PHYTOCHEMICAL PROFILES AND BIOEFFICACY OF SELECTED ORGANIC LEAF EXTRACTS OF *Tithonia diversifolia* AND *Vernonia lasiopus* AGAINST *Sitophilus zeamais*

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DECLARATION

I, Stephen Maina Gitahi, duly declare that this thesis is my original work and has not been presented for a degree in any other university or for any other award

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This work is dedicated to my dear wife Consolata Nyambura and our children Mary Njeri, Winnie Munyutha, Alvin Gitahi and Ivy Wanjiku. You were all my source of inspiration throughout my study period

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ABBREVIATIONS AND ACRONYMS

AATF	African Agricultural Technology Foundation
ACh	Acetylcholine
AChE	Acetlycholinesterase
AChEIs	Acetylcholinesterase Inhibitors
ACTI	Acetylthiocholide Iodide
AD	Alzihmers Disease
AE	Adult Weevil Emergence
ANOVA	Analysis of Variance
BBC	British Broadcasting Corporation
BHT	Butylated Hydroxytoluene
BSA	Bovine Serum Albumin
Bt	*Bacillus thuringiensis*
BuChE	Butyrylcholinesterase
ChE	Cholinesterase
ChEIs	Cholinesterase Inhibitors
CIMMYT	Centro Internacional de Mejoramiento de Maíz y Trigo (Spanish); International Maize and Wheat Improvement Center (English)
Cm	Centimeters
CMs	Carbamates
CNS	Central Nervous System
C_n	Number in Control Experiment
CRD	Completely Randomized Design
CSI	Chitin Synthesis Inhibitors
DAT	Days after Treatment
DCM	Dicholomethane
DDT	Dichlorodiphenyltrichloroethane
DEET	N-diethyl-3-methylbenzamide
DGE	Discontinuous Gas Exchange
DMNT	Dimethy-1,3,7-nonatriene
DMS	Document Management System
DNA	Deoxyribonucleic Acid
DT	Drought Tolerant
DTMA	Drought Torelant Maize for Africa
DTNB	5, 5’ dithiobis-2-nitrobenzoic acid
DTT	Dithiothreital
EDTA	Ethylenediaminetetraacetic acid

EPN	Enthomopathogenic Nematode
ESI-MS	Electrospray Ionization Mass spectrometry
ESA	East and Southern Africa
EtOAc	Ethyl Acetate
EU	European Union
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistical Database
F_1	First Filial Generation
GABA	Gamma Aminobutyric Acid
GC	Gas Chromatography
GLS	Gray Leaf Spot
GM	Genetically Modified Crops
GRAS	Generally Regarded as Safe
GPS	Geographical Position
IC_{50}	Inhibition Concentration (50%)
LC_{50}	Lethal Concentration (50%)
ICIPE	International Centre for Insect Physiology and Ecology
ICU	Intensive Care Unit
IGRs	Insect Growth Regulators
IITA	International Institute of Tropical Agriculture
IPM	Integrated Pest Management Programs
IR	Inhibition Rate
IWCM	Integrated Weevil Control and Management
JH	Juvinile Hormone
KNH	Kenyatta National Hospital
LC-MS	Liquid Chromatography-Mass Spectrophotometry
MCP	Method of Counting and Weighing
MDGs	Millennium Development Goals
MeOH	Methanol
MLN	Maize Lethal Necrotic Disease
MAO	Monoamine Oxidase
MRSA	Methicillin Resistant Staphylococcus Aureus
MS	Mass Spectrometry
MSV	Maize Streak Virus
MSVW	Method of Standard Volumetric Weight
MT	Metric Tons
NCPB	National Cereals and Produce Board
NIST	National Institute Standard and Technology
NIOSH	National Institute for Ocupational Safety and Health
NPIC	National Pesticide Information Center

NUE	Nutrients Utilization Efficiency
OD	Oviposition Deterrence
OPs	Organophosphates
PAF	Pakstan Air Force Academy
PD	Percentage Weevil Damage to Grains
Ph	Hydrogen Potential
PIR	Percentage Inhibition Rate
PLA	Polylactide
PM	Percentage Mortality/Weevil Reduction
PMD	p-menthane-3, 8-diol
PR	Percent Repulsion
Pr	Corrected Mortality Percentages
QPM	Quality Protein Maize
RBC	Reduced Red Blood Cells
RCD	Randomized Controlled Study Design
RNS	Reactive Nitrogen Species
ROS	Reactive Oxidative Speciess
RT	Retention Time
SD	Standard Deviation
SEA	Southern and Eastern Africa
SEM	Standard Error of Mean
SSA	South Saharah Africa
SSB	Spotted Stem Borer
TIEAGC	Tegemeo Institute and East African Grain Council
TLB	Turcicum Leaf Blight
$\mathbf{T_n}$	Number in the Treatment Experiment
TRPA-1	Transient Receptor Potential Ankyrin-1
UNEP	United Nations Environmental Programme
UTM	Urchin Tracking Module
UV	Ultraviolet Rays
WHO	World Health Organization
WPI	Weevil Perforation Index

ABSTRACT

Maize is among the worlds most important crops. Uses of maize include human consumption, livestock feed as well as raw material for industrial processing. There are a number of pest that attack maize and contribute to grain loss in the tropics. Weevils infestation has been recognized as an increasingly constraint to maize production causing an estimated annual loss of 30 - 50% of stored maize grains in tropical Africa. The damage caused by *S. zeamais* can therefore, lead to food insecurity. Conventional use of synthetic insecticides is the most common and effective method insect pest control around the world. However, these chemicals are expensive and are arguably associated with deleterious side effects on environment. They kill even the non-targeted organisms and pose severe hazards to human health that were not anticipated at the time of their introduction. Therefore, to achieve sound management of stored cereals, there is an increasing interest in medicinal plants as complementary and alternative pesticides owing to their affordability, assumed safety and eco-friendly status due to their highly reduced negative effects on environment. This study therefore, evaluated the efficacy of selected organic leaf extracts of *Tithonia diversifolia* and *Vernonia lasiopus* against *S. zeamais* on maize grains. The phytochemical profile of *T. diversifolia* and *V. lasiopus* was determined using Gas Chroma-tography Mass Spectrophotometer (GC-MS). The extracts were assayed for their insecticidal abilities through fumigant and direct-contact toxicity-induced mortalities of *S. zeamais* exposed to treated grains for the period of 96 hour. The repellency effects of these extracts on weevils were experimenttally determined using area preference method. The effects of *T. diversifolia* and *V. lasiopus* on weevils' reproduction and development were tested through determination of their rate of oviposition and the first filial (F_1) progeny emergence in the treated grains. The extracts' antifeedant potential parameters determined included the reduction in insects' damage and grain weight loss on treated grains. Crude acetlycholinesterase (AChE) was extracted from adult weevils and the effects of the extracts on enzyme activity determined using Ellman method. The results indicated that *T. diversifolia* and *V. lasiopus* leaf extracts exhibited potent pesticidal effects against *S. zeamais*. They significantly exhibited insecticidal abilities that ranged between 1.67 to 99.98% and 32.45 and 98.7% through fumigant and contact toxicity-induced mortalities respectively. The extracts also manifested repellency effects of between 26-96% on weevils. Moreover, they showed oviposition deterrence (12.18-100%) and F_1 progeny emergence inhibition of weevils (38.38-100%). Further, it was evident that the extracts have antifeedant potentials as indicated by reduction in damage by insects (weevil perforation indices (WPI) below 50) and reduction in grain weight loss (2.7-15.4%) of treated maize grains. The studied organic leaf extracts of *T. diversifolia* and *V. lasiopus* showed potent AChE inhibitory effects (10.75-89.25%) *in Vitro*. The GC-MS analysis revealed the presence of bioactive compound in *T. diversifolia* and *V. lasiopus* which are associated with the pesticidal and the antiacetylcholinesterase activities observed. This study, therefore, scientifically confirms the traditional use of the *T. diversifolia* and *V. lasiopus.* It provides important data and a platform for further study on *T. diversifolia* and *V. lasiopus* as bio-resource of pesticides that could serve as novel postharvest protectant of maize grains against *S. zeamais*. However, there is need for bioassay-guided fractionation and purification of specific phytochemicals in *T. diversifolia* and *V. lasiopus* associated with specific insecticidal effects witnessed. Besides, further investigations are recommended to establish whether there are non-target effects on other insects, especially the weevil predators.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Maize (*Zea mays* L.) also known as corn, is one of the few most important crops in the world together with wheat and rice. It is a non-branching annual cereal (starchy grain) crop in the grass family (Poaceae) and is related to wheat, rice, oat and barley (Ranum *et al.,* 2014). Maize was first domesticated about 10,000 years ago in what is now Mexico and was introduced into Africa by the Portuguese in the 16th century (Longe and Ofuya, 2010; Ranum *et al.*, 2014). In developing countries, tropical white maize tends to be more important than the temperate yellow maize that represents the bulk of maize grown worldwide (Tahir and Habib, 2013; Caitlin *et al.,* 2015). Generally, maize grows well in areas with average rainfall of between 2900-1700 mm and temperatures of between 18^{o} C and 27^{o} C (Mutegi *et al*., 2012).

The global production of maize is estimated to be over 785 million tons, with the largest producer, the United States, producing 42% (IITA, 2013). Africa produces about 6.5% and the largest African maize producer is Nigeria with nearly eight million tons followed by South Africa. In Kenya, the national food security is basically pegged on availability and adequate supplies of maize to meet domestic demand. Small scale farmers account for 70% of the total national production and over 80% of the total maize area (Oluoch-Kosura, 2011). Maize production is expected to increase steadily because of increasing demand due to increasing human population (Shiferaw, 2011; Tecla and Ngeywo, 2015).

Globally, maize is grown primarily for feeding livestock (about 66%), for human consumption (25%) and for industrial purposes (9%). In Africa, 95% of maize produced is primarily grown for human consumption (IITA, 2013; Ranum *et al*., 2014). In Kenya, maize is the most important stable food crop. It contributes 3% of Kenya's Gross Domestic Product (GDP) and 21% of the total value of primary agricultural commodities (Nyakio, 2015). Maize provides a major source of carbohydrates, iron, vitamins, proteins, and minerals to human body (Munyiri *et al.*, 2013).

Maize grains constitute a major ingredient in the production of livestock feed (mash) while the green leaves of maize plant can also be fed as fodder to livestock, or in the form of hay/silage (Longe, 2016). Products resulting from industrial processing of maize include starch, high fructose syrup, dextrose, corn oil, ethanol, cosmetic or skin care products, beverages, crayons, soaps, absorbent material for diapers, food additives, biodegradable plastics and food supplements (Caitlin *et al.*, 2015).

In view of its increasing importance, betterment of agronomical aspects of maize has received big attention globally and a lot still needs to be done to increase maize production (Oganda *et al.*, 2011). Most importantly, loss of maize grains after harvesting must be reduced for its sustained availability to meet the increased demand as well as for food security to be realized in the developing countries in line with their increasing human populations (FAO, 2015).

Despite the great importance of maize, there are many constraints, comprising both abiotic and biotic factors affecting maize production while in fields and in stores (FAO, 2015; FAO, 2018). Some of these problems include drought, salinity, costly farm inputs, weeds, floods, strong winds, pests, and diseases (Nyoro *et al*., 2004).

Postharvest deterioration because of poor storage, fungal and insect infestation is a major problem faced by maize farmers in sub- Saharan Africa (Ojo and Omoloye, 2012). Worldwide annual maize grain losses caused by insect pests have been estimated to be between 5 and 10% (Weaver and Petroff, 2005). It is further estimated that 14 to 50% of the total maize produced each season in developing countries is lost due to insect pest-inflicted damage, as compared to only 1 to 2% in the developed countries (Ojo and Omoloye, 2012; Parwada *et al.*, 2018).

In Kenya, post-harvest losses due to insect infestation especially during storage have been one of the major problems encountered in agriculture. In fact, the entire tremendous efforts by the governments to increase maize production will be lost in the absence of crop protection resulting in financial losses to the farmers and unavailability of the grains (Ashamo *et al*., 2013).

The major pests that cause post-harvest infestation and loss in maize include, the maize weevil (*Sitophilus zeamais*), larger grain borer (*Prostephorus truncates*) and granary weevil (*Sitophilus granamus*) (Harris and Lindblad, 2008). *Sitophilus zeamais* is the most predominant and notorious storage insect pest of maize. Maize weevils lower the grain quantity and sometimes even contaminate the grains, thereby making it unfit for human consumption (Adijah *et al.,* 2011).

Maize weevil (*Sitophilus zeamais*), are small snout beetles (Coleoptera: Curculionidae) measuring between 2.5 and 4.5 mm in length. They are virtually found in every part of the world, especially tropical regions where maize is grown (Mugo *et al.,* 2012). Weevils can live up to 12 months, depending on environmental conditions (Ojo and Owoloye, 2012). They are the most predominant and destructive insect pests of maize grains both in the field and within the store causing a weight loss of stored grain up to 18.3% (Mugo *et al.,* 2012).

Conventional weevil control relies on use of synthetic insecticides such as Actellic^{TM}, Centynal^{TM} EC, Diacon$^{®}$ IGR PLUS and PBO-8$^{®}$ because of their high effectiveness (Udo, 2011). These chemicals stand as “Miracle Weapons” in the frontline of defense of stored grains but they are not free from demerits. Their successful use is greatly affected by the fact that they invoke several adverse effects including residual effects on non-target organisms’ health and environmental hazards as well as pest resistance, among others (Chengala and Singh, 2017). That synthetic insecticide is not readily available, costly and has several adverse effects have necessitated search for alternative means of controlling insect pests (Ashamo and Akinneye, 2009).

There are increasing efforts to understand and adopt indigenous strategies for protecting stored maize against pests, with a view to revive and modernize their use. The rural storage techniques remain remote if not scientifically used in agriculture. Further, it is high time to protect such methodologies, provide proper documentation and create wide publicity (Mkenda *et al*., 2015).

Indigenous strategies for protecting stored maize against pests include techniques which can alter either the temperature or the humidity. Some of these strategies, however, have detectable adverse effects on maize. For instance, over exposure to heat source during grain drying can cause breakage of seeds, damage to seed coat, bleaching, scorching, discoloration, and loss of germination power and nutritional changes of the grains (Odeyemi and Daramola, 2000). Admixture of grains with inert dust material such as clays, diatomite, wood ash, silicates and sand make the sorting out and grading of maize grains cumbersome hence reduce grain marketability (De Lima, 1987; Golob, 1997).

Plants have been used traditionally as medicines for generations throughout the world (Belmain *et al*., 2001). Botanicals form a rich source of novel insecticides. There is several well-known plant-derived insecticides including pyrethrin, found in *Chrysanthemum spp.,* which led to the development of the mostly used class of synthetic insecticides (pyrethroids). Neem (*Azadirachta indica*) is another example of a botanical insecticide presently used throughout the world. Insecticidal extracts from tobacco and rotenone have also been studied extensively for their pesticidal activities (Moreira *et al.*, 2007).

Plant secondary metabolites like flavonoids, alkaloids, saponins, terpenes, limonoids, phenols, stilbenes and coumarins have been reported to possess toxic, repellent, anti-feedant, growth inhibiting or reproduction inhibiting effects and hence protectant effects on stored grains against insect pests (Govindachari *et al.*, 1999; Bina *et al.*, 2004; Sunita and Lalijee, 2008).

Many other plant species, especially from tropical regions, have the potential to be used either as botanical insecticides or as sources of pesticidal bioactive compounds. However, empirical research data is scanty on insecticidal and/or pesticidal potential of these plants (Shaalan *et al*., 2005).

Herbal insecticides have been encouraged since they are believed to be safer to the environment, biodegradable, generally affordable, readily available, effective, and are easily processed thus can be used by farmers and small-scale industries (Habib *et al.*, 2011; Ashamo *et al*., 2011). Pests do not develop resistance to botanical pesticides quickly (Belmain *et al*., 2013).

Biopestcides are generally regarded as safe (GRAS) and are generally certified for organic production methods because rapid degradation of their active compounds is convenient as it reduces the risk of residues on food. Botanical pesticides also have a great advantage of being compatible with other acceptable low risk options (Singh, 2008). In general, the use of botanical pesticides can potentially surmount the problems resulting from use of synthetic pesticides. This has, therefore, led to more focused research on pesticidal plants for development of new botanical pesticides (Anjarwalla *et al.*, 2016).

Pesticidal plants, *T. diversifolia* and *V. lasiopus*, are used by indigenous communities particularly the Embu and Mbeere communities in management of several field and store-grain pests. However, their pesticidal potential has not yet been scientifically and empirically evaluated. In this regard, the present study was carried out to bioscreen selected organic extracts of *T. diversifolia* and *V. lasiopus* as possible plant-derived pestcides against weevils with predicted mechanisms of action. This work provide alternative or complementary for a remarkable shift from the conventional weevil control approach. The data collected on phytochemical composition provided information about insecticidal phytochemicals, which can further be purified and developed into plant-derived bio-pesticides for crop protection. The study also revealed research gaps that need to be explored further.

1.2 Statement of Problem

The post-harvest damage caused by *S. zeamais* reduces maize quantity and quality, thereby rendering it unfit for consumption. It is estimated that 14 to 50% of the total maize produced each season in developing countries is lost due to insect depredation of stored maize grains, particularly *S. zeamais* (FAO, 2011). Weevil infestation has, therefore, constituted a major constraining factor militating against successful maize production and availability. Grain losses attributed to maize weevils are enormous and have, therefore, threatened food security in Kenya and other developing countries (Tecla and Ngeywo, 2015).

All maize production enhancement efforts will be of no use if the produced maize grains are not effectively and safely protected from the weevils. This recalls the proverb “a grain saved is a grain equally produced”. It is imperative; therefore, that greater attention be paid to protection of the maize grains against weevils during storage to make them available for use throughout the year.

Kenyan farmers majorly use synthetic insecticides like actellic to control weevils. However, the adoption of these synthetic insecticides especially among subsistence and small holder farmers, who produce the bulk of maize in Sub Saharan Africa (SSA) still remain slow and has increasingly become unpopular (Odogola and Henriksson, 1991; Lyon., 2000). This is particularly so because they have been highly associated with potential risk of human health hazards due to *in vivo* toxicity, carcinogenicity and mutagenic effects. Most of indigenous strategies for protecting stored maize against pests including use of inert materials and drying under the fire have also been associated with some adverse effects on the stored grains (Odeyemi and Daramola, 2000).

1.3 Justification of Study

Due to demerits associated with traditional and/or indigenous weevil control strategies as well as the increasing negative public concerns of synthetic pesticides in terms of environmental pollution, human health hazards and development of insecticide resistance in some insect groups, use of bio-insecticides of plant origin is becoming more popular (Dales, 1996; Nabil, 2013). This is because botanicals provide not only effective but are also assumed to be safe and locally available. Furthermore, some of the effective pesticides commonly used by farmers are plant-derived eg. Pyrethroids (Dubey, 2008; Lengai *et al.*, 2020).

There is need to exploit other potential plant botanicals as effective sources of pest control agents. Recently, several research works have proved botanicals as potential bio-pesticides (Ogendo *et al.,* 2008). According to Adedire and Akinneye (2004), the leaves of both *T. diversifolia* and *V. lasiopus* have been traditionally used against weevils in stored maize in the various parts of Embu south regions. Wanzala *et al.* (2012) also reported the insecticidal use of *Vernonia lasiopus* by farmers in Western Kenya. However, the bio insecticidal value of the two plants has not been scientifically explored or validated and their mode of action is unknown (Wanzala *et al.,* 2012).

There is a great need to have scientific information on whether these two plants should be treated as potential sources of alternative bio-pesticides that can be of high efficacy against weevils. This study therefore, sought to validate and document, in a systematic way, the pesticidal properties of the genus *T. diversifolia* and *V. lasiopus* used for many years by many people of the world. These findings provide yet another example of the validity of making more advantageous structural modifications of seemingly rather thoroughly examined pesticidal agent in further researches.

The crude extracts demonstrating insecticidal activity could lead to the discovery of novel pesticidal agents. Therefore, *T. diversifolia* and *V. lasiopus* can further be used for generating a plant derived pesticide for maize weevil control in stores. The study also provides valuable information for further phytochemical isolation and characterization studies of active compounds, necessary for the development of new pesticide.

1.4 Research Questions

i. What are the contact and fumigant toxicity effects of selected organic leaf extracts of *T. diversifolia* and *V. lasiopus* against *S. zeamais?*

ii. What are the repellency effects of selected organic leaf extracts of *T. diversifolia* and *V. lasiopus* on *S. zeamais?*

iii. What are the oviposition deterrence and F_1 progeny emergence inhibition effects of selected organic leaf extracts of *T. diversifolia* and *V. lasiopus* on *S. zeamais*?

iv. What are the effects of selected organic leaf extracts of *T. diversifolia* and *V. lasiopus* on grain loss of stored maize grains?

v. What are the *ex-vivo* effects of selected organic leaf extracts of *T. diversifolia* and *V. lasiopus* on acetylcholinesterase (AChE) activity in *S. zeamais?*

vi. What are the quantitative phytochemical compositions of the selected organic leaf extracts of *T. diversifolia* and *V. lasiopus*?

1.5 Research Objectives

1.5.1 General Objective

To evaluate the quantitative phytochemical profiles and bioefficacy of selected organic leaf extracts of *T. diversifolia* and *V. lasiopus* against *S. zeamais.*

1.5.2 Specific Objectives

i. To determine the contact and fumigant toxicity effects of selected organic leaf extracts of *T. diversifolia* and *V. lasiopus* against *S. zeamais*

ii. To evaluate the repellency effect of selected organic leaf extracts of *T. diversifolia* and *V. lasiopus* on *S. zeamais*

iii. To determine the oviposition deterrence and F_1 progeny emergence inhibition effects of selected organic leaf extracts of *T. diversifolia* and *V. lasiopus* on *S. zeamais*

iv. To determine the grain loss protection effects of selected organic leaf extracts of *T. diversifolia* and *V. lasiopus*

v. To assess the *ex-vivo* effects of selected organic leaf extracts of *T. diversifolia* and *V. lasiopus* on acetylcholinesterase (AChE) activity in *S. zeamais*

vi. To evaluate the quantitative phytochemical composition of selected organic leaf extracts of *T. diversifolia* and *V. lasiopus.*

CHAPTER TWO

LITERATURE REVIEW

2.1 Maize (*Zea mays*)

2.1.1 Introduction

Maize also known as corn, is a domesticated form of teosinte, a wild grass occurring naturally in isolated patches of central Mexico. It is a non-branching annual cereal (starchy grain) crop in the grass family (Poaceae) related to wheat, rice, oat and barley. It is a caryopsis that belongs to the order *Poales*, sub-family Panicoideae and tribe Andropogoneae (Ranum *et al.*, 2014). In developing countries, tropical white maize tends to be more important than the temperate yellow maize that represents the bulk of maize grown worldwide. The difference in their appearance is typically due to the presence or absence of a carotene pigment in the temperate yellow maize and tropical white maize, respectively (Ranum *et al.*, 2014) (Figure 2.1).

Figure 2.1: Tropical White and Temperate Yellow Maize Cobs
In SSA, Tropical Maize is Predominantly White in Color Selected Probably as a Result of Gastronomy as Compared to Temperate Maize that is Predominantly Yellow in Color

Maize was first domesticated about 10,000 years ago in what is currently Mexico and was introduced into Africa by the Portuguese in the 16th century, but since then it has now become the most important staple food crop in many parts of Africa (Longe and Ofuya, 2009; Ranum *et al.,* 2014).

2.1.2 Global Maize Production

The crop is grown in more countries than any other cereal with the developed countries producing more maize than the developing countries (Tahir and Habib, 2013). It is annually planted over an area of 15.5 million hectares in SSA and Latin America (Lobell, 2013; IITA, 2013). In fact, although tropical maize has been customarily the main staple of the diet in SSA, its consumption exceeds its production (Morris, 2001). It is estimated that in 2012, the total world production of maize was 875,226,630 tons, (FAO, 2012) with United States, China and Brazil harvesting 31, 24 and 8% of total production of maize respectively.

African countries produce only about 7% of the world's maize with South Africa and Egypt being the main producers (FAO, 2007). In 2013, Africa produced only 6.5% and imported 28% of the required maize from countries outside the continent (Lobell, 2013; Smale *et al.,* 2011). The crop covers more than 33 million ha (17%) of sub-Sahara Africa's estimated 200 million ha of cultivated land and is produced in diverse production environments and consumed by people with varying food preferences and socio-economic backgrounds (FAO, 2015). The top 20 countries, namely South Africa, Nigeria, Ethiopia, Tanzania, Malawi, Kenya, Zambia, Uganda, Ghana, Mozambique, Cameroon, Mali, Burkina Faso, Benin, DRC, Angola, Zimbabwe, Togo, and Cote d'Ivoire, account for 96% of the total maize production in SSA (FAO, 2015). However, in all these countries excluding South Africa, the bulk of maize produced is by small holder farmers in individual or in groups (Lyon, 2000).

In Kenya, 90% of the rural households grow maize with the national maize production ranging between 24 and 33 million bags per annum which does not keep pace with the domestic consumption levels. Kenyan counties that produce large maize surplus are in the Rift Valley region mainly Trans Nzoia and Uasin Gishu Counties primarily on medium and large farm (Nyakio *et al.*, 2015). Actually, in recent years there has been an expansion of land used for maize production in Kenya, as evidenced by 1.7 million hectares in 2008 and 1.8 million hectares in 2009 (Jayne *et al.*, 2001; Nyoro *et al.*, 2004; Adijah *et al.*, 2011; Nyakio *et al.*, 2015).

2.1.3 Economic Importance of Maize

Maize (*Zea mays*) is considered as the queen of cereals. It is one of the most important crops in the world with highest production and productivity under both irrigated and rain fed agricultural system in the semi-arid and arid tropics especially in sub-Saharan Africa (Mazunda, 2013; Lobell, 2013). Almost every part of the maize plant is utilized (MAFAP, 2013a).

Globaly, about 66% of all maize is used for feeding livestock, 25% for human consumption and 9% for industrial purposes. In the developing world, about 50% of all maize is consumed by humans as food while 43% is fed to livestock and the remainder (7%) for industrial purposes (IITA, 2003).

Maize is a major staple food crop grown in diverse agro-ecological zones and farming systems and consumed by people with varying food preferences and socio-economic backgrounds in the world. In Mexico where it originated, virtually every dish in Mexican cuisine uses maize. It is a major stable crop in Africa with more than 300 million Africans depending on it as their main food source. In fact, of the 22 countries in the world where maize forms the highest percentage of calorie intake in the national diet, 16 are in Africa (Nuss and Tanumihardjo, 2011; Munyiri *et al.,* 2013). The central role of maize as a staple food in SSA is comparable to that of rice or wheat in Asia, with consumption rates being the highest in eastern and southern Africa (ESA) (Longe, 2016).

In Kenya, maize provides a major source of carbohydrates, iron, vitamins, proteins and minerals to human body (Munyiri *et al.,* 2013; Kumar and Jhariya, 2013). According to Chaudhary (1983), maize grains have great nutritional value as they contain 72% starch, 10% protein, 4.8% oil, 8.5% fiber, 3.0% sugar and 1.7% ash (Munyiri *et al.,* 2013).

Maize is a very rich source of highly digestible carbohydrate; which if eaten in the immature state, provides useful quantities of Vitamin C. The temperate yellow grain varieties also contain vitamin A (Anyanwu *et al.,* 1998). Prior to the development of quality protein maize (QPM) the superiority of maize over the other cereals was limited by its low protein content (FAO, 2007: IITA, 2007).

Fresh immature or even mature whole grain of maize can be boiled, roasted or cooked with beans to prepare “Githeri”, as called in Kenya and served as food. It can also be consumed on the cob when boiled or roasted when fresh. Very dry maize or kernels can be processed by milling or powdering it to give maize flour that eventually can be used to prepare various foods, such as semovita, bread among other snacks. In Kenya, maize is grounded to flour and consumed as a largely renowned “ugali” dish or porridge upon mixing with hot water. Maize flour also serves as the main raw material to produce the nonconventional local brews known in Kenya as “Changaa” and “Busaa”. These brinks are largel revered in local communities and are part and parcel of traditional ceremonies.

Maize grains can also be grounded and prepared into paste for making palp; or fried in oil into 'corn-cake’ (Longe, 2016). Dry grains of a particularly variety, *Zea mays* everta is roasted to expand and puff up into a popular refreshment known as popcorns or ‘pop-corn’ (Longe, 2016). Maize remains the most important cereal fodder. The grains constitute a major ingredient in the production of livestock feed (mash), particularly, for poultry and pigs. The green leaves of maize plant can also be fed as fodder to livestock, or in the form of hay/silage (Longe, 2016). While in developing countries, such as Kenya it is mainly used for human consumption, the bulk of maize produced is used as livestock feed as well as raw material for industrial processing in developed countries (Aquino *et al.*, 2001; MAFAP, 2013a).

Maize trade forms an important source of income and livelihood to several households, importers and millers of maize grains, its products or by products in our local and international markets (IITA, 2016). It is used in many different and major commercial ways such as human food (maize flour), livestock feed (mash) and industrially as raw materials for manufacture of various products explained below **(**Alhassana and Kumahb, 2018).

Products resulting from industrial processing of maize include starch, high fructose syrup, dextrose, corn oil, ethanol, cosmetic or skin care products, beverages, crayons, soaps, absorbent material for diapers, food additives, biodegradable plastics and food supplements. Other products are livestock feed and other components such as fuel (Halm *et al.*, 1996; Cardona *et al.*, 2007).

Corn starch is used mainly as a thickener or as a stabilizer of other ingredients such as baking powder, candies, puddings and other prepared food mixes. Starch from maize can also be made into plastics, fabrics, adhesives, and many other chemical products. Paper and textile industries also utilize corn starch. It is also used as a carrier for some vitamins and medicines (Dupoint *et al.*, 1990). The chrysanthemin substance found in purple corn is used as a food coloring. Other industrial products of maize include cereal baby foods, corn cooking oil, glucose, syrup, gum, soap, starch and alcohol. Corn oil is used for making salad, as cooking oil and in the production of margarine. Dextrose is utilized in the bakery industries where it serves as a yeast nutrient and provides some sweetness and browning of the crust on baked products. Other major uses of dextrose are in food canning, frozen packaged foods, ketchup, jams and jellies, soft drinks, wines and malt liquors (Dass *et al.*, 2016).

The corn steep liquor is a watery by product of maize wet milling process, widely used in the biochemical industry and research as a culture medium to grow many kinds of microorganisms (Manueke *et al.,* 2015). Maize is also increasingly being used as a feedstock for the production of ethanol and biodiesel fuel. This ethanol fuel is mixed with gasoline to decrease the amount of pollutants emitted when used to fuel motor vehicles in the U.S.A and Germany (Kumar and Jhariya, 2013; Ranum *et al*., 2014).

2.1.4 Storage and Handling of Maize

Preservation of quality in maize grains during long term storage is a challenge in many parts of the world. Generally, inappropriate postharvest handling of grain leads to an estimated 20% avoidable losses in the postharvest stages (Gras *et al.,* 2000). An estimated 8 to 10% of total grains stored in warehouses or in silos is lost as a result of inappropriate storage conditions yearly (Adijah *et al.,* 2011).

Infestation of maize stock comes either from outside or inside where the grains are stored and the extent of the infestation of various insect pests is variable under different storage structure, and practices. Storage of food grains by the local people is mainly traditional. The traditional methods have been used for many years with little or no modification and are successful because of the application of improved methods, though unaware. The selection of a traditional storage system by the farmers is often related to local natural resources (Hall, 1970).

Storage loss is directly related to structural design and structural material of stores. Under bad hygiene and in poorly designed stores, it is likely to occur considerable damage and absorption of moisture in grain would be higher (Adijah *et al.,* 2011). The nature of storage method and the duration of the storage period is influenced by the quantity of grain produced in a season. Maize storage in Africa is predominantly in traditional granaries, with cobs drying out gradually through natural ventilation (Owusu, 1981; Nicol *et al.*, 1997; Mannan and Tarannum, 2011).

Farmers use traditional storage containers for storing grains for their own need only. These storage structures were comparatively cheap, ecofriendly, make use of locally available material and impart high shelf life to the stored maize grains (Karani *et al.*, 2017). These traditional storage systems could be applied in modem storage areas with modification. This could save food produces that would otherwise be damaged by insects. Rural people have knowledge on conservation and utilization of natural resources (Mannan and Tarannum, 2011).

There are three main traditional storage systems based on type and location and these are; indoor, outdoor and underground systems (Osei-Akrasi, 1999). The indoor and outdoor structures are usually used to store both shelled and unshelled maize, but the underground storage is for shelled maize and it is used in drier regions. Thus, maize storage structures tend to be specific to a climatic zone and are constructed to meet the requirements of that particular area. Small quantities of seed maize are usually stored indoors using calabashes, gourds and earthenware clay pots at the rural household level. On the large-scale maize is stored in jute sacks or bins in large warehouses after shelling, drying and treating with the recommended pesticides (Nicol *et al.*, 1997).

Many farmers store their maize cobs with the husk on, which does not significantly affect the rate of grain drying in cribs. Undehusked maize and grains on the cob are less susceptible to *S. zeamais* attack than the shelled, but shelled maize suffers less damage from pest such as *Prostephanus truncatus* Horn (Coleoptera: Bostrychidae) than maize stored on the cob (Hodges, 1985; Meikle, 1998; FAO, 2007).

2.1.5 Constraints to Maize Production

2.1.5.1 Abiotic Factors

Maize crop often suffers from abiotic stresses like moisture stress (low/excess soil moisture), temperature (high/low), salt/salinity, nutrient in the soil, etc. Climate change and resulting drought (water deficit) is the most important abiotic problem that may undoubtedly worsen global food crises and exacerbate decreased maize production caused by the activities of the notorious post-harvest pests like *S. zeamais* (FAO, 2002). Persisted drought may result in 100% crop loss in affected areas because it deprives crops of water which is a vital component of all living cells. Water plays a component role in growth and maintains of various metabolic process of plant life (Austin, 2008; Aslam *et al.*, 2013). Maize is mainly produced under rain-fed agriculture particularly in Africa. Hence, irregular rainfall pattern and drought occasioned by climate change has greatly affected the maize crop performance (Aslam *et al.*, 2015).

High temperature stress is the second major abiotic problem after drought that reduces grain yield more than 15%. Spring season is most suitable time for the cultivation of maize in India but transitory or constantly high temperatures cause an array of morphological, anatomical, physiological and biochemical changes, which affect plant growth and development. Especially more effects at reproductive stage through pollen abortion, silk desiccation and reduced grain set, ultimately leading to reduction in economic yield (Adijah *et al.,* 2011; Dass *et al.,* 2016).

Maize is highly sensitive to frost and moderately sensitive to chilling especially during early autotrophic growth. The extremely cold temperatures affect the maize growth in a number of ways right from emergence till flowering and seed setting. Seemingly, photosynthesis is strongly affected due to the inhibition of certain enzymes of the C4 and the Calvin cycle. The reduced photosynthetic activity promotes dissipative mechanisms and affects the anti-oxidative defense in maize leaves (Adijah *et al.,* 2011; Kostandini *et al.,* 2015; Dass *et al.,* 2016; Liu *et al.,* 2017).

Distinct drought tolerant (DT) maize varieties have been developed to reduce maize venerability to drought. For instance, more than 180 distinct drought tolerant (DT) maize varieties have been developed and released through DTMA, and nearly 52,000 metric tons (MT) of seed was produced and delivered across 13 target countries in SSA in 2014 alone. Many DT and NUE varieties are being scaled-up in eastern, southern and West African countries, with significant present and potential impacts (Alene *et al.,* 2009; Liu *et al.,* 2017).

Soil salinity affects maize production in many parts of the world, particularly on irrigated land. Osmotic stress (drought problem), ion imbalance, particularly with Ca+2, K+ and the direct toxic effects of ions on the metabolic process are the most important and widely studied physiological impairments caused by salt stress (Munns *et al.*, 2006).

Salt stress, like many abiotic stress factors, also induces oxidative damage to plant cells catalyzed by reactive oxygen species (Azevedo-Neto *et al.,* 2006). Excessive buildup of Na^+ and Cl damages root membrane and causes ionic imbalances that may discourage potassium uptake by plants (Gadallah, 1999). Sodium (Na^+) and chloride (Cl^-) concentration in different plant parts, tissues, cells and cell organelles increases by increasing salinity. Nitrogen fertilization not only promotes plant growth it may also lessen the effects of salt on plants. Plants take up nitrogen mainly as NO_3 and NH_4^+ forms but when both are present in solution then NH_4^+ is preferred (Flores *et al.,* 2001).

Under salinity nitrate consumption is slowed down and salinity reduces NO_3 absorption with the possible consequence of nitrogen insufficient in the plant (Oliel *et al.*, 2005). It is difficult to study plant response to fertilizers under saline conditions due to high concentration of salts and nutritional imbalances. Studies showed that application of fertilizers in saline soils might result in increased, decreased or unchanged plant salt tolerance. In other words, plant response to fertilizers depends on severity of salt stress in the root zone (Oliel *et al.,* 2005; Dass *et al.,* 2016).

Maize is considered as a moderately salt-sensitive plant. Salt tolerance is a multi-genic trait, a complex phenomenon that involves biochemical and physiological process as well as morphological and developmental changes that allows maize plant to grow under salt stress condition by maintaining salt balance through osmotic adjustment without reduction in economic yield (Yuan, 2018).

2.1.5.2 Biotic Factors

2.1.5.2.1 Weeds and Weed Control

Weeds compete with the crop for essential resources such as nutrients, water and light. In Africa, it is estimated that invasive weeds together with associated pathogens are responsible for approximately between 7.5 and 8.5% reduction of the crop yield (Mutambara *et al.*, 2013). Generally weeds reduce crop yields by competing for light, nutrients, water and carbon dioxide as well as interfering with harvesting and increasing the cost involved in crop production. Different weed control methods have been used to manage the weeds but mechanical and chemical methods are more frequently used for the control of weeds than any other control methods (Iken and Amusa, 2004). Application of herbicides and weeding are the most common ways of weed control (Chikoye *et al.*, 2007).

A common parasitic weed *Striga hermonthica* has affected maize production overtime. Its infestation has caused severe losses to the maize farmers (Anderson and Halvarsson, 2011). Striga is reported to have infested 210,000 hectares of maize farms in western part of Kenya alone (Bedada *et al.*, 2011). According to the African Agricultural Technology

Foundation (AATF), Striga costs African farmers across the continent about $1 billion per year (AATF, 2006). Development and deployment of striga tolerant improved maize varieties together with other improved crop management practices, including cereal-legume rotation assists to control striga (Anderson and Halvarsson, 2011; Runo *et al.,*2011).

2.1.5.2.2 Maize Diseases

Maize is susceptible to many diseases mainly maize lethal necrosis (MLN), maize streak virus (MSV), turcicum leaf blight (TLB), gray leaf spot (GLS), southern leaf rust and blight, that contributes to approximately between 6 and 10% maize yield loss annually (Subedi *et al.*, 2015). The major diseases that attack maize crop also include leaf rust, leaf blight, maize streak disease and grey leaf spot. Among all these, maize streak disease is recognized as a major virus disease of the monocotyledons crops in SSA (Suleiman and Rosentrater, 2015).

Maize lethal necrosis (MLN) disease has emerged as a notorious threat to food security in east and central African region since 2011. Kenya’s loss was estimated at 0.3 million tons per year, or 23% of the average annual production estimated at US$ 110 million (US$ 365/ton). The situation is particularly critical as more than 95% of the commercial maize varieties in eastern African seed market are vulnerable to MLN (Mahuku *et al.*, 2015). An integrated approach using agronomic, nutritive, or chemical controls are adopted for an effective disease management (Agrios, 1988; Tefera, 2012; Subedi *et al.,* 2015).

2.1.5.2.3 Pests

There are a number of pest that attack maize and contribute to grain loss both at the farmer and consumer level in the tropics (Lale and Ofuya, 2001; Adedire, 2003). These are either field pest or storage insect pest. Some of the most common field pest are army worms (*Spodoptera exempta, Persectetamia ewingii* and *Mythimna connecta*) and stalk borer (Busseola *fusk*) (Suleiman and Kurt, 2015).

Storage pests form a major cause of grain loss (Muyinza, 1998). The most common pests of stored grain are the larger grain borer and maize weevils (Vowotor *et al.,* 2005; Suleiman and Kurt, 2015). Post-harvest losses to storage insect pests have been recognized as an increasingly important constraint to maize production in Africa (Markham *et al.,* 1994). *Sitophilus zeamais* is the most important, most predominant and destructive of all these pests whose infestation cause an estimated annual loss of between 30 and 50 % of stored maize grains in tropical Africa (Adedire, 2001; Vowoter *et al.*, 2005; Suleiman *et al.*, 2015; Khaliq *et al.,* 2018).

2.2 Maize Weevils (*Sitophilus zeamais*)

2.2.1 Biology and Description

The maize weevil, *S. zeamais*, (Coleoptera: Curculionidae) is a small agricultural insect pest in the beetle family, Curculionidae (snout beetles). Adult *S. zeamais* is quite small, measuring from 3 to 5 mm in length (Prasanna *et al.,* 2015; Suleiman *et al.*, 2015). It is dark-brown or black in color with four distinct pale reddish-brown oval markings, (spots), or patches on the elytra (Appert, 1987; Prasanna *et al.,* 2015).

The head has capitate (rounded) and elbowed antennae with 8 segments that are often carried in an extended position when the insect is walking. The fore-wings are hard with well-developed meta- thoracic flight wings and a prothorax with circular punctures and which gives the adult weevil a greater ability and tendency to fly to the ripening crop in the field and establish an infestation in the grains before harvest, than other species (Appert, 1987; Odeyemi and Daramola, 2000; Prasanna *et al.,* 2015).

The adult maize weevil is characterized and often distinguishable from all other common stored product pests by its long beak (or rostrum), which is a forward snout-like extension of the head. The rostrum carries the mouthparts in a position that is ideal for penetrating commodities. Possession of a long rostrum is the main diagnostic feature of all curculionids called weevils (Odeyemi and Daramola, 2000; Adedire, 2001).

Generally, they are small snout beetles that vary from red-brown to black and sometimes with yellow spots. Their average size is approximately between 2.5 and 4.5 mm long. The legs are prominent, and the wings are well developed making them good fliers (Tefera, 2011; Mugo *et al.,* 2012). Maize weevils are virtually found in every part of the world, especially tropical regions where maize is grown (Mugo *et al.,* 2012).

The weevils are morphologically sexed by examining the rostrum and abdominal shape of the insects. The rostrum of the male *S. zeamais* is rough, distinctly shorter and wider than that of the female, while the rostrum of the female is smooth, shiny, distinctly longer and narrower than that of the male (Halstead, 1963; Ojo and Owoloye, 2012).

Figure 2.2: Mature Adult Maize Weevil, *Sitophilus zeamais*

2.2.2 Life Cycle of *Sitophilus zeamais*

Maize weevil, *S. zeamais* undergo complete metamorphosis from egg to larvae to pupa and finally to adult in its life cycle. The adult weevils may appear on maize cobs in the field as soon it reaches the roasting ear stage but the adult female weevils only begin to lay eggs once the maize cob ear become firm.

2.2.2.1 Oviposition

Upon grain infestation, the female weevils bore minute holes into the grains in which the females deposit their eggs. The holes are sealed with mucilaginous material secreted by the female (Hill, 2008). Their eggs are naturally white and oval in shape measuring 0.7 mm by 0.3 mm and each female may deposit as many as five eggs per day laying a total of 150 to 400 eggs during its entire life span (Bosque-Perez, 1992; Arthur *et al.,* 1998).

2.2.2.2 Larvae Development

Under optimal conditions of between 27 and 30ºC and approximately 71% relative humidity the larval development takes about twenty-five days before pupae emerge but under unfavorable environmental conditions, the larval stage may last for up to 98 days (Mattah, 2001). First, the eggs require four to nine days to hatch into tiny legless, thick-bodied grubs which are white in color with brown head and strong jaws (Kiritani, 1965).

2.2.2.3 Pupation

Upon hatching, larvae bore into the grain and after developing through several pupal stages emerge as adults (Ojo and Omuloye, 2016). The pupae stage (pupation) lasts for approximately three to six days while still within the grain after which adults emerge from the pupae. These newly emerged adults remain inside the grains for few days before they move out. Lastly after pupation, the newly developed adult chews its way out of infested grain through a fairly large characteristic circular emergence perforations made on the outer coat of the grain (Longstaff, 1981; Hugh, 1988; Chilio *et al.,* 2004; Ojo and Omuloye, 2016).

2.2.2.4 Generation Time

Weevils can live up to 12 months, depending on environmental conditions (Longstaff, 1981). The complete development time and adult emergence of *S. zeamais* from seeds under optimal conditions of 30°C and 70% relative humidity, occurs between 35 and 60 days after eggs have been laid; but may be up to 110 days at sub-optimal conditions such as temperatures above 32^{o}C with less than 14% maize moisture content (Kiritani, 1965).

Chilio *et al.* (2004) demonstrated that weevil is unable to survive at temperatures above 32 °C (Odeyemi and Daramola, 2000).

Unlike *C. maculatus* adults which do not feed, adults of *S. zeamais* feed (grubbing) inside the maize grains; leaving powdery residues afterwards. This feeding habit helps to enhance long-life in the weevils allowing them a longevity period of up to 5 months or even l year. Emerged adults are good fliers (Adedire, 2001). They re-enter holed maize seeds at will, either to feed or to lay eggs. When the adults emerge, the females move to a high surface and release sex pheromones. Males are then attracted to this pheromone for mating to occur (Mason, 2003).

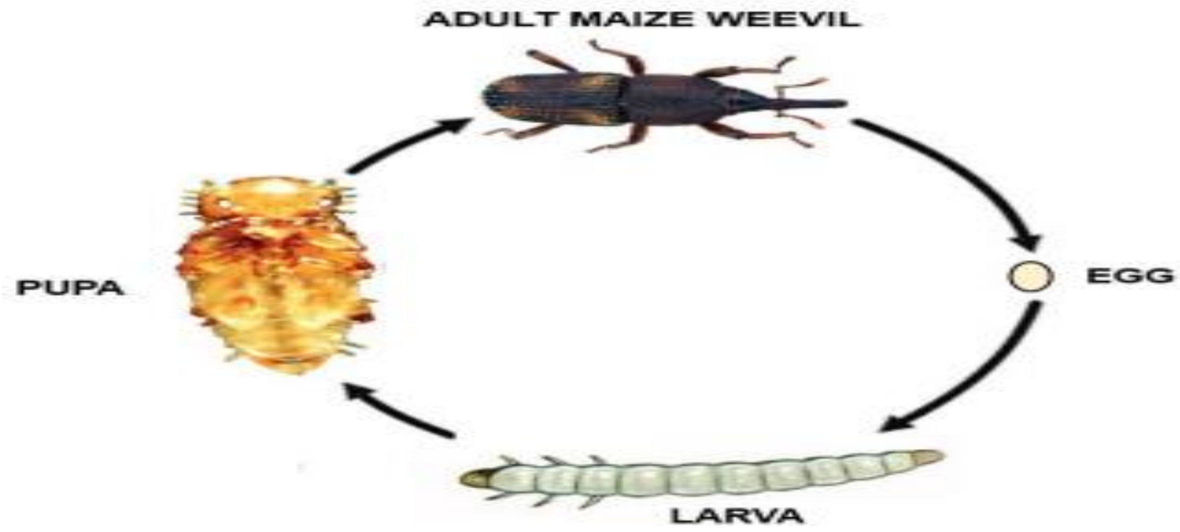

Figure 2.3: Life Cycle Model for Sitophilus zeamais. Adapted from http: // www. budgetpestcontrolpgh. Com /crawling-insects/grainbeetle control/)

2.2.3 Damage Done by *Sitophilus zeamais* to Maize Kernels

The damage done to stored maize by *S. zeamais* in the tropics is enormous. It bores holes in the grain, consumes a large portion of the endosperm, destroys the germ and thus reduces the nutritive value and viability of the grain (Compton and Sherington, 1999).

The maize weevil, *S. zeamais* is a highly polyphagous pest of many stored grains. Both adults and larvae feed causing substantial grain losses. Adult weevils and larvae feed on undamaged grains and reduce them to powdery form (Adedire, 2001). Presence of *S. zeamais* in the grain lowers its quality and value. Heavy infestation by *S. zeamais* may cause weight losses of as much as between 30 and 40% of produce (CABI, 2005).

Maize weevil infestation results in substantial reduction in the quantity and considerable deterioration of quality of the grains. Additionally, the kernel damage caused by the pest enables other species, the external feeders, which are not capable of infesting sound grain, so increase the damage rapidly (Abebe *et al*., 2009).

The weevils are unique because their attack tends to increase the moisture content of infested cereal grains to appreciable levels that consequently leads to caking and promotes rapid growth of micro-flora especially fungal growth which constitutes quality loss. It has been reported that weevil infestation also brings about the establishment of mound infestations including *Aspergillus* spp. which produces aflatoxins making the grain unsuitable for food and feed (Kling, 1991). The grain is left with foul odor, the excrement of insects and micro-organisms leading to the production of mycotoxins thereby lowering the quality and rendering it undesirable for consumption (Mejia, 2007).

Grain damage by weevils and subsequent loss in maize quality during storage is a very serious problem for farmers and traders (Onekutu, 2015). Damaged and depreciated maize grains are unsuitable for human consumption and cannot be used for agricultural and commercial purposes (Adedire *et al*., 2011; Onekutu *et al*., 2015). Maize weevil infestation culminates to outright rejection of the product at the local and international markets (Nwosu and Nwosu, 2012).

Weevils have a relatively short developmental period and hence high populations can easily be built up. Thus, unless control measures are taken, heavy infestations occur and massive grain damage may take place within a short period of storage (Suleiman, *et al.*, 2015). Adesuyi (1997) reported that after only six months of storage in a traditional storage structure about 24.7% of stored maize was lost through attack by the maize weevil, which feeds on the germ of maize seeds.

2.3 Methods of Crop Pests Control

2.3.1 Cultural Pests Control

Cultural pest control methods are practices that reduce pest establishment, reproduction, dispersal, and survival. They are pest management tools and activities that make the crop habitat less favorable for pests to survive or cause crop damage (Summy, 1997). The method includes all the practices that may make the crop or crop habitat inhospitable to pests directly or indirectly. Crop rotation programs that directly deny overwintering pests their preferred food source that ultimately starve them to death. Indirectly cultural practices encourage natural enemies, predators and parasitoids to enhance biological control (Summy, 1997).

Intercropping maize with aromatic herbs *Allium cepa* (onion) and *Allium sativa* (garlic) is one of the cultural methods of pest control, which is used to repel insects like *C. partellus* in maize (Njoroge *et al.,* 2016). Intercropping of vegetables with lemon grass and onions has been used to control *Myzus persicae* (Aliyu *et al.,* 2015). These methods have not been practical in large scale production (Pauline *et al.,* 1992). Trap plants which attract maize pests like the stem borers are used so that they do not destroy maize and other host crops (Shelton and Badenes-Perez, 2005; Holden *et al.,* 2012). Push-pull strategy is another method of controlling insect pests and increasing crop production (Cook *et al.,* 2007; Hassanali *et al.,* 2008). By using narrow row spacing, the canopy closes over the soil faster, reducing the attractiveness of the crop to host-seeking corn earworm moths (Ognakossan *et al.,* 2013; Tounou *et al.,* 2013).

Hygienic pest control methods involve consistent cleaning and disinfestation of storage vessel to remove debris, laid eggs, larvae and dead insects left after consumption of the grain. Moreover, before new maize grains are taken into the storage kernels, the infested grains are removed and burnt in order to limit immediate infestation of the new grains (Newman, 2008). As a cultural practice, cracks and crevices within the grain storage structures are filled up and the walls painted so as to repel insect pests away from grain stores. High temperatures applied for a long period of up to 48 hours ensure total disinfestation of the stores (Upadhyay and Ahmed, 2011; Frank *et al.,* 2018).

2.3.2 Physical Pest Control

Farmers control pests physically by killing pests directly or modifying the environment in which insects live, so that it becomes deleterious or unsuitable for the survival of the target pest species. Mechanical pest control include use of traps for rodents and barriers such as screens to keep birds or insects away (Frank *et al.,* 2018). Physical pest control also involves such techniques as the manipulation of abiotic factors such as temperature, humidity and the amount of available oxygen, carbon dioxide or carbon monoxide. Insects and mites generally have narrow environmental limits within which they survive and breed successfully. Any technique which can alter either the temperature or the humidity, without any detectable adverse effect on the commodity will therefore provide adequate protection against insect and mite pests (Upadhyay and Ahmed, 2011). Physical control measures and storage systems been adopted in maize grains storage, includes the following:

2.3.2.1 Sun Drying (Solarization)

Drying is a critical aspect in grains storage. This is basically because moisture is an important factor in grains deterioration during storage. Products must be sun dried to sufficiently low moisture content (<14%) before storage (Wembly and Thomsan, 1999). Weevil infestation on grains reduces due to the heat of the sun which may kill or shoo them away. Furthermore, high temperature due to direct solar radiation may kill the developing larvae in the grains (Zare and Ranjbaran, 2012).

2.3.2.2 Dry Heat Treatment and Storage in Fireplace

High temperature can destroy several life stages of store product since they cannot tolerate extreme temperatures. Superheating of these grains ensure extra-protection without applying any insecticides. Dry heat treatment has been found to be an effective control against all developmental stages of *S. zeamais* (Mohammed-Dawd and Morallo-Rejesus, 2000). When commodities are dried above fire, care must be taken to prevent cracking of the grains and loss of viability. Heat exposure of grains therefore should not be more than 35^0C (Odeyemi and Daramola, 2000).

2.3.2.3 Use of Low Temperature (Freezing)

The best and reasonable way to kill weevil is to freeze your maize grains. As soon as you buy any food item, freeze it for at least four days. Food can be stored in a freezer for the longer period (Carrillo *et al*., 2006). Most research workers store or disinfect maize and cowpea grains which have been attacked by weevils and bruchids respectively, by deep-freezing (Nakakita *et al.*, 1997). Nakakita *et al.* (1997) reported that both hatching and metamorphosis of *S. zeamais* were completely inhibited at 10°C; a small number of adult *S. zeamais* emerged at 15°C.

2.3.2.4 Irradiation

This involves subjecting the grains to both gamma and beta radiation to control grain insects. Gamma and beta radiation are generated from Cobalt 60 and electric source respectively. When done in closed chambers, it eliminates all life stages of insects without affecting the nutritive value of the grains. However, it requires high start-up costs (Hallman, 2013). There is also misconception about irradiated food by the public. In addition, at doses recommended for use, the irradiation does not cause acute mortality (Phillip, 2006; Philip and Throne, 2010).

2.3.2.5 Hermetic Technology (Controlled Atmosphere)

The hermetic technique is an air-tight technology for grain storage that functions on the principle of respiration (Suleiman and Rosentrater, 2016). As the insects respire and use up oxygen that is contained in the air-tight storage device, carbon dioxide builds up (Jonfia-Essien *et al.,* 2010). The insects will die from reduced oxygen (hypoxia), lack of oxygen (anoxia) and eventual carbon dioxide build up and poisoning (hypercarbia). Sealed polythene bags can be used for partial hermetic storage of grains (Longe and Ofuya, 2016). This technology requires structures that are airtight, filled as completely as possible and kept at uniform cool temperature (Longe and Ofuya, 2016; Bhushan *et al.,* 2017).

2.3.2.6 Use of Inert Materials

Besides the use of inert dusts such as calcium oxide, gypsum, kaolinite and attapulgite as diluents or carriers in the formulation of pesticides, these so-called inert materials also exhibit insecticidal property when used alone (Sonja *et al.,* 2018). Inert materials act as a physical barrier preventing adult weevils from locating mates or the grain as well as descicants (Golob, 1982; De Lima, 1987).

Permualand le Patourel (1990) and Lorini *et al.* (2003) observed that acid activated clay treatment caused significant adult mortality of several stored grain pests within 24 hours after treatment. Attapulgite based clay dusts have good insecticidal property causing 90% mortality in *C. cephalonica, T. castaneum* and *S. oryzae* within 48 hours after treatment (Soujanya *et al.,* 2016). Yevoor (2003) opined that kaolinite at 10% caused up to 90% mortality of adult at 28 days after release and much less percentage of grain damage and weight loss after up to 90 days of storage. The use wood ash, kaolinite and clay for the control of curculionids, particularly *S. zeamais*, has also been investigated (De Lima 1987; Mahmoud *et al.,* 2010).

Sivasrinivasu (2001) found significant percent mortality of rice weevil at 28 days after storage in sorghum admixtured with 30% ash and observed no weight loss for 90 days of storage period. However, Yevoor (2003) reported that ash was not effective to maize grains which showed less than 50% adult mortality, 30 days after treatment and zero percent adult mortality at 150 and 180 days after treatment.

Application of sand in India is an ages old practice to protect stored grains from insect attack (Pruthi and Singh, 1950; Chitra and Subramanian, 2016). Sand at 30% effectively prevented the infestation by *S. zeamais* and *S. cerealellela* in maize (Golob *et al.*, 1982).

When sawdust was mixed with wheat grains, the reduction in the pest population was related to the ratio of sawdust to wheat grains with greatest reduction being obtained at ratio of 4:5 (Perisic *et al.*, 2018). Yevoor (2003) also reported that sawdust at 10% was not effective in controlling rice weevil damage to maize grains.

2.3.4 Chemical Pest Control

Use of synthetic chemical pesticides is the mainstay of insect crop pest control dominating most of the countries around the world (Jeschke *et al.*, 2002). It is the most commonly used and the most effective method. In fact, the protection of plants from insect pests is currently dependent on synthetic pesticides (Teodoro *et al.,* 2017).

The worldwide use of synthetic pesticides has increased in recent years with 2.5 million tonnes of pesticides being used annually (Koul *et al.,* 2008). This is mainly because of their effectiveness and simplicity in dealing with the pest. It includes the use of contact dust admixtures and fumigating with phosphine gas and it is recommended when grains are stored at high temperatures (between 25 and 35ºC) or for a longer period exceeding three months (Giga and Mazarura, 1990; Brooker *et al.*, 1992).

The storage pest insecticides most commonly used include aluminium phosphide, lindane, methyl bromide, ethylene dibromide, edifenphos, pirimiphos methyl, permethrin, Malathion, sumithion, chlorpyrifos methyl, chlorpyrifos, propoxur, fenithrothion, dichlorvos, bromophos, fenvalerate, bioresmethrin, phenothrin and deltamethrin (Lale 2002; Denloye, 2010; Rahman, 2011).

2.3.4.1 Classification of Synthetic Pesticides

Synthetic pesticides are widely grouped into organophosphates, carbamates, pyrethroids and neonicotinoids. However, depending on the method of application and the way they enter the insect's body, they are further classified into either contact or systemic pesticides (Chung and Chen, 2011). Contact insecticides are sprayed or dusted on the insect's body. The poison is absorbed through the body wall/cuticle. Most soft-bodied insects like aphids are vulnerable to contact insecticides. However, unlike systemic insecticides contact insecticides have no residue activity (Newman, 2008).

Systemic insecticides are absorbed by plant tissues and become distributed systematically throughout the whole plant so that when insects feed on the sap they are poisoned. Some insecticides change to a vapor quite readily (fumigants) and enter the insect's trachea apparatus. This type of pesticide is useful in enclosed areas where the vapors can remain concentrated, such as greenhouses or storage bins, but usually do not work well in open fields. However, some insecticides may create a bit of fumigant activity at the time an insect is moving across the treated surface (Massango *et al.*, 2017). Fumigation is the main and effective means by which synthetic insecticides are applied to the cereals. Once taken up through the spiracles, their fumes spread to the trachea and finally combine with hemolymph components. However, most synthetic pesticides like organophosphates have both contact and systemic/fumigant activities against the target insects (Newman, 2008).

Organophosphates (OP) and carbamates are the main insecticides in use today (Toutant, 1989). Organophosphate class of pesticides include; chlorpyrifos, diazinon, isofenphos, parathion, malathion, dichlorvos and trichlorfon while carbamates include bendiocarb and carbaryl. They are used against insects in all developmental stagess, but mainly to kill mature insects because both affect insect nervous systems in the same way (Abo-Elghar *et al*., 2004; Clark, 2006). However, Organophosphate and carbamates have high avian and mammalian toxicity (Tomizawa and Casida, 2005).

Pyrethroids are synthetic analogues of pyrethrin, a natural compound found among chrysanthemum species like pyrethrum. In contrast to organophosphate insecticides, pyrethroids exhibit low toxicity to mammals and birds, while also demonstrating strong selectivity for insects and invertebrates (Fishel, 2005). Chronic animal feeding studies have produced high no-effect levels of pyrethroids with indication of both a low potential to bioaccumulate and proficient detoxicification of these chemicals in mammalian receptors. Furthermore, they are reportedly practically nontoxic to birds (Fishel, 2005). In addition to low avian and mammalian toxicity, physical properties of pyrethroids also are an improvement over their predecessor organophosphates and carbamates (Oros and Werner, 2005).

Neonicotinoids is a class of insecticides which are highly systemic with long-term persistence. Acting on contact and systemic, the neonicotinoids are particularly suited for controlling many insects with biting and sucking mouth parts especially if swallowed (Bogusław *et al.,* 2019). They are also used in seed dressing for protection from soil insects; they are absorbed by the radical apparatus and are then distributed evenly, maintaining an effective concentration of active substance in young plants (Daniela *et al.,* 2008).

The neonicotinoids have higher selectivity factors for insects versus mammals than most insecticides apart from pyrethroids (Tomizawa and Casida, 2005). Several neonicotinoids, however, show very strong toxicity to pollinating insects, causing also other effects which are seldom easily identifiable, such as behavioral disturbances, orientation difficulties and impairment of social activities (Guez *et al.*, 2001; Bortolotti *et al.*, 2003; Tomizawa and Casida, 2005).

2.3.4.2 Mode of Action of Pesticides

The mode of action describes how the pesticide kills the pests or makes them inactive. According to Bloomquist (2008), mode of action refers specifically to the biological process in body of the pests that the pesticide interrupts. Different pesticides have different mode of action. Understanding the mode of action is important because one can know whether an insecticide will be toxic to non-target organisms such as fish, birds and mammals or not (Njoroge *et al.,* 2016). Most insecticides have mode of action that can be classified into three categories namely: neurotoxins, respiratory inhibitors and growth regulators (IGR’s) (Song and Scharf, 2009).

Neurotoxic insecticides such as organophosphates cause overstimulation of the nervous system leading to paralysis and death. The chemicals may achieve this effect by inhibiting cholinesterase activity or by acting as sodium/chloride channel modulators

(Tomizawa and Casida, 2003; Brown, 2006). Organophosphate and carbamate insectcides are both acetylcholinesterase inhibiting pesticides. They interfere with the transmitssion of nerve impulses across the synaptic gap between two nerve cells by preventing the breakdown of the predominant neurotransmitter, acetylcholine (Abo-Elghar *et al.*, 2004; Pascual-Villalobos, 2010).

Neonicotinoids permanently bind to nicotinic receptors of acetylcholine (ACh), blocking them and consequently the passage of nerve impulses. In particular, the chloronicotinyls nitroguanidine family of neonicotinoid insecticides such as Acetamiprid, Clothianidin, Dinotefuran and Thiacloprid blocks the receptor sites to which ACh attaches. Although they are considered nontoxic to humans gastrointestinal symptoms along with respiratory distress and neuropsychiatric features are still reported following accidental inhalational exposure by human (Kumar *et al.*, 2013).

Some neurotoxic pesticides affect insect nervous systems at a different point by disruption of voltage-gated channels on nerves; hence delay the opening of this channels and eventually results in improper coordination, convulsions of muscle and paralysis. Synthetic pyrethroids act on the voltage-gated sodium channel (Tundis, 2015). On the other hand, Fipronil and Avermectins are relatively new chemical insecticides used to control some crop pests by blocking the GABA-regulated chloride channel of nerve cells thus disrupts normal nerve function (Bedford *et al.*, 1998).

Insect growth regulators (IGRs) pesticides mimic hormones in young insects and hence disrupt how insects grow and reproduce. They attack endocrine system of the insects which produces the hormones needed for growth and development into adult form. The growth regulator effect may be seen in several ways. First are those molecules inhibiting metamorphosis, in other words, compounds preventing metamorphosis from appropriately taking place at the right time. Other IGRs pesticides compounds force the insect to go through an early metamorphosis, so that development takes place at a time not favorable for the insect (Aktar *et al.*, 2009). Lastly, it has been observed that certain molecules may alter hormones related to this function so that insects suffer malformations, are sterile, or die. Insects poisoned with IGRs cannot moult or reproduce and they eventually die since hormones play various roles in moulting (Elsheikha and Khan, 2011). Insect growth regulators (IGRs) pesticides are safe and effective at low concentrations and remain effective for a long time. They have good safety margin to non-target biota and are much less likely to be toxic to mammals (Arjen, 2009).

Some target insect growth and development processes through interfering with hormones and others through blocking the production of a structural component of the exoskeleton (chitin). For instance, Chitin Synthesis Inhibitors (CSI) pesticides block the production of chitin. An insect poisoned with a CSI cannot make chitin and so cannot moult to reach maturity (the adult stage), hence cannot reproduce (Najat, 2015). CSIs have selective insect toxicities and as such are considered “soft insecticides” (Brown, 2006).

IGRs do not kill target insects immediately; do not expect to see dead insects the day after you apply an IGR. Instead, they have a long-term debilitating effect. It may be 2 or 3 weeks before you notice any effect on the target population (Wildboltz, 1988). Other pesticides inhibit metabolic processes of insects by causing mechanical suffocation which affects insect respiratory patterns and metabolic rate. They include soaps and oils pesticides (Toshiyuki and Hitoshi, 2016).

2.3.4.3 Effects of Pesticides on Insect Pests

2.3.4.3.1 Toxicity Effects

Toxicity of a pesticide chemical practically imposes harmful effects that kill or retard the growth of pests. The sub-lethal effects of pesticides on insects like weevils have different possible physiological end points including behavioral and respiratory patterns, metabolic rate, and water loss rate. They can manifest themselves through changes in motor activity, behavior or learning ability, in the case of social insects. The changes in the function of neural or muscular tissues lead to insufficiency in autonomic processes such as thermoregulation (Belzunces *et al.*, 2012), respiration (Kestler, 1991) and water balance (Sláma and Miller 1987).

The most common synthetic insecticides (organophosphorus compounds, pyrethroids, and neonicotinoids), botanical extracts, bacterial toxins, bio-fungicides and inert powders target the nervous system, which controls autonomic functions in an organism (Muljar *et al.*, 2012; Hatjina *et al.*, 2013; Zheng *et al.*, 2013; Kivimagi *et al.*, 2013; Karise *et al.*, 2015). Other toxicants cause an increase in metabolic rate, frequency of the respiratory contraction, frequency of DGE (discontinuous gas exchange) cycles or even replacement of DGE with continuous respiration. These nerve poisons induce rapid or delayed disappearance of DGE rhythms due to inhibition of muscle work at different levels. Losing control over ventilatory muscle work leads to higher water loss and eventual death of the insect (Karise *et al.,* 2015). The point of entry of these toxins into insects is one of the important factors which affect their toxicity effects on insects. Pesticides can be inhaled (fumigants), ingested or absorbed via insect body surface due to the abrasive effect on the pest cuticle as contact toxicants (Regnault-Roger, 1997).

Weevils may encounter insecticides in several ways. Perhaps the most common way of exposure to insecticide is by direct contact. In this case, insecticide residues remain on the surface of the treated grains and weevils encounter the pesticide as they walk across the treated maize grain surfaces. The insecticide, hence, enters the insect body through its feet and then makes its way to the site of action like nerve cells or hormone sites. Again, if the weevils are present at the time of insecticide application, the powder or spray may cover the insect body and eventually penetrate its body directly to the site of action (Chowański *et al.,* 2016).

Fumigants, on the other hand, are pesticides that change into vapor quite readily. They enter the insect's breathing apparatus, the spiracle, and then make their way to the site of action. These kinds of pesticides are particularly useful in enclosed areas where the vapors can remain concentrated, such as in greenhouses or storage bins, but usually do not work well in open landscapes (Karise *et al.,* 2015). In most cases, an insecticide creates a bit of fumigant activity at the time an insect is moving across the treated surface. The insect hence gets a double exposure to the insecticide and thereby experiences both contact and fumigant insecticidal activities. This combined effect of contact and fumigant activities is difficult for the insect pests to overcome (Padin *et al.*, 2013).

In some cases, an insect will feed on treated grain surfaces. The insect ingests the insecticide and absorbs it through the stomach lining. In this case, the insecticide is able to attack the site of action more quickly than when the insect simply walks across the treated surface (contact activity). Although with some exceptions, an ingested insecticide will induce a more severe response than the same amount of insecticide encountered by an insect through direct contact (Padin *et al.*, 2013; Karise *et al.*, 2015; Chowański *et al.*, 2016).

2.3.4.3.2 Repellent Effects

The use of topical repellents provides an effective arthropod control (Kareru *et al.*, 2013). Examples of such repellent materials include, but are not limited to *N*,*N*-diethyl-3-methylbenzamide (DEET), p-menthane-3, 8-diol (PMD), permethrin, allethrin, piperonyl butoxide, lemongrass oil, citronella oil, eucalyptus oil, camphor, geranium oil, ethyl hexanediol, ethyl butylacetylaminopropionate and hydroxyethyl–isobutyl-piperidine. Repellents commonly available to consumers contain the active ingredients DEET, a few repellents contain permethrin, while none or very few repellents contain botanical compounds (New York State Department of Health (2737/04) 2004; Abtew *et al.*, 2015). The use of repellents to prevent insects from infesting food crops and grains is also a very common practice among Kenyan small-scale farmers (Githinji, 2018). Use of plants as repellents has been achieved by plant compounds having odor with irritant effects on insects as is the case for garlic and hot peppers (Ali *et al.,* 2014; Hieu, 2015; Showler, 2017; Parwada *et al.,* 2018). There is need, therefore, to focus research on the development of effective and safe weevil repellents that can be incorporated in the existing Integrated Weevil Management (IWM) for stored grains protection programs (Misra, 2014; Mishra *et al.,* 2018). Commercial repellent products containing botanical oils, such those of geranium, cedar, lemon grass, soy and or citronella have been available (Abtew *et al.,* 2015; Lengai *et al.,* 2019).

2.3.4.3.3 Oviposition Deterrence and F_1 Progeny Emergence Inhibition Effects

Under suitable conditions, the adult female weevils lay their eggs within or on the maize grains, which later develop into new adults, thereby increasing grain infestation and subsequently cause more grain damage. Therefore, when considering long term protection of the maize grains, weevil reproduction control through inhibition of oviposition and eventual F_1 progeny emergence forms the best management strategy (Jovanovi *et al.,* 2007; Ouko *et al.,* 2017). Such effects like oviposition deterrence, increased larval mortality and progeny emergence inhibition enhances the weevil reproduction control. These reproduction control processes do not cause the pest resistance, while their effect is satisfying in the reduction of future populations (Jovanovi *et al.,* 2007). Notably insecticides are usually the most effective against certain stages of the target insect. Typically, these are the small immature stages.

2.3.4.3.4 Anti-feedant Activities

According to Dancewicz *et al.* (2006), a feeding deterrent (anti-feedant) is a compound that once probed by the insect, causes it to stop feeding and therefore starve to death. Anti-feedants are substances that partly or completely inhibit feeding or disrupt insect feeding, making the treated plants unattractive or unpalatable. Antifeedants inhibit insect feeding, through sensory perception, but they do not directly kill insects like the way toxicants do (Li *et al.,* 2005). The anti-feedant effects of a certain treatment can be assessed by evaluating the weight losses caused by insect activities and feeding of insects on grains.

2.3.4.4 Challenges Facing Use of Synthetic Pesticides

Even though this method is effective, the repeated and indiscriminate use of synthetic chemical pesticides has given rise to many well-known and serious concerns. Synthetic chemicals are prone to user abuse, have low shelf life, and farmers generally lack the technical expertise in handling and applying them. Accessibility of synthetic pesticides for pest control remains limited for many farmers due to their cost and restricted distribution networks that lead to the products being frequently adulterated by unscrupulous traders (Giga and Mazarura, 1990).

Most synthetic grain protectants are hardly effective against all the insect species of interest. In most cases, their efficacy varies among species and developmental stages of a species. For example, eggs and pupae of *Sitophilus* species have been established as tolerant (Weller and Morton, 2001; Daglish *et al.,* 2002). However, in the recent past, pharmaceutical companies have increased development of pesticides that target a broad spectrum of pests (Chagnon, 2015). Acording to Luo and Zhang (2011), synthetic pyrethroids have a broad-spectrum of activity but are however becoming increasingly unpopular due to other limitations and bans.

Synthetic pesticides have negative effects on environment. They cause water, soil, and atmospheric pollution as well as intoxication of the fauna and flora. Synthetic insecticides have long-term/ residual activity. Compared to pyrethrins, which are extremely sensitive to high temperatures, light and moisture, the synthetic pyrethroids are stable and persist in the surroundings much longer (Yang, 2012; Chagnon, 2015).

Synthetic pesticides kill even the non-targeted organisms due to the fact that they are majorly unselective hence may cause various biochemical changes even in non-target animals. Over 98% of sprayed insecticides exert their effect on non-targeted species because they spread across the whole agricultural field (George, 2004). That excessive use of insecticides destroys many beneficial insects, birds and small mammals is occasioned by the high mobility of most of these chemicals through runoff and wind which carry and spread them to other locations (Damalas and Eleftherohorinos, 2011).

Synthetic pesticides pose potential severe hazards to human health that were not anticipated at the time of their introduction. The residues of these pesticides sprayed on kernels can persist for long time thus posing more risk to the users (Anjarwalla *et al.*, 2016). A number of chronic respiratory symptoms such as asthma and chronic obstructive pulmonary disease have been commonly observed among people with long-term exposure to these chemicals (Hoppin, 2009). Organophosphates and organochlorine insecticides have also been closely associated with the risk of lung cancer (Ye, 2013).

World Health Organization (WHO) estimates that 200,000 people are killed worldwide every year as a direct result of pesticide poisoning (Belmain *et al.*, 2013). Consumption of synthetic pesticide in Africa accounts for 24% of the global pesticide market of US$31 billion (Sola *et al.,* 2014; Stevenson *et al.,* 2017). Africa has the highest reported human mortality risks related to misuse of pesticides (Williamson *et al*., 2008). More than 20,000 accidental deaths and three million cases of pesticide poisoning are reported in the world every year (Gunnell and Eddleston, 2004).

According to a study by Kahuho (1980) on the patients admitted with chemical poisonings in the intensive care unit (ICU) of Kenyatta National Hospital (KNH), most of the admissions were as a result of exposure to organophosphates (33.3%) of whom 45.9% died. Another study in the same hospital, this time looking at hospital admissions during 2002-2003, identified that pesticide poisoning was the cause of 43% of poisoning admissions, with a predominance of organophosphates and rodenticides (Nyamu *et al.*, 2012).

Some cytogenetic studies revealed the existence of genetic perturbations related to cancer amongst users of these pesticides. Globally, those studies have shown an elevation in the frequency of damage to the DNA (Simoniello *et al.,* 2008), chromosomal deviations such as broken and translocation deviations (Sailaja *et al.*, 2006; Muniz *et al.*, 2008), existence of micronuclei, DNA adducts in peripheral blood lymphocytes and increase in the 8- OH -dG oxidized bases in the plasma (Muniz *et al.*, 2008).

The synthetic pesticides invoke resistance over time. Pests resistance has been found in different insecticides groups for example, 291 species have developed cyclodiene resistance, followed by dichlorodiphenyltrichloroethane (DDT) (263 species), organophosphates (260 species), carbamates (85 species), pyrethroids (48 species), fumigants (12 species), and other (40 species) (Lorini and Galley, 2000). The *S. zeamais* and *S. oryzae* have also been found to be resistant to DDT and phoxim as well as to both Malathion and Pirimiphos-methyl and to pyrethroids (Lorini and Galley, 2001).

In short, global ecology is facing severe threat from the use of pesticides so the search for ecologically safe methods to control insect pest of food products is an awe-inspiring field of research. To achieve sound management of stored cereals there is an increasing interest in biological control, which will prove eco-friendly with highly reduced negative effects on environment (Arbogast 1984, Guedes 1990, Brower *et al*. 1996).

Intrestingly, although the problems concerning the pest resistance and a healthy environment are in the focus of public interest in the recent days, the protection of stored grain using the conventional insecticides is still the prevailing method of insect control (Subramanyam and Hagstrum, 1995; Benelli *et al.*, 2018). Nonetheless, due to these and many concerns, there is an increased interest in alternatives to chemical insecticides.

Problems with synthetic pesticides have resuscitated the idea of botanical insecticide as a promising alternative to pest control. There is an interest in finding alternative ways for stored products protection, especially within the scope of organic, biological and natural products that are popularly used for same purpose in the ethno-tradition. This has therefore led to more focused research and development of pesticidal plants (Belmain *et al.*, 2001; Anjarwalla *et al.,* 2016).

2.3.5 Biological Pests Control

Biological control is the use of natural enemies to manage crop pest populations. Organisms employed are the natural enemies of the pest species; or individuals of the pest species, modified such that they destroy members of their own species (Kamali *et al.*, 2013). The major areas of application of natural enemies are in the suppression of both Coleopteran and Lepidopteran insects in stores (Anjarwalla *et al.,* 2016). Most biological agents used against these insect pests include parasitoids, predators and pathogens. Biological pests control is the beneficial action of predators, parasites, pathogens, and competitors in controlling pests and their damage (Dreistadt, 2007).

Biological pests' control was started by International Centre of Insect Physiology and Ecology (ICIPE) in an attempt to reduce maize pests in production. The larval parasitoid Cotesia flavines was introduced from Asia into Kenya and was released at the coast in 1993 to control the invasive exotic maize stem borer *C. partellus* (Swinhoe) (Sanda *et al.*, 2014). *Metahrizium anisopliae* and Enthomopathogenic Nematode (EPN) as biological control were also reported to be effective against termites (Abonyo *et al.,* 2019).

Examples of different parasites used to control stored grain pest are indicated on Appendix 1.5.

Unlike synthetic chemical method, biological pest control is safe, species specific and has a long-term action on specific target pests only (Nafiu and Mustapha, 2014). Consequently, the interest in biological control has increased over the recent past with farmers as well as consumers increasing concern and demand for products that are grown in a sustainable manner and free of insecticide residue. However, there are also a number of shortcomings associated with the use of these macro organisms as control agents. Since they rarely eliminate the pest, it may be more expensive than available chemical control measures. They are also not suitable when dealing with heavy infestations and macro organisms have limited “shelf life” (Scholler, 2010).

2.3.6 Bio-Technological Pest Control and Use of Resistant Varieties

Choosing resistant or tolerant varieties as a pest preventive measure is enough to keep the pest population below the control threshold (Barzman *et al.,* 2015). Resistance to pests occurs in three different forms; chemical, morphological and nutritional. Chemical resistance occurs when the plant develops substances which attract (attractants) or repel pests (repellants) (Stankovic *et al.,* 2020).

Morphological resistance is whereby plants may develop some structural features which favour the crop but impede the development of a pest (Bradshaw and Holzapfel, 2011). Such characteristics include: the plant's leaf size, shape and colour; the presence or absence of secretory glands; toughness of the tissue or coat of plant/seed and the presence of epidermal hairs which impair the mobility of insects (Stein, 1991; Lamichhane *et al.,* 2015).

Nutritional resistance occurs when there is the combination of nutrients within a plant genotype, which can, in turn, make a plant appear suitable for a pest as a source of food or as a place to lay eggs (Stankovic *et al.,* 2020). Bergvinson (2001) reported that there were strong correlations between the insect resistance, kernel hardness and elevated levels of diphenolic acids located within the pericarp of the kernel (Garcia-Lara *et al.,* 2004).

Tripsacorn, hybrid maize developed from a perennial teosinte, *Zea diploperennis* L. and eastern gamagrass, *Tripsacum dactyloides* L., may have resistance to storage insect pests that could be incorporated into commercial maize hybrids (Thronea and Eubanks, 2002). Whole Tripsacorn grains are not attacked by *S. zeamais*. The grains are difficult to grind because of the hardness of the fruit case, and the inability of the weevil to lay eggs is also attributed to this same factor. There is also the possibility that the fruit case of the maize contains a repellent that deters oviposition (Thronea and Eubanks, 2002).

Chicken avidin has been known to possess insecticidal property causing mortality in many species of stored-product insects by preventing the absorption of dietary biotin (Midega *et al.,* 2016). The avidin gene has been incorporated into maize plants and avidin maize grains are resistant to insects, especially when the grains are ground into a meal or powder. When avidin content in transgenic maize grains reached about 100 ppm or higher, it inhibited the development of almost all insect pests that damage grain during storage, including the maize weevil, *S. zeamais* (Midega *et al.*, 2016).

Bacillus thuringiensis is known as the most successful microbial insecticide against different insect pests in agriculture. It is against this background that *Bt* toxin genes also have been efficiently used to enhance resistance to insect pests in genetically modified (GM) crops (Jouzani *et al.*, 2017). According to Ivbiljaro (2009) resistant maize cultivars can reduce losses due to weevil infestation but no maize grain was immune to attack by the weevil. The use of resistant varieties alone may not provide a permanent solution to the problems of maize storage but rather may contribute to integrated pest management (Credland *et al.*, 2003; Gerema *et al.*, 2017; Khakata *et al.*, 2018).

2.3.6 Use of Botanical Pesticides

Mankind has used plant parts or extracts to control insects since ancient times. Botanical products have played important roles in traditional storage pest control in the tropics (Hassanali *et al.*, 2008; Belmain *et al.*, 2013). Several local communities in tropical regions have used local flora as a means of protecting stored grains against insect pests (Owusu, 2000; Stathers *et al.*, 2008).

Plant derived products have received increased attention from scientists and more than 2000 plant species are already known to have insecticidal properties (Sukamar *et al.*, 1991). Furthermore, natural insecticides such as pyrethrum, nicotine and rotenone, among others, have been extensively used for insect control (Adeniyi *et al.*, 2012).

2.3.6.1 Challenges Facing Adoption of Botanical Pesticides

Regulatory barriers are among the many challenges that appear to limit the success of botanicals as alternative pest control. There are no legal registrations establishing their use (Anjarwalla *et al*., 2013; 2015). It has been claimed that most of these products are not truly insecticides since they are merely insect deterrents and their effectiveness against pests is slow with rapid degradation by UV light so that their residual action is short (Anjarwalla *et al*., 2015; 2016). Botanicals are not necessarily available season long. Most of them have no established residue tolerances. Not all recommendations followed by growers have been scientifically verified (Isman, 2006; 2011). Even though plant origin insecticides are superbly advantageous option from an ecological point of view, it would be unreasonable to think they will completely replace synthetic insecticides (Anjarwalla *et al.*, 2015). On the contrary, it will be quite logical to expect a complementary use and the coexistence of the two kinds of compounds. This happens today with pyrethrum and synthetic pyrethroids in some IPM programs (Sola *et al.*, 2014).

2.3.6.2 Classification of Botanical Pesticides

2.4.1 First Generation Botanical Pesticides

2.4.1.1 Nicotine

Nicotine is an alkaloid obtained from the foliage of tobacco plants (*Nicotiana tabacum*) and related species; it has a long history as an insecticide. Nicotine and two closely related alkaloids, nornicotine and anabasine, are synaptic poisons that mimic the neurotransmitter acetylcholine. As such, they cause symptoms of poisoning similar to those seen with organophosphate and carbamate insecticides (Regnault-Roger *et al.*, 2008). In insects, the action of nicotine is selective, and only certain types of insects are affected (Nabil, 2012; Moghbel *et al.*, 2017).

2.4.1.2 Rotenone

Rotenone is one of several isoflavonoids produced in the roots or rhizomes of the tropical legumes *Derris*, *Lonchocarpus*, and *Tephrosia* (Showler, 2017). Most rotenone used at present comes from *Lonchocarpus* grown in Venezuela and Peru and is often called cubè root. Extraction of the root with organic solvents yields resins containing as much as 45 % total rotenoids; studies indicate that the major constituents are rotenone (44%) and deguelin (22%) (Ling, 2003). Rotenone is extremely toxic to fish and is often used as a fish poison in water management programs (Ling, 2003; Cabizza *et al.*, 2004; Isman, 2006).

2.4.1.3 Sabadilla

Sabadilla is a botanical pesticide obtained from the seeds of the South American lily *Schoenocaulon offiinale*. In purity, the active principles, cevadine-type alkaloids, are extremely toxic to mammals, but commercial preparations typically contain less than 1% active ingredient, providing a margin of safety. These alkaloids are remarkably similar to that of the pyrethrins, despite their lack of structural similarity. Sabadilla kills insects of some species immediately, while others may survive in a state of paralysis for several days before dying (Isman, 2006).

2.4.1.4 Pyrethrum

Pyrethrum is the powdered, dried flower head of the pyrethrum daisy, *Chrysanthemum cinerariaefolium* (Asteraceae). The term “pyrethrum” is the name for crude flower dust itself, and the term “pyrethrins” refers to the six related insecticidal compounds that occur naturally in the crude material, the pyrethrum flowers. The flowers are ground to a powder and then extracted with hexane or a similar nonpolar solvent, removal of the solvent yields an orange-colored liquid that contains the active principles (Casida and Quistad, 1995; Zhang *et al.,* 2018). The mechanism of action of pyrethrins is qualitatively like that of DDT and many synthetic organochlorine pesticides. In purity, pyrethrins are moderately toxic to mammals, but technical grade pyrethrum is considerably less toxic (Kostyukovsky *et al.*, 2002; Zhang *et al.*, 2018).

2.4.1.5 Plants Essential Oils

Steam distillation of aromatic plants yields essential oils, has for long been used as fragrances and flavorings in the perfume and food industries, respectively, and more recently for aroma therapy and as herbal medicines (Buckle, 2003; Abdallah *et al.*, 2004). The rapid action against some pests by plant essential oils is an indication of a neurotoxic mode of action, and there is evidence for interference with the neuromodulator octopamine by some oils and with GABA-gated chloride channels by others (Jankowska, *et al.*, 2018).

2.4.2 Second Generation Botanical Pesticides

2.4.2.1 Neem Products (Azadrachtin)

Botanical pesticides can be obtained from seeds of the neem tree, *Azadirachta indica* (Meliaceae). Neem oil, which is obtained by cold-pressing seeds, can be effective against soft-bodied insects and mites but is also useful in the management of phyto-pathogens (Dimetry, 2012). Azadirachtin has two profound effects on insects. Azadirachtin blocks the synthesis and release of moulting hormones leading to incomplete ecdysis in immature insects. In adult female insects, a similar mechanism of action leads to sterility (Nabil, 2013). In addition, azadirachtin is a potent antifeedant to many insects. Neem has been effectively used to control stem borers including *C. partellus* and termites (Shiberu *et al.*, 2014).

2.4.2.2 Melia Extracts

The remarkable bioactivity of azadirachtin from the Indian neem tree (*Azadirachta indica*) led to the search for natural pesticides in the most closely related genus, *Melia* (Karar *et al.*, 2017). Seeds from the chinaberry tree, *Melia azedarach*, contain several triterpenoids, the melia carpins that are similar but not identical to the azadirachtins, and these also have insect growth regulating bioactivities (Muturi *et al.*, 2018; Sarma *et al.*, 2019).

2.4.2.3 Synthetic Pyrethroids

The earliest synthetic pyrethroids retained most of the beneficial properties. Synthetic pyrethroids are generally recognized as neurotoxicants that act directly on excitable membranes. These compounds induce intense repetitive activity in sense organs and in myelinated nerve fibers. Pyrethroids are also known to cause prolongation of the sodium current together with repetitive activity in nerve fibers of invertebrates (Henk and Bercken, 1990).

2.5 Plant Species Under Study

2.5.1 *Tithonia diversifolia* (Hemsl.) A. Gray

2.5.1.1 Description and Geographical Distribution

Tithonia diversifolia (Hemsley) A. Gray is an impressive herbaceous flowering plant in the sunflower family, Asteraceae. Its scientific Synonym is *Mirasolia diversifolia* (Hemsley) A. Gray (Muoghalu, 2008; Obiakara and Fourcade, 2018). Its preferred common name is Tithonia with Mexican sunflower; tree marigold (English) and Guasmara; Jalacate (Spanish) as its international common names. Its other vernacular/common names in Kenya include Amaua maroro (Kisii), Maruru (Kikuyu), Ilaa (Kamba), Maua makech, akech or Maua madungo (Luo) and Maua amalulu (Luhya) (Chukwuka *et al.,* 2007; CABI, 2017).

It is native to Mexico, Central America, Cuba and the West Indies, although it has been introduced and now naturalized in tropical parts of Asia and Africa (Robert *et al.,* 2017). It is a common shrub on field boundaries, grasslands and disturbed lands in East Africa. In Kenya it was introduced from Central America as an ornamental plant, it is now found in Western and Central provinces, in coastal regions and parts of Rift Valley. It grows in regions of between 550 and 1950 m altitude and with mean annual temperature of between 15 and 31° C and mean annual rainfall of between 100 and 2000 mm (Chukwuka *et al.*, 2007; Wen, 2015).

Tithonia diversifolia is a woody herb or succulent shrub, between 1.2 and 3 m tall. Opposite leaves (between 3 and 5), attenuate base, acute apex, crenate margin. Leaf size is from 5 to 17 x 5 to 12 cm, densely pubescent beneath, palmate venation. Occasionally upper leaves are unloaded (Chukwuka *et al.*, 2007; Muoghalu, 2008).

Flowers are yellow in color; their ray size is 306 cm x 5 to 18 mm. The flower heads are solitary on a peduncle from 6 to 13 cm long. Each mature stem may bear several flowers at the top of branches. *Tithonia diversifolia* flowers and produces seeds throughout the year and at different times in its separate habitats and mainly during the rainy seasons in east Africa. *Tithonia diversifolia* is a prolific seeder which retains its seeds until the plant dries in the dry season when the seeds disperse and are spread by wind, water and the movement of people, livestock and vehicles. The plant flowers and produces seeds throughout the year (Chukwuka *et al.*, 2007; Wen, 2015; Obiakara and Fourcade, 2018).

The ‘seeds’ (achenes) are from 4 to 8 mm long and topped with a ring (pappus) of scales and two awns (5 mm long). These ‘seeds’ are covered in close-lying hairs (they are appressed pubescent), blackish in colour, and are somewhat four-angled. The lightweight seeds can be dispersed by wind, water and animals (Muoghalu, 2008).

Figure 2.4: A photomicrograph of Aerial Part of a Mature Flowering *T. diversifolia* Plant taken on July 2017 at Siakago Sub County, Embu County, Kenya

Tithonia diversifolia can be propagated from seeds and cuttings. Seeds germinate readily when sown fresh, without treatment, all year round. Seeds are sprinkled directly on soil or only just cover with soil. Stem cuttings of between 20 and 40 cm length establish readily, regardless of the angle at which they are inserted into the soil. Cuttings buried horizontally in the soil will sprout, but they are less effective than cuttings inserted either upright or at an angle into soil. The cuttings should be planted into moist soil immediately after collection and not allowed to sun dry (Muoghalu, 2008; CABI, 2017).

2.5.1.2 Cultural Uses

In the traditional methods of treatment, infusion of *T. diversifolia* is suitable for constipation, stomach pains, indigestion, sore throat, liver pains and to treat malaria. The extracts *T. diversifolia* are traditionally used for the treatment of diabetes, diarrhea, menstrual pain, malaria, hematomas, hepatitis, hepatomas and wound healing (Rungeler *et al.,* 1998; Duarte and Empinotti, 2012).

In the 1970s, farmers grew more *T. diversifolia* as green manure but abandoned it with the emergence of chemical fertilizers. However, farmers still use its leaves, soft branches and flowers as a fodder crop believed to have high nutritive-quality index. It is also traditionally used as a source of fuel by providing firewood to farmers (Tona *et al.,* 2000).

In Uganda and some parts of Kenya farmers use it both in pre and postharvest pest management although there is no published work to report evidence for these effects. Water leaf powder extract was used against broad range of field crop insects while the whole leaves were traditionally layered in stored cobs against weevil in stored maize (Bitam *et al.,* 2010). In Ikolomani Division of Kakamega County, Kenya herbalists use boiled *T. diversifolia* concoction to clean jigger infested areas of human body (Shisanya, 2011; Githinji *et al.,* 2018). The residents of Kandara Sub-County, Murang’a County also consider using the plant concoction to control fleas and jigger infestation (Mwangi *et al.,* 2015; Zabron, 2017).

2.5.1.3 Medicinal Uses

A number of scientific studies has investigated and reported activities of *T. diversifolia* leaves as antifungal, anti-inflammatory, analgesic, antimalarial, antiviral, antidiabetic, antidiarrhoeal, antimicrobial, antispasmodic, vasorelaxant and cancer-chemopreventive and antibacterial activities (Njoroge and Bussmann, 2006; Linthoingambi and Mutum, 2013).

In an investigation of the ethanolic extracts of the aerial part of *T. diversifolia* and the stem bark of *Crossopteryx febrifuga* against early, residual (repository) and established malaria infections *in vivo*, *T. diversifolia* was found to possess anti-malarial properties in Swiss albino mice at a dose range of 50 and 400 mg/kg per day (Elufioye and Agbedahunsi, 2004).

According to a study conducted by Githinji *et al.* (2018) the aqueous flowers and leaf extracts of *T. diversifolia* exhibited 93% *in vitro* antiflea activity. According to Adedire and Akinneye (2004) the powder and ethanol extract of *T. diversifolia* leaves were tested for their efficacy at five different concentrations (0.0, 0.5, 1.0, 1.5 and 2.0%) on bruchid mortality, oviposition and adult emergence of cowpea seed beetle *Callosobruchus maculatus* at ambient tropical storage conditions of temperature and relative humidity. Results showed that the leaf extract of *Tithonia diversifolia* have a high bioactivity on oviposition, adult emergence and mortality of *C. maculatus*. The results of this study revealed *T. diversifolia* as a potential candidate for bioinsecticide preparations because of antiovipositional, ovicidal and knockdown properties of its products, which have some volatile components (Adedire and Akinneye, 2004; Githinji *et al.*, 2020).

2.5.2 *Vernonia lasiopus* (O. Hoffman)

2.5.2.1 Description and Geographical Distribution

Vernonia lasiopus is an erect sparsely branched sub-shrub that belongs to the sunflower family, Asteraceae and grows up to a height of three meters. It is an angiosperm, of the order Asterales, of the family Asteraceae, genus *Vernonia* and species *V. amygdalina.* The full binomial name is *Vernonia lasiopus* (O. Hoffman) (Dharani and Yenesew, 2010; Mercader *et al.*, 2018).

Vernonia lasiopus is commonly known as vernonia. In the African local communities, it is known by different common names including; muata (Meru), muhasha (Swahili), muvatha (Kamba), Mucatha (Kikuyu), Olusia (Luo), Ol-euguru (Maasai) and Nkaputi (Samburu) (Kokwaro, 2009).

This plant is a shrub that grows up to 3 meters high mainly in the African tropics. The stem is woody at the base and reaches three centimeters in diameter. The bark is greyish brown and smooth. It has oval-shaped and densely hairy leaves. Flowers are pale mauve or white, in heads, flat or slightly rounded, 5 to 10 mm across (Figure 2.5). The plant is found in disturbed areas, bush land, grassland and riverine woodland or forest, growing at an altitude between 1,000 and 2,500 m (Wanzala *et al.*, 2017)

Figure 2.5: A photomicrograph of Aerial Part of a Mature Flowering *V. lasiopu*s Plant taken on July 2017 at Siakago Sub-County, Embu County, Kenya

2.5.2.2 Cultural Uses

Aliyu *et al.* (2008) documented that the leaves of *V. lasiopus* are culturally used as a valuable source of food and medicine for the prevention of illness and maintenance of human health. The plant is locally reputed to have several health benefits. Traditionally an infusion of its powdered leaves is used to cure indigestion, severe stomach-ache, malaria and as a purgative. A root decoction of *V. lasiopus* is said to be one of the most effective treatments for stomachache (Dharani and Yenesew, 2010).

Further, *V. lasiopus* is also traditionally used for the treatment of other varied diseases among communities in Kenya. In Western Kenya (Bungoma), farmers apply the whole plant on the animal’s body to control livestock ticks (Wanzala *et al.,* 2012). Leaves and stem decoction of *V. lasiopus* are also used traditionally to treat worms and nonbacterial infections by some Kenyan communities (Kareru *et al.,* 2008; Wanzala *et al.,* 2012).

According to Muregi *et al.* (2007), *V. lasiopus* among other herbal plants have been used traditionally for many years as a remedy against malaria symptoms among other medical conditions by Kikuyu people of central Kenya (Kokwaro, 2009). Tanzanians also prepare a bitter decoction from the whole plant and used in treatment of epilepsy, indigestion and in childbirth, root extracts are used to facilitate parturition. Leaf infusion is used as an antihelmintic, leaves are used as purgative, root decoction is used as aphrodisiac for human males and leaf decoction is used in treatment of epilepsy and abdominal disorders (Muregi *et al.,* 2007; Kokwaro, 2009).

The Kamba people of eastern Kenya use it against scabies, the Luo of western Kenya used it leaves against venereal diseases, while pounded leaves are applied to sores by Maasai of southern Kenya (Erasto and Grierson, 2001; Kareru *et al.,* 2008). Kenyan farmers traditionally use it in postharvest weevil management where maize grains are stored in a sack which is then put in another sisal bag which had been soaked with *V.*

lasiopus extract. However, there is no published work to report evidence of this described effect (Kokwaro, 2009). This study was therefore scientifically crucial because it sought to validate the described effects of *V. lasiopus* against maize grains.

2.5.2.3 Medicinal Uses

The organic fraction extracts of the *V. lasiopus* were shown to possess sedative, analgesic (leave and seeds), anti-ulcerogenic (leaves and seeds) and membrane stabilizing activity (leaves and roots) shown by reduced red blood cells (RBC) lies (Erasto and Grierson, 2001). Elemanolides, epivernodalol and lasiopulide chemical compounds, isolated after chromatographic separation of the alcoholic extract of the dried aerial parts of the *V. lasiopus* showed scientific *in vitro* cytotoxicity against human cancer cell lines in culture. Sesquiterpene lactones vernodalol and demethyl acroylated vernodalol, which have been found present in other species of *Vernonia*, such as *V. amygdalina* (Koul *et al.,* 2003; Dharani and Yenesew, 2010). In their study, Muriithi *et al.* (2015) scientifically confirmed and supported the traditional use of leaves of *V. lasiopus* in enhancing hematological parameters towards boosting and improving health (Muriithi *et al.*, 2015).

The methanolic leaf extracts of *V. lasiopus* plant materials have showed antifungal activity against clinical isolate of *Candida albicans* (Lachuonyo *et al.,* 2016). In the study, extracts from *V. lasiopus* (O. Hoffman) proved that the plant could be used as an antifungal agent against fungal infections and promised to be a potent raw material for manufacturing of conventionally used antifungal agents (Lachuonyo *et al.,* 2016).

The plant was found to possess larvicidal activity against *A. gambiae.* The methanolic extract of *V. lasiopus* has been found to have significant antimalarial activity *in vivo* tests (Muregi *et al.,* 2007). The organic extracts of leaves of *V. lasiopus* show significant antimalarial activity (Erasto and Grierson, 2001). In another study by Irungu *et al.* (2007), *V. lasiopus* showed high antiplasmodial activity ranging between 1.4 and 35 µg/ml. These studies recommend that *V. lasiopus* warrants further evaluation in the search for novel antimalarial agents against drug resistant malaria (Irungu *et al.,* 2007).

The organic fraction extracts of the plant were shown to possess sedative, analgesic (leave and seeds), anti ulcerogenic (leaves and seeds) and membrane stabilizing activity (leaves and roots) shown by reduced RBC lies (Erasto and Grierson, 2001). Research work has shown that the roots and leaves of *V. lasiopus* contain active insecticidal compounds including steroids, flavonoids, terpenoids, cardiac glycosides, saponins and tannins (Muregi *et al.*, 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant Sample Collection

Fresh leaves of *T. diversifolia* and *V. lasiopus* were collected from Makunguru village, Siakago sub-location, Nthawa location, Mbeere North Sub-county of Embu County, Kenya (Figure 3.1). The GPS coordinates of the exact collection sites are Latitude: 0^{0}35’39’’S and Longitude: 37^{0}38’10’’E (for *T. diversifolia*) and Latitude: 0^{0}35’39.51’’S and Longitude: 37^{0}38’23.62’’E (for *V. lasiopus*) (Table 3.1). The plants were identified with the help of local herbalists. The folklore information obtained included the local name of the plants, part of plant harvested, season of harvesting, method of preparation and other medicinal importance of the plants.

The plant samples were sorted out, cleaned and transported to Kenyatta University, in the Biochemistry, Microbiology and Biotechnology (BMB) departmental laboratories (Latitude: 1°10'50.0"S, Longitude: 36°55'41.0"E) where this study was undertaken. The plant samples were identified by a Taxonomist for botanical authentication and voucher specimens deposited in the Pharmacy and Complementary or Alternative Medicine research herbaria at of Kenyatta University. The specimens were assigned voucher numbers as SMG-V1/17 and SMG-V2/17 for *T. diversifolia* and *V. lasiopus,* respectively.

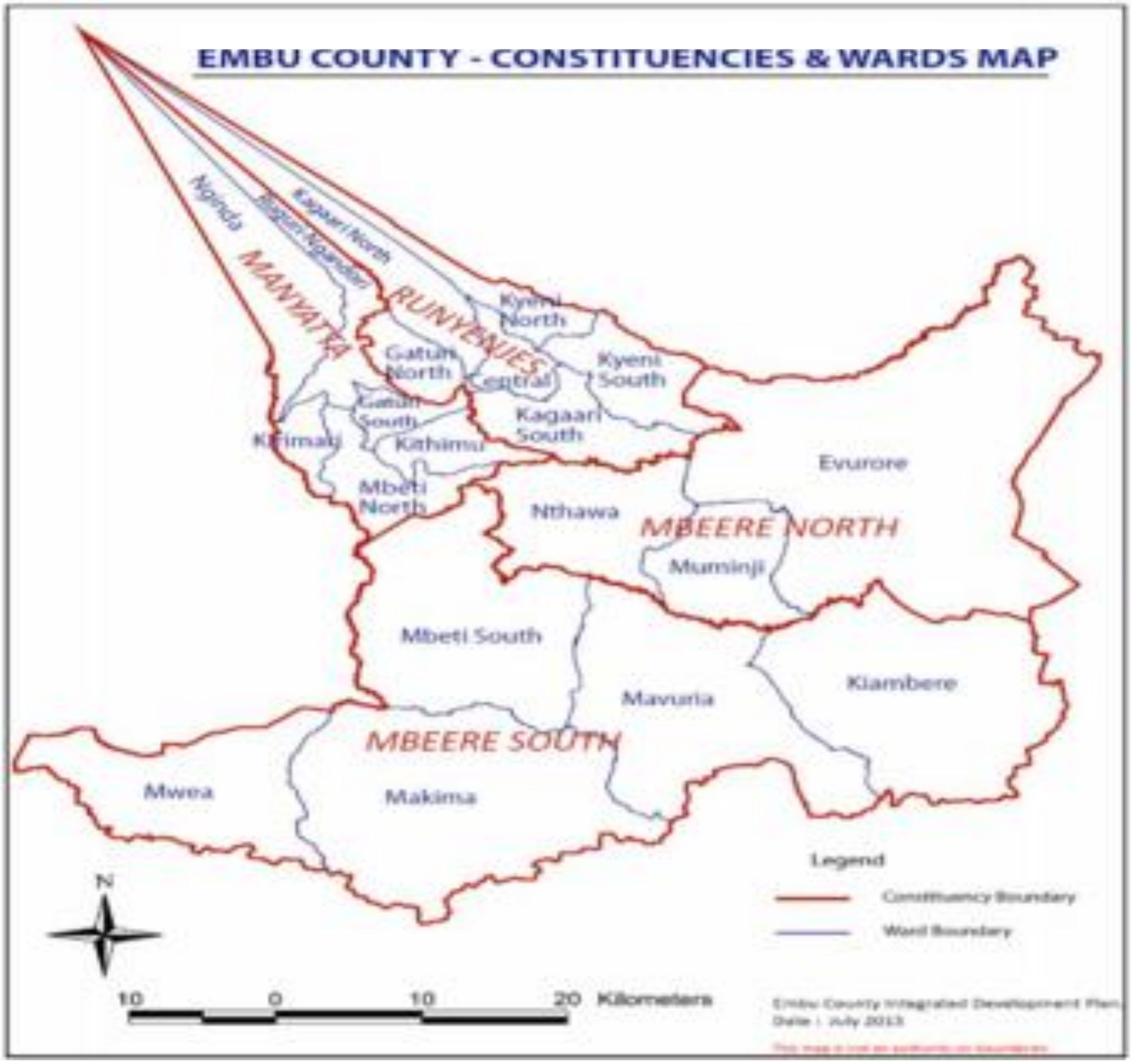

Figure 3.1: **Map Showing Nthawa Location, Mbeere North Sub-County, Embu County, Kenya**

Table 3.1: **GPS Coordinates of the Site of Collection of *T. diversifolia* and *V. lasiopus* Plant Samples**

Plant Species	UTM Eastings	UTM Northings	Latitude DMS	Longitude DMS
T. diversifolia	348,632.42	9936,033.76	0^{0}35’39’’S	37^{0}38’10’’E
V. lasiopus	348,899.23	9936,145.99	0^{0}35’39.51’’S	37^{0}38’23.62’’E

UTM =Universal Trasverse Mercator; DMS= Degrees Minutes Seconds

3.2 Sample Preparation and Extraction

The leaves of *T. diversifolia* and *V. lasiopus* were air dried separately under shade and room temperature for a period of two weeks. They were then ground into fine powder using a grinding electric mill and sieved using a 300 µm mesh. The powder was used for organic extraction by following the guideline used by Singh *et al.* (2005). Extraction with the two solvents (DCM and EtOAc) with different polarity was done to ensure maximum extraction of a wide polarity of compounds, because extraction of phytochemicals depends on the polarity of extracting solvent and polarity of the chemical being extracted (King, 2006; Tiwari *et al.*, 2011).

A weight of 200 g of each plant powder was soaked in 200 ml of the respective solvents (DCM and EtOAc) for 2 hours. The extracts were decanted and 200 ml of the respective solvents (DCM and EtOAc) added and set for 24 hours. After 24 hours filtration was done again, 200 ml solvent added for the final extraction until 48 hours when the last filtrate was obtained. Occasional swirling was done at an interval of 30 minutes to ensure thorough extraction. Aluminium foil and cotton wool was always used to cover the flasks to prevent escape of solvents.

Muslin cloth and Whatman No. 1 paper was used for the filtrations of the extracts. The extract filtrates were then concentrated in vacuum using Heidolph rotary evaporator at the vapourization temperature of 39.6 and 77.11 ^{0}C DCM and EtOAc, respectively and solvent was recovered. The concentrates were further allowed to dry to remove traces of the solvent into a semi solid state. All the extracts were later kept in labelled sample bottles and refrigerated awaiting use in quantitative phytochemical screening and other subsequent experiments undertaken in this study.

3.3 Determination of Percentage Plant Extract Yields

The percent yields of the DCM and EtOAc leaf extracts of the two plants were determined using the formula shown below.

$$\%\ Extract\ Yield = \frac{mass\ of\ extract\ obtained\ (g)}{mass\ of\ sample\ (g)}\ x100$$

3.4 Determination of Quantitative Phytochemical Composition

Sample for phytochemical analysis was prepared as follows. A 1 mg weight of the dried crude organic leaf extracts of *T. diversifolia* and *V. lasiopus* was dissolved in 1 ml of the respective extraction solvent (Sigma Aldrich gc-grade). The sample were vortexed for 30 s and sonicated in an ultra-bath for 15 min before being centrifuged at 14,000 rpm for 5 min. The supernatant obtained were passed through anhydrous Na_2SO_4 to remove moisture. The resultant stock solution (1 mg/ml) was used to prepare experimental sampls whose final concentrations were 100 ng/µl. The plant samples were prepared in triplicates.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the DCM and EtOAc leaf extracts of *T. diversifolia* and *V. lasiopus* was performed using the procedure previously used by Dar *et al.* (2012). Analysis of sample was carried out using GC-MS (7890/5975 Agilent Technologies, Inc., Beijing, China) consisting of a gas-chromatograph interfaced to a mass spectrometer.

The GC-MS was equipped with a HP-5 MS (5% phenyl methyl siloxane) low bleed capillary column of 30 mm length, 0.25 mm diameter and 0.25 µm film thicknesses. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. The carrier gas used was helium (99.99%) at a constant flow rate of 1.25 ml/min in split mode. The injector and mass transfer line temperature were set at 250 and 200°C respectively, and an injection volume of 1 µl was employed.

The oven temperature was programmed from 35°C for 5minutes, with an increase of 10°C/min to 28°C for 10.5 minutes, then 50°C/min to 285°C for 29.9 minutes with a run time of 70 minutes. The MS operating parameters were: Ionization energy; 70eV; Ion source temperature; 230°C, Solvent cut time; 3.3 min, Scan speed 1666 µ/sec; Scan ranged between 40 and 550 m/z and the interface temperature was 250°C.

Interpretation of mass-spectrum from GC-MS analysis was performed using the central database of the National Institute Standard and Technology (NIST) which contains more than 62,000 patterns. Therefore, as for the unknown components, their spectrum were compared with those which are known from the NIST library (Dar *et al.,* 2012). Identity of phytochemicals was proposed based on their general fragmentation pattern and using reference spectra published by library-MS databases (National Institute of Standards and Technology (NIST); 05, 08) together with Adams and Chem ecol-L mass spectral databases. The retention time indices were determined using hydrocarbons range of between C5 and C32.

The identity of the spectra above 60% of the library match was required for the identification of phytochemicals. The relative amounts of each component were expressed as percentage with peak-area normalization and compound concentration (ng/mg) was also calculated. The phytochemical compound name, molecular weight, chemical class and structure of the components of the plant extracts were also ascertained.

3.5 Preparation of Maize Grains

To eliminate the effect of varietal resistance to *S. zeamais* infestation, a known susceptible maize variety (SC Punda milia 53- SC513) was obtained as a test variety for use in this study (Adedire *et al.,* 2011). The experimental grains were cleaned and standardized using the method of Sulherie *et al.* (2003). The damaged kernels were sorted out and the clean ones put in a deep freezer at -20^{0}C for three days to eliminate any eggs, larvae, pupae, or adult weevils.

The dead weevils were sieved out and grains aired for 72 hours prior to use. This acclimatization of the maize grains stabilized moisture content at between 12 and 13%, thereby ensuring its suitability for the feeding by the weevils (Abebe *et al.,* 2009; Adeyemi and Osipitan, 2014). Cracked grains were avoided because they attract female weevils during oviposition (Walgenbach *et al.,* 1987).

3.6 Mass Rearing and Sexing of Weevils

A stock culture of *S. zeamais* was initiated by collecting adult weevils from the infested maize grains at Marigiti-market in Maragua town, Kenya and cultured in their food media of susceptible whole maize grains. Two hundred pairs of weevils were introduced into each of the five, two litre containers, with 500 g of maize. The insects could oviposit for seven days after which they were sieved out using medium size U.S standard mesh size of 4.75 mm and subsequently used for the bioassay experiments. The insect stock culture was further maintained in glass bottles of two litre capacity containing the maize grains throughout the test period.

The weevils were reared subsequently by replacing devoured and infested grains with fresh, clean, uninfected maize grains in containers covered with muslin cloth to allow for air circulation and prevent escape of insects. The muslin cloths covering the containers were held in place with rubber bands. The maize dust was periodically sieved to prevent the growth of mould, which may lead to the caking of grains and ultimate death of the insects. *Sitophilus zeamais* breeding and the experiments were conducted at ambient temperature of 27 ± 2^{o}C, relative humidity of 75±5.5% and suitable photoperiod (LD 12:12). The prepared culture was maintained and used throughout the period of this study.

The weevils were sexed morphologically under dissecting microscope using the methods of Ojo and Owoloye, 2012 by examining the rostrum and abdominal shape of the insects. Male *S. zeamais* were identified with rough, distinctly shorter and wider rostrum while the female was identified with smooth, shiny, distinctly longer and narrower rostrum than that of the male. The male and female weevils were hence separated into different insect stock jars.

3.7 Preparation of Extract Concentrations for Bioassay Studies

Four concentrations of the extracts were used for determination of fumigant and contact toxicity, repellency, oviposition deterrence and F_1 progeny emergence inhibition as well as antifeedant activities. The concentrations were: 25% (v/v), 50% (v/v), 75% (v/v) and 100% (v/v). These extract concentrations were prepared as follows; The extract concentrates were diluted with respective solvents at a concentration of 1 gml^{-1} and this was termed as stock solution (100% w/v concentration) as described by Deshmukh and Borie (1975) with limited modifications. The 25% (v/v) concentration was prepared by diluting 1ml of the stock solution with 3 ml of solvent to make up 4 ml. The 50% (v/v) concentration was prepared by diluting 2 ml of stock solution with 2 ml of the solvent to make up 4 ml while for the 75% (v/v) concentration, 1ml of the solvent was added to 3 ml of stock solution to make up 4 ml.

For the determination of the antiacetylcholinesterase activities, both *T. diversifolia* and *V. lasiopus* fresh leaf extracts were weighed and diluted (w/v) with respective solvents; dichloromethane and ethyl-acetate into six concentrations of 2.5, 5.0, 7.5, 25, 50 and 75 mg/ml. All the different plant extract concentrations were kept in an airtight well labeled sample bottles and stored at -4^0C before use in bioassay studies.

3.8 Determination of Fumigant and Contact Toxicity Effects

These tests were carried out using a randomized controlled study design as described by (Don-Pedro, 1996) with slight modifications. Fumigant toxicity was determined using the closed container method. One milliliter of extract was uniformly applied on 6 cm Whatmann No. 1 filter papers using a micropipette. Twenty adult male *S. zeamais* were introduced into each of the plastic vials and then covered with lid.

The impregnated filter papers were quickly hung with a thread in the vials. The vials were then sealed airtight to ensure fumigation in the vials. In the negative control group, filter papers were impregnated with one milliliter of respective solvent only. For the positive control group, the filter papers were treated with a conventional standard pesticide (Actellic Super ^{TM}) at a recommended dose of 50 g/90 kg. The experiments were set up into six independent treatment groups (as shown in Table 3.2) with four replicates per treatment group.

Table 3.2: Treatment Protocol for Determination of Fumigant Toxicity Effects of Selected Organic Leaf Extracts of *T. diversifolia* and *V. lasiopus* on *S. zeamais*

Group	**Treatment**
I (Negative Control)	Solvent Only
II (Positive Control)	Actellic Super ^{TM}
III (Experimental Group A)	25% v/v) Plant Leaf Extract
IV (Experimental Group B)	50% (v/v) Plant Leaf Extract
V (Experimental Group C)	75% (v/v) Plant Leaf Extract
VI (Experimental Group D)	100% (v/v) Plant Leaf Extract

Solvent; DCM, EtOAc used were analytical grade (95.5%); Actellic super ^{TM} (50g/90kg)

Weevil mortality because of fumigants toxicity was assessed 6, 24, 48, 72 and 96 hours after the insects were exposed to the extracts. After these test observation periods, the plastic vials were opened, and the weevils transferred into an open recovery tray for five minutes before mortality was assessed. The insects were confirmed dead if they could not move their appendages when probed with a sharp pin at the abdomen (Ileke and Bulus, 2012). Corrected mortality percentages were then computed using Abbort formula (1925).

$$P_r = \frac{\mathrm{Pt} - \mathrm{Pc}}{100 - \mathrm{Pc}} \times 100$$

Where: P_r = corrected mortality

P_t = % mortality in various extracts treatments $(\frac{\mathrm{Dn}}{\mathrm{Tn}} \times \frac{100}{1})$

P_c = % mortality in solvent control $(\frac{\mathrm{Dn}}{\mathrm{Tn}} \times \frac{100}{1})$

D_n = Number of dead insects

T_n = Total number of insects

On the other hand, the determination of contact toxicitity tests was done as follows; twenty grams of maize grains were weighed and put into 50 ml plastic vials. 1.0 ml of each plant extract at predetermined concentrations of 25, 50, 75 and 100% was added. The mixture was shaken gently to ensure uniform coating of grains. After the grains and extracts were thoroughly mixed, the set ups were air-dried for 2 hours to evaporate all traces of solvents.

Twenty male adult *S. zeamais* were introduced into each plastic vial and then tightly covered with a lid. Several tiny openings were made on the lid of plastic vials to ensure ventilation. In negative control, maize grains were mixed with one milliliter of respective solvent only while for the positive control group, the grains were treated with Actellic Super $^{\mathrm{TM}}$ as the standard pesticide. The experiments were set up with 4 replicates for each treatment group. This assay was therefore set up into six independent treatment groups as shown in Table 3.3.

Table 3.3: Treatment Protocol for Determination of Contact Toxicity Effects of Selected Organic Leaf Extracts of *T. diversifolia* and *V. lasiopus* on *S. zeamais*

Group	Treatment
I (Negative control)	Solvent only
II (Positive control)	Actellic Super ™
III (Experimental group A)	25% (v/v) plant leaf extract
IV (Experimental group B)	50% (v/v) plant leaf extract
V (Experimental group C)	75% (v/v) plant leaf extract
VI (Experimental group D)	100% (v/v) plant leaf extract

Solvent; DCM, EtOAc used were analytical grade (95.5%); Actellic super ™ (50 g/90kg)

Weevil mortality was assessed 6, 24, 48, 72 and 96 hours after the insects were exposed to the extracts. Those weevils that did not respond to five jabs with a blunt dissecting probe of the abdomen after five minutes recovery period was presumed dead (Ileke and Bulus, 2012). Corrected mortality percentages (Pr) were calculated using the formula described by Abbort formula (1925).

$$P_r = \frac{\mathrm{Pt} - \mathrm{Pc}}{100 - \mathrm{Pc}} \times 100$$

Where: P_r = corrected mortality
P_t = % mortality in various extracts treatments $(\frac{\mathrm{Dn}}{\mathrm{Tn}} \times \frac{100}{1})$
P_c = % mortality in solvent control $(\frac{\mathrm{Dn}}{\mathrm{Tn}} \times \frac{100}{1})$
D_n = Number of dead insects
T_n = Total number of insects

3.9 Determination of the Repellent Activity

The repellent assessment adopted a randomized controlled study design (RCD). The study used the area preference method as described by Obeng-Ofori *et al*. (1998). To create the two experimental areas, Whatmann No. 1 filter paper circles of 10 cm in diameter were cut into two halves. One milliliter of each extract treatment at predetermined concentrations of 25, 50, 75 and 100% were uniformly applied with a pipette to a half-filter-paper disc as uniformly as possible. This half filter paper circle formed the treated test area of the experiments. The other half circle was treated with solvent only to serve as negative control area. All the discs were then air dried to evaporate solvents completely (Obeng-Ofori *et al*., 1998). For positive control, a conventional pesticide Actellic®25EC was applied on the treated area at the recommended rate of 2 µg/ml.

A full filter paper was then re-made by attaching the treated halves with the untreated halves with cellotape. The treated and the untreated half-circles were hence placed contiguously on the petri dishes and ten live weevils carefully introduced at the centre of each filter paper disc in the Petri dish and covered well. Each treatment was replicated four times. The treatments were set up into six independent treatment groups as shown in Table 3.4. Each of all the six experiments including the control treatments were set out with four replications.

Table 3.4: Treatment Protocol for Determination of Repellency Activities of Selected Organic Leaf Extracts of *T. diversifolia* and *V. lasiopus* on *S. zeamais*

Group	Treated Area	Control Area
I Experimental group A	25% Plant Extract (v/v)	Solvent Only
II Experimental group B	50% Plant Extract (v/v)	Solvent Only
III Experimental group C	75% Plant Extract (v/v)	Solvent Only
IV Experimental group D	100% Plant Extract (v/v)	Solvent Only
V Positive control	Actellic Super ™	Solvent Only

Solvent; DCM, EtOAc used were analytical grade (95.5%); Actellic®25EC (2 μg/ml).

The number of weevils in the treated (N_t) and control (N_c) areas of preference was counted and recorded after every one hour for a total of five hours. These numbers were then used to calculate percent repellency (PR) of each extract by using the formula described by Thien *et al.* (2013) as shown below.

$$\text{PR (Percentage of Repellency)} = \frac{(\text{Nc} - \text{Nt})}{(\text{Nc} + \text{Nt})} \times \frac{100}{1}$$

Where:

Nc = Represent the number of insects recorded in the control area
Nt = Represent the number of insects recorded in the treated area

The index of repellency (IR) was then calculated using the formula described by Nerio *et al.* (2010).

$$\text{IR (Index of Repellency)} = \frac{2\text{T}}{\text{T}+\text{C}}$$

Where:

C and T represent the number of insects on the untreated and treated areas of preference, respectively. The repellency index values were classified as Repellency (Values <1), Neutral (Values = 1) and Attractant (Values >1) (Nerio *et al.*, 2010)

3.10 Determination of Oviposition Deterrence and F_1 Progeny Emergence Inhibition Effects

In this study, the determination of oviposition deterrence and F_1 progeny emergence inhibition experiments adopted a randomized controlled study design (RCD). The negative control group was set up using the appropriate solvent without any plant extract while the positive control was set up using Actellic Super ^{TM} as a conventional reference pesticide. The experimental groups were set up by treating the 20 g grains with plant extracts at concentrations of 25, 50, 75 and 100%. Each set up was replicated four times, appropriately labelled and arranged on laboratory bench. In these assessment tests, the treatments were therefore set up into six independent groups as shown in Table 3.5.

Table 3.5: Treatment Protocol for Determination of Oviposition Deterrence and F_1 Progeny Emergence Inhibition Effects of Selected Organic Leaf Extracts of *T. diversifolia* and *V. lasiopus* on *S. zeamais*

Group	Treatment
I (Negative Control)	Solvent Only
II (Positive Control)	Actellic Super ^{TM}
III (Experimental Group A)	25% (v/v) plant leaf extract
IV (Experimental Group B)	50% (v/v) plant leaf extract
V (Experimental Group C)	75% (v/v) plant leaf extract
VI (Experimental Group D)	100% (v/v) plant leaf extract

Solvent; DCM, EtOAc used were analytical grade (95.5%); Actellic super ^{TM} (50g/90kg)

Maize grains were mixed with different test solution for 1 min and solvent allowed to evaporate for 20 min. Twenty grams of treated maize grains was placed in plastic vials (9 cm high and 7 cm in diameter). The treated grain samples in each plastic vial were infested with 10 male and 10 female weevils collected from the insect stock jars. The containers were covered with muslin cloth and strongly fastened with cut edge of plastic vial lids to allow for aeration while still preventing exit of weevils and entry of any unwanted organisms. The infested grains were left for seven days under same experiment condition during which the weevils fed, mated, and laid eggs freely (Abebe *et al.*, 2009).

After seven days post infestation, both dead (those that did not respond to abdominal probe with a pin) and live weevils were sieved out and discarded. The total number of eggs laid was counted on ten randomly selected grains from each sample using the method described by Frankenfeld (1948) with slight modification.

Grains were examined using a hand lens to locate the transparent gelatinous egg plugs on grains which made the spots to appear shinny white. It was assumed that each plug covered only one egg and each egg was covered by one plug. The weevils feeding puncture and mechanical injuries were distinguished from the gelatinous egg plugs by the shiny white covering surface of the egg plugs (Adedire *et al.*, 2011).

The number of eggs laid by the females on the treated (Tn) and the control (Cn) groups counted with aid of a hand lens and recorded appropriately. The effects of selected organic extracts of *T. diversifolia* and *V. lasiopus* against oviposition traits of *S. zeamais* on treated grains were examined and calculated using the formula described by Arivoli and Tennyson (2013).

Percent oviposition deterrence (% OD) = $\frac{\mathrm{Cn}-\mathrm{Tn}}{\mathrm{Cn}+\mathrm{Tn}} \times \frac{100}{1}$

Where:

Cn = Represent the number of eggs laid on grains in the negative control sample
Tn = Represent the number of eggs laid on grains in respective treatment sample

For the determination of F_1 progeny emergence inhibition, maize grains were treated and infested with weevils as described above. However, after seven days of feeding, mating, and oviposition, all the 20 weevils, both dead and live, were sieved out. The setups were then left for 35 more days to monitor for the emergence of F_1 progeny. Then, right at the beginning of the emergence (35 days after the eggs were laid), the number of emerged adults were counted after every 2 days until the end of the emergence.

Based on the indications of piloted life cycle of weevils on untreated maize (normal control), the counting period of F_1 progeny was established to avoid an overlap of population generations. Furthermore, by the fact that all emerging weevils were sieved, counted, and discarded from the treated grains ensured that no F_2 generation progeny was realized in the experiment until the last F_1 progeny emerged.

The number of F_1 progeny produced was recorded after every 2 days for two weeks from the time of first adult emergence. Cumulative number of the adults that emerged from every set of experiment was calculated and recorded. The inhibitory effects of the selected organic extracts of *T. diversifolia* and *V. lasiopus* against emergence of *S. zeamais* (F_1 progeny) was hence examined and calculated using the formula described by Tapandjuo *et al.* (2002).

$$\text{IR (Emergence inhibition rate)} = \frac{(\mathrm{Cn}-\mathrm{Tn})}{\mathrm{Cn}} \times \frac{100}{1}$$

Where:

Cn = Represent the number of emerged weevils in the negative control sample
Tn = Represent the number of emerged weevils in respective treatment sample

3.11 Determination of Anti-Feedant Effects

The evaluation of antifeendant effects of *T. diversifolia* and *V. lasiopus* experiments adopted a randomized controlled study design (RCD). In these assessment tests, the treatments were set up into six independent groups as shown in Table 3.6. The experimental groups were set up by treating 20 g of maize grains with plant extracts at the different concentrations. The negative control group was treated with the appropriate solvent without any plant extract while the positive control was treated using Actellic Super ^{TM} as a conventional reference pesticide. Each set up was replicated four times.

Table 3.6: Treatment Protocol for the Determination of Anti-Feedant Effects of Selected Organic Leaf Extracts of *T. diversifolia* and *V. lasiopus*

Group	**Treatment**
I (Negative Control)	Solvent Only
II (Positive Control)	Actellic Super ^{TM}
III (Experimental Group A)	25% (v/v) Plant Leaf Extract
IV (Experimental Group B)	50% (v/v) Plant Leaf Extract
V (Experimental Group C)	75% (v/v) Plant Leaf Extract
VI (Experimental Group D)	100% (v/v) Plant Leaf Extract

Solvent; DCM, EtOAc used were analytical grade (95.5%); Actellic super ^{TM} (50 g/90kg)

After thorough mixing and evaporation of the solvent, the 20 g treated maize grains were put in different 30 ml plastic vials. Twenty grams (recorded as initial weight) of maize grain was impregnated with different test extracts in each of four replicates. All the test materials were applied within the same time of one hour. Each plastic vial with treated maize samples was then infested with ten adult male weevils and allowed to feed for 7 days (Keita *et al.*, 2001; Mahdi and Rahman, 2008). After the feeding period of 7 days, the maize grains were reweighed. This was recorded as the final weight of grains.

To determine grain weight loss reduction effects, the final weight of the maize grains was compared with the initial weight (Arannilewa *et al.*, 2006). The percent weight loss (PWL) was, therefore, computed using the formula described by Mahdi and Rahman (2008).

$$\text{PWL} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

Where:

PWL = Represent the Percentage Weight Loss

The damage to the maize grains by *S. zemais* was also assessed in each sample after seven days from the time of infestation. All the weevils, dead and alive were first sieved out. Samples of 50 grains were randomly taken from treated and untreated grains. The number of maize grains with and without exit holes/perforations was established. The number of insect-damaged grains was hence assessed and recorded as the number of damaged and undamaged grains.

The percent grain damage (PD) for each treatment was calculated using the formula described by Odeyemi and Daramola (2000) while the weevil perforation index (WPI) was calculated using the formula described by Fatope *et al.* (1995) and quoted by Arannilewa *et al.* (2006).

$$\text{PD} = \frac{\text{Total Treated grains Perforated}}{\text{Total Number of grains}} \times 100$$

$$\text{WPI} = \frac{\text{Percentage of Treated Grains Perforated}}{\text{Percentage of Control Grains Perforated} + \text{Percentage Treated Grains Perforated}} \times 100$$

Where:

PD = Represent the percentage of the damaged maize grains
WPI = Represent the weevil perforation index value

Weevil Perforation Index (WPI) value above 50 was regarded as an index of negative stored maize grain protecting ability (Enhancement of infestation by weevils) (Odeyemi and Daramola, 2000).

3.12 Determination of the Antiacetylcholinesterase Activity

3.12.1 Assay Reagents

Bovine Serum Albumin (BSA), Coomassie Brilliant Blue G-250, 95% ethanol and 85% phosphoric acid were used for determination of protein content in the isolated acetylcholinesterase. The acteylcholinesterase assay kit (colourmetric) ab138871, which contains acetylcholinesterase standard, assay buffer, 5, 5′-dithio-bis (2- nitrobenzoic acid) (DTNB) and acetylthiocholine, were purchased from Abcam (UK), bovine serum albumin (BSA), Commassie brilliant blue and Phosphoric acid (Sigma aldrich, USA), Triton X-100 (Lobal Chemie, India) and EDTA (Duchefa Haarlem, Netherlands).

3.12.2 Extraction of Crude Acetylcholinesterase

The crude acetlycholinesterase (AChE) enzyme was isolated from *Sitophilus zeamais* adults reared in the laboratory as described earlier in section 3.6 of this chapter. The adult insects were first washed three times with distilled water. The crude AChE enzyme was isolated by collecting about 0.5 g of live adult maize weevils and homogenizing them for 3 min in 6 ml sodium phosphate buffer (pH 8.0, 0.01 M), containing 1 mM EDTA, 1% Triton X-100, 1 M NaC1) according to Carter *et al.* (2007) and Tarhoni *et al.* (2011).

After filtration through Whatman number 1 filter paper, the homogenate was centrifuged at 15,000 g for 10 min at 4^oC. The resulting supernatant was used as an enzyme source. The concentration of the isolated AChE was determined in the supernatant using the Bradford (1976) method.

3.12.3 Determination of Protein Content

The protein content of the isolated acetylcholinesterase was determined according to the protein-dye-binding method of Bradford (1976) using bovine serum albumin (BSA) as standard. The dye solution was made by dissolving 100mg of Coomassie Brilliant Blue G-250 in 50 ml 95% ethanol and 100 ml 85% (w/v) phosphoric acid. The rates of hydrolysis by AChE were monitored spectrophotometrically at 595 nm.

Serial dilutions of 1 mg/µl, 20 µg/µl, 30 µg/µl, 40 µg/µl, 50 µg/µl, 60 µg/µl, 70 µg/µl and 80 µg/µl were prepared from the stock solution of 1 mg/ml of BSA. The absorbance was measured in a 72N39 spectrophotometer (at 595 nm) after mixing samples in 3 ml cuvettes against the blank. The protein concentration was estimated from a standard curve of concentration of BSA (Bradford, 1976).

3.12.4 Experimental Design

The antiacetylcholinesterase assessment bioassays also adopted a randomized controlled study design (RCD). However, this study bioassayed six extracts concentration levels for the DCM and ethyl-acetate leaf extracts of both *T. diversifolia* and *V. lasiopus* (section 3.6). Therefore, to accomplish this, the treatments were set up into eight independent treatment groups as shown in Table 3.7.

The experimental groups were set up by treating crude enzyme extract (AChE) with plant extracts at the different concentrations (5.0, 7.5, 10.0, 12.5, 15.0 and 17.5 mg/ml). The normal control constituted the same reagents without any plant extract. The positive control was set up using actellic as an established reference pesticide. All the treatments were conducted in triplicates.

Table 3.7: Treatment Protocol for the Determination of *Ex Vivo* Anti-Acetylcholinesterase Effects of Selected Organic Leaf Exracts of *T. diversifolia* and *V. lasiopus* on *S. zeamais*

Group	Treatment
Normal	(No plant extract + buffer) + crude enzyme extract (AChE) + ATCI+ DTNB
Positive	Actellic super^{TM} +crude enzyme (AChE) + buffer+ ATCI + DTNB
Group A	5.0mg/ml of plant extract + crude enzyme extract (AChE) + buffer + ATCI + DTNB
Group B	7.5mg/ml of plant extract + crude enzyme extract (AChE) + buffer + ATCI + DTNB
Group C	10.0mg/ml of plant extract + crude enzyme extract (AChE) + buffer + ATCI + DTNB
Group D	12.5mg/ml of plant extract + crude enzyme extract (AChE) + buffer + ATCI + DTNB
Group E	15.0mg/ml of plant extract + crude enzyme extract (AChE) + buffer + ATCI + DTNB
Group F	17.5mg/ml of plant extract + crude enzyme extract (AChE) + buffer + ATCI + DTNB

ACTI: Acetylthiocholine iodide; DTNB: 5-5 dithiobis-2-nitrobenzoic acid; AChE: Acetylcholinesterase

The inhibitory activities of selected organic leaf extracts of *T. diversifolia* and *V. lasiopus* against maize weevil acetylcholinesterase (AChE) activities were determined as described by Ellman's method (Ellman *et al*., 1961) (Appendix 8.1), with some modification which allowed use of 1 ml cuvette glass. Assay reactions with plant extracts were all performed in triplicates at six concentrations of 5.0, 7.5, 10.0, 12.5, 15.0 and 17.5 mg/ml. In a 2 ml eppendorf tube, 150 µl of 0.1 M sodium phosphate buffer (pH 8) was put, in which 10 µl of the plant extract was added, followed by 20 µl crude enzyme.

The reaction was initiated by addition of 10 µl of 14 mM of acetylthiocholine iodide (ATCI) as a substrate. The enzyme hydrolyzes the substrate ATCI to thiocholine and acetic acid. The eppendorf tube with the mixture was incubated for 30 min at 25^{o}C. Thereafter, 10 µl of 10 mm of DTNB was added, which was used for the measurement of AChE activity. The reaction mixture was incubated for 5 min at room temperature (25^{o}C).

The absorbance was read after one minute (initial absorbance) and then at the fourth minutes (final absorbance). Then the change in optical density with time (OD/min) was recorded after 3 min at room temperature (25^{o}C) to estimate substrate hydrolysis over time. These changes in absorbance were used to calculate acetylcholinesterase enzyme activities as well as the percent enzyme inhibition rates.

The acetylcholinesterase enzyme activity was, therefore, calculated from the absorbance value using Beer Lamberts law. One unit of AChE activity was defined as 1µl of substrate hydrolyzed per minute.

$$\text{Enzyme activity} = \frac{(\Delta As - \Delta\text{Ac})\text{X vol. of cuvette X } 10^{6}}{\varepsilon \text{ X time X vol. of the sample X protein conc.}}$$

Where.

ΔAs	Represents the change in the absorbance of test sample from the beginning to the end of the measurement period
ΔAc	Represents the change in the absorbance of normal control from the beginning to the end of the measurement period
Cuvette vol.	Absorbance is proportional to concentration and must be multiplied by the volume to calculate the absolute number of moles product formed,
10^{6}	This converts the moles of ε to µmoles.
ε	The molar extinction coefficient converts absorbance values to concentrations.
Time	Enzymatic activity is expressed per unit time and this represents the time interval the absorbance was measured.

Sample vol.	All the parameters above represent the activity in the cuvette. Therefore, it is necessary to divide by the sample volume so that the activity is expressed per unit volume of the sample,
Protein	Dividing by the protein concentration will provide information about the amount of enzyme activity per unit protein.

Further, the percent inhibition properties of the selected organic leaf extracts of *T. diversifolia* and *V. lasiopus* were calculated using the formula adopted from Njoroge *et al.* (2016).

$$\text{Percent inhibition} = \frac{(\text{Rate without inhibitor} - \text{Rate with inhibitor})}{\text{Rate without inhibitor}} \text{X } 100$$

The value of median inhibition concentration (IC_{50}) for the inhibitors was also calculated based on log (extract/inhibitor concentration) versus probit (percentage of inhibition) linear regression. Concentration-response curve of percent inhibition against concentration was plotted to show the sensitivity of the enzyme to the extracts.

3.13 Data Management and Statistical Analysis

Experimental data obtained was recorded and later tabulated on abroad sheet. Data were formulated and computed using Ms Excel program. The data was checked for normality using Kolomogorov-Smirnov test and then analyzed through descriptive statistics and presented as Mean ± Standard Error of Mean (SEM).

The data from different treatment groups was also subjected to inferential statistics using one-way ANOVA followed by Tukey's and Fisher LSD post hoc tests for separation and pairwise comparisons of means in the fumigant and contact toxicities, repellency, oviposition deterrence and F_1 progeny emergence inhibition, antifeedant and anti-acetylcholinesterase assays.

Unpaired student t-test was used for pairwise separation and comparison of means between different treatment groups for the two plants. The significant difference between the treatment groups were reported at $p \leq 0.005$ for the fumigant and contact toxicities, repellency, oviposition deterrence and F_1 progeny emergence inhibition, antifeedant assays and $p \leq 0.001$ for the anti-acetylcholinesterase assays. All the analyses were conducted using Minitab® Version ^{17} software as the statistical tool. The resulting data of this study were presented in form of tables and bar graphs. Photographs were also taken to show the experiment set ups.

CHAPTER FOUR

RESULTS

4.1 Percentage Extract Yields

The results of the percentage yields of organic leaf extracts of *T. diversifolia* and *V. lasiopus* are tabulated in Table 4.1. The dichloromethanolic extracts of *T. diversifolia* had the highest yield of 1.69% followed by ethyl acetate extracts *T. diversifolia* (1.44%), (Table 4.1). The dichloromethane and ethyl acetate extracts of *V. lasiopus* had the lowest yield of 0.56 and 0.54%, respectively (Table 4.1).

Table 4.1: The Percentage Crude Yields of Selected Organic Leaf Extracts of *T. diversifolia* and *V. lasiopus*

Plant	Extract	Mass of Crude Extract (g)	Percent Yield of Crude Extract (%)
T. diversifolia	DCM Extract	3.38	1.69
	EtOAc Extract	2.88	1.44
V. lasiopus	DCM Extract	1.12	0.56
	EtOAc Extract	1.07	0.54

DCM, EtOAc used were analytical grade (95.5%)

4.2 Phytochemical Analysis of the Extracts of *T. diversifolia* and *V. lasiopus*

4.2.1 Phytochemical Analysis of DCM Extracts of *T. diversifolia* and *V. lasiopus*

The GC-MS analysis revealed presence of 34 bioactive phytocompounds in the DCM extract of *T. diversifolia.* The retention time (min), molecular formula, chemical class, relative abundance (%) and concentration of the identified compounds of *T. diversifolia*, were presented in Table 4.2 and Appendix 2.1.

From these results, it was found that the DCM extract of *T. diversifolia* contained five triterpenoids (Table 4.2). B-amyrin was the most abundant triterpenoid (522.35 ng/g) followed by squalene (392.50 ng/g), α-amyrin (177.89 ng/g), ursonic acid (61.17 ng/g) and friedooleanan-3-ol (41.40 ng/g) (Table 4.2). It was also observed that phytol acetate was the most abundant diterpenoid (254.59 ng/g) followed by methyl linoleate (164.40ng/g), phytol (159.82 ng/g), phytol acetate (60.92 ng/g) and isophytol (7.05 ng/g) (Table 4.2). It was further revealed that hexadecanoic acid (204.48 ng/g) was the most abundant of all fatty acids and their derivatives in the *T. diversifolia* extract, while dodecanoic acid (7.15 ng/g) was the least abundant (Table 4.2).

The highest concentrated phenolic compound in the *T. diversifolia* extract was benzofuranone (14.86 ng/g) while phenol, 2,4-bis (1,1-dimethylethyl) was the least abundant phenol (4.88 ng/g) (Table 4.2). Trans-beta-ionone (trans-β-ionone) was the most abundant monoterpenoids (13.11 ng/g) found present in the *T. diversifolia* extract followed by linolool (7.34 ng/g) and octadiene6-diol (4.02 ng/g) (Table 4.2). These results also revealed two phytosterol compounds; ergostadienol and ergosta-dien-3-ol in the order of concentration (Table 4.2). A single sesquiterpenoid (α-copaene) was identified in the DCM extract of *T. Diversifolia* (Table 4.2).

Table 4.2: Quantitative Phytochemical Composition the DCM Leaf Extract of *T. diversifolia*

Class of Compounds	Compound Name	Molecular Formula	Retention Time (Min)	Relative Abundance (%)	Conc. (ng/g)
Triterpenoids	β-amyrin	$C_{30}H_{50}O$	38.39	14.45	522.35
	Squalene	$C_{30}H_{50}$	30.85	10.84	392.50
	α-amyrin	$C_{30}H_{50}O$	39.27	4.88	177.89
	Ursonic acid	$C_{30}H_{48}O_3$	38.78	1.64	61.17
	Friedooleanan-3-ol	$C_{30}H_{50}O$	41.73	1.09	41.40
Diterpenoids	Phytol, acetate	$C_{22}H_{42}O_2$	22.29	7.01	254.59
	Methyl linoleate	$C_{19}H_{34}O_2$	25.15	4.50	164.40
	Phytol	$C_{20}H_{40}O$	24.96	4.38	159.82
	Isophytol	$C_{20}H_{40}O$	23.38	0.13	7.05
Fatty acids and Derivatives	Hexadecanoic acid	$C_{16}H_{31}O_2$	23.52	11.12	402.48
	Octadecanoic acid	$CH_3(CH_2)_{16}COOH$	25.38	3.82	139.90
	Ethyl hexadecanoate	$C_{18}H_{36}O_2$	23.82	2.80	102.90
	Ethyl octadecanoate	$C_{20}H_{40}O_2$	25.66	1.37	51.58
	Docosanoic acid	$C_{22}H_{44}O_2$	28.70	0.99	37.88
	Pentadecanone	$C_{18}H_{36}O$	22.35	0.82	31.94
	Tetradecanoic acid	$C_{14}H_{28}O_2$	21.42	0.40	16.80
	Heptadecanoic acid	$CH_3(CH_2)_{15}COOH$	24.43	0.36	15.21
	Tetradecanal	$C_{14}H_{28}O$	19.81	0.21	9.75
	Pentadecanoic acid	$C_{15}H_{30}O_2$	22.46	0.20	9.50

Table 4.2: Cont'd

Class of Compounds	Compound Name	Molecular Formula	Retention Time (Min)	Relative Abundance (%)	Conc. (ng/g)
Fatty acids	Tridecanal	$C_{13}H_{26}O$	20.96	0.18	8.60
	Dodecanoic acid	$C1_{2}H_{24}O_{2}$	19.16	0.14	7.15
Polyphenols	Benzofuranone	$C_{11}H_{16}O_{2}$	18.99	0.35	14.86
	Eugenol	$C_{10}H_{12}O_{2}$	16.68	0.20	9.53
	Phenyl ethyl alcohol	$C_{8}H_{10}O$	13.04	0.09	5.60
	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	18.62	0.07	4.88
Benzyl Derivatives	Benzaldehyde, 2,4-dimethyl-	$C_{9}H_{10}O$	14.68	0.17	8.49
	p-xylene	$C_{6}H_{4}(CH_{3})_{2}$	8.07	0.07	4.69
Monoterpenoids	Trans-beta-Ionone (trans-β-ionone)	$C_{13}H_{20}O$	18.37	0.30	13.11
	Linalool	$C_{10}H_{18}O$	12.80	0.14	7.34
	Octadiene-2,6-diol	$C_{10}H_{18}O_{2}$	14.22	0.05	4.02
Phytosterols	Ergostadienol	$C_{28}H_{46}O$	37.65	8.32	301.79
	Ergosta-5,22-dien-3-ol, (3-beta.,22E,24S)	$C_{29}H_{48}O$	36.65	2.12	78.46
Sesquiterpenoids	α-copaene	$C_{15}H_{24}$	16.99	0.06	4.32

Summary of compounds identified in the DCM extract of *T. diversifolia* with their RT: Retention time (Min); Relative Abundance (%); Compound Concentration (ng/g); Molecular Formula and Relative Molecular Mass. Compounds are listed in the order of concentration in respective chemical class

On the other hand, the GC-MS analysis of DCM leaf extract of *V. lasiopus* revealed the presence of 22 constituent compounds as presented in Table 4.2.2 and Appendix 2.2. Based on these results, the phytochemical compounds identified in this extract can be categorized into six major categories; monoterpenoids, diterpenoids, triterpenoids, phytosterols, flavonoids (phenolics) and fatty acids and their derivatives (Table 4.3).

It was observed that β-amyrin was the highest concentrated triterpenoid (183.79 ng/g) while α-amyrin was the least concentrated triterpenoid (48.08 ng/g) (Table 4.3). There were two diterpenoids; phytol acetate and phytol with concentrations of 170.93 and 51.17 ng/g, respectively (Table 4.3).

The GC-MS analysis of DCM leaf extract of *V. lasiopus* revealed three phytosterols; chondrillasterol (67.52 ng/g), stigmasterol (13.74 ng/g) and nonadecene (3.18 ng/g) (Table 4.3). Among the fatty acids and their derivatives, palmitic acid was the most abundant (65.37 ng/g) while ethyl tetradecanoate was the least abundant (3.9 ng/g) (Table 4.3). The most abundant polyphenol compound in the DCM leaf extract of *V. lasiopus* was eugenol (5.3 ng/g) followed by indanol (5.21ng/g), whereas phenol, 2,5-bis (1,1-dimethylethyl) (3.5 ng/g) had the lowest concentration (Table 4.3).

The class of monoterpenoids had 7-dimethyl-1, 6-octadien-3-ol with the highest concentration while ionone was the least concentrated (Table 4.3). The GC-MS chromatogram of dichloromethane leaf extract of *V. lasiopus* revealed the presence of various compounds with corresponding peaks at different retention time (Appendix 3.2).

Table 4.3: Quantitative Phytochemical Composition the DCM Leaf Extract of *V. lasiopus*

Class of Compounds	Compound Name	Molecular Formula	Retention Time (Min)	Relative Abundance (%)	Conc. (ng/g)
Triterpenoids	β-amyrin	$C_{30}H_{50}O$	38.36	17.95	183.79
	α-Amyrin	$C_{30}H_{50}O$	39.26	4.53	48.08
	Sqaalene	$C_{30}H_{50}$	30.85	14.60	149.90
Diterpenoids	Phytol acetate	$C_{22}H_{42}O_2$	22.29	16.68	170.93
	Phytol	$C_{20}H_{40}O$	24.96	4.84	51.17
Phyto-sterols	Chondrillasterol	$C_{29}H_{48}O$	37.63	6.45	67.52
	Stigmasterol	$C_{29}H_{48}O$	36.64	1.14	13.74
	Nonadecene	$C_{19}H_{38}$	21.12	0.09	3.18
Fatty Acids	Hexadecanoic acid (palmitic acid)	$C_{16}H_{32}O_2$	23.49	6.24	65.37
	Octadecatrienoic acid	$C_{18}H_{30}O_2$	25.18	5.19	54.70
	Ethyl hexadecanoate	$C_{18}H_{36}O_2$	23.82	5.01	52.91
	Methyl linoleate	$C_{19}H_{34}O_2$	25.39	4.03	43.03
	Octadecatrienoic acid, ethyl ester	$C_{19}H_{34}O_2$	25.46	2.95	32.12
	Trimethyl-pentadecanone,	$C_{19}H_{34}O_2$	22.35	1.56	17.99
	Methyl 19-methyl-eicosanoate	$C_{21}H_{40}O_2$	27.36	1.01	12.46
	Tetradecanoic acid	$C_{14}H_{28}O_2$	21.41	0.32	5.53
	Ethyl tetradecanoate	$C_{16}H_{32}O_2$	21.80	0.16	3.90
Polyphenols	Eugenol	$C_{10}H_{12}O_2$	16.71	0.30	5.30
	Indanol	$C_9H_{10}O$	14.68	0.29	5.21
	Phenol, 2,5-bis (1,1-dimethylethyl)	$C_{14}H_{22}O$	18.62	0.12	3.50
Monoterpenoids	Octadien-3-ol	$C_{10}H_{18}O$	12.80	0.18	4.09
	Ionone	$C_{13}H_{20}O$	18.37	0.10	3.25

Summary of compounds identified in the DCM extract of *V. lasiopus* with their RT: Retention time (Min); Relative Abundance (%); Compound Concentration (ng/g); Molecular Formula and Relative Molecular Mass. Compounds are listed in the order of concentration in respective chemical class

A comparative analysis of the phytochemical composition of the DCM leaf extracts of *T. diversifolia* and *V. lasiopus* revealed that certain compounds were present in both extracts as shown in Table 4.4. However, it was noted that the concentrations of all the common compounds were lower in the *V. lasiopus* extract compared to their concentrations in the *T. diversifolia* extract (Table 4.4).

Table 4.4: Phytochemicals Present in the DCM Leaf Extracts of both *T. diversifolia* and *V. lasiopus*

Name of Compounds	Molecular Formula	Chemical Class	Concentration (ng/g)	
			T. diversifolia	***V. lasiopus***
β-amyrin	$C_{30}H_{50}O$	TT	522.35	183.79
Phytol acetate	$C_{22}H_{42}O_2$	DT	254.59	170.93
Squalene	$C_{30}H_{50}$	TT	392.50	149.90
Hexadecanoic acid	$C_{16}H_{32}O_2$	FAD	402.48	65.37
Octadecatrienoic acid	$C_{18}H_{30}O_2$	FAD	321.74	54.70
Ethyl hexadecanoate	$C_{18}H_{36}O_2$	FAD	102.90	52.91
Phytol	$C_{20}H_{40}O$	DT	159.82	51.17
α-amyrin	$C_{30}H_{50}O$	TT	177.89	48.08
Methyl linoleate	$C_{19}H_{34}O_2$	FAD	164.40	43.03
Tetradecanoic acid	$C_{14}H_{28}O_2$	FAD	16.80	5.53
Eugenol	$C_{10}H_{12}O_2$	P	9.53	5.30
Phenol, 2,5-bis	$C_{14}H_{22}O$	P	4.88	3.50

P stands for phenolic, ST for sesquiterpenoid, TT for triterpenoid, DT for diterpenoid and FAD for fatty acid derivative

4.2.2 Phytochemical Analysis of the EtOAc Extracts of *T. diversifolia* and *V. lasiopus*

Phytochemical analysis of the ethyl acetate extracts of *T. diversifolia* and *V. lasiopus* was done using GC-MS. The analysis revealed presence of 57 compounds in the ethyl acetate extracts of *T. diversifolia* (Table 4.5; Appendix 2.3). These phytochemical compounds can be categorized into twelve groups with the majority being terpenes and terpenoids (Table 4.5).

The most abundant of the fatty acid and derivative compounds in the ethyl acetate leaf extract of *T. diversifolia* was hexadecanoic acid (789.58 ng/g) while the least abundant fatty acids was crotonic acid (5.83 ng/g) (Table 4.5). The most abundant sesquiterpenoid compound was widdrol (110.29 ng/g) while α-bulnesene sesquiterpene had the least concentration (4.57 ng/g) (Table 4.5). Benzofuranone was the most concentrated among the monoterpenoids in this extract, while terpineol (3.47 ng/g) was the least concentrated monoterpenoid (Table 4.5). There were three diterpenoids found in the *T. diversifolia* extract namely phytol, phytol acetate and 10-dimethyl-5,9-Undecadien-2-one in the order of concentration (Table 4.5). Squalene was found to be the most abundant triterpenoid while α-amyrin was the least concentrated triterpenoid in this extract of *T. diversifolia* (Table 4.5).

The GCMS analysis further showed that the phytosterols in the *T. Diversifolia* extracts included ergosta-5,22-dien-3-ol, campesterol, ergosta-7,22-diene-3,5,6-triol and Selin-11-en-4-alpha-ol (kongol) in the order of concentration (Table 4.5). There were three phenolic compounds in this extract namely indanol (16.03 ng/g), 3-propanetriol (14.3ng/g) and Phenol, 2,4-bis (1,1-dimethylethyl) (13.88 ng/g) (Table 4.5). Ketones class of compounds was also found in this extract. They included 1-cyano-d-camphidine, benzaldehyde and vanillin in the order of their concentration. The two alkaloids identified in this plant extract were indole (11.33 ng/g) and diisopropylethylamine (5.16 ng/g) (Table 4.5). Finally, this study also revealed only one naphthalene derivative (naphthalene, 4-dimethyl) and only one coumarin (2-coumaranone) (Table 4.5).

Table 4.5: Quantitative Phytochemical Composition of the Ethyl Acetate Leaf Extract of *T. diversifolia*

Class of Compounds	Compound Name	Molecular Formula	Retention Time (Min)	Relative Abundance (%)	Conc. (ng/g)
Fatty acids and Derivatives	Hexadecanoic acid (palmitic acid)	$C_{16}H_{32}O_2$	23.562	13.72	789.58
	Octadecatrienoic acid	$C_{18}H_{30}O2$	25.2468	7.96	459.03
	Methyl linoleate	$C_{19}H_{34}O_2$	25.1766	6.41	370.28
	Propanoic acid, 2-methyl-	$C_4H_8O_2$	6.3687	4.63	267.83
	3-Tetradecyne	$C_{14}H_{28}$	22.7254	3.47	201.41
	Octadecanoic acid (steric acid)	$C_{17}H_{35}$ CO_2H	25.4048	3.25	188.86
	Docosanoic acid	$C_{22}H_{44}O_2$	28.7217	2.34	136.70
	Citronellyl isobutyrate	$C_{14}H_{26}O_2$	22.5382	1.99	116.29
	Hexadecanoic acid	$C_{18}H_{36}O_2$	23.8135	1.31	77.30
	Pentadecanone	$C_{18}H_{36}O$	22.3569	0.94	56.01
	Tetradecanoic acid	$C_{14}H_{28}O_2$	21.4326	0.75	45.02
	Tridecenol	$C_{10}H_{20}O$	24.0885	0.46	28.87
	Pentadecanol	$C_{15}H_{32}O$	18.3496	0.33	21.38
	Tetradecanal	$C_{14}H_{28}O$	20.9587	0.23	15.46
	1-Nonadecene	$C_{19}H_{38}$	21.1225	0.16	11.49
	Octanoic acid	$C_8H_{16}O_2$	13.927	0.12	9.40
	Hexanoic acid	$C_6H_{12}O_2$	10.651	0.12	9.22
	Nonanoic acid	$C_9H_{18}O_2$	15.3368	0.10	8.05
	Crotonic acid	$C_4H_6O_2$	6.5208	0.06	5.83
Triterpenoids	Squalene	$C_{30}H_{50}$	30.8511	7.77	448.08
	β-amyrin	$C_{30}H_{50}O$	38.3801	5.15	297.63
	Octamethyl-octadecahydro-2H-picen-3-one	$C_{30}H_{48}O$	40.5154	1.89	110.92

Table 4.5: Cont'd

Class of Compounds	Compound Name	Molecular Formula	Retention Time (Min)	Relative Abundance (%)	Conc. (ng/g)
Triterpenoids	Olean-12-ene	$C_{30}H_{50}$	37.9004	1.37	80.86
	α-amyrin	$C_{30}H_{50}O$	41.5801	0.32	20.78
Sesquiterpenoids	Widdrol	$C_{15}H_{26}O$	33.4076	1.88	110.29
	Nerolidol	$C_{15}H_{26}O$	19.268	1.47	86.54
	Italicene epoxide	$C_{15}H_{24}$	24.2464	0.45	28.20
	α-copaene	$C_{15}H_{24}$	16.9807	0.34	21.54
	Caryophyllene oxide	$C_{15}H_{24}O$	19.6483	0.30	19.33
	Modheph-2-ene	$C_{15}H_{24}$	17.0743	0.09	7.65
	α-isocomene	$C_{15}H_{24}$	17.1679	0.08	7.05
	α-bulnesene	$C_{15}H_{24}$	17.4545	0.04	4.57
Monoterpenoids	Benzofuranone	$C_{11}H_{16}O_3$	18.9931	0.36	22.85
	2-carene	$C_{10}H_{16}$	13.067	0.30	19.68
	Isoeugenol	$C_{10}H_{12}O_2$	16.6765	0.26	16.89
	1,6-octadien-3-ol,	$C_{10}H_{18}O$	12.7921	0.13	9.89
	Terpinen-4-ol	$C_{10}H_{18}O$	14.0849	0.08	6.58
	L-α-terpineol	$C_{14}H_{22}$	14.2897	0.02	3.47
Diterpenoids	Phytol	$C_{20}H_{40}O$	24.966	5.56	321.37
	Phytol acetate	$C_{22}H_{42}O_2$	25.902	1.49	87.95
	10-dimethyl-5,9-Undecadien-2-one	$C_{13}H_{22}$	17.8933	0.05	5.35
Alkaloids	Indole	C_8H_7N	15.8107	0.16	11.33
	Diisopropylethylamine	$C_8H_{19}N$	15.9277	0.05	5.16
Phytosterols	Ergosta-5,22-dien-3-ol, (3-beta, 22E,24S)-	$C_{28}H_{46}O$	36.6719	3.53	204.89
	Campesterol	$C_{28}H_{48}O$	36.672	1.49	87.95

Table 4.5: Cont'd

Class of Compounds	Compound Name	Molecular Formula	Retention Time (Min)	Relative Abundance (%)	Conc. (ng/g)
Phytosterols	Ergosta-7,22-diene-3,5,6-triol	$C_{26}H_{46}O_3$	35.227	0.41	25.92
	Selin-11-en-4-alpha-ol (kongol)	$C_{15}H_{26}O$	20.4615	0.38	23.85
Polyphenolics	Indanol	$C_9H_{10}O$	14.6699	0.24	16.03
	3-propanetriol, 1-acetate	$C_7H_{12}O_5$	15.0268	0.21	14.30
	Phenol, 2,4-bis (1-dimethylethyl)	$C_{22}H_{22}O$	18.6245	0.20	13.88
	3-Methoxy 6-trimethyl cyclohex	$C_9H_{14}O$	20.6662	0.14	10.51
Ketones and Aldehydes	1-cyano-d-camphidine	$C_6H_{10}N_2$	21.3507	0.20	13.81
	Benzaldehyde	C_7H_6O	10.2064	0.06	5.56
	p-methyl acetophenone	$C_9H_{10}O$	14.2019	0.10	7.74
Naphthalene	Naphthalene, 1,4-dimethyl-	$C_{12}H_{12}$	17.3434	0.07	6.48
Coumarins	2-coumaranone	$C_8H_6O_2$	14.939	0.04	4.60

Summary of compounds identified in the ethyl acetate extract of *T. diversifolia* with their RT: Retention time (Min); Relative Abundance (%); Compound Concentration (ng/g); Molecular Formula and Relative Molecular Mass. Compounds are listed in the order of concentration in respective chemical class

On the other hand, the GC-MS analysis of ethyl acetate leaf extract of *V. lasiopus* revealed 40 compounds (Table 4.6; Appendix 2.4). The fatty acids and their derivatives were the most abundant class of compounds having 0.11-10.56% relative abundance followed by diterpenoids (0.92-7.65%) and triterpenoids (0.81-3.97%), (Table 4.6). n-hexadecanoic acid (321.66 ng/g) had the highest concentration of all fatty acids and their derivatives identified in this extract while 3-hexenoic acid was the least concentrated fatty acid (5.49 ng/g) (Table 4.6).

The highest concentrated phytosterol compound identified in this plant extract was ergosta-5,22-dien-3-ol (85.26 ng/g) while γ-ergostenol was the least concentrated phytosterol compound (24.19 ng/g) (Table 4.6). Only two diterpenenes (phytol acetate; 233.7 ng/g and phytol; 60.40 ng/g) were identified in this plant extract (Table 4.6). Among the ketone and aldehyde compounds found present in the ethyl acetate leaf extract of *V. lasiopus*, benzaldehyde was the most abundant (4.11 ng/g) while benzaldehyde, 4-dimethyl was the least abundant (8.01 ng/g), (Table 4.6).

This study revealed several sesquiterpenoids with nerolidol being the most abundant (56.13 ng/g) and α-isocomene being the least abundant (5.21 ng/g) (Table 4.6). The ethyl acetate leaf extract of *V. lasiopus* was also found to contain several monoterpenoids including the highest concentrated cis-b-ocimene (11.55 ng/g) and the least concentrated α-pinene (4.53 ng/g) (Table 4.6).

The triterpenoids identified in this extract included squalene, β-amyrin and 19-cycloergost-24 (28)-en-3-ol in the order of their concentration (Table 4.6). The GCMS analysis of ethyl acetate leaf extract of *V. lasiopus* also revealed a furanone compound, benzofuranone and a phenolic compound (phenol,2,4-bis (1,1-dimethylethyl) (Table 4.6).

Table 4.6: Quantitative Phytochemical Composition of the Ethyl Acetate Leaf Extract of *V. lasiopus*

Class of Compounds	Name and Class of Compound	Molecular Formula	Retention Time (Min)	Relative Abundance (%)	Conc. (ng/g)
Fatty Acids and Derivatives	Hexadecanoic acid	$C_{16}H_{31}O_2$	23.51	10.56	321.66
	Octadecatrienoic acid	$C_{18}H_{30}O_2$	25.19	6.14	187.89
	Propanoic acid, 2-methyl	$C_4H_8O_2$	6.02	4.56	140.28
	Methyl linoleate	$C_{19}H_{34}O_2$	25.13	3.94	121.30
	Octadecanoic acid (stearic acid)	$C_{18}H_{36}O_2$	25.37	2.78	86.26
	Heptadecanoic acid	$C_{19}H_{38}O_2$	24.43	0.80	26.37
	Ethyl hexadecanoate	$C_{18}H_{36}O_2$	23.81	0.72	24.05
	Pentadecanol	$C_{15}H_{32}O$	18.35	0.50	17.35
	Tetradecanoic acid	$C_{14}H_{28}O_2$	21.42	0.37	13.41
	3-Hexenoic acid	$C_6H_{10}O_2$	10.97	0.11	5.49
Phytosterols	Ergosta-5,22-dien-3-ol	$C_{28}H_{46}O$	36.65	2.74	85.26
	γ-ergostenol	$C_{28}H_{48}O$	38.75	0.73	24.19
	19-cycloergost-24(28)-en-3-olalpha. 5, alpha.)-	$C_{32}H_{52}O_2$	39.15	0.81	26.76
Diterpenoids	Phytol acetate	$C_{22}H_{42}O_2$	22.28	7.65	233.70
	Phytol	$C_{20}H_{40}O$	24.95	1.92	60.40
Ketones	Ethanone, 1-(4-methylphenyl)-	$C_9H_{10}O$	14.21	0.08	4.55
Phenolics	Phenol, 2,4-bis(1-dimethylethyl)-	$C_{22}H_{22}O$	18.62	0.11	5.48
Triterpenoids	Squalene	$C_{30}H_{50}$	30.84	3.97	122.41
	β-amyrin	$C_{30}H_{50}O$	38.34	1.25	39.98

Table 4.6: Cont'd

Class of Compounds	Name and Class of Compound	Molecular Formula	Retention Time (Min)	Relative Abundance (%)	Conc. (ng/g)
Sesquiterpenoids	Nerolidol	$C_{15}H_{26}O$	19.27	1.78	56.13
	Pentadecanone	$C_{18}H_{36}O$	22.35	0.90	29.40
	Dehydroxy-isocalamendiol	$C_{15}H_{26}O_2$	24.36	0.74	24.66
	α-copaene	$C_{15}H_{24}$	16.99	0.42	14.93
	Neointermedeol	$C_{16}H_{26}O$	20.46	0.37	13.55
	Caryophyllene oxide	$C_{15}H_{24}O$	19.65	0.32	11.87
	α-isocomene	$C_{15}H_{24}$	17.17	0.10	5.21
Monoterpenoids	cis-b-ccimene	$C_{10}H_{16}$	13.07	0.31	11.55
	Linalool	$C_{10}H_{18}O_0$	12.79	0.18	7.58
	Modheph-2-ene	$C_{10}H_{20}$	17.07	0.12	5.93
	Terpinen-4-ol	$C_{10}H_{18}O_0$	14.08	0.12	5.84
	Limonene	$C_{10}H_{16}$	11.55	0.09	5.06
	Sabinene	$C_{10}H_{18}O$	12.25	0.08	4.57
	α-pinene	$C_{10}H_{16}$	9.59	0.08	4.53
Furan and Furanones	Benzofuranone	$C_{11}H_{16}O_2$	18.99	0.23	9.31
Others	Butanoic acid, 2-methyl-	$C_5H_{10}O_2$	7.59	0.08	4.53
	p-xylene	$C_6H_4(CH_3)_2$	8.08	0.17	7.42
	Benzeneacetic acid	$C_8H_8O_2$	15.06	0.14	6.37

Summary of compounds identified in the ethyl acetate extract of *V. lasiopus* with their RT: Retention time (Min); Relative Abundance (%); Compound Concentration (ng/g); Molecular Formula and Relative Molecular Mass. Compounds are listed in the order of concentration in respective chemical class

This study found that several compounds were common in ethyl acetate leaf extracts of both *T. diversifolia* and *V. lasiopus* (Table 4.7). The compounds were expressed in different concentrations (ng/g) as summarized in Table 4.7. Notably the phytochemical analysis of the DCM leaf extracts of the two plants revealed that the *T. diversifolia* extract had the highest concentration of all the common compounds found present in the extracts of two plants (Table 4.7).

Table 4.7: Phytochemicals Present in the Ethyl Acetate Leaf Extracts of both *T. diversifolia* and *V. lasiopus*

Compound Name	**Molecular Formula**	**Chemical Class**	**Concentration (ng/g)**	
			T. diversifolia	***V. lasiopus***
Octadecatrienoic acid	$C_{18}H_{30}O_2$	FAD	459.03	187.89
Propanoic acid, 2-methyl	$C_4H_8O_2$	FAD	267.83	140.28
Squalene	$C_{30}H_{50}$	TT	448.08	122.41
Methyl linoleate	$C_{19}H_{34}O_2$	FAD	370.28	121.30
Octadecanoic acid	$C_{18}H_{36}O_2$	FAD	188.86	86.26
Ergosta-5,22-dien-3-ol,	$C_{28}H_{46}O$	S	204.89	85.26
Phytol	$C_{20}H_{40}O$	DT	321.37	60.40
Nerolidol	$C_{15}H_{26}O$	ST	86.54	56.13
β-amyrin	$C_{30}H_{50}O$	TT	297.63	39.98
2-pentadecanone	$C_{18}H_{36}O$	ST	56.01	29.40
Pentadecanol	$C_{15}H_{32}O$	FAD	21.38	17.35
α-copaene	$C_{15}H_{24}$	ST	21.54	14.93
Tetradecanoic acid	$C_{14}H_{28}O_2$	FAD	45.02	13.41
Caryophyllene oxide	$C_{15}H_{24}O$	ST	19.33	11.87

P stands for phenolic, ST for sesquiterpenoid, TT for triterpenoid, DT for diterpenoid, MT for monoterpenoid, S for phytosterol, AD for aldehyde and FAD for fatty acid derivatives

4.3 Fumigant Toxicity Effects of the Extracts of *T. diversifolia* and *V. lasiopus*

4.3.1 Fumigant Toxicity Effects of DCM Extracts of *T. diversifolia* and *V. lasiopus*

Generally, the DCM leaf extracts of *T. diversifolia* and *V. lasiopus* caused death of *S. zeamais*, upon fumigation. The number of dead weevils increased with increase in the extract concentration (Table 4.8; Appendix 3.1 and 3.2). The effects of extracts on the weevils also continued to increase gradually with exposure time. None of the *S. zeamais* introduced in the negative control group died throughout the test period (Table 4.8; Appendix 3.1 and 3.2).

Six hours after treatment, the DCM leaf extract of *T. diversifolia* caused comparable ($p>0.005$) weevil mortality of 23.73, 31.23, 27.48 and 28.73% at the tested dose levels of 25, 50, 75 and 100%, respectively (Table 4.8). Although the four dose levels demonstrated significantly higher fumigant-induced mortality of *S. zeamais* than the negative control sample ($p<0.005$; Table 4.8; Appendix 3.1), their effects were significantly lower than that of the reference pesticide, Actellic Super ^{TM} ($p<0.005$; Table 4.8).

Twenty-four hours post-treatment, the DCM leaf extracts of *T. diversifolia*, at the concentration level of 100%, caused significantly more deaths of *S. zeamais* when compared to the effects of other extracts concentrations ($p<0.005$; Table 4.8). The percent mortalities recorded by the 25, 50 and 75% extract concentrations were not significantly different at this test period ($p>0.005$; Table 4.8).

The extracts at the dose level of 100% remained the most toxic to *S. zeamais* among all the extract concentrations (Table 4.8; Appendix 3.1). Further, the effectiveness of this extract dose level was statistically comparable to that of the standard pesticide, Actellic Super $^{\text{TM}}$ after 72 hours of exposure to weevils ($p>0.005$; Table 4.8). Fumigant toxicity effects of *T. diversifolia* extract at lower dose levels of 25 and 50% were more effective than the unprotected grain sample ($p<0.005$; Table 4.8).

Following 72 and 96 hours of exposure to the weevils, the DCM extract of *T. diversifolia*, at the dose level of 75% also recorded remarkably high weevil mortalities that were not significantly different from that caused by the extract dose level of 100% as well as by the reference pesticide ($p>0.005$; Table 4.8).

Table 4.8: Fumigant Toxicity Effects of DCM Leaf Extracts of *T. diversifolia* Against *S. zeamais*

Group	**Treatment**	**Mean % of Corrected Mortality (Pr) ± S.E with Exposure Period (hours)**				
	(% Extract)	6hr	24hr	48hr	72hr	96hr
Negative Control	Solvent Only	00.00 ± 0.00^{cA}	00.00 ± 0.00^{dA}	00.00 ± 0.00^{dA}	00.00 ± 0.00^{cA}	00.00 ± 0.00^{cA}
Positive Control	Actellic Super ™	77.50 ± 1.44^{aB}	81.22 ± 1.23^{aB}	82.47 ± 1.43^{aB}	96.18 ± 2.42^{aA}	97.45 ± 2.51^{aA}
DCM Leaf Extract	25% (v/v)	23.73 ± 2.37^{bC}	27.48 ± 3.25^{cC}	42.48 ± 3.22^{bcB}	43.67 ± 2.40^{bB}	57.42 ± 3.23^{bA}
	50% (v/v)	31.23 ± 2.42^{bB}	34.98 ± 2.04^{bcB}	37.48 ± 1.46^{cB}	52.42 ± 3.22^{bA}	66.17 ± 2.42^{bA}
	75% (v/v)	27.48 ± 1.46^{bC}	33.73 ± 1.24^{bcC}	56.23 ± 3.15^{bB}	88.68 ± 3.80^{aA}	93.68 ± 2.40^{aA}
	100% (v/v)	34.98 ± 2.04^{bB}	43.73 ± 1.27^{bB}	91.22 ± 2.40^{aA}	91.18 ± 2.37^{aA}	94.93 ± 2.04^{aA}

Values expressed as mean ± SEM for four replicates per group (n=4). Statistical comparisons were made within each column and row. The values followed by the same superscripts along the row (upper case) and column (lower case) are not significantly different by one-way ANOVA ($p>0.005$) followed by Tukey's post hoc test.

On the other hand, it was observed that, after six hours of treatment, DCM leaf extract of *V. lasiopus,* at extract concentrations of 25, 50 and 75%, caused low weevil mortalities, which were not significantly different from the effects of the solvent (negative control) ($p>0.005$; Table 4.9). After 24 hours of exposure of insects to the fumigants, the three higher extract concentrations (50, 75 and 100%) continued to cause higher weevil mortalities, which were statistically similar ($p>0.005$) to each other but lower ($p<0.005$) than that caused by the dose of 100% as well as that caused by Actellic Super TM (standard pesticide). All the extract dosages remained comparable ($p>0.005$) to the unprotected grain samples except that of 100% concentrations (Table 4.9).

Fumigant toxicities of DCM leaf extract of *V. lasiopus* were more pronounced at concentration level of 100 percent killing between 28.73 and 77.43% of adult weevils throughout the test period (Table 4.9). However, after 72 and 96 hours of exposure of extract to the insects the dose of 75% caused equally high weevil mortality ($p>0.005$; Table 4.9) compared with that at concentration level of 100 percent. None of the *V. lasiopus* leaf extract concentrations matched the effectiveness of the standard synthetic pesticide, Actellic Super TM ($p<0.005$; Table 4.9) at any of the test periods.

Table 4.9: Fumigant Toxicity Effects of DCM Leaf Extract of *V. lasiopus* (O. Hoffman) Against *S. zeamais*

Group	**Treatment**	**Mean % of Corrected Mortality (Pr) ± S.E with Exposure Period (hours)**				
	(% Extract)	6hr	24hr	48hr	72hr	96hr
Negative Control	Solvent Only	00.00 ± 0.00^{cA}	00.00 ± 0.00^{dA}	00.00 ± 0.00^{dA}	00.00 ± 0.00^{dA}	00.00 ± 0.00^{dA}
Positive Control	Actellic Super ™	77.47 ± 1.43^{aB}	81.22 ± 1.23^{aB}	82.47 ± 1.43^{aB}	96.18 ± 2.42^{aA}	97.43 ± 2.51^{aA}
DCM Leaf Extract	25% (v/v)	01.23 ± 1.23^{cC}	11.23 ± 1.26^{cdB}	21.23 ± 1.26^{cB}	33.67 ± 1.29^{cA}	38.67 ± 1.29^{cA}
	50% (v/v)	04.97 ± 2.04^{cC}	16.23 ± 3.12^{cB}	31.23 ± 4.27^{cA}	38.67 ± 1.26^{cA}	41.17 ± 1.28^{cA}
	75% (v/v)	04.97 ± 2.06^{cC}	11.23 ± 1.26^{cdC}	34.98 ± 2.87^{cB}	62.43 ± 3.23^{bA}	66.18 ± 3.17^{bA}
	100% (v/v)	28.73 ± 2.41^{bD}	43.73 ± 2.41^{bC}	63.73 ± 2.41^{bB}	68.68 ± 2.43^{bAB}	77.43 ± 1.46^{bA}

Values expressed as mean ± SEM for four replicates per group (n=4). Statistical comparisons were made within each column and row. The values followed by the same superscripts along the row (upper case) and column (lower case) are not significantly different by one-way ANOVA ($p>0.005$) followed by Tukey’s post hoc test.

Generally, the DCM extract of *T. diversifolia* was found to be more efficacious than that of *V. lasiopus* (p<0.005). *Tithonia diversifolia* caused higher weevil mortalities at all dose levels as compared to mortalities caused by *V. lasiopus* (Figure 4.1). However, the two plants exhibited comparable weevil mortalities at the dose levels of 100% following 24 hours of exposure to weevils (p>0.005; Figure 4.1). At the extract dose of 50%, *T. diversifolia* caused high weevil mortality relative to *V. lasiopus*. Similar observations were also made at the extract dose of 25 and 50% after 72 hours of exposure of the extract to weevils (p>0.005; Figure 4.1).

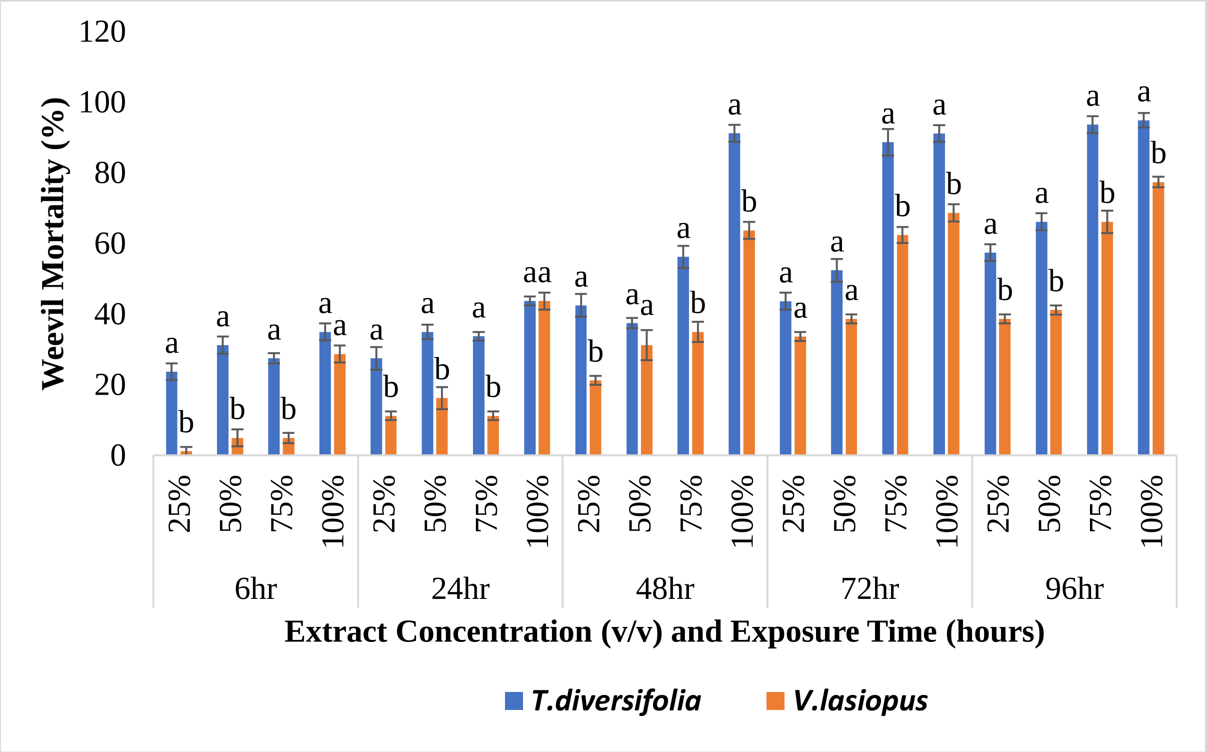

Figure 4.1: Comparison of the Fumigant Toxicity Effects (Corrected Percent Mortality) of the DCM Leaf Extracts of *T. diversifolia* and *V. lasiopus* Against *S. zeamais*

Bars with different superscripts within the same concentration (%) are significantly different calculated by un-paired student *t*- test at the Respective Test Period (hours; p<0.005)

4.3.2 Fumigant Toxicity Effects of EtOAc Extracts of *T. diversifolia* and *V. lasiopus*

In general, the ethyl acetate extracts of *T. diversifolia* and *V. lasiopus* caused increase in weevil mortality with increase in time of exposure (Table 4.10 and 4.11; Appendix 3.3 and 3.4). No weevil mortality was reported by the solvent (ethyl acetate) in the negative control group after its exposure to *S. zeamais* throughout the whole experiment period (Table 4.10 and 4.11).

After six hours of exposure to weevils, the *T. diversifolia* extract, at the doses of 25, 50, 75 and 100% manifested comparable weevil mortalities of 20.00, 28.75, 17.50 and 21.25%, respectively ($p>0.005$; Table 4.10). These results were significantly higher than that caused by the negative control sample ($p<0.005$). However, these effects were significantly lower than the effect of the standard pesticide, Actellic Super ^{TM} ($p<0.005$; Table 4.10).

After 24 hours of exposure to weevils, the *T. diversifolia* extract at the higher concentrations (75 and 100%), produced high fumigant-induced mortalities of 83.72 and 76.22%, respectively, which was statistically comparable to the weevil mortality caused by Actellic Super ^{TM} (81.22%) ($p>0.005$; Table 4.10). Following 48 hours of exposure to weevils, the dose of 100% manifested a significantly better weevil killing ability (97.47%) as compared to that caused by the standard pesticide, Actellic Super ^{TM} (82.22%); ($p<0.005$; Table 4.10).

It was further observed that the efficacy of the *T. diversifolia* extract concentrations of 75 and 100% consistently remained statistically the same throughout the test period ($p>0.005$; Table 4.10). At 50% extract concentration, the *T. diversifolia* extract caused weevil mortality of at 68.70% which was similar at 72 and 96 hours post exposure to weevils (Table 4.10).

Table 4.10: Fumigant Toxicity Effects of Ethyl Acetate Leaf Extract of *T. diversifolia* Against *S. zeamais*

Group	**Treatment** (% Extract)	**Mean Percent Corrected Mortality (Pr) with Exposure Period (hours)**				
		6hr	24hr	48hr	72hr	96hr
Negative Control	Solvent Only	00.00 ± 0.00^{cA}	00.00 ± 0.00^{dA}	00.00 ± 0.00^{dA}	00.00 ± 0.00^{cA}	00.00 ± 0.00^{cA}
Positive Control	Actellic Super ™	77.47 ± 1.43^{aB}	81.22 ± 1.23^{aB}	82.47 ± 1.43^{bB}	96.18 ± 2.42^{aA}	97.43 ± 2.51^{aA}
Ethyl Acetate Extract	25% (v/v)	20.00 ± 2.04^{bC}	31.23 ± 2.40^{cC}	54.98 ± 2.06^{cB}	62.45 ± 3.20^{bAB}	69.95 ± 2.02^{bA}
	50% (v/v)	28.75 ± 2.39^{bC}	56.23 ± 2.38^{bB}	64.97 ± 2.02^{cAB}	68.70 ± 1.27^{bA}	68.70 ± 1.27^{bA}
	75% (v/v)	17.50 ± 3.23^{bC}	83.72 ± 3.14^{aB}	92.47 ± 3.22^{abAB}	96.20 ± 2.40^{aA}	97.45 ± 1.44^{aA}
	100% (v/v)	21.25 ± 2.39^{bC}	76.22 ± 2.42^{aB}	97.47 ± 1.46^{aA}	97.45 ± 2.48^{aA}	98.70 ± 1.23^{aA}

Values expressed as mean ± SEM for four replicates per group (n=4). Statistical comparisons were made within each column and row. The values followed by the same superscripts along the row (upper case) and column (lower case) are not significantly different by one-way ANOVA ($p>0.005$) followed by Tukey’s post hoc test.

On the other hand, ethyl acetate leaf extracts of *V. lasiopus* was noted to have remarkable weevil killing potential throughout the experimental period (Table 4.11; Appendix 3.4). The effectiveness of the *V. lasiopus* extract at all the tested doses was significantly higher than that of the negative control sample, treated with solvent only ($p<0.005$; Table 4.11). However, this was in exception of the 25% dose, where very low mortality was reported, not statistically different from that caused by solvent, after 48 hours of exposure to weevils ($p>0.005$; Table 4.11).

The mortality evoked by the *V. lasiopus* extract dose of 100% (67.47%) was not significantly different from that caused by Actellic Super ^{TM} (82.47%) after 48 hours of exposure to weevils ($p>0.005$; Table 4.11). Nevertheless, none of the rest of *V. lasiopus* extract dosages manifested comparable mortalities with Actellic Super ^{TM}, throughout the test period ($p<0.005$; Table 4.11).

Table 4.11: Fumigant Toxicity Effects of Ethyl Acetate Leaf Extract of *V. lasiopus* Against *S. zeamais*

Group	**Treatment**	**Mean Percent Corrected Mortality (Pr) with Exposure Period (hours)**				
	(% Extract)	6hr	24hr	48hr	72hr	96hr
Negative Control	Solvent Only	00.00 ± 0.00^{cA}	00.00 ± 0.00^{dA}	00.00 ± 0.00^{dA}	00.00 ± 0.00^{dA}	00.00 ± 0.00^{eA}
Positive Control	Actellic Super ^{TM}	77.47 ± 1.43^{aB}	81.22 ± 1.23^{aB}	82.47 ± 1.43^{aB}	96.18 ± 2.42^{aA}	97.43 ± 2.51^{aA}
Ethyl Acetate Leaf Extract	25% (v/v)	13.75 ± 1.25^{bB}	16.23 ± 1.26^{cB}	18.73 ± 1.24^{cdB}	29.95 ± 2.06^{cA}	32.45 ± 2.52^{dA}
	50% (v/v)	20.00 ± 2.04^{bC}	27.48 ± 3.25^{cBC}	37.48 ± 3.25^{bcA}	42.45 ± 4.35^{cA}	32.45 ± 2.52^{cdAB}
	75% (v/v)	22.50 ± 3.23^{bB}	26.23 ± 3.74^{cB}	39.98 ± 4.55^{bA}	44.95 ± 2.04^{cA}	49.95 ± 2.04^{cA}
	100% (v/v)	26.25 ± 3.15^{bC}	46.23 ± 2.38^{bB}	67.47 ± 4.77^{aA}	69.95 ± 4.06^{bA}	72.45 ± 3.21^{bA}

Values expressed as mean ± SEM for four replicates per group (n=4). Statistical comparisons were made within each column and row. The values followed by the same superscripts along the row (upper case) and column (lower case) are not significantly different by one-way ANOVA ($p>0.005$) followed by Tukey's post hoc test.

Generally, a comparison between ethyl acetate leaf extract of *T. diversifolia* indicated a more effective weevils killing potential as compared to the *V. lasiopus* extract (Figure 4.2). Six hours after exposure of the extracts to the weevils, the two plants showed no statistical difference ($p>0.05$) in their effectiveness against *S. zeamais* (Figure 4.2). However, during the rest of the experiment period, the ethyl acetate extract of *T. diversifolia* remained significantly the most toxic on weevils upon fumigation ($p<0.005$; Figure 4.2).

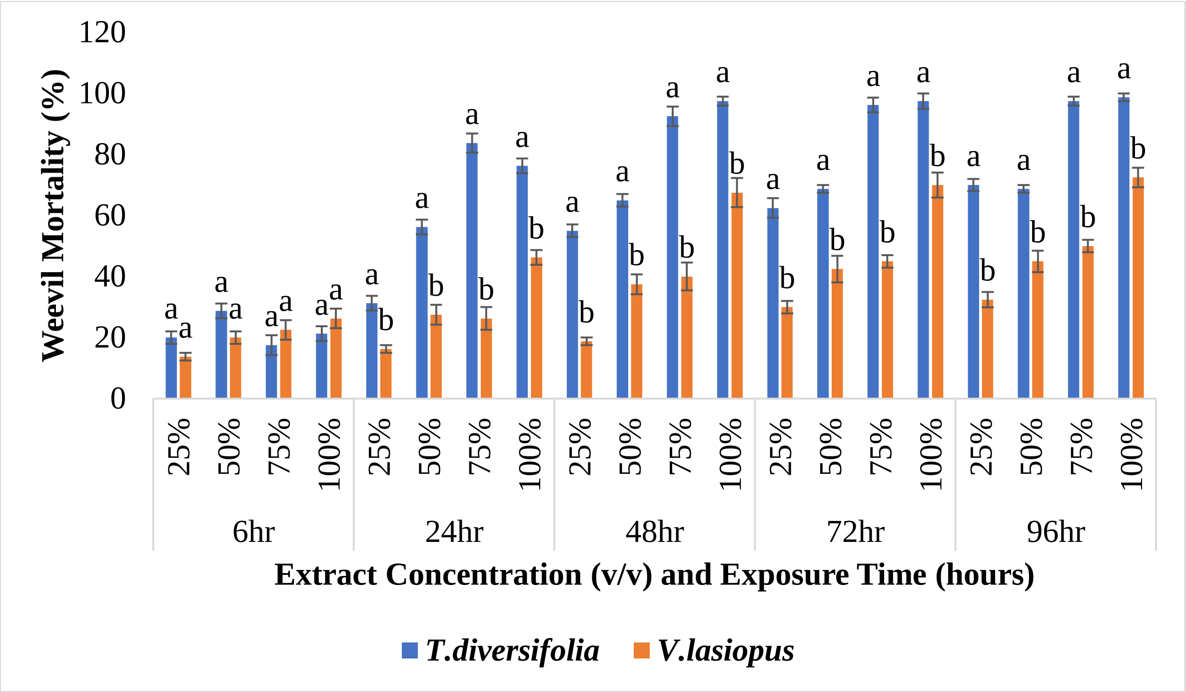

Figure 4.2: Comparison of the Fumigant Toxicity Effects (Corrected Percent Mortality) of the Ethyl Acetate Leaf Extracts of *T. diversifolia* and *V. lasiopus* Against *S. zeamais*

Bars with different superscripts within the same concentration (%) are significantly different calculated by un-paired student *t*- test at the Respective Test Period (hours; $p<0.005$)

4.4 Contact Toxicity Effects of Extracts of *T. diversifolia* and *V. lasiopus*

4.4.1 Contact Toxicity Effects of DCM Extracts of *T. diversifolia* and *V. lasiopus*

As Tables 4.12 and 4.13 shows, the DCM leaf extracts of *T. diversifolia* and *V. lasiopus* generally caused remarkable weevil mortality upon contact in all the tested doses (Tables 4.12 and 4.13; Appendix 4.1 and 4.2). It was observed that, the two plants showed an increase in weevil mortality with increase in exposure time to weevils throughout the experimental period (Tables 4.12 and 4.13). In this study, the solvent (Dichloromethane) was reported to have no weevil killing potential throughout the experimental period (Tables 4.12 and 4.13).

Twenty-four hours post-exposure to weevils, the *T. diversifolia* extract dose of 75% caused the least weevil mortality (72.47%), which was significantly lower than the effects caused by the other dosages ($p<0.005$; Table 4.12). At the same time, the *T. diversifolia* extract doses of 25, 50 and 100% evoked statistically similar weevil killing abilities, which also matched the effects caused by Actellic Super ^{TM} ($p>0.005$; Table 4.12).

Following 48, 72 and 96 hours of exposure to weevils, the contact toxicity effects of the *T. diversifolia* extract, at all concentrations was comparable to each other ($p>0.005$; Table 4.12). Further, the effects caused by all the *T. diversifolia* extracts concentrations were found to be statistically comparable to the effects of the standard pesticide, Actellic Super ^{TM} after long durations of exposure to *S. zemails* ($p>0.005$; Table 4.12).

Table 4.12: Contact Toxicity Effects of DCM Leaf Extracts of *T. diversifolia* Against *S. zeamais*

Group	**Treatment**	**Mean Percent Corrected Mortality (Pr) with Exposure Period (hours)**				
	(% Extract)	6hr	24hr	48hr	72hr	96hr
Negative Control	Solvent Only	00.00 ± 0.00^{cA}	00.00 ± 0.00^{cA}	00.00 ± 0.00^{bA}	00.00 ± 0.00^{bA}	00.00 ± 0.00^{bA}
Positive Control	Actellic Super ^{TM}	95.00 ± 2.04^{aA}	97.47 ± 2.49^{aA}	99.98 ± 0.25^{aA}	99.95 ± 0.29^{aA}	99.93 ± 0.03^{aA}
DCM Leaf Extract	25% (v/v)	25.00 ± 0.00^{bB}	92.50 ± 2.50^{aA}	92.50 ± 2.50^{aA}	94.95 ± 0.05^{aA}	97.40 ± 2.50^{aA}
	50% (v/v)	33.75 ± 2.39^{bB}	89.97 ± 4.56^{aA}	94.97 ± 2.87^{aA}	97.45 ± 1.41^{aA}	98.68 ± 1.23^{aA}
	75% (v/v)	36.25 ± 3.15^{bC}	72.47 ± 3.23^{bB}	94.97 ± 2.06^{aA}	98.70 ± 1.27^{aA}	99.93 ± 0.03^{aA}
	100% (v/v)	33.75 ± 4.27^{bC}	98.72 ± 1.24^{aB}	99.98 ± 0.03^{aA}	99.95 ± 0.02^{aA}	99.93 ± 0.03^{aA}

Values expressed as mean ± SEM for four replicates per group (n=4). Statistical comparisons were made within each column and row. The values followed by the same superscripts along the row (upper case) and column (lower case) are not significantly different by one-way ANOVA ($p>0.005$) followed by Tukey's post hoc test.

In this study, the DCM leaf extracts of *V. lasiopus* also demonstrated considerable dose dependent contact-induced weevil mortalities (Table 4.13; Appendix 4.6). However, it was apparent that none of the *V. lasiopus* extracts caused weevil mortality in a comparable fashion to that caused by the standard pesticide, Actellic Super ^{TM} ($p<0.005$; Table 4.13).

The *V. lasiopus* extract concentrations of 25 and 50% caused comparable weevil mortalities throughout the experimental period ($p>0.005$; Table 4.13). The two highest extract concentrations (75 and 100%) caused significantly different weevil mortalities upon contact compared with the rest of tested doses ($p<0.005$; Table 4.13). This was only in exception of the observation noted after 72 hours of exposure to weevils, when the two dosages caused statistically similar mortalities of 43.7 and 57.45%, respectively ($p>0.005$; Table 4.13). However, these affects were significantly lower than the effects of the reference pesticide, Actellic Super ^{TM} ($p<0.005$; Table 4.13).

Table 4.13: Contact Toxicity Effects of DCM Leaf Extracts of *V. lasiopus* Against *S. zeamais*

Group	**Treatment**	**Mean Percent Corrected Mortality (Pr) with Exposure Period (hours)**				
	(% Extract)	6hr	24hr	48hr	72hr	96hr
Negative Control	Solvent Only	00.00 ± 0.00^{dA}	00.00 ± 0.00^{dA}	00.00 ± 0.00^{eA}	00.00 ± 0.00^{dA}	00.00 ± 0.00^{eA}
Positive Control	Actellic Super ^{TM}	95.00 ± 2.04^{aA}	97.47 ± 2.49^{aA}	99.98 ± 0.25^{aA}	99.95 ± 0.29^{aA}	99.93 ± 0.03^{aA}
DCM Leaf Extract	25% (v/v)	01.67 ± 1.67^{dC}	06.63 ± 1.63^{dC}	18.30 ± 3.30^{dB}	24.93 ± 4.98^{cAB}	31.57 ± 1.67^{dA}
	50% (v/v)	02.50 ± 2.50^{dC}	11.23 ± 2.38^{dBC}	18.73 ± 2.39^{dB}	26.20 ± 3.15^{cAB}	32.43 ± 1.43^{dA}
	75% (v/v)	25.00 ± 2.04^{cB}	29.98 ± 2.06^{cB}	31.23 ± 2.42^{cB}	43.70 ± 3.14^{bA}	54.93 ± 2.06^{cA}
	100% (v/v)	36.25 ± 1.25^{bC}	46.23 ± 1.23^{bBC}	47.48 ± 1.43^{bB}	57.45 ± 3.20^{bA}	66.18 ± 1.24^{bA}

Values expressed as mean ± SEM for four replicates per group (n=4). Statistical comparisons were made within each column and row. The values followed by the same superscripts along the row (upper case) and column (lower case) are not significantly different by one-way ANOVA ($p>0.005$) followed by Tukey’s post hoc test.

In comparison, it was observed that, the DCM leaf extract of *T. diversifolia* generally evoked a more effective weevils killing potential as compared to the *V. lasiopus* extract (Figure 4.3). Nevertheless, following 6 hours of exposure to weevils, the extracts of both *T. diversifolia* and *V. lasiopus*, at the doses of 75 and 100%, showed no significant difference in their effectiveness ($p>0.005$; Figure 4.3). However, during the rest of the experimental period, the *T. diversifolia* extract remained significantly the most potent plant against *S. zeamais* across the tested concentration ($p<0.005$; Figure 4.3).

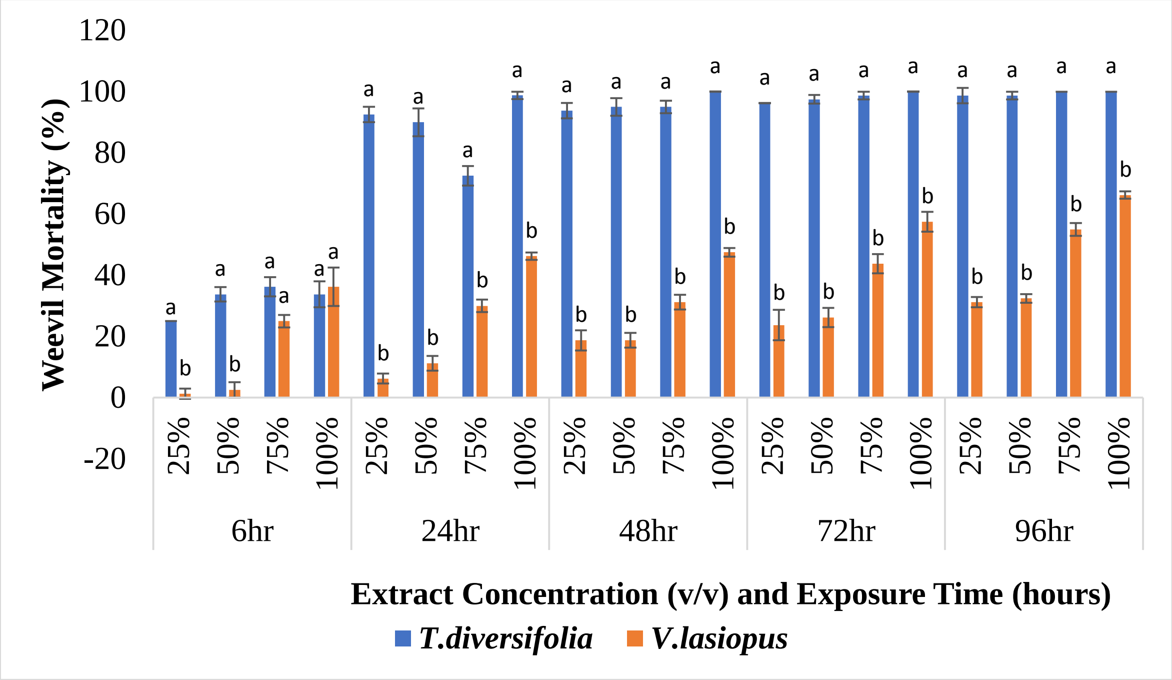

Figure 4.3: Comparison of the Contact Toxicity Effects (Corrected Percent Mortality) of the DCM Leaf Extracts of *T. diversifolia* and *V. lasiopus* Against *S. zeamais*

Bars with different superscripts within the same concentration (%) are significantly different calculated by un-paired student *t*- test at the Respective Test Period (hours; $p<0.005$)

4.4.2 Contact Toxicity Effects of EtOAc Extracts of *T. diversifolia* and *V. lasiopus*

In general, the ethyl acetate leaf extracts of *T. diversifolia* and *V. lasiopus* demonstrated weevil killing abilities, which increased with increase in extract dosage only after 6 hour exposure time (Tables 4.14 and 4.15; Appendices 4.3 and 4.4). The effectiveness of these plant extracts was also found to increase with increase in exposure time to maize weevils (Tables 4.14 and 4.15). All the weevils in the negative control group were found alive, showing zero percent mortality (Tables 4.14 and 4.15).

This study demonstrated that, apart from the *T. diversifolia* extract dose of 100%, none of the other dosages showed weevil mortalities comparable to that caused by Actellic Super $^{\text{TM}}$ following a short duration (six hours) of exposure to weevils ($p<0.005$; Tables 4.14). In the rest of the experimental period, it was found that the *T. diversifolia* extract doses of 75 and 100% were as effective as the standard pesticide, Actellic Super $^{\text{TM}}$ ($p>0.005$; Table 4.14).

After 48 and 72 hours of exposure to weevils, the effectiveness of the *T. diversifolia* extract doses of 25 and 50% were found to be comparable to each other ($p>0.005$) though significantly lower than the rest of extract treatments ($p<0.005$; Table 4.14). At the last observation time (96 hours post exposure), the *T. diversifolia* extract dose of 50% was found to be equally effective as the extract doses of 75 and 100% as well as the reference pesticide, Actellic Super $^{\text{TM}}$ ($p>0.005$; Table 4.14).

Table 4.14: Contact Toxicity Effects of Ethyl Acetate Leaf Extract of *T. diversifolia* Against *S. zeamais*

Group	**Treatment**	**Mean Percent Corrected Mortality (Pr) with Exposure Period (hours)**				
	(% Extract)	6hr	24hr	48hr	72hr	96hr
Negative Control	Solvent Only	00.00± 0.00^{eA}	00.00± 0.00^{dA}	00.00± 0.00^{cA}	00.00± 0.00^{cA}	00.00± 0.00^{cA}
Positive Control	Actellic Super ^{TM}	95.00 ± 2.04^{aA}	97.47 ± 2.49^{aA}	99.98 ± 0.25^{aA}	99.95 ± 0.29^{aA}	99.93 ± 0.03^{aA}
Ethyl Acetate Extract	25% (v/v)	31.67 ± 1.67^{Db}	33.30 ± 1.65^{cB}	35.30 ± 1.65^{bB}	61.60 ± 1.65^{bB}	74.93 ± 2.86^{bA}
	50% (v/v)	47.50 ± 1.44^{cD}	54.98 ± 2.04^{bC}	62.48 ± 5.22^{bBC}	66.20 ± 1.27^{bB}	98.65 ± 1.25^{aA}
	75% (v/v)	66.25 ± 1.25^{bC}	87.47 ± 1.43^{aB}	92.47 ± 1.43^{aAB}	97.45 ± 2.52^{aAB}	98.65 ± 1.25^{aA}
	100% (v/v)	86.25 ± 3.15^{aB}	92.47 ± 2.52^{aAB}	96.22 ± 2.42^{aAB}	98.70 ± 1.23^{aA}	99.90 ± 0.04^{aA}

Values expressed as mean ± SEM for four replicates per group (n=4). Statistical comparisons were made within each column and row. The values followed by the same superscripts along the row (upper case) and column (lower case) are not significantly different by one-way ANOVA ($p>0.005$) followed by Tukey’s post hoc test.

On the other hand, 6 hours after exposure to weevils, the *V. lasiopus* extract evoked weevil mortalities of 12.50, 23.75, 32.50 and 41.25% at doses of 25, 50, 75 and 100%, respectively (Table 4.15). These effects were significantly different from those reported by both positive and unprotected grains samples ($p<0.005$; Table 4.15).

It was apparent that the effectiveness of the *V. lasiopus* extract at low doses of 25 and 50% were not significantly different from each other ($p>0.005$) after 6, 24, 72 and 96 hours exposure time except after 48 hours of exposure (Table 4.15). Likewise, the high extract doses (75 and 100%), evoked statistically similar weevil mortalities after 6, 48, 72 and 96 hour exposure time of the *V. lasiopus* extract exposure to weevils ($p>0.005$; Table 4.15).

This study further demonstrated that the effect of the *V. lasiopus* extract did not match the effectiveness of synthetic pesticide after a short duration of exposure to weevils (<48 hours) ($p<0.005$; Table 4.15). However, the higher extract dosages of *V. lasiopus* (75 and 100%) evoked mortalities which were comparable to each other as well to the effect caused by Actellic Super ^{TM} following exposure to weevils for 72 and 96 hours ($p>0.005$; Table 4.15). It was also evident that after long durations of exposure to weevils (72 and 96 hours), the *V. lasiopus* extract doses of 25 and 50% remained equally effective ($p>0.005$) but significantly lower than other treatments ($p<0.005$; Table 4.15).

Table 4.15: Contact Toxicity Effects of Ethyl Acetate Leaf Extracts of *V. lasiopus* Against *S. zeamais*

Group	**Treatment**	**Mean Percent Corrected Mortality (Pr) with Exposure Period (hours)**				
	(% Extract)	6hr	24hr	48hr	72hr	96hr
Negative Control	Solvent Only	00.00 ± 0.00^{eA}	00.00 ± 0.00^{eA}	00.00 ± 0.00^{eA}	00.00 ± 0.00^{cA}	00.00 ± 0.00^{cA}
Positive Control	Actellic Super ™	95.00 ± 2.04^{aA}	97.47 ± 2.49^{aA}	99.98 ± 0.25^{aA}	99.95 ± 0.29^{aA}	99.93 ± 0.03^{aA}
Ethyl Acetate Extract	25% (v/v)	12.50 ± 1.44^{dBA}	13.73 ± 2.41^{deB}	19.98 ± 2.04^{dB}	28.70 ± 2.42^{bB}	63.65 ± 4.28^{bA}
	50% (v/v)	23.75 ± 2.39^{cdC}	26.23 ± 4.29^{cdC}	37.46 ± 1.43^{cB}	43.70 ± 3.76^{bB}	68.6 ± 3.17^{bA}
	75% (v/v)	32.50 ± 2.50^{bcD}	38.73 ± 1.24^{cD}	71.22 ± 3.12^{bC}	82.45 ± 3.21^{aB}	93.65 ± 2.36^{aA}
	100% (v/v)	41.25 ± 2.39^{bD}	54.98 ± 2.04^{bC}	77.47 ± 4.80^{bB}	84.95 ± 7.34^{aB}	97.40 ± 1.44^{aA}

Values expressed as mean ± SEM for four replicates per group (n=4). Values expressed as mean ± SEM for four replicates per group (n=4). Statistical comparisons were made within each column and row. The values followed by the same superscripts along the row (upper case) and column (lower case) are not significantly different by one-way ANOVA (p>0.005) followed by Tukey’s post hoc test.

Upon comparison between the ethyl acetate leaf extract of *T. diversifolia* and that of *V. lasiopus*, results indicated that *T. diversifolia* generally demonstrated more potent weevils killing abilities as compared to the *V. lasiopus* extract (Figure 4.4). All the tested concentrations of the *T. diversifolia* extract showed significantly higher weevil mortality after 6, 24 and 48 hours of exposure to the weevils than the effects of the *V. lasiopus* extract ($p \leq 0.05$; Figure 4.4).

However, following 72 hours of exposure to weevils, the 100% dose of the two plants showed no significant statistical difference in their effectiveness against *S. zeamais* ($p > 0.05$; Figure 4.4). At the observation time of 96 hours, the two exracts, at extract concentrations of 25, 75 and 100% were equally effective against *S. zeamais* ($p > 0.05$; Figure 4.4).

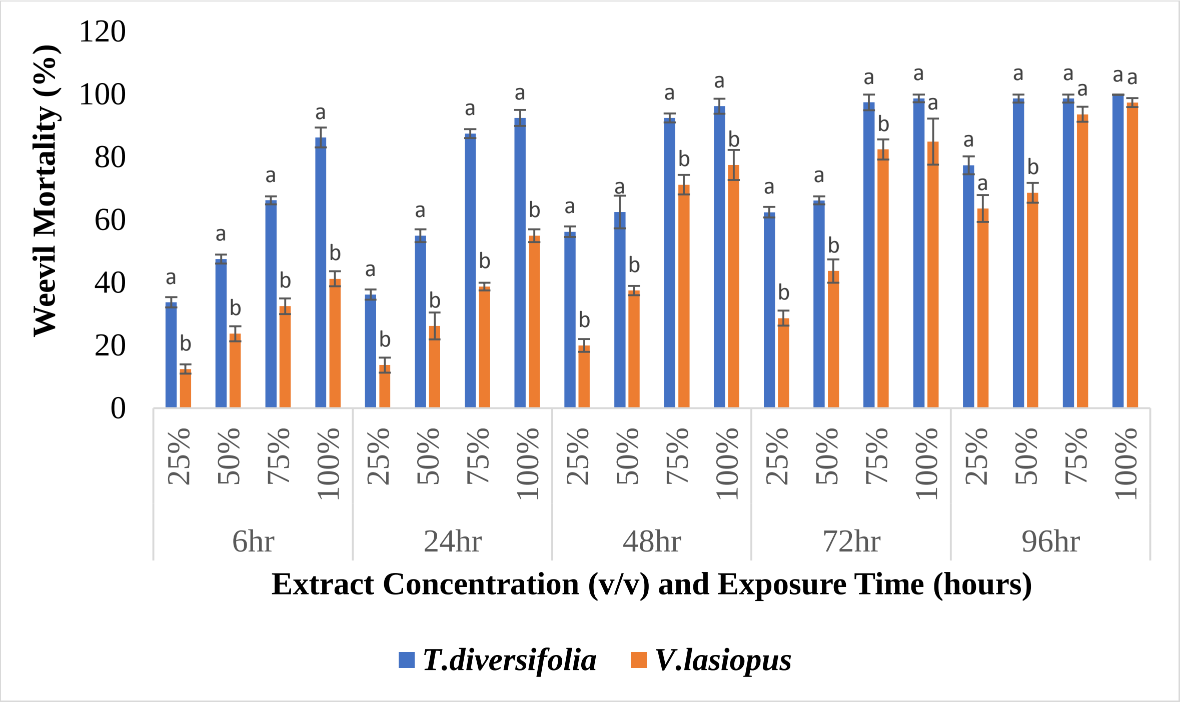

Figure 4.4: **Comparison of the Contact Toxicity Effects (Corrected Percent Mortality) of Ethyl Acetate Leaf Extracts of *T. diversifolia* and *V. lasiopus* Against *S. zeamais***

Bars with different superscripts within the same concentration (%) are significantly different calculated by un-paired student *t*- test at the Respective Test Period (hours; $p<0.005$)

4.5 Repellent Activity of Extracts of *T. diversifolia* and *V. lasiopus*

4.5.1 Repellent Activity of DCM Extracts of *T. diversifolia* and *V. lasiopus*

Generally, the DCM leaf extracts of *T. diversifolia* and *V. lasiopus* repelled *S. zeamais* with repellency index (IP) values of less than one (Table 4.16 and 4.17). Overall, the average repellency activities of the *T. diversifolia* and *V. lasiopus* extracts were largely dose dependent, as they resulted in a regular pattern of repellency, from the lowest to the highest dosages (Table 4.16 and 4.17). In the first hour of experimental period, The *T. diversifolia* extracts dose of 75% evoked a greater repellency (100%) as compared to that of 100% extract concentration (95%), (Table 4.16).

After two hours of exposure to weevils, the *T. diversifolia* extracts doses of 25 and 50% remained comparable to each other ($p>0.005$; Table 4.16) but significantly different from the rest of treatment samples ($p<0.005$; Table 4.16). The *T. diversifolia* extract dose of 100% caused a 100% repellent effect after the second and third hours of exposure to weevils. It was noted that these effects were not significantly different from that caused by the standard pesticide, Actellic Super ^{TM} ($p>0.005$; Table 4.16). The *T. diversifolia* extract dose of 25 and 50% caused the least repellent effects on weevils at the fourth hour of the experimental period as compared with 75 and 100% as well as actellic treated weevils (Table 4.16).

It was observed that the mean percentage repellent effects of the *T. diversifolia* extracts after the 5 hours experimental period ranged between 33 and 96% (Table 4.16). Only the *T. diversifolia* extract doses of 75% and 100% manifested repellency activities that were not significantly different from the effects caused by Actellic Super ^{TM} ($p>0.005$; Table 4.16).

Table 4.16: Repellency Activity of the DCM Leaf Extracts of *T. diversifolia* Against *S. zeamais*

Concentration	**PR (Mean % ± S.E.M) ^{m} with Exposure Time (hours)**					**PR**	**IP**
(% Extract)	1hr	2hr	3hr	4hr	5hr	**(Mean±S.E.M) ^{n}**	**(Mean±S.E.M) ^{n}**
25	35.00 ± 5.00^{cAB} (0.65)	45.00 ± 5.00^{bA} (0.55)	45.00 ± 5.00^{bA} (0.55)	15.00 ± 9.57^{bBC} (0.85)	25.00 ± 5.00^{bB} (0.75)	33.00 ± 5.83^{b}	0.67 ± 0.0583^{a}
50	65.00 ± 5.00^{bA} (0.35)	55.00 ± 5.00^{bAB} (0.45)	50.00 ± 5.77^{bB} (0.50)	50.00 ± 5.77^{bB} (0.50)	35.00 ± 5.00^{bC} (0.65)	51.00 ± 4.84^{b}	0.49 ± 0.0485^{a}
75	100.00 ± 0.00^{aA} (0.00)	95.00 ± 5.00^{aA} (0.05)	95.00 ± 5.00^{aA} (0.05)	90.00 ± 5.77^{aA} (0.10)	95.00 ± 5.00^{aA} (0.05)	95.00 ± 1.56^{a}	0.05 ± 0.0158^{b}
100	95.00 ± 5.00^{a} (0.05)	100.00 ± 0.0^{aA} (0.00)	100.00 ± 0.00^{aA} (0.00)	90.00 ± 5.77^{aA} (0.10)	95.00 ± 5.00^{aA} (0.05)	96.00 ± 1.87^{a}	0.04 ± 0.0187^{b}
Actellic	100.00 ± 0.00^{a} (0.00)	95.00 ± 5.00^{aA} (0.05)	100.00 ± 0.00^{aA} (0.00)	100.00 ± 0.00^{aA} (0.00)	95.00 ± 5.00^{aA} (0.05)	98.00 ± 1.22^{a}	0.02 ± 0.0122^{b}

Statistical comparisons were made within each column and row. The values followed by the same superscripts along the row (upper case) and column (lower case) are not significantly different by one-way ANOVA ($p>0.005$) followed by Tukey's post hoc test.Superscript ^{m} values were means based on four extract concentrations, four replicates ($n=4$). Superscript ^{n} values were means obtained over the 5hours test duration ($n=5$). Values in parenthesis indicate repellency index (IP<1 for Repellency, IP=1 for Neutral and IP>1for Attractant).

On the other hand, the DCM leaf extract of *V. lasiopus* remarkably repelled *S. zeamais* by an average of between 51 and 91% (Table 4.17; Appendices 5.2) after the 5hours duration of the experiment. The *V. lasiopus* extract concentration of 100%, achieved the highest weevil repelling activity of 80% after one hour of exposure, which later decreased to 85% by the end of fifth hour of exposure to weevils (Table 4.17).

The least repellency activity was manifested by the *V. lasiopus* extract concentration of 50 % at the fourth hour of exposure to weevils (Table 4.17). Like the lower doses of the *V. lasiopus* extract (25 and 50%), the higher doses of 75% and 100% also exhibited comparable weevil repellent effects throughout the experimental period ($p>0.005$; Table 4.17).

It was also observed that the *V. lasiopus* extract dose of 25% produced high repellent effects of 70% and 65% after the second and fifth hours of exposure respectively (Table 4.17). This effectiveness was not significantly different from the effects caused by high extract doses (75 and 100%) as well as the positive control ($p>0.005$; Table 4.17).

On average, after the five hours of this experiment, it was observed that only the *V. lasiopus* extract concentration of 100% manifested repellency effects (0.09), which was not significantly different from that portrayed by synthetic pesticide, Actellic Super ^{TM} (0.02) ($p>0.005$; Table 4.17).

Table 4.17: Repellent Activity of DCM Leaf Extracts of *V. lasiopus* Against *S. zeamais*

Concentration (% Extract)	**PR (Mean % ± S.E.M)^{m} with Exposure Time (hours) Posttreatment**					**PR (Mean± S.E.M)^{n}**	**IP (Mean± S.E.M)^{n}**
	1hr	2hr	3hr	4hr	5hr		
25	55.00±5.00^{bB} (0.45)	70.00±5.77^{abA} (0.30)	55.00±9.57^{bB} (0.45)	65.00±9.57^{bcAB} (0.35)	65.00±5.00^{abAB} (0.35)	62.00±3.00^{bc}	0.38±0.0670^{ab}
50	60.00±8.16^{bA} (0.40)	55.00±5.00^{bAB} (0.45)	50.00±5.77^{bBC} (0.50)	40.00±0.00^{cC} (0.60)	50.00±5.77^{bBC} (0.50)	51.00±3.32^{c}	0.49±0.0332^{a}
75	75.00±5.00^{abAB} (0.25)	70.00±5.77^{abB} (0.30)	85.00±5.00^{abA} (0.15)	70.00±5.77^{abcB} (0.30)	70.00±5.77^{abB} (0.30)	74.00±2.92^{b}	0.26±0.0292^{b}
100	95.00±5.00^{aA} (0.05)	90.00±5.77^{aA} (0.10)	95.00±5.00^{aA} (0.05)	90.00±5.77^{abA} (0.10)	85.00±5.00^{aA} (0.15)	91.00±1.87^{a}	0.09±0.0187^{c}
Actellic	100.00±0.00^{aA} (0.00)	95.00±5.00^{aA} (0.05)	100.00±0.00^{aA} (0.00)	100.00±0.00^{aA} (0.00)	95.00±5.00^{aA} (0.05)	98.00±1.22^{a}	0.02±0.0122^{c}

Statistical comparisons were made within each column and row. The values followed by the same superscripts along the row (upper case) and column (lower case) are not significantly different by one-way ANOVA ($p>0.005$) followed by Tukey's post hoc test.Superscript ^{m} values were means based on four extract concentrations, four replicates (n=4). Superscript ^{n} values were means obtained over the 5-hour test duration (n=5). Figures in parenthesis indicate repellency index (IP<1 for repellency, IP=1 for neutral and IP>1for attractant).

The comparison of the repellent effects of DCM Leaf Extracts of *T. diversifolia* and *V. lasiopus* indicated that, the *V. lasiopus* extract was significantly more effective than the *T. diversifolia* extract at the extracts dose of 25% ($p<0.005$; Figure 4.5). However, the *T. diversifolia* extract dose of 75% manifested a significantly higher effectiveness than the *V. lasiopus* extract at similar concentration ($p<0.005$; Figure 4.5). It was also evident that there was no significant statistical difference in the repellent activities of the two plant extracts at the dose level of 50 and 100% ($p>0.005$; Figure 4.5). Both plant extracts had equal mean weevil repellency of 51% at dose of 50% (Figure 4.5).

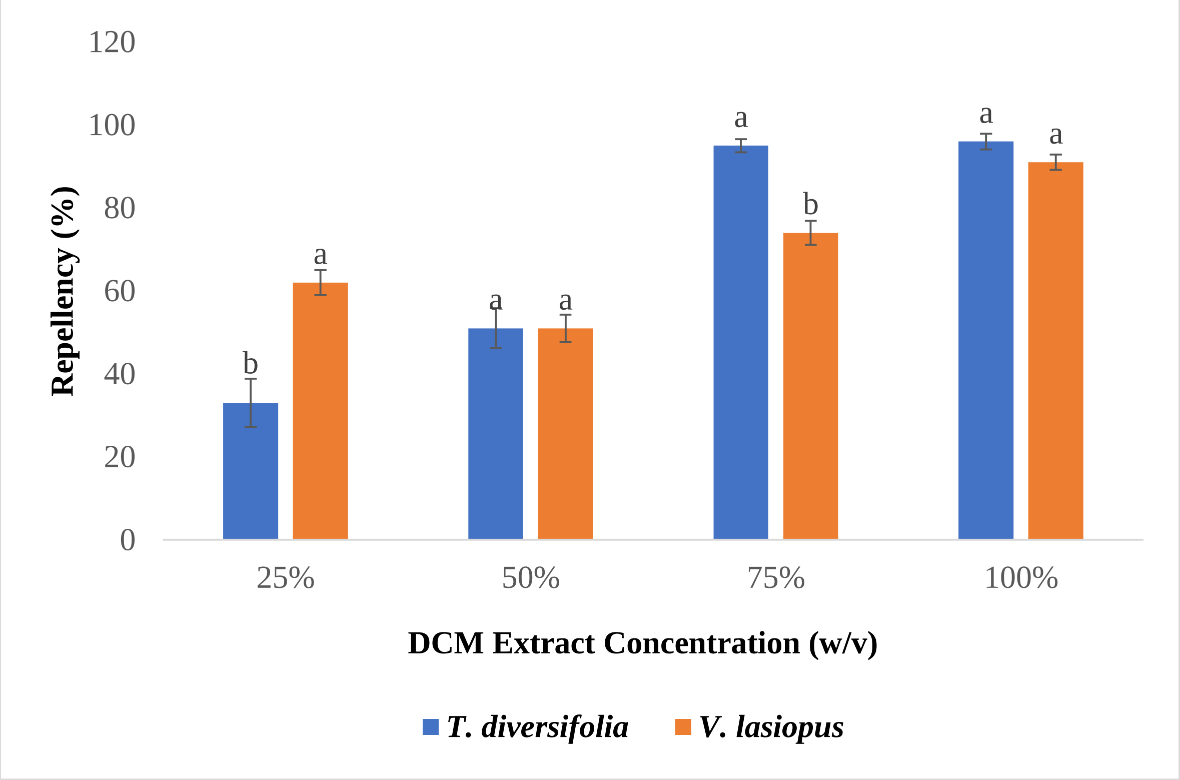

Figure 4.5: **Comparison of the Repellent Activities (Percent Repellency) of the DCM Leaf Extracts of *T. diversifolia and V. lasiopus* Against *S. zeamais***

Bars with different superscripts within the same concentration (%) are significantly different calculated by un-paired student *t*- test ($p<0.005$)

4.5.2 Repellent Activity of EtOAc Extracts of *T. diversifolia* and *V. lasiopus*

Generally, this study clearly showed that the repulsion of weevils by ethyl acetate leaf extracts of *T. diversifolia* and *V. lasiopus* increased with increase in the extract concentration (Table 4.18 and 4.19). The repellent activities of the two plant extracts were independent of the duration of exposure to weevils (Table 4.18 and 4.19; Appendices 5.3 and 5.4). All the *T. diversifolia* extract concentrations (25, 50, 75 and 100%) caused repellent activities of above 40% within the first hour of exposure to weevils (Table 4.18). However, at the least extract concentration level of 25%, the repellent activity gradually reduced with exposure time to 25% by the end of the experimental period (Table 4.18).

It was also evident that only the extract dose of 100% exhibited a repellent effect not statistically different from that caused by Actellic Super ^{TM} after a short time (1 hour) of exposure to weevils ($p>0.005$; Table 4.18). The effectiveness of the *T. diversifolia* extract doses of 50 and 75% were comparable to each other, while the repellency caused by the dose of 25% was significantly different from the rest of treatments after such a short period of exposure to weevils ($p<0.005$; Table 4.18).

In the second, third and fourth hours after exposure to weevils, the *T. diversifolia* doses of 25 and 50% showed comparable effects while doses of 75 and 100% similarly showed comparable effects to each other as well as to the effects caused by the conventional pesticide, Actellic Super ^{TM} ($p>0.005$; Table 4.18). All the extract concentrations showed statistically similar effects during the last hour of the test period ($p>0.005$; Table 4.18).

Table 4.18: Repellent Activity of Ethyl Acetate Extracts of *T. diversifolia* Against *S. zeamais*

Concentration	**PR (Mean % ± S.E.M)** ^{m} **with Exposure Time (hours) Posttreatment**					**PR**	**IP**
(% Extract)	1hr	2hr	3hr	4hr	5hr	**(Mean± S.E.M)** ^{n}	**(Mean± S.E.M)** ^{n}
25	40.00±0.00^{cA} (0.60)	45.00±9.57^{bA} (0.55)	35.00±5.00^{bAB} (0.65)	30.00±5.77^{bB} (0.70)	25.00±5.00^{bA} (0.75)	35.00±3.54^{b}	0.65±0.0354^{a}
50	65.00±5.00^{bA} (0.35)	50.00±5.77^{bB} (0.50)	50.00±5.77^{bB} (0.50)	45.00±5.00^{bB} (0.55)	65.00±5.00^{aA} (0.35)	55.00±4.18^{b}	0.45±0.0418^{a}
75	65.00±5.00^{bC} (0.25)	95.00±5.00^{aA} (0.05)	90.00±5.77^{aA} .10)	85.00±9.57^{aB} (0.15)	95.00±5.00^{aA} (0.05)	86.00±5.57^{a}	0.14±0.0557^{b}
100	90.00±5.77^{Aa} (0.10)	85.00±5.00^{aB} (0.15)	95.00±5.00^{aA} (0.05)	95.00±5.00^{aA} (0.05)	95.00±5.00^{aA} (0.05)	92.00±2.00^{a}	0.08±0.0200^{b}
Actellic	100.00±0.00^{aA} (0.00)	95.00±5.00^{aA} (0.05)	100.00±0.00^{aA} (0.00)	100.0±0.00^{aA} (0.00)	95.00±5.00^{aA} (0.05)	98.00±1.22^{a}	0.02±0.0122^{b}

Statistical comparisons were made within each column and row. The values followed by the same superscripts along the row (upper case) and column (lower case) are not significantly different by one-way ANOVA ($p>0.005$) followed by Tukey's post hoc test.Superscript ^{m} values were means based on four extract concentrations, four replicates (n=4). Superscript ^{n} values were means obtained over the 5-hour test duration (n=5). Figures in parenthesis indicate repellency index (IP<1 for repellency, IP=1 for neutral and IP>1for attractant)

On the other hand, the ethyl acetate leaf extract of *V. lasiopus* also showed remarkable repellent effects against maize weevils (Table 4.19). The *V. lasiopus* extract concentration of 100% manifested the highest repellency (90%) after the second, third and fourth hours of exposure to weevil (Table 4.19). The lowest repellency of only 10% was manifested by the *V. lasiopus* extract concentration of 25% at the last hour of the experiment period (Table 4.19).

The *V. lasiopus* extract concentrations of 25 and 50% induced comparable repellent effects on *S. zeamais* throughout the test period ($p>0.05$; Table 4.19). None of the *V. lasiopus* extract concentrations achieved repellent effect comparable to the effect caused by the reference pesticide, Actellic Super ^{TM} after 1 hour of exposure to weevils ($p<0.05$; Table 4.19). However, the highest test concentrations of 100% demonstrated effectiveness that was comparable ($p>0.05$; Table 4.19) to that of the standard pesticide at 2nd, 3rd, 4th and 5th hours of the experiment period. The *V. lasiopus* extract concentrations of 75% also induced repellent activity comparable to that caused by Actellic Super ^{TM} in the second and fifth hours of the test periods ($p>0.05$; Table 4.19).

It was observed that only the *V. lasiopus* extract dose of 75 and 100% exhibited effectiveness with repellency index value of 0.37 and 0.19, respectively that was not significantly different from that portrayed by the positive control, Actellic Super ^{TM} (IP 0.02) ($p>0.05$; Table 4.19).

Table 4.19: Repellent Activity of Ethyl Acetate Extracts of *V. lasiopus* Against *S. zeamais*

Concentration	**PR (Mean % ± S.E.M)^{m} with Exposure Time (hours) Posttreatment**					**PR**	**IP**
(% Extract)	1hr	2hr	3hr	4hr	5hr	**(Mean±S.E.M)^{n}**	**(Mean±S.E.M)^{n}**
25	45.00±5.00^{bcA} (0.55)	30.00±5.77^{cB} (0.70)	30.00±5.77^{bB} (0.70)	15.00±5.00^{cC} (0.85)	10.00±5.77^{c} (0.9)	26.00±6.20^{c}	0.74±0.0620^{a}
50	30.00±5.77^{cB} (0.7)	45.00±5.00^{bcA} (0.55)	25.00±5.00^{bB} (0.75)	20.00±8.16^{cC} (0.80)	35.00±5.00^{bcAB} (0.65)	31.00±4.30^{c}	0.69±0.0430^{a}
75	65.00±5.00^{bB} (0.35)	65.00±5.00^{abB} (0.35)	50.00±5.77^{bC} (0.50)	60.00±0.00^{bB} (0.40)	75.00±5.00^{aA} (0.25)	63.00±4.06^{b}	0.37±0.0406^{b}
100	70.00±5.77^{bB} (0.30)	90.00±5.77^{aA} (0.10)	90.00±5.77^{aA} (0.10)	90.00±5.77^{abA} (0.10)	65.00±5.00^{abB} (0.35)	81.00±5.57^{ab}	0.19±0.0557^{bc}
Actellic	100.00±0.00^{aA} (0.00)	95.00±5.00^{aA} (0.05)	100.0±0.00^{aA} (0.00)	10.00±0.00^{aA} (0.00)	95.00±5.00^{aA} (0.05)	98.00±1.22^{a}	0.02±0.0122^{c}

Statistical comparisons were made within each column and row. The values followed by the same superscripts along the row (upper case) and column (lower case) are not significantly different by one-way ANOVA ($p>0.005$) followed by Tukey's post hoc test.Superscript ^{m} values were means based on four extract concentrations, four replicates (n=4). Superscript ^{n} values were means obtained over the 5-hour test duration (n=5). Figures in parenthesis indicate repellency index (IP<1 for repellency, IP=1 for neutral and IP>1for attractant)

The comparative contrast between ethyl acetate extracts of *T. diversifolia* and *V. lasiopus* indicated that the *T. diversifolia* extract generally manifested the strongest weevil repelling potential (Figure 4.6). The *T. diversifolia* extract doses of 50 and 75% showed significantly more repellent abilities as compared to the *V. lasiopus* extract ($p<0.005$; Figure 4.6). However, the *T. diversifolia* and *V. lasiopus* extract doses of 25 and 100% manifested statistically similar effectiveness in repellency ($p>0.005$; Figure 4.6).

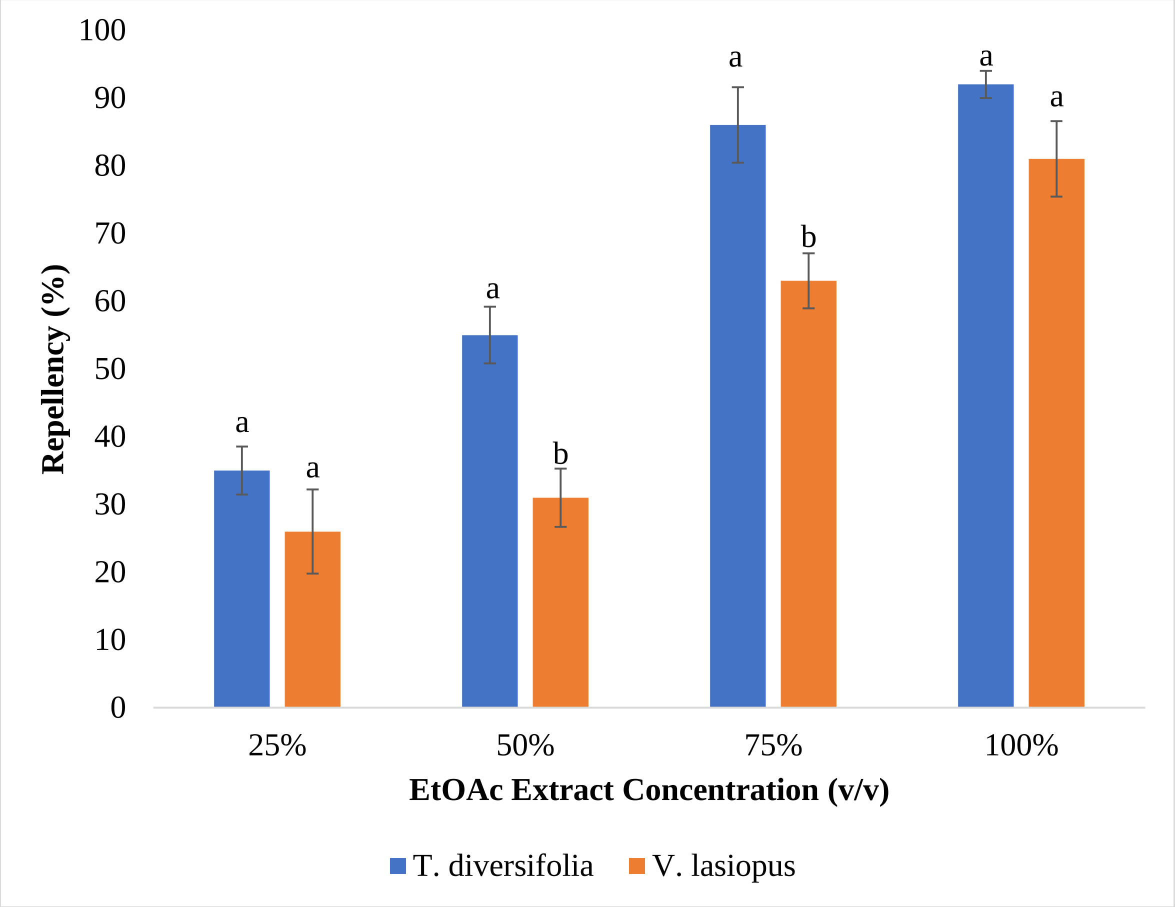

Figure 4.6: Comparison of the Repellent Activities (Percent Repellency) of the Ethyl Acetate Extracts of *T. diversifolia and V. lasiopus* Against *S. zeamais*

Bar graphs with different superscripts within the same concentration (%) are significantly different by un-paired student *t*-test ($p<0.005$)

4.6 Oviposition Deterrence Effects of Extracts of *T. diversifolia* and *V. lasiopus*

4.6.1 Oviposition Deterrence of DCM Extracts of *T. diversifolia* and *V. lasiopus*

Generally, the DCM leaf extracts of *T. diversifolia* and *V. lasiopus* reduced oviposition by *S. zeamais* on maize grains (Table 4.20; Appendices 5.1 and 5.2). Overall, the oviposition deterrence activities of the DCM leaf extracts *T. diversifolia* and *V. lasiopus* extracts were dose dependent, as they resulted in a regular pattern of repellency, from the lowest to the highest dosages (Tables 4.20; Appendices 5.1 and 5.2). However, it was noted that all the DCM leaf extracts of *T. diversifolia* and *V. lasiopus* manifested oviposition reduction activities that were significantly lower than the effects of by Actellic Super ^{TM} ($p<0.005$; Table 4.20).

The DCM extract of *T. diversifolia* showed percentage oviposition deterrence effects of 14.92, 18.44, 25.45 and 37.27% at the extract concentrations of 25, 50, 75 and 100% respectively (Table 4.20; Appendix 5.1). At lower dose levels of 25 and 50%, the *T. diversifolia* extracts exhibited statistically similar oviposition reduction effects ($p>0.005$; Table 4.20). However, the higher extract concentrations of 75 and 100% produced significantly different activities ($p<0.005$; Table 4.20).

On the other hand, the DCM leaf extract of *V. lasiopus* manifested the highest oviposition deterrence of 64.39% at the highest test dose of 100% and the least oviposition deterrence effect of 12.19% at the least extract concentration of 25% (Table 4.20; Appendix 5.2). The extract lower doses of 25 and 50% also induced statistically comparable weevils oviposition deterrent effects ($p>0.005$; Table 4.20).

Table 4.20: Percentage Oviposition Deterrence Effects of DCM Extracts of *T. diversifolia* and *V. lasiopus* against *S. zeamais*

Group	Treatment	Plant Leaf Extract	
		T. diversifolia	***V. lasiopus***
I (Negative Control)	Solvent Only	00.004±0.385^{e}	00.004±0.385^{e}
II (Positive Control)	Actellic Super ™	99.075±0.926^{a}	99.075±0.926^{a}
III (Experimental Group A)	25% v/v	14.921±0.778^{d}	12.185±0.766^{d}
IV (Experimental Group B)	50% v/v	18.439±0.382^{d}	17.469±0.813^{d}
V (Experimental Group C)	75% v/v	25.448±0.429^{c}	28.889±0.754^{c}
VI (Experimental Group D)	100% v/v	37.270±1.530^{b}	64.390±0.650^{b}

Values expressed as mean ± SEM based on four extract concentrations, four replicates (n=4). Means followed by the same superscripts along the column are not significantly different by one-way ANOVA (p>0.005) followed by Tukey's post hoc test.

In comparison, the DCM leaf extracts of *T. diversifolia* and *V. lasiopus* elicited statistically similar oviposition deterrent effects at the extracts dose levels of 25, 50 and 75% (p>0.005; Figure 4.7). However, it was noted that at the highest extract concentration of 100%, the *V. lasiopus* extract evoked significantly higher oviposition reduction potential of 64.390% as compared to that caused by the extracts of *T. diversifolia* extract (37.270%) (p<0.005; Figure 4.7).

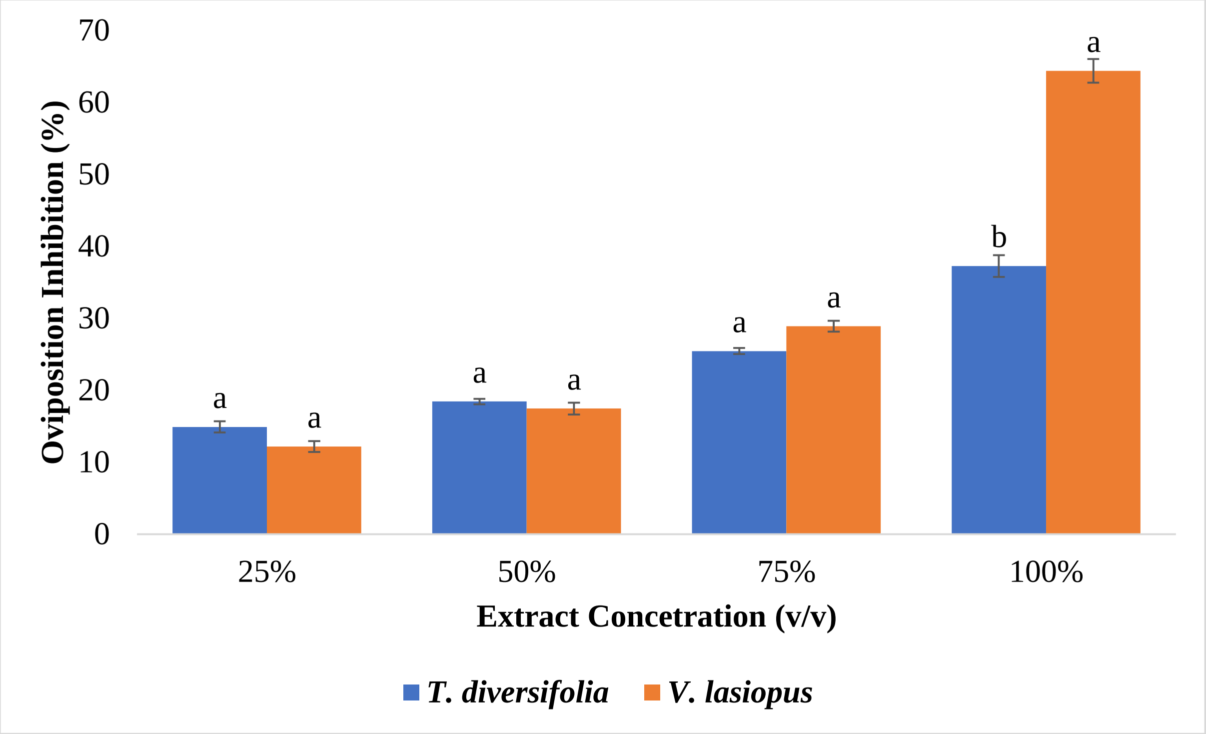

Figure 4.7: Comparison of Oviposition Deterrence Activities of DCM Extract *T. diversifolia* and *V. lasiopus* Against *S. zeamais*
Bars with different superscripts within the same concentration (%) are significantly different calculated by un-paired student *t*- test (hours; $p<0.005$)

4.6.2 Oviposition Deterrence of EtOAc Extracts of *T. diversifolia* and *V. lasiopus*

As table 4.21 shows, the ethyl acetate leaf extracts of *T. diversifolia* and *V. lasiopus* showed a dose dependent reduction in oviposition of *S. zeamais* (Table 4.21; Appendices 5.3 and 5.4). The number eggs laid on all the leaf extract treated grains was significantly lower than the number of eggs laid on the negative control samples ($p<0.005$; Table 4.21; Appendices 5.3 and 5.4). It was also observed that the ethyl acetate extracts of the two plants manifested oviposition reduction effects that were significantly lower than the effects caused by actellic Super $^{\text{TM}}$ at all tested concentration ($p<0.005$; Table 4.21; Appendices 5.3 and 5.4).

The oviposition deterrence activity of the *T. diversifolia* extract at the dose level of 50% (31.28%) was not significantly different from that caused by the extract doses of 25% (25.47% OD) and 75% (35.49% OD) ($p>0.005$; Table 4.21). However, the ethyl acetate extract dose levels of 25% and 75% showed significantly different oviposition deterrence activities ($p<0.05$; Table 4.21).

On the other hand, the ethyl acetate extract of *V. lasiopus* demonstrated a maximum oviposition deterrence of 87.625% at the extract concentration of 100% and the least oviposition deterrence of 17.79% at the extract concentration of 25% (Table 4.21; Appendix 5.4). It was evident that all the extract concentrations of *V. lasiopus* manifested significantly different oviposition reduction potential ($p<0.005$; Table 4.21).

Table 4.21: Percentage Oviposition Deterrence Effects of Ethyl Acetate Extracts of *T. diversifolia* and *V. lasiopus* Against *S. zeamais*

Group	Treatment	Plant Leaf Extract	
		T. diversifolia	***V. lasiopus***
I (Negative Control)	Solvent Only	00.004 ± 0.385^{e}	00.004 ± 0.385^{f}
II (Positive Control)	Actellic Super ™	99.075 ± 0.926^{a}	99.075 ± 0.926^{a}
III (Experimental Group A)	25% v/v	25.466 ± 0.959^{d}	17.785 ± 0.534^{e}
IV (Experimental Group B)	50% v/v	31.283 ± 0.774^{cd}	25.819 ± 0.371^{d}
V (Experimental Group C)	75% v/v	35.490 ± 1.080^{c}	43.790 ± 1.650^{c}
VI (Experimental Group D)	100% v/v	53.120 ± 1.650^{b}	87.625 ± 0.959^{b}

Values expressed as mean ± SEM based on four extract concentrations, four replicates (n=4). Means followed by the same superscripts along the column are not significantly different by one-way ANOVA ($p>0.005$) followed by Tukey's post hoc tests

The comparison of the oviposition deterrence effects of ethyl acetate leaf extracts of *T. diversifolia* and *V. lasiopus* showed that the *T. diversifolia* extract elicited significantly higher activities at the lower extract concentrations of 25 and 50% ($p<0.005$; Figure 4.8).

However, at higher extract concentrations of (75 and 100%), the *V. lasiopus* extract manifested significantly higher weevils' oviposition reduction effects ($p<0.005$; Figure 4.7).

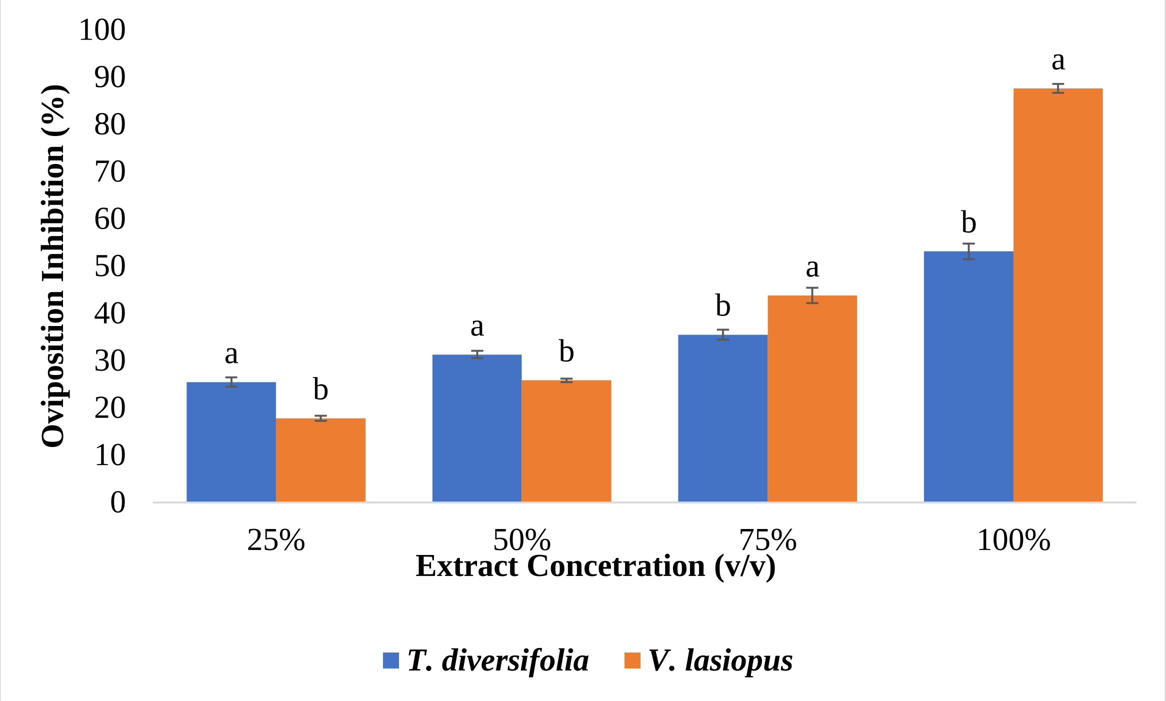

Figure 4.8: Comparison of Oviposition Deterrence Activities of Ethyl Acetate Extract of *T. diversifolia* and *V. lasiopus* Against *S. zeamais*
Bars with different superscripts within the same concentration (%) are significantly different calculated by un-paired student *t*- test (hours; $p<0.005$)

4.7 Progeny (F_1) Emergence Inhibition of Extracts of *T. diversifolia* and *V. lasiopus*

4.7.1 F_1 Emergence Inhibition of DCM Extracts of *T. diversifolia* and *V. lasiopus*

Generally, it was observed that the DCM leaf extracts of *T. diversifolia* and *V. lasiopus* caused inhibition of *S. zeamais* F_1 progeny emergence 56 days post treatment. These effects were found to increase with increase in extract concentrations (Table 4.22; Appendices 5.1 and 5.2). The effects observed for the two plant extracts were higher than that observed in the negative control ($p<0.005$; Table 4.22). Overall, no F_1 progeny emerged in the sample treated with Actellic Super ^{TM} (the standard conventional pesticide) (Table 4.22; Appendices 5.1 and 5.2).

The DCM extract of *T. diversifolia* demonstrated mean percent inhibition of F_1 progeny emergence activities that ranged between 38.38 and 84.85% in a dose dependent manner (Table 4.22; Appendix 5.1). The effects induced by all the DCM leaf extract of *T. diversifolia* were significantly different from each other ($p<0.005$; Table 4.22).

The DCM extract of *V. lasiopus* also exhibited remarkable F_1 progeny emergence inhibition activities during the 56 days study period (Table 4.22; Appendix 5.1). The inhibition of F_1 progeny emergence activities of *V. lasiopus* ranged from 38.39% to 80.81% in a dose dependent manner (Table 4.22; Appendix 5.1).

Table 4.22: Percentage F_1 Progeny Emergence Inhibition Effects of DCM Extracts of *T. diversifolia* and *V. lasiopus* Against *S. zeamais*

Group	Treatment	Plant Leaf Extract	
		T. diversifolia	***V. lasiopus***
I (Negative Control)	Solvent Only	00.00 ± 0.583^{f}	00.00 ± 0.583^{f}
II (Positive Control)	Actellic Super ^{TM}	100.0 ± 0.000^{a}	100.0 ± 0.000^{a}
III (Experimental Group A)	25% v/v	38.38 ± 1.300^{e}	38.89 ± 1.730^{e}
IV (Experimental Group B)	50% v/v	64.65 ± 0.583^{d}	65.15 ± 0.967^{d}
V (Experimental Group C)	75% v/v	73.74 ± 0.825^{c}	72.22 ± 0.967^{c}
VI (Experimental Group D)	100% v/v	84.85 ± 1.010^{b}	80.81 ± 0.583^{b}

Values expressed as mean ± SEM based on four extract concentrations, four replicates (n=4). Means followed by the same superscripts along the column are not significantly different by one-way ANOVA ($p>0.005$) followed by Tukey's post hoc test

In comparison, it was noted that at all the concentrations, the effectiveness of DCM leaf extracts of *T. diversifolia* and *V. lasiopus* against *S. zeamais* F_1 progeny emergence was not statistically different (p<0.005; Figure 4.9).

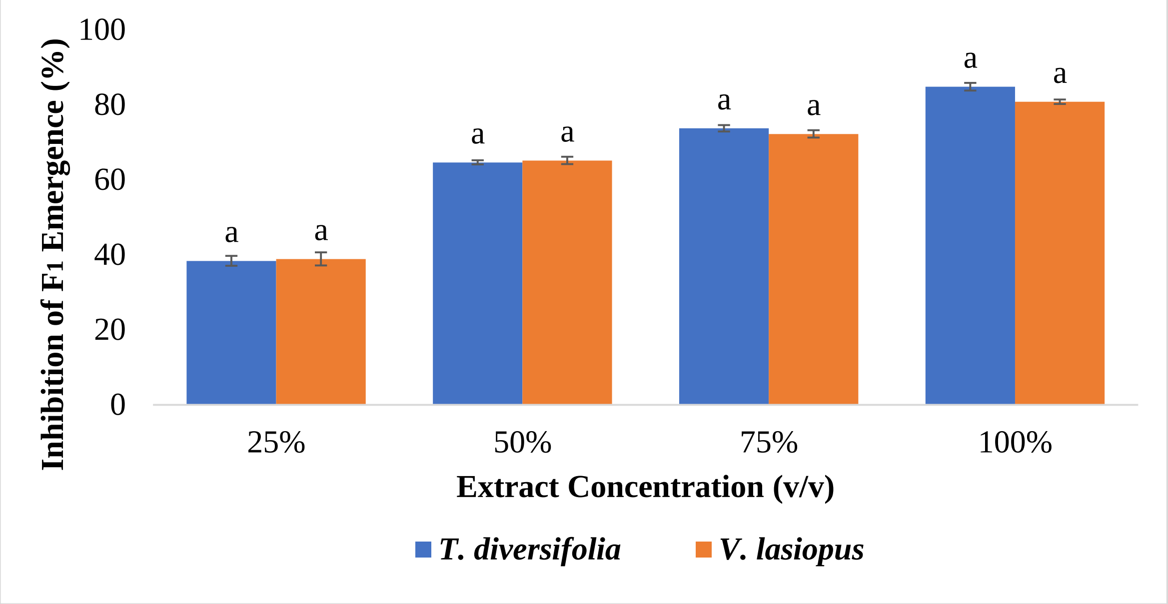

Figure 4.9: **Comparison of the F_1 Progeny Emergence Inhibition Activities of DCM Extract *T. diversifolia* and *V. lasiopus* against *S. zeamais***
Bars with different superscripts within the same concentration are significantly different calculated by *t*-test (p<0.005)

4.7.2 F_1 Emergence Inhibition Effects of the EtOAc Extracts of *T. diversifolia* and *V. lasiopus*

On the other hand, it was observed that the F_1 progeny emergence of weevils was generally suppressed by all the ethyl acetate leaf extracts of both *T. diversifolia* and *V. lasiopus* (Table 4.23; Appendices 5.3 and 5.4). All the four tested extracts concentrations of the two plants elicited F_1 emergence inhibition effects that were significantly higher than the effects caused by solvent in the negative control group (p<0.005; Table 4.23).

All the *T. diversifolia* extract concentrations caused significantly different weevils progeny emergence inhibition effects that range between 57.07 and 88.89% ($p<0.005$; Table 4.23). All the ethyl acetate extracts concentrations caused F_1 progeny emergence inhibition effects that was significantly lower than the effects caused by the reference pesticide, Actellic Super ^{TM} ($p<0.005$; Table 4.23).

This study found out that the ethyl acetate extract of *V. lasiopus* induced significantly different progeny emergence inhibition effects of 60.1, 75.25, 81.31 and 97.45% at extract concentrations of 25, 50, 75 and 100%, respectively ($p<0.005$; Table 4.23; Appendix 5.4). It was evident that although the inhibition effects induced by lower extract doses (25, 50 and 75%) was statistically lower than that of the standard pesticide, the effectiveness of the highest extract concentration (100%) was comparable to the effects caused by the standard pesticide, Actellic super ^{TM} ($p>0.005$; Table 4.23).

Table 4.23: Percentage F_1 Progeny Emergence Inhibition Effects of Ethyl Acetate Extracts of *T. diversifolia* and *V. lasiopus* against *S. zeamais*

Group	**Treatment**	**Plant Leaf Extract**	
		T. diversifolia	***V. lasiopus***
I (Negative Control)	Solvent Only	00.00 ± 0.583^f	00.00 ± 0.583^e
II (Positive Control)	Actellic Super ^{TM}	100.0 ± 0.000^a	100.0 ± 0.000^a
III (Experimental Group A)	25% w/v	57.07 ± 0.505^e	60.10 ± 1.520^d
IV (Experimental Group B)	50% w/v	66.67 ± 1.300^d	75.25 ± 0.967^c
V (Experimental Group C)	75% w/v	83.84 ± 0.825^c	81.31 ± 0.505^b
VI (Experimental Group D)	100% w/v	88.89 ± 0.583^b	97.48 ± 0.505^a

Values expressed as mean ± SEM based on four extract concentrations, four replicates (n=4). Means followed by the same superscripts along the column are not significantly different by one-way ANOVA ($p>0.005$) followed by Tukey's post hoc test

The comparative contrast of the effects of the ethyl acetate extracts of the two plants showed that the *V. lasiopus* extracts evoked significantly higher weevil progeny emergence suppression activities at the extract concentrations of 50 and 100% ($p<0.005$; Figure 4.10). However, no significant difference in the inhibition effects was noted between the two plant extracts at the extract dosages of 25 and 75% ($p>0.005$; Figure 4.10).

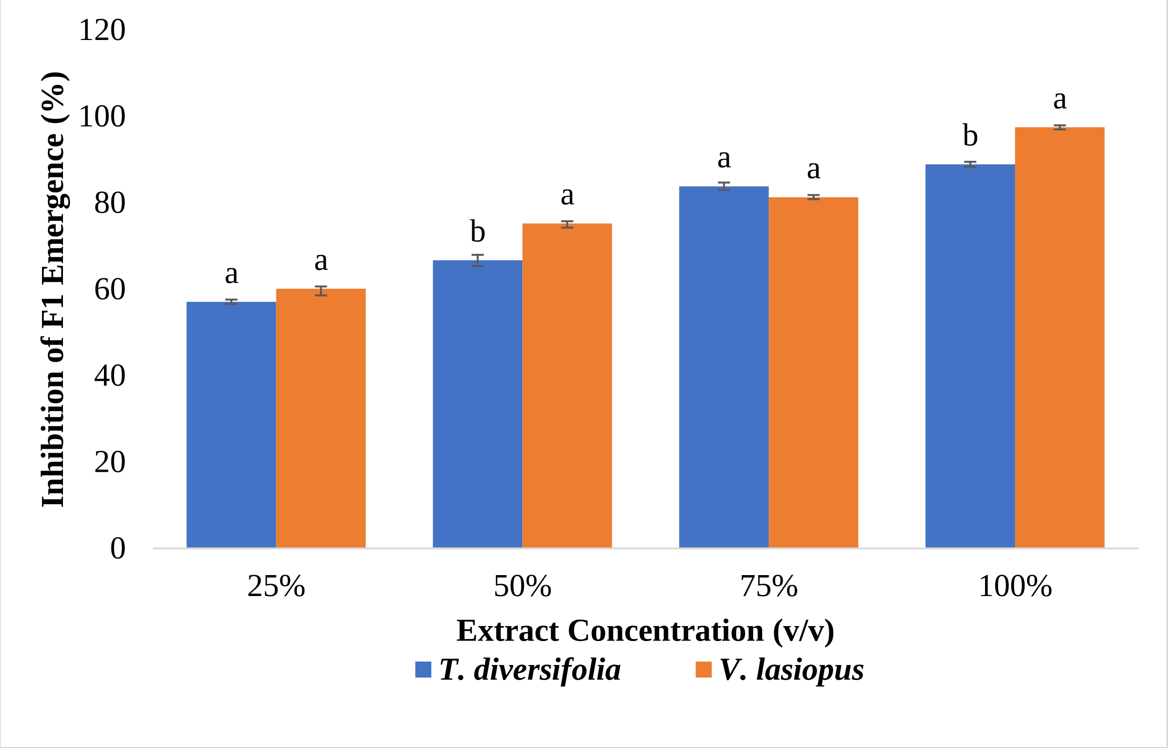

Figure 4.10: Comparison of F1 Progeny Inhibition Activities of Ethyl Acetate Extract of *T. diversifolia* and *V. lasiopus* against S. zeamais

Bars with different superscripts within the same concentration (%) are significantly different calculated by un-paired student *t*- test (hours; $p<0.005$)

4.8 Grain Weight Loss Reduction Effects of Extracts of *T. diversifolia* and *V. lasiopus*

4.8.1 Grain Weight Loss Reduction Effects of DCM Extracts of *T. diversifolia* and *V. lasiopus*

Generally, the DCM leaf extracts of *T. diversifolia* and *V. lasiopus* reduced maize grain weight loss effects following *S. zeamais* infestation (Table 4.24; Appendices 6.1 and 6.2). The maize grains treated with the DCM extracts of *T. diversifolia* showed grain loss of between 02.7 and 13.7% while maize grains treated with DCM extracts of *V. lasiopus* exhibited grain weight loss of between 4.7 and 15.4% (Table 4.24; Appendices 6.1 and 6.2). The results also revealed that grain weight loss reduction effects of the DCM leaf extracts of the two plants were dose dependent (Table 4.24; Appendices 6.1 and 6.2).

Overall, as the amount of the plant extracts was increased to 100%, their protectant abilities against weevils also increased (Table 4.24; Appendices 6.1 and 6.2). At the low extract concentrations (25 and 50%), the DCM leaf extracts of *T. diversifolia* and *V. lasiopus* demonstrated low grain loss reduction effects (Table 4.24; Appendices 6.1 and 6.2). However, it was noted that at the highest tested extract concentration of 100%, the DCM leaf extracts of *T. diversifolia* and *V. lasiopus* manifested effective grain loss reduction activities that were comparable to the effects of the standard pesticide, Actellic Super ^{TM} ($p>0.005$; Table 4.24).

Table 4.24: Percentage Reduction of Maize Grain Weight Loss by the DCM Extracts of *T. diversifolia* and *V. lasiopus* following *S. zeamais* Infestation

Group	Treatment	Mean % Weight Loss (PWL)	
		T. diversifolia	***V. lasiopus***
I (Negative control)	Solvent only	16.210 ± 1.640^{a}	16.210 ± 1.640^{a}
II (Positive control)	Actellic Super ™	00.916 ± 0.140^{c}	00.916 ± 0.140^{d}
III (Experimental group A)	25% v/v	13.700 ± 0.348^{a}	15.368 ± 0.342^{ab}
IV (Experimental group B)	50% v/v	12.350 ± 0.348^{ab}	12.675 ± 0.171^{ab}
V (Experimental group C)	75% v/v	08.037 ± 0.521^{b}	09.925 ± 0.695^{bc}
VI (Experimental group D)	100% v/v	02.720 ± 1.080^{c}	04.700 ± 1.600^{cd}

Values expressed as mean ± SEM based on four extract concentrations, four replicates (n=4). Means followed by the same superscripts along the same column are not significantly different by One Way ANOVA followed by Tukey's post hoc test (p>0.005)

In comparison, the DCM extract of *T. diversifolia* was found to be more efficacious in protecting maize grains against weight loss due to *S. zeamais* infestation compared to the DCM extract of *V. lasiopus* (p<0.005; Figure 4.11). However, the two plant extracts exhibited comparable grain weight loss reduction effects at the dose level of 50% (p>0.005; Figure 4.11).

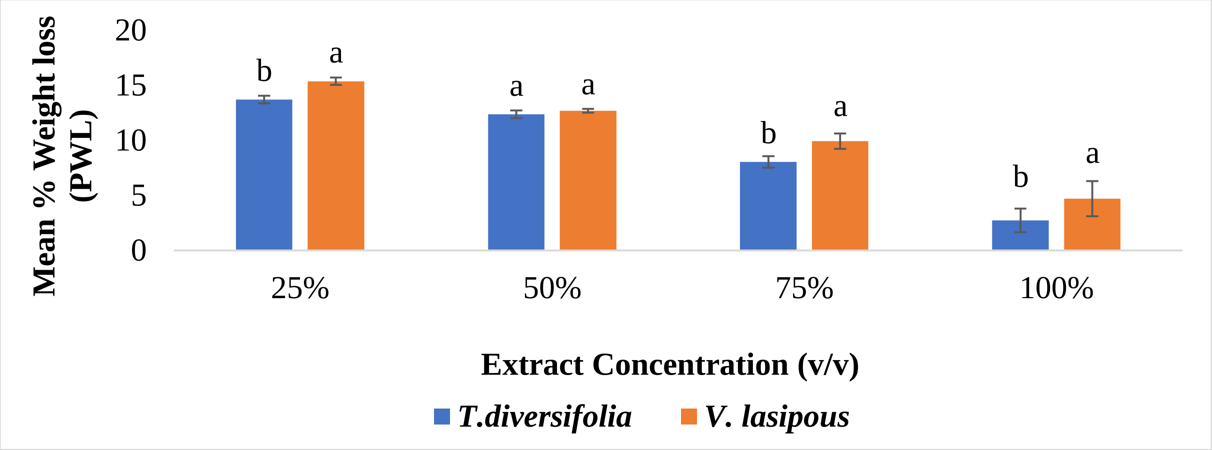

Figure 4.11: Comparison of the Maize Grain Weight Loss Reduction Effects by the DCM Extracts of *T. diversifolia* and *V. lasiopus* following S. zeamais Infestation

Bars with different superscripts within the same concentration (%) are significantly different calculated by un-paired student *t*- test (hours; p<0.005)

7.8.2 Grain Weight Loss Reduction Effects of EtOAc Extracts of *T. diversifolia* and *V. lasiopus*

It was evident that the ethyl acetate leaf extracts of *T. diversifolia* and *V. lasiopus* also reduced grain weight loss following weevil infestation (Table 4.25; Appendices 6.3 and 6.4). Overall, it was observed that exposure of the maize grain samples to the EtOAc extracts of *T. diversifolia* and *V. lasiopus* resulted in a regular pattern of reduced grain weight loss, from the lowest to the highest dosages (Table 4.25; Appendices 6.3 and 6.4).

It was noted that the two plant extracts, at the lowest extract concentrations (25%), did not significantly reduce high grain loss effects as they were comparable to the grain loss observed in the unprotected grains (p>0.005; Table 4.25). The effectiveness of DCM leaf extracts of *T. diversifolia,* at the concentrations of 75 and 100%, and *V. lasiopus,* at the extract concentration of 100%, were comparable to the effectiveness of standard pesticide (p>0.005; Table 4.25).

Table 4.25: Percentage Reduction of Maize Grain Weight Loss by EtOAc Extracts of *T. diversifolia* and *V. lasiopus* following *S. zeamais* Infestation

Group	**Treatment**	**Mean % Weight Loss (PWL)**	
		T. diversifolia	***V. lasiopus***
I (Negative Control)	Solvent Only	16.210 ± 1.640^{a}	16.210 ± 1.640^{a}
II (Positive Control)	Actellic Super	00.916 ± 0.140^{c}	00.916 ± 0.140^{c}
III (Experimental Group A)	25% v/v	11.163 ± 0.101^{ab}	12.462 ± 0.579^{ab}
IV (Experimental Group B)	50% v/v	08.850 ± 0.478^{b}	10.938 ± 0.629^{ab}
V (Experimental Group C)	75% v/v	02.760 ± 1.300^{c}	08.637 ± 0.208^{b}
VI (Experimental Group D)	100% v/v	00.488 ± 0.357^{c}	02.810 ± 1.020^{c}

Values expressed as mean ± SEM based on four extract concentrations, four replicates (n=4). Means followed by the same superscripts along the same column are not significantly different calculated by One Way ANOVA followed by Tukey’s post hoc test (p>0.005)

The comparison of the percent reduction of maize grain weight loss by the ethyl acetate leaf extracts of *T. diversifolia* and *V. lasiopus* indicated that the *T. diversifolia* extract was significantly more effective than the *V. lasiopus* extract (p<0.005; Figure 4.8.2).

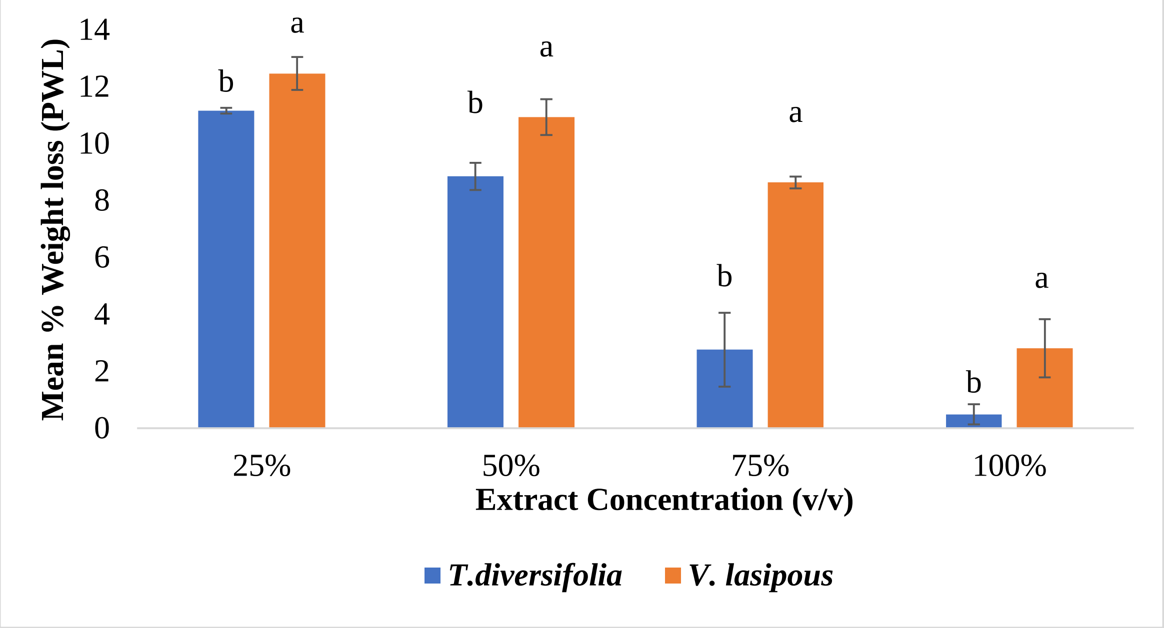

Figure 4.12: Comparison of the Maize Grain Weight Loss Reduction Effects by the EtOAc Extracts of *T. diversifolia* and *V. lasiopus* following *S. zeamais* Infestation

Bars with different superscripts within the same concentration (%) are significantly different calculated by un-paired student *t*- test (hours; p<0.005)

7.9 Grain Damage Deterrence Effects of Extracts of *T. diversifolia* and *V. lasiopus*

7.9.1 Grain Damage Deterrence Effects of DCM Extracts of *T. diversifolia* and *V. lasiopus*

The results of the weevil Perforation Index (WPI) clearly indicated that the DCM leaf extracts of *T. diversifolia* and *V. lasiopus* had significant effects as grain protectants against weevils as the percentage of grain damage was reduced following application of the extracts (Table 4.26; Appendices 7.1 and 7.2). Moreover, the efficacy was observed to be directly proportional to the concentration of the applied extract (Table 4.26; Appendices 7.1 and 7.2). All the DCM leaf extract concentrations (25%, 50%, 75% and 100%) caused weevil perforation index (WPI) below 50. It was, therefore, evident that the extracts discouraged weevils from feeding on the treated grains hence protecting the grains (Table 4.26; Appendices 7.1 and 7.2).

On the contrary, the grain samples treated with solvent only manifested approximate weevil perforation index (WPI) values of 50 (Table 4.26). This is regarded as an index of negative stored maize grain protecting ability.

The effectiveness of DCM leaf extracts of *T. diversifolia* and *V. lasiopus* at the higher tested concentrations of 75 and 100% was comparable to the effectiveness of standard pesticide, Actellic Super ^{TM} ($p>0.005$; Table 4.26).

Table 4.26: Grain Damage Deterrence Effects of DCM Extract of *T. diversifolia* and *V. lasiopus* following *S. zeamais* Infestation

Group	**Treatment**	**Mean % Damaged/ Perforated**	
		T. diversifolia	***V. lasiopus***
I (Negative Control)	Solvent Only	47.50±2.220^{a} (49.92±1.18)	47.50±2.220^{a} (49.92±1.18)
II (Positive Control)	Actellic Super ™	01.00±0.577^{c} (02.02±1.17)	01.00±0.577^{d} (02.02±1.17)
III (Experimental Group A)	25% v/v	10.50±0.957^{b} (18.04±1.37)	12.50±1.710^{b} (20.64±2.31)
IV (Experimental Group B)	50% v/v	09.50±1.500^{b} (16.50±2.09)	10.50±2.060^{bc} (17.79±2.95)
V (Experimental Group C)	75% v/v	04.50±1.260^{bc} (08.50±2.16)	04.50±1.260^{cd} (08.50±2.16)
VI (Experimental Group D)	100% v/v	01.50±0.957^{c} (02.95±1.87)	03.50±0.957^{cd} (06.77±1.72)

Values expressed as mean ± SEM based on four extract concentrations, four replicates (n=4). Means followed by the same superscripts along the column are not significantly different culculated by One Way ANOVA followed by Tukey's post hoc test (p>0.005). Figures in parenthesis indicate Weevil Perforation Index (WPI) values.

The comparative contrast between the DCM extracts of *T. diversifolia* and *V. lasiopus* indicated that the *T. diversifolia* extract generally exhibited the strongest antifeedant potential (Figure 4.13). The *T. diversifolia* extract doses of 25 and 100% showed significantly lower weevil perforation Index (WPI) as compared to the *V. lasiopus* extract (p<00.005; Figure 4.13). However, the *T. diversifolia* and *V. lasiopus* extract concentrations of 50 and 75% manifested comparable effectiveness (p>0.005; Figure 4.13).

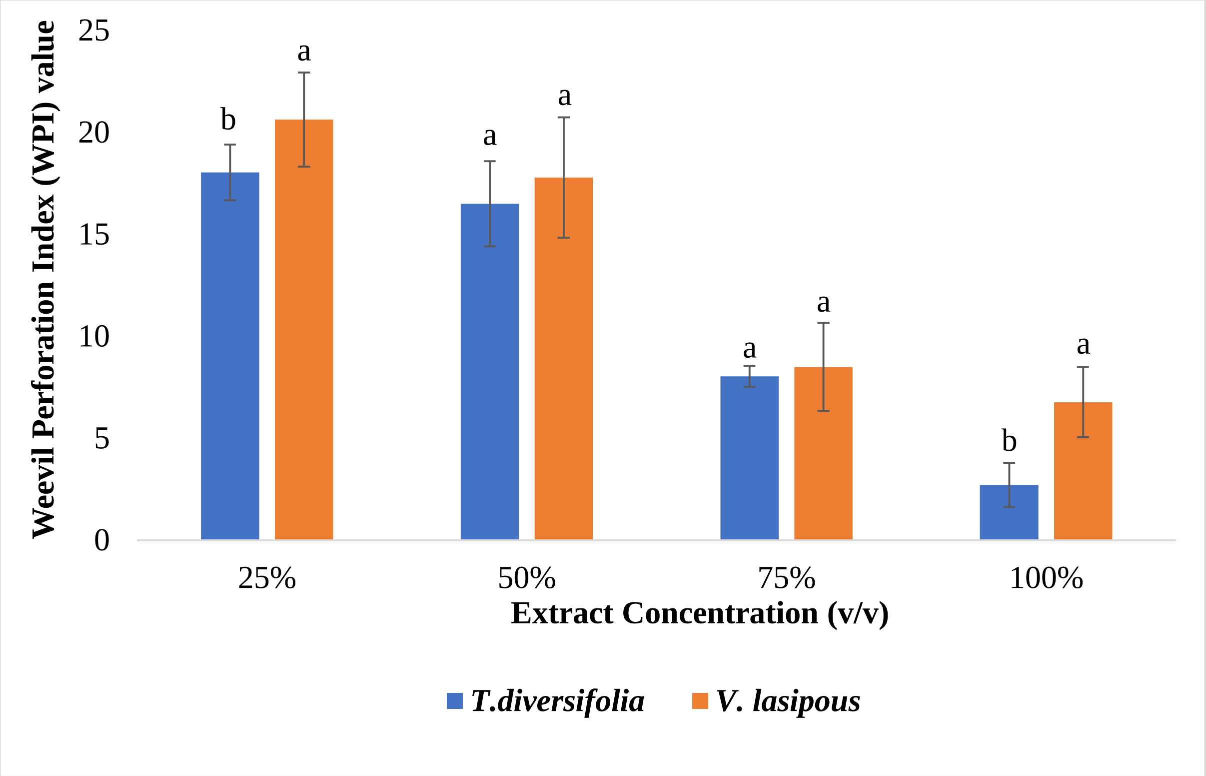

Figure 4.13: Comparison of the Maize Grain Damage Deterrence Effects of the DCM Extracts of *T. diversifolia* and *V. lasiopus* following S. zeamais Infestation

Bars with different superscripts within the same concentration (%) are significantly different calculated by un-paired student *t*- test (hours; $p<0.005$)

7.9.2 Grain Damage Deterrence Effects of EtOAc Extracts of *T. diversifolia* and *V. lasiopus*

On the other hand, the ethyl acetate leaf extract of *T. diversifolia* and *V. lasiopus* also showed remarkable antifeedant effects against maize weevils (Table 4.27; Appendices 7.3 and 7.4). The damage caused by weevils on the treated maize grains ranged from 2 to 11% (Table 4.27; Appendices 7.3 and 7.3). The resultant WPI was also less than 50 at all the varying extract doses used, showing its positive protectant ability of maize grains against *S. zeamais* (Table 4.27). The least damage by weevils was observed in the grains treated with *T. diversifolia* at extract concentration of 100% while the grains treated with 25%

extract concentration of *V. lasiopus* showed the greatest damage by weevils ($p<0.005$; Table 4.27).

This damage on the treated grains was significantly reduced as compared to the damage caused by weevils on the negative control sample ($p<00.005$; Table 4.27). It was also evident that all the EtOAc leaf extracts of *T. diversifolia* and *V. lasiopus* achieved grains damage reduction effects that was comparable to that caused by the reference pesticide, Actellic Super $^{\text{TM}}$ except at their lowest tested extract concentration of 25% ($p<0.005$; Table 4.27).

Table 4.27: Grain Damage Deterrence Effects of the EtOAc Extract of *T. diversifolia* and *V. lasiopus* following *S. zeamais*

Group	**Treatment**	**% Damage/ Perforations caused**	
		T. diversifolia	***V. lasiopus***
I (Negative Control)	Solvent Only	47.50 ± 2.220^{a} (49.92±1.18)	47.50 ± 2.220^{a} (49.92±1.18)
II (Positive Control)	Actellic Super $^{\text{TM}}$	01.00 ± 0.577^{c} (02.02±1.17)	1.000 ± 0.577^{c} (02.02±1.17)
III (Experimental Group A)	25% v/v	08.00 ± 2.160^{b} (14.04±3.21)	11.00 ± 1.290^{b} (18.68±1.80)
IV (Experimental Group B)	50% v/v	06.50 ± 1.500^{bc} (11.83±2.43)	06.50 ± 1.260^{bc} (11.90±2.00)
V (Experimental Group C)	75% v/v	03.50 ± 0.957^{bc} (06.77±1.72)	05.50 ± 0.500^{bc} (10.36±0.86)
VI (Experimental Group D)	100% v/v	02.00 ± 0.816^{bc} (03.96±1.59)	02.50 ± 0.957^{c} (04.89±1.85)

Values expressed as mean ± SEM based on four extract concentrations, four replicates (n=4). Means followed by the same superscripts along the column are not significantly different by One Way ANOVA followed by Tukey's post hoc test ($p>0.005$). Figures in parenthesis indicate Weevil Perforation Index (WPI) value

The analyzed results of this study demonstrated that the EtOAc leaf extracts of *T. diversifolia* generally exhibited comparatively more effective antifeedant activities on weevils than EtOAc leaf extracts of *V. lasiopus* at 25 and 75% extract doses (Figure 4.14). The grain samples treated with *T. diversifolia* extract at doses of 25 and 75% showed statistically lower weevil perforation Index (WPI) as compared to the effectiveness of *V. lasiopus* extract ($p<00.005$; Figure 4.14). However, the *T. diversifolia* and *V. lasiopus* extract concentrations of 50 and 100% manifested comparable effectiveness of reducing weevils damage on the treated grains ($p>0.005$; Figure 4.14).

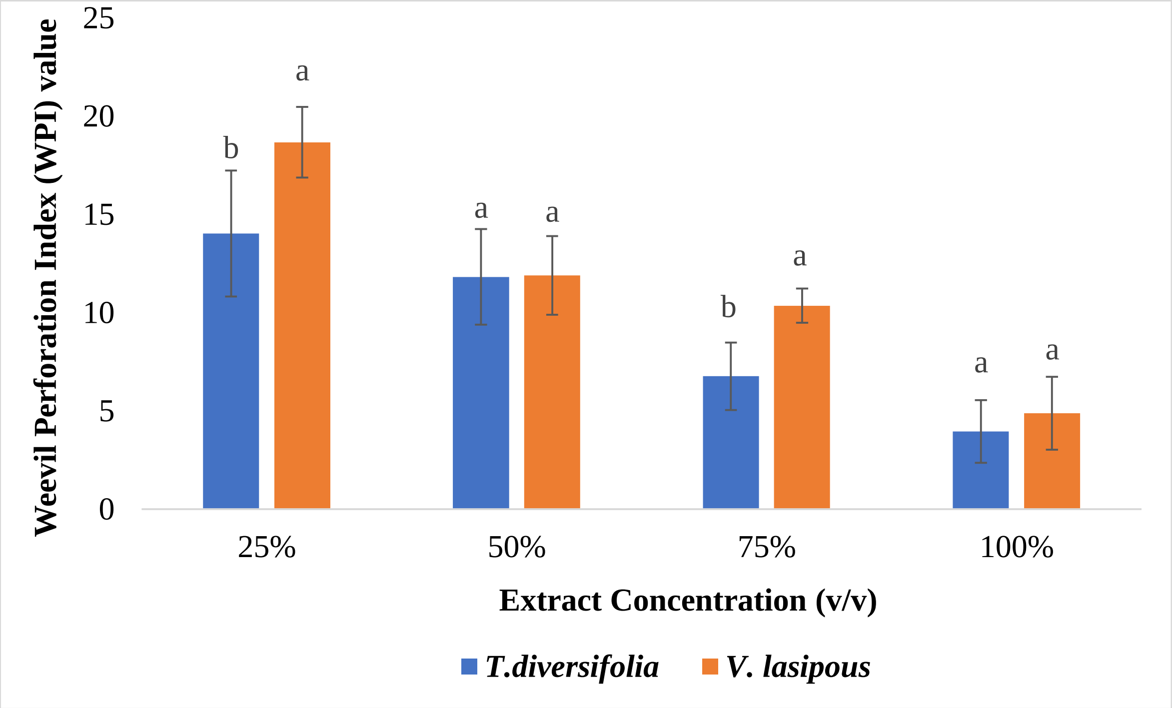

Figure 4.14: Comparison of the Maize Grain Damage Deterrence Effects of the EtOAc Leaf Extracts of *T. diversifolia* and *V. lasiopus* Against *S. zeamais*

Bars with different superscripts within the same concentration (%) are significantly different calculated by un-paired student *t*- test (hours; $p<0.005$)

4.10 *Ex-Vivo* Anti-ChE Effects of Extracts of *T. diversifolia* and *V. lasiopus*

4.10.1 Estimated Acetylcholinesterase Concentration

The isolated protein concentration was estimated from a standard curve of BSA concentration versus mean absorbance (Figure 4.15). The protein content of the isolated acetylcholinesterase was 8.47758mg/ml.

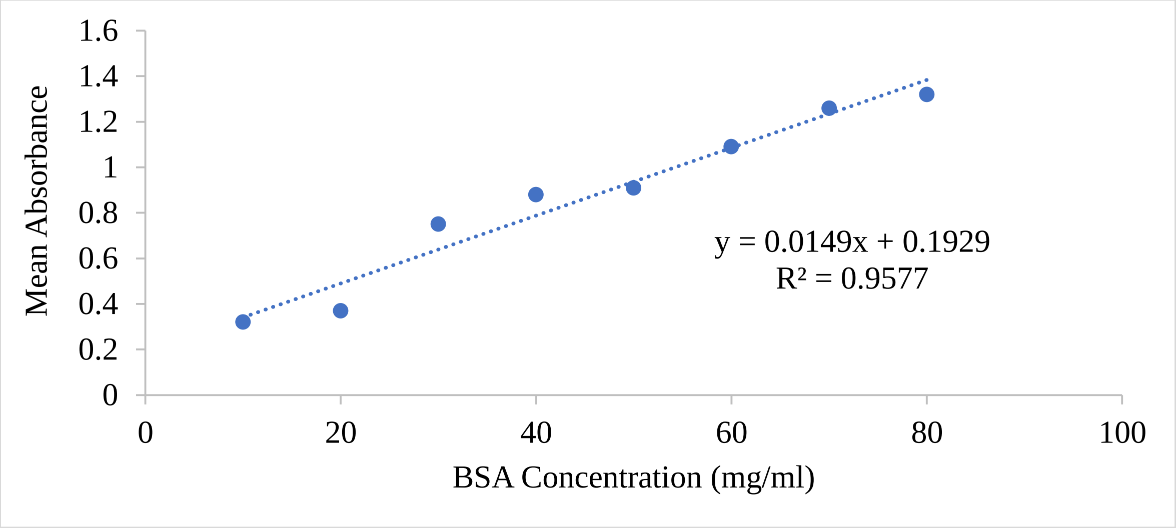

Figure 4.15: Linear Regression from Which Protein Concentration (mg/ml) was calculated

4.10.2 *Ex-Vivo* Anti-ChE Effects of DCM Extracts of *T. diversifolia* and *V. lasiopus*

Generally, the DCM leaf extracts of *T. diversifolia* and *V. lasiopus* successfully inhibited acetylcholinesterase enzyme activity, which was indicated by reduction in enzyme activity by various extract concentrations (Tables 4.28 and 4.29; Appendix 8.2). It was apparent, that small changes in absorbance were associated with higher inhibition of enzyme activity (Tables 4.28 and 4.29; Figure 4.16 and 4.17; Appendix 8.2). The acetylcholinesterase enzyme inhibitory activity of DCM leaf extracts of *T. diversifolia* increased with increasing concentration (Tables 4.28).

The extracts manifested enzyme inhibitory activities that ranged between 27.96 and 77.42% (Tables 4.28; Appendix 8.2). Analysis of enzyme activity demonstrated that the DCM leaf extracts of *T. diversifolia* at higher tested concentrations levels of 12.5, 15 and 17.5 mg/ml, showed inhibitions of 55.91, 72.04 and 77.42%, respectively which were comparable to the effect of the standard pesticide, Actellic (95.38%) (p>0.001; Table 4.28; Appendix 8.2). However, it was observed that the DCM leaf extract of *T. diversifolia,* at the extract concentration levels of 5, 7.5 and 10 mg/ml, were notably not effective as they were not statistically different from the effects seen in the negative control setup (p>0.001; Table 4.28; Figure 4.10.2).

Table 4.28: *Ex-Vivo* Anti-Acetylcholinesterase Activities of DCM Leaf Extracts of *T. diversifolia*

Treatment (mg/ml)	Mean Enzyme Activity (µmoles/min/mg protein)	Mean Inhibition of Enzyme Activity (%)
Negative Control	13.44 ± 3.55^{a}	00.00 ± 0.00^{d}
05.0mg/ml	09.69 ± 0.29^{ab}	27.96 ± 2.15^{cd}
07.5mg/ml	09.11 ± 0.43^{abc}	32.26 ± 3.23^{bcd}
1.00mg/ml	07.08 ± 0.77^{bc}	47.31 ± 1.08^{bc}
12.5mg/ml	05.92 ± 0.77^{bcd}	55.91 ± 2.15^{abc}
15.0mg/ml	03.76 ± 0.77^{bcd}	72.04 ± 2.15^{abc}
17.5mg/ml	03.04 ± 1.00^{cd}	77.42 ± 7.45^{ab}
Actellic	00.63 ± 0.62^{d}	95.38 ± 4.66^{a}

Values are expressed as mean ± SEM for triplicate readings (n=3). Values with different superscripts along the same column are significantly different calculated by One Way ANOVA followed by Fisher LSD post hoc test (p<0.001)

On the other hand, the DCM leaf extracts of *V. lasiopus* also showed appreciable efficacy against AChE enzyme from *S. zeamais* as was indicated by remarkable AChE enzyme activity inhibition by the various extract concentrations (Table 4.29; Figure 4.16; Appendix 8.2). The extract manifested inhibitory effects of between 10.75 and 72.04% (Table 4.29). All the tested extract concentrations of *V. lasiopus* possessed the ability to inhibit acetylcholinesterase of *S. zeamais* in a dose dependent manner (Table 4.29; Figure 4.16; Appendix 8.2).

Low extract concentrations of 5 and 7.5 mg/ml were notably ineffective in hindering AChE enzyme activity as they showed low inhibitory effects of 10.75 and 30.11% respectively, which was statistically comparable to the effects observed in the negative control group ($p<0.005$; Table 4.29; Appendix 8.2). However, the rest of extract concentrations evidently manifested effectiveness, which were comparable to each other as well as to the effects manifested by Actellic (95.38%), ($p<0.005$; Table 4.29).

Table 4.29: *Ex-Vivo* Anti-acetylcholinesterase Activities of DCM Leaf Extracts of *V. lasiopus*

Treatment (mg/ml)	**Mean Enzyme Activity (µmoles/min/mg protein)**	**Mean Inhibition of Enzyme Activity (%)**
Negative Control	13.44 ± 3.55^{a}	00.00 ± 00.00^{d}
05.0mg/ml	11.99 ± 0.63^{ab}	10.75 ± 04.69^{cd}
07.5mg/ml	09.39 ± 0.72^{abc}	30.11 ± 05.38^{bcd}
1.00mg/ml	07.66 ± 1.38^{bc}	43.00 ± 10.30^{bc}
12.5mg/ml	05.64 ± 0.75^{bcd}	58.06 ± 01.86^{abc}
15.0mg/ml	05.06 ± 0.77^{cd}	62.37 ± 02.15^{ab}
17.5mg/ml	03.76 ± 0.81^{cd}	72.04 ± 02.84^{ab}
Actellic	00.63 ± 0.62^{d}	95.38 ± 04.66^{a}

Values are expressed as mean ± SEM for triplicate readings (n=3). Values with different superscripts along the same column are significantly different calculated by One Way ANOVA followed by Fisher LSD post hoc test ($p<0.001$)

In comparison, the DCM leaf extract of *T. diversifolia* was found to be more effective at inhibiting acetylcholinesterase enzyme of *S. zeamais* as compared to *V. lasiopus*, at the least tested extract concentration (5 mg/ml), ($p<0.001$; Figure 4.16). However, the two plant extracts exhibited comparable enzyme inhibitory effects at the rest of the plant extract concentrations ($p>0.001$; Figure 4.16).

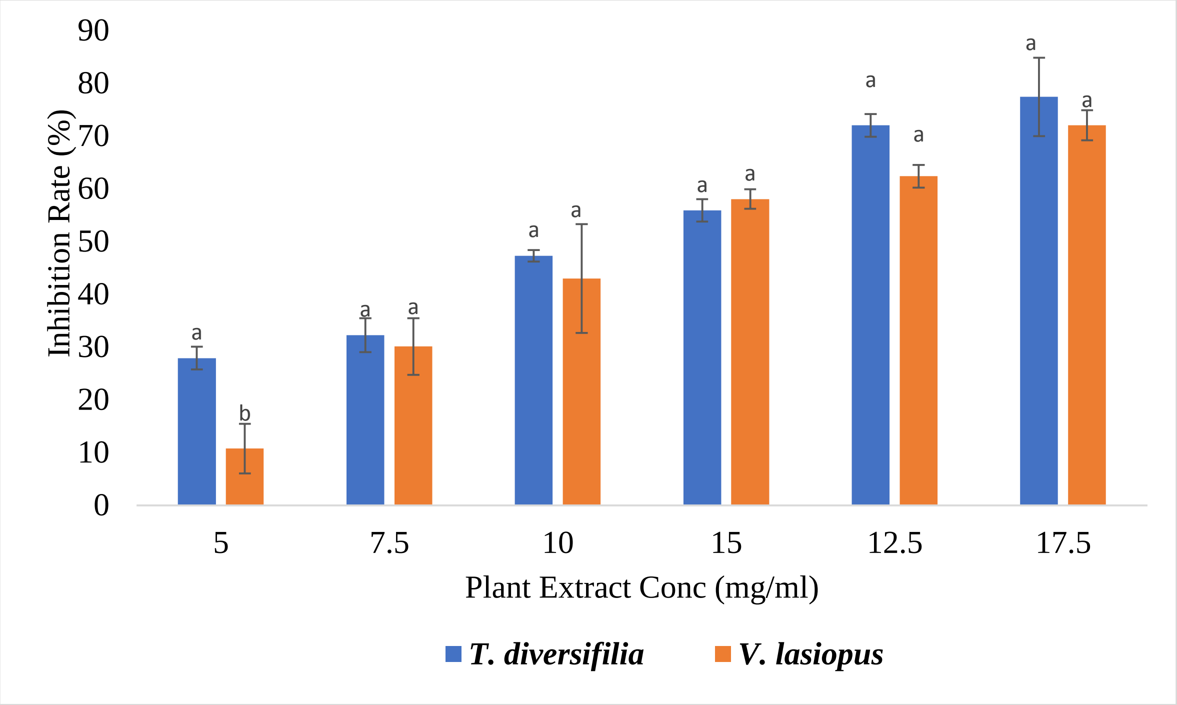

Figure 4.16: **Comparison of Percent Enzyme Inhibition at Various Concentrations of DCM Leaf Extracts of *T. diversifolia* and *V. lasiopus* Against Acetylcholinesterase Enzyme of S. zeamais**
Bars with different superscripts within the same concentration (%) are significantly different calculated by un-paired student *t*- test (hours; $p<0.001$)

4.10.3 *Ex-vivo* Anti-Acetylcholinesterase Activities of Ethyl Acetate Leaf Extracts of *T. diversifolia* and *V. lasiopus*

Overall, it was observed that the EtOAc extracts of *T. diversifolia* and *V. lasiopus* caused a regular pattern of enzyme inhibition, which was indicated by reduction in enzyme activity by various extract concentrations (Tables 4.30 and 4.31; Figure 4.17; Appendix 8.3). Small changes in absorbance were associated with higher inhibition of enzyme activity (Tables 4.30 and 4.31).

The EtOAc leaf extracts of *T. diversifolia* showed effective efficacy against AChE enzyme as was indicated by remarkable AChE inhibitory effects, which were statistically different from the effects seen in the negative control group (Tables 4.30). The extracts exhibited enzyme inhibitory activities ranging between 45.81 and 89.25% while the negative control group had no effect against the enzyme activity (Table 4.30).

The percent inhibition of AChE activity by the EtOAc extracts of *T. diversifolia* at the concentration of 5 mg/ml was significantly different from the effectiveness of Actellic ($p<0.001$; Table 4.30). However, the inhibitory effects of the rest of the extract concentrations (7.5, 10, 12.5, 15 and 17.5 mg/ml) were statistically equipotent to the effectiveness observed in the standard pesticide (Actellic) ($p>0.001$; Table 4.30).

Table 4.30: *Ex-vivo* Anti-Acetylcholinesterase Effects of Ethyl Acetate Leaf Extracts of *T. diversifolia*

Treatment (mg/ml)	**Mean Enzyme Activity (µmoles/min/mg AChE Protein)**	**Mean Inhibition of Enzyme Activity (%)**
Negative Control	13.44 ± 3.55^{a}	00.00 ± 0.00^{d}
05.0mg/ml	07.29 ± 0.48^{b}	45.81 ± 3.59^{c}
07.5mg/ml	05.78 ± 1.04^{bc}	56.99 ± 7.75^{bc}
10.00mg/ml	04.19 ± 1.28^{bcd}	68.82 ± 9.56^{abc}
12.5mg/ml	02.89 ± 0.58^{bcd}	78.49 ± 4.30^{abc}
15.0mg/ml	02.02 ± 1.18^{cd}	84.95 ± 8.80^{ab}
17.5mg/ml	01.45 ± 0.58^{cd}	89.25 ± 4.30^{ab}
Actellic	00.63 ± 0.62^{d}	95.38 ± 4.66^{a}

Values are expressed as mean ± SEM for triplicate readings (n=3). Values with different superscripts along the same column are significantly different calculated by One Way ANOVA followed by Fisher LSD post hoc test ($p<0.001$)

On the other hand, the EtOAc leaf extracts of *V. lasiopus* reduced AChE enzyme activity with remarkable inhibition rates of between 25.81 and 82.80% (Table 4.31; Figure 17; Appendix 8.3). The extract at higher tested concentrations of 10, 12.5, 15.0 and 17.5

mg/ml, manifested effective AChE enzyme inhibitory effects, which were statistically equipotent to each other as well as to the effects caused by Actellic ($p>0.005$; Table 4.31). However, it was observed that the extract concentrations of 5 and 7.5 mg/ml were not effective as they were not statistically different from the effects seen in the negative control set up ($p>0.005$; Table 4.31).

Table 4.31: *Ex-vivo* Anti-Acetylcholinesterase Effects of Ethyl Acetate Leaf Extracts of *V. Lasiopus*

Treatment (mg/ml)	Mean Enzyme Activity (µmoles/min/mg Protein)	Mean Inhibition of Enzyme Activity (%)
Negative Control	13.44 ± 3.55^{a}	00.00 ± 0.00^{d}
05.0mg/ml	09.98 ± 0.75^{ab}	25.81 ± 5.59^{cd}
07.5mg/ml	09.11 ± 0.43^{abc}	32.26 ± 3.23^{bcd}
10.00mg/ml	06.79 ± 0.52^{bcd}	49.46 ± 1.08^{abc}
12.5mg/ml	04.34 ± 0.87^{bcd}	67.74 ± 6.45^{abc}
15.0mg/ml	03.04 ± 0.90^{cd}	75.27 ± 2.84^{ab}
17.5mg/ml	02.31 ± 0.63^{d}	82.80 ± 1.08^{a}
Actellic	00.63 ± 0.62^{d}	95.38 ± 4.66^{a}

Values are expressed as mean ± SEM for triplicate readings (n=3). Values with different superscripts along the same column are significantly different calculated by One Way ANOVA followed by Fisher LSD post hoc test ($p<0.001$)

A comparative contrast between the ethyl acetate extracts of *T. diversifolia* and *V. lasiopus* revealed that the *T. diversifolia* extract generally exhibited the strongest acetyl-cholinesterase enzyme inhibition potential at 5, 7.5 and 10 mg/ml (Figure 4.17). The *T. diversifolia* extract, at the doses of 5, 7.5 and 10 mg/ml, showed significantly higher enzyme inhibitory effects as compared to the *V. lasiopus* extract ($p<00.005$; Figure 4.17). However, the *T. diversifolia* and *V. lasiopus*, at the higher tested extract concentrations of

12.5, 15 and 17.5 mg/ml, manifested comparable effectiveness against acetylcholinesterase enzyme activity (p>0.005; Figure 4.10.3).

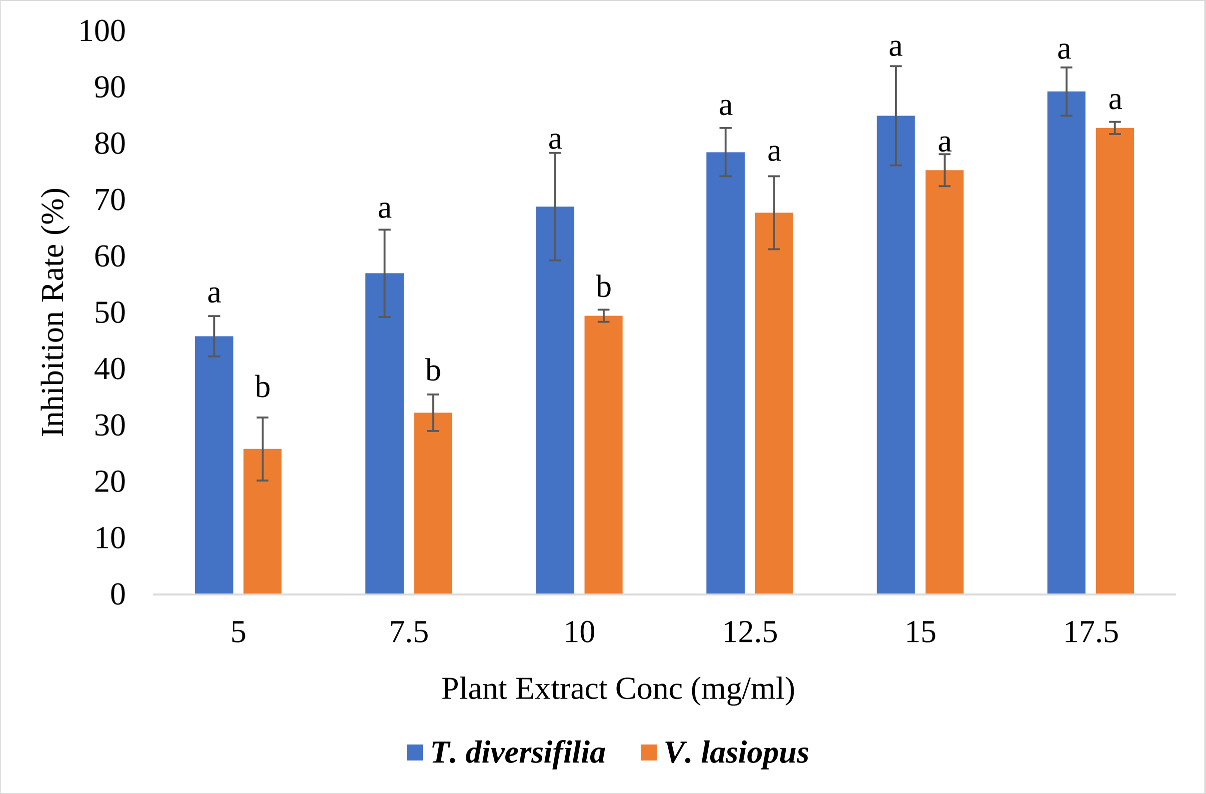

Figure 4.17: Comparison of Percent Enzyme Inhibition at Various Concentrations of Ethyl Acetate Leaf Extracts of *T. diversifolia* and *V. lasiopus* Against AChE of *S. zeamais*

Bars with different superscripts within the same concentration (%) are significantly different calculated by un-paired student *t*- test (hours; p<0.005)

4.10.4 Median Inhibitory Concentrations of Plant Extracts

From the calculation of percent inhibition of enzyme activity versus log concentrations of the tested plant leaf extracts, it was found that the IC_{50} of DCM extracts of *T. diversifolia* and *V. lasiopus* were 11.24 and 10.05 mg/ml ,respectively while those of EtOAc extracts of the two plants were 10.38 and 9.98 mg/ml, respectively (Table 4.32). This information provided an estimation of the plant extract concentrations required to inhibit acetylcholinesterase enzyme activity by 50%. The EtOAc extracts of *V. lasiopus* had the least IC_{50} (9.98 mg/ml) and hence established to be the most effective of the tested extracts, there was no significant statistical difference among the IC_{50} values of all the tested plant leaf extracts (p>0.005; Table 4.32).

Table 4.32: IC_{50}'s Calculated from Percent (%) Inhibition of Crude AChE Activity by Selected Extracts of *T. diversifolia* and *V. lasiopus Ex-vivo*

Plant Extract	**IC_{50} Values**
DCM leaf extract of *T. diversifolia*	11.24 ± 1.48^{a}
DCM leaf extract of *V. lasiopus*	10.05 ± 0.67^{a}
Ethyl acetate Leaf Extract of *T. diversifolia*	10.38 ± 0.36^{a}
Ethyl acetate Leaf Extract of *V. lasiopus*	09.98 ± 1.25^{a}

The IC_{50} Values are expressed as mean ±SEM for triplicate test readings (n=3). Values with similar superscripts along the same column were not significantly different calculated by One Way ANOVA followed by Fisher LSD post hoc test (p>0.001)

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Phytochemical Analysis of Extracts of *T. diversifolia* and *V. lasiopus*

This study evaluated the presence and identity of biologically active components of dichloromethane and ethyl acetate leaf extracts of *T. diversifolia and V. lasiopus* using GC-MS. The gas chromatogram revealed relative concentrations of various phytochemicals eluted at different retention times (Apendices 2.1, 2.2, 2.3 and 2.4). The heights of the peaks are indicative of relative abundance of the components present in the plant extracts (Apendices 2.1, 2.2, 2.3 and 2.4).

The fragmentation pattern indicated disintegration of large fragments into smaller compounds that gave rise to appearance of peaks at different m/z ratios. Mass spectra provided a blueprint of phytocompounds of the organic leaf extracts of *T. diversifolia* and *V. lasiopus,* which were identified from the National Institute of Standards and Technology (NIST) data library (Apendices 2.1, 2.2, 2.3 and 2.4).

This study identified 41 and 25 phyto-compounds in the DCM leaf extracts of *T. diversifolia* and *V. lasiopus* respectively (Tables 4.2 and 4.3) while the ethyl acetate leaf extracts of *T. diversifolia* and *V. lasiopus* was found to contain 66 and 40 bioactive compounds respectively (Tables 4.2 and 4.3). These compounds were found at different concentrations, featured by the major components at high concentrations (above 100 (ng/g) compared to the other compounds, which were present in smaller quantities (Pichersky, 2006).

The low extract yields observed in this study for the two plants may be since the plant materials were air dried contrary to the fresh samples of the same plants used in other studies (Chukwuka and Ojo, 2014). Furthermore, the organic extracts yields from members of this family (Asteraceae) are low compared to those of members of other plant families. For instance, lamiaceae family members have been reported as among the top extract producers with yields of up to 3% w/w (Telci and Sahbaz, 2005; Agostini *et al.*, 2009). These results agree with the findings of other earlier studies that reported low extract yields of up to 0.1%w/w from the samples of these plants (Moronkola *et al*., 2007; Chukwuka and Ojo, 2014). Likewise, Hădărugă *et al.* (2009) reported a high extract yield of 0.5% w/w for many plants in the Asteraceae family.

The GC-MS results of the present study showed the presence of active insecticidal compounds in *T. diversifolia* and *V. lasiopus.* These compounds include lipids (fatty acid esters and phytosterols), terpenoids (monoterpene, sesquiterpenoids diterpenes and triterpenes) phenolic compounds and alkaloids. This is not different from the findings of previous studies using the two plants albeit using different extraction solvents (Adedire and Ajayi, 1996; Chon *et al.,* 2000; Waliwitiya *et al.,* 2005; Muregi *et al.,* 2007; Kouninki *et al.,* 2007; Nyamador *et al.,* 2010; Olutobi and Olasupo, 2012; Ngamo *et al.,* 2017).

Limonene was specifically identified in the ethyl acetate leaf extract of *V. lasiopus*. Limonene is a terpene, usually referred to as d- limonene, which is its main chemical form.

Today, limonene is often used as a natural treatment for a variety of health issues. It has been studied for its potential anti-inflammatory (Yu *et al.,* 2015), antioxidant (Gabriele *et al.,* 2017), anticancer properties (Jessica *et al.,* 2013) and against heart diseases (Jing *et al.,* 2014) among other health benefits. It has a strong aroma that protects plants by deterring predators and pests (Miguel, 2010). In Califonia, limonene extracted from cloves and peppermint is heavily used for controlling structural pests such as termites (Liu *et al.,* 2011).

Limonene contained in *Citrus sinensis* was reported to be effective against *Myzus persicae* at LC_{50} 57.7 µl ml^{-1}, whereas its essential oil, at a concentration of 3.3 µl ml^{-1}, caused significant mortality of *B. brassicae* (Jahan *et al.,* 2016). It is also a component of lemongrass and citrus peels, which are extensively used for the control of fleas and ticks in the companion animals (Isman, 2014). Limonoids such as azadirachtin present in species from the Meliaceae are recognized for their toxic effects on insects and are used in several insecticide formulations in many parts of the world (Harve and Kamath, 2004). Limonene in the essential oil of *Cymbopogon giganteus* has demonstrated insecticidal activity against *C. maculatus* and *C. subinnotatus* (Nyamador, 2010).

Phytochemical analysis of organic leaf extracts of *T. diversifolia* and *V. lasiopus* also revealed the presence of linalool. Linalool from leaves of *P. angolensis* have been demonstrated to have pesticidal effects against several insects including *Sitophilus zeamais*, *Rhyzopertha dominica* and *Callosobruchus maculatus* (Ketoh *et al.,* 2007;

Noudogbessi *et al.,* 2013). According to the findings of Liu *et al.* (2011) linalool extracted from *F. Africana* and *T. Vogelii* is toxic to insects and hence considered insecticidal.

Eugenol is an interesting naturally occurring phenolic monoterpene belonging to phenylpropanoids group, which was also identified in the organic leaf extracts of *T. diversifolia* and *V. lasiopus*. It has anti-inflammatory as well as antioxidant potential (Joice *et al.,* 2018). Eugenol has versatile actions against insects, bacteria, pathogens and other harmful microorganisms (Bendre *et al.,* 2016). Furthermore, eugenol or its mixture with citronellal has been patented for pesticidal activities against cockroaches and aphids (Ping, 2007) as well as against house flies (*Musca domestica*) (Radhika *et al.,* 2011).

Squalene is a triterpene that is an intermediate in the cholesterol biosynthesis pathway (Popa *et al.,* 2015). It was also found in the organic leaf extracts of *T. diversifolia* and *V. lasiopus*. Many other polyprenyl compounds structurally like squalene include β-carotene, coenzyme Q10 (ubiquinone) and vitamins A, E and K (Zih-Rou *et al.,* 2009). Squalene has been reported to have antitumor effects (Desai *et al.,* 1996; Senthilkumar *et al.,* 2006), antioxidant activity (Aioi *et al.,* 2005), protective and moisturizing role in the skin (Ashida *et al.,* 2005; Fuller *et al.,* 2005) as well as improving the immune response to vaccines (activating antigen-presenting cells and T-cells (Cardeno *et al.,* 2015).

The results of the GC-MS analysis of *T. diversifolia* and *V. lasiopus* also revealed the presence of amyrin. Amyrins are three (α-amyrin, β-amyrin and δ-amyrin) closely related pentacyclic triterpenol compounds (Saimaru *et al.,* 2007). In plant, α-amyrin is the precursor of ursolic acid and β-amyrin is the precursor of oleanolic acid (Berni *et al.,* 2019)

α-amyrin are common phytocompounds found in medicinal plants. Previous studies have shown that α-amyrin exhibit antioxidant, anti-inflammatory and anti-lipoxygenase activities. The use of *C. gigantean* to treat asthma is associated with anti-lipoxygenase activity as well as antioxidant effects of α-amyrin (Bulani *et al.,* 2011). The compound has been suggested to play a key role in the anti-inflammatory effects of *B. Simaruba* extracts (Carretero *et al.,* 2008). α-amyrin has the potential to combat acute pancreatitis by acting as an anti-inflammatory and antioxidant agent (Melo *et al.*, 2010). It has also been proposed as possible biomarkers for the fungal resistance of grape-vine leaves (*Vitis vinifera*) (Batovska *et al.*, 2008).

The β-amyrins was also identified from the organic extracts of *T. diversifolia* and *V. lasiopus*. Both *in vitro* and in *vivo* studies have shown that β- amyrin also exhibit important biological functions including anti-microbial, anti-inflammatory, antifungal, anti-lipoxygenase, antioxidant (Rivero-Cruz *et al.*, 2008; Jabeen *et al.,* 2011; Bulani *et al.,* 2011), anti-ulcer (Rosas-Acevedo *et al.,* 2011) and other interesting biological activities. β-amyrin was found to exhibit weak cytotoxic activities against NTUB$_1$ cells, human bladder cancer cells (Mauricio *et al.,* 2018).

The results of the GC-MS analysis of ethyl acetate leaf extract of *V. lasiopus* revealed the presence of sabinene. Sabinene is a bicyclic monoterpene that contributes to the spiciness of black pepper (Jeleń and Gracka, 2015). It exhibits anti-inflammatory, antioxidant and insecticidal activities among other biological properties (Hammer *et al.*, 2012).

α-bulnesene is a major component of essential oil insectcides also found present in the extracts used in the present study. α-bulnesene is known to exhibit anti- inflammatory activity through its PAF receptor antagonistic effect as well as an anti-platelet aggregation property (Hui-Chun *et al.*, 2016). Previously, α-bulnesene has been reported to demonstrate pesticidal properties such as insecticidal, repellence and pupicidal activities on different insects (Gokulakrishnan *et al.*, 2013; Senthil-Nathan, 2020).

Terpinen-4-ol was only identified in the ethyl acetate leaf extract of *T. diversifolia.* It is an isomer of terpineol with a molecular weight of 154.249 micro grams. It has insecticidal as well as antibacterial and antifungal effects (Hammer *et al.,* 2012). It is considered the primary active ingredient of tea tree oil. It is also the compound of highest concentration in the essential oil of nutmeg. Terpinen-4-ol occurs in *Juniperus communis* and is thought to be the reason this wood is highly resistant to rot. Norwegian farmers used to exploit this by making fence poles out of juniper especially alpine subspecies (Hammer *et al.,* 2012). Purified terpineols is reported to have insecticidal and molluscicidal effects (Khaleel *et al*., 2081; Abdelgaleil, 2010).

The phytochemical analysis of DCM and ethyl acetate leaf extracts of *T. diversifolia* and *V. lasiopus* revealed the presence of caryophyllene oxide. Caryophyllene oxide is a sesquiterpene that possesses pesticidal activities. Caryophyllene oxide identifed from

Melaleuca styphelioides exhibited strong insecticidal activities against *Aphis spiraecola*, *Aphis gossypii* and *M. persicae* (Amoabeng *et al.*, 2013). (E)-caryophyllene myrecene contained in *C. sativa*, showed effective insecticidal activities on *Aulacorthum solani* (Benelli *et al.*, 2016) and *M. persicae* (Benelli *et al.*, 2018).

The insecticidal activities of chloroform extract of *P. nigrum* and petroleum ether extract of *J. curcas* was mainly related to caryophyllene oxide among other sesquiterpenes (Adebowale and Adedire, 2006; Lee *et al.*, 2011). *Tithoni diversifolia* has been found to be highly anti-plasmodial due to the presence of caryophyllene oxides among other sesquiterpenes (Ajaiyeoba *et al.*, 2006).

The results of the GC-MS analysis of *T. diversifolia* and *V. lasiopus* revealed the presence of copaenes, which is a chemically tricyclic sesquiterpenes. α-copaene is rare but is found in small amounts in some plants. The name, α-copaene, is derived from that of the resin-producing tropical copaiba tree (*Copaifera langsdorfii*), from which the compound was first isolated in 1914. Its structure, including the chirality, was determined in 1963 (Zhang *et al.*, 2011). The double-bond isomer with an exocyclic-methylene group, β-copaene, was first reported in 1967. α-copaene is significant because it strongly attracts Mediterranean fruit fly (*Ceratitis capitata*) which is an important agricultural pest (Zhang *et al.*, 2011).

GC-MS analysis of organic leaf extracts of *T. diversifolia* and *V. lasiopus* also revealed the presence of α-pinene. α-pinene is a insecticidal monoterpenes whose pesticidal effects have been demonstrated against several insects. The leaves of *P. angolensis* were reported to contain α-pinene that was credited for the plant's pesticidal effects against *Sitophilus zeamais*, *Rhyzopertha dominica* and *Callosobruchus maculatus* (Ketoh *et al.*, 2006; Noudogbessi *et al.,* 2009). Further, α-pinene in *C. sativa*, showed effective insecticidal activity on *Aulacorthum solani* (Benelli *et al.,* 2016) and *M. persicae* (Benelli *et al.*, 2018).

Widdrol is an odorous compound, which was also identified in the ethyl acetate leaf extract of *T. diversifolia.* It is widely used in traditional medicine to treat fever, inflammation and cancer. It has previously been reported that widdrol has *in vitro* antitumor activity affected through apoptosis induction in cancer cells. However, reports of its pesticidal activity remain elusive (Jin *et al.,* 2015).

The GC-MS profile of the two organic leaf extracts indicated the presence of long-chain polyunsaturated fatty acids that includes omega-3 and omega-9 fatty acids. Omega-3 fatty acids included alpha linolenic acid, hexadecanoic acid, docosanoic acid, pentadecanol, crotonic acid, dodecanoic acid, Pentadecanol, Tetradecanoic acid and Octadecatrienoic acid among others. The identified omega-9 fatty acids included olean-12-ene acid. The omega-3 fatty acids are derived from linolenic acid while the omega-9 fatty acids from oleic acid (Pereira *et al*., 2011). The alpha linolenic acid is usually converted into docosanoic acid and other more usable forms of essential fatty acids (Pereira *et al*., 2011; Cave *et al*., 2020).

Tetradecanal is a long-chain fatty aldehyde that is tetradecane in which two hydrogens attached to a terminal carbon are replaced by an oxo group. Tetradecanal is found to exhibit strong anti-inflammatory and antibacterial activity (Resch *et al.,* 2001; Cardeno *et al.*, 2015; Diksha *et al.,* 2019). Further, methyl linoleate is a fatty acid whose pesticidal effectiveness has been reported against *Zabrotes subfasciatus* (Boheman) that attack stored beans (Hill and Schoonhoven, 1981).

Oleic acid and linoleic acid were reported to have insecticidal effects against larvae of *Aedes aegyptii, Helicoverpa zea*, *Lymantria dispar*, *Orgyia leucostigma* and *Malacosoma disstria* (Ramsewak *et al.*, 2001). Cockroaches are known to release "smell of death" (oleic and linoleic acid) upon death. Olenolic acid is reported to inhibit bacterial growth (Rivero-Cruz *et al.,* 2008). These compounds deter and prevent other roaches from entering the area. The similar mechanism is found in ants, bees and other insects to keep away other insects upon their death (Pascal *et al.*, 2005; Poul *et al.*, 2016).

Citronellyl isobutyrate is another fatty acid compound identified in the ethyl acetate leaf extract of *T. diversifolia*. Citronellal, cotronellol and citonellyl or mixtures of these monoterpenoids have been patented as a pesticide against lice in human (Ping, 2007; Lucia *et al.* 2017). The antioxidant and antimicrobial activity *Pelargonium graveolens* essential oil was characterized by the predominance of citronellol (Wissal *et al.*, 2013).

Phytochemical analysis of *T. diversifolia* and *V. lasiopus* also revealed the presence of phenolic compounds like 5-indanol, phenol, 2,4-bis (1,1-dimethylethyl) among others. Phenols are the simplest secondary metabolites with benzene rings and containing hydroxyl substituents. They are synthesized through pathways of shikimic acid, pentose phosphate and phenylpropanoid metabolism in plants (Randhir *et al*., 2004).

Phenolic acids contain a hydroxyl group bonded to a carbon atom that is part of an aromatic ring (Jensen, 2009). They occur in form of glycosides or esters conjugated with different natural compounds such as alcohols, hydroxy fatty acids, flavonoids and sterols (Giacomelli *et al.*, 2004; Jensen, 2009). Doughari (2009; 2012) pointed out that the role of phenols in *T. diversifolia* is in defense system of the plant against pathogens and pests and hence its pesticidal properties.

The GC-MS analysis of ethyl acetate extracts of *T. diversifolia* also indicated the presence of alkaloids including indole and diisopropylethylamine. This is in agreement with results of Olayinka *et al.* (2015) who also indicated that *T. diversifolia* leaves contain insecticidal alkaloids. Alkaloids are reported to confer pesticidal activities by affecting the antioxidant system in insects (Chowanski *et al.*, 2016). They generate reactive oxygen species (ROS), which induce metabolic stress in cells. Consequently, ROS cause processes such as the peroxidation of membrane lipids, protein damage or the disruption of mitochondrial membrane potential. This eventually leads to the death of organism (Omayio *et al.*, 2016).

Alkaloids (glycoalkaloids) are insecticidal and act as phytoalexins protecting crops against insect pests attack. Adamski *et al.* (2014) evaluated and confirmed oxidative damage by steroidal glycoalkaloid-α-solanine from potato leaves on adult *Galleria mellonella* (L.) leading to their death. The insecticidal activities of organic extract of *P. nigrum* and *J.*

curcas extracts were mainly related to their alkaloid components (Adebowale and Adedire, 2006; Lee *et al.*, 2011).

Indanol is a unique alkaloid, which is associated with several important biological properties such as antimicrobial, anti-fungal, antiviral, antitubercular, anti-inflammatory, anti-HIV, CNS depressant, analgesic, hypnotics and antipsychotic activities. Indanols also possess insecticidal activities (Strehlke *et al.*, 1975; Christelle *et al.*, 1998; Wang *et al.*, 2016).

Major phytosterols were also revealed in the GCMS analysis of the organic leaf extracts of *T. diversifolia* and *V. lasiopus.* Stigmasterol is among the phytosterol compounds structurally similar to cholestrerol. It is reported to lower cholesterol absorption in the intestines hence showing potential to reduce the risk of cardiovascular diseases (Abooducker and Suza, 2019). However, recent studies demonstrated that stigmasterol accumulation can lead to cardiac injury and promote mortality in a rodent model of phytosterolemia. Chondrillasterol, also identified in the extracts, has been previously associated with both antibacterial and antibiofilm activities against *S. aureus*, *K. pneumoniae* and *P. aeruginosa* (Abooducker and Suza, 2019; Mozirandi *et al.*, 2019).

Nerolidol (3,7,11-trimethyl-1,6,10-dodecatrien-3-ol) is another compound identified in the ethyl acetate leaf extracts of *V. lasiopus*. It is a naturally occurring sesquiterpene alcohol that is present in various plants with a floral odor. It is synthesized as an intermediate in the pro duction of (3E)-4,8-dimethy-1,3,7-nonatriene (DMNT), a herbivore-induced volatile that protects plants from herbivore damage (Weng-Keong *et al.,* 2016). Nerolidol exhibits a floral odor and is used as a flavoring agent in perfumery owing to its floral fruity fragrance and rose apple note (Jirovetz *et al.,* 2007). It is also used in non-cosmetic products as cleansers and detergents asides from its health effects such as antimicrobial, antifungal, antioxidant and anticancer effects (Chan *et al.,* 2016).

Phytochemical analysis of *T. diversifolia* and *V. lasiopus* also revealed the presence of phytol. Phytol is an acyclic diterpene alcohol that can be used as a precursor for the manufacture of synthetic forms of vitamin E (Werner, 2007) and vitamin K_1 (Daines, 2003). Phytol is used in the fragrance industry to make cosmetics, shampoos, toilet saops, household cleaners, detergents as well as in vape fluid (Apostolidis *et al.,* 2002). Insects, such as the sumac flea beetle, are reported to use phytol chemical and its metabolite compounds originating from the host plants such as phytanic acid as chemical deterrents against predation (Vencl and Morton, 1998). Phytol is therefore considered pesticidal in nature.

p-Xylene (para-xylene) is an aromatic hydrocarbon also identified in the organic leaf extracts of *T. diversifolia* and *V. lasiopus*. It is one of the three isomers of dimethylbenzene known collectively as xylenes. The *p*- stands for *para*-, indicating that the two methyl groups in *p*-xylene occupy the diametrically opposite substituent position 1 and 4. It is the positions of the two methyl groups, their arena substitution pattern, which makes it differ from the other isomers, *o*-xylene and *m*-xylene (Sabatini *et al.,* 2008**)**. All xylene isomers are colorless and highly flammable. Its effects vary with animal and xylene isomer in question (Hino *et al.*, 2008). *p*-xylene can affect the central nervous system if swallowed and could cause chemical pneumonitis when breathed into the lungs. Furthermore, previous studies have suggested that *p*-xylene could cause damage to development and reproductive systems (Rajanand Malathi, 2014). Therefore, as results in this chapter show, the two plants have potent and bioactive phytocompounds associated with many biological activities including but not limited to pesticidal activities.

5.1.2 Fumigant and Contact Toxicity Effects of Extracts of *T. diversifolia* and *V. lasiopus*

The present study was designed to assess the toxicity effects of organic leaf extracts of *T. diversifolia* and *V. lasiopus* against *S. zeamais*. It was apparent that the two plants possess both contact and fumigant toxic properties on adult weevils hence showed insecticidal potential against the insect pests. The extracts showed fumigant toxicity ratings that ranged between low toxicity of 41.17% (in 50% DCM extract concentration of *V. lasiopus*) and high toxicity of 98.7% at 100% EtOAc extract concentration of *T. diversifolia* extract after 96 hours of exposure to the extracts. On the other hand, the extracts had contact toxicity ratings that ranged between moderately low toxicity of 31.57% at 50 % DCM leaf extract of *V. lasiopus* and very high toxicity of 99.93% at 75 and 100% DCM extract dose levels of *T. diversifolia* after 96 hours of exposure to the extracts.

There is immense scientific literature on both crude extracts of plants and isolated phytochemicals with insecticidal effects against storage pests and hence fronted for consideration as bioresourse for developing new botanical biopesticide agents. Many plant species such as *O. basilicum, M. piperita, P. anisum, M. pulegium, A. indica* and *F.*

vulgare among others, have shown outstanding effectiveness against insects in both contact and fumigation assays (Ikbal and Pavela, 2019).

The insecticidal results of the present study are therefore, in accordance with many other botanical studies which have reported to be an effective control against major pest species of stored grain system. Trivedi *et al.* (2017) demonstrated fumigant toxicity against the stored grain pest *Callosobruchus chinensis*. The essential oils of cinnamon, clove, rosemary, bergamot and Japanese mint also showed effective fumigants toxicity against pulse beetle (Malik *et al.*, 2012; Iqbal *et al.*, 2015).

Similarly, the present findings support the results of Stoll (2001) who reported organic leaf extracts of various plants have effective toxicity against insect pests of various crops on the field and in store. In consonance with the findings of the present study, the extracts of *P. amarus*, *A. albida* and *T. diversifolia* also demonstrated insect mortalities of between 40 and 56%, 24 and 60% as well as 42 and 88%, respectively (Oyedokun *et al.*, 2011).

Ouko *et al.* (2017) carried out similar laboratory-based tests to determine the toxicities of methanolic, hexane and methanolic: hexane blend extracts of *Allium sativum* on maize weevils using the four concentrations levels of 25, 50, 75 and 100%. To evaluate the contact toxicity effects of the alcoholic leaf extracts of *T. diversifolia* on termites Oyedokun *et al.* (2011) used similar test dosages used in this study and demonstrated equally high toxicity effects of *T. diversifolia* on termites

The synthetic insecticide used in this study, Actellic, is a broad-spectrum insecticide. It is used for control of large grain borer, weevils, and other insects and mites of stored grains and pulses. It contains permethrin (3 g/kg) and pirimiphos-methyl (16 g/kg) as its active ingredients which give Actellic an effective control against storage pests. Pirimiphos-methyl, which is taken by the insect through its respiratory system, affect the pests through its fumigant and repellence activity. On the other hand, permethrin can penetrate the insect cuticle and hence affect its contact and stomach activity on pests (Taylor, 1989).

It is worth noting that, during 48 hours test observation time, the fumigant activity of EtOAc and DCM leaf extract of *T. diversifolia* at the highest tested extract concentration of 100% was marginally more effective (97.47 and 91.22%, respectively) than actellic super ™ (82.47%). This suggests a possibly better insecticidal mechanism of the extracts or mimicry of actellic mode of action by the active phytochemicals in the extracts. It is also possible that the EtOAc and DCM leaf extract of *T. diversifolia* were efficiently inhibiting alternative mechanisms for killing weevils.

The results of this study showed a direct relationship between the level at which the plant extract treatments were applied and their effectiveness on *S. zeamais***.** The effects of the treatments at different concentration levels on the S. zeamais were notably different from each other. In general, the fumigant bioactivities of DCM and EtOAc leaf extracts of *T. diversifolia* and *V. lasiopus* were directly proportional to the extract concentrations. The higher the plant extract dosage, the more potent the extract. The number of dead weevils increased with increasing the concentration.

This could be due to the increase in bioactive components as the concentration of the extract increases such that, it is likely that at the lower dose there was simply not a sufficient concentration of the active principle(s). This variability can also be explained by the fact that the probabilities of feeding on the botanical insecticidal compounds along with extract particle increases with increase in concentration.

This trend of mortality rate in correlation with extract concentration was consistent with the findings of Cheubey (2012) and Ouko *et al.* (2017) that showed a positive concentration dependent correlation of *A. sativum* verses mortality in pulse beetle and maize weevils respectively. The correlation suggests that organic extracts of the two plant can best be applied at 100% v/v concentration in order to have a better kill of maize weevils by fumigation. This may be due to the mixture of the active compounds that were in the best proportional mixture for insecticidal activities at 100 % v/v concentration (Ouko *et al.,* 2017).

The toxicity activities of the two plants on the weevils varied according to the solvent used during extraction. The content and the activity of the extracted phytochemicals depend on the polarity of the solvents and the solubility of the bioactive compounds in the extracting solvent. Therefore, the extracting solvents plays an important role in the biocidal potency of plant crude extracts as shown in this study (Dai and Mumper, 2010; Dehkharghanian *et al.,* 2010).

Previous studies have shown that non-polar organic solvents like ethyl acetate extract pesticidal non-polar compounds such as terpenoids and phytosterols (Cowan, 1999).

Medium-polar solvents such as dichloromethane effectively extract flavonoids, terpenoids, phytosterols, fatty acids, alkaloids and phenols (Houghton and Raman, 1998; Cowan, 1999) which also exhibit pesticidal properties. Polar organic solvents like methanol usually extracts polar compounds such as amino acids, sugars and glycosides which are not particularly associated with pesticidal activities (Houghton and Raman, 1998; Yu *et al.*, 2009).

Therefore, the variation in toxicity effect of the extracts in this study can be attributed to the varying phytochemical composition of the extracts. The higher mortality exhibited by the ethyl acetate leaf extracts indicated that this organic solvent extracted more active compounds with insecticidal activities than the DCM leaf extracts. Therefore, where in this study, the fumigant activity of EtOAc and DCM leaf extracts of *T. diversifolia* exhibited no significant difference suggests that using either of the two extraction solvents renders no difference.

That the extracts manifested a higher mortality with an increased exposure time of the weevils to the treated maize grains, could be explained by the fact that increase in exposure time allows for more contact time with the target site and hence permits an increase in uptake of active constituents hence the observed higher mortality with longer exposure span.

The GC-MS analysis (Tables 3.3, 3.4, 3.5 and 3.6) revealed that the organic leaf extracts of *T. diversifolioa* and *V. lasiopus* contains phytochemical compounds which are toxic to insect pests and parasites. These compounds include citronellol, 1, 8-cineole, linalool, α-terpineol, and eugenol among others (Lalla *et al.*, 2013; Lucia *et al.*, 2017).

When inhaled, ingested or absorbed through the insect body surface, these compounds interfered with basic metabolic, biochemical, physiological and behavioral functions of adult *S. zemails*. The phytochemicals with insecticidal properties in this extract could have acted individually or in synergy thus potentiating its toxicity effects on weevils (Omolo *et al.*, 2004; Liu *et al.*, 2006).

Fatty acids identified by GC-MS analysis of the selected organic leaf extracts of *T. diversifolia* and *V. lasiopus* have been demonstrated to have insecticidal effectiveness against *S. zeamais* among many insect pests (Hill and Schoonhoven, 1981; Adebowale and Adedire, 2006; Lee *et al.,* 2011). It is therefore not strange that the extracts killed the adult weevils in the present assays. Furthermore, insecticidal effects of these fatty acids have been suggested to enhance the efficacy of the microbial insecticides such as *Bacillus thuringiensis* (Gaudet and Puritch, 1989).

Major Phytosterols also revealed in the GC-MS analysis of the organic leaf extracts of *T. diversifolia* and *V. lasiopus* can be associated to the extract's toxicity effects on the weevils. Stigmasterols are among the phytosterol compounds whose accumulation in body lead to cardiac injury and hence promote mortality (Tao *et al.,* 2019).

p-xylene may cause death of organisms through affecting the central nervous system if swallowed or causing chemical pneumonitis when breathed into the lungs. This suggests that *p*-xylene found in the organic leaf extracts of *T. diversifolia* and *V. lasiopus* could be responsible of both fumigant and contact toxicity effects of the extracts on weevils in the present study. Furthermore, previous studies have suggested that *p*-xylene could cause damage to development and reproductive systems (NIOSH, 2013).

The pesticidal effects of the selected organic leaf extracts of *T. diversifolia and V. lasiopus* could also be due to the presence of α-pinene in these extracts. Benelli *et al.* (2016) reported that α-pinene contained in the organic leaf extract of *C. sativa*, contributed to the 98.20% insect mortality. Furthermore, the results of Benelli *et al.* (2018) also indicated a similarly high mortality of *M. persicae* also associated with presence of α-pinene in the organic extracts of *Aulacorthum solani*.

Uptake of eugenol from the plant extracts may also have contributed to the high mortality of the adult insects in this study. Eugenol has been reported to have effective effects against insects such as aphids, house flies and cockroaches (Ping, 2007; Radhika *et al.*, 2011). The insecticidal activity of *C. cinnamomum* and *C. cymbopogon* species against *M. domestica* was also largely associated to the predominance (77%) of eugenol in their essential oils (Radhika *et al.,* 2011).

According to the GC-MS analysis, the organic leaf extracts of *T. diversifolia* and *V. lasiopus* contains limonene whose insecticidal activities have been are extensively reported against various insect such as *C. maculatus*, *C. subinnotatus, B. brassicae,* fleas and ticks among others (Nyamador, 2010; Isman, 2011; Jahan *et al.*, 2016). The toxicity effects of these extracts could also be as result of the presence of linalool. Few reports have been published regarding the mode of action of linalool in insects. However, like limonene, linalool is thought to cause death of insects by affecting the activity of nerves in insects (Abdelgaleil and El-Aswad, 2005; Abdelgaleil *et al.*, 2008; Rattan, 2010).

The insecticidal activity of the organic leaf extracts of *M. lucida* was attributed to sabinene among other known major components of oxygenated monoterpenes in the extract (Hammer *et al.,* 2012). It is, therefore, likely that the toxicity effects of organic leaf extracts of *T. diversifolia* and *V. lasiopus* on adult weevils in this study was contributed by sabinene which was also identified during phytochemical analysis of these extracts.

The toxicity of the extracts on adult weevils in the present study could also be linked to the presence of α-Bulnesene in the organic leaf extracts of *T. diversifolia* and *V. lasiopus* (Gokulakrishnan *et al.,* 2013). According to Albuquerque *et al.* (2007), α-Bulnesene extracted from *Pogostemon cablin* exhibits insecticidal activities against various urban ant species.

Also present in the extracts is Caryophyllene oxide which is an insecticidal sesquiterpene and hence probably responsible of the toxicity of the organic leaf extracts of *T. diversifolia* and *V. lasiopus* on weevils. Caryophyllene oxide identifed from *Melaleuca styphelioides* exhibited strong insecticidal activities against *Aphis spiraecola, Aphis gossypii* and *M.*

persicae (Amoabeng *et al.*, 2013). Further, a closely similar (E)-caryophyllene myrecene that was extracted from *C. sativa*, was particularly reported to exhibit effective insects killing potential on *A. solani* and *M. persicae* (Benelli *et al.*, 2016; Benelli *et al.*, 2018).

Insecticidal activity of organic leaf extracts of *T. diversifolia* and *V. lasiopus* could be as a result of caryophyllene oxide that was found to be highly present in the extracts. Acording to Zhang *et al.* (2014), sesquiterpenes like (E)-caryophyllene oxide are naturally pesticidal. (E)-caryophyllene perecence in the root bark of Chinese bittersweet, *Celastrus angulatus* Max, was largely associated with the toxicity effects of the plant extract against insects such as *Mythimna separate*.

It is also noteworthy, that as much as the extracts of *V. lasiopus* showed presence of insecticidal phytochemicals such as terpenoids, phenolics, phytosterols, fatty acids and alkaloids, their levels were low in the organic leaf extracts of *V. lasiopus* to induce an effective mortality on weevils as compared to *T. diversifolioa* extracts. That the organic leaf extracts of *V. lasiopus* had lower contact toxicity against maize weevils could be attributed to presence of these potent compounds in low concentration.

The possible cause of toxicity of these extracts on weevils is inhibition of acetylcholinesterase enzyme in the insects by the present phytochemicals. Many phytochemicals affect neurotransmission and signal transduction in organisms. Binding of these antagonists to the acetylcholinesterase receptors cause physiological and biochemical disturbances and blockage. The subsequently observed effects include restlessness, lack of coordination, unconsciousness and eventual death of the insect as similarly observed in the present study (Wink, 1993).

Njoroge *et al.* (2016) suggested that the aqueous and DCM leaf extracts of *G. glauca*leaves possess acetylcholinesterase enzyme inhibitory activity in *Chilo partellus* larvae at concentrations of 0.25, 5 and 7 mg/mL due to the presence of phenols, terpenoids, alkaloids and steroids in the extracts.

However, the observed toxicity of the extracts on weevils could also be that the active constituents in these extracts targeted voltage-gated sodium channels. These channels are vital for electrical signaling in most excitable cells. Pesticidal alkaloids from sabadilla and pyrethrins from *Tanacetum cinerariae folium* targets these channels, binds to specific receptors in the sodium channels and hence alters their functions (Silver *et al.,* 2014). Likewise, the pesticidal phytocompounds in this study could have acted by blocking the sodium channel pores and altered their gating function. This may have resulted to the cell being re-excited, hence inhibiting the generation of action potential leading to paralysis and ultimately the death of the weevils (BASF, 2013; Khater, 2013).

Other probable mechanisms that caused the death of adult weevils in this study could include DNA intercalation, interference of protein biosynthesis and disruption of membrane stability in the insect by allelochemicals of the plant. There is a positive correlation between the degree of DNA intercalation and inhibition of DNA polymerase I, reverse transcriptase and translation at the molecular level and with toxicity against insects at organismic level (Wink *et al.* 1998)

This study therefore indicates that the extracts exhibit considerably diverse insecticidal properties via fumigant and contact toxicity effects which were dose and exposure duration dependent. The study hence revealed the possible potency of *T. diversifolia and V. lasiopus* extracts at managing the population of *S. zeamais* on stored grains.

5.1.3 Repellent Activity of Organic Leaf Extracts of *T. diversifolia* and *V. lasiopus*

This study was designed to evaluate repellent properties of crude organic leaf extracts of *T. diversifolia* and *V. lasiopus* on *S. zeamais*. All the studied organic leaf extracts of *T. diversifolia* and *V. lasiopus* demonstrated potent repellent potential on *S. zeamais*. By the end of 5h of test period, it was evident that all the test samples turned out to simply repel *S. zeamais* from attacking the grains made them craw away from the extracts treated areas. Most of the insects stayed on the untreated areas of petri dishes and evaded the extracts-treated areas. The extracts were able to induce insect repellency of between 10 and 100% within 5 hours of experimental period.

With a minimum of 80% pesticidal action required for test substance to be considered successful (Kay *et al.,* 2019), both plant organic leaf extracts largely exhibited potential repellent actions against the weevils. The repellent index value of all the organic leaf extracts of *T. diversifolia* and *V. lasiopus* against *S. zeamais* adults was lower than 1, thus they were classified as insect repellents and not attractants. The present finding correlated with that of other plant extracts like *Aframomum melegueta* and *Zingiber officinale* which repelled adults *S. zeamais* (Ukeh *et al.*, 2009; Mmbone *et al.,* 2014; Ismail *et al.*, 2019).

Consistent with these findings, the hexane-ethyl acetate extracts of *C. capitatum* exhibited between 90 and 98% repellency activities against stored grain pests *S. oryzae, R. dominica*, and *T. castaneum* (Pudin *et al.,* 2013). Acetone seed extract of *Aphanamixis pofystachya* showed 100% repellent effects on red flour beetles (Talukder and Howse, 1995). Kumar *et al.* (2004) found a maximum of 91.2% of repellency with an extract of protein enriched bean flour on weevils, *S. oryzae* after 48 h of the test period. Similarly, high repellency was observed with treatments of *Zanthoxylum* sp. and *Eucalyptus camaldulensis* against facultative blowfly species, *Lucilia sericata* (Meigen) and *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae) (Vasanthakumar *et al.*, 2012; Kay *et al.*, 2019).

The high insect repellent results seen in this study are also supported by Boateng and Kusi (2008) who showed that *J. curcas* seed extracts could repel up to 95% of the *C. maculatus* and *D. basalis.* Likewise, Yoon *et al.* (2012) also reported that caraway and grapefruit successfully repelled weevils at such high rates. Acetone seed extract of *Aphanamixis pofystachya* showed 100% repellent effects on red flour beetles (Talukder and Howse, 1995). The ethanol extracts of *Urtica dioica* and *Taraxacum officinale* respectively showed 99.4% and 98.8% repellency, after 48 h of the study period (Jovanavic *et al.*, 2007).

Several other plants have been demonstrated to exhibit good repellent activities against *S. zeamais.* Ishii *et al*. (2010) reported high susceptibility of *S. zeamais* to extracts and essential oils of common spices. Members of alliaceae family like garlic have previously been reported to possess repellent properties on *S. zeamais* (Yang *et al*., 2012; Karavina *et al.*, 2014; Ouko *et al.*, 2017). The extracts of *M. nodosa*, *O. surinamensis* and *L. aurea* also showed repellent effects on *S. zeamais* (Fouad *et al*., 2012).

The present findings correlated with repellecy effects of diethyl ether extracts of *A. melegueta* and *Z. officinale* on *S. zeamais* (Ukeh *et al.*, 2009). Trivedi *et al.* (2017) further demonstrated repellent activities of essential oils of cinnamon, clove, rosemary, bergamot, and Japanese mint against pulse beetle (*Callosobruchus chinensis*). Many other related studies have also documented repellent potential of various plants against other post-harvest pests (Jilani and Su 1983; Prakash and Rao 1997; Juan *et al.*, 2008; Ogendo *et al.*, 2008; Benzi *et al.*, 2009; Nerio *et al.*, 2010; Auamcharoen *et al.*, 2012; Niroumond *et al.*, 2016).

In contrast, Tavares and Vendramim (2005) reported a lack of repellent activity of insecticidal extracts of *C. ambrosioides* on *S. zeamais*. Further, contrary to the findings of the present study, Jovanović *et al*. (2007) previously achieved very low repellent activities of herbal extracts of *T. officinale* (100%) and *U. dioica* (100%) against bean weevils. These findings do not agree with the results observed in the present work, in which all the studied extracts showed remarkable repellent activities on the target insect. This is probably due to performance of extracts derived from plants of different families from the presently studied extracts.

A free choice (area preference) bioassay model was used in this study for it is easily applicable and reliable. Similar laboratory tests were carried out using extracts of 13 plants to assess their repellent properties against the banana weevil (Emosairue *et al.,* 2005). A free choice bioassay system was also used to evaluate repellency effects of extracts and fractions from leaves of *C. capitatum* against three major stored grain insect pests viz; *S. oryzae*, *R. dominica*, and *T. castaneum* (Adesina *et al.,* 2019).

The extract concentration ranges used in this study were within the dose ranges used by Oyewole *et al.* (2008), Ouko *et al.* (2017) and Acero (2017; 2019). The works of Acero (2017) and Acero (2019) used extract concentrations of 25%, 50% and 75% in evaluating the pesticidal properties of *A. heterophyllus* and *C. odorota* against a closely similar weevil, *S. oryzae*. Ouko *et al.* (2017) also carried out similar laboratory experiments to determine the insect repellency of methanolic, hexane and methanolic: hexane blend extracts of *A. sativum* and *O. basilicum* on maize weevils using extract concentrations levels of used in this study.

The levels of repulsion of target insects in the present study were generally proportional to the extract concentrations. An increase in extract concentration resulted in an increase in the repulsion of *S. zeamais*. This could be due to the increase in bioactive components as the concentration of the extract increased. There was no appropriate concentration of the active principle(s) at the lower extract dose levels. It is also likely that at a lower dose, there is simply not a sufficient concentration of the active principle(s).

That the effectiveness of the extracts was dependent on extract concentrations is in agreement with earlier researches of Chaieb *et al.* (2007), Kafle and Shih (2013), Cortés-Rojas *et al.* (2014), Nattudurai *et al.* (2015) and Ismail *et al.* (2019) among others. In a related study, Marimuthu (2011) indicated plant extracts distilled from *C. citrates*, *C. zeylanicum*, *R. officinalis* and *Z. officinale* had promising dose-dependent repellent properties against *Culex tritaeniorhynchus* and *Anopheles subpictus*.

Interestingly, the effectiveness of *V. lasiopus* extracts at low concentrations (25 and 50%) was not significantly different from the effects caused by high extract doses (75 and 100%) as well as the positive control, Actellic, during the first 2 hours of the test period. This may be since, even at low extract concentration, the combination of repellent compounds was also in the appropriate proportional mixture to repel the target insects.

This observation was like the previous results for other plant extracts against different insect pests including *S. zeamais*, *T. casteneum* and *S. oryzae* (Udo, 2001; Mobki *et al.*, 2014). Similarly, the organic extracts of *Eucalyptus globulus*, *Citrullus colocynthis* and *O. basilicum* have also showed strong repellency against *S. oryzae, C. maculatus* and *T. casteneum* at remarkably lower concentrations (Mishra *et al.*, 2012; Kosini and Nukenine, 2017).

The findings of this study demonstrated no trend with exposure time. In fact, high repellent ratings were scantly noted at both the initial and final hours of the experiment. This is could be due to a possibility that *S. zeamias* were equally sensitive to odor of the extracts even at low concentration. Other studies have also indicated such trends where repellent activities of plant extracts on insects were independent of exposure time (Khani *et al*., 2014; Ouko *et al.,* 2017).

On the contrary, Mobki *et al*. (2014) reported that repellent activity of garlic extracts to *S. zeamais* increased with duration of exposure. However, where an increase in repellency activities was noted for the first 3 hours of the test period followed by decrease in repellency is interesting. This decrease may be caused by evaporation of the active volatile compound(s).

It was evident that repellent activities were more pronounced for *T. diversifolia* than *V. lasiopus* extracts. This variation could be attributed to unequal distribution of chemical constituents within these plant species. The high repulsive activity of *T. diversifolia* extracts is an indication of higher concentration of phytochemicals with repellent activities as compared to *V. lasiopus* extracts. After all, the chemical variations in plant extracts composition are rather common even within the same species. Mainly, this depends on the type of genotype, plant organ, harvest, region, season, climatic conditions and plant nutritional status (Webster *et al.,* 2010; Dhif *et al.,* 2016).

The contrasts between solvents extractives of the two plants indicated DCM extract as being significantly less potent than ethyl acetate extracts. The higher effectiveness exhibited by EtOAc leaf extracts indicated that this solvent captured more actively repellent compounds within the extract than the DCM. This finding mirrors the observation by Kosini and Nukenine (2017), who reported that EtOAc extract of *Citrullus colocynthis* and *Gnidia kaussiana* (Thymeleaceae) exhibited a higher repellent activity against cowpea weevil than hexane and methanol extracts.

The plant extracts acted as repellents by driving the insects away due to their smell or taste. Arthropods like insects and mites will tend to evade areas with pungent odor (Acero, 2017; 2019). The Repulsion of weevils by these extracts was possibly through stimulation of olfactory receptors (Rajashekar, 2012). They have several olfactory receptor cells (ORCs) in their antennae (Kain *et al*., 2013). The ORCs have a coded pattern of behavior for the specific quality and quantity of semiochemicals in different complex mixtures present in their environment.

In response to an odor substance emitted into insects' environment, chemical message is decoded and integrated into the olfactory centers of the central nervous system (CNS) (Eisner and Meinwald, 1995). Hydrocarbons especially monoterpenes and oxygenated compounds like phenols and esters determine distinctive odor of plants and hence the plants' repellent effect on insects (Nerio *et al.*, 2010; Karavina *et al.*, 2014; Nwachukwu and Asawalam, 2014). To produce an odor sensation, the phytochemical substance must be volatile, and its molecules must come into contact with the olfactory end organ in the insect pest (Wang *et al*., 2008; Moore, 2014).

This ultimately causes olfactory-induced changes in the behavior of the insects, which is of considerable importance in relation to the mechanism of insect attractancy and repellency

(Eisner and Meinwald, 1995). A similar mechanism of action could have been used by the phytochemicals in *T. diversifolia* and *V. lasiopus* leaf extracts to induce repulsion activity of *S. zeamais*.

The repellent phytochemicals could also have acted by interfering with the perception of host-attractant signals. These phytochemicals could have induced excitement of receptors responsible for an opposite or competing behavior rather than food-attractancy behaviour (Kain *et al*., 2013). This resulted to switching of the sensory message from attraction to repulsion. Several different receptor systems were hence activated so that the normal and meaningful sensory information, was “jammed” by the enhanced repellent effect and exciting the repellent (noxious substance/phytocompound) receptors (Eisner and Meinwald, 1995; Kain *et al*., 2013; Acero, 2017).

The observed repellent activity in the present study could partly be attributed to the presence of plant volatile bioactive constituents, which are well-known repellents of insects by acting in the vapour form on the olfactory receptors (Wang *et al*., 2008; Kain *et al*., 2013). This has been partly due to the lack of any secure correlation between the odors of phytochemical and the chemical constitution, reactivity, physical shapes or electrical properties of the odorous molecules (Eisner and Meinwald, 1995; Kain *et al*., 2013). Although much of explanation for repellency is generally agreed, there has been no accepted theory of the triggering process by which the odorous molecule beyond this, towards initiation and discharge of the olfactory nerve (Kain *et al*., 2013). However, the mechanism of interaction of the olfactory receptors and the phytochemicals is still obscure.

The GC-MS analysis revealed a range of volatile phytochemical compounds in the tested plant extracts including alkaloids, terpenoids, fatty acids, phytosterols, and benzaldehyde, among others. These phytochemical compounds could be responsible for the observed repellency activities against the *S. zemails*. The concentrations of the major repellant terpenoids observed in *T. diversifolia* and *V. lasiopus* organic extracts are consistent with the demonstrated properties of these plants as an insect repellent.

Terpenes are widely linked to insect repellent or attractant properties. Their presence is speculated to be associated with fragrance and repellent activities of essential oils. Several studies have also indicated terpenoids as arthropod-repellent compounds (Nerio *et al*, 2010). However, terpenes from ponderosa pine bark have been characterized as attractants to bark beetle (*Ips confusus*) and iso-thiocyanates from the seeds of crucifera are attractants to insects seeking food and site for oviposition (Mahulikar and Chavan, 2007).

The repellent activities of *T. diversifolia* and *V. lasiopus* extracts could have been due presence of monoterpenes. Monoterpenes such as eugenol, limonene, camphor and thymol commonly found in basil have strong repellent activities against insects (Yang *et al.*, 2004). Odalo *et al*. (2005) also found out monoterpenes components of basil (labiate) as effective repellents against *A. gambiae* (Diptera).

Widdrol is an odorous phytocompound, whose presence in the ethyl acetate leaf extract of *T. diversifolia* is likely to have contributed to the strong odor of the plant extracts. It is such smells in the extracts that are thought to drive the insects away especially because insects will always tend to evade places with such pungent odors (Acero, 2019). However, it still remains difficult to make a precise association of widdrol with the repellent activities of plant extracts (Jin *et al.,* 2015).

The presence of vanillin (a phenolic aldehyde) in the studied extracts could also have contributed to the observed repellency activities. Vanilla extract has been reported as significant insect repellent due to its distinct aroma and flavor (Lewis, 2004). It works well in safeguarding homes and body skin against insects such as mosquitoes, flies, and gnats (Gupta and Sharma 2014; Abuirneileh *et al.,* 2015). Vanilla extract is usually effective as an insect repellent for about half an hour. Hence, requires reapplication more especially when exposed to very high temperatures which may cause it to evaporate quickly or in cases of being diluted with water elements like rain (Abuirneileh *et al.,* 2015).

The GC-MS analysis also revealed that the organic leaf extracts of *T. diversifolioa* and *V. lasiopus* contain odorous oleic and linoleic acids, which could also be associated with their repellent properties. Arthropods are known to release oleic and linoleic acids upon death usually referred to as “smell of death”. The smell of these compounds is believed to repel other insects, thereby keeping them away from approaching their death zone. Previous study by Ramsewak *et al.* (2001) attributed the botanical repellent effects on *Aedes aegyptii*, *Helicoverpa zea*, *Lymantria dispar*, *Orgyia leucostigma* and *Malacosoma disstria* to the strong smell of oleic and linoleic acids.

The repellent activities of the extracts on adult weevils in the present study could also be linked to the presence of α-Bulnesene in the organic leaf extracts of *T. diversifolia* and *V. lasiopus*. According to Gokulakrishnan *et al.* (2013), α-Bulnesene extracted from *Pogostemon cablin* exhibits repellent activities against various urban ant species. Similarly, the presence of α-Bulnesene in *P. cablin* was associated with the plant's repellent activities against selected important vectors of mosquitoes including *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* (Senthil-Nathan, 2020).

The repellency of the organic leaf extracts of *T. diversifolia* and *V. lasiopus* against *S. zeamais* may be explained by the revealed presence of benzaldehyde in these extracts. Benzaldehyde is also the main chemical compound in *M. nodosa,* which was reported to be the cause of moderate repellency against *S. zeamais*, *O. surinamensis* and *amblyomma cajennense* (Tresvenzol *et al*., 2010; Soares *et al*., 2010; Fouad *et al*., 2012).

Additionally, benzaldehyde was also extracted from *Tanaecium nocturnum* (Bignoneaceae), which caused repellency to *Sitophilus oryzae* (Coleoptera: Curculionidae), *Rhyzopertha dominica* (Coleoptera: Bostrichidae) and *T. castaneum* (Jillani and Saxena 1990). Futhermore, benzaldehyde has been found to be lethal on *S. zeamais* and *Tenebrio molitor* (Coleoptera: Tenebrionidae), confirming its bioactivity against stored grain pests (Fazolin *et al*., 2007; 2010).

The repellent properties of these extracts could also be attributed to specific compounds among the many that GCMS analysis revealed in this study. However, synergistic or additive effects as a result of combination and interaction between phytochemicals cannot be ignored (Omolo *et al.*, 2004; Liu *et al.*, 2006; 2007; 2012). Furthermore, the repellent activities of the plant volatiles may not be limited only to its major constituents; it could also be due to some minor constituents or a synergistic effect of several constituents (Adesina *et al.*, 2019).

Actellic Super ^{TM}, is a broad-spectrum insecticide. It is conventionally used for the control of storage pests in bulk-stored grains, bagged grains and storage surfaces. It is also effectively used for the control of insect pests in pineapples, citrus, bananas, potatoes and vegetables. It contains 250 g/ml pirimiphos-methyl which is taken by the insect through its respiratory system and affects the pests through its repellence effects. Although Actellic is actually a contact insecticide, it contains permethrin which was recently studied and suggested as an insecticide as well as an insect repellent (Khater, 2012; 2013; Islam *et al.*, 2017). In fact, in the US Actellic is registered as both repellent and insecticide (Khater, 2012; 2013).

It is worth noting that on average, after 5 hours test period, all extracts of *T. diversifolia* and *V. lasiopus* produced appreciable repellent activities against weevils, which compare well with the standard chemical Actellic. This suggests a possible mimicry of Actellic mode of action by active phytochemicals in the studied crude extracts in repelling the weevils. Therefore, the findings of this study evidently show that the organic leaf extracts of *T. diversifolia* and *V. lasiopus* can be used as an effective repellent against *S. zeamais* on stored maize grains.

5.1.4 Oviposition Deterrence and Progeny (F_1) Emergence Enhibition effects of the Extracts of *T. diversifolia* and *V. lasiopus*

The results of this study revealed a good potency of *T. diversifolia* and *V. lasiopus* at managing the population of *S. zeamais* through oviposition deterrence (ovicidal) as well as F_1 progeny emergence inhibition (larvicidal) properties. Unlike the killing and repellence aspects of pesticides, ovicidal and larvicidal are quite important especially when considering long term protection of the grain. This is because ovicidal and larvicidal properties lead to the population and generation control of the insects decrease adult emergence by ovicidal and larvicidal efects leads to a reduction in the insects population (Sarwar and Salman 2015; El-Sheikh *et al.*, 2016; Ali *et al.*, 2017). From the results of this study, selected organic leaf extracts of *T. diversifolia* and *V. lasiopus* manifested oviposition deterrence that ranged between 12.18 to 100%. The extracts also exhibited F_1 progeny emergence inhibition properties that ranged between moderately low inhibition of 38.38% and very high toxicity of 100% inhibition.

Several botanicals have also been previously shown to inhibit similar oviposition deterrence of a range of lepidopteran pests including *S. zeamias* in treated grains. Pierre also exhibited oviposition deterrence on beetles (Kumar *et al.*, 2006; Pavela and Herda, 2007; Belide *et al.*, 2010). In addition, oviposition deterrence results of this study corroborated with the findings of Habib *et al.* (2012), who observed that essential oil of *C. zeylanicum* showed good effects on decreasing eggs laid by female insects on treated surfaces.

Essential oils of *P. lentiscus* L. portlayed significant mating inhibitions, reductions of longevity, fecundity and egg hatching on insects such as *Ephestia kuehniella* Zeller and *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae) (Bachrouch *et al*., 2010). Other previous studies in harmony with the present findings include studies by Adedire and Lajide (1999) and Arannilewa *et al*. (2006; 2010).

A number of research findings also support the results of the current study showing the ability of organic leaf extracts of *T. diversifolia* and *V. lasiopus* to impair the progeny development and consequently reduce adult emergence. Abbasipour *et al.* (2010) stated that *Peganum harmala* seed extract decreased oviposition of *P. xylostella* (L.) on leaves of cauliflower. Extract and essential oils of *Azadirachta indica* are known to possess ovicidal activities against various stored-product insects (Desmarchelier, 1994; Hill and Schoonhoven, 1981; Shaaya *et al.,* 1997). Application of *Tagetes minuta* essential oils also interfered with the egg hatching process of the coleopteran beetle, *Tribolium castaneum* also delayed the moulting process of *Hyalomma rufipes* (Krishna *et al.,* 2005; Nchu *et al.,* 2012).

A good reduction of F_1 progeny was achieved by application of the leaf extracts from *T. officinale*, *U. dioica* and *A. millefolium* against bean weevil (Jovanovi *et al.,* 2007). Previous studies on effects of *A. sativum* components on adult emergence of *C. chinensis* (coleoptera; bruchidae) also recorded high progeny inhibition rates (Chaubey, 2012). Other evaluation studies done using acetone extracts of garlic and its various components against red flour beetle and grain moth have also reported greater reduction of adult emergence in treated grain (Yang *et al.*, 2012; Ali *et al.*, 2014).

The results from the current study also find support from a study by Agboka *et al.* (2009), who showed that the hatching of *M. nigrivenella* eggs was adversely affected by “neem” (*A. indica*) and *Jatropha curcas* oils and decreased with increasing concentration of applied oils. Adebowale and Adedire (2006) reported that all tested *J. curcas* oil concentrations completely prevented emergence of adult *C. maculatus.* Petroleum ether and chloroform extracts of *P. nigrum* and petroleum ether extract of *J. curcas* extracts also showed strong deterrence to progeny emergence in *S. oryzae* (Khani *et al.*, 2011).

That all the tested extract concentrations exhibited a remarkable inhibition of emergence of adult *S. zeamais* agrees with other previous studies. Keita *et al.* (2000) observed zero emergences of *C. maculatus* F_1 progeny in cowpea treated with *O. basilicum* extracts. A closely related study conducted by Vanmathi *et al.* (2010) also reported that aqueous extracts of *O. tenuiflorum* greatly reduced F_1 adult emergence of coleopterons. However, Vanmathi *et al*. (2010) reported much lower inhibition rates of *O. tenuiflorum* extracts against *C. maculatus*, which is contrary to the findings of the present study.

The oviposition deterrence and F_1 progeny emergence inhibition activity increased with the increase in extract concentrations. This could be due to the increase in the constituent active phytochemicals as the extract concentration increases. Alternatively, the combinations of the responsible phytocompounds were in the best proportion at 100% v/v concentration. On the other hand, the lower extract concentrations had insufficient active principle (s) hence, manifested a lower action than higher leaf extracts concentrations.

Increasing doses of essential oils of *Premna angolensis* and *Premna quadrifolia* leaves also proportionally reduced the emergence of adults of *Sitotroga cerealella*, 50 days after infestation rates (Adjalian *et al.,* 2015). Furthermore, this observation is consistent with previous findings on the effects organic plant extracts on various pest insects including *S. zemails*, *S. oryzae*, *C. maculatus* and *T. castaneum* among other insects (Khani *et al.*, 2011; Habib *et al.,* 2012; Ashamo *et al.*, 2013; Akinbuluma *et al.*, 2015). It is therefore, suggested that the two plant extracts should be applied at 100% v/v for a better potency on maize weevils to be realized.

That ethyl acetate leaf extracts of the two plants demonstrated greater activities of as compared to the DCM leaf extracts of *T. diversifolia* and *V. lasiopus* may be due to the differences in the type of active constituents present in the extracts (Jeyaseelan *et al.*, 2012). The higher oviposition deterrence as well as F_1 progeny emergence inhibition exhibited by the ethyl acetate leaf extracts indicated that EtOAc extracted more active compounds with these activities than the DCM leaf extracts of the same plants. This observation is in consonance with the findings of other previous studies. For instance, a study conducted by Nethravathi *et al.* (2009) showed that EtOAc leaf extract of *G. glauca* possessed relatively better larvicidal potential against *Aedes aegypti* than the other organic leaf extracts of *G. glauca* **(**Lakshmi *et al.*, 2018).

The EtOAc leaf extract of *V. lasiopus*, at the highest tested extract concentration of 100%, was comparably effective (97.48%) to actellic super^{TM} (100%). This suggests a mimicry of actellic mode of action by the active phytochemicals in the EtOAc leaf extract of *V. lasiopus*. However, the possibility of the EtOAc leaf extract of *V. lasiopus* possessing alternative efficacy mechanisms cannot be ruled out.

Maize weevils, among other insects, depend on the chemical odors and pheromones not only in order to locate their mates, but also the suitable ovipositional sites and even oviposition itself. Therefore, the low egg laying capacity (oviposition deterrence) of *S. zeamais* within the grain might have been brought about by the change in behavior and physiology of the insects to locate their mates and suitable ovipositional sites due to chemical nature of extract treatment (Masse *et al.*, 2009). Therefore, it is possible that the chemical adors/ scents of some phytochemicals such as terpenoids and fatty acids in the studied extracts may have blocked the pheromone receptors in the target insects. This compromised the social communication among the weevils thereby adversely affecting mating and oviposition.

The stifling effect of the extracts could have derailed sexual communication among the female and male adult insects. It led to the disruption of mating activities among weevils and hence lowered oviposition (Emeasor *et al.*, 2005; Ileke *et al.*, 2013). Mating disruption is usually effective in area of low pest density since with high pest density, there is a greater probability that the males and females will get each other by chance or by visual intimation signals/ cues.

It is speculated that the stifling effects of the extracts could have reduced mating competition among male weevils for female weevils. On the other hand, the extracts could also have reduced the receptiveness of females for males. In addition, exposure of *S. zeamais* to the organic leaf extracts of *T. diversifolia* and *V. lasiopus* could have weakened both male and female weevils and hindered successiful copulation. Consequently, there was a reduction in oviposition by the female weevils (Chebet, 2013). This mating disruption strategy decreased the chances of successful mate finding thus reducing the number of fertilized eggs laid and subsequently reducing progeny in the subsequent generation.

According to a study by Ofuya and Osadahun (2005), organic extracts of *E. aromatic* lowered both the receptiveness of female *C. maculatus* for males and the mating competition of males for females in this weevil. However, all these factors may collectively have led to low egg laying capacity by adult weevils within the treated maize grains (Adesina *et al.,* 2015).

The effectiveness of this extract in deterring oviposition could also be linked to its significant potency to induce both fumigant and contact mortalities on the adult *S. zeamais* (Table 3.3; Table 3.4; Table 3.5; Table 3.6; Chapter 3) (Ileke and Ogungbite, 2014). On this account, the high toxicity and hence mortality induced by the organic leaf extracts of *T. diversifolia* and *V. lasiopus* affected the number of adult female weevils available and capable of laying eggs. Therefore, oviposition deterrence action of the studied extracts could be explained by this operative mechanism as postulated by Ofuya (1990).

The oviposition deterrence exhibited by the organic leaf extracts of *T. diversifolia* and *V. lasiopus* in the preset study, could also be associated with the mode of application of extracts (as contact insecticides) during the assay (Emeasor *et al.,* 2005). The nature of these extracts in terms of consistency and texture (sticky), probably inhibited movement of the weevils in the study and, therefore, disrupting their mating.

The suppression of progeny development and consequent reduction of F_1 progeny emergence observed in the present study could also be ascribed to the fact that metabolically toxic *T. diversifolia* and *V. lasiopus* extracts may have penetrated the eggs through a short funnel-like structure at their posterior ends of weevils' eggs (Credland, 1992) and hence chemically induced death of the eggs or the first instar larvae of *S. zeamais* (Don-Pedro, 1996). The plant extracts could have physically occluded the funnel-like feature on the eggs of weevils. Such occlusion cuts off the supply of gas to the larvae. The death of the developing embryo, therefore, may also have occurred due to asphyxiation (Credland, 1992).

Another possible mechanism of progeny suppression is that *T. diversifolia* and *V. lasiopus* extracts could have disrupted the hormonal and biochemical processes hence interfering with the normal embryonic and post-embryonic development of the weevil. Ecdysis is a complex physiological process that occurs periodically in insects under tight hormonal regulation (Nation, 2008). A delicate balance of molting hormones (prothoracicotropic hormone, molting hormone and juvenile hormone) must be sustained for proper metamorphosis and insect development (Hartfelder, 2000).

A compromise in molting hormone synthesis and secretion may have affected the weevils' transition to adulthood. It worth noting that some phytochemical compounds such as limonene and other terpenoids can act as Juvenile Hormone (JH) mimics, hence inhibitors of chitin biosynthesis (Dinan *et al.,* 2001). They can also act as ecdysone receptors agonists/antagonist and even inhibitors of Acetyl CoA carboxylase, thereby, interfering with proper insect metamorphosis. Consequently, pupation and adult emergence is altered or inhibited by the phytochemicals, resulting in progeny suppression as observed in this study (Dinan *et al.,* 2001).

The GC-MS analysis revealed that the organic leaf extracts of *T. diversifolioa* and *V. lasiopus* contain several phytochemical compounds which have previously been associated with the pesticidal effects (Ge *et al.,* 2015). These phytochemicals include terpenoids, alkaloid, phenolics and fatty acids among others. These results agree with the findings of Yang (2012), Lalla *et al.* (2013) and Lucia *et al.* (2017). These phytochemicals could have either demonstrated synergistic or additive effects when used in such a crude form (Mohamed *et al*., 2010).

The abundance of phenolic substances in the organic leaf extracts of *T. diversifolia* and *V. lasiopus* might be responsible for the inhibition of oviposition and F_1 progeny emergence effects against weevils. A study by Wójcicka (2010) showed that the total phenols and o-dihydroxyphenols present in triticale caused a reduction in fecundity of cereal aphids. Phenolic compounds of basil have been reported in other studies to have larvicidal and nymphicidal properties (Belong *et al.,* 2013).

Major Terpenoids also revealed in the GCMS analysis of the organic leaf extracts of *T. diversifolia* and *V. lasiopus* can be associated with the inhibition of oviposition and F_1 progeny emergence effects on the weevils (Chebet *et al.,* 2013). Terpenoids have been reported to inhibit reproduction and fertility among coleopterans (Chebet *et al.,* 2013; Adesina *et al.,* 2015). According to Adjalian *et al.* (2015) the reduction in F_1 progeny emergence rates resulted from the manifestation of ovicidal or larvicidal volatile extract components, which destroyed the development of some eggs or larvae probably due to mono-, sesqui-, di- and triterpenoids compounds and synergy among themselves or with minor compounds.

The observed reduction in F_1 progeny emergence of weevils may also be due to α-Bulnesene, which was also found in the studied extracts. The presence of α-Bulnesene in *P. cablin* was associated with the plant's pupicidal activities against selected important vectors of mosquitoes including *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* (Gokulakrishnan *et al.*, 2013).

Limonoids also present in the studied extracts are known for a range of biological activities, including insect growth-regulating properties, which might have contributed to the observed inhibition of weevil F_1 progeny emergence by these plant extracts (Mulholland *et al.,* 2000). Toosendanin, a limonoid constituent of *M. azedarach*, which has been commercialized in China, is an effective oviposition deterrent against *Trichoplusia Ni* Hübner (cabbage looper), and a growth inhibitor against *Ostrinia nubilalis* Hübner (European corn borer) (Koul *et al.,* 2000; Mitchell *et al.*, 2004).

The inhibition in weevil F_1 progeny emergence could also have been caused by alkaloids found present in these plant extracts. Alkaloids have been found to disrupt growth and reduce larval survival by hindering loss of exoskeleton during larval development. Alkaloids from extracts of *Pergulariato mentosa* exhibited larvicidal effects against fifth instar larvae of migratory locust, *Locustami gratoria* (Acheuk, 2013).

A study conducted by Ge *et al.* (2015) demonstrated effects of alkaloids (antofine N-oxide and tylophorin) from *Cynanchum mongolicum* on the molting hormone of *S. litura* larvae. Higher dosages of alkaloids resulted in higher mortality and greater disruption of development, 72hours post-treatment. Treatment of *S. litura* with 800mg/L of *C. mongolicum* extract resulted in more than half of the pupae not molting into adults. Sun *et al.* (2012) further reported that treatment of larvae with alkaloids elongated the insect development time from the 3rd instar to emergence.

The inhibition in weevil F_1 progeny emergence could also be as a result of linalool, oleic acid and linoleic acid. According to the findings of Liu *et al.* (2011) linalool extracted from *F. africana* and *T. vogelii* is toxic to the eggs and larvae of insects. Oleic acid and linoleic acid were reported with insecticidal effect against fourth instar *Aedes aegyptii* larvae (Ramsewak *et al.,* 2001). Therefore, methyl linoleate, linalool, oleic acid and linoleic acid identified in this study could be associated with the plants' oviposition inhibition activities on weevils.

The standard pesticide used in this study, actellic super ^{TM}, has been previously reported to inhibit insect oviposition and F_1 progeny emergence (Lale, 2000; Khater, 2013; Ouko, 2017). It was evident that the standard pesticide exhibited significantly higher F_1 progeny emergence properties compared to the tested plant extracts. An exception was only found in the case of ethyl acetate extract of *V. lasiopus* at the extract concentration of 100%. This finding was not strange because, in an earlier study, Lale (2000) and Ouko (2017) also reported such superiority of this synthetic insecticide, actellic super ^{TM} (pirimiphos methyl) in reducing oviposition and F_1 progeny emergence of *C. maculatus* and *S. zeamais*, respectively.

The fact that the organic leaf extracts of *T. diversifolia* and *V. lasiopus* against *S. zeamais* produced high oviposition deterrence as well as F_1 progeny emergence is a welcome scientific development based on the promise it holds for the rationalized use of botanical pesticides for long-term protection of stored durable agricultural products.

5.1.5 Anti-feedant Effects of Extracts of *T. diversifolia* and *V. lasiopus*

The present study was designed to assess the capacity of organic leaf extracts of *T. diversifolia* and *V. lasiopus* to reduce maize grain damage following by *S. zeamais* infestation during storage. It was evident that the studied organic leaf extracts of *T. diversifolia* and *V. lasiopus* has insect controlling properties that made the maize grains less favourable host and less prone to attack and infestation by weevils. The results obtained indicated attractive antifeedant potentials of the studied extracts with PWL in the range of 2.7 to 15.4% and PD of from 0.8 to 11.3%. The significant reduction in damage of the maize grains suggest that the plant extracts acted as antifeedants.

Results of this study agree with the findings of Ngatia and Kimondo (2011), who reported maize grain weight loss results in the range of 0.3 to 13.4%. These results also agree with previously reported percent weight loss in maize and wheat (Alonso and Avila, 2011). Miguel and Jorge (2011) also reported percent weight loss range of 2.3 to 14.5% while Braga-Caneppele *et al.* (2003) found 2.3 to 21% as percent maize grain losses.

It was observed that the reduction in grains damage was coupled with high mortality of weevils as reported earlier in Chapter 4 of this study. Similarly, several authors have reported that, the deleterious effects of crude plant extracts on insects are manifested in several ways, including toxicity/mortality (Hiremath *et al.,* 1997) and feeding inhibition (Klepzig and Schlyter, 1999; Wheeler and Isman, 2001). A study by Adesina and Ileke (2014) reported that crude extract of *Momordica charantia* and *Mitrocarpus villosus* exhibited both feeding deterrence and toxic activities (>80%) against the flea beetles Podagrica spp. Jacq. (Coleoptera: Chysomelidae). Similarly, Peta and Pathipati (2008) reported that *Momordica charantia* exhibited both toxic effects and feeding deterrence effects on larvae, *S. litura* and *A. Janata*. Oboho *et al.* (2016) also reported grains protectant efficacy of *Cymbopogon citratus* Stapf leaf extract against *S.*

zeamais Motschulsky (Coleoptera: Curculionidae) on stored maize (*Zea mays* L.) by both toxic and feeding deterrence effects.

The antifeedant findings of the present study corroborate with other scientific literature on use of botanicals against storage pests (Kortbeek *et al.*, 2019). For instance, the antifeedant properties of *A. indica* are well established, particularly for a range of lepidopteran pests, which are closely related to *S. zeamais* (Martinez *et al.*, 1999; Liang *et al.*, 2003; Roel *et al.*, 2010). According to Khani *et al.* (2011), petroleum ether and chloroform extracts of *P. nigrum* and petroleum ether extract of *J. curcas* showed strong antifeedant activities against rice weevil, *S. oryzae.*

Ohazurike *et al*. (2003) had also reported the antifeedant activity against *S. zeamais* in the stored maize grains by *J. curcas* seed extracts. The essential oils isolated from *C. nardus*, *C. flexuosus* and *C. martini* exhibited strong antifeedant activities against *Acharia fusca* and *Euprosterna elaeasa* (Hernández-Lambraño *et al.*, 2014). Further, the findings of this study are in line with earlier findings of Schmutterer (1995), Nawrot *et al*. (1998), Harborne and William (2000) and Inyang and Emosirue (2005).

The grain weight loss reduced with extract concentrations. The number of holes bored on treated grains also appeared to follow a similar trend and hence both effects appeared to be dose dependent. This is in consonance with the potent feeding deterrence evaluated using glass-fibre discs that showed activities of *Azadirachta indica*, *Ocimum basilicum*, *Cymbopogon citratus*, *Lippia javanica Cymbopogon citratus* and *Azadirachta indica* against the fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) (Phambala *et al.*, 2020).

This trend could be due to the increase in bioactive phytochemicals as the extract concentration increased or may be due to the fact that the combination of the active phytochemicals was in the best proportion for optimum effects at 100% v/v concentration. It is also likely that at the lower dose, there was no sufficient concentration of the active principle (s) to cause as high effects as at higher dosages. Therefore, this correlation suggests that the extracts can best be applied at 100% v/v concentration in order to have better antifeedant effects on weevils (Ouko *et al.*, 2017).

The strongest antifeedant effects occurred with the ethyl acetate extract of *T. diversifolia* followed by the DCM extracts of the same species. This corroborates with the study of Truong, *et al.* (2019), who reported a higher effectiveness of ethyl acetate extract as compared to the other organic extracts. These results further correspond with the findings of Momeni *et al*. (2005) and Kagucia *et al.* (2018). The higher antifeedant effects exhibited by the EtOAc leaf extracts indicated that this solvent extracted more active compounds with antifeedant activities than DCM.

It is generally accepted that the assessment procedure of storage losses is difficult. However, the two criteria used in the present laboratory study (damage of attacked grains and weight loss thereof) are the most commonly used method of assessment of the grain damage (Kumar and Kalita, 2017). The standard weight method is simpler than volumetric techniques in that it only involves measuring the dry weight of grains at the beginning of the storage period and comparing it with the dry weight of the same volume of grain at the end of the storage period (Boxall, 2001; Kagucia *et al.*, 2018). Therefore, grain loss can most readily be valued. It also needs smaller samples than volumetric techniques with a maximum of about 1,000 grains and it is, therefore, a useful, quick and realistic method (Irshad and Javed, 1990;). Amelot and Avila-Nunez (2011) noted that acceptable post-harvest grain loss assessment methods should yield realistic results.

The principle underlying this method is that unlike surface-feeding insects, weevils develop inside the grains. The shape of the grains will remain intact and the damaged grains will occupy the same volume but will weigh less (Ngatia and Kimondo 2011). The main disadvantage of this method is that the insects may show a preference for grains of certain dimensions, composition or moisture content and, consequently, the mean weight of insect-damaged grains before damage may be different from the mean weight of grains in the undamaged sample (Harris and Lindblad, 1978; Irshad and Javed, 1990; Amelot and Avila-Nunez, 2011).

On the other hand, an indirect method of evaluating the number of emergent holes/perforations per grain was also applied. This method also used a factor related to weight loss, which could easily be measured and then transformed into an estimate of loss, using a formula (Isman, 2006). It was done using the most common factor of percentage damaged grain as it is easily measured. Unfortunately, a visibly undamaged grain may have had a hidden infestation, which leads to an underestimate of the loss (Compton and Sherington, 1999; Neto *et al.*, 2006).

With respect to the observed antifeedant properties, it is worth noting the presence of a number of bioactive compounds in the studied extracts maybe responsible for the effects upon detection by weevils. According to Vencl and Morton (1998), insects such as the sumac flea beetle, are reported to use phytochemicals originating from the host plants such as chemical deterrents against predation.

Phytochemicals possibly contributing to the antifeedant results obtained in this study included triterpenoids, alkaloids, coumarins, polyphenols and fatty acids. Similar phytochemicals extracted from other plants have previously been reported by various authors as excellent feeding deterrents against insect pests (Shadia *et al.*, 2007; Ekeh *et al.*, 2013).

Many sesquiterpene lactones exhibit high antifeedant activities against storage pests (Nawrot and Harmatha, 2012). Different sesquiterpene including aguerin, chlorojanerin, syringing and lactones were isolated and identified from different Asteraceae family members like *Rhaponticum pulchrum* are reported to inhibit feeding in different coleopteran pests including *Sitophilus granarius*, *Trogoderma granarium* and *Tribolium confusum* (Bruno, *et al.*, 2005; Rosselli, *et al.*, 2006). The DCM bark extracts of *Cinnamosma fragrans* and *Warburgia ugandensis* (Canellaceae), which are enriched with pungent drimane sesquieterpane, were found to be antifeedant against mosquitoes (Inocente *et al.*, 2018; 2019).

Paruch *et al*. (2000; 2001) also reported that terpenoid lactones show antifeeding activity towards grain storage pests including the granary weevil beetle (*S. granarius*), the khapra beetle (*T. granarium*), and the confused flour beetle (*T. confusum*).

The presence of bitter-tasting compounds found in flowering woody plants like jackfruit plant among others, make the plants to use the characteristic as defense against predators (Acero, 2019). According to Gabrys *et al.* (2001) and Sandjo *et al.* (2008; 2013), the presence of limonoids, which are highly oxygenated triterpenoids, could be responsible for the bitterness of plant extracts, which justifies the bitterness of *T. diversifolia* and *V. lasiopus* as well as effectiveness of their extracts as antifeedants.

The antifeedant effectiveness of organic leaf extracts of *T. diversifolia* and *V. lasiopus* is also probably due to the fatty acids content of the plants. Schmutterer (1995) and Harborne and William (2000) linked the presence of fatty acids and esters in plants with antifeedant activities against insects. Oleic and linoleic acids were reported to exhibit potent feeding deterrent activity against larvae of *Helicoverpa zea*, *Lymantria dispar*, *Orgyia leucostigma* and *Malacosoma disstria* (Ramsewak *et al.*, 2001). Therefore, fatty acids such as methyl linoleate, oleic acid and linoleic acid identified in the studied plant extracts could be associated with the plants anti-feeding activities against weevils. According to Isman

(2006), the antifeedant benefits of majority of plants can be linked to their insecticidal properties than to merely the changing of the insect behavior.

It is also worth noting that the fact that more weevils responded to the treatments could also be due to the presence of repellent phytochemicals in the organic extracts of the two plants as described in chapter 4 of this study. It is, therefore, postulated that the plant extracts in the present study may have had such effective antifeedant effects because they disrupt the insects’ odor code. The odorant receptors (OR) function to detect special odor signals that enable the insect to locate a potential host or food source. Antifeedant chemicals act on very specific sensory cells (antifeedant receptors) in the insect pest. The neurons associated with these antifeedant receptors either prevent insect feeding (feeding deterrent effect) or cause cessation or slowing of further feeding (feeding suppressant effect) (Isman, 2006; Wang *et al.*, 2008; Moore, 2014).

The likely mode of antifeedant action of the studied plant extracts may also be through the blockage of the insects’ recognition of attracting food odor signals or by causing an active avoidance behavior in weevils. Some antifeedants are known to have an apparent ability to block the function of a herbivore's feeding-stimulant receptors, or an ability to bind directly to its normal feeding cues, such as sugars and amino acids (Kain *et al*., 2013; Acero, 2017). The action of DEET (*N*, *N*-diethyl-*m*-toluamide) is a perfect example of this mechanism. The DEETS in the repellent body lotions deter arthropods by blocking their ability to perceive feeding stimulants in the host (Ditzen, *et al*., 2008; Kain *et al*., 2013; Acero, 2017). Similary, the very potent antifeedant azadirachtin, in neem plant, also acts by reducing the sensitivity of sugar-sensing cells in insects and thus causing the insects to incorrectly assess nutritional adequacy or attractancy of the treated host leaves (Eisner and Meinwald, 1995; Moore, 2014).

The influence of phytochemicals in the studied plant extracts may have corrupted the messages from attractive scents in the grains and as a consequence, weevils lost their orientation. They hence masked the attractant natural maize volatiles and diverted the weevils away from their prospective food.

It is possible that the phytochemicals in the studied extracts can be used to digress the weevils while on the maize grains, and adversely affect their feeding habits, through which they cause grain loss. Therefore, the use of organic leaf extracts of the two plants may be an attractive strategy to incorporate into the existing integrated weevil management program for stored grain pest control and management.

5.1.6 *Ex Vivo* Anti-Acetylcholinesterase Effects of Extracts of *T. diversifolia* and *V. lasiopus*

The present study was also designed to evaluate the possible inhibition effects of selected organic leaf extracts of *T. diversifolia* and *V. lasiopus* against acetylcholinesterase enzymes in maize weevils. All the extracts demonstrated enzyme inhibitory effects that ranged between 10.75 and 89.25%. These results were comparable to those of a study done by Kiendrebeogo *et al.* (2011), which revealed that the *Eucalyptus camaldulensis* and *Ocimum canum* had percent acetylcholinesterase inhibition rates of 83% and 72% respectively.

The inactivated acetylcholinesterase enzyme is no longer capable of hydrolyzing acetylcholine, resulting in the buildup of ACh in the nerve synapse, leading to death (O'Brien, 1967; Pang, 2006). Acetylcholinesterase is a key enzyme that terminates nerve impulses by catalyzing the hydrolysis of neurotransmitter acetylcholine (Seo *et al.*, 2014). Therefore, inhibition of acetylcholinesterase causes death due to failure of acetylcholine hydrolysis and hence its accumulation in the nervous system of various organisms. Artificial irreversible inhibitors of acetylcholinesterase such as organophosphates and carbamates have been developed as insecticides that apply these principles to kill insect pests (Preveena and Sanjayan, 2010).

The Ellman's method (Ellman *et al*., 1961) was used to determine the inhibitory activity of selected organic leaf extracts of *T. diversifolia* and *V. lasiopus* against maize weevil acetylcholinesterase (AChE) activity. The principle of this method is the measurement of the rate of production of thiocholine from the hydrolysis of acetylthiocholine iodide (ATCI). The rates of hydrolysis by AChE are monitored spectrophotometrically. Thiocholine can react with DTNB. This reaction resulted in the development of a yellow colored anion 5-thio-2-nitrobenzonic acid. The color intensity of this product was measured at 412nm and was taken to be proportionate of the enzyme activity (Appendix 8.2).

Extracts from different plants have been known to possess anti-AChE properties against a wide range of insect pests**.** The inhibition of AChE in the present study is in line with previous studies by other researchers. The crude fruits sap extract of *S. incanum* inhibited the acetylcholinesterase of green peach aphids (Umar *et al.,* 2015). Aqueous and DCM leaf extracts from *C. glaucescens* and *G. glauca* have the potential of anti- acetlycholinesterase activity (Njoroge *et al.,* 2015). The AChE inhibitory activity of aqueous extract of *Emblica officinalis* (Family: Euphorbiaceae) was reported in an earlier study (Shekhar and Kumar, 2015). The hexane extract of *S. lavandulifolia* also showed the highest AChE inhibitory activity with an IC_{50} value of 13.7l g/mL (Tundis *et al.,* 2015).

The inhibition of AChE was observed in the cockroach, *Periplaneta americana* L. (Shafeek *et al.,* 2004) and the snail, *Limnaea acuminate* Lamarck, at 40 and 80% concentrations of neem oil (Singh and Singh 2000). Adsersen *et al.* (2006) and Kim *et al.* (1999) found that a methanolic extract of the tuber of *Corydalis ternate* showed significant inhibition of AChE activity. A similar study was conducted by Orhan *et al*. (2004), which demonstrated that *Fumaris* species showed potent inhibitory activity against AChE. Report by Chaiyana and Okonogi (2012) also revealed inhibition of cholinesterase by essential oils of leaf and fruit peels of *C. aurantifolia*. The extracts of *C. sinensis*, *C. aurantifolia, Z. vulgaris, B. nigra, and R. damascene* were screened for AChE inhibitory activity using Ellman's spectrophotometric method and were found to show excellent and active AChE inhibitory property (Jazayeri *et al.*, 2014).

In this study, the organic leaf extracts of *T. diversifolia* and *V. lasiopus* exhibited appreciable acetylcholinesterase inhibitory activities in a dose dependent fashion. That the acetylcholinesterase activity of the organic extracts of plants in this study decreased with the increase in the extract concentration was consistent with the other previous studies.

Umar *et al.,* 2015 reported that crude fruits sap extract of *S. incanum* inhibited the acetylcholinesterase of green peach aphids was dose dependent.

That the acetylcholinesterase inhibitory activity increased with the increase in the extract concentration could be due to the increase in anti-AChE phytochemical components as the concentration of the extract increased. At high extract concentrations, there was apparently a sufficient concentration of the enzyme inhibitory active principle(s). It is also likely that there was no appropriate concentration of these active principle(s) at the lower extract dose levels.

The observed anti acetylcholinesterase activity in the present study could be attributed to the presence of plant phytochemical compounds. Such constituents revealed by GC-MS analysis in the tested plant extracts include phenols, alkaloids, terpenoids and fatty acids among others, which are well-known enzyme inhibitors of acetylcholinesterase in insects.

Most likely, the presence of phenols in the extracts could have contributed to the inhibition of AChE activity. These results are comparable with those reported by Nwidu *et al*. (2017) and Elufioye *et al*. (2019), who reported a positive correlation of phenolic content with acetylcholinesterase inhibitory activities. A research by Kadri *et al.* (2010) evaluated *C. schoenanthus* and found they were rich in phenols and they had good AChE inhibitory activities comparable to butylated hydroxytoluene (BHT), a known standard. Phenolic acids such as caffeic acid, chlorogenic acids, and catechin have been reported to be potent inhibitors of both AChE and BChE (Olasehinde *et al.,* 2017; Jabir *et al.,* 2018). Previously, AChE inhibitory activity has been reported for green tea leaves infusion of *Camellia sinensis* probably due to the occurrence of polyphenols which are well documented in different preparations of *C. sinensis* (Eksi *et al.,* 2019). Therefore, the observed AChE inhibitory properties in this study could be attributed to the fact that phenols were present in the organic leaf extracts of *T. diversifolia* and *V. lasiopus* (Puupponen-Pimia *et al.,* 2008; Kadri *et al.*, 2010).

The presence of diterpenoids in the studied extracts could also have contributed to the observed anti acetylcholinesterase activities. Four diterpenes; dihydrotanshinone, cryptotanshinone, tanshinone I and tashinone IIA, isolated from the acetone extract of the dried root of *Salvia militiorrhiza,* were previously associated with the anti-acetylcholinesterase activity of *S. militiorrhiza* (Ren *et al.*, 2004; Orhan and Aslan, 2009). Similarly, Tundis *et al.* (2015) reported that essential oils and extracts, rich in terpenes, exhibit strong AChE and BChE inhibitory activities.

Alkaloids along with terpenoids, phenols, flavonoids, tannins and steroids have been suggested to constitute part of the plant defenses against phytophagous animals and are insecticidal at low concentrations. The mechanism of action of their insecticidal abilities is by affecting acetylcholine receptors in the nervous system (Hartmann, 1996; Kubo, 2006; Cox, 2007; Rattan, 2010). Orhan *et al.* (2004) reported that since most of the acetylcholinesterase inhibitors are known to contain nitrogen, the higher activity of these extracts may be due to their alkaloidal content. Furthermore, a research conducted by

Fatma and Bahia (2013), reports that alkaloids interfere with neuroendocrine control by inactivating the acetylcholinesterase in treated larvae.

Alkaloids from some plants have a similar effect of blocking the acetylcholinesterase enzyme (Filho *et al.*, 2006; Eksi *et al.*, 2019). Aqueous extract of *Cola acuminata* was repoted to inhibit AChE as a result of the presence of an alkaloid called caffeine which have already been characterized in these extracts (Ahmed *et al.*, 2003; Murray *et al.*, 2013). Studies have shown that caffeine is a noncompetitive inhibitor of acetylcholinesterase but not BChE (Silva *et al.*, 2012).

That actellic exhibited superior effectiveness is in agreement with the previous studies by Manju *et al*. (2014). A number of previous experimental studies have also indicated that organophosphate pesticides *viz* Actellic inhibit AChE activity in organisms but different organisms show different sensitivities to these pesticides (Manju *et al*., 2014). That the standard chemical, Actellic has superior effectiveness is due to the fact that the pure and fully developed active principles are more effective as compared to those in the crude forms (Gitahi *et al.*, 2015).

In conclusion, *T. diversifolia* and *V. lasiopus* were found to possess anti-AChE in this study. The extracts of the two plants have capacity indicative of their potential utilisation toward development of a biopesticide capable of inhibiting acetylcholinesterase activity, provide a characteristic AChE deficit and termination of impulse transmission on *S.*

zeamais. This kills *S. zeamais* thereby protecting and controling infestation of stored grains by the pests.

5.2 Conclusions

In conclusion, the present study demonstrated that:

i. The DCM and EtOAc leaf extract of *T. diversifolia* and *V. lasiopus* have phytochemicals that are associated with pesticidal activities against *S. zemails*.
ii. The DCM and EtOAc leaf extract of *T. diversifolia* and *V. lasiopus* displayed significant contact and fumigant toxicity activities.
iii. The DCM and EtOAc leaf extract of *T. diversifolia* and *V. lasiopus* displayed remarkable repellent effects on *S. zemails.*
iv. The DCM and EtOAc leaf extract of *T. diversifolia* and *V. lasiopus* have remarkable oviposition deterrence and F_1 progeny emergence inhibition effects on *S. zemails.*
v. The DCM and EtOAc leaf extract of *T. diversifolia* and *V. lasiopus* have appreciable antifeedant activities against *S. zeamais*.
vi. The DCM and EtOAc leaf extract of *T. diversifolia* and *V. lasiopus* have significant *in vitro* anti-AChE activities in *S. zeamais*.

Therefore, the research questions formulated in this study were answered in affirmative.

5.3 Recommendations

5.3.1 Recommendations from the Study

From findings of this study the following recommendations are suggested:

i. The use of concentrations higher than 50% of *T. diversifolia* and *V. lasiopus* organic extracts for effective control of *S. zeamais*.

ii. This study recommends the use of non-polar solvents (DCM and EtOAc) extracts of the *T. diversifolia* and *V. lasiopus* to be used by local communities in protection of stored maize grains against weevils.

iii. The use of the *T. diversifolia* and *V. lasiopus* organic extracts as insect repellents rather than attractants. Findings of this research indicate that *T. diversifolia* and *V. lasiopus* can be a good source of natural repellents for use in food stores against insects.

iv. This study scientifically validates the use of the selected organic leaf extracts of *T. diversifolia* and *V. lasiopus* in the management of storage pests of maize. It can serve as alternative/ complement to conventional ways of managing weevils.

v. Considering toxicity, repellency, antifeedant and offspring reduction effects as desirable modes of action against many stored products pests, this study therefore suggest the scientific rationale of using extracts from *T. diversifolia* and *V. lasiopus* as natural control agents against maize weevil.

vi. There is need for efficient conservation strategies for the Kenyan populations of *T. diversifolia* and *V. lasiopus* due to their high bioactivity and abundance in phytochemicals.

vii. Anti-acetylcholinesterase activities exhibited by extracts from *T. diversifolia* and *V. lasiopus* can be associated with their observed insecticidal properties.

viii. In addition to their use in the management of storage insect pests, the selected organic leaf extracts of *T. diversifolia* and *V. lasiopus* can also be bioscreened for use as a complementary acetylcholinesterase inhibitory therapy (AChEIs) as a strategy for symptomatic treatment of Alzheimer's disease (AD).

5.3.2 Recommendations for Further Work

i. There is need to isolate, purify and bioscreen the individual phytochemical compounds of organic leaf extracts of *T. diversifolia* and *V. lasiopus* in order to associate specific phytochemicals with specific insecticidal effects witnessed. Different combinations of such phytochemicals should also be tested for any synergistic or additive effects.

ii. Undertake the study using aqueous extraction of *T. diversifolia* and *V. lasiopus* to establish how its insecticidal activities can be compared with observations made from the selected organic extracts.

iii. Further research should be done to elucidate more possible mechanisms of insecticidal activities of *T. diversifolia* and *V. lasiopus* extracts other than acting on acetylcholinesterase. This will provide more knowledge on better ways of the crude extracts application hence create a break-through in management of maize weevils.

iv. Evaluation of application formulations of *T. diversifolia* and *V. lasiopus* that would be more effective against *S. zeamais* and cost friendly. Study should be carried out to assess whether the plant extract possess the same activity when applied in powder form.

v. Further investigations are necessary to establish whether there are non-target effects on other insects, especially the weevil predators.

vi. A study should be carried out to determine the residual effect of crude leaves extract of *T. diversifolia* and *V. lasiopus*

vii. X-ray crystallography to be done to get the identity of the unknown compounds in ethyl acetate extracts of the *T. diversifolia* and *V. lasiopus* leaf extracts which could not be identified by mass spectrometry.

viii. *In vivo* study of the anti-acetylcholinesterase activities of the extracts to confirm the observations obtained from *in vitro* studies.

ix. Purification of the adult maize weevils' acetylcholinesterase. This will help in removing unwanted debris from the enzyme and maybe the activity will be better than that the crude enzyme.

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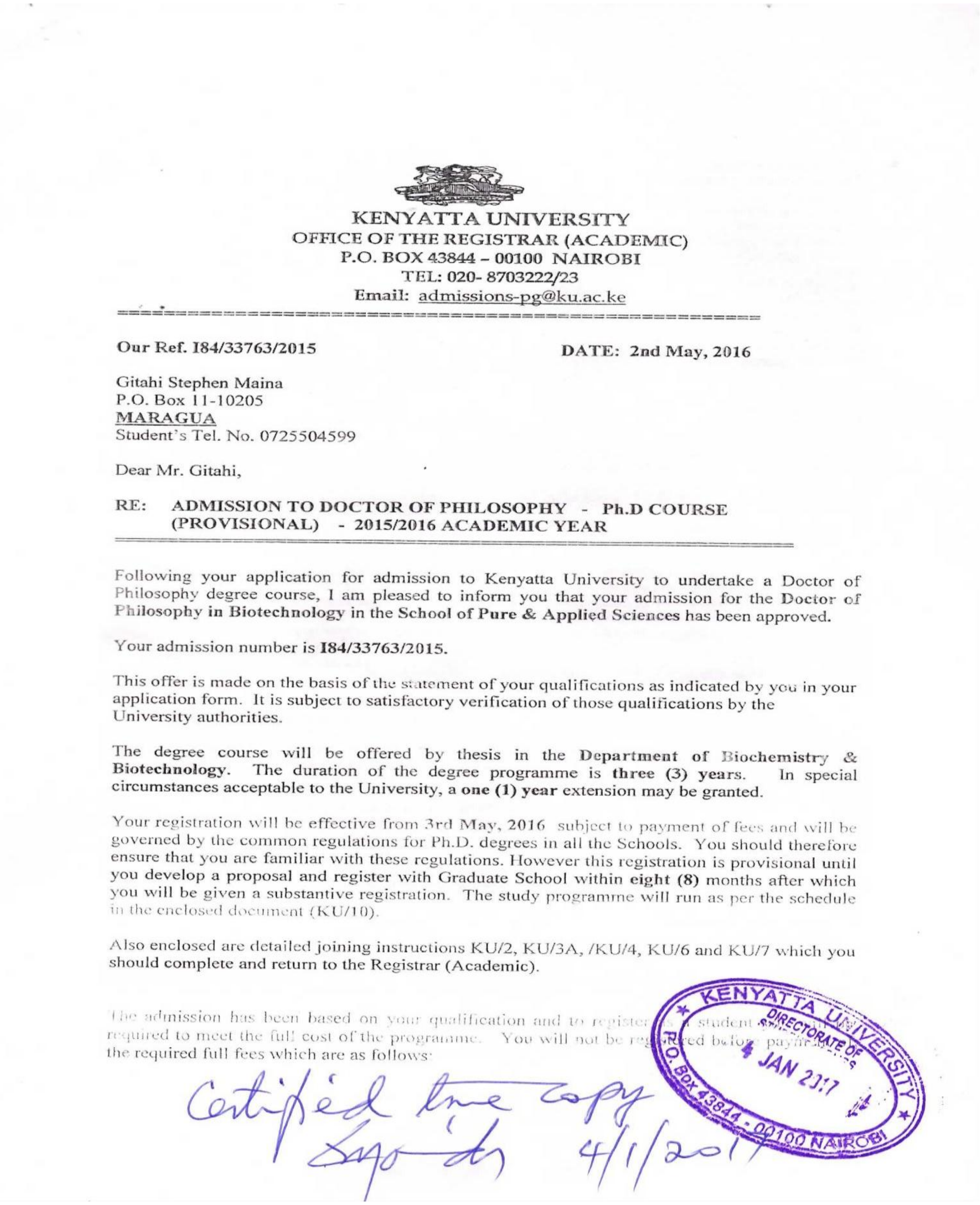
APPENDICES

Appendix 1.1: Letter of Admission to Doctor of Philosophy (Biotechnology) of Kenyatta University



KENYATTA UNIVERSITY
OFFICE OF THE REGISTRAR (ACADEMIC)
P.O. BOX 43844 – 00100 NAIROBI
TEL: 020- 8703222/23
Email: admissions-pg@ku.ac.ke

Our Ref. I84/33763/2015 **DATE: 2nd May, 2016**

Gitahi Stephen Maina
P.O. Box 11-10205
MARAGUA
Student's Tel. No. 0725504599

Dear Mr. Gitahi,

RE: ADMISSION TO DOCTOR OF PHILOSOPHY - Ph.D COURSE (PROVISIONAL) - 2015/2016 ACADEMIC YEAR

Following your application for admission to Kenyatta University to undertake a Doctor of Philosophy degree course, I am pleased to inform you that your admission for the Doctor of Philosophy **in Biotechnology** in the **School of Pure & Applied Sciences** has been approved.

Your admission number is **I84/33763/2015.**

This offer is made on the basis of the statement of your qualifications as indicated by you in your application form. It is subject to satisfactory verification of those qualifications by the University authorities.

The degree course will be offered by thesis in the **Department of Biochemistry & Biotechnology.** The duration of the degree programme is **three (3) years.** In special circumstances acceptable to the University, a **one (1) year** extension may be granted.

Your registration will be effective from 3rd May, 2016 subject to payment of fees and will be governed by the common regulations for Ph.D. degrees in all the Schools. You should therefore ensure that you are familiar with these regulations. However this registration is provisional until you develop a proposal and register with Graduate School within **eight (8)** months after which you will be given a substantive registration. The study programme will run as per the schedule in the enclosed document (KU/10).

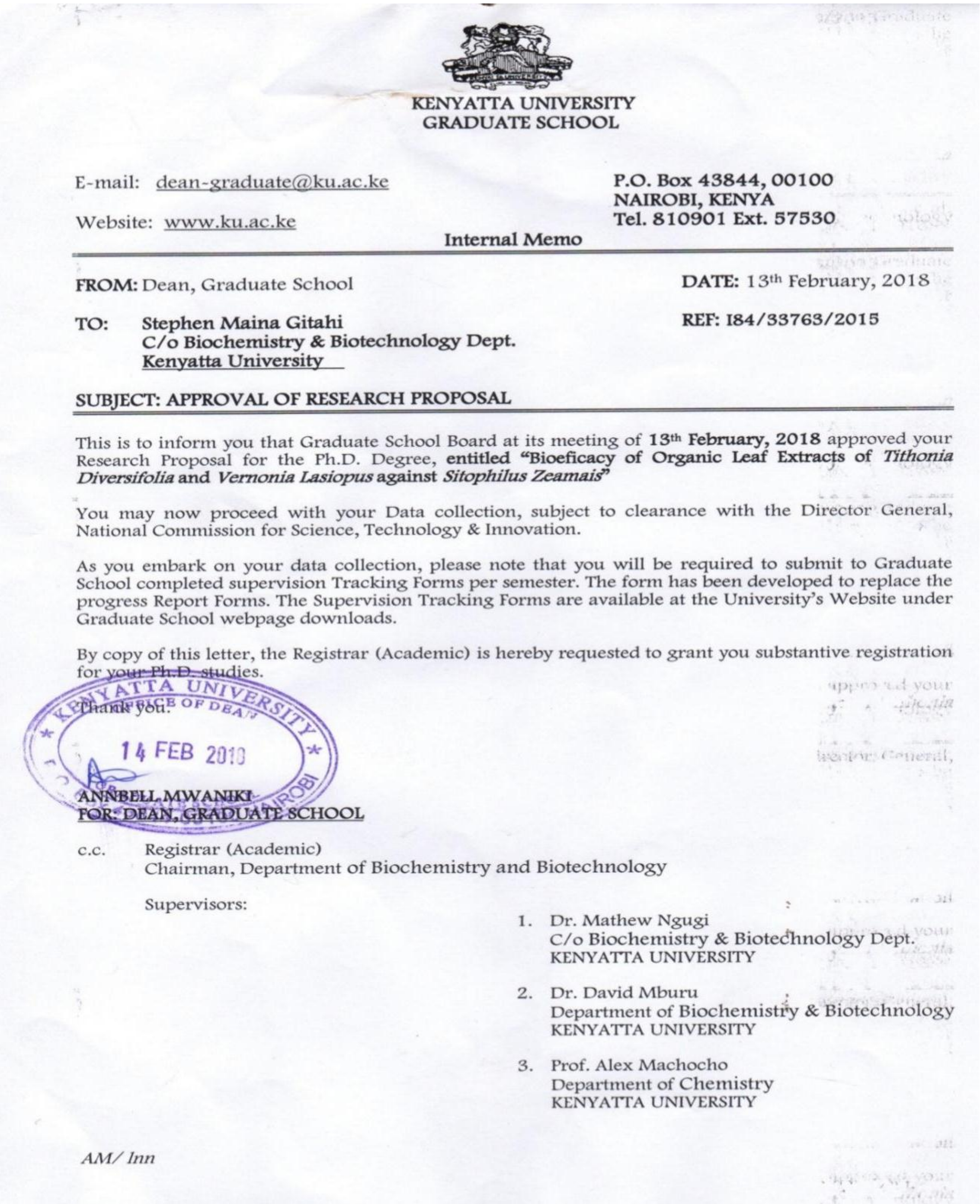
Also enclosed are detailed joining instructions KU/2, KU/3A, /KU/4, KU/6 and KU/7 which you should complete and return to the Registrar (Academic).

The admission has been based on your qualification and to register as a student [illegible] required to meet the full cost of the programme. You will not be registered before payment of the required full fees which are as follows:

DIRECTORATE OF
4 JAN 2017

Certified true copy
4/1/2017

Appendix 1.2: Letter of Approval of Research Proposal by Graduate School of Kenyatta University



KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School

DATE: 13th February, 2018

TO: **Stephen Maina Gitahi**
C/o Biochemistry & Biotechnology Dept.
Kenyatta University

REF: I84/33763/2015

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board at its meeting of **13th February, 2018** approved your Research Proposal for the Ph.D. Degree, **entitled "Bioeficacy of Organic Leaf Extracts of *Tithonia Diversifolia* and *Vernonia Lasiopus* against *Sitophilus Zeamais*"**

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed supervision Tracking Forms per semester. The form has been developed to replace the progress Report Forms. The Supervision Tracking Forms are available at the University's Website under Graduate School webpage downloads.

By copy of this letter, the Registrar (Academic) is hereby requested to grant you substantive registration for your Ph.D. studies.

Thank you.

14 FEB 2018

ANNBELL MWANIKI
FOR: DEAN, GRADUATE SCHOOL

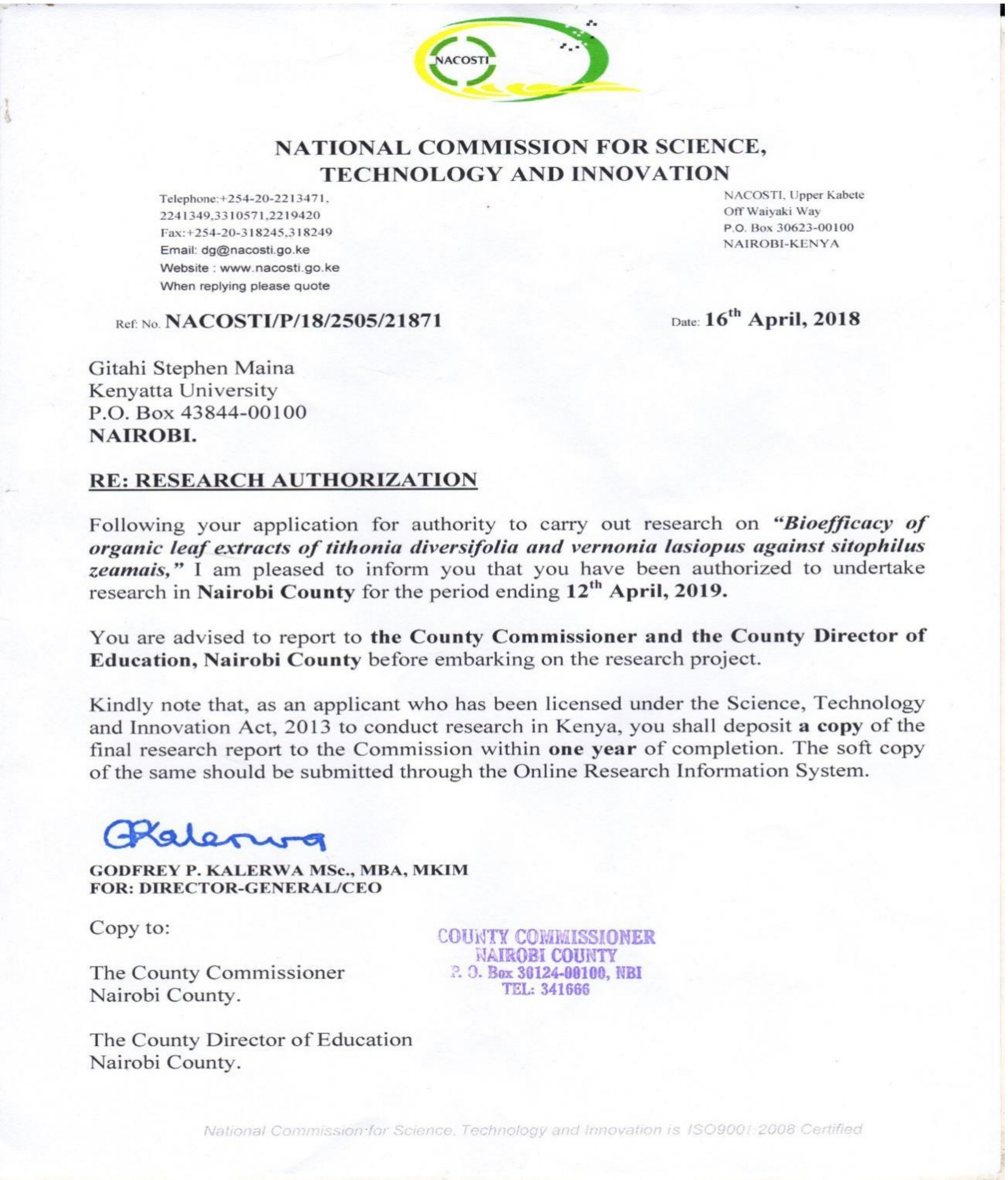
c.c. Registrar (Academic)
Chairman, Department of Biochemistry and Biotechnology

Supervisors:

1. Dr. Mathew Ngugi
   C/o Biochemistry & Biotechnology Dept.
   KENYATTA UNIVERSITY
2. Dr. David Mburu
   Department of Biochemistry & Biotechnology
   KENYATTA UNIVERSITY
3. Prof. Alex Machocho
   Department of Chemistry
   KENYATTA UNIVERSITY

AM/ Inn

Appendix 1.3: Letter of Research Authorization by National Commission for Science, Technology and Innovation (NACOSTI), Kenya



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/18/2505/21871**

Date: **16th April, 2018**

Gitahi Stephen Maina
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

<u>RE: RESEARCH AUTHORIZATION</u>

Following your application for authority to carry out research on ***"Bioefficacy of organic leaf extracts of tithonia diversifolia and vernonia lasiopus against sitophilus zeamais,"*** I am pleased to inform you that you have been authorized to undertake research in **Nairobi County** for the period ending **12th April, 2019.**

You are advised to report to **the County Commissioner and the County Director of Education, Nairobi 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.

Kalerwa

GODFREY P. KALERWA MSc., MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Nairobi County.

COUNTY COMMISSIONER
NAIROBI COUNTY
P. O. Box 30124-00100, NBI
TEL: 341666

The County Director of Education
Nairobi County.

National Commission for Science, Technology and Innovation is ISO9001:2008 Certified

Appendix 1.4: Letter of Research Authorization by the Ministry of Education, Kenya

Republic of Kenya
MINISTRY OF EDUCATION
STATE DEPARTMENT OF BASIC EDUCATION

Telegrams: "SCHOOLING", Nairobi
Telephone: Nairobi 020 2453699
Email: rcenairobi@gmail.com
cdenairobi@gmail.com
When replying please quote

REGIONAL COORDINATOR OF EDUCATION
NAIROBI REGION
NYAYO HOUSE
P.O. Box 74629 – 00200
NAIROBI

Ref: **RCE/NRB/GEN/1/VOL. 1**

DATE: **18th October, 2017**

Giotahi Stephen Maina
Kenyatta University
P O Box 43844-00100
NAIROBI

RE: <u>RESEARCH AUTHORIZATION</u>

We are in receipt of a letter from the National Commission for Science, Technology and Innovation regarding research authorization in Nairobi County on "**Bioefficacy of organic leaf extracts of tithonia diversifolia and vernonia lasiopus against sitophilus zeanais.**"

This office has no objection and authority is hereby granted for a period ending **12th April, 2019** as indicated in the request letter.

Kindly inform the Sub County Director of Education of the Sub County you intend to visit.

18 APR 2018

JAMES KIMOTHO
FOR: REGIONAL COORDINATOR OF EDUCATION
NAIROBI

C.C
Director General/CEO
Nation Commission for Science, Technology and Innovation
NAIROBI

Appendix 1.5: Different Parasites used to Control Stored Grain Pest

Name of parasite	Family	Order	Pest Controlled
***Bracon hebetor* say**	Braconidae	Hymenoptera	*Oryzaphilus surinamensis*
Venturia canescens	Pyralidae	Lepidoptera	*Plodia interpunctella* (Hubner)
V. canescens	Ichneumonidae	Hymenoptera	*Oryzaephilus surinamensis L.*
L. distinguendus	Ptermalidae	Hymenoptera	*Sitophilus granarius L.*
***A. clandrae* (Howard)**	Pteromalidae	Hymenoptera	*O. surinamensis L*
***Xylocriflavipes* (Reuter)**	Anthocoridae	Hemiptera	*Plodia interpunctella* (Hubner)
Lyctocoris spp	Anthocoridae	Hemiptera	*S. oryzae*
***A. wenat (*Klug)**	Reduviidae	Hemiptera	*S. oryzae*
***A. calandrae* (Howard)**	Pteromalidae	Hymenoptera	*Cephalonomia waterstoni*
P. biannulipes	Reduviidae	Hemiptera	*Sitophilus granarius L.*
Scenopinus fenetralis	Scenopinidae	Diptera	*Cephalonomia waterstoni*
***V.* canescens**	Ichneumonidae	Hymenoptera	*S. oryzae*
***Bracon hebetor* Say**	Braconidae	Hymenoptera	*S. oryzae*
Antrocephalus spp	Chalcididae	Hymenoptera	*S. oryzae*

Adapted from Upadhyay and Ahmad, 2011

Appendix 1.6: Examples of Some Common Pesticidal Plants Used to Control Different Insect Pests Globally

Botanical pesticides	Insect Pest	References
Nicotine	Aphids, thrips, caterpillars	Casanova *et al.*, 2002
Rotenone	Bugs, aphids, potato beetles, spider mites, carpenter ants	Cabizza *et al.*, 2004
Ryania	Codling moths, potato aphids, onion trips, corn earworms, silkworm	Isman, 2006, Copping and Menn 2000
Sabadilla	Grasshoppers, codling moths, armyworms, aphids, cabbage loopers, squash bugs	Bloomquist 1996, 2003
Pyrethrum	Caterpillars, aphids, leafhoppers, spider mites, bugs, cabbage worms, beetles	Casida 1973; Glynne Jones 2001)
Essential oils	Caterpillars, cabbage worms, aphids, white flies Land snails	Enan 2005
Neem products	Armyworms, cutworms, stemborers, bollworms, leaf miners, caterpillars, aphids, whitefles, leafhoppers, psyllids, scales, mites and thrips	Dimetry *et al.*, 2010

Appendix 1.7: Examples of Common Plant Essential Oils with Insecticidal Activities (Adapted from Asgar Edadollahi, 2013)

Plant species	Activity and tested insect	Reference
Ammi visnaga	Ovicidal activity against *Mayetiola destructor*. Adulticidal and ovicidal activity against *Callosobruchus maculatus*.	Lamiri *et al*., 2001 Tripathi *et al*., 2001b
Anethum graveolense	Adulticidal activity on *C. chinensis* Fumigant toxicity against *C. chinensis, C. chinensis* Fumigant activity against *Reticulitermes speratus*.	Upadhyay *et al*., 2007 Chaubey, 2008 Seo *et al*., 2009
Angelica sylvestris	Fumigant toxicity against *Acanthoscelides obtectus*.	Papachristos , 2002
Angelica dahurica	Fumigant toxicity against *S. oryzae* and *C. chinensis*.	Kim e*t al*., 2003b
Azilia eryngioides	Fumigant on *S. granarius* and *Tribolium castaneum*.	Ebadollahi and Mahboubi, 2011
Bunium persicum	Fumigant toxicity against adults of *T. castaneum*.	Moravej *et al*., 2009
Carum carvi	Toxicity on *S. zeamais, S. oryzae* and *T. castaneum*	Fang, 2010; Sahaf *et al*., 2007
Carum copticum	Toxicity against adults of *T. confusum, Rhyzopertha dominica* and *Oryzaphilus surinamensis*.	Habashi *et al*., 2011
Cnidium officinale	Toxicity against *S. oryzae* and *C. chinensis*.	Kim *et al*., 2003b
Cuminum cyminum	Fumigant activity on *S. oryzae, C. maculatus* adults. .	Ebadollahi e*t al*., 2012
Peucedanum officinale	Fumigant activity against adults of *T. castaneum*.	Lee *et al*., 2002
Prangos acaulis	Adulticidal and larvicidal against *C. maculatus*.	Sarikolaei et al.,, 2010

Adapted from Asgar Edadollahi, 2013

Appendix 1.8: Ethnomedicinal Uses of *Vernonia lasiopus* (O. Hoffman)

Part used	Country	Use in Ethnomedicine	Reference
L, St	Kenya	Malaria and Worms	Kareru *et al.,* 2008
L	Rwanda	bacteria, virus	Vlietinck *et al.,* 1995
L	Uganda	Febrile convulsions	Ssegawa and Kasenene, 2007
L, B, R	Kenya	Malaria	Muregi *et al.*, 2007a, 2007b
N/I	Mexico	GIT parasites	Heinrich, 1996
L, R	Kenya	Heart burn, crop pests and	Gakuya *et al.*, 2012
L	Kenya	Skin diseases	Njoroge and Bussmann, 2007
WP, R	Tanzania	Epilepsy, indigestion,	Burkill, 1985
L	Rwanda	Hepatitis, wounds and	Mukazayire *et al.*, 2010
L, R	Tanzania	Reproductive health,	Hedberg *et al.*,1982
L	Kenya	Epilepsy, Anti-plasmodial	Irungu *et al.*, 2007

B-Bark; L-Leaf; R-Root; S-Seed; St-Stem; WP-Whole plant

Appendix 1.9: *In-vitro* Studies carried out on the Bioactivity of *T. diversifolia*

Study	Activity/Results	Reference
Antibacterial	NA	Vlietinck *et al.,* 1995
Antiviral, Antifungal	A	Vlietinck *et al.,* 1995
Antiplasmodial	IC_{50}=1.0 µg/ml	Muregi *et al.*, 2003)
Cytotoxicity	A	Koul *et al.*, 2003)
Antimalarial/Cytotoxicity	IC_{50}=1.4 µg/ml	Irungu *et al.*, 2007)
Hepatoprotective	1 mg/ml	Mukazayire *et al.*, 2010)

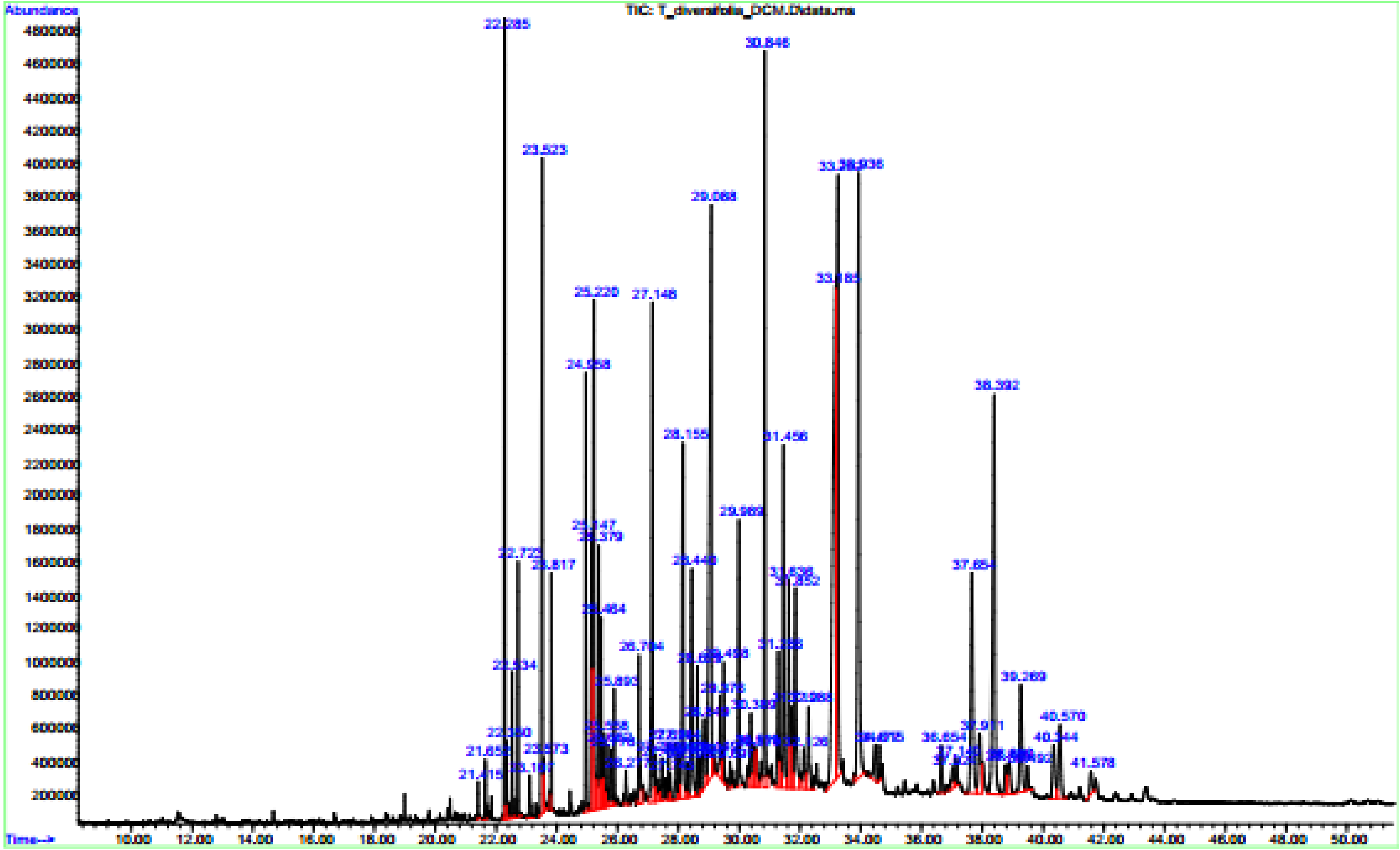
A=Active; NA=not active; NI=Not indicate

Appendix 1.10: ***In-vitro* Studies carried out on the Bioactivity of *V. lasiopus***

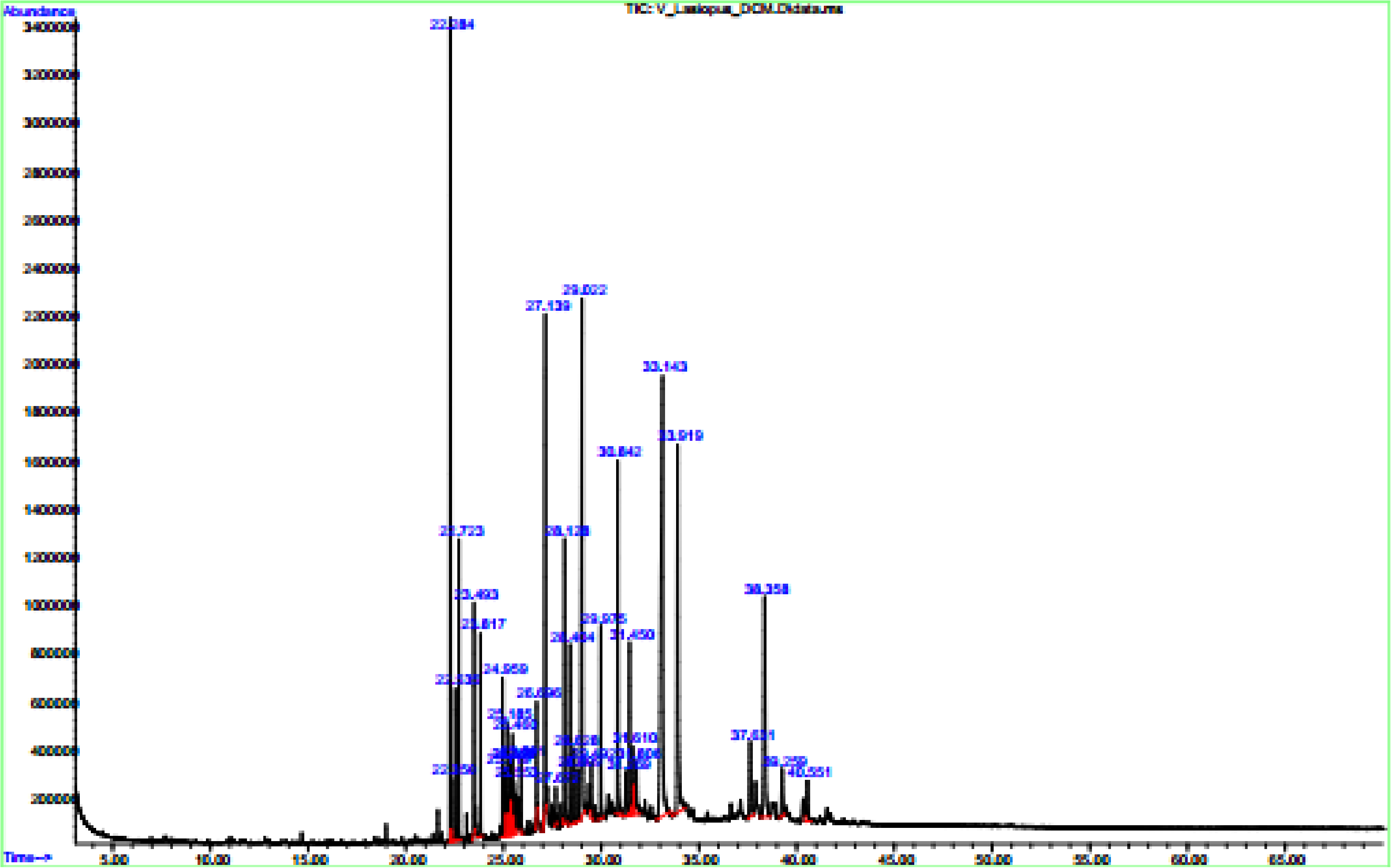
Study	Activity/Results	Reference
Antibacterial	NA	Vlietinck *et al.,* 1995
Antiviral, Antifungal	A	Vlietinck *et al.,* 1995
Antiplasmodial	IC_{50}=1.0 µg/ml	Muregi *et al.,* 2003)
Cytotoxicity	A	Koul *et al.,* 2003)
Antimalarial/Cytotoxicity	IC_{50}=1.4 µg/ml	Irungu *et al.,* 2007)
Hepatoprotective	1 mg/ml	Mukazayire *et al.,* 2010)

A=Active; NA=not active; NI=Not indicate

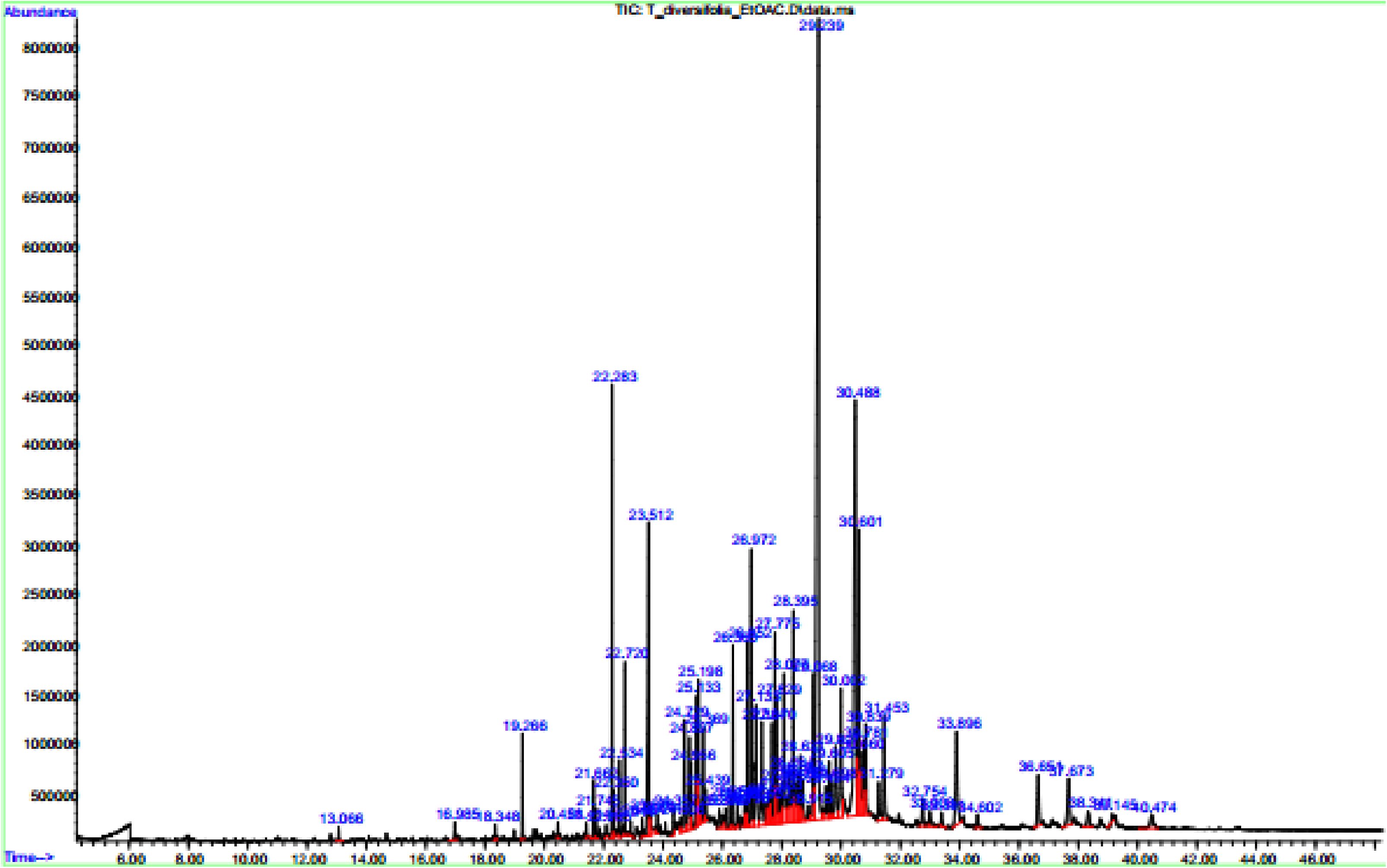
Appendix 2.1: **Representative Total Ion GCMS Chromatogram of DCM Leaf Extract of *T. diversifolia* with Retention Time (Min) for the Identified Phytochemicals Concentration Determination**



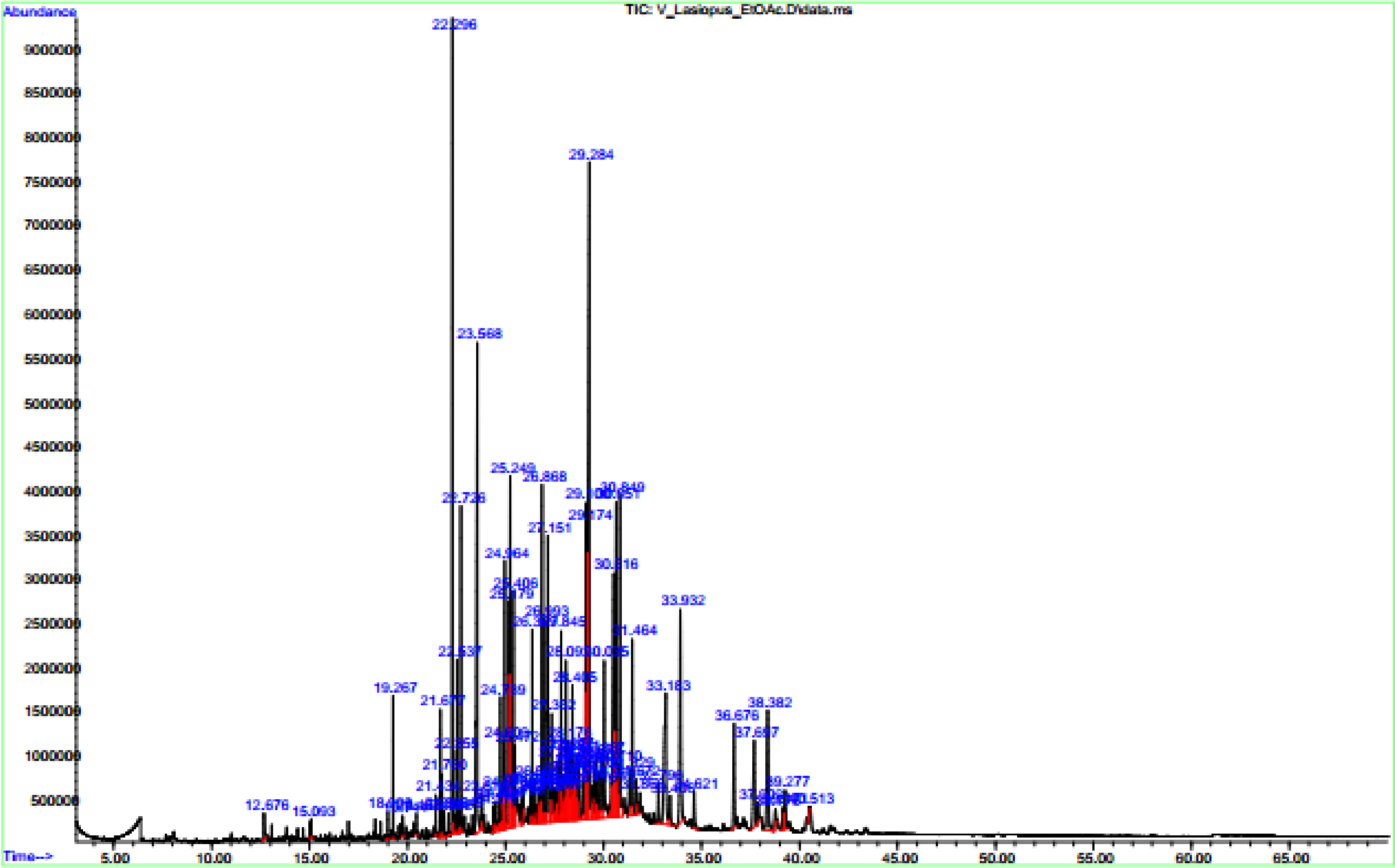
Appendix 2.2: The GC-MS Total Ion Chromatogram of DCM Leaf Extract of *V. lasiopus* with Retention Time for Phytochemicals Concentration Determination



Appendix 2.3: The GC-MS Total Ion Chromatogram of Ethyl Acetate Leaf Extract of *T. diversifolia* with Retention Time for Phytochemicals Concentration Determination

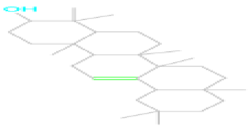


Appendix 2.4: **The GC-MS Total Ion Chromatogram of Ethyl Acetate Leaf Extract of *V. lasiopus* with Retention Time for Phytochemicals Concentration Determination**



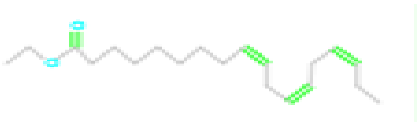
Appendix 2.5: Chemical Structures of Insecticidal Phytochemical Components Identified in the Leaf Extracts of *T. diversifolia* and *V. lasiopus*

β-Amyrin



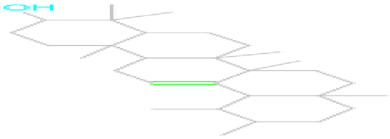
α-Copaene

Octadecatrienoic acid

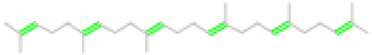


Pentadecanone

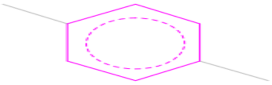
α-Amyrin



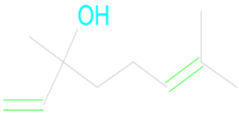
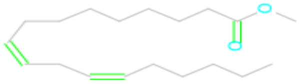
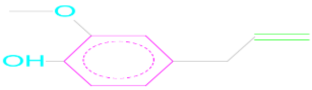
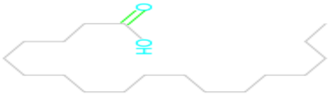
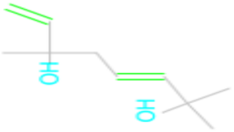
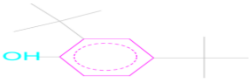
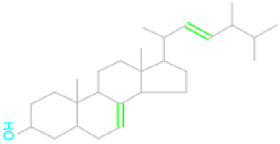
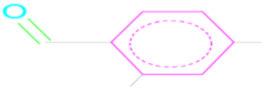
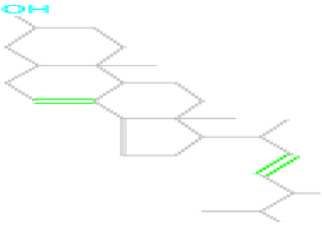
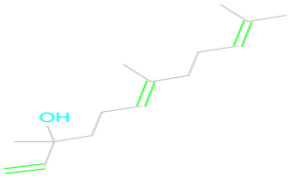
Squalene

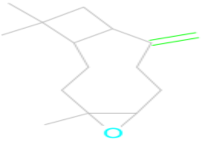
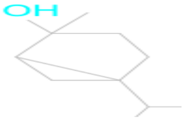
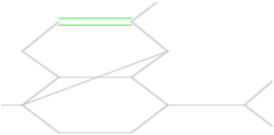
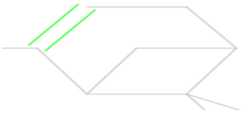
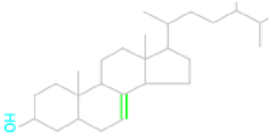
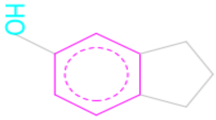
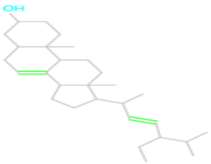
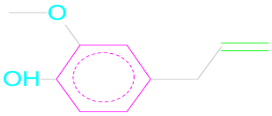


p-Xylene

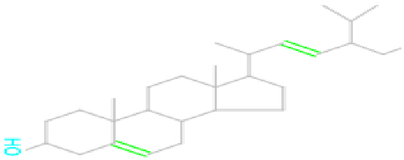


Phytol

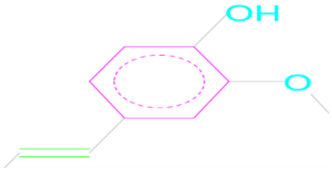
Linalool 	**Methyl linoleate** 
Eugenol 	**Octadecanoic acid** 
Octadiene- ol 	**Phenol, dimethylethyl** 
Ergostadienol 	**Benzaldehyde, 2,4-dimethyl-** 
Ergostadienol 	**(E)-Nerolidol** 

Caryophyllene oxide  	**Limonene** 
Sabinene  	**Terpinen-4-ol** 
α-Copaene 	**α-Pinene** 
γ-Ergostenol  	**Indanol** 
Chondrillasterol  	**Eugenol** 

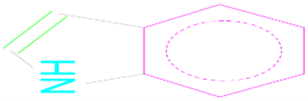
Stigmasterol



(E)-Isoeugenol

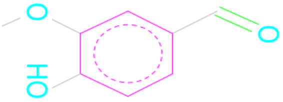


Indole

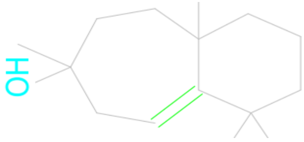


L-α-Terpineol

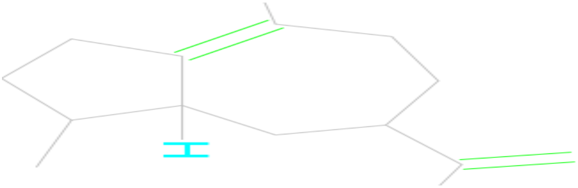
Vanillin



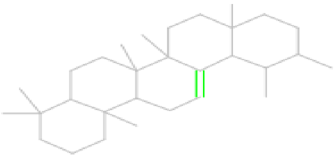
Widdrol



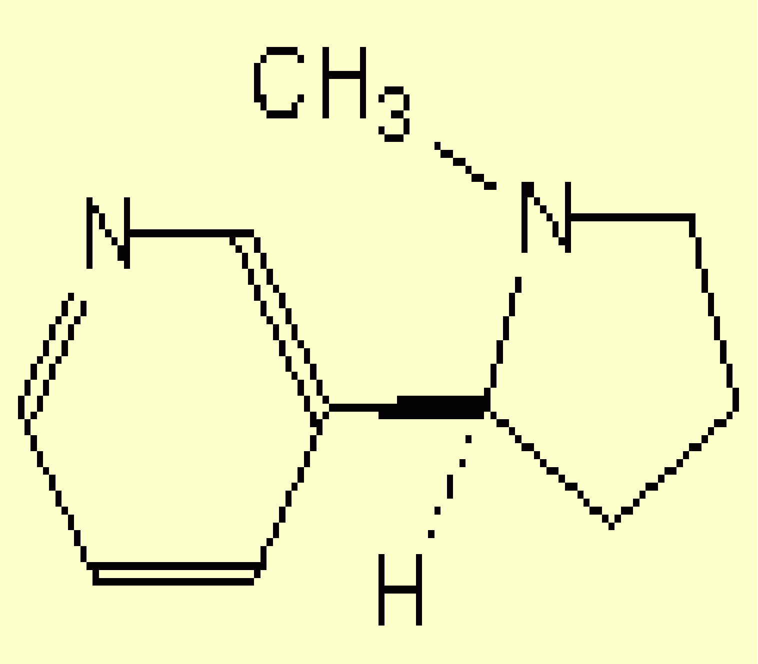
α-Bulnesene



Urs-12-ene



Appendix 2.6: **Molecular Structure of First Generation Botanical Pesticides**



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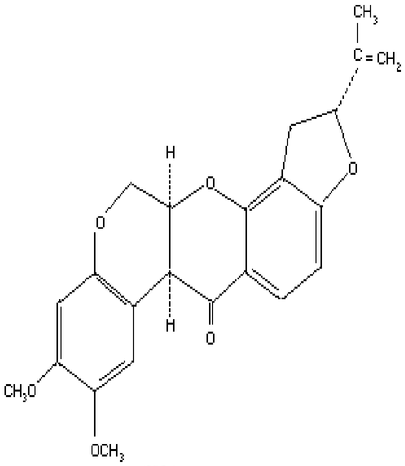
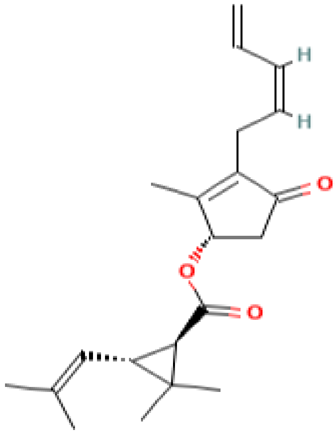
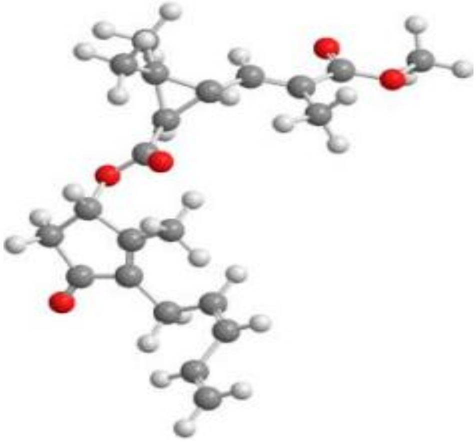
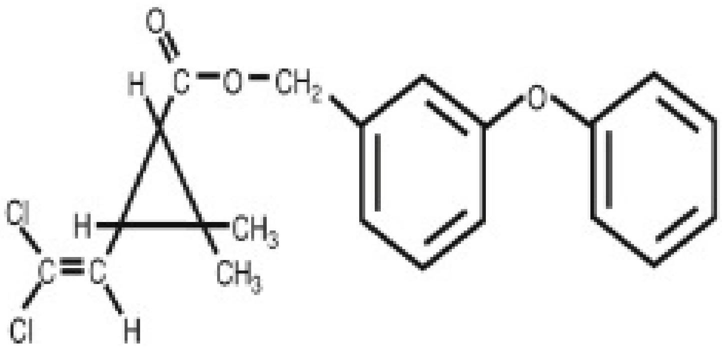
Molecular Structure of Nicotine



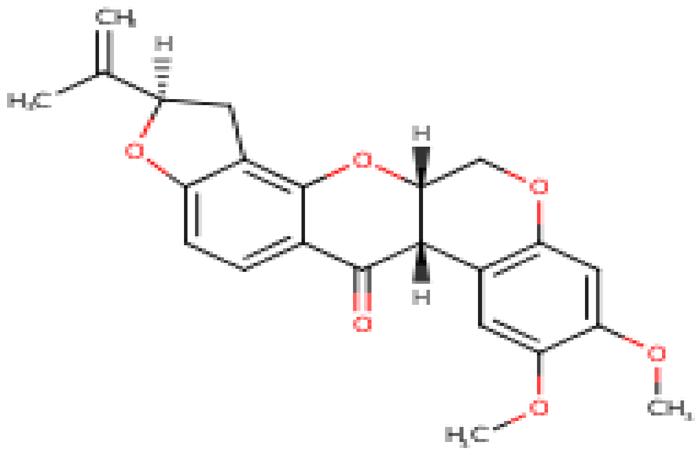




Molecular Structure of Permethrin



Molecular Structure of Rotenone



Molecular Structure of Sabadilla

Appendix 3.1: Fumigant Toxicity Effects of DCM Leaf Extracts of *T. diversifolia* against *S. zeamais*

Group	Treatment	Mean % Mortality (PM) ± S.E with Exposure Period (hours)				
		6h	24h	48h	72h	96h
I (Negative Control)	Solvent Only	000.00±0.00	001.25±1.25	001.25±1.25	002.50±1.44	003.75±1.25
II (Experimental Group A)	25% Extract	023.75±2.39	027.50±3.23	042.50±2.39	043.75±2.39	057.50±3.23
III (Experimental Group B)	50% Extract	031.25±2.39	035.00±2.04	037.50±1.44	052.50±3.23	066.26±2.39
IV (Experimental Group C)	75% Extract	027.50±1.44	033.75±1.25	056.25±3.15	088.75±3.75	093.75±2.39
V (Experimental Group D)	100% Extract	028.75±2.39	043.75±1.25	091.25±2.39	091.25±2.39	095.00±2.04
VI (Positive Control)	Actellic	092.50±3.23	097.50±2.50	100.00±0.00	100.00±0.00	100.00±0.00

Appendix 3.2: Fumigant Toxicity Effects of DCM Leaf Extracts of *V. lasiopus* against *S. zeamais*

Group	**Treatment**	**Mean % Mortality (PM) ± S.E with Exposure Period (hours)**				
		6h	24h	48h	72h	96h
I (Negative Control)	Solvent Only	000.00±0.00	001.25±1.25	001.25±1.25	002.50±1.44	002.50±1.44
II (Experimental Group A)	25% Extract	035.00±2.04	043.75±2.39	063.75±2.39	068.75±2.39	077.50±1.44
III (Experimental Group B)	50% Extract	005.00±2.04	011.25±1.25	035.00±2.89	062.50±3.23	066.25±3.15
IV (Experimental Group C)	75% Extract	005.00±2.04	016.25±3.15	031.25±4.27	038.75±1.25	041.25±1.25
V (Experimental Group D)	100% Extract	001.25±1.25	011.25±1.25	021.25±1.25	033.75±1.25	038.75±1.25
VI (Positive Control)	Actellic	092.50±4.33	097.50±2.50	100.00±0.00	100.00±0.00	100.00±0.00

Appendix 3.3: Fumigant Toxicity Effects of Ethyl Acetate Leaf Extracts of T. diversifolia against S. zeamais

Group	Treatment	Mean % Mortality (PM) ± S.E with Exposure Period (hours)				
		6h	24h	48h	72h	96h
I (Negative Control)	Solvent Only	000.00±0.00	001.25±1.25	002.50±1.44	002.50±1.44	002.50±1.44
II (Experimental Group A)	25% Extract	020.00±2.04	031.25±2.39	055.00±2.04	062.50±3.23	070.00±2.04
III (Experimental Group B)	50% Extract	028.75±2.39	056.25±2.39	065.00±2.04	068.75±1.25	068.75±1.25
IV (Experimental Group C)	75% Extract	017.50±3.23	083.75±3.15	092.50±3.23	096.25±2.39	097.50±1.44
V (Experimental Group D)	100% Extract	021.25±2.39	076.25±2.39	097.50±1.44	097.50±2.50	098.75±1.25
VI (Positive Control)	Actellic	092.50±3.23	097.50±1.44	100.00±0.00	100.00±0.00	100.00±0.00

Appendix 3.4: Fumigant Toxicity Effects of Ethyl Acetate Leaf Extracts of *V. lasiopus* against *S. zeamais*

Group	**Treatment**	**Mean % Mortality (PM) ± S.E with Exposure Period (hours)**				
		6h	24h	48h	72h	96h
I (Negative Control)	Solvent Only	1.25±1.25	1.25±1.25	1.25±1.25	3.75±2.39	3.75±2.39
II (Experimental Group A)	25% Extract	13.75±1.25	16.25±1.25	18.75±1.25	30.00±2.04	32.50±2.50
III (Experimental Group B)	50% Extract	20.00±2.04	27.50±3.23	37.50±3.23	42.50±4.33	45.00±3.54
IV (Experimental Group C)	75% Extract	22.50±3.23	26.25±3.75	40.00±4.56	45.00±2.04	50.00±2.04
V (Experimental Group D)	100% Extract	26.25±3.15	46.25±2.39	67.50±4.79	70.00±408	72.50±3.23
VI (Positive Control)	Actellic	92.50±4.33	97.50±2.50	100.00±0.00	100.00±0.00	100.00±0.00

Appendix 4.1: Contact Toxicity Effects of DCM Leaf Extracts of *T. diversifolia* against *S. zeamais*

Group	**Treatment**	**Mean % Mortality (PM) ± S.E with Exposure Period (hours)**				
		6h	24h	48h	72h	96h
I (Negative Control)	Solvent Only	000.00±0.00	001.25±1.25	001.25±1.25	002.50±1.44	003.75±1.25
II (Experimental Group A)	25% Extract	020.00±0.00	092.50±2.50	092.50±2.50	095.00±0.00	097.50±2.50
III (Experimental Group B)	50% Extract	033.75±2.39	090.00±4.56	095.00±2.89	097.50±1.44	098.75±1.25
IV (Experimental Group C)	75% Extract	036.25±3.15	072.50±3.23	095.00±2.04	098.75±1.25	100.00±0.00
V (Experimental Group D)	100% Extract	033.75±4.27	093.75±1.25	100.00±0.00	100.00±0.00	100.00±0.00
VI (Positive Control)	Actellic	096.00±2.04	097.50±2.50	100.00±0.00	100.00±0.00	100.00±0.00

Appendix 4.2: Contact Toxicity Effects of DCM Leaf Extracts of *V. lasiopus* against *S. zeamais*

Group	**Treatment**	**Mean % Mortality (PM) ± S.E with Exposure Period (hours)**				
		6h	24h	48h	72h	96h
I (Negative Control)	Solvent Only	000.00±0.00	001.25±1.25	001.25±1.25	002.50±1.4	003.75±1.25
II (Experimental Group A)	25% Extract	001.67±1.67	001.67±1.67	018.33±3.33	025.00±5.0	031.67±1.67
III (Experimental Group B)	50% Extract	002.50±2.50	011.25±2.39	018.75±2.39	026.25±3.5	032.50±1.44
IV (Experimental Group C)	75% Extract	025.00±2.04	030.00±2.04	031.25±2.39	043.75±3.5	055.00±2.04
V (Experimental Group D)	100% Extract	036.25±1.25	046.25±1.25	047.50±1.44	057.50±3.3	066.25±1.25
VI (Positive Control)	Actellic	095.00±2.04	097.50±2.50	100.00±0.00	100.00±0.0	100.00±0.00

Appendix 4.3: Contact Toxicity Effects of Ethyl Acetate Leaf Extracts of *T. diversifolia* against *S. zeamais*

Group	Treatment	Mean % Mortality (PM) ± S.E with Exposure Period (hours)				
		6h	24h	48h	72h	96h
I (Negative Control)	Solvent Only	000.00±0.00	001.25±1.25	001.25±1.25	002.50±1.44	005.00±2.04
II (Experimental Group A)	25% Extract	031.67±1.67	033.33±1.67	053.33±1.67	061.67±1.67	075.00±2.89
III (Experimental Group B)	50% Extract	047.50±1.44	055.00±2.04	062.50±5.20	066.25±1.25	098.75±1.25
IV (Experimental Group C)	75% Extract	066.25±1.25	087.50±1.44	092.50±1.44	097.50±2.50	098.75±1.25
V (Experimental Group D)	100% Extract	086.25±3.15	092.50±2.50	096.25±2.39	098.75±1.25	100.00±0.00
VI (Positive Control)	Actellic	095.00±2.04	097.50±2.50	100.00±0.00	100.00±0.00	100.00±0.00

Appendix 4.4: Contact Toxicity Effects of Ethyl Acetate Leaf Extracts of *V. lasiopus* against *S. zeamais*

Group	**Treatment**	**Mean % Mortality (PM) ± S.E with Exposure Period (hours)**				
		6h	24h	48h	72h	96h
I (Negative Control)	Solvent Only	000.00±0.00	000.00±0.00	000.00±0.00	001.67±1.67	005.00±2.89
II (Experimental Group A)	25% Extract	012.50±1.44	013.75±2.39	020.00±2.04	028.75±2.39	063.75±4.27
III (Experimental Group B)	50% Extract	023.75±2.39	026.25±4.27	037.50±1.44	043.75±3.75	068.75±3.15
IV (Experimental Group C)	75% Extract	032.50±2.50	038.75±1.25	071.25±3.15	082.50±3.23	093.75±2.39
V (Experimental Group D)	100% Extract	041.25±2.39	055.00±2.04	077.50±4.79	085.00±7.36	097.50±1.44
VI (Positive Control)	Actellic	095.00±2.04	097.50±2.50	100.00±0.00	100.00±0.00	100.00±0.00

Appendix 5.1: **Oviposition Deterrence and F_1 Progeny Emergence Inhibition Effects of DCM Leaf Extracts of *T. diversifolia* against *S. zeamais***

Group	Treatment	Eggs Hutched	% Oviposition Deterrence	Embryo Mortality	Larval Mortality	F_1 Progeny	% F_1 Emer. Inhb.
I (Negative Control)	Solvent Only	51.00 ± 0.41^{a}	00.004 ± 0.385^{e}	003.77 ± 0.77^{d}	02.93 ± 0.55^{c}	49.50 ± 0.29^{a}	000.00 ± 0.58^{f}
II(Positive Control)	Actellic	00.00 ± 0.00^{f}	99.075 ± 0.926^{a}	100.11 ± 0.00^{a}	-	00.00 ± 0.00^{f}	100.00 ± 0.00^{a}
III (Exp. Group A)	25% v/v	33.25 ± 0.48^{b}	14.921 ± 0.778^{d}	015.17 ± 2.47^{c}	08.20 ± 2.47^{c}	30.50 ± 0.65^{b}	038.38 ± 1.30^{e}
IV (Exp.Group B)	50% v/v	29.00 ± 0.58^{c}	18.439 ± 0.382^{d}	020.57 ± 0.95^{bc}	39.64 ± 0.21^{b}	17.50 ± 0.29^{c}	064.65 ± 0.59^{d}
V (Exp. Group C)	75% v/v	22.75 ± 0.25^{d}	25.448 ± 0.429^{c}	027.77 ± 0.69^{b}	42.89 ± 1.30^{b}	13.00 ± 0.41^{d}	073.74 ± 0.83^{c}
VI (Exp. Group D)	100% v/v	18.25 ± 0.75^{e}	37.270 ± 1.530^{b}	024.54 ± 3.41^{bc}	58.89 ± 2.44^{a}	07.50 ± 0.5^{e}	084.85 ± 1.01^{b}

Values expressed as mean ± SEM based on four extract concentrations, four replicates (n=4). Means followed by the same superscripts along the column are not significantly different by one-way ANOVA (p>0.005) followed by Tukey's post hoc test

Appendix 5.2: Oviposition Deterrence and F$_1$ Progeny Emergence Inhibition Effects of DCM Leaf Extracts of *V. lasiopus* against *S. zeamais*

Group	Treatment	Eggs Hutched	% Oviposition Deterrence	Embryo Mortality	Larval Mortality	F$_1$ Progeny	% F$_1$ Emer. Inhb.
I (Negative Control)	Solvent Only	51.00±0.408^{a}	00.004±0.385^{e}	003.78±0.77^{c}	02.93±0.55^{b}	49.50±0.29^{a}	000.00±0.58^{f}
II(Positive Control)	Actellic	0.00±0.00^{f}	99.075±0.926^{a}	100.00±0.00^{a}	-	00.00±0.00^{f}	100.00±0.00^{a}
III (Exp. Group A)	25% v/v	36.25±0.479^{b}	12.185±0.766^{d}	012.65±1.15^{b}	16.48±2.91^{b}	30.25±0.85^{b}	038.39±1.73^{e}
IV (Exp.Group B)	50% v/v	32.00±0.408^{c}	17.469±0.813^{d}	014.67±1.09^{b}	46.04±1.91^{a}	17.25±0.48^{c}	065.15±0.97^{d}
V (Exp. Group C)	75% v/v	24.25±0.479^{d}	28.889±0.754^{c}	016.95±1.62^{b}	34.11±3.15^{a}	13.75±0.48^{d}	072.22±0.97^{c}
VI (Exp. Group D)	100% v/v	10.25±0.25^{e}	64.390±0.650^{b}	010.87±2.17^{c}	07.05±4.40^{b}	09.50±0.29^{e}	080.81±0.58^{b}

Values expressed as mean ± SEM based on four extract concentrations, four replicates (n=4). Means followed by the same superscripts along the column are not significantly different by one-way ANOVA ($p>0.005$) followed by Tukey's post hoc test

Appendix 5.3: Oviposition Deterrence and F_1 Progeny Emergence Inhibition Effects of Ethyl Acetate Leaf Extract of *T. diversifolia* against *S. zeamais*

Group	**Treatment**	**Eggs Hutched**	**% Oviposition Deterrence**	**Embryo Mortality**	**Larval Mortality**	**F_1 Progeny**	**% F_1 Emer. Inhb.**
I (Negative Control)	Solvent Only	51.00±0.41^{a}	00.004±0.385^{e}	003.77±0.77^{c}	02.93±0.55^{c}	49.50±0.29^{a}	000.00±0.58^{f}
II(Positive Control)	Actellic	00.00±0.00^{e}	99.075±0.926^{a}	100.00±0.00^{a}	-	00.00±0.00^{f}	100.00±0.00^{a}
III (Exp. Group A)	25% v/v	28.00±0.41^{b}	25.466±0.959^{d}	010.95±2.79^{c}	24.03±1.93^{b}	21.25±0.25^{b}	057.07±0.51^{e}
IV (Exp.Group B)	50% v/v	23.25±0.63^{c}	31.283±0.774^{cd}	016.25±1.15^{bc}	29.04±1.96^{b}	16.50±0.65^{c}	066.67±1.30^{d}
V (Exp. Group C)	75% v/v	18.00±0.41^{d}	35.490±1.080^{c}	028.58±2.38^{b}	55.56±1.96^{a}	08.0±00.48^{d}	083.84±0.83^{c}
VI (Exp. Group D)	100% v/v	15.25±0.63^{d}	53.120±1.650^{b}	005.56±5.56^{c}	67.91±1.40^{a}	05.5±00.29^{e}	088.89±0.58^{b}

Values expressed as mean ± SEM based on four extract concentrations, four replicates (n=4). Means followed by the same superscripts along the column are not significantly different by one-way ANOVA ($p>0.005$) followed by Tukey's post hoc test

Appendix 5.4: Oviposition Deterrence and F_1 Progeny Emergence Inhibition Effects of Ethyl Acetate Leaf Extracts of *V. lasiopus* against *S. zeamais*

Group	**Treatment**	**Eggs Hutched**	**% Oviposition Deterrence**	**Embryo Mortality**	**Larval Mortality**	**F_1 Progeny**	**% F_1 Emer. Inhb.**
I (Negative Control)	Solvent Only	51.00 ± 0.408^{a}	00.004 ± 0.385^{f}	003.77 ± 00.77^{c}	02.93 ± 0.55^{b}	49.50 ± 0.29^{a}	000.00 ± 5.83^{e}
II(Positive Control)	Actellic	00.00 ± 0.000^{f}	99.075 ± 0.926^{a}	100.00 ± 00.00^{a}	-	00.00 ± 0.00^{e}	100.00 ± 0.00^{a}
III (Exp. Group A)	25% v/v	33.50 ± 0.645^{b}	17.785 ± 0.534^{e}	009.46 ± 10.74^{bc}	40.94 ± 2.79^{a}	19.75 ± 0.75^{b}	060.10 ± 1.52^{d}
IV (Exp.Group B)	50% v/v	27.25 ± 0.479^{c}	25.819 ± 0.371^{d}	012.80 ± 01.53^{b}	54.93 ± 2.45^{a}	12.25 ± 0.48^{c}	075.25 ± 0.98^{c}
V (Exp. Group C)	75% v/v	18.00 ± 0.408^{d}	43.790 ± 1.650^{c}	013.25 ± 01.97^{b}	48.45 ± 2.50^{a}	09.25 ± 0.25^{d}	081.31 ± 0.51^{b}
VI (Exp. Group D)	100% v/v	03.00 ± 0.000^{e}	87.625 ± 0.959^{b}	014.29 ± 00.00^{b}	58.33 ± 8.33^{a}	01.25 ± 0.25^{e}	097.48 ± 0.51^{a}

Values expressed as mean ± SEM based on four extract concentrations, four replicates (n=4). Means followed by the same superscripts along the column are not significantly different by one-way ANOVA ($p>0.005$) followed by Tukey's post hoc test

Appendix 6.1: Grain Loss Effects of the DCM Leaf Extract of *T. diversifolia* against *S. zeamais*

Group	**Treatment**	**Initial Grain Weight**	**Final grain Weight**	**Weight Lost**	**% Weight Loss (PWL)**
I (Negative Control)	Solvent Only	20.000±0.00	16.758±0.328	3.242±0.328	16.210±1.640^{a}
II(Positive Control)	Actellic	20.000±0.00	19.817±0.028	0.183±0.028	00.916±0.140^{c}
II (Experimental Group A)	25% Extract	20.000±0.00	17.260±0.070	2.740±0.070	13.700±0.348^{a}
III (Experimental Group B)	50% Extract	20.000±0.00	17.530±0.064	2.470±0.064	12.350±0.348^{ab}
IV (Experimental Group C)	75% Extract	20.000±0.00	18.392±0.104	1.607±0.104	08.037±0.521^{b}
V (Experimental Group D)	100% Extract	20.000±0.00	19.455±0.215	0.545±0.215	02.720±1.080^{c}

Appendix 6.2: Grain Loss Effects of the DCM Leaf Extract of *V. lasiopus* against *S. zeamais*

Group	Treatment	Initial Grain Weight	Final Grain Weight	Weight Lost	% Weight Loss (PWL)
I (Negative Control)	Solvent Only	20.000±0.00	16.758±0.328	3.242±0.328	16.210 ± 1.640^{a}
II(Positive Control)	Actellic	20.000±0.00	19.817±0.028	0.183±0.028	00.916 ± 0.140^{d}
II (Experimental Group A)	25% Extract	20.000±0.00	16.927±0.068	3.074±0.068	15.368 ± 0.342^{ab}
III (Experimental Group B)	50% Extract	20.000±0.00	17.465±0.034	2.535±0.034	12.675 ± 0.171^{ab}
IV (Experimental Group C)	75% Extract	20.000±0.00	18.015±0.139	1.985±0.139	09.925 ± 0.695^{bc}
V (Experimental Group D)	100% Extract	20.000±0.00	19.060±0.321	0.940±0.321	04.700 ± 1.600^{cd}

Appendix 6.3: Grain Loss Effects of the EtOAc Leaf Extract of *T. diversifolia* against *S. zeamais*

Group	Treatment	InitialGrain Weight	Final Grain Weight	Weight Lost	% Weight Loss (PWL)
I (Negative Control)	Solvent Only	20.000±0.00	16.758±0.328	3.242±0.328	16.210±1.640[a]
II(Positive Control)	Actellic	20.000±0.00	19.817±0.028	0.183±0.028	00.916±0.140[c]
II (Experimental Group A)	25% Extract	20.000±0.00	17.767±0.020	2.233±0.020	11.163±0.101[ab]
III (Experimental Group B)	50% Extract	20.000±0.00	18.230±0.096	1.770±0.096	08.850±0.478[b]
IV (Experimental Group C)	75% Extract	20.000±0.00	19.447±0.260	0.553±0.260	02.760±1.300[c]
V (Experimental Group D)	100% Extract	20.000±0.00	19.903±0.071	0.098±0.071	00.488±0.357[c]

Appendix 6.4: Grain Loss Effects of the EtOAc Leaf Extract of *V. lasiopus* against *S. zeamais*

Group	Treatment	InitialGrain Weight	Final Grain Weight	Weight Lost	% Weight Loss (PWL)
I (Negative Control)	Solvent Only	20.000±0.00	16.758±0.328	3.242±0.328	16.210±1.640^{a}
II(Positive Control)	Actellic	20.000±0.00	19.817±0.028	0.183±0.028	00.916±0.140^{c}
II (Experimental Group A)	25% Extract	20.000±0.00	17.508±0.116	2.492±0.116	12.462±0.579^{ab}
III (Experimental Group B)	50% Extract	20.000±0.00	17.813±0.126	2.188±0.126	10.938±0.629^{ab}
IV (Experimental Group C)	75% Extract	20.000±0.00	18.273±0.042	1.728±0.042	08.637±0.208^{b}
V (Experimental Group D)	100% Extract	20.000±0.00	19.438±0.205	0.563±0.205	02.810±1.020^{c}

Appendix 7.1: **Grain Damage Deterrence Effects of the DCM Leaf Extract of *T. diversifolia* against *S. zeamais***

Group	Treatment	Grains Sampled	Damaged Grains	Undamaged Grains	% Damage Caused (PD)	WPI
I (Negative Control)	Solvent Only	50.000±0.00	23.75±1.110	26.25±1.110	47.50±2.220^{a}	49.92±1.18^{a}
II(Positive Control)	Actellic	50.000±0.00	0.500±0.289	49.50±0.289	01.00±0.577^{c}	02.02±1.17^{c}
II (Experimental Group A)	25% Extract	50.000±0.00	5.250±0.479	44.75±0.479	10.50±0.957^{b}	18.04±1.37^{b}
III (Experimental Group B)	50% Extract	50.000±0.00	4.750±0.750	45.25±0.750	09.50±1.500^{b}	16.50±2.09^{b}
IV (Experimental Group C)	75% Extract	50.000±0.00	2.250±0.629	47.75±0.629	04.50±1.260^{bc}	08.50±2.16^{c}
V (Experimental Group D)	100% Extract	50.000±0.00	0.750±0.479	49.25±0.479	01.50±0.957^{c}	02.95±1.87^{c}

Appendix 7.2: Grain Damage Deterrence of the DCM Leaf Extract of *V. lasiopus* against *S. zeamais*

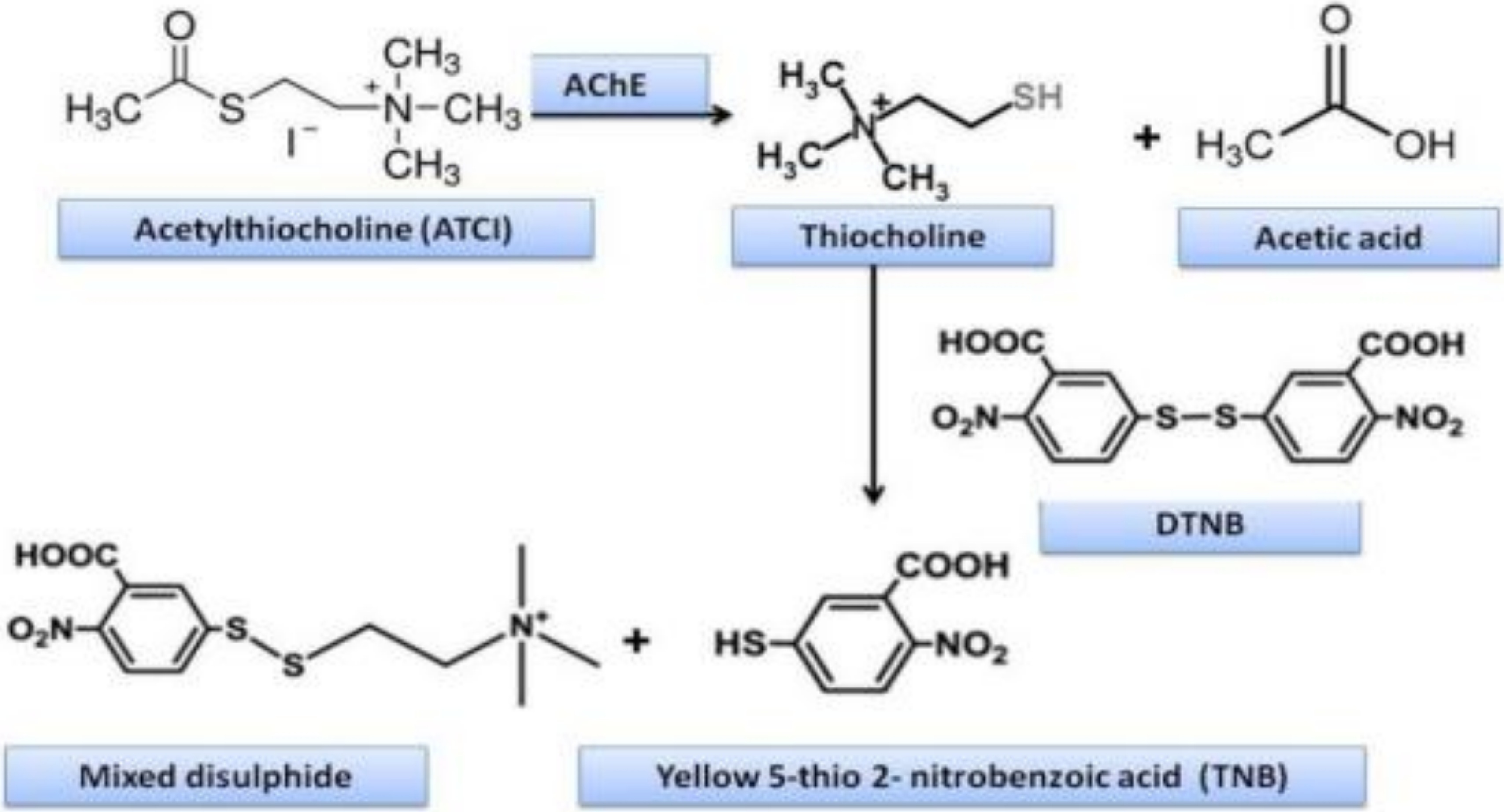
Group	Treatment	Grains Sampled	Damaged Grains	Undamaged Grains	% Damage Caused (PD)	WPI
I (Negative Control)	Solvent Only	50.000±0.00	23.75±1.110	26.250±1.110	47.50±2.220[a]	49.92±1.18[a]
II(Positive Control)	Actellic	50.000±0.00	0.500±0.289	49.500±0.289	01.00±0.577[d]	02.02±1.17[c]
II (Experimental Group A)	25% Extract	50.000±0.00	6.250±0.854	43.750±0.854	12.50±1.710[b]	20.64±2.31[b]
III (Experimental Group B)	50% Extract	50.000±0.00	5.250±1.030	44.750±1.030	10.50±2.060[c]	17.79±2.95[b]
IV (Experimental Group C)	75% Extract	50.000±0.00	2.250±0.629	47.750±0.629	04.50±1.260[d]	08.50±2.16[c]
V (Experimental Group D)	100% Extract	50.000±0.00	1.750±0.479	48.250±0.479	03.50±0.957[d]	06.77±1.72[c]

Appendix 7.3: Grain Damage Deterrence Effects of the EtOAc Leaf Extract of *T. diversifolia* against *S. zeamais*

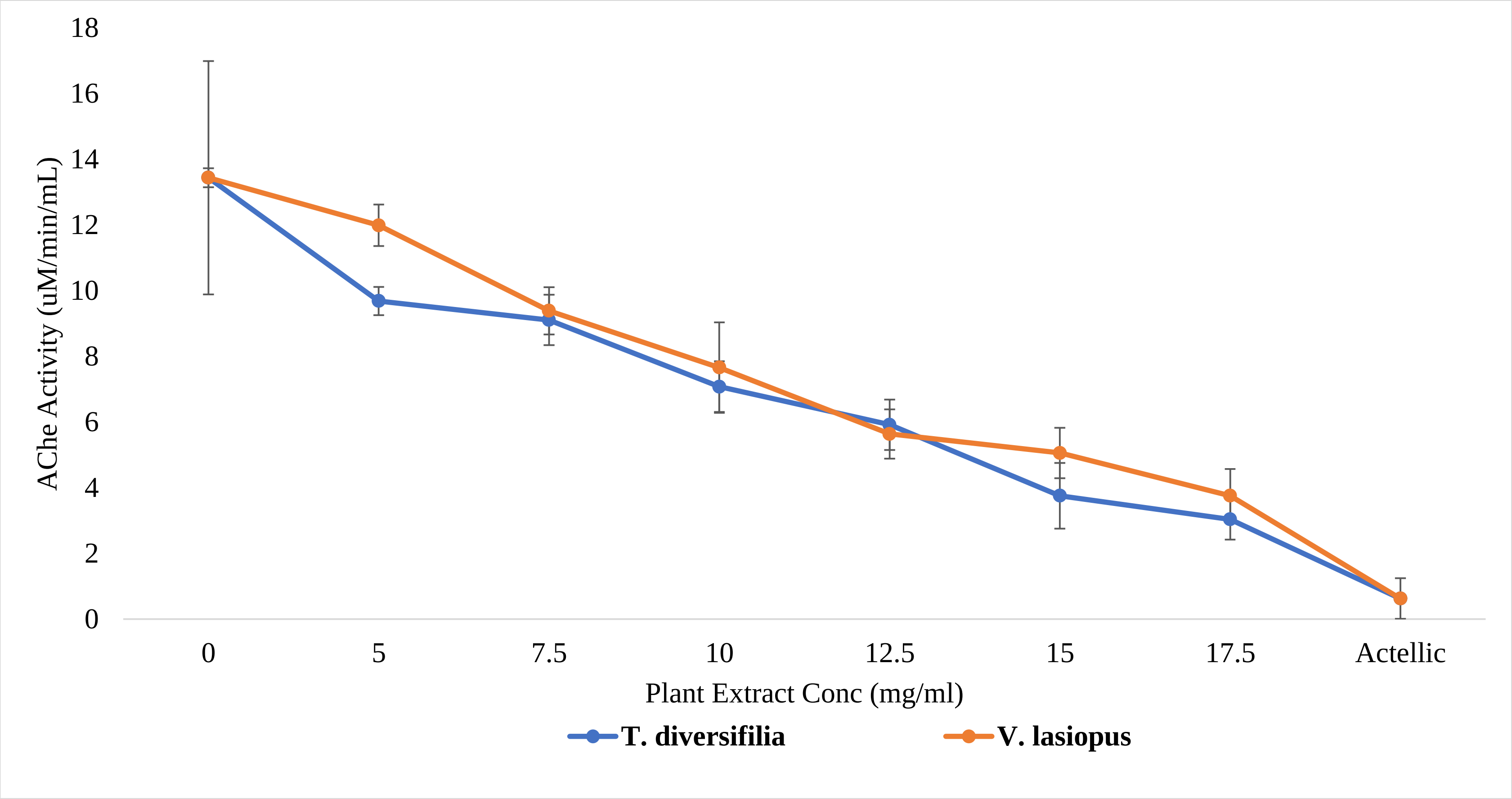
Group	Treatment	Grains Sampled	Damaged Grains	Undamaged Grains	% Damage Caused (PD)	WPI
I (Negative Control)	Solvent Only	50.000±0.00	23.75±1.110	26.250±1.110	47.50±2.220[a]	49.92±1.18[a]
II(Positive Control)	Actellic	50.000±0.00	0.500±0.289	49.500±0.289	01.00±0.577[c]	02.02±1.17[b]
II (Experimental Group A)	25% Extract	50.000±0.00	4.000±1.080	46.000±1.080	08.00±2.160[b]	14.04±3.21[b]
III (Experimental Group B)	50% Extract	50.000±0.00	3.250±0.750	46.750±0.750	06.50±1.500[bc]	11.83±2.43[bc]
IV (Experimental Group C)	75% Extract	50.000±0.00	1.750±0.479	48.250±0.479	03.50±0.957[bc]	06.77±1.72[bcd]
V (Experimental Group D)	100% Extract	50.000±0.00	1.000±0.408	49.000±0.408	02.00±0.816[bc]	03.96±1.59[cd]

Appendix 7.4: Grain Damage Deterrence Effects of the EtOAc Leaf Extract of *V. lasiopus* against *S. zeamais*

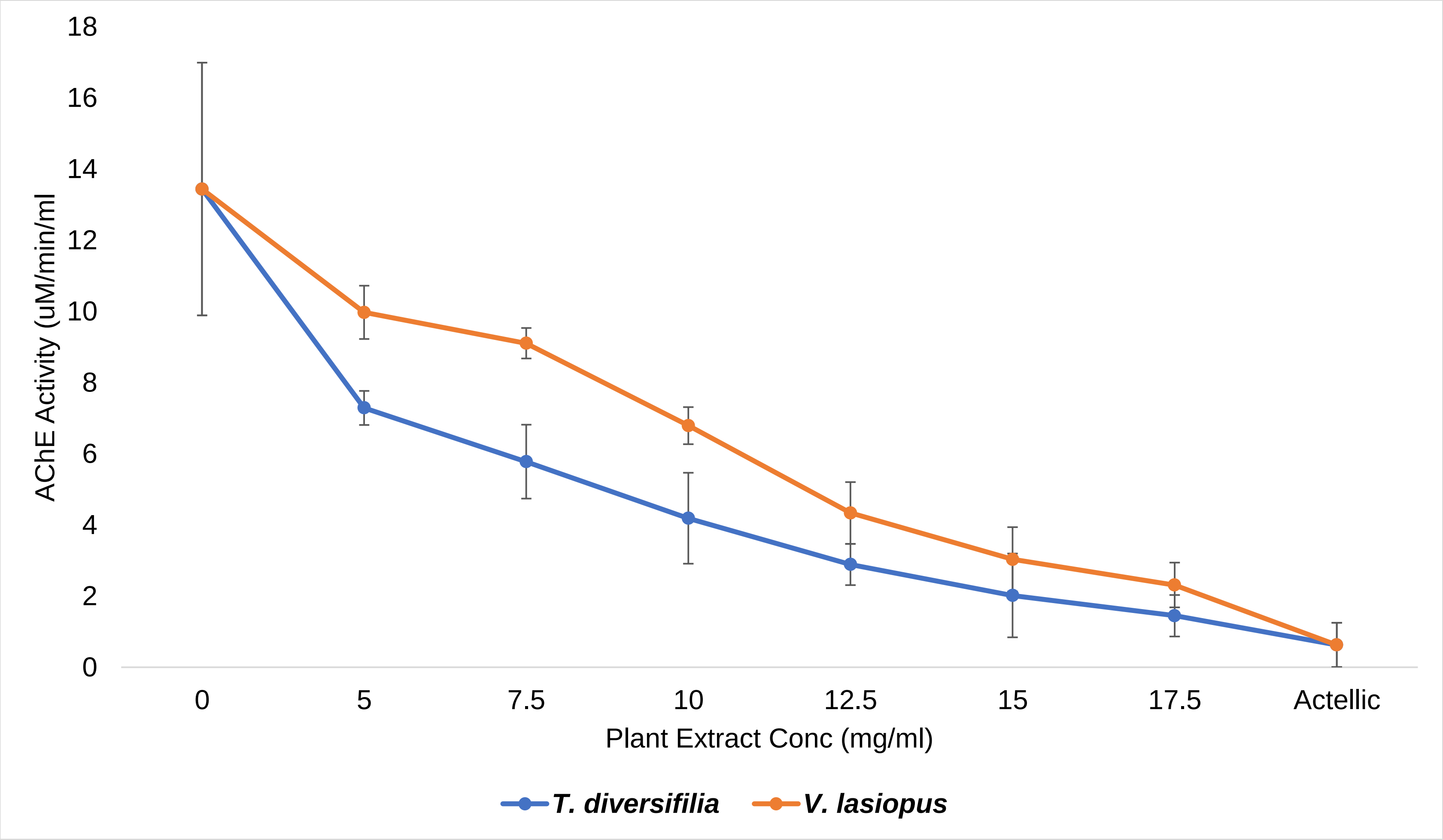
Group	Treatment	Grains Sampled	Damaged Grains	Undamaged Grains	% Damage Caused (PD)	WPI
I (Negative Control)	Solvent Only	50.000±0.00	23.75±1.110	26.250±1.110	47.50±2.220^{a}	49.92±1.18^{a}
II(Positive Control)	Actellic	50.000±0.00	0.500±0.289	49.500±0.289	1.000±0.577^{c}	02.02±1.17^{e}
II (Experimental Group A)	25% Extract	50.000±0.00	5.500±0.645	44.500±0.645	11.00±1.290^{b}	18.68±1.80^{b}
III (Experimental Group B)	50% Extract	50.000±0.00	3.250±0.629	46.750±0.629	06.50±1.260^{bc}	11.90±2.00^{bc}
IV (Experimental Group C)	75% Extract	50.000±0.00	2.750±0.250	47.250±0.250	05.50±0.500^{bc}	10.36±0.87^{cd}
V (Experimental Group D)	100% Extract	50.000±0.00	1.250±0.479	48.750±0.479	02.50±0.957^{c}	04.89±1.85^{de}



Appendix 8.1: **Principle of Allman's Acetylcholinesterase Assay (Mohammed *et al.,* 2014)**

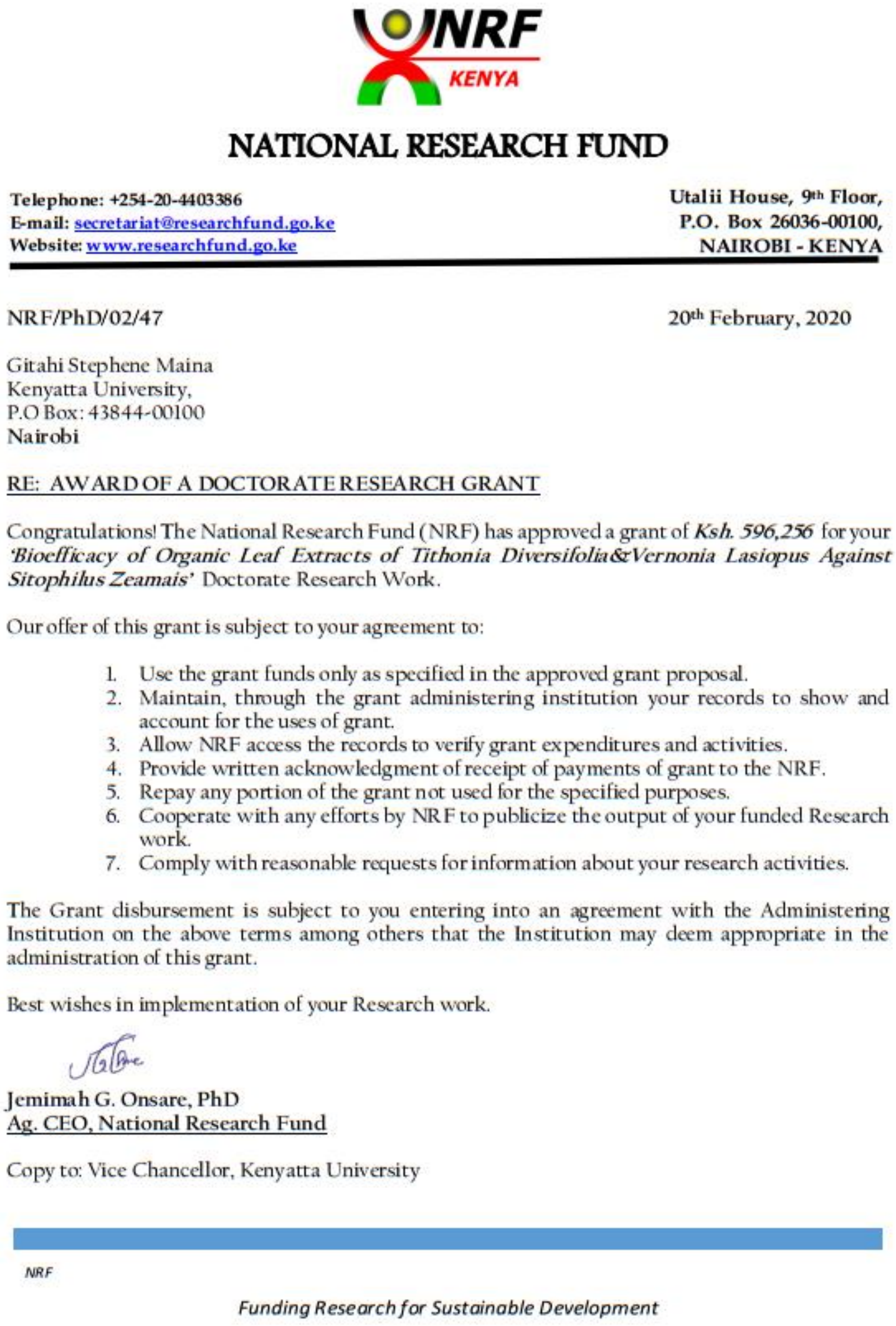


Appendix 8.2: ***Ex Vivo*** **Anti-Acetylcholinesterase Activity of DCM Leaf Extracts of** ***T. diversifolia*** **and** ***V. lasiopus***



Appendix 8.3: ***Ex vivo*** **Anti-Acetylcholinesterase Activity of Ethyl Acetate Leaf Extracts of *T. diversifolia* and *V. lasiopus***

Appendix 8.4: Award of a Doctorate Research Grant



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NRF/PhD/02/47

20th February, 2020

Gitahi Stephene Maina
Kenyatta University,
P.O Box: 43844-00100
Nairobi

RE: AWARD OF A DOCTORATE RESEARCH GRANT

Congratulations! The National Research Fund (NRF) has approved a grant of ***Ksh. 596,256*** for your ***'Bioefficacy of Organic Leaf Extracts of Tithonia Diversifolia&Vernonia Lasiopus Against Sitophilus Zeamais'*** Doctorate Research Work.

Our offer of this grant is subject to your agreement to:

1. Use the grant funds only as specified in the approved grant proposal.
2. Maintain, through the grant administering institution your records to show and account for the uses of grant.
3. Allow NRF access the records to verify grant expenditures and activities.
4. Provide written acknowledgment of receipt of payments of grant to the NRF.
5. Repay any portion of the grant not used for the specified purposes.
6. Cooperate with any efforts by NRF to publicize the output of your funded Research work.
7. Comply with reasonable requests for information about your research activities.

The Grant disbursement is subject to you entering into an agreement with the Administering Institution on the above terms among others that the Institution may deem appropriate in the administration of this grant.

Best wishes in implementation of your Research work.

Jemimah G. Onsare, PhD
Ag. CEO, National Research Fund

Copy to: Vice Chancellor, Kenyatta University

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