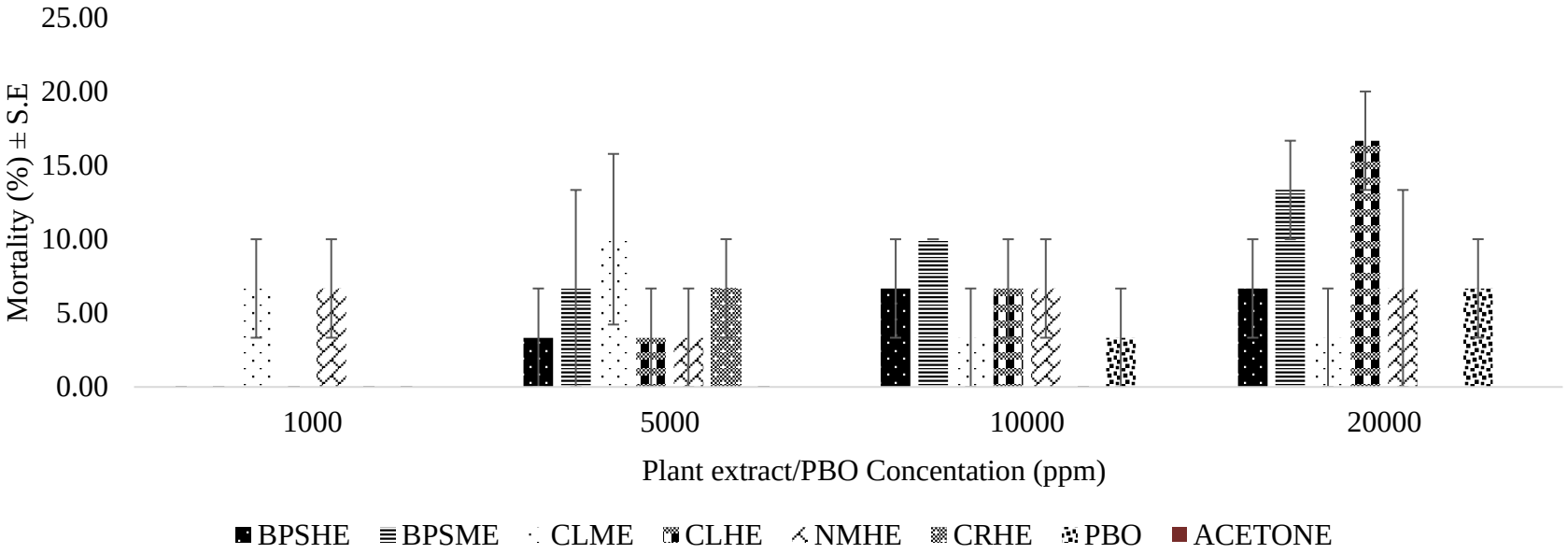
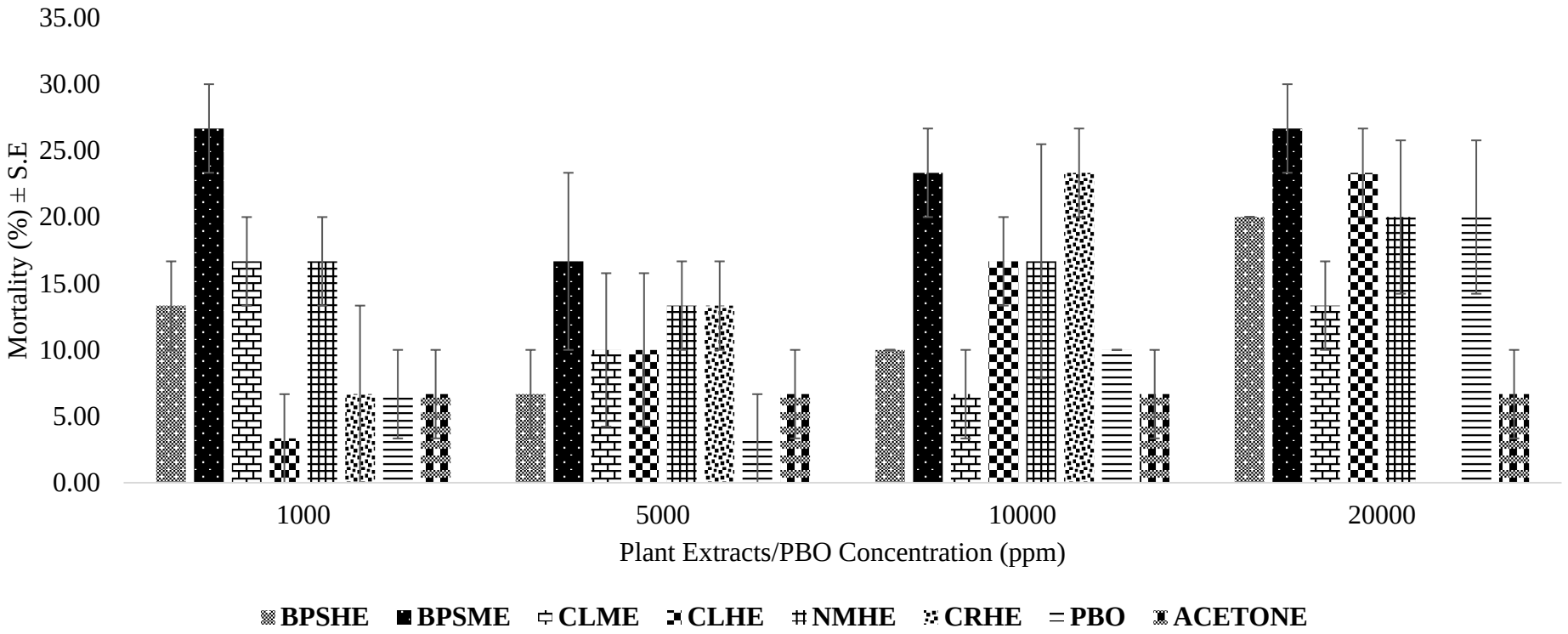
APPENDICES

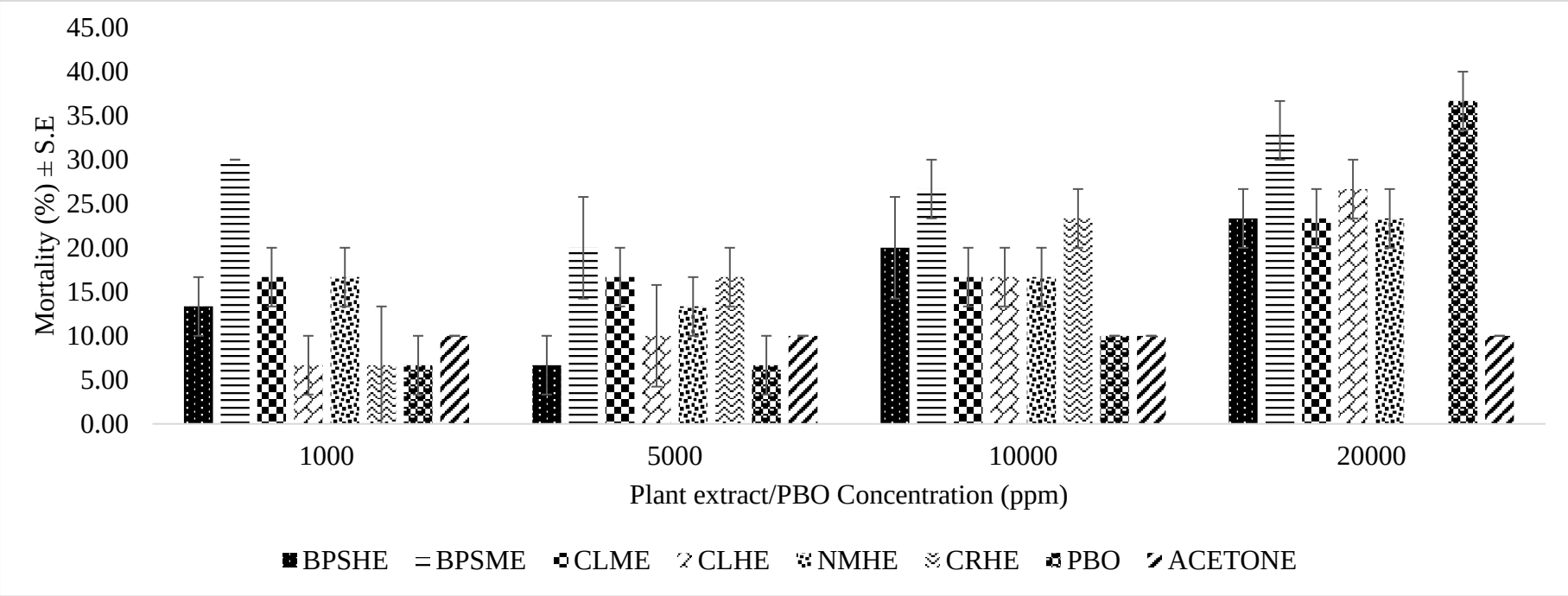
Appendix I: Mean Percentage Mortality (± S.E) of *S. zea-mais adults* at 24 h After Topical Application of Each Plant Extract at Four Concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm), PBO and Acetone as The Controls.



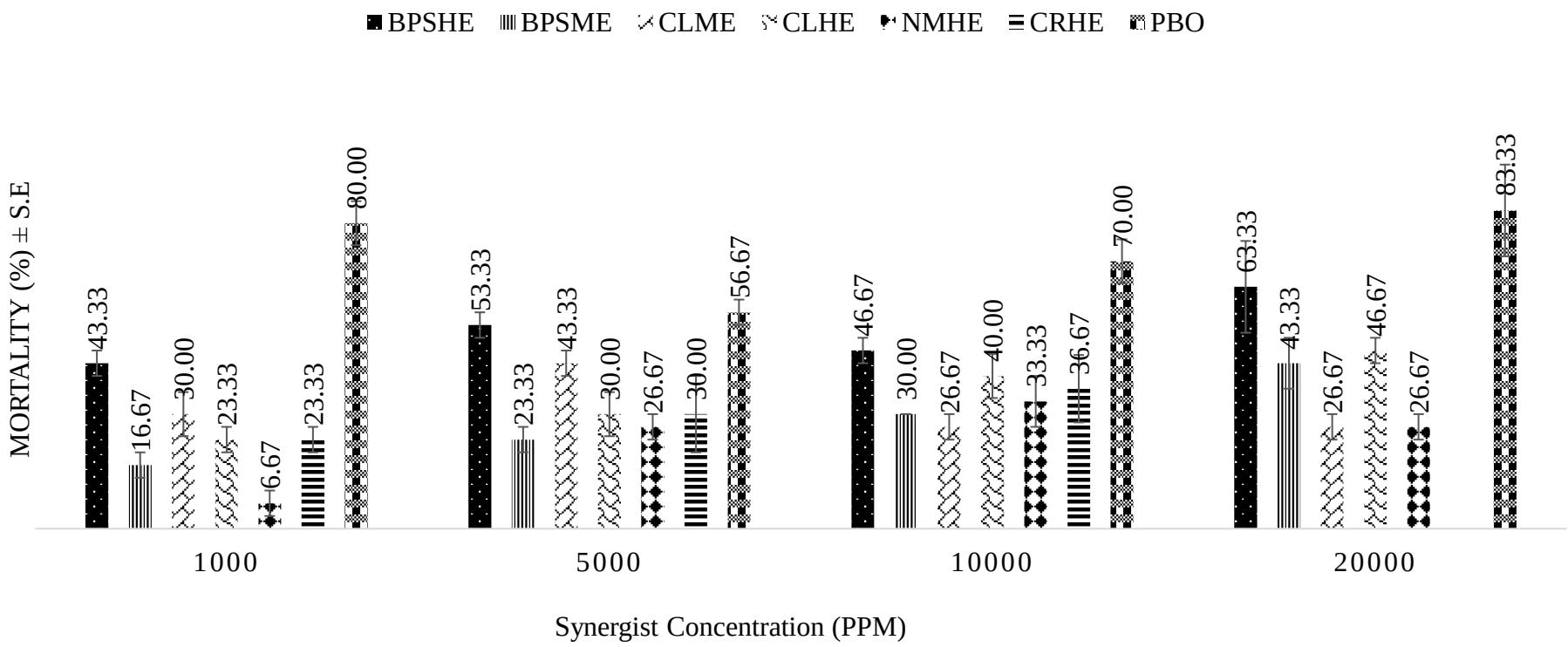
Appendix II: Mean Percentage Mortality (± S.E) of *S. zea-mais adults* at 48 h after Topical Application of each Plant Extract at Four Concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm), PBO and Acetone as the Controls.



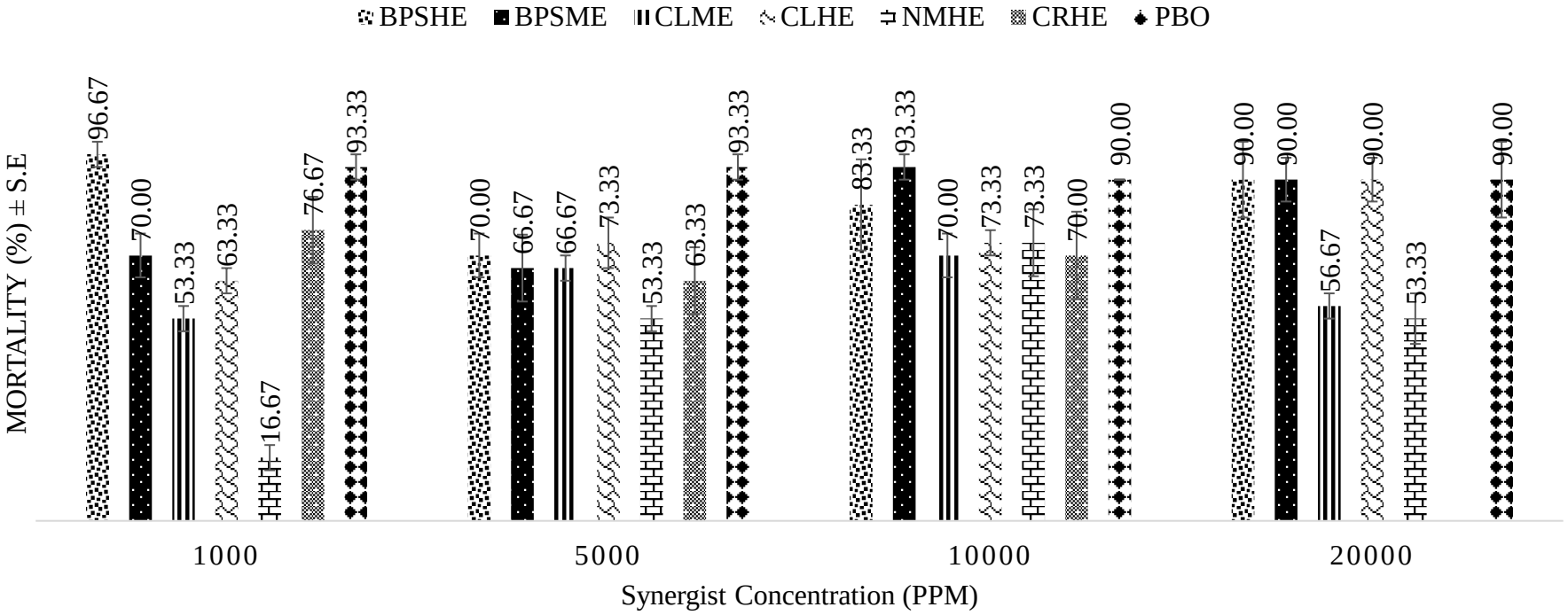
Appendix III: Mean Percentage Mortality (± S.E) of *S. zea-mais adults* at 72 h after Topical Application of Each Plant Extract at Four Concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm), PBO and acetone as the controls.



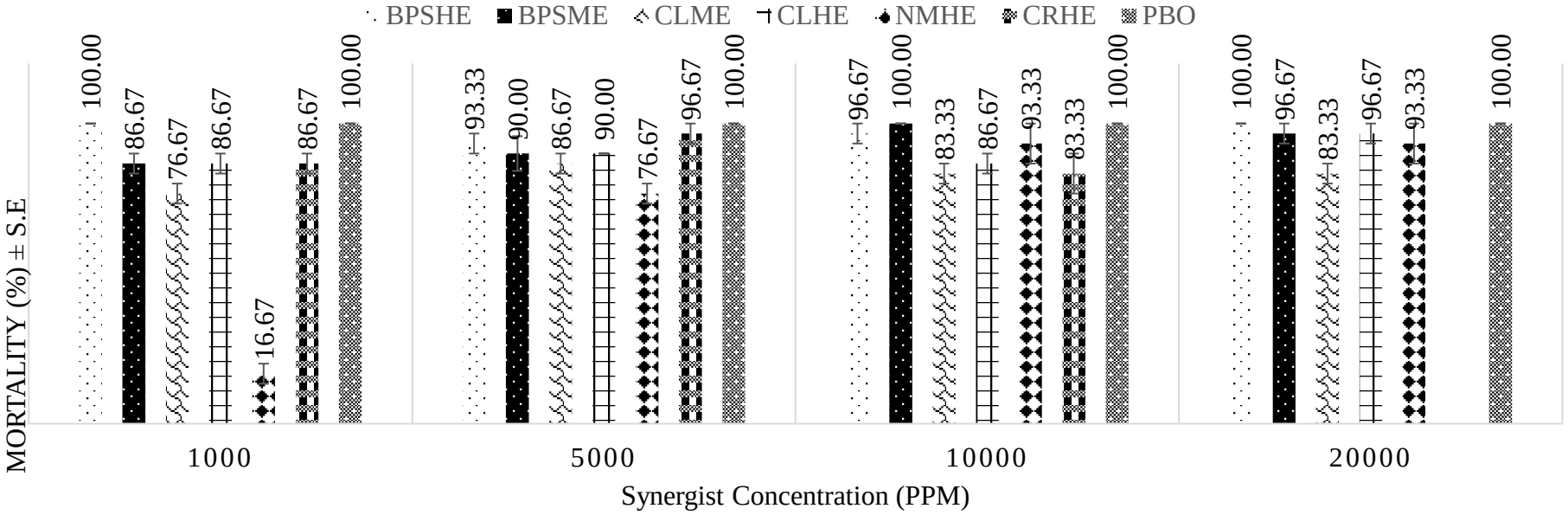
Appendix IV: Mean Percentage Mortality (± S.E) in *S. zea-mais adults* at 24 h Exposure after Application of Synergists at Four Concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) in Combination with LC_{20} pyrethrins (2,200 ppm) at the Ratio of 1:1 (Synergist: Pyrethrins).



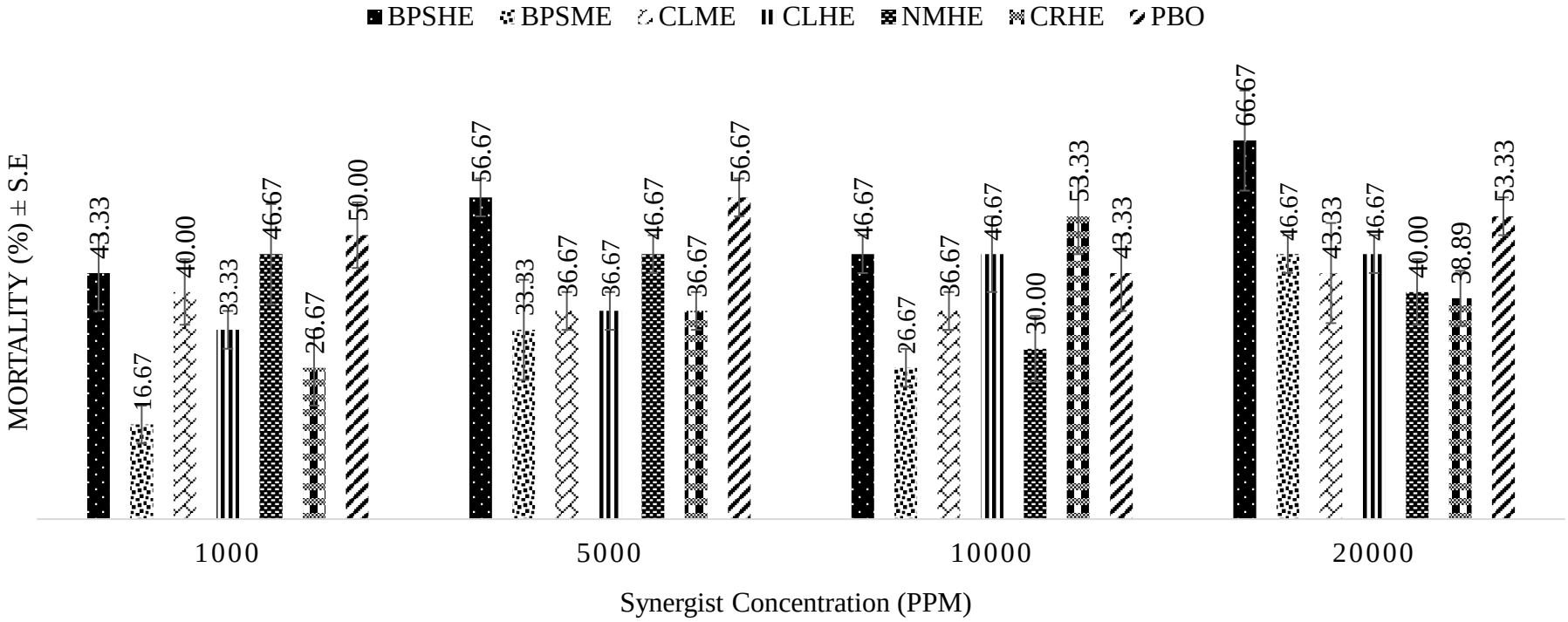
Appendix V: Mean Percentage Mortality (± S.E) in *S. zea-mais adults* at 48 h Exposure After Application of Synergists at Four Concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) in Combination with LC_{20} pyrethrins (2,200 ppm) at the Ratio of 1:1 (synergist: pyrethrins).



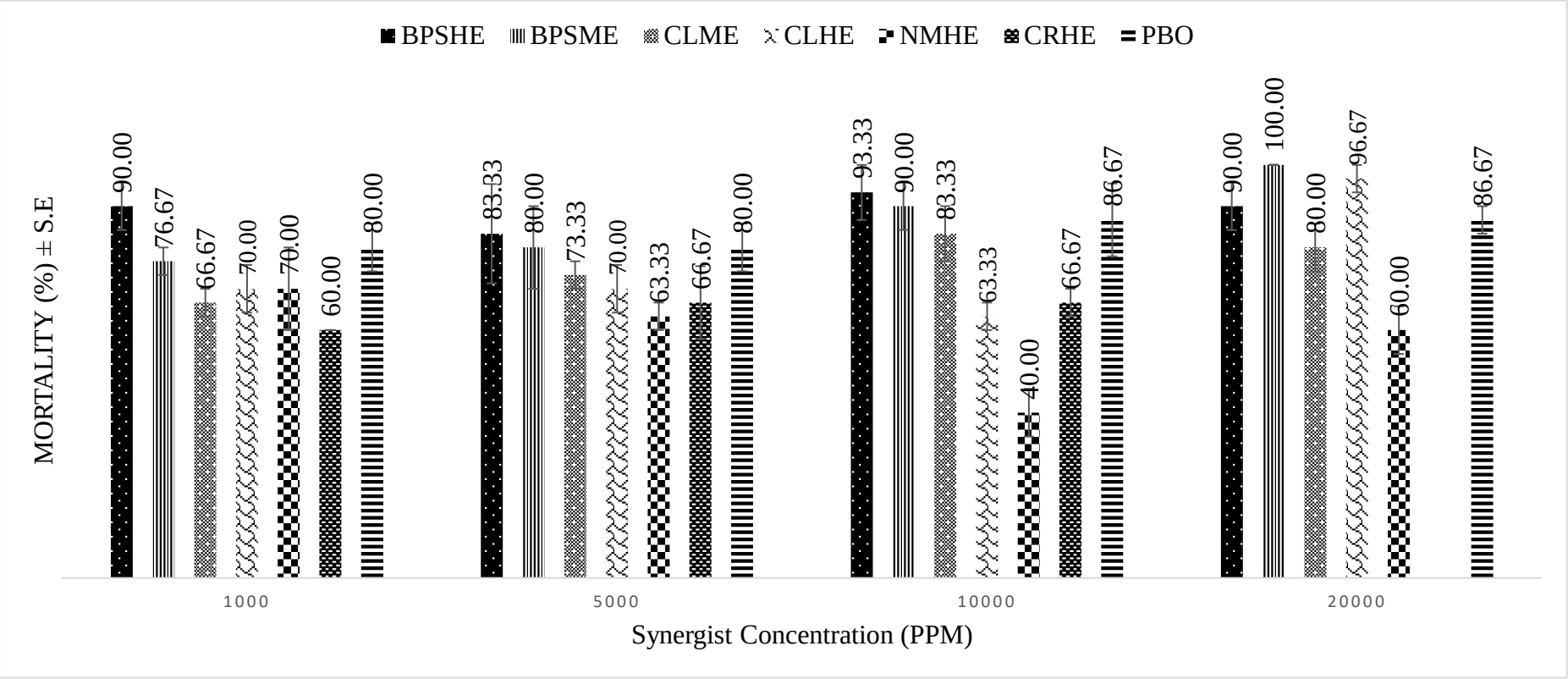
Appendix VI: Mean Percentage Mortality (± S.E) in *S. zea-mais adults* at 72 h Exposure After Application of Synergists at Four Concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) in Combination with LC_{20} pyrethrins (2,200 ppm) at the Ratio of 1:1 (synergist: pyrethrins).



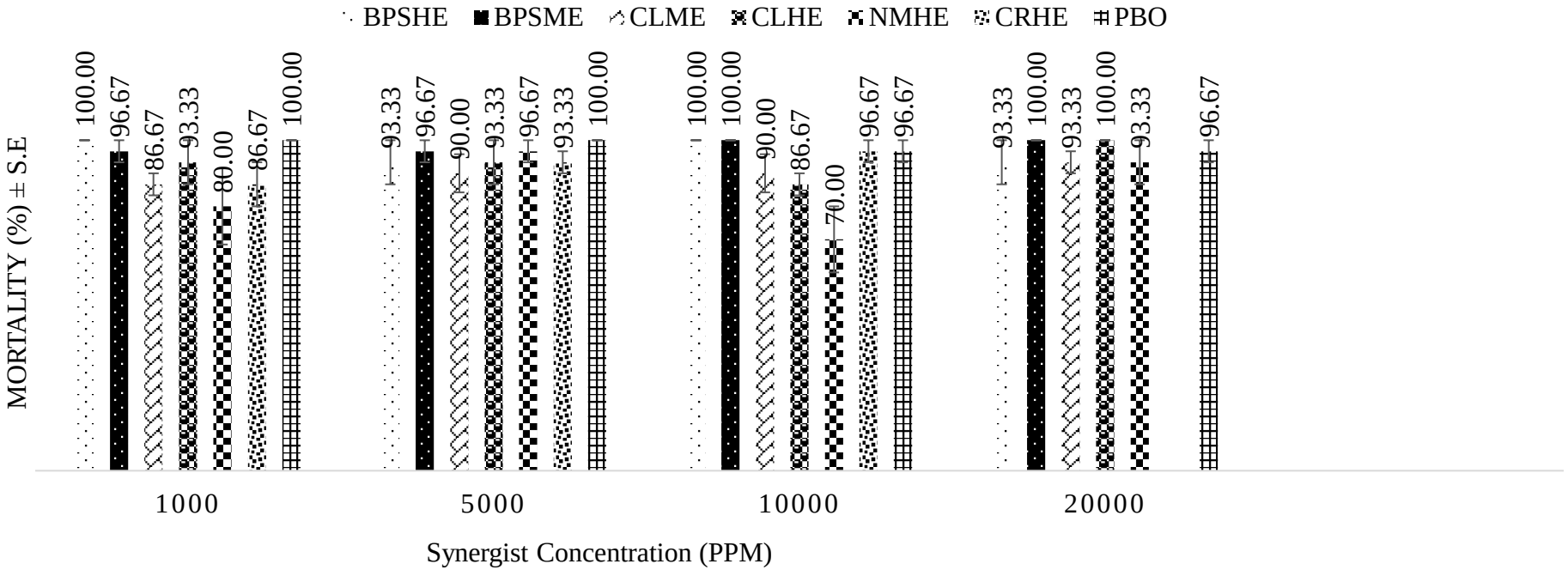
Appendix VII: Mean Percentage Mortality (± S.E) in *S. zea-mais adults* at 24 h exposure after Application of Synergists at Four Concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) in Combination with LC_{20} pyrethrins (2,200 ppm) at the ratio of 2:1 (Synergist: Pyrethrins).



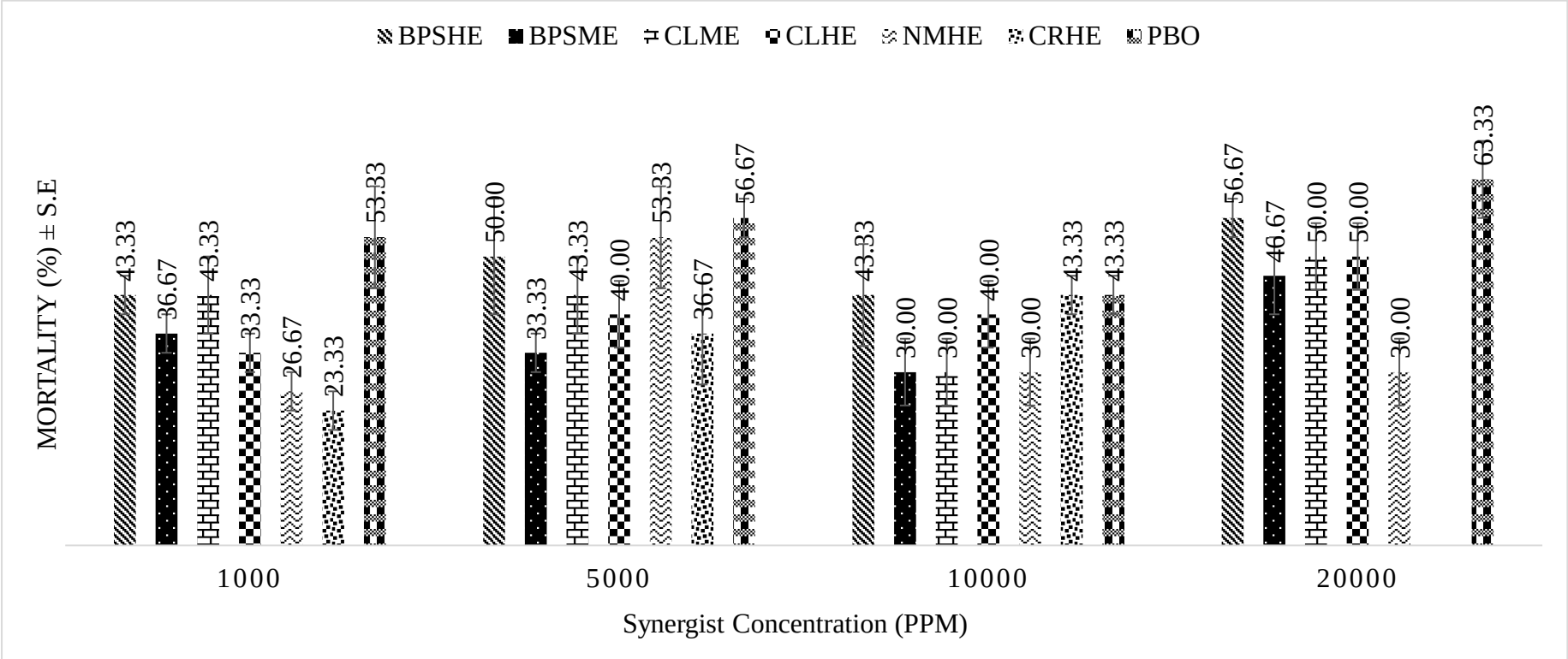
Appendix VIII: Mean Percentage Mortality (± S.E) in *S. zea-mais adults* at 48 h Exposure After Application of Synergists at Four Concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) in combination with LC_{20} pyrethrins (2,200 ppm) at the Ratio of 2:1 (synergist: pyrethrins).



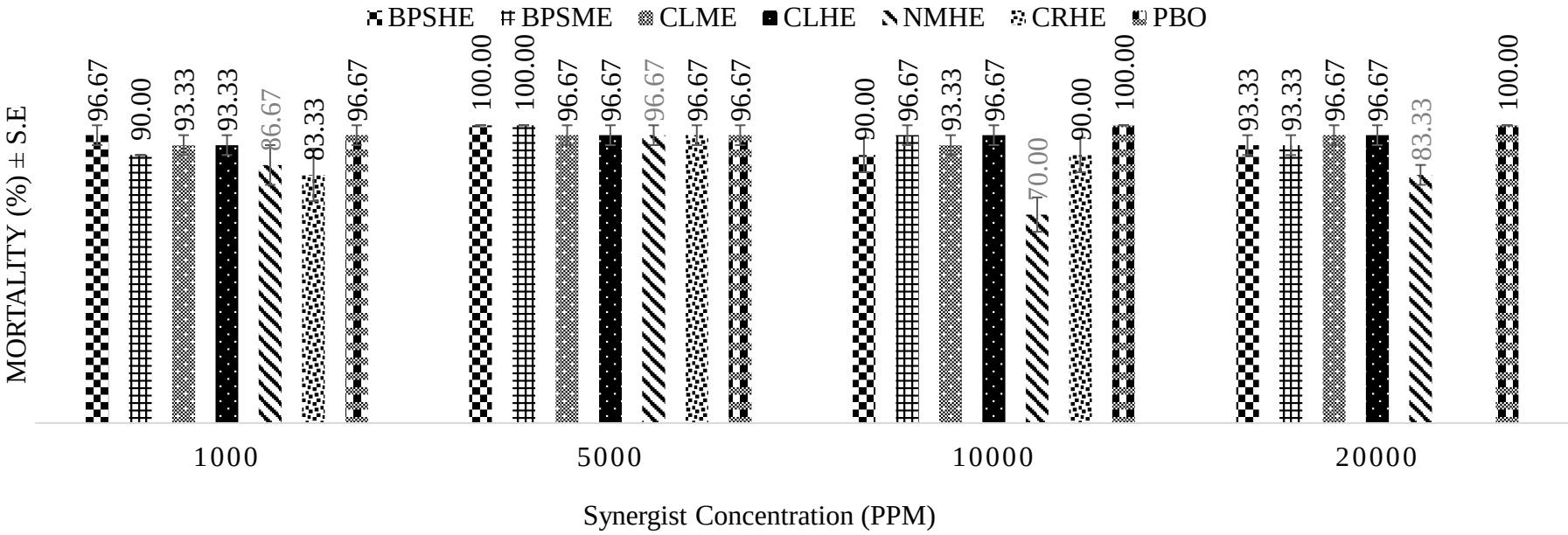
Appendix IX: Mean Percentage Mortality (± S.E) in *S. zea-mais adults* at 72 h exposure after Application of Synergists at Four Concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) in Combination with LC_{20} pyrethrins (2,200 ppm) at the Ratio of 2:1 (synergist: pyrethrins).



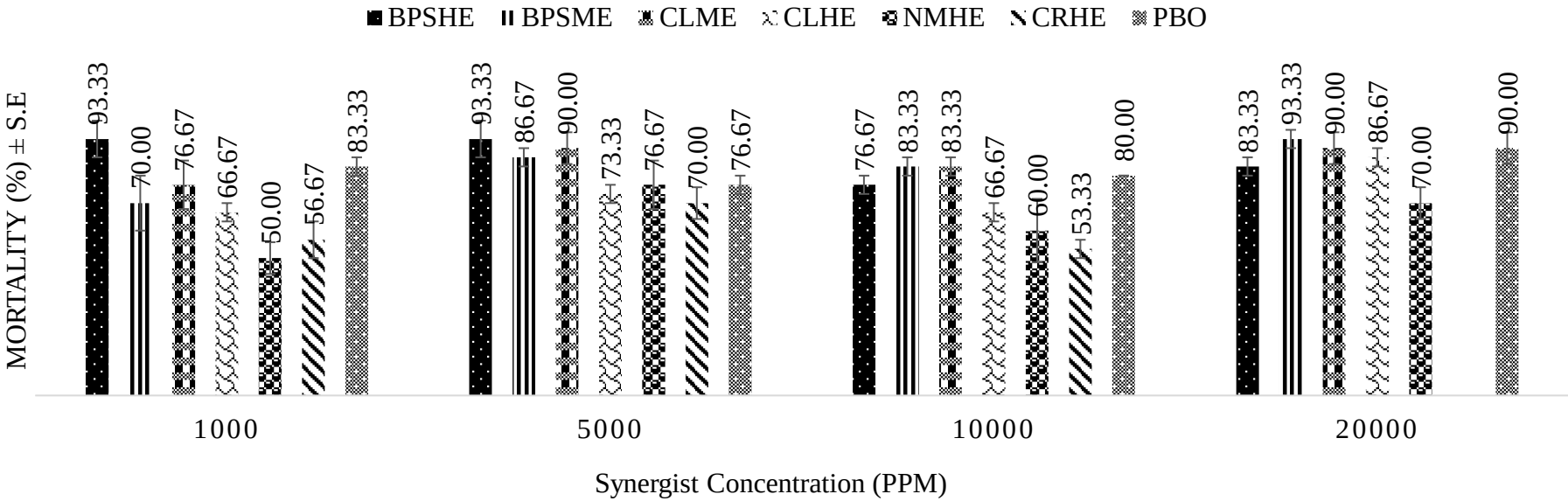
Appendix X: Mean Percentage Mortality (± S.E) in *S. zea-mais adults* at 24 h Exposure after Application of Synergists at Four Concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) in combination with LC_{20} Pyrethrins (2,200 ppm) at the Ratio of 3:1 (synergist: pyrethrins).



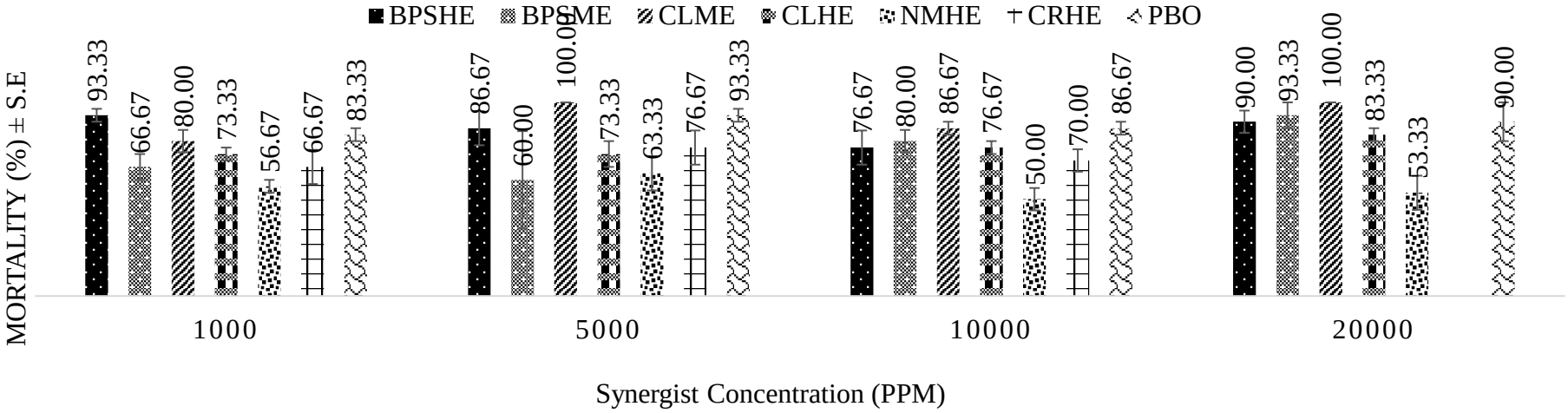
Appendix XI: Mean Percentage Mortality (± S.E) in *S. zea-mais adults* at 72 h Exposure after application of Synergists at four Concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) in Combination with LC_{20} pyrethrins (2,200 ppm) at the Ratio of 3:1 (Synergist: Pyrethrins).



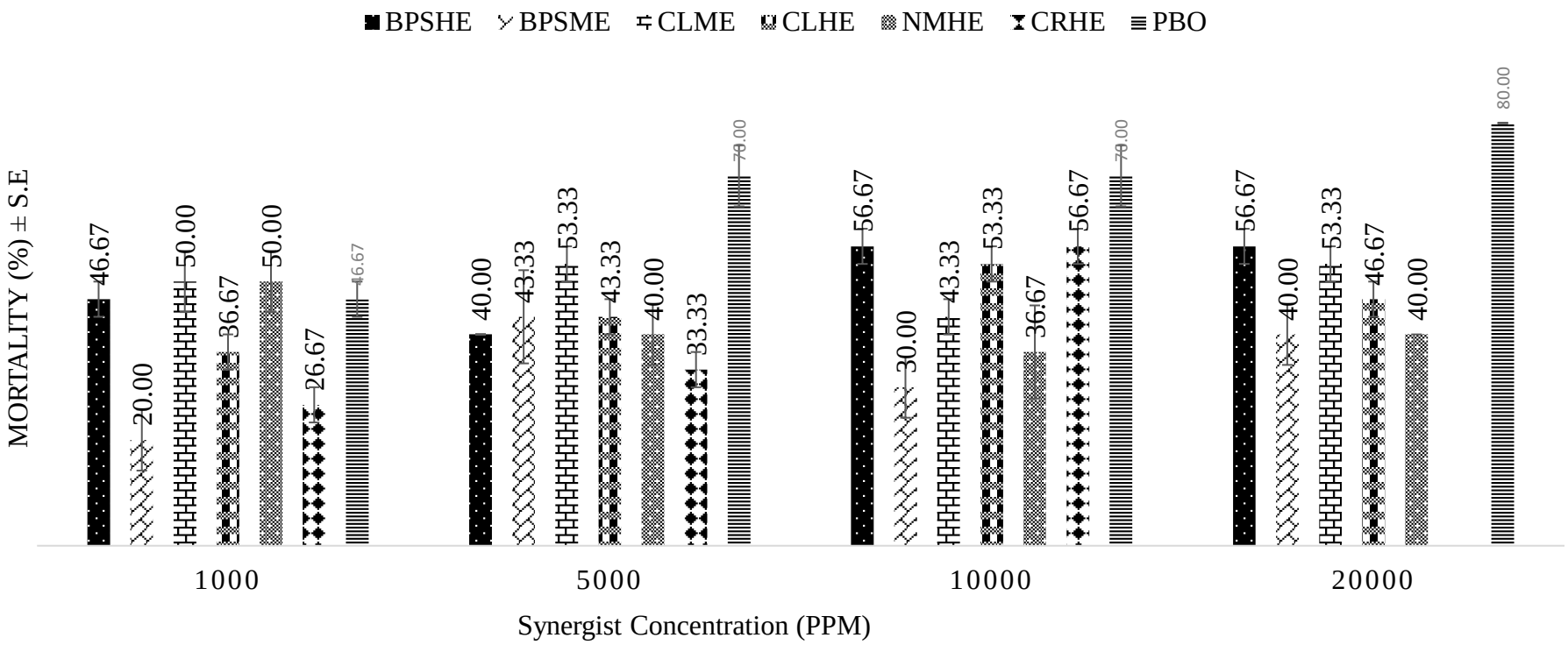
Appendix XII: Mean Percentage Mortality (± S.E) in *S. zea-mais adults* at 48 h exposure After Application of Synergists at Four Concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) in Combination with LC_{20} pyrethrins (2,200 ppm) at the Ratio of 3:1 (Synergist: Pyrethrins).



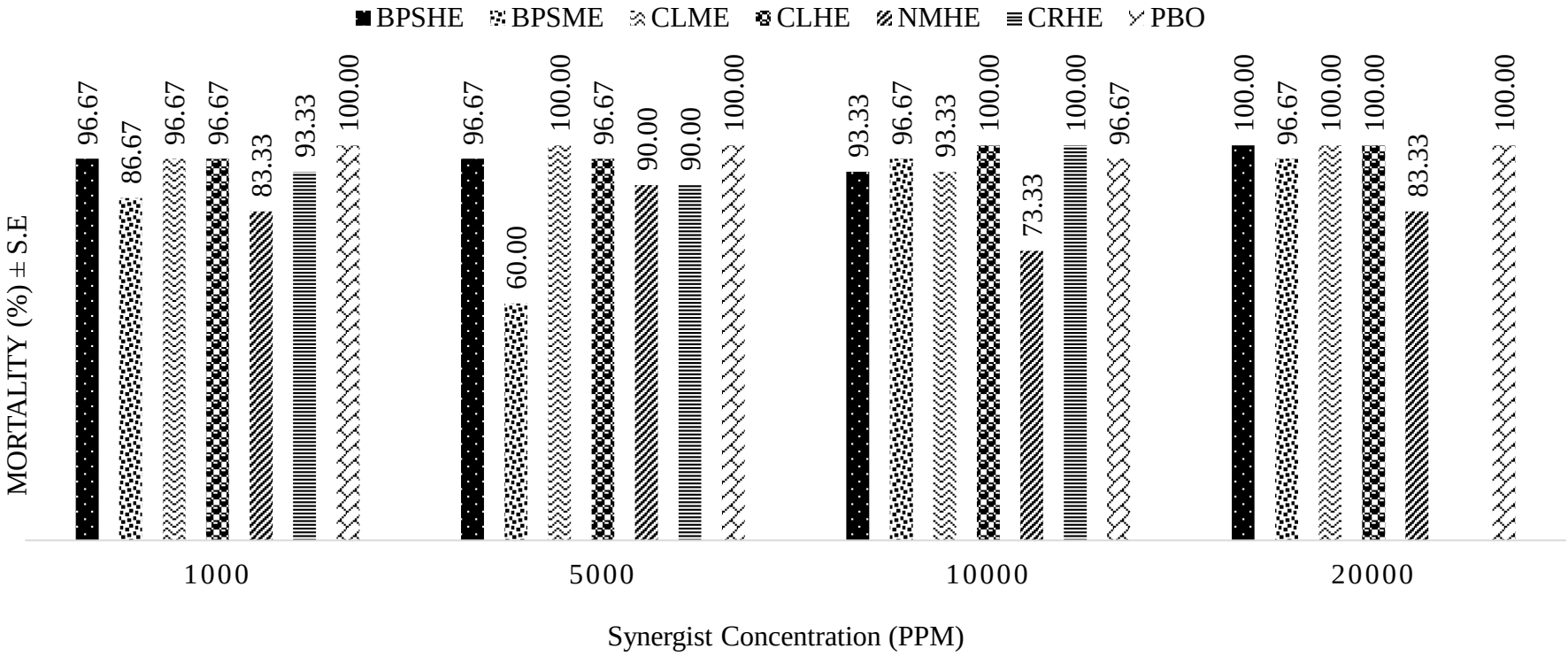
Appendix XIII: Mean Percentage Mortality (± S.E) in *S. zea-mais adults* at 24 h Exposure After Application of Synergists at Four Concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) in combination with LC_{20} pyrethrins (2,200 ppm) at the Ratio of 4:1 (Synergist: Pyrethrins).



Appendix XIV: Mean Percentage Mortality (± S.E) in *S. zea-mais adults* at 48 h exposure after Application of Synergists at Four Concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) in combination with LC_{20} pyrethrins (2,200 ppm) at the Ratio of 4:1 (synergist: pyrethrins).



Appendix XV: Mean Percentage Mortality (± S.E) in *S. zea-mais adults* at 72 h Exposure after Application of Synergists at four Concentrations (1,000 ppm, 5,000 ppm, 10,000 ppm and 20,000 ppm) in Combination with LC_{20} pyrethrins (2,200 ppm) at the Ratio of 4:1 (Synergist: Pyrethrins).



Appendix XVI: Co-toxicity Factors Calculated on the Basis of LC_{20} Pyrethrins and Synergists applied on *S. zea-mais* at Ratio 2:1 (Synergist: Pyrethrins) 48 h Exposure.

	Synergist at 1000ppm			Synergist at 5000ppm			Synergist at 10000ppm			Synergist at 20000ppm		
	% Mortality			% Mortality			% Mortality			% Mortality		
	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor
BPSHE	33	90	172.7	27	83	208.6	30	93	211.1	40	90	125
BPSME	47	77	63.12	37	80	116.2	43	90	109.3	47	100	112.8
CLME	37	67	80.18	30	73	144.4	27	83	208.6	33	80	142.4
CLHE	23	70	204.3	30	70	133.3	37	63	71.17	43	97	124.8
NMHE	37	70	89.19	33	63	91.92	37	40	8.108	40	60	50
CRHE	27	60	122.2	33	67	102	43	67	55.04			
PBO	27	80	196.3	23	80	247.8	30	87	188.9	40	87	116.7

- Values lower than -20 suggest antagonism, values between -20 and 20 suggest additive character and values greater than 20 suggest synergism
- The expected value is contributed by the constant 20% mortality of *S. zea-mais*due to pyrethrins (LC20) and toxicity of the plant extract/synergist alone while observed mortality is due to the synergist-pyrethrins formulation

Appendix XVII: Co-toxicity Factors Calculated on the basis of LC_{20} Pyrethrins and Synergists Applied on *S. zea-mais* at Ratio 2:1 (Synergist: Pyrethrins) 72 h Exposure.

	Synergist at 1000ppm			Synergist at 5000ppm			Synergist at 10000ppm			Synergist at 20000ppm		
	% Mortality			% Mortality			% Mortality			% Mortality		
	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor
BPSHE	33	100	203	27	93	245.7	40	100	150	43	93	117.1
BPSME	50	97	93.33	40	97	141.7	47	100	112.8	53	100	88.68
CLME	37	87	134.2	37	90	143.2	37	90	143.2	43	93	117.1
CLHE	27	93	245.7	30	93	211.1	37	87	134.2	47	100	112.8
NMHE	37	80	116.2	33	97	192.9	37	70	89.19	43	93	117.1
CRHE	27	87	221	37	93	152.3	43	97	124.8			
PBO	27	100	270.4	27	100	270.4	30	97	222.2	57	97	69.59

- Values lower than -20 suggest antagonism, values between -20 and 20 suggest additive character and values greater than 20 suggest synergism
- The expected value is contributed by the constant 20% mortality of *S. zea-mais*due to pyrethrins (LC20) and toxicity of the plant extract/synergist alone while observed mortality is due to the synergist-pyrethrins formulation

Appendix XVIII: Co-toxicity Factors Calculated on the basis of LC_{20} pyrethrins and Synergists Applied on *S. zea-maisat* ratio 3:1 (Synergist: Pyrethrins) 48 h Exposure.

	Synergist at 1000ppm			Synergist at 5000ppm			Synergist at 10000ppm			Synergist at 20000ppm		
	% Mortality			% Mortality			% Mortality			% Mortality		
	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor
BPSHE	33	93	182.8	27	93	245.7	30	77	155.6	40	83	108.3
BPSME	47	70	48.94	37	87	134.2	43	83	93.8	47	93	98.58
CLME	37	77	107.2	30	90	200	27	83	208.6	33	90	172.7
CLHE	23	67	189.9	30	73	144.4	37	67	80.18	43	87	101.6
NMHE	37	50	35.14	33	77	132.3	37	60	62.16	40	70	75
CRHE	27	57	109.9	33	70	112.1	43	53	24.03			
PBO	27	83	208.6	23	77	233.3	30	80	166.7	40	90	125

- Values lower than -20 suggest antagonism, values between -20 and 20 suggest additive character and values greater than 20 suggest synergism
- The expected value is contributed by the constant 20% mortality of *S. zea-mais*due to pyrethrins (LC20) and toxicity of the plant extract/synergist alone while observed mortality is due to the synergist-pyrethrins formulation

Appendix XIX: Co-toxicity Factors Calculated on the basis of LC_{20} Pyrethrins and Synergists applied on *S. zea-maisat* ratio 3:1 (Synergist: Pyrethrins) 72 h Exposure.

	Synergist at 1000ppm			Synergist at 5000ppm			Synergist at 10000ppm			Synergist at 20000ppm		
	% Mortality			% Mortality			% Mortality			% Mortality		
	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor
BPSHE	33	97	192.9	27	100	270.4	40	90	125	43	93	117.1
BPSME	50	90	80	40	100	150	47	97	105.7	53	93	76.1
CLME	37	93	152.3	37	97	161.3	37	93	152.3	43	97	124.8
CLHE	27	93	245.7	30	97	222.2	37	97	161.3	47	97	105.7
NMHE	37	87	134.2	33	97	192.9	37	70	89.19	43	83	93.8
CRHE	27	83	208.6	37	97	161.3	43	90	109.3			
PBO	27	97	258	27	97	258	30	100	233.3	57	100	75.44

- Values lower than -20 suggest antagonism, values between -20 and 20 suggest additive character and values greater than 20 suggest synergism
- The expected value is contributed by the constant 20% mortality of *S. zea-mais*due to pyrethrins (LC20) and toxicity of the plant extract/synergist alone while observed mortality is due to the synergist-pyrethrins formulation

Appendix XX: Co-toxicity Factors Calculated on the basis of LC_{20} Pyrethrins and Synergists Applied on *S. zea-maisat* ratio 4:1 (Synergist: Pyrethrins) 48 h Exposure.

	Synergist at 1000ppm			Synergist at 5000ppm			Synergist at 10000ppm			Synergist at 20000ppm		
	% Mortality			% Mortality			% Mortality			% Mortality		
	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor
BPSHE	33	93	182.8	27	87	221	30	77	155.6	40	90	125
BPSME	47	67	41.84	37	60	62.16	43	80	86.05	47	93	98.58
CLME	37	80	116.2	30	100	233.3	27	87	221	33	100	203
CLHE	23	73	218.8	30	73	144.4	37	77	107.2	43	83	93.8
NMHE	37	57	53.15	33	63	91.92	37	50	35.14	40	53	33.33
CRHE	27	67	146.9	33	77	132.3	43	70	62.79			
PBO	27	83	208.6	23	93	305.8	30	87	188.9	40	90	125

- Values lower than -20 suggest antagonism, values between -20 and 20 suggest additive character and values greater than 20 suggest synergism
- The expected value is contributed by the constant 20% mortality of *S. zea-mais*due to pyrethrins (LC20) and toxicity of the plant extract/synergist alone while observed mortality is due to the synergist-pyrethrins formulation

Appendix XXI: Co-toxicity Factors Calculated on the Basis of LC_{20} Pyrethrins and Synergists applied on *S. zea-maisat* ratio 4:1 (Synergist: Pyrethrins) 72 h Exposure.

	Synergist at 1000ppm			Synergist at 5000ppm			Synergist at 10000ppm			Synergist at 20000ppm		
	% Mortality			% Mortality			% Mortality			% Mortality		
	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor	Expected	Observed	Co-toxicity factor
BPSHE	33	97	192.9	27	97	258	40	93	133.3	43	100	132.6
BPSME	50	87	73.33	40	60	50	47	97	105.7	53	97	82.39
CLME	37	97	161.3	37	100	170.3	37	93	152.3	43	100	132.6
CLHE	27	97	258	30	97	222.2	37	100	170.3	47	100	112.8
NMHE	37	83	125.2	33	90	172.7	37	73	98.2	43	83	93.8
CRHE	27	93	245.7	37	90	143.2	43	100	132.6			
PBO	27	100	270.4	27	100	270.4	30	97	222.2	57	100	75.44

- Values lower than -20 suggest antagonism, values between -20 and 20 suggest additive character and values greater than 20 suggest synergism
- The expected value is contributed by the constant 20% mortality of *S. zea-mais*due to pyrethrins (LC20) and toxicity of the plant extract/synergist alone while observed mortality is due to the synergist-pyrethrins formulation

Appendix XXII: Letter from University Ethics Commitee

CHUKA UNIVERSITY

Telephones: 020 2310512
020 2310518

P.O. Box 109
Chuka

OFFICE OF THE CHAIRMAN
INSTITUTIONAL ETHICS REVIEW COMMITTEE

Our Ref: CU/IERC/NCST/18/23 14th March, 2018

THE CHIEF EXECUTIVE OFFICER
NATIONAL COMMISION FOR SCIENCE, TECHNOLOGY AND INNOVATION
P.O. BOX 30623-00100
NAIROBI

Dear Sir/Madam,

RE: RESEARCH CLEARANCE AND AUTHORIZATION FOR MICHURA ANNE JEROTICH GARRY. REG NO SD14/11708/13

The above matter refers:

The Institutional Ethics Review Committee of Chuka University met and reviewed the above PhD Research Proposal Synergistic Effect of Piperine on Potency of Botanical Fomulations against Coleopteran Maize Pests" The Supervisors are Prof. Adiel Magana, Dr. Ochieng Ombaka and Dr. Kennedy Gachoka.

The committee recommended that after candidate amends the issues highlighted in the Attached research clearance and authorization check list, the permit be issued.

Attached please find copies of the minutes, research clearance and authorization check list for your perusal. Kindly assist the student get the research permit.

Yours faithfully,

Prof. Adiel Magana
CHAIR
INSTITUTIONAL ETHICS REVIEW COMMITTEE
cc: BPGS

Appendix XXIII: Research Authorisation (NACOSTI)

NATIONAL COMMISSION FOR SCIENCE,
TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471,
2241349,3310571,2219420
Fax:+254-20-318245,318249
Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. **NACOSTI/P/18/34976/22333**

Date: **15th May, 2018**

Anne Jerotich Garry Michura
Chuka University
P.O. Box 109-60400
CHUKA.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Synergistic effect of piperine on potency of botanical formulations against coleopteran grain pests,"*** I am pleased to inform you that you have been authorized to undertake research in **Tharaka Nithi County** for the period ending **15th May, 2019.**

You are advised to report to **the County Commissioner and the County Director of Education, Tharaka Nithi County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

DR. STEPHEN K. KIBIRU, PhD.
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Tharaka Nithi County.

The County Director of Education
Tharaka Nithi County.

Appendix XXIV: Research Permit

THIS IS TO CERTIFY THAT:
MS. ANNE JEROTICH GARRY MICHURA
of CHUKA UNIVERSITY, 20158-20100
NAKURU,has been permitted to conduct
research in *Tharaka-Nithi County*

on the topic: *SYNERGISTIC EFFECT OF PIPERINE ON POTENCY OF BOTANICAL FORMULATIONS AGAINST COLEOPTERAN GRAIN PESTS*

for the period ending:
15th May,2019

Permit No : NACOSTI/P/18/34976/22333
Date Of Issue : 15th May,2018
Fee Recieved :Ksh 2000

.....
Applicant's Signature

.....
Director General
National Commission for Science, Technology & Innovation

Appendix XXV: ANOVA for Formulations at Ratio 2:1

DUNCAN'S MULTIPLE RANGE TEST RESULTS

ANOVA

BPSME 24 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1,000.000	3	333.333	3.077	.091
Within Groups	866.667	8	108.333		
Total	1866.667	11			

Duncan[a]

CONC	N	Subset for alpha = 0.05	
		1	2
1,000	3	43.33	
1,0000	3	46.67	46.67
5,000	3	56.67	56.67
20,000	3		66.67
Sig.		.171	.054

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA

BPSME 48 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	158.333	3	52.778	.275	.842
Within Groups	1533.333	8	191.667		
Total	1691.667	11			

Duncan

		Subset for alpha = 0.05
CONC	N	1
5,000	3	83.33
1,000	3	90.00
20,000	3	90.00
1,0000	3	93.33
Sig.		.428

Means for groups in homogeneous subsets are displayed

a. Uses Harmonic Mean Sample Size = 3.000

ANOVA

BPSME 72 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	133.333	3	44.444	.667	.596
Within Groups	533.333	8	66.667		
Total	666.667	11			

Duncan

		Subset for alpha = 0.05
CONC	N	1
5,000	3	93.33
20,000	3	93.33
1,000	3	100.00
1,0000	3	100.00
Sig.		.373

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

ANOVA

BPSHE 24 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1425.000	3	475.000	5.700	.022
Within Groups	666.667	8	83.333		
Total	2091.667	11			

Duncan[a]

		Subset for alpha = 0.05	
CONC	N	1	2
1,000	3	16.67	
1,0000	3	26.67	
5,000	3	33.33	33.33
20,000	3		46.67
Sig.		.064	.111

Means for groups in homogeneous subsets are displayed

a. Uses Harmonic Mean Sample Size = 3.000

ANOVA

BPSHE 48 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1,000.000	3	333.333	3.077	.091
Within Groups	866.667	8	108.333		
Total	1866.667	11			

Duncan

		Subset for alpha = 0.05	
CONC	N	1	2
1,000	3	76.67	
5,000	3	80.00	80.00
1,0000	3	90.00	90.00
20,000	3		100.00
Sig.		.171	.054

Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA

BPSHE 72 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	33.333	3	11.111	.667	.596
Within Groups	133.333	8	16.667		
Total	166.667	11			

Duncan[a]

		Subset for alpha = 0.05
CONC	N	1
1,000	3	96.67
5,000	3	96.67
1,0000	3	100.00
20,000	3	100.00
Sig.		.373

Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

ANOVA

CLME 24 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	91.667	3	30.556	.306	.821
Within Groups	800.000	8	100.000		
Total	891.667	11			

Duncan

		Subset for alpha = 0.05
CONC	N	1
5,000	3	36.67
1,0000	3	36.67
1,000	3	40.00
20,000	3	43.33
Sig.		.463

Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA
CLME 48 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	491.667	3	163.889	2.185	.168
Within Groups	600.000	8	75.000		
Total	1091.667	11			

Duncan[a]

		Subset for alpha = 0.05
CONC	N	1
1,000	3	66.67
5,000	3	73.33
20,000	3	80.00
1,0000	3	83.33
Sig.		.058

Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA
CLME 72 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	66.667	3	22.222	.333	.802
Within Groups	533.333	8	66.667		
Total	600.000	11			

Duncan

		Subset for alpha = 0.05
CONC	N	1
1,000	3	86.67
5,000	3	90.00
1,0000	3	90.00
20,000	3	93.33
Sig.		.373

Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA
CLHE 24 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	425.000	3	141.667	2.429	.140
Within Groups	466.667	8	58.333		
Total	891.667	11			

Duncan[a]

		Subset for alpha = 0.05
CONC	N	1
1,000	3	33.33
5,000	3	36.67
1,0000	3	46.67
20,000	3	46.67
Sig.		.080

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA
CLHE 48 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1966.667	3	655.556	9.833	.005
Within Groups	533.333	8	66.667		
Total	2500.000	11			

Duncan[a]

		Subset for alpha = 0.05	
CONC	N	1	2
1,0000	3	63.33	
1,000	3	70.00	
5,000	3	70.00	
20,000	3		96.67
Sig.		.365	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA

CRHE 72 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	266.667	3	88.889	1.185	.375
Within Groups	600.000	8	75.000		
Total	866.667	11			

Duncan[a]

		Subset for alpha = 0.05
CONC	N	1
1,0000	3	86.67
1,000	3	93.33
5,000	3	93.33
20,000	3	100.00
Sig.		.114

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA

NMHE 24 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	558.333	3	186.111	1.595	.265
Within Groups	933.333	8	116.667		
Total	1491.667	11			

Duncan[a]

		Subset for alpha = 0.05
CONC	N	1
1,0000	3	30.00
20,000	3	40.00
1,000	3	46.67
5,000	3	46.67
Sig.		.114

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA

NMHE 48 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1500.000	3	500.000	3.750	.060
Within Groups	1066.667	8	133.333		
Total	2566.667	11			

Duncan[a]

		Subset for alpha = 0.05	
CONC	N	1	2
1,0000	3	40.00	
20,000	3	60.00	60.00
5,000	3		63.33
1,000	3		70.00
Sig.		.067	.339

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA

NMHE 72 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1366.667	3	455.556	2.103	.178
Within Groups	1733.333	8	216.667		
Total	3100.000	11			

Duncan[a]

		Subset for alpha = 0.05
CONC	N	1
1,0000	3	70.00
1,000	3	80.00
20,000	3	93.33
5,000	3	96.67
Sig.		.071

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA

CRHE 24 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1088.889	2	544.444	5.444	.045
Within Groups	600.000	6	100.000		
Total	1688.889	8			

Duncan[a]

		Subset for alpha = 0.05	
CONC	N	1	2
1,000	3	26.67	
5,000	3	36.67	36.67
1,0000	3		53.33
Sig.		.267	.087

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA
CRHE 48 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	88.889	2	44.444	.500	.630
Within Groups	533.333	6	88.889		
Total	622.222	8			

Duncan[a]

		Subset for alpha = 0.05
CONC	N	1
1,000	3	60.00
5,000	3	66.67
1,0000	3	66.67
Sig.		.434

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA
CRHE 72 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	155.556	2	77.778	1.167	.373
Within Groups	400.000	6	66.667		
Total	555.556	8			

Duncan[a]

		Subset for alpha = 0.05
CONC	N	1
1,000	3	86.67
5,000	3	93.33
1,0000	3	96.67
Sig.		.197

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA

PBO 24 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	291.667	3	97.222	1.296	.341
Within Groups	600.000	8	75.000		
Total	891.667	11			

Duncana

		Subset for alpha = 0.05
CONC	N	1
1,0000	3	43.33
1,000	3	50.00
20,000	3	53.33
5,000	3	56.67
Sig.		.114

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA

PBO 48 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	133.333	3	44.444	.381	.770
Within Groups	933.333	8	116.667		
Total	1066.667	11			

Duncan[a]

		Subset for alpha = 0.05
CONC	N	1
1,000	3	80.00
5,000	3	80.00
1,0000	3	86.67
20,000	3	86.67
Sig.		.496

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA

PBO 72 h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	33.333	3	11.111	.667	.596
Within Groups	133.333	8	16.667		
Total	166.667	11			

Duncan[a]

		Subset for alpha = 0.05
CONC	N	1
1,0000	3	96.67
20,000	3	96.67
1,000	3	100.00
5,000	3	100.00
Sig.		.373

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.