AN ASSESSMENT OF THE EFFECTS OF SOME PLANT OILS ON *DERMESTES MACULATUS* (DEGEER) LARVAE IN SMOKE-DRIED *PROTOPTERUS ANNECTENS* (OWEN)

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DECEMBER, 2015.

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BY

Folasade Damilola OGUNDUYILE, B.Tech Fisheries (FUTA) 2010
M.Sc/SCI/12409/2011-2012

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES AHMADU BELLO UNIVERSITY ZARIA IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR THE AWARD OF A MASTER OF SCIENCE DEGREE (M.Sc) IN FISHERIES

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DECEMBER, 2015.

DECLARATION

I declare that the work in this thesis titled "**AN ASSESSMENT OF THE EFFECTS OF SOME PLANT OILS ON *DERMESTES MACULATUS* (DEGEER) LARVAE IN SMOKE-DRIED *PROTOPTERUS ANNECTENS*(OWEN)**" was performed by me in the Department of Biological Sciences under the supervision of Prof. T.O.L. Aken'Ova and Prof. S.J. Oniye. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has previously been presented for the award of degree or diploma at this or any other institution.

Folasade Damilola OGUNDUYILE _____ _____

Signature Date

CERTIFICATION

This thesis titled "**AN ASSESSMENT OF THE EFFECTS OF SOME PLANT OILS ON *DERMESTES MACULATUS* (DEGEER) LARVAE IN SMOKE-DRIED *PROTOPTERUS ANNECTENS*(OWEN)**" by Folasade Damilola OGUNDUYILE meets the regulations governing the award of a Master of Science degree (M.Sc) in fisheries of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to my husband, Lt. J.D. Amulejoye and children, Oluwafunmilayo and Oluwaseyifunmi

ACKNOWLEDGEMENTS

My appreciation goes the Almighty God for sustaining me through the course of this study.

My utmost gratitude goes to my supervisors Professor T.O.L Aken'Ova and Professor S.J. Oniye for their assistance, guidance and support. I am also grateful to Mr David Adebote for his contribution and support.

To MrsM.O. Hassan-Adeyemi, Messers M. Gero and S.O Aribido of the National Research Institute of Chemical Technology, Zaria, I am grateful for your support and contributions in making this thesis a success. May the Almighty God bless you.

To my family and friends that contributed immensely during the course of this study, I am grateful.

ABSTRACT

The toxicity and repellency of *Dermestes maculatus* larvae by the oils of *Allium sativum*(Garlic), *Cocos nucifera* (Coconut), *Zingiber officinale* (Ginger), *Jatropha curcas* (Jatropha) and *Azadirachta indica* (Neem) were evaluated in the laboratory. Late instar larvae of *D. maculatus* were introduced into Kilner jars containing fish treated with these oils, whose physicochemical properties and fatty acid composition had been previously determined. Jars with untreated fishserved as control. Results obtained showed that the oils caused significant mortality ($P<0.05$) in *D. maculatus*larvae on treated fish compared to the untreated fish at 96h after infestation. 100% mortality was recorded for all the oils at the highest concentration (0.256ml/g of fish) by the 96^{th} hour of exposure. Repellency was highest in *A. sativum* oil (87%) and lowest in *A. indica* oil (59%) at the highest concentration (0.256ml/g of fish) within 24 hours of exposure. The physicochemical properties of the oils showed that acid values of the oils were below 10 mg/KOHg except in *A. indica* which had 12.75mg/KOHg (potassium hydroxide). Saponification values ranged from 190.74 (*A. sativum*) to 251.05 (*C. nucifera*). *Zingiber officinale*oil had the highest peroxide value 2.96mg/100g while *J.curcas* oil had the lowest (0.65mg/100g). Refractive index values ranged from 1.24 (*J. curcas*) to 1.49 (*A. sativum*). The fatty acids detected in the oils were: oleic (in *J. curcas* and *A. indica*); octadecanoic (in *Z. officinale* and *C. nucifera*);stearic and palmitic acids (in *A. indica* and *C. nucifera*);lauric, linoleic and myristic acids (in *C. nucifera*); octadecanoic acid (in *J. curcas*) and cyclopropanepentanoic acid (in *Z. officinale*). The occurrence of these fatty acids might be responsible for the insecticidal properties exhibited by the oils. The larvicidal and repellent properties of the oils in offering effective protection against infestation and damage by *D. maculatus* have been clearly demonstrated in this study; the oils are therefore recommended for utilization in post-harvest preservation of smoke-dried fish.

TABLE OF CONTENTS

Title		Page
Title Page	- - - - - - - - -	**-i**
Declaration	- - - - - - - - -	**-ii**
Certification	- - - - - - - - -	**-iii**
Dedication	- - - - - - - - -	**-iv**
Acknowledgments	- - - - - - - - -	**-v**
Abstract	- - - - - - - - -	**-vi**
Table of Contents	- - - - - - - - -	**-vii**
List of Tables	- - - - - - - - -	**-xi**
List of Figures	- - - - - - - - -	**-xii**
List of Plates	- - - - - - - - -	**-xiii**
CHAPTER ONE		
1.0 INTRODUCTION	- - - - - - - -	**-1**
1.1 Background of Study	- - - - - - -	**-1**
1.2 Statement of Research Problem	- - - - - -	**-4**
1.3 Justification	- - - - - - - - -	**-5**
1.4 Aim of the Study	- - - - - - - -	**-6**
1.5 Objectives of the Study	- - - - - - -	**-7**
1.6 Hypotheses	- - - - - - - - -	**-7**
CHAPTER TWO		
2.0 LITERATURE REVIEW	- - - - - - -	**-8**
2.1 *Protopterus annectens* (Owen)	- - - - -	**-8**

Title **Page**

2.1.1 Biology and distribution - - - - - - -8

2.2 *Dermestes maculatus* (Degeer 1774)- - - - - -10

2.2.1 Biology - - - - - - - - -10

2.2.2 Distribution - - - - - - - - -10

2.2.3 Description - - - - - - - - -10

2.2.4 Life-cycle - - - - - - - - -11

2.3 Economic Importance - - - - - - -12

2.4 General Control of *Dermestes maculatus* - - - - -12

2.5 Use of Plant Extracts for control of (storage) insect pests - -15

2.5.1 *Allium sativum* (Garlic) - - - - - - -15

2.5.2 *Jatropha curcas*(Jatropha) - - - - - - -16

2.5.3 *Zingiber officinale* (Ginger) - - - - - - -18

2.5.4 *Azadirachta indica*(Neem) - - - - - - -20

2.5.5 *Cocos nucifera* (Coconut) - - - - - - -21

CHAPTER THREE

3.0 MATERIALS AND METHODS - - - - - -23

3.1 Sources of Plant Materials - - - - - - -23

3.1.1 Processing of plant materials - - - - - - -23

3.1.2 Soxhlet extraction of oils - - - - - - -23

3.2 Physico-Chemical Properties of the Oils - - - - -24

3.2.1 Determination of specific gravity - - - - - -24

3.2.2 Determination of refractive index - - - - - -31

3.2.3 Determination of saponification value - - - - -31

3.2.4 Determination of peroxide value - - - - - -33

Title **Page**

3.2.5 Determination of iodine value - - - - - -33

3.2.6 Determination of acid value - - - - - - -34

3.3 Insect Maintenance and Culture - - - - - -35

3.4 Collection of Smoke-Dried Fish - - - - - -35

3.5 Pilot Study - - - - - - - - - -35

3.6 Bioassay - - - - - - - - - -38

3.6.1 Mortality test - - - - - - - - -38

3.6.2 Repellency test- - - - - - - - -38

3.7 Gas Chromatography/Mass Spectrometry Analysis - - -39

3.8 Data Analysis- - - - - - - - - -39

CHAPTER FOUR

4.0 RESULTS - - - - - - - - - -41

4.1 Physicochemical Properties and Fatty Acid Composition - - -41

4.1.1 Physicochemical properties of oils - - - - - -41

4.1.2 Fatty acid composition of the oils - - - - - -41

4.1.3 Metabolites detected in the plant oils - - - - - -41

4.2 Larval Mortality of *Dermestes maculatus*Larvae caused by the Oils -45

4.2.1 Larval mortality at 0.001ml/g Oil Concentration - - - -45

4.2.2 Larval mortality at 0.004ml/gOil Concentration - - - -45

4.2.3 Larval mortality at 0.016ml/gOil Concentration - - - -48

4.2.4 Larval mortality at 0.064ml/gOil Concentration - - - -48

4.2.5 Larval mortality at 0.256ml/gOil Concentration - - - -48

4.3 Median Lethal Concentration (LC_{50}) of the Plant Oils - - -52

4.4 Repellency of the Oils - - - - - - -52

Title **Page**

CHAPTER FIVE

5.0 DISCUSSION - - - - - - - -59

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS - - - -67

6.1 Conclusions - - - - - - - - -67

6.2 Recommendations - - - - - - - -67

REFERENCES - - - - - - - - -69

APPENDIX - - - - - - - - - -80

LIST OF TABLES

Table **Page**

4.1: Physicochemical properties of the oils of *Allium sativum, Cocos nucifera, Jatropha curcas, Zingiber officinale* and *Azadirachta indica* - - - - - - -42

4.2: Fatty acid composition (%) of the plant oils of *Allium sativum, Cocos nucifera, Jatropha curcas, Zingiber officinale* and *Azadirachta indica* - - - - - - -43

4.3: Metabolites (%) detected in the plant oils of *Allium sativum, Cocos nucifera, Jatropha curcas, Zingiber officinale* and *Azadirachta indica* - - - - - - - 44

4.5: Repulsion of*D. maculatus* larvae by the Oils of *Azadirachta indica,Jatropha curcas, Zingiber officinale, Allium sativum, Cocos nucifera*- - - - - - - - 58

LIST OF FIGURES

Figure		Page
4.1:	Mortality of *Dermestes maculatus* larvae exposed to 0.001ml/g test plant oils - - - - -	-46
4.2:	Mortality of *Dermestes maculatus* larvae exposed to 0.004ml/g test plant oils - - - - -	-47
4.3:	Mortality of *Dermestes maculatus* larvae exposed to 0.016ml/g test plant oils - - - - -	-49
4.4:	Mortality of *Dermestes maculatus* larvae exposed to 0.064ml/g test plant oils - - - - -	-50
4.5:	Mortality of *Dermestes maculatus* larvae exposed to 0.256ml/g test plant oils - - - - -	-51
4.6:	Probit graph for determining the LC_{50} of *Azadirachta indica* oil against the larvae of *Dermestes maculatus* - - -	-53
4.7:	Probit graph for determining the LC_{50} of *Jatropha curcas* oil against the larvae of *Dermestes maculatus* - - -	-54
4.8:	Probit graph for determining the LC_{50} of *Zingiber officinale* oil against the larvae of *Dermestes maculatus* - - -	-55
4.9:	Probit graph for determining the LC_{50} of *Allium sativum* oil against the larvae of *Dermestes maculatus* - - -	-56
4.10:	Probit graph for determining the LC_{50} of *Cocos nucifera* oil against the larvae of *Dermestes maculatus* - - -	-57

LIST OF PLATES

Plate		Page
I:	*Protopterus annectens*(Owen) - - - - -	- 9
II:	Soxhlet Apparatus - - - - - -	- 25
III:	*Allium sativum*. (a) Whole Bulbs (b) Peeled Cloves - -	- 26
IV:	*Jatropha curcas.*(a) Leaves (b) Fruits (c) Dried seeds- -	-27
V:	*Zingiber officinale*. (a) Whole Rhizomes (b) Peeled and Sliced Rhizomes - - - - - -	-28
VI:	*Azadirachta indica*. (a) Leaves(b) Fruits (c) Dried Seeds -	- 29
VII:	(a) Whole *Cocos nucifera* (Coconut) and (b) Cracked Coconut Fruit - - - - - - - -	-30
VIII:	Refractometer - - - - - - -	-32
IX:	Smoke-Dried *Protopterus annectens* - - - -	-36
X:	*Dermestes maculatus.*(a) Larva(b) Adult - - -	-37
XI:	Gas Chromatography/MassSpectrometry System - -	-40

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Fish is one of themajoranimal protein sources and it is being used increasingly because of its availability and nutritional value; it is also used to correct protein deficiency in human diets in the tropics. With its high protein content, it serves as a natural supplement for cerealsand red meat staples in the human diet. Consumption of fish provides important nutrients to a large number of people worldwide and thus makes a very significant contribution to nutrition (Fasakin and Aberejo, 2002; Azam *et al.,* 2004).A decline in fish availability will have a detrimental effect on the nutritional status in places where fish contributes significantly to the protein intake of the people (Omojowo *et al*., 2009a).

According to Olaoye and Oloruntoba (2011), Nigerians consume a lot of fish and offer the largest market for fish and fisheries products in Africa.Fish demand in Nigeria is put at about 1.2 million metric tons per annum, and the total domestic fish production is only 511,700 metric tons (Nwankwo, 2005).The high demand for fishery products has arisen from the awareness of its significance in the local diet and its low-pricecompared to its substitutes (Bowen and Jones,2005). Since aquatic resources are finite, although renewable, every effort should be geared towards increased fish production through improved resource management, resource conservation and intensive aquaculture practices. This should be matched with post-harvest fish handling, preservation and processing to prevent spoilage and subsequent loss (Akinneye *et al.*, 2007).

Capture fisheries and aquaculture supplied the world with about 148 million tonnes of fish in 2010 (with a total value of US$217.5 billion), of which about 128 million tonnes was utilized as food for people. With sustained growth in fish production and improved distribution channels, world fish food supply has grown dramatically in the last five decades, with an average growth rate of 3.2 percent per year in the period 1961–2009, outpacing the increase of 1.7 percent per year in the world's population (FAOUN, 2012). World per capita food fish supply increased from an average of 9.9 kg (live weight equivalent) in the 1960s to 18.4 kg in 2009, and preliminary estimates for 2010 point to a further increase in fish consumption to 18.6 kg (FAOUN, 2012).

Fish is easily perishable in tropical climates and needs to be preserved quickly after capture (Omojowo *et al.,* 2009b). Drying is one of the most popular means of preservationand dried fish is highly favoured in many traditional Nigerian dishes. Most of the dried fish available to fish consumers in the tropics is smoke-dried, salted, fermented and sun dried. Dried fish is often a good alternative to fresh fish, and it is readily available in many inland communities because of improved transportation andpreservation methods. However, drying of fish does not guarantee the end to the problems of preservation, storage and distribution. Dried fish stored in damp warehouses absorb moisture so rapidly that theybecome susceptibleto infestation by beetles, fungi and mites.Besides, in most cases fish are not dried properly resulting in pest infestation (Mohammed and Yussuf, 2001).Insects invade dried fish during storage and feed on it extensively causing up to 50% loss in weight (FAO, 1981).Dried fish can suffer considerable loss of weight due to damage caused by insects and mites (Eke *et*

al., 2008). These losses in quantity and quality often lead to lowering orreduction of commercial value.

Pests commonly found on dried fish are beetles, flies and mites (FAO, 1989). In Nigeria, fly genera such as*Calliphora*, *Chrysomia*, *Lucillia* and *Musca*and the beetles, *Dermestesmaculatus*Degeer and *Necrobia rufipes*Degeer have been reported as pests of dried fish (Osuji, 1985). *Dermestes maculatus* is widely recognized as a cosmopolitan pest of stored commodities especially those containing animal protein. The hide beetle feeds on hides, skins, feathers and horns, and it is a known pest of dried fish. Losses in quality and quantity of dried fish during storage have been attributed to *D. maculatus* infestation (Fasakin and Aberejo, 2002).

*Dermestes maculatus*accounts for about 71.5% of dried fish infestation in most of the producing areas with a substantial loss in dry weight of about 43-62.7% from both larvae and adults (Osuji,1974).Lale and Sastawa (1996) and Odeyemi *et al.* (2000) put the losses due to susceptibility to pest infestation and deterioration of smoked fish products at 50% during storage, leading to reduction of nutritive quality and market price of smoked fish.The extent and value of quantitative losses caused to dried fish by *Dermestes*sp.have been assessed by various investigators and estimates range from negligible to 50% weight loss, depending on length of storage, salt content, moisture content, climatic conditions and general hygiene duringprocessing and storage(Fasunwon *et al.,* 2011)

To control insect infestation,fish merchants spray insecticides and chemicals which are hazardous to human health.Chemical insecticides such as malathion,

pirimiphos methyl used traditionally in pest control cause pest resistance, environmental and food contamination and toxicity to non-target organisms (Kamrin, 1997).Khan and Khan (2001) reported that fish curers apply different types of insecticides such as dichlorvos, malathion, gamaxine, endrine and DDT on dried fish to protect them from infestation, thus contravening the recommendations of Codex Alimentaris or FAO/ WHO Joint Meeting Pesticide Residue Committee (JMPRC). One promising area to control pest infestation is the use of plant-derived pest control agents. According to Akinwumi (2011), there are some active ingredients in plant materials which protect fish from beetles and reported 100% toxicity of different plant powders on the adult and larvae of *D. maculatus.* Several natural plant products, including oils derived from plant materials, have been tested as protectants of dried fish from insect infestation. The fruit oil of *Piper guineese* Schum `and Thonn (Uziza) wasfound to be effective as protectant of dried fish against larval and adult stages of *D.maculatus* (Amusan and Okorie, 2002).

Protopterus annectens commonly known as the African lungfish is the only species of the primitive family Lepidosirenidae found in West African fresh waters (Reed *et al.*,1967). It is a highly prized food fish in Nigeria (Otuogbai, 2001). It is distributed in shallow parts of rivers and lakes of some West African countries whereit contributes to a relatively high percentage of artisanal fisheries (Okafor, 2004).

1.2 Statement of Research Problem

Dermestes maculatus is a major pest of stored fish in Nigeria accounting for 50% weight loss in dried fish (FAO, 1981).Large scale deterioration in quality

and quantity of dried fish is attributed to dermestid infestation.Dermestid larvae and adults, unlike many other beetles, are less susceptible to chemical insecticides that normally attack pests of stored products and the use of such insecticides renders smoked fish unattractive to fish consumers(Amusan and Okorie, 2002). The pest proliferates and grows on the dried fish, thus changing its appearance making it unfit for marketing. In addition to this, insect pests of fish often transmit *Escherichia coli* and mold spores; heat and moisture produced by heavy infestation can create conditions suitable for fungi growth on dried fish (Jackson and Ayub, 2013). The limitations of highly toxic and persistent chemicals used as fish protectants include: health hazard, cost of purchase, development of highly resistant strains, and environmental contamination among others.Adult *Dermestes* fly and are easily dispersed to new sources of food where they cause damage. Infestation of dried fish by insects and mites may have public health implications as transmitters of disease-causing pathogens to humans, which may cause intestinal disorders and allergies (Busvine, 1980; Osuji, 1985, Gram *et al*., 2000).

1.3 Justification

The need to protect dried fish from pests is imperative because of the crucial role fish plays in ensuring food security, income generation and employment opportunities. The indiscriminate use of synthetic insecticide and chemicals to control these insect pests result in resistance, food and food product contamination with toxic residues, increased cost of application, environmental contamination with toxic residues and handling hazards. The increased public awareness and concern for environmental safety, increased regulatory constraints and ventilation restrictions in storage are the negative effects of

thesynthetic chemicals (Oppert *et al.,* 2010). Thus, the search for eco-friendly, cost-effective, easily available insect pest management options is intensified worldwide to reduce the use of synthetic chemicals in pest management. Among the benign alternative insect pest management options is the use of plant-based products to curtail the menace caused by insect pests in storage. Broadly speaking, plant-based products are effective and benign tools in insect pest management with broad spectrum action (Muhammad, 2009).Plant-derived materials are more readily degradable, less toxic to mammals, retard the development of resistance, easy and cheap to produce by farmers and small scale industries as crude, or partially purified extracts. Plantsand plant products were said to be useful and desirable tools in pestmanagement programmes because they are effective and complement for the actions of natural enemies (Schmutterer, 1990).The potential pesticideactivities of *Azadirachta indica*A. Juss, *Anchomanes difformis*Blume, *Aframomum meleguata*Schum, *Zingiber officinale*Roscoe, *Jatrophacurcas*Linnaeus and *Annona muricata*(Linnaeus) products have been reported (Iloba and Ekrakene, 2006).

1.4 Aim of the Study

The aim of this study is to evaluate the adverse effects of the oils of *Allium sativum* (Garlic),*Azadirachta indica* (Neem), *Cocos nucifera* (Coconut), *Jatropha curcas* (Jatropha) and*Zingiber officinale* (Ginger) and on the larvae of *D. maculatus*.

1.5 Objectives of the Study

The objectives of this study are;

i. To determine the physicochemical parameters of the oils of *A. sativum* (Garlic),*A. indica* (Neem),*C. nucifera* (Coconut), *J. curcas* (Jatropha) and*Z. officinale* (Ginger).

ii. To determine the toxicity and repellent effects of the oils of*A. sativum* (Garlic),*A. indica* (Neem),*C. nucifera* (Coconut), *J. curcas* (Jatropha) and*Z. officinale* (Ginger) on *D. maculatus*larvae.

iii. To compare the performance of the oils of *A. sativum* (Garlic), *A. indica* (Neem),*C. nucifera* (Coconut), *J. curcas* (Jatropha) and*Z. officinale* (Ginger)on *D. maculatus* larvae.

1.6 Hypotheses

i. There are no differences in the physicochemical parameters of the oils of *A. sativum* (Garlic),*A. indica* (Neem),*C. nucifera* (Coconut), *J. curcas* (Jatropha) and*Z. officinale* (Ginger).

ii. The oils of *A. sativum* (Garlic),*A. indica* (Neem),*C. nucifera* (Coconut), *J. curcas* (Jatropha) and*Z. officinale* (Ginger)have no larvicidal and repellent effects on the larvae of *D. maculatus*.

iii. There are no significant differences in the performance of the oils of *A. sativum* (Garlic),*A. indica* (Neem),*C. nucifera* (Coconut), *J. curcas* (Jatropha) and*Z. officinale* (Ginger)against the larvae of *D. maculatus*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Protopterus annectens*(Owen)

Protopterus annectens has a prominent snout and small eyes; its body is long and eel-like (Plate I). It has two pairs of long, filamentous fins. The pectoral fins have a basal fringe and are about three times the head length, while its pelvic fins are about twice the head length. In general, three external gills are inserted posterior to the gill slits and above the pectoral fins.It has cycloid scales embedded in the skin, of which 40-50 occur between the operculum and the anus, and 36-40 around the body, before the origin of the dorsal fin. The dorsal side is olive or brown in color and the ventral side is lighter with blackish or brownish spots on the body and fins except on its belly.*P. annectens* belongs to the ClassSarcopterygii, OrderLepidosireniformes and FamilyProtopteridae(Kees *et al.,* 2002; Froese and Pauly,2013).

2.1.1 Biology and distribution

*Protopterus annectens*lives in marginal swamps and backwaters of rivers and lakes. It is strongly associated with life of aquatic plants in terms of breeding and feeding ecologies and its nests are made in weedy areas (Kees *et al.,* 2002). The lungfish has two modes of life; the active state that occurs in the water during the rainy season and the dormant state that occurs under the ground during the dry season when it aestivates in the mud. During aestivation, the fish secretes from its skin a slimy fluid which soon congeals to form a water-proof covering called cocoon that wraps properly the entire body of the fish throughout the period (Giuisi *et al.,* 2011). To hibernate, the fish literally chews



PLATE I: *Protopterus annectens*(Owen)

Source: Froese and Pauly (2013)

its wayinto the substrate ejecting mud from its gill openings; it may reach a depth of 3-25 cm below the bottom depending on the length of the fish.Lungfish are periodically exposed to water with low oxygen content or situations in which their aquatic environment dries up.Their adaptation for dealing with these conditions is an outpocketing of the gut, related to the swim bladder of fishes that serves as lungs (Bruton *et al.*,1998).

2.2 *Dermestes maculatus*(DeGeer 1774)

2.2.1 Biology

The hide beetle, *Dermestes maculatus*is a coleopteran of the family Dermestidae that feeds on carrion and dry animal products. These beetles form aggregations around resources where individuals feed and mate; the femalesare attracted by pheromones secreted by males. Aggregations can vary in size, but small sources of food usually have approximately one to 13 beetles (McNamara*et al.*,2008). The adult beetles have forensic significance in helping to estimate the post mortem interval in suicide, or homicide unattended death cases (Richardson and Goff, 2001).

2.2.2 Distribution

Dermestes maculatus is a species of beetle with a worldwide distribution being present in all continents except Antartica (Kulshrestha and Satpathy, 2001).

2.2.3 Description

The eggs of *Dermestes maculatus* are typically laid in batchesof three to 20. The amount of eggs a single female canlay over a lifetime varies greatly, ranging from 198 to 845(Veer *et al*., 1996).The bodies of the larvae are covered in rows ofhairs of different lengths, called setae. The underside ofthe abdomen is

typically yellowish-brown while the dorsalsurface is typically dark brown, usually with a centralyellow line (Haines and Rees, 1989). The last larval skin will usually provide a protective covering for the pupa. The end of the pupal chamber toward the surface can be closed by either debris from the skin (Veer *et al*., 1996). The pupae are an oval shape, usuallysmaller than the larvae, and do not have the many longhair-like projections (Kulshrestha and Satpathy, 2001).*Dermestes maculatus* adults range in size from 5.5to 10.0 mm. Each side of the thorax has a band of whitehairs. The underside of the abdomen is primarily white with black spots at the sides, and a large black patch on thelast segment. The antennae areshort and segmented with clubbed tips. The edges of theabdominal end of the elytra are serrated and end in a small spine projecting straight out (Nath and Pande, 1998).

2.2.4 **Life-cycle**

The life-cycle of *D. maculatus* in dry stored animal products requires approximately five to seven weeks under optimum conditions.Pheromones secreted by males through a gland onthe base of the abdomen, are used to attract females. Males and females will mate multiple times and the female willlay eggs within 24 hours of the first mating (Jones *et al.*, 2006). Eggs are laid in cracks of the matter on which theyare feeding. Females are capable oflaying eggs continuously. Larvae will pass through five to 11 instars, the number of instars increasing with unfavorable conditions. During the last 10 days ofthe final instar, the larvae will seek out a place to pupate, typically within animal product or a non-food substance such aswood. Exposed pupae that have failed to find a suitable pupal chamber are often cannibalized by larvae. Larvae without a suitable place to pupate can delay pupation byover 20 days (Archer and Elgar,

1998).Survivorship for individuals is highest between 25°Cand30°C (Richardson and Goff, 2001). Once adults, the beetles can disperse to other food sourcesby flying. Adult beetles typicallylive between four and six months.

2.3 Economic Importance

Veer *et al.* (1996) reported that *Dermestes maculatus* was a vector for pebrine disease which caused the closure of the silkworm industry in many countries in 1931 and that in 1987, 20% loss in silk production was recorded in India as a result of the disease. The primary cause of loss was due to the feeding of *D. maculatus* larvae, boring into the cocoons to eat the silkworm pupae, and thus the cocoons could not be used for reeling. Other commodities that *D. maculatus* can damage include stored animal products such as dried fish, cheese, hide, fur, bacon, and dog treats. The larvae can cause considerable damage to timber, cork, plaster, linen, and cotton into which they bore to pupate (Cloud and Collison, 1986).

2.4 General Control of *Dermestes maculatus*

The important control measures may be physical, chemical or biological. The physical method involves the use of a physical barrier, such as a screen or a sealed box, to prevent the insects from gaining access to the stored item. Chemical methods involve the use of insecticides or fumigants to deter or destroy the insects.Biological method involves the use of other organisms against the insect.These methods have not met with sufficient success, as the causative factors of pest infestation have not been tackled. One solution to these problems might be to replace synthetic chemicals with compounds, which occur naturally in plants. Studies by Walker and Wood (1986) and Okorie *et al.*

(1990)foundthat plant extracts show much promise as protectants of dried fish, as well as the use of salt (Osuji, 1975), proper hygiene practices, smoking, solar driers, packaging and irradiation (Proctor, 1977).

Plant extracts have been reported as being toxic to several insect pests. Abdullahi *et al.* (2010) recorded 100% mortality in*Dermestes maculatus* exposed to nutmeg powder (*Monodira myristica*) within 72 hours.Okorie *et al.* (1990) reported a 93% mortality in*D. maculatus* larvae and total mortality in adults exposed to 2g of neem seed powder per 25g of dried *Tilapia*. It was observed that the powder affected the oviposition and hatchability of eggs of *Dermestes maculatus* and also killed the adults. Incubation period was prolonged and the larvae did not develop beyond the 2nd instar. Ofuya and Dawodu (2001) reported the susceptibility of different stages of*Callosobruchus maculatus*Fabriciusto*Piper guineense*(pepper) powder. Fasakin (2003) reported that crude extract of *Monodora myristica*Dunal (nutmeg) at different concentrationswaseffectiveagainst *Dermestes maculatus*. Okonkwo and Okoye (2011) reported 100% mortality of larvae of *D. maculatus* when treated with the extracts of *Dennettia tripetala*(pepper fruit) and *Piper guineense*. The oil of *Detarium microcarpum*Guill and Perr (Tallow tree) caused high mortality and repellency to the larvae of *Dermestes maculatus* and even at a low concentration protected dried fish against dermestid attack (Adebote *et al*., 2006).

The efficacies of the powders of four plant materials, *Dennettia tripetala* (Baker), *Eugenia aromatic* (Hook),*Monodoramyristica*(Dunal) and *Piper guineense*(Schum and Thonn) were evaluated at different concentrations on smoked *Clarias gariepinus* for the control of*Dermestes maculatus*. Each of the

four plant powders caused significantly high mortality in both the adults and larvae at all concentrations when compared to the control, and was effective in inhibiting progeny development in treated fish (Akinwumi, 2011). Amusan and Okorie (2002) reported that *Piper guineense* fruit oil at dosages of 0.125ml/25g fish and 0.150 ml/25g were effective in the control of larval and adult stages of *D.maculatus* in dried fish. Owoade(2008) also exposed *Dermestes maculatus*larvae (24 – 48 hours old) to three different concentrations of *Piper guineense, Aframomum melequeta, Zingiber officinale* and *Capsicum annuum,* for over 25 days. Mortalities monitored for five days showed that 100% of the beetles exposed to*P. guineense* diedat the end of 24 hours, and by the end of 72 hours, 100% mortality were recorded in all three concentrations in *P. guineense* and*A. melegueta*gave the second best result (53.3%) at 25g/kg.The spices at the end of the experiment were shown to have slowed down or extended time of development of the larvae to the adult, especially at the two concentrations of 20g/kg and 25g/kg.

Akpotu and Adebote (2013) reported significant repellent effect of theoils extracted from five plants:*Albizia lebbeck, Anacardium occidentale, Citrullus colocynthis, Citrullus vulgaris* and *Khaya senegalensis* against the larvae of *D. maculatus* in smoke-dried fish. They observed 100% repellency in 24 hours, using 4.13ml of the oils per 17g of fish. Adedire and Lajide (2000)reported that the powdered fruit and bark of *Dennettia tripetala* had ovicidal, larvacidal and insecticidal effects on *Callosobrunchus maculatus* in stored cowpea.

Vegetable oils have also been reported to be effective in controlling some pests of stored pulses (Golob and Webley, 1980). Nigerian fish merchants rub

groundnut and other vegetable oils on dried fish for protective or cosmetic reasons (Don-Pedro 1989 and 1990). However, results obtained from an experiment in the Lake Turkana region in Kenya, in which bottled cod-liver was applied to dried*Tilapia* (*Orechromis niloticus*) at a treatment level of 44ml per kg showed that after 45 days storage there was little difference in insect infestation between treated and untreated samples (Walker and Wood, 1986). When *Dermestes maculatus* eggs were assayed against groundnut oil, freshly applied on dried trout surfaces, the LC_{50} value was found to be as low as 18.29 per kg. It was observed that absorption of surface oils by fish muscle over time reduced activity against eggs.Generally, the oils were shown to act mainly against eggs and have no direct toxicity against active stages of the insect (Don-Pedro, 1989). Gupta *et al*. (2000) evaluated the efficacy of different vegetable oils such as castor, mustard, linseed, soybean, coconut, groundnut and sesame against the rice weevil *Sitophilus oryzae*. It was observed that all the oils offered protection over a period of around 120 days.

2.5 Use of Plant Extracts for Control of Storage Insect Pests.

2.5.1 *Allium sativum*(Garlic)

*Allium sativum*Linnaeusconsist of sulphur-containing compounds such as allicin, alliin, ajoene, diallyl disulphide, dithiin and S-Allylcysteine (Schaumberg and Paris, 1977).These large numbers of sulfur compounds contribute to the smell and taste of garlic. Diallyl disulphide is believed to be an important odour component in garlic. Allicin has been found to be the compound most responsible for the spiciness of the raw garlic and being a powerful antibiotic and antifungal compound, it is believed to be the agent responsible for the speed recovery from sore throat or other mild ailments when garlic is used (Block,

1992). Garlic oil has numerous uses in the world today which include the flavouring some cuisine like salads, and sauces (Amagase *et al*., 2001). The regular consumption of garlic oil is said to reduce blood pressure; prevent heart disease including atherosclerosis, high cholesterol and cancer. Garlic oil is an effective antibiotic, anti-viral, anti-fungal agent, which could be used to prevent nausea, diarrhea, ease coughs, it has also been used in the treatment of conditions such as malaria and cholera, an immune system enhancementand some studies have found the oil to lower rates of certain types of cancer in people (Turner, 2004).

Teklay *et al.* (2012) studied the insecticidal and repellent properties of the extracts of *A. sativum*, tobacco, *A. indica* and *Aloe vera* collected from Sofoho, Axum, North East Africa. The result obtained showed that 100% mortality was observed in garlic, 86.7% in tobacco, 80% in neem and 53.5% in *Aloe vera* extracts. It was observed that all the plant extracts repelled the insects completely. Lalla *et al.* (2013) in their study on chemical composition and biological activity of *Allium sativum* essential oils against *Callosobruchus maculatus* revealed that garlic essential oils exert a strong toxicity on bruchid. In fact after 24 hours of exposure of adult bruchids to the different concentrations tested, LC_{50} and LC_{99} were 2.50 and 23.30µl/l of air for females and 2.56 and 46.07µl/l of air for males, respectively.

2.5.2 *Jatropha curcas (*Jatropha)

Jatropha curcas belongto the family Euphorbiaceae. It is cultivated in central and South America, Southeast Asia, India and Africa (Gübitz*et al.*, 1999). *J. curcas* tree which can easily be propagatedby cutting is widely planted as a

hedge to protect the field's erosion, as it is not browsed by cattle. *J. curcas* can grow wellunder adverse condition because of its low moisture demands, fertility requirements and tolerance to high temperatures (Kaushik *et al.,* 2007). All parts of *J. curcas* plant have their own uses. Like many other *Jatropha* species, *J. curcas*is a succulent tree that sheds its leaves during the dry season. It is well adapted to aridand semi-arid conditions and often used for erosion control(Openshaw, 2000). The leaves are used in traditional medicine against coughs or as antiseptics after birth, and the branches as chewing sticks (Gübitz*et al.,* 1999). The seed oil is found useful for medicinal and veterinary purposes, as insecticide, for soap production and as fuel substitute (Gübitz*et al.*,1999). The chemical composition of the oil varies according to climate and locality.The plant contains terpenes;diterpenes from different species of *Jatropha* have shown a wide range ofbiological activities such ascytotoxic, anti-inflammatory, anti-tumor, molluscicidal, insecticidal, and fungicidal activities. Aqueous extract of leaves is reported to have insecticidal properties. In Ghana, *J.curcas*aqueous extract of leaves are used for fumigation of houses against bed bugs. The ether extract shows antibiotic activity against *Staphylococcus aureus* and *Escherichia coli*. *Jatropha* oil has a very high saponification value and it is extensively used for making soap in India and other countries.

Adebowale and Adedire (2006) reported that high unsaponifiable matter of 3.8% was found in the oil of *J.curcas*which couldserve as a potential insecticide. This is because unsaponifiable matter contains sterols and triterpene alcohols which are responsible for the insecticidal properties of fixed oils (Haftmann, 1970).Neelu and Sushilkumar (2008) evaluated the anti-termite activity of *J. curcas*and its toxic fraction at different dilutions i.e., 1%, 5%,10%,20% against

Microcerotermes beesoni. The maximum wood protection against termites of both the treatments was obtained at their highest concentration i.e. 20%. All the treatments proved to be effective over the control (50.84%).

2.5.3 *Zingiber officinale*(Ginger)

Zingiber officinale Roscoe, commonly known as ginger belongs to the family Zingiberaceae. The ginger family consists of more than 1,300 plant species in 52 genera. Ginger is widely used around the world in foods as a spice. Native to tropical Asia, ginger is a perennial cultivated in the tropical climates of Australia, Brazil, China, India, Jamaica, West Africa, and parts of the United States (Langner *et al.,* 1998).

Ginger isa herbaceous rhizomatous perennial, reaching up to 90 cm in height under cultivation. Rhizomes are aromatic, thick-lobed, pale-yellowish, bearing simple alternate distichous narrow oblong lanceolate leaves. The herb develops several lateral shoots in clumps, which begin to dry when the plant matures. Leaves are long and 2-3 cm broad with sheathing bases, the blade gradually tapering to a point, inflorescence solitary with lateral radical pedunculate oblong cylindrical spikes. Flowers are rare, rather small, calyx superior, gamosepalous, three-toothed; open-splitting on one side, corolla of three sub-equal oblong to lanceolate connate greenish segments (Wang and Wang, 2005).

Ginger has been shown to produce antioxidant effects (Fuhrman *et al*., 2000). 6-gingerol appears to be the antioxidant constituent present in ginger, as it was shown to protect HL-60 cells from oxidative stress (Wang *et al*., 2003). Ginger oil has dominative protective effects on DNA damage induced by hydrogen

peroxide. Ginger oil might act as a scavenger of oxygen radical and might be used as an antioxidant (Ma *et al.*, 2004). Ginger oil has been used in the treatment of fractures, rheumatism, arthritis, bruising, carbuncles, nausea, hangovers, travel and sea sickness, colds and flu, catarrh, congestion, coughs, sinusitis, skin sores, sore throat, diarrhea, colic, cramps, chills and fever. Also, ginger oil is used for cooking, as a flavouring in cookies, biscuits and cake (Grzanna *et al.*, 2005).

The active ingredients in ginger are thought to reside in its volatile oils, which comprise approximately 1-3% of its weight. The major active ingredients in the oil are the sesquiterpenes: bisapolene, zingiberene, and zingiberol (Connell and Sutherland, 1969; Yoshikawa *et al.*, 1993). The concentrations of active ingredients vary with the growing conditions. The active ingredients have a variety of physiological effects, for example, the gingerols have analgesic, sedative, antipyretic and antibacterial effects *in vitro* and in animals (Mascolo, 1989).The characteristic smell and flavour are caused by a mixture of zingerone, shogaols and gingerols, volatile oils that comprise one to 3% of the weight of fresh ginger. In laboratory animals, the gingerols increase the motility of the gastrointestinal tract and have analgesic, sedative, antipyretic and antibacterial properties. Ginger oil has been shown to prevent skin cancer in mice and a study at the University of Michigan demonstrated that gingerols can kill ovarian cancer cells. 6-Gingerol (1-[4'-hydroxy-3'-methoxyphenyl]-5-hydroxy- 3-decanone) is the major pungent principle of ginger. The chemo-preventive potentials of [6]-gingerol present a promising future alternative to expensive and toxic therapeutic agents.The pungent taste of ginger is due to non-volatile phenylpropanoid derived compounds, particularly gingerols and shogaols, which

are formed from gingerols when ginger is dried or cooked (Chrubasik *et al*., 2007).

2.5.4 *Azadirachta indica*(Neem)

The neem tree, *Azadirachta indica,* is a member of the mahogany family Meliaceae. It is indigenous to arid regions of Southern and South Eastem Asia. The tree is fast growing and may reach a height of 25m;it is best grown under tropical conditions and can tolerate an extended dry season. It can tolerate severe droughts and poor shallow soils (Koul *et al.,* 1990; Schmutterer, 1990). It ispropagated easily by seed and 9 to 12 month old seedlings transplant well.The neem treehas an amazing variety of uses and this is one of the main reasons that it has been planted in so many parts of the world. Products from the neem tree have been used for centuries in traditional medicine and agriculture(Koul*et al.,* 1990). Neem oil, extracted from the seeds, was used in traditional Indian medicine to treat leprosy, skin diseases, and malaria(Baladrin *et al.,* 1988). However, the efficacy of these treatments has not been documented. Neem leaves have been used as feed for cattle and sheep, organic manure and soil conditioner. Crude neem extracts, mainly from the seeds and leaves, have been shown to exhibit antibacterial, antifùngal, and antiviral properties, as well as a range of anti-insect properties. In some localities, neem trees have been planted for shade, reforestation and erosion control. The quality of the wood makes it excellent for timber and in some cases it is used for fuel (Koul *et al.,*1990; Schmutterer, 1990).

Over 300 compounds have been isolated from neem seeds, one third of these beingtetranortritepenoids (limnoids) (Kumar *et al.,* 1996). These limnoids include the most active,azadirachtin-A (Schroeder and Nakanishi, 1987), and

eight other highly active isomers,azadirachtinB-1, moderately active nimbandiol, salannin, and the less active gedunin, vilasinin, azadirone and azadiradione (Sundaram, 1996).The compound azadirachtin, the most polar and highly oxygenated of these limnoids(Johnson *et al.,* 1996), has received the most attention because of its importance as an environmentally friendly insect control agent. It is generally believed that the bio-activity of neem preparations is mainly the result of their azadirachtin content (including all the azadirachtinisomers) (Govindachari *et al.,*1996; Isman, 1997). The oil extracted from the seeds is commonly used for insect pest control, although there are problems with its large droplet size and oily nature (Ascher, 1993).

2.5.5 *Cocos nucifera*(Coconut)

The coconut palm, *Cocos nucifera* Linnaeus is in the family Arecaceae (palm family), it is the only species in the genus*Cocos* and is found throughout the tropics and subtropics. The coconut is known for its great versatility as seen in its many domestic, commercial and industrial uses. Coconuts are part of the daily diets of many people and are different from any other fruits because they contain a large quantity of water and when immature they are known as tender-nuts or jelly-nuts. When dried, the coconut flesh is called copra. The oil and milk derived from it are commonly used in cooking and frying; coconut oil is also widely used in soaps and cosmetics. The clear liquid coconut water within is a refreshing drink. The husks and leaves can be used as material to make a variety of products for furnishing and decorating. It also has cultural and religious significance in many societies that use it.

Coconut oil has been used extensively for edible and non-edible purposes all over the world. Detergent industries depend mainly on coconut and palm kernel oils for lauric acid. It is one of the world's most desirable natural oils for confectioneries, bakery goods, deep fat frying and for non-edible purposes. This is due to two of its special characteristics, high degree of saturationand good stability. It has also been used as a vehicle for the preparation of control agents against many disease vectors and it has shown some degree of repellency against mosquitoes (Sylla *et al.,* 2003).

Physically, coconut oil is hard and brittle at lower temperatures with a melting point below 30°C. The oil is hydrolysed commercially and the short chain fatty acids are fractionally distilled into two end products of various degrees of purity, which in turn are transformed into alcohols, amides and esters for a large variety of specialized end uses in detergents, plastics, e.t.c.The composition of crude coconut oil includes triacylglycerides, free fatty acids, partial glycerides, phospholipids, sterols, tocopherols, pigments, trace metals and oxidized products. About 90% of the fatty acids of coconut oil are saturated and consist mainly of lauric, myristic and palmitic acid (Makkar *et al*., 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

The tests were conducted in the Fisheries Research Laboratory, Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria.

3.1 Sources of Plant Materials

Garlic bulbs (Plate III), Gingerrhizomes (Plate V)and Coconut(Plate VII) were purchased from Sabon-Gari market, Zaria.*Jatropha*(Plate IV) and Neem seeds (Plate VI) were obtained from *Jatropha* and neem trees in the premises of National Research Institute of Chemical Technology, Bassawa, Zaria, Kaduna State.All seeds were properly identified and authenticated at the herbarium of the Department of Biological Sciences,Ahmadu Bello University, Zaria.

3.1.1 Processing of plant materials

Jatropha, coconut and neem seeds obtained were sundried for two (2) weeks. The garlic cloves and ginger rhizomes were peeled and sun-dried for two weeks. The seeds, bulbs and rhizomes were pulverized using a mortar and pestle. The resulting powder was sieved using 2mm mesh size and stored in pre-labelled cleanplastic bags. Oils were extracted from the resulting powders using n-hexane as solvent.

3.1.2 Soxhlet extraction of oils

100g of each plant powder in muslin cloth was introduced separately into the soxhlet chamber for oil extraction (Plate II). In the round-bottom flask, 350ml of n-Hexane was introduced as solvent. The extraction was done at 60-80°C until the solvent in the soxhlet chamber became transparent. A rotary evaporator was

used to evaporate excess solvent from the oil. Extracted plant oils were stored in separate labelled bottles until required for bioassay.

3.2 Physico-Chemical Properties of The Oils

The specific gravity, refractive index and saponification, peroxide, acid and iodine values were determined according to standard procedures (Official Methods of Analysis, 1998; Oils and Fats, 2012).

3.2.2 Determination of specific gravity

The pycnometer was filled with the test oil in such a manner as to prevent entrapment of air bubbles after removing the cap of the side arm. The stopper was inserted and immersed in a water bath at 300°Cfor 30minutes. The cap on the side of the pynometer was then removed and weighed quickly to ensure that the temperature did not fall below 30°C.Excess oil was wiped from the capillary opening and the bottle was removed from the bath, then cleaned and dried.

$$\text{Specific gravity at } 30°C = \frac{A - B}{C - B}$$

Where,

A = weight (g) of specific gravity bottle with oil at 30°C

B = weight (g) of specific gravity bottle at 30°C

C = weight (g) of specific gravity bottle with water at 30°C



PLATE II:Soxhlet Apparatus

Note: (a) Round bottom flask (b) Extraction chamber (c) Heat source



Magnification approximately ×1



Magnification ×0.8

Plate III. *Allium sativum*.(a)Whole Bulbs (b)Peeled Cloves.



Magnification ×0.6

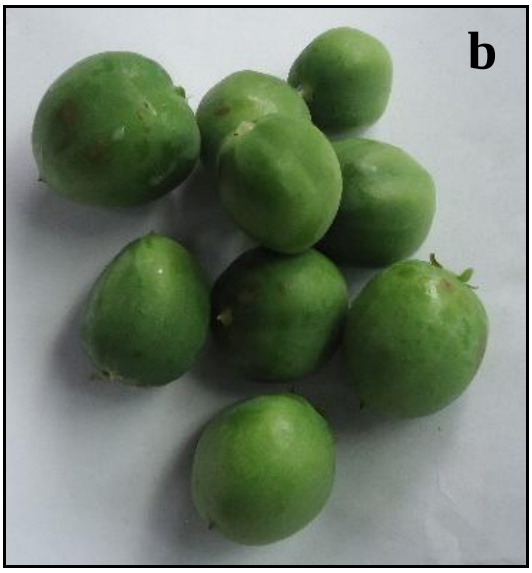




Plate IV:*Jatropha curcas.* (a) Leaves (b)Fruits (c) Dried seeds





Magnification ×0.6

Plate V:*Zingiber officinale*. (a) Whole Rhizomes (b) Peeled and Sliced Rhizomes



Magnification ×0.3

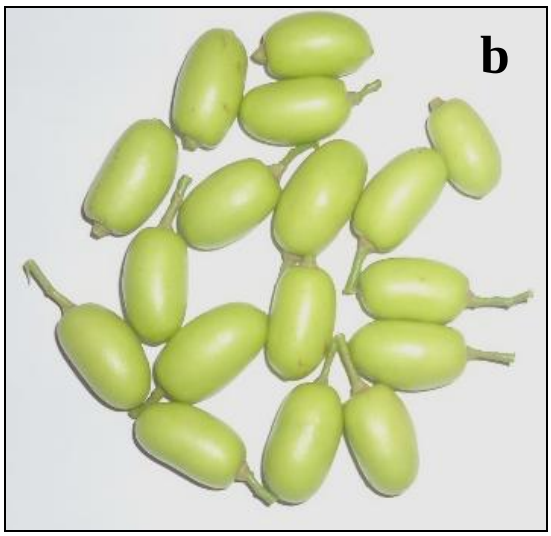
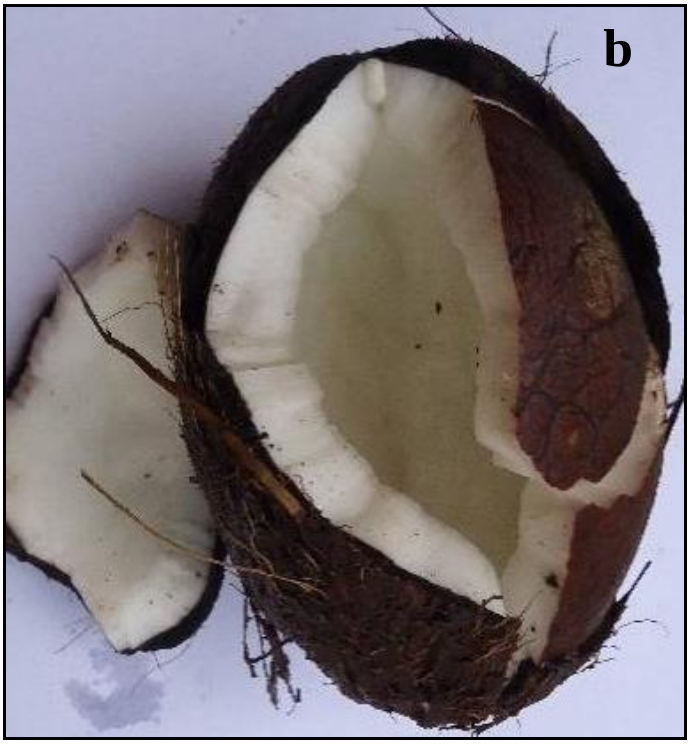




Plate VI:*Azadirachta indica.*(a) Leaves(b) Fruits(c) Dried Seeds





Magnification ×0.8

Plate VII:(a) Whole *Cocos nucifera* (Coconut); (b) Cracked Coconut Fruit

3.2.3 Determination of refractive index

Refractive index is the ratio of light in vacuum to the velocity of light in the oil. This was determined using theRefractometer (Plate VIII). The instrument was charged by opening the prism surface and a drop of oil was placed on the prism. The instrument was allowed to stand for few minutes before taking the reading so that the temperature of the test sample and instrument are the same. The prism was cleaned between readings by wiping with ethyl alcohol-moistened cotton pads and allowed to dry.

3.2.4 Determination of saponification value

Saponification value is the number of mg of potassium hydroxide required to saponify 1gram of oil. 2g of the oil sample was mixed thoroughly and weighed into a ground neck conical flask and 25ml of alcoholic potassium hydroxide solution was added. Antibumping was added and reflux condenser was fitted and boiled for one hour. After 60 minutes, 1ml of phenolphthalein indicator was added and titrated against 0.5M hydrochloric acid. A blank test was carried out simultaneously.

Calculation:

$$S.V = \frac{56.1\,(B - S)N}{W}$$

Where,

B= volume in ml of standard hydrochloric acid required for the blank

S= volume in ml of standard hydrochloric acid required for the sample

N= normality of the standard hydrochloric acid

W= weight in gram of the oil taken for the test

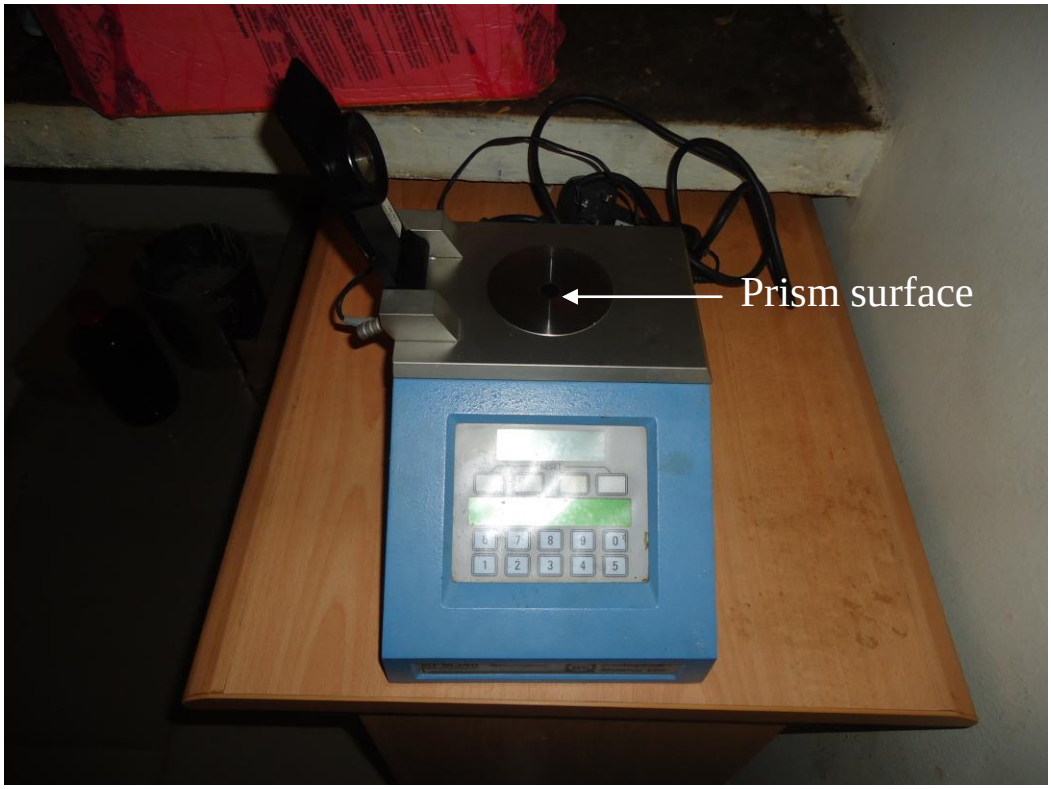


Plate VIII: Refractometer

3.2.5 Determination of peroxide value

2.0g of oil extracted was added to 22ml of a mixture of 12mlchloroform and 10ml acetic acid. 0.5ml of saturated potassium iodide was added to the flask. The flask was corked and allowed to stand, with occasional agitation for 1 minute. 30ml of distilled water was then added to the mixture and titratedagainst 0.1M of sodium sulphite until the yellow colour had almost gone. 0.5ml of starch indicator was quickly added and titration continued until the blue colour just disappeared. A blank titration was also carried out.

$$\text{Peroxide value} = \frac{(S - B) \times N \times 1000}{W}$$

Where: Peroxide value =Milliequivalent peroxide per 100g of sample

S = volume of titrant (ml) for sample

B = volume of titrant (ml) for blank

N = molarity of Na_2SO_3 solution (mEq/ml)

1000 = conversion of units (g/kg)

W = Weight of oil sample

3.2.6 Determination of iodine value

0.4g of the oil sample was weighed into a conical flask and 20ml of carbon tetrachloride was added to dissolve the oil. Then 25ml of wij's reagent was added to the flask using a safety pipette in fume chamber. The content of the flask was vigorously swirled. The flask was then placed in the dark for 2 hours 30 minutes. At the end of which 20ml of 10% aqueous potassium iodide and 125ml of water were added using a measuring cylinder. The content was titrated against 0.1M sodium-thiosulphate solutions until the yellow colour almost disappeared. A few drops of 1% starch indicator was added and the titration

continued by adding thiosulphate drop wise until the blue coloration disappeared after vigorous shaking. The same procedure was used for the blank test and other samples.

The iodine value (I.V) was expressed as follows:

$$\text{Iodine Value} = \frac{12.69C\,(V1 - V2)}{W}$$

Where C = Concentration of sodium thiosulphate

V1 = Volume of sodium thiosulphate used for blank

V2 = Volume of sodium thiosulphate used for determination

W = Weight in gram of the sample

3.2.7 Determination of acid value

25ml of 5% ethanol was boiled in a water bath; the heating is to ensure the removal of dissolved gases. 2.5g of oil was added to 25mlof hot ethanol and the mixture was heated to boiling. Then a few drops of 1% phenolphthalein indicator was added and titrated against 0.1M potassium hydroxide with constant agitation until a permanent pink colour was obtained, the acid value was expressed as:

$$\text{Acid Value} = \frac{56.1 \times M \times V}{W}$$

Where M = Concentration of potassium hydroxide

V = Titre value

56.1 = Molecular weight of potassium hydroxide

W = weight of oil sample

3.3 Insect Maintenance and Culture

Adults of *D. maculatus*(Plate X) were obtained from infested fish concentrates purchased from Sabon-gari market, Zaria and were identified morphologically using taxonomic key. The identified adults were kept in Kilner jars containing dried fish with wet cotton wool at room temperature and 70-80% relative humidity. The culture jars were kept inside boxes to prevent escape of the insects. After two weeks, the adults were removed ffrom the Kilner jars. The larvae reached a size useful for the experiment after four weeks.

3.4 Collection of Smoke-Dried Fish

Smoke-dried *Protopterus annectens*(Plate IX) were purchased from Sabon-gari market, Zaria.

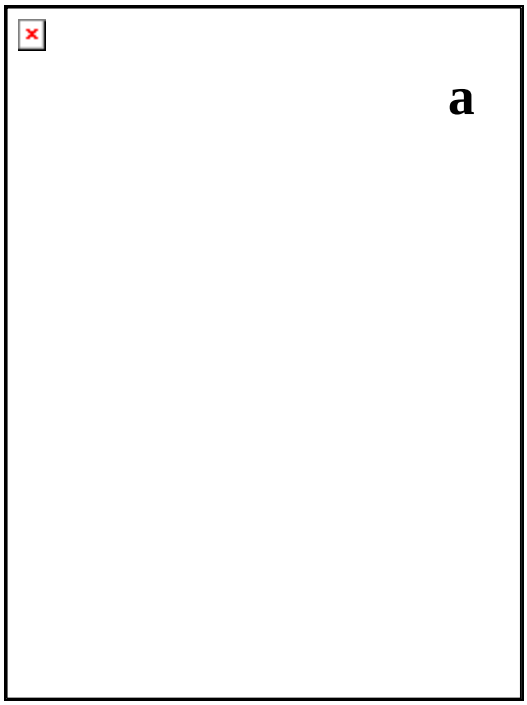
3.5 Pilot Study

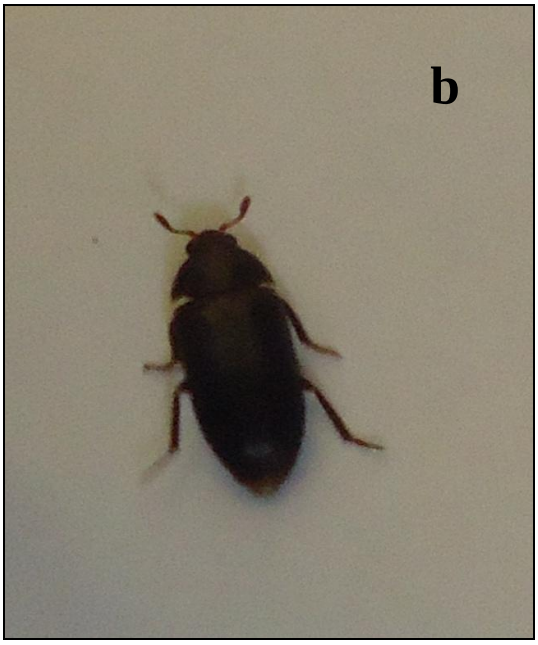
Range-finding tests were conducted to determine the concentration range of each oil to be used for the definitive tests (bioassay). This was done by applying five nominal concentrations (0.001 to 0.256ml/g of fish) of the oil to the heat-sterilized fish and placing them in Kilner jars. Twenty (20) late instar larvae of *D. maculatus* were then introduced into each jar. The jars were observed at 24, 48, 72 and 96 hours to establish total and zero mortality/minimal mortality range. Five concentrations between 0.001 and 0.256ml/g of fish were chosen for the bioassay test.



Magnification(×0.8)

Plate IX: Smoke-dried *Protopterus annectens* (Dorsalview)





Magnification ×5

Plate X:*Dermestes maculatus.*(a) Larva(b) Adult

Bioassay

3.6.1 Mortality test

The smoke-dried fish was heat-sterilized in the oven at 60°C for one hour and allowed to cool down to room temperature after which they wereweighed and tagged.The oils were applied to the fish at concentrations of 0.001ml/g of fish, 0.004ml/g, 0.016ml/g, 0.064ml/g and 0.256ml/g. The oil-treated fish were then introduced into separate Kilner jars.For the control experiment, fish without oil treatment were introduced into separate Kilner jars. 20 larvae of *D. maculatus* were introduced into each jar containing treated fish including the control and labelled for oil type, oil volume and time of larval inoculation. The experiment was replicated thrice for each oil volume. The setup was maintained for 96 hours and mortality of larvae in treatments and control was recorded after 24, 48, 72 and 96 hours exposure period at room temperature. Larvae that failed to respond to the gentle touch of a small pair of forceps were considered dead.

3.6.2 Repellency test

A rectangular container (18cm×12cm×10cm) was used for the repellent test. The heat-sterilized fish was weighed and tagged. At concentrations of 0.001ml/g of fish, 0.004ml/g, 0.016ml/g, 0.064ml/g and 0.256ml/g, the oil was applied on the fish. The treated fish was placed at the one end of the container and the control fish (untreated fish) was placed at the other end. The experiment was replicated thrice. The repellency of the larvae to the oil was determined by counting the number of larvae that moved away from the treated fish after 24 hours exposure period in an experimentalrectangular glass container(Egwunyenga *et al.,* 1998).

3.6 Gas Chromatography/Mass Spectrometry Analysis

The oils of all plant samples wereanalyzed on a Hewlett-Packard (Model: 6890NGas Chromatography/ Mass Spectrometry) system (Agilent Technologies) (Plate XI) coupled to a mass spectrometer (Model: HP5973). Separations were carried out using DB-5 capillary column (30m × 0.25mm i.d., 0.25µm film thickness).The injector and detector temperatures were maintained at 220°C and 280°C respectively. Column temperature was initially kept at 60°C for 2minutes and then gradually increased to 240°C at the rate of 50°C/min. Helium wasused as carrier gas at a constant flow of 1.0mL/minand an injection volume of 1µL was employed. The Mass Spectrometry scan parameters included electron impact ionization voltage of 70eV, a mass range of 40-750m/z and a scan interval of 0.5s. Samples diluted in n-hexane were injected manually in the splitless mode. The identification of the components was based on comparison of their mass spectra with those of National Institute of Standards and Technology (NIST) libraries provided with the computer-controlling GC-MS system.

3.7 Data Analysis

All repellency and mortality data were subjected to Analysis of Variance at $P<0.05$ to determine any significant difference among treatments. Duncan Multiple Range Test (DMRT) was employed to separate the means. Mortality data from all the oils were subjected to Probit Analysis (Finney, 1964) to determine their median lethal concentration (LC_{50}).



Plate XI: Gas Chromatography/Mass Spectrometry System

CHAPTER FOUR

4.0 RESULTS

4.1 Physicochemical Properties and Fatty Acid Composition

4.1.1 Physicochemical properties of the oils

The acid values of the oils were below 10mg KOHg except for *A. indica* which had 12.75mg KOHg. The saponification values of the oils ranged from 190.74 (*A. sativum*) to 251.05 (*C. nucifera*). *Zingiber officinale* oil had the highest peroxide value (2.96mg/100g) while *J. curcas* oil had the lowest value (0.65mg/100g). The values of the refractive indices ranged from 1.24 (*J. curcas*) to 1.49 (*A. sativum*) (Table 4.1).

4.1.2 Fatty acid composition of the oils

Table 4.2 shows the fatty acid composition of the five plant oils. Oleic acid was found only in the oils of *J. curcas* and *A. indica*; octadecanoic acid in *Z. officinale* and *C. nucifera.* Stearic and palmitic acids in *A. indica* and *C. nucifera;* lauric, linoleic and myristic acids *C. nucifera* only; octadecanoic acid in *J. curcas* and cyclopropanepentanoic acid in *Z. officinale*.

4.1.3 Metabolites detected in the plant oils

The metabolites found in the oils include α-Zingiberene (1.58%), α-pinene (1%), trimethlmethylene (6.38%), 2,4-decadienal (10.47%), α-curcumene (1.42%), β-farnesene (1.25%) and β-sesquiphellandrene (1.43%) in *Z. officinale*. Hexane was detected in the oils of *A. indica* and *C. nucifera* with values of 6.87% and 2.36% respectively. Tetrahydrofuran (4.51%) and ethylvinyl (0.85%) were detected in *A. sativum*. Cyclobutanol (5.26%), ethylbenzene (7.14%) and pentacyclo octacosa (56.10%) were detected in *J. curcas* (Table 4.3).

Table 4.1: Physicochemical properties of the oils of *Allium sativum, Cocos nucifera, Jatropha curcas, Zingiber officinale* and *Azadirachta indica*

Oils	Relative density	Refractive Index	Saponification value	iodine value	peroxide value	Acid value
Cocos nucifera	0.94±0.00^{a}	1.46±0.01^{a}	251.05±1.27^{a}	7.61±0.16^{e}	2.22±0.00^{c}	2.02±0.05^{e}
Allium sativum	0.88±0.12^{a}	1.49±0.03^{a}	190.74±3.09^{d}	12.05±3.27^{d}	2.53±0.00^{b}	4.21±2.08^{d}
Zingiber officinale	0.94±0.05^{a}	1.47±0.02^{a}	215.98±1.47^{b}	15.86±2.49^{c}	2.96±0.78^{a}	4.60±0.00^{c}
Jatropha curcas	0.91±0.00^{a}	1.24±0.12^{b}	196.15±0.87^{cd}	62.18±6.08^{b}	0.65±1.00^{e}	5.60±0.20^{b}
Azadirachta indica	0.89±0.02^{a}	1.47±0.02^{a}	199.16±1.34^{c}	85.03±10.16^{a}	0.88±0.02^{d}	12.75±0.01^{a}

Means along column with the same alphabet are not significant (P≥0.05).

Table 4.2: Fatty acid composition (%) of the plant oils of *Allium sativum, Cocos nucifera, Jatropha curcas, Zingiber officinale* and *Azadirachta indica*

	Oil type (%)±S.E.				
Fatty Acids	**A. sativum**	**Z. officinale**	**J. curcas**	**A. indica**	**C.nucifera**
Pentadecanoic acid (U)	5.6±0.25	_	6.20±0.01	_	_
Palmitic acid (S)	7.57±0.18	_	_	20.09±00	12.84±0.69
Elaidic acid (M)	12.68±0.54	_	_	9.87±0.98	-
Stearic acid (S)	5.91±1.97	32.38±5.60	_	2.96±0.17	3.24±0.17
Hexadecanoic acid (S)	47.60±6.56	_	_	_	_
Pentanoic acid (S)	5.36±0.43	8.87±0.01	_	_	_
Oleic acid (M)	_	_	25.30±0.09	60.13±2.8	_
Lauric acid (S)	_	_	_	_	1.76±0.00
Myristic acid (S)	_	_	_	_	0.88±0.19
Linoleic acid (U)	_	_	_	_	47.54±4.75
Total	**84.72±9.93**	**74.80±6.82**	**31.50±0.10**	**93.05±3.95**	**73.39±5.80**

U= Unsaturated, M= Monounsaturated,
S=Saturated

Table 4.3: Metabolites (%) detected in the plant oils of *Alliumsativum, Cocos nucifera, Jatropha curcas, Zingiber officinale* and *Azadirachta indica*

	Mean±S.E.				
Metabolites	***A. sativum***	***Z. officinale***	***J. curcas***	***A. indica***	***C. nucifera***
Hexanal	_	_	5.26±0.14	_	_
Linolein	-	-	-	-	2.31±0.14
Ethylbenzene	_	_	7.14±0.00	_	_
Pentacyclo	_	_	56.10±6.47	_	_
Hexane	_	_	_	6.87±0.20	2.36±0.12
α-pinene	_	1.00±0.19	_	_	_
Trimethylmethylene	_	6.38±2.80	_	_	_
2,4-Decadienal	_	10.47±0.00	_	_	_
α-curcumene	_	1.42±1.92	_	_	_
α-zingiberene	_	1.58±0.08	_	_	_
β-farnesene	_	1.25±0.52	_	_	_
β-sesquiphellandrene	_	1.43±0.02	_	_	_
Tetrahydrofuran	4.51±0.18	_	_	_	_
Ethylvinyl	0.85±2.87	_	_	_	_

4.2 Mortality of *Dermestes maculatus*Larvae caused by the Oils

The oils from *Allium sativum* (garlic), *Cocos nucifera* (coconut), *Zingiberofficinale* (ginger), *Jatropha curcas* (Jatropha)and *Azadiracta indica* (neem) caused significant mortality of *Dermestes maculatus* larvae in smoke-dried *Protopterus annectens* (Figures 4.1 to 4.5).

4.2.1 Larval mortality at 0.001ml/g Oil Concentration

The mortality at 0.001ml/g of fish for all the oils is presented in Figure 4.1. After 96 hours of exposure, *A. sativum* oil was the most effective with a mortality of 15% while *A. indica* oil had the lowest mortality (5%). *C. nucifera* and *J. curcas* had the same mortality of 11.67% at 96 hours of exposure.

4.2.2 Larval mortality at 0.004ml/g Oil Concentration

Allium sativum oil had the highest mortality at this concentration with 10% and 21.67% at 24 hours and 96 hours exposure respectively. Although, no significant difference (>>0.05) at 24 hours exposure in comparison with the oil of *C. nucifera* as shown in Figure 4.2, the lowest mortality of 1.67% was observed in *Z. officinale* at 24 hours while *A. indica* had the lowest mortality from 48 hours (5%) to 96 hours (6.67%) exposure.

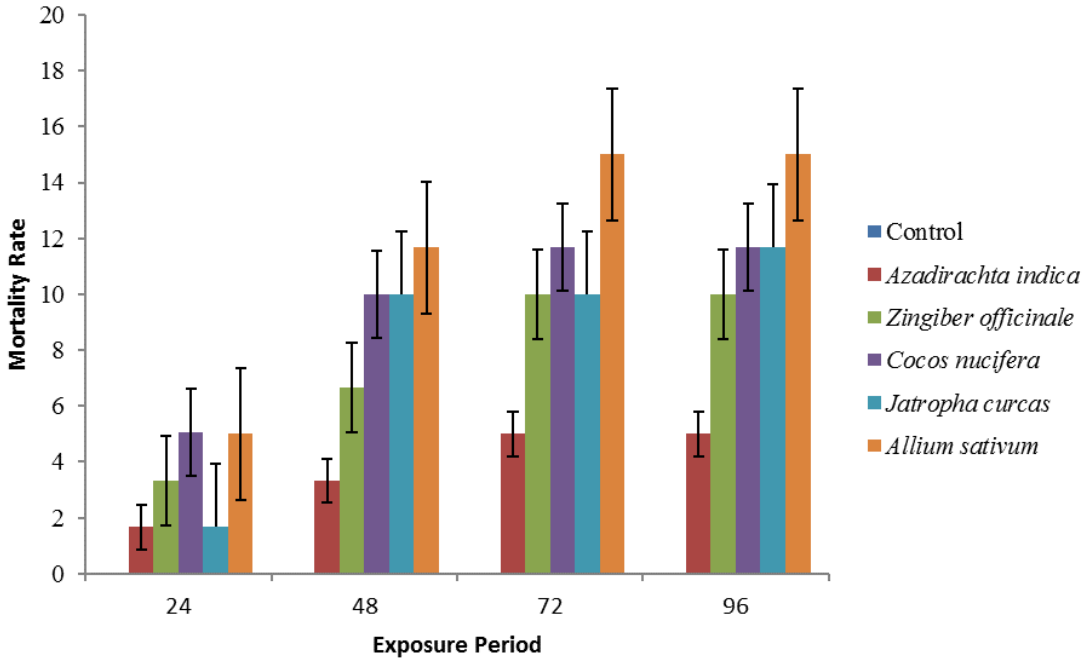


Figure 4.1: Mortality of *Dermestes maculatus* larvae exposed to 0.001ml/g test plant oils.

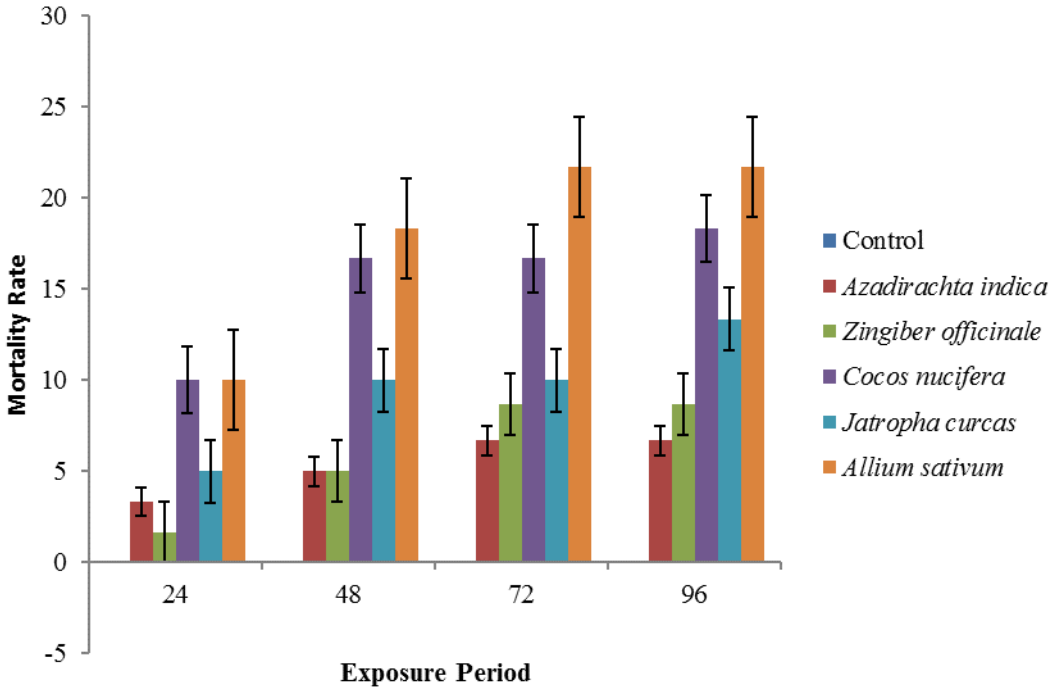


Figure 4.2: Mortality of *Dermestes maculatus* larvae exposed to 0.004ml/g test plant oils

4.2.3 Larval mortality at 0.016ml/g Oil Concentration

The mortality at 0.016ml/g for all the oils is presented in Figure 4.3. The highest mortality was observed in *A. sativum* from 24 hours (35.00%) to 96 hours (53.33%) exposure, which was significantly different ($P \leq 0.05$) from the other plant oils. *A. indica* had the lowest mortality from 24 hours (8.33 %) to 96 hours (10.00%) exposure; while *J. curcas* had the same mortality as *A. indica* at 24 hours and *Z. officinale* at 96 hours (23.33%) exposure.

4.2.4 Larval mortality at 0.064ml/g Oil Concentration

The mortality at 0.064ml/g of all the oils is shown in Figure 4.4. *Allium sativum* gave the highest mortality from 24 hours (70.00%) to 96 hours (88.33%) of exposure. *C. nucifera* gave the same mortality as *A. sativum* at 48 hours (76.67%) exposure. The lowest mortality was observed in *A. indica* oil from 24 hours (18.33%) to 96 hours (20.00%) exposure.

4.2.5 Larval mortality at 0.256ml/g Oil Concentration

The mortality of all the oils at 0.256ml/g is shown in Figure 4.5. 100% mortality was recorded in *A. sativum*, *J. curcas* and *C. nucifera*. There was no significant difference ($P>0.05$) among the plant oils from 48 hours to 96 hours, although *A. indica* had the lowest mortality at 24 hours (83.33%) exposure which is significantly different from the other plant oils.

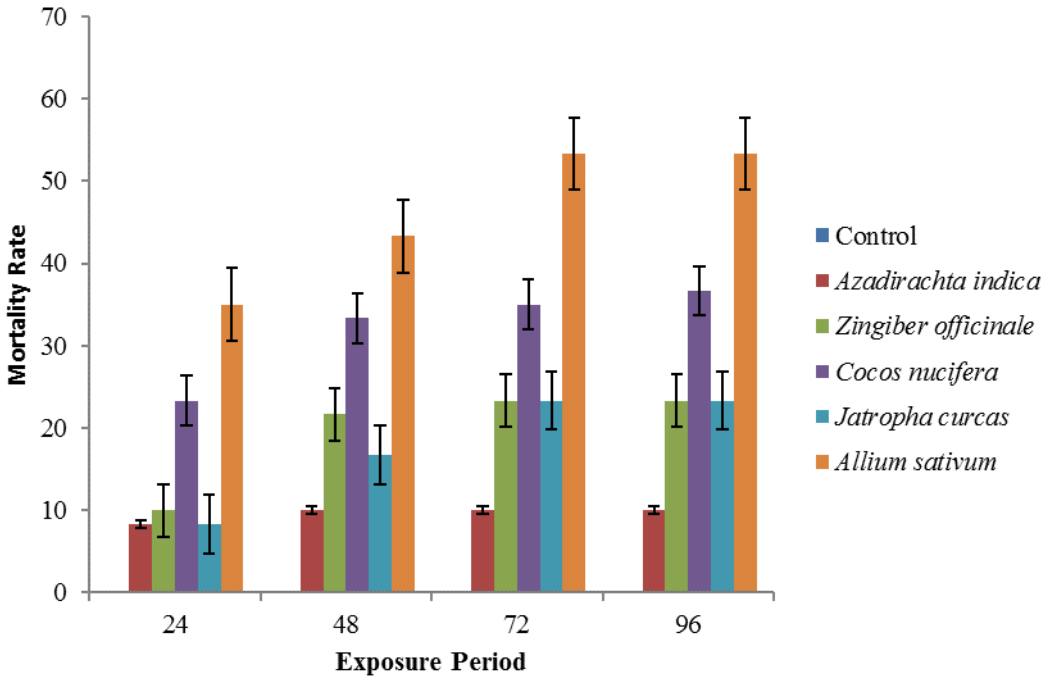


Figure 4.3: Mortality of *Dermestes maculatus* larvae exposed to 0.016ml/g test plant oils.

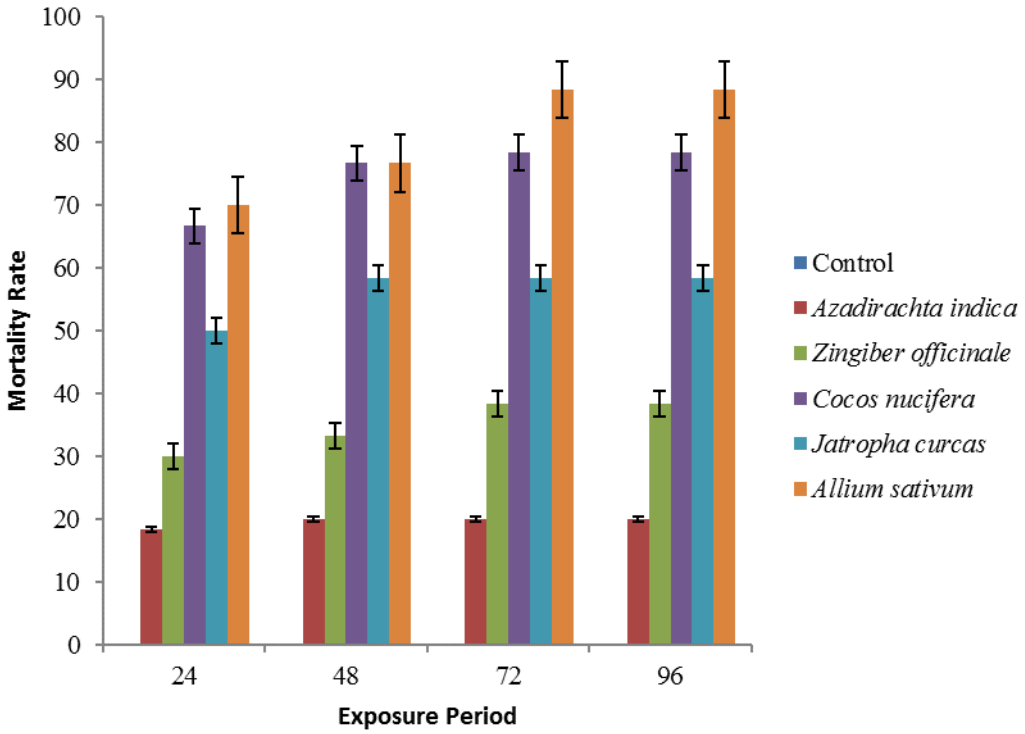


Figure 4.4: Mortality of *Dermestes maculatus* larvae exposed to 0.064ml/g test plant oils.

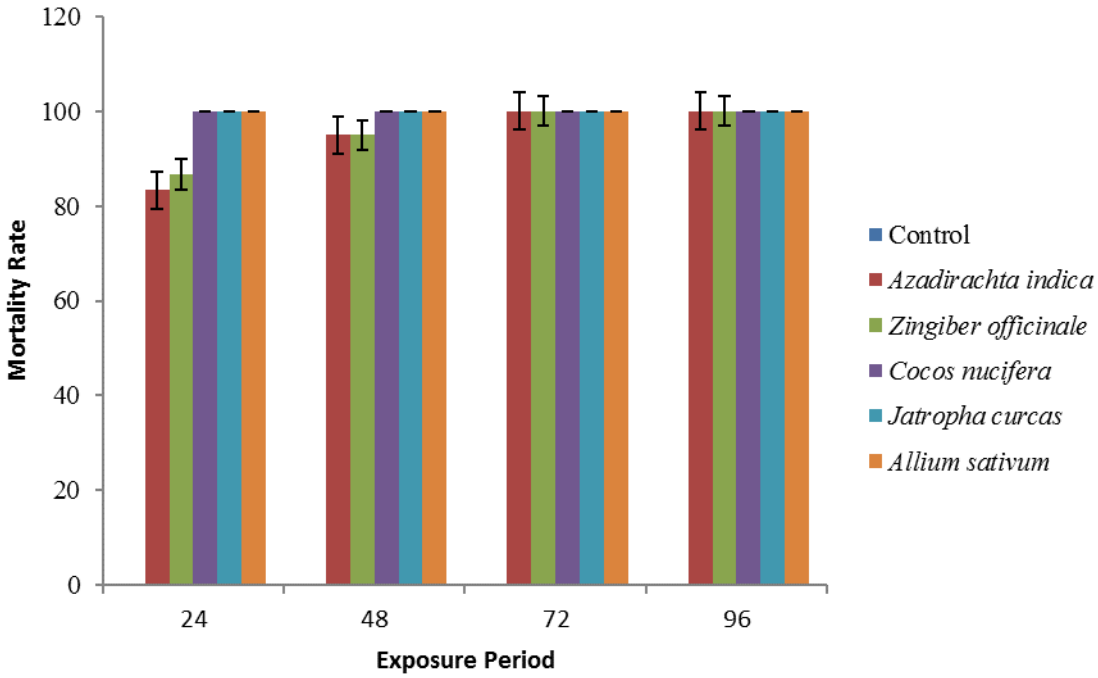


Figure 4.5: Mortality of *Dermestes maculatus* larvae exposed to 0.256ml/g test plant oils.

4.3 Median Lethal Concentration (LC_{50}) of the Plant Oils

As exposure time proceeds, there was increase in the toxicity of the oil to the larvae of *Dermestesmaculatus*. The LC_{50} values of the oils were obtained from the graphs Figure 4.6 – Figure 4.10. The values obtained include; 5.495 for *A. indica,* 0.011 for *A. sativum,* 0.021 for *C. nucifera* 0.066 for *J. curcas* and 0.200 for *Z. officinale*. A lower LC_{50} is indicative of increased toxicity, which shows that *A. sativum* was of higher toxicity to *D. maculatus*larvae.

4.4 Repellency of the Oils

Table 4.4 shows that all the five plant oils repelled the larvae of *Dermestesmaculatus*. There were significant differences at $P<0.05$ among the oils during the period of 24 hours of exposure to the insects. The highest degree of repellency of *D. maculatus* larvae (95%) was obtained with *A. sativum* oil which significantly repelled the larvae even at low concentration while *A. indica* oil showed the lowest repellency (58.33%) at the highest concentration. *Zingiber officinale* (80%) and *C. nucifera* (86.67%) showed a greater repellency than *J. curcas* oil(68.33%) against larvae of *D. maculatus*. The repellent activity of all the five plant oils increased with increase in concentration.

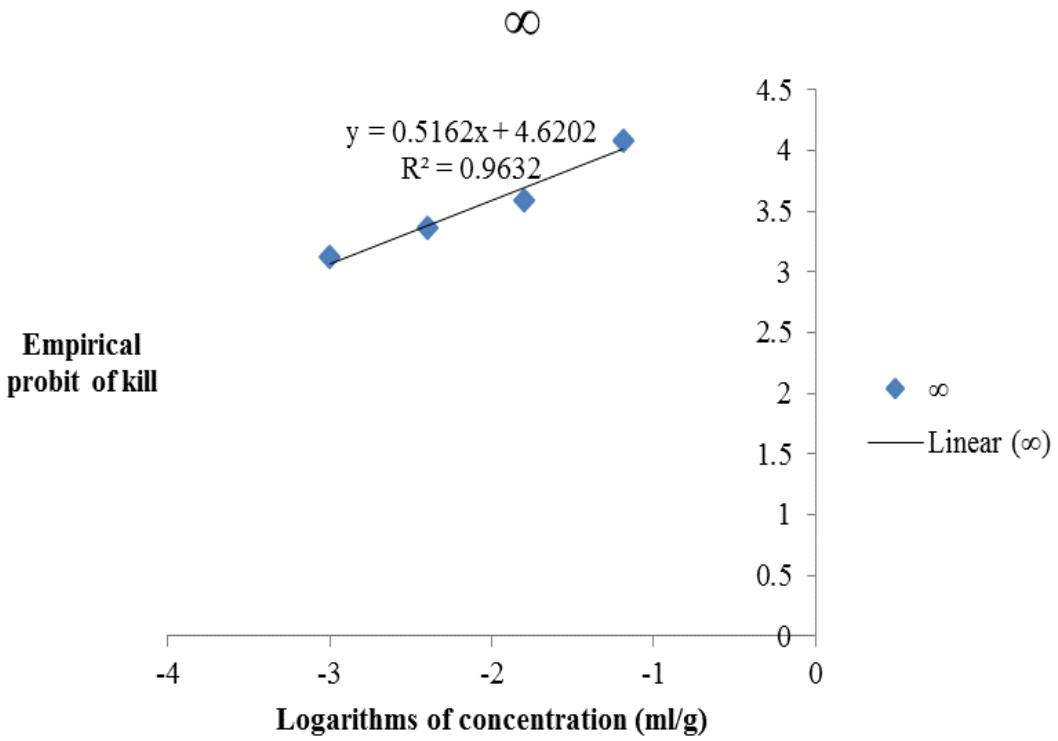


Figure 4.6: Probit determining the LC_{50} of *Azadirachta indica* oil against the larvae of *Dermestes maculatus*

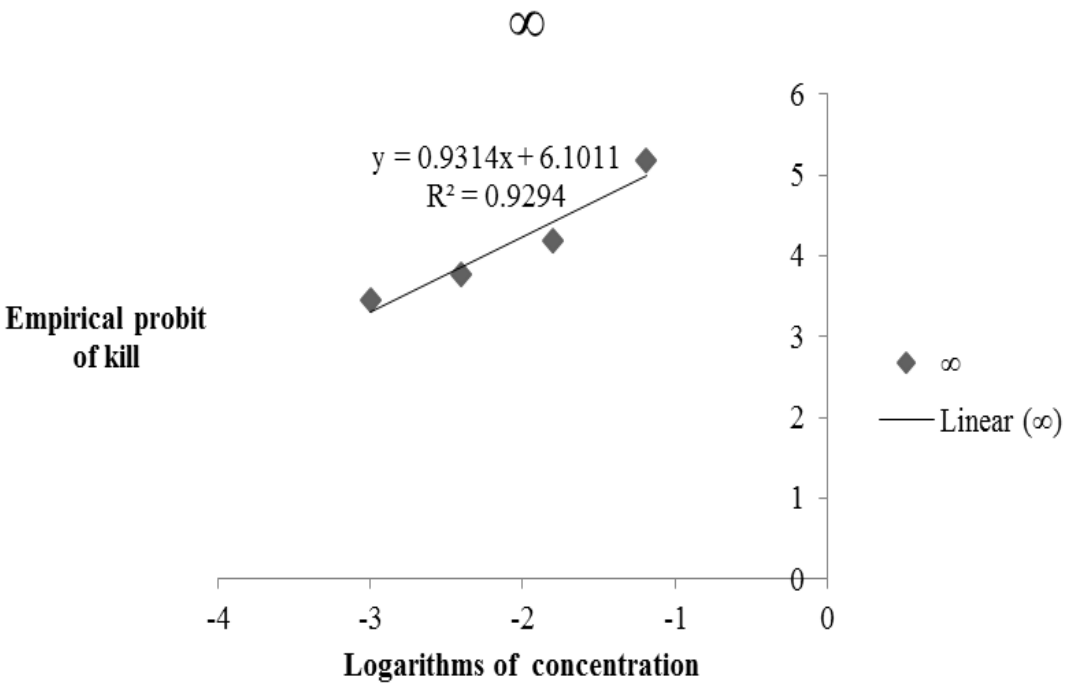


Figure 4.7: Probit determining the LC_{50} of *Jatropha curcas* oil against the larvae of *Dermestes maculatus*

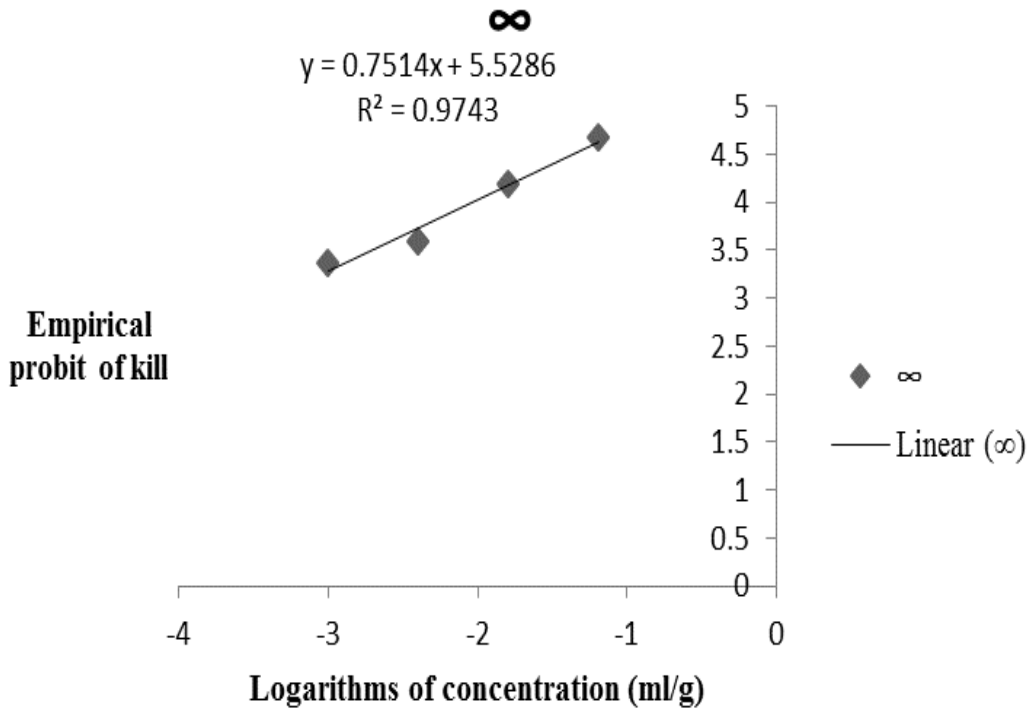


Figure 4.8: Probit determining the LC_{50} of *Zingiber officinale* oil against the larvae of *Dermestes maculatus*

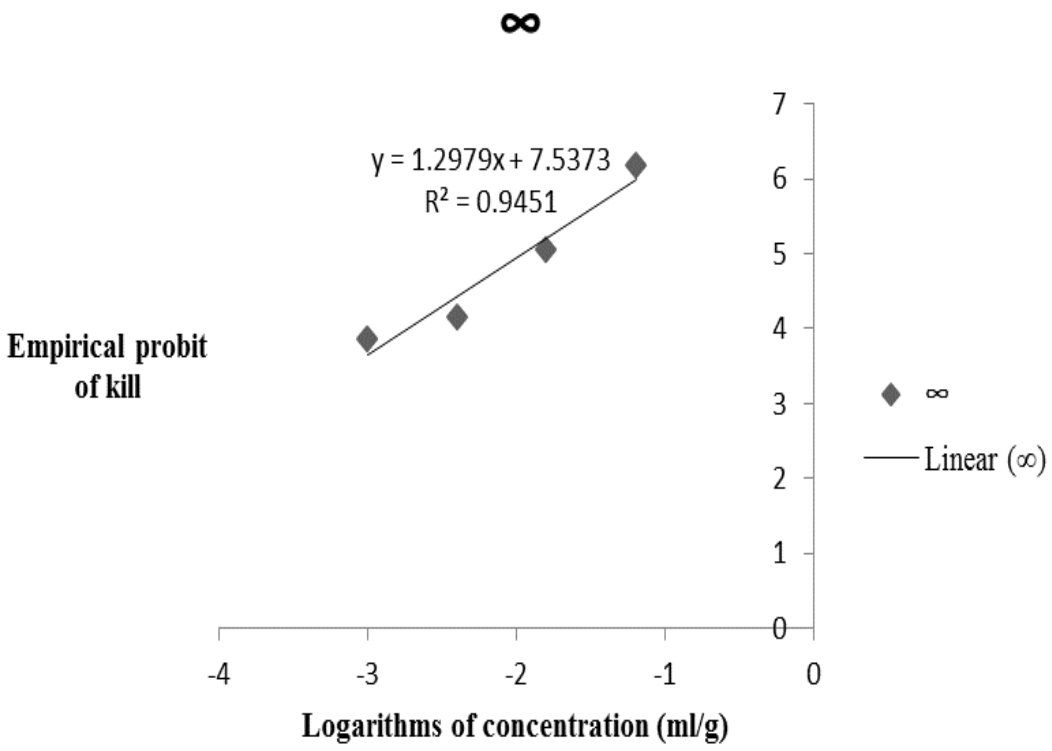


Figure 4.9: Probit determining the LC_{50} of *Allium sativum* oil against the larvae of *Dermestes maculatus*

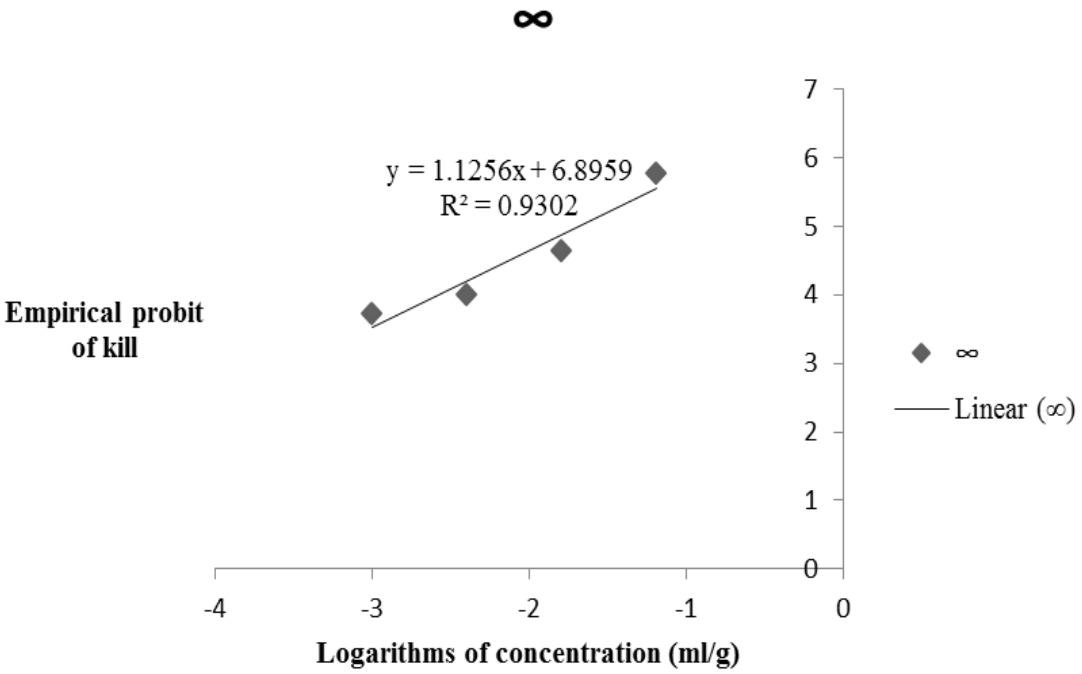


Figure 4.10: Probit graph for determining the LC_{50} of *Cocos nucifera* oil against the larvae of *Dermestes maculatus*

Table 4.4: Repulsion of*D. maculatus* larvae by the Oils of *Azadirachta indica*, *Jatropha curcas*, *Zingiber officinale*, *Allium sativum*, *Cocos nucifera* (n=20)

Concentration (mL/g of fish)	**Oil type (Repellency %)**				
	A. indica	***J. curcas***	***Z. officinale***	***A. sativum***	***C. nucifera***
0.001	16.67±0.46^{d}	40.00±3.14^{b}	25.00±3.06^{c}	50.00±2.88^{a}	33.33±2.16^{b}
0.004	41.67±0.50^{d}	45.00±0.42^{c}	41.67±0.50^{d}	53.33±0.57^{a}	48.33±0.42^{b}
0.016	46.67±0.71^{c}	48.33±2.59^{c}	53.33±1.86^{b}	63.33±0.60^{a}	56.67±0.75^{b}
0.064	50.00±2.10^{d}	56.67±1.48^{c}	65.00±1.52^{b}	75.00±2.10^{a}	58.33±0.39^{c}
0.256	58.33±0.47^{e}	68.33±0.99^{d}	80.00±0.54^{c}	95.00±2.66^{a}	86.67±0.56^{b}

Means along the row with the same alphabet are not significant (P≥0.05).

CHAPTER FIVE

5.0 DISCUSSION

The iodine values of the oils extracted from this study were lower than 100, classifying the oils as non-drying. Oils are classified into drying, semi-drying and non-drying according to their iodine values. Those with iodine value less than 110 are non-drying oil, 125 are drying oils and 110-140 are classified as semi-drying oil (Ali *et al.*, 2008). The iodine values of the oils of *A. sativum*, *Z. officinale* and *C. nucifera* were lower than that of *J. curcas* and *A. indica*. The high values of the oils *J. curcas* and *C. nucifera* is due to the high content of unsaturated fatty acids in the oils. *C. nucifera* oil had a high degree of saturation with high saturated fatty acid content which made it highly resistant to oxidative rancidity. The high iodine value of oil indicates high degree of unsaturation (Pearson, 1981). This implies that the oils of *A. sativum*, *Z.officinale* and *C. nucifera* will last longer on the fish, therefore offer lasting protection to the smoke-dried fish against insect pest.

The highest percentage of acid was found in *A. indica* oil, this indicates a higher rancidity than in the other oils. Acid value is used to determine the quality, storage ability, age, edibility and suitability of oils for use in industries for paint production (Demain, 1990).The acid values obtained in this study show that the oils have good storage ability. The higher the acid value of an oil, the lower its storage ability (Nielsen, 1998).

The refractive index of light in a vacuum is 1.00.The refractive indices of the oils in this study were greater than 1 which allows the oils to spread easily over the surface of the fish to which they are applied. Peroxide value is an indicator

of the deterioration of lipids due to oxidation at the double bond of an unsaturated fatty acid which causes rancidity. The low peroxide value associated with the oils of *A. sativum, C. nucifera, A. indica, Z. officinale* and *J. curcas* implies that its use to protect fish might prevent oxidative rancidity at room temperature (Anhwange *et al.,* 2005).

A higher saponification value was obtained in *C.nucifera* oil and *Z.officinale* oil than in the other oils. High saponification value indicated the presence of greater number of ester bonds, suggesting that the fat molecules were intact (Denniston *et al.,* 2004). This implies that they would serve as important raw materials for the manufacture of low toxic insecticides, cosmetics, lubricants and candles (Anhwange *et al.,* 2005).

Azadirachta indica oil had the highest fatty acid composition (93.05±3.94), this does not confer a more larvicidal and repellent power on the oil.This research findings shows the highest larval mortality caused by the oils of *A. sativum* and this was closely followed by *C. nucifera*, this could be ascribed to the presence of fatty acids in the oils, this include hexadecanoic acid (47.6%) found in *A. sativum* and linoleic acid (47.54%) found in *C. nucifera* which were the major composition of the oils. The fatty acids found in *A. sativum* oil have been reported to show larvicidal activities when isolated. Rahuman *et al*. (2000), it was reported that n-hexadecanoic acid in *Feronia limonia* dried leaves was toxic against fourth instar larvae of *Culex quinquefasciatus*, *Anopheles stephensi* and *A. aegypti*. Salud*et al*. (2011)reported that oleic, palmitic and stearic acids extracted from *Carica papaya* showed insecticidal activity against *Spodoptera frugiperda* (armyworm).Ramsewak *et al*. (2001) found that both linoleic and

oleic acids isolated from hexane of *Dirca palustris*(leatherwood) seeds were toxic when tested against fourth instar larvae of *A. aegypti* at 24hours exposure. Various amounts of oleic and linoleic acid were detected in the oils except *A. sativum* oil, the presence of the fatty acids could have contributed to theefficacy of the oils against *D. maculatus*. According to Rollo *et al.* (1995),oleic and linoleic acids have been indicated in death recognition and death aversion (repellency) in cockroaches.

The higher content of unsaturated fatty acids detected in the oils under study keeps them liquid at room temperature while the saturated fatty acids are very stable and less susceptible to oxidation. The detection of lauric, myristic, linoleic and palmitic acids in *C. nucifera* oil in this study, agrees with the findings of Rossell *et al.* (1985)and Makkar *et al*. (2010). These fatty acids could have repelled or killed the insect larvae that were exposed to them. Mohamad *et al.* (2013) reported 100% mortality of aphids at 0.5% concentration of lauricacid. The detection of hexane (which is said to have a disagreeable odour) in the oils of *A. indica* and *C. nucifera* is likely to have contributed to the repulsion of the larvae *D. maculatus.*

The tocopherols, triterpenes and sesquiterpenoids detected in *Z. offinicale* oil are natural antioxidants which are also found in most vegetable oils.Pentacyclo octacosa was also detected in the oil of *J. curcas* which might have been responsible for the toxicity of the oil to *D. maculatus* larvae. Shankaranarayana *et al.*(1980) reported and identified an insect growth-inhibiting properties and chemosterilant activity of a pentacyclic triterpenoid extracted from the bark of *Santalum album.* The presence of sesquiterpenoids in the oil of *Maclura*

pomifera (Osage orange) were repellent to German cockroach (Peterson *et al.*, 2000). Tetrahydrofuran was detected in *A. sativum* oil in this study, this compound showed insecticidal activity against the larvae of *Spodoptera exigua*(beet armyworm)by retarding their growth and also killing them (Rodriguez-Saona *et al*., 2000).

It has been established in this study that oils obtained from *A. sativum*, *A. indica*, *C. nucifera*,*J. curcas* and*Z.officinale*induce high mortality in the larvae of *Dermestes maculatus* infesting smoke-dried *P. annectens*,particularly at the highest tested concentration (0.256ml/g).The toxicity of the oils must be due to some of their components with insecticidal properties; these components may also act in synergy to enhance the toxic substances present in the oils either by increasing or prolonging their effects. The effectiveness of these plant oils may stem from their impact on the respiratory system of the insect larvae by blocking the spiracles and preventing oxygen inhalation, leading to death.Death of stored product pests due to asphyxia when oils are applied to grains has been reported by Cooping and Menn (2000).

The oil of *Allium sativum* was highly toxic against*D.maculatus*larvae,inducingthe highest mortality from the lowest (0.001ml/g)to the highest concentration (0.256ml/g) and resulting in 100% mortality atthe highest concentration. This finding agrees with that of Teklay *et al.* (2012) who reported that the bulb extract of *Allium sativum* was highly toxic to different insects compared to extracts from *Aloe vera* and *Azadirachtaindica*. Teklay *et al*. (2012)attributedthe insecticidal and toxic properties of garlic oilto its salphone hydroxyl ion which penetrates the blood-brain barrier and acts as a

poison to insects and evenhigher animal life. Douri *et al.*(2013) also reported 100% mortality of the eggs of *Callosobruchus maculatus*treated with the lowest concentration of *A.sativum* oil; they also observed that no adult*C.maculatus*emerged from the chickpeas at the different concentrations, although they emerged from the control seeds. They observed that *A.sativum* oil was toxic to*C.maculatus* at the larval and nymphal stages.

In this study, *A. indica* oil was the least effective of the oils although it was highly toxic at the highest concentration.The mode of action could be as a result of the oil blocking the intake of oxygen by *D. maculatus*which probably occurred when the larval cuticle became coated with the oil. The low mortality of*D. maculatus* larvae exposed to*A.indica* oil from the lowest concentration 0.001ml/g to 0.0064ml/g could be attributed to its slow effect on the larvae as the insects donot die immediately after the application of the oil.Schmutterer (1990) and Ascher (1993) reported that one of the major disadvantages of neem products compared to synthetic pesticides is the slow rate of mortality they induce on insect pests.The significant increase of insect mortality with increasing oil concentration observed in this study agrees with the finding of Ajao (2012) that neem seed oil substantially reduced oviposition in *D. maculatus* at the lowest concentration (53%) and totally inhibited oviposition at the highest concentration (99%).

The mortality of *D.maculatus* larvae on application of *J. curcas* oil observed in this study might be attributed to the fatty acid composition of the oil and chemicals such as terpenoids and triterpenes present in the oil. Adebowale and Adedire (2006) reported that *J.curcas*oil contained sterols and triterpene

alcohols which have insecticidal properties of the oil and that they also acted as growth inhibitors.They observed that *J.curcas* oil reduced the number of eggs laid by *Callosobruchusmaculatus* which they said was probably due to suffocation and/or lethal chemical poisoning of the immature forms by the oil. Nayak and Patel(2010) attributed the toxicity of the oil to the disagreeable odour of the *J. curcas* seed.

Cocos nucifera oil showed a high level of toxicity in this study. From analysis, lauric, myristic, linoleic, oleic and palmitic acids occur in *C.nucifera*, agreeing with the finding of Dales (1996). Dales (1996) noted that higher plants contain a vast amount of secondary metabolites including acids, alcohols, aldehydes, alkaloids, esters, fatty acids, flavones, glycosides, hydrocarbons, lactones, nitrogen-containing compounds, sterols, phenols and terpenoids, which are insecticidal.

Some species of the Zingiberaceae (Ginger) family have been used for medicinal purposes and some have been reported to have biologically active compounds (Ohsawa *et al.,* 1994). In this study, active compounds such as α-curcumene, β-farnesene, β-sesquiphellandrene and α-pinene were present in the oil of *Z.officinale*. This is in agreement with Purseglove (1972) who reported that *Z.officinale* containedsesquiterpenes carbon and that the pungent odour appeared to be responsible for its toxicity and repulsion in insect pests.According to Agarwal *et al.* (2001), ginger oil has insect growth regulatory and antifeedant activity against larvae of the arctiid *Spilosoma oblique* (Jute hairy caterpillar).

The oils of *A. sativum*, *C. nucifera*, *J. curcas*, *Z. officinale* and *A. indica*were significantly repellent to *D. maculatus*larvae in the treated fish.The

repulsionobserved in this study could be associated with the presence of monoterpenoids and sesquiterpenes. Curcumene and α-pinenethat were found in the oil of *Z.officinale*have been reported to repel ticks, mosquitoes and some insect pests. Tunon *et al.*(2006) reported that α-pinene which was isolated from the essential oil of *Dianthus caryophyllum*strongly repelled ticks. Weston *et al.* (1989) reported that the levels of curcumene in several accessions of *Lycopersicon hirsutum* (wild tomato)are positively correlated with the repellency of *L. hirsutum* foliage to*Tetranychus urticae.*

Theoils in this study showed different repellent activities, this may be as a result of the differences in the active compounds of the oils. This agrees with the finding of Ngamo *et al.*(2007) that the essential oils of different plants did not have same efficacy on the pests considered in their study and that the insects reacted differently to different oils. The rating of the plant oils as having good repulsive properties in this study is partly in agreement with Egwunyenga *et al.* (1998) who also attributed the repellency of *D. maculatus*to*Dennetia tripetala* from admixed fish to olfactory and gustatory sensations.

The repellent properties of *A.sativum, J.curcas* and *Z.officinale*on the larvae of *D. maculatus*could be attributed to their strong smell. Amusan and Okorie (2002) attributed the repellent properties of *Piper guineense* and *Dennetia tripetala* to their pungent smell, and also stated that the smell and pepperish taste could asphyxiate insects by blocking their spiracles.The repulsion of *D. maculatus* larvae by*A.indica* oil could be attributed to the chemosensory impact of neem oil on the olfactory or gustatory receptors and also the active

compounds of neem, particularly allelochemical azadirachtin that is well known for anti-insect properties (Mordue and Blackwell, 1993).

Some plant oils have been observed to interfere with basic metabolites and the biochemical, physiological and behavioural functions of insects. Insects inhale, ingest or absorb essential oils; the rapid action of these oils against some pests is indicative of neurotoxicity as there is evidence for interference with the neuromodulator octopaminen(Khater, 2012). Some essential oils are larvicidal and have the capacity to delay development and suppress the emergence of adults of insects (Khater and Shalaby, 2008).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The oils of*Allium sativum,Zingiber officinale, Jatropha curcas, Azadiractha indica* and *Cocos nucifera*differ significantly in their saponification, iodine, peroxide and acid values.The physicochemical properties of the oilsof*Allium sativum,Zingiber officinale, Jatropha curcas, Azadiractha indica* and *Cocos nucifera*indicate that they are non-drying, stable and have the capability of being storedwithout going rancid.

The oils of *Cocos nucifera,Zingiber officinale, Jatropha curcas, Azadirachta indica* and *Allium sativum* have larvicidal and repellent effects on the larvae of *D. maculatus*.The oil of*Allium sativum*causes the highest mortality and repellency whereas that of *Azadirachta indica*causes the least.The oils cause high mortality and repellency in the larvae of *Dermestes maculatus* at different concentrations.

The performance of the oils of *Allium sativum,Zingiber officinale, Jatropha curcas, Azadiractha indica* and *Cocos nucifera*are significantly different (P<0.05) from one another.The plant oil extractshave the potential of protecting smoke-dried fish against*D. maculatus* infestation and suppressing damage to the fish.

6.2 Recommendations

The most effective of the oils, *Allium sativum* and *Cocos nucifera* are recommended for the protection of smoke-dried fish against insect pest infestation, as good alternatives to synthetic insecticides. The toxicity of the oils on adult *Dermestes maculatus*, *Dermestes lardarius* and other pests of fish should be investigated. Further studies should be conducted to obtain information regarding the practical effectiveness of the oils under natural conditions. Also, further studies on toxicity of *Cocos nucifera* on other stored product pests is recommended since much work has not been done on the toxicity of this oil on insect pests.

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Appendix

Appendix I: Median Lethal Concentration of *Azadirachta indica*

Conc (ml/g)	Log of Conc.	Number Tested	Number Dead	Percentage Mortality	Abbott's Corrected Mortality	Empirical Probit of Kill
0.256	-0.59	60	60	100	100	∞
0.064	-1.19	60	12	20	18	4.08
0.016	-1.8	60	6	10	8	3.59
0.004	-2.4	60	4	7	5	3.36
0.001	-3	60	3	5	3	3.12
0.000		60	1	2	0	

Appendix II: Median Lethal Concentration of *Jatropha curcas* Oil

Conc (ml/g)	Log of Conc.	Number Tested	Number Dead	Percentage Mortality	Abbott's Corrected Mortality	Empirical Probit of Kill
0.256	-0.59	60	60	100	100	∞
0.064	-1.19	60	35	58	57	5.18
0.016	-1.8	60	14	23	21	4.19
0.004	-2.4	60	8	13	11	3.77
0.001	-3	60	5	8	6	3.45
0.000		60	1	2	0	

Appendix III: Median Lethal Concentration of *Zingiber officinale* Oil

Conc (ml/g)	Log of Conc.	Number Tested	Number Dead	Percentage Mortality	Abbott's Corrected Mortality	Empirical Probit of Kill
0.256	-0.59	60	60	100	100	∞
0.064	-1.19	60	23	38	37	4.67
0.016	-1.8	60	14	23	21	4.19
0.004	-2.4	60	6	10	8	3.59
0.001	-3	60	4	7	5	3.36
0.000		60	1	2	0	

Appendix IV: MedianLethal Concentration of *Allium sativum* Oil

Conc (ml/g)	Log of Conc.	Number Tested	Number Dead	Percentage Mortality	Abbott's Corrected Mortality	Empirical Probit of Kill
0.256	-0.59	60	60	100	100	∞
0.064	-1.19	60	53	88	88	6.18
0.016	-1.8	60	32	53	52	5.05
0.004	-2.4	60	13	22	20	4.16
0.001	-3	60	9	15	13	3.87
0.000		60	1	2	0	

Appendix V: Median Lethal Concentration of *Cocos nucifera* Oil

Conc (ml/g)	Log of Conc.	Number Tested	Number Dead	Percentage Mortality	Abbott's Corrected Mortality	Empirical Probit of Kill
0.256	-0.59	60	60	100	100	∞
0.064	-1.19	60	47	78	78	5.77
0.016	-1.8	60	22	37	36	4.64
0.004	-2.4	60	11	18	16	4.01
0.001	-3	60	7	12	10	3.72
0.000		60	0	0	0	