TOXIC AND REPELLENT EFFECTS OF SEED OIL OF FIVE PLANTS ON THE LARVAE OF *DERMESTES MACULATUS* (Degeer) IN TREATED SMOKE-DRIED AFRICAN CATFISH, *CLARIAS GARIEPINUS* (L.)

BY

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Declaration

I declare that the work in this thesis entitled "Toxic and repellent effects of the seed oil of five plants on the larvae of *Dermestesmaculatus* (Degeer) in treated smoke – dried African catfish, *Clarias gariepinus* (L.)" was conducted by me in the Department of Biological Sciences under the supervision of Professor S.J. Oniye and Dr. D.S. Abolude. The information derived from literatures has been duly acknowledged in the text and on the list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other University.

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Certification

This thesis entitled "Toxic and repellent effects of the seed oil of five plants on the larvae of *Dermestesmaculatus* (Degeer) in treated smoke – dried African catfish, *Clarias gariepinus* (L.)" is a study conducted in the Department of Biological Sciences, Ahmadu Bello University, Zaria by ONORIODE JOSEPH AKPOTU and it meets the regulations governing the award of Masters of Science (M.Sc) degree in Fisheries of the Department of Biological Sciences, Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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Dedication

This thesis is wholeheartedly dedicated to the Almighty God for his mercies and eternal faithfulness and for preserving my life to complete the programme. I give him all the glory.

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Abstract

This study was designed to investigate the toxicological effect of the seed oil of five plants namely; *Albizia lebbeck* (Woman's tongue), *Anacardiumoccidentale* (Cashew), *Citrulluscolocynthis* (Melon), *Citrullus vulgaris* (Watermelon) and *Khayasenegalensis* (Mahogany) on the larvae of *Dermestesmaculatus*. The oils of these plants were extracted by soxhlet extraction. The physicochemical properties of the oils were determined by standard procedure. The result showed that *K. senegalensis* had the least(2.71) while *C. vulgaris* had the highest value (8.98) of the acid value property. The iodine value was highest (114) for *A. lebbeck* and least (42.40) for *A. occidentale*. The peroxide value was highest (10.75) for *C.vulgaris* and least (2.94) for *C. colocynthis*. There was no difference in the relative densities and also in the refractive indices of all the oils. The fatty acid composition of the oils was determined by GCMS procedure. Linoleic acid was present in all the oil samples analyzed. Behenic acid, Linolenic acid, Palmitoleic acid and Lignoceric acid were only seen in *A. occidentale*. Arachidic acid was present in *A. lebbeck* and *A. occidentale*. Oleic acid was present in *A. occidentale* and *K. senegalensis*. Palmitic acid was present in all the oils of these plants while Stearic acid was absent in *C. vulgaris*. The result showed that highest value of repellency rate (77.78%) was obtained in *A. occidentale* while the lowest value of repellency rate (44.44%) was obtained for *C. colocynthis*at 1.38ml application rate. The result also indicated that repellency was absolute (100%) at application level of 4.13ml of all the oils tested. The mortality effect of cashew.13ml and lowest (44.44%) at 0.46ml application rate. The interaction effect of 4.13ml of all the five seed oils at 96h exposure gave absolute kill (100%) at $p<0.05$. The interaction effect of 1.38ml cashew seed oil

and 72h exposure gave 87.78% kill, which was significant ($p<0.05$) greater than the percent kill of the other concentrations and exposure period. Similarly, the *C. colocynthis* and *A. lebbeck* at 1.38ml killed 81.11% of *D. maculatus* larvae, seed oil at tested applications against the insect larvae was dose dependent, highest (100%) at 4which was significant ($p<0.05$) compared to the control and the other concentrations of the same oil used in the experiment. *C. vulgaris* gave 91.11% kill at the same application level. The exposure of *D. maculatus* larvae to the fish treated with 1.38ml of *K. senegalensis* seed oil for 72h had a significant effect on its mortality (86.67% kill). The observed insecticidal effect of these oils on exposed *D. maculatus* larvae was dose and exposure dependent, *A. occidentale* had the greatest mortality effect while *C. colocynthis* had the least effect. No significant difference was observed in the performance of *K. senegalensis* and *A. lebbeck* seed oils which were significantly higher than that recorded for *C. vulgaris*. It is concluded that the seed oils of *A. lebbeck*, *A. occidentale*, *C. colocynthis*, *C. vulgaris* and *K. senegalensis*have repellent and larvicidal effect on the larvae of *D. maculatus*.

CHAPTER ONE

1.0 INTRODUCTION

In recent years, the fishery sector has been a source of income and livelihood for millions of people around the world. FAO (2010) reported that capture fisheries and aquaculture constitute the sources of fisheries to humanity and that Inland fisheries are a vital component in the livelihoods of people in many parts of the world, in both developing and developed countries. However, irresponsible fishing practices, habitat loss and degradation, water abstraction, drainage of wetlands, dam construction, and pollution (including eutrophication) often act together, thereby compounding their effects resulting in substantial decline and other changes in inland fishery resources.

Aquaculture continues to be the fastest growing animal food-producing sector which is set to overtake capture fisheries as a source of food fish (FAO, 2010). Fish is a highly perishable commodity, but with significant capacity for processing to lengthen its shelflife, availability and relevance to consumers. However, developing countries still lack adequate infrastructure including hygienic landing centres, electric power supply, potable water, roads, long supply chains as well as services such as ice, ice plants, cold rooms and refridgerated transport. These factors linked with tropical temperatures cause a high percentage of post-harvest losses and quality deterioration with consequent risk to the health of consumers. Due to inadequate infrastructures such as hygienic landing centres, electric power supply, e.t.c and established consumer habits, fish in developing countries is traded primarily in live or fresh form or after curing through drying, smoking or fermentation (FAO, 2010).

The high cost of beef has made low income earners to patronize fish as an alternative source of cheaper animal protein; this accounts for the growth recorded in the fishery industry, particularly in aquaculture in recent ten years in a country like Nigeria (FAO, 2003). In addition, demand for smoked fish abroad particularly in the United Kingdom has remained constant throughout the year (FAO, 2003). These fishes however are susceptible to post – harvest deterioration by high tropical temperatures and microorganisms if not quickly consumed. Nigeria accounts for 30 – 40% post – harvest

losses of landed catches of fish, for this reason, landed catches of fish are subjected to a variety of processing methods; one of such is fish smoking and drying. Forty five percent (45%) of total fish catch in Nigeria are utilized as smoked fish and it is one of the most widely distributed and cheapest animal protein products in Nigeria (FAO, 2002; 2004).

Stored products like grains, cheese, hide, fur, bacon, dried fish, meat, and other protein-containing concentrates, have been known to be destroyed by insect pests. Insect infestation of cured fish by blowflies and hide beetles is an important cause of post – harvest losses in many developing countries (Johnson and Esser, 2000). Fish is susceptible to attack by insect pests throughout processing and storage. The principal pests are blowflies (Diptera: Calliphoridae and Sarcophagidae) and hide beetles (Coleoptera: Dermestidae and Cleridae). Losses caused by infestation could be physical, economical and nutritional in nature (Johnson and Esser, 2000).

During the onset of the rainy season, dermestid pests move in-door unto their substrate, find mating partners, mate, make a crack on the substrate and lay eggs. In most cases, the larvae that result can be extremely destructive. The humid conditions in the tropics favour infestation by dermestids which is a group of important pest of smoke – dried fish. According to Osuji (1974) and Awoyemi (1989), large scale deterioration in quality and quantity of dried fish is attributed to dermestid infestation. Veer *et al*.(1996) reported that larvae of *Dermestes maculatus* can cause considerable damage to timber, cork, plaster, linen, and cotton when they bore into these materials to pupate.

Prominent insecticide families used in the control of pest of stored products include organochlorines, organophosphates, and carbamates. Organochlorine hydrocarbons (e.g. DDT) could be separated into dichlorodiphenylethanes, cyclodiene compounds, and other related compounds.They operate by disrupting the sodium/potassium balance of the nerve fiber, forcing the nerve to transmit continuously. Their toxicities vary greatly, but they have been phased out because of their persistence and potential to bioaccumulate (Kamrin, 1997).Most pesticides work by poisoning pests.Systemic insecticides, which poison pollen and nectar in the flowers may kill bees and other needed pollinators (Pettis *et al*., 2013).Some insecticides kill or harm other creatures in

addition to those they are intended to kill. For example, birds may be poisoned when they eat food that was recently sprayed with insecticides or when they mistake insecticide granules on the ground for food and eat it.Some insecticides have been banned due to the fact that they are persistent toxins which have adverse effects on animals and humans.Sprayed insecticides may drift from the area to which it is applied into wildlife areas, especially when it is sprayed aerially.The high cost of synthetic pesticides, the danger of pesticide misuse and toxic residues in food have resulted in rapid development and assessment of plants as alternatives for chemical control of stored product pests (Shaaya*et al.,* 1997).

1.1 Statement of Research Problem

Smoke drying of fish is a valuable method of preserving fish against spoilage. The heat and dryness associated with hot smoking reduces the water activity of the food (fish) thereby limiting microorganisms, the prerequisite for spoilage. However, smoke – dried fish are vulnerable to insect pest attack, thereby reducing their economic value generally.

Several coleopteran insects have been notable threats of stored products.Unlike most Dermistidae, Khapra beetle will attack almost any kind of material such as dried blood, fish meal, dried milk, goat skin, wool, etc. They can feed on products with as little as 2% moisture content. *Dermestes spp*, especially its larvae, can seriously damage natural animal fibers, foodstuff, furnishing and zoological collections.The eating habits of *Dermestes maculatus*can cause a dead animal to become just a skeleton (http://en.wikipedia.org/wiki/Dermestes_maculatus).This makes *Dermestes maculatus*to constitute the most insect pest of smoke – dried fish.

Synthetic chemical insecticides (Malathion, Phosphine, Lindane, DDT, e .t. c) often used to control *D. maculatus* infestation of smoked fish could result in human poisoning and other objectionable food and health hazards. Jurewicz and Hanke (2008) reported that children's exposure to pesticides may bring about impairments in their neurobehavioral development. Children exposed to organophosphate pesticides (OP), both prenatally and during childhood, may have difficulties

performing tasks that involve short-term memory, and may show increased reaction time, impaired mental development or pervasive developmental problems. In newborns, the effects of OP exposure are manifested mainly by an increased number of abnormal reflexes, while in adolescents, by mental and emotional problems.Acute health problems may occur in workers that handle pesticides, such as abdominal pain, dizziness, headaches, nausea, vomiting, as well as skin and eye problems (Ecobichon, 1996).

Smoke – dried fish treated with synthetic chemicals could also cause poisoning when employed in feed formulation thereby leading to economic loss in the livestock industry. This arises from a long-term low-level exposure, which livestock are exposed to from sources such as pesticide residues in food as well as contact with pesticide residues in the air, water, soil, sediment, food materials, plants and animals.

1.2 Justification of the Study

Attack on smoked catfishby*D. maculatus*and*N. rufipes*significantly reduces thequantity and quality of fish flesh destined for both humanconsumption and economic purposes. Osuji (1974)reported that about71.5% of dried fish infestation was caused by*D. maculatus*. Inaddition, adult*Dermestes*and*Necrobia*can fly and are thuseasily dispersed to new sources of food where they cause fooddamage, and their infestation also predisposes the infested fish to microbial attack (Adedire and Lajide, 2000). Therefore control of *D. maculatus* infestation of smoke – dried fish is vital for economic sustainability in the fish industry worldwide. This is necessary to reduce fish wastage and enhance the availability of protein sources to humanity.

Several plant oils as well as powders tested against insect pests of smoke – dried fish were effective as protectants (Akinwumi *et al*., 2007; Amusan and Okorie, 2002). These toxicological studies of plant oils to economic pest like *D. maculatus* would expand the range of tools available for pest management and also establish larvicidal prospect of plant oil in the management of *D. maculatus* infestation of smoke – dried fish. This could provide the much needed replacement of the use of synthetic chemicals to protect human food.

To stem the dangers associated with the misuse of chemicals in edible fish protection, there is need to find alternatives to synthetic insecticides and these alternatives could be derived from the fixed oils in edible plant seeds hence the need for this research on the larvicidal prospect of seed oils against *D. maculatus.*

This study may therefore identify natural fixed oil from plants that are lethal and repellent to *D. maculatus* larvae with the ability of protecting smoke – dried fish in a cheaper, safer and eco – friendly manner than synthetic chemicals.

1.3 Aims of the Study

The general aim of this study is to determine the toxicological effect of the seed oil of five plants,*Khaya senegalensis* (Mahogany), *Albizia lebbeck* (Woman's tongue), *Anacardium occidentale* (Cashew), *Citrullus colocynthis* (Melon), and *Citrullus vulgaris* (Watermelon) on the larvae of *Dermestes maculatus.*

1.4 Objectives of the Study

The specific objectives of this study include:

●To determine the fatty acid composition and physical properties of the seed oils of *Khaya senegalensis*, *Albizia lebbeck*, *Anacardium occidentale*, *Citrullus colocynthis* and *Citrullus vulgaris.*

●To determine the larvicidal and repellent effect of *Khaya senegalensis*, *Albizia lebbeck*, *Anacardium occidentale*, *Citrullus colocynthis* and *Citrullus vulgaris* on the larvae of *D. maculatus.*

●To compare the quality of the seed oils of *Khaya senegalensis*, *Albizia lebbeck*, *Anacardium occidentale*, *Citrullus colocynthis* and *Citrullus vulgaris*as repellent and larvicide on the larvae of *D. maculatus.*

1.5 Hypotheses

●The seed oils of *Khaya senegalensis*, *Albizia lebbeck*, *Anacardium occidentale*, *Citrullus colocynthis* and *Citrullus vulgaris*do not differ in fatty acid composition and physical properties.

●The seed oil of *Khaya senegalensis*, *Albizia lebbeck*, *Anacardium occidentale*, *Citrullus colocynthis* and *Citrullus vulgaris* has no larvicidal and repellent effect on the larvae of *D. maculatus.*

●There is no significant difference in the quality of the seed oils of *Khaya senegalensis*, *Albizia lebbeck*, *Anacardium occidentale*, *Citrullus colocynthis* and *Citrullus vulgaris*as repellent and larvicide on the larvae of *D. maculatus.*

CHAPTER TWO

2.0 LITERATUREREVIEW

2.1 Medicinal Plants

Over the years, there has been a growing concern about the high cost of synthetic pesticides, the danger of pesticide misuse and toxic residues of these synthetic products in food. Also, the number of fumigants used for insect control has decreased drastically due to the development of resistance by the target insects and the mounting social pressures against the use of toxic chemicals have limited the introduction of new compounds (Snelson, 1987). Methyl bromide and phosphine are the two officially recognized fumigants in use in Nigeria as at now with both having attendant consequences; methyl bromide is reported as being responsible in the ozone layer depletion (WMO, 1995) while phosphine have been reported to have mutagenic effects on occupational fumigators (Garry *et al*., 1989). As a result of drawbacks in the use of conventional fumigants, efforts are geared towards the development of new non – toxic compounds, preferably from plant origin, for control purposes. Unlike synthetic chemical insecticides that kill both pests and non – target organisms, natural insecticides including botanicals are relatively target specific (Isman, 1997). These have led to the search for plants which have toxic properties, environmentally friendly, easily biodegradable and that can serve as ready alternative for the control of pests of stored products (Shaaya *et al*., 1997).

Plant materials such as spices, vegetable oils, extracts, powder or ash (Keita *et al*., 2001; Akinkurolere *et al*., 2006; Adedire *et al*., 2011) have been reported for their insecticidal efficacy. The efficacy of roots, leaves and bark of many botanical plants with medicinal properties have been tested and still being tested against insect vectors of disease and economically important pest that destroy stored products like grains, cheese, dried fish and dried meat (Don Pedro, 1989, 1990; Okorie *et al*., 1990). Recently, essential oil extracts from these plants have taken a leading role in toxicity research.

2.1.1 *Citrullus vulgaris* and *Citrullus colocynthis*

Cucurbitaceae is a large plant family which consists of nearly 100 genera and 750 species. This plant family is known for its great genetic diversity and widespread adaptation in tropical and subtropical regions, arid deserts and temperate locations (Oluba *et al.,* 2008b). Curcubits are known for their high protein and oil content. Seeds of curcubits are sources of oils and protein with about 50% oil and up to 35% protein (Achu *et al.,* 2005). Specifically for these reasons they are cultivated and consumed world over. Eguisi (*Citrullus colocynthis* L.) and watermelon (*Citrullus vulgaris* L.) belongs to the species of the genus *Citrullus* of curcubitaceae family, which usually consists of a large number of varieties that are generally known as melons, (Mabaleha *et al.,* 2007). Watermelon is the most popular fruit in Serbia, grown for its fruit, while Eguisi is among the 300 species of melon found in tropical Africa and it is cultivated for its seeds, which are rich in oil (53%) and protein (28%) (Ntui *et al.,* 2009). The regions of its cultivations are Middle East, West Africa (Nigeria, Ghana, Togo, Benin) and other African countries for the food in the seeds and as a crop inter – planted with maize, cassava and yam (Uruakpa and Aluko, 2004). In Nigeria only, Eguisi is cultivated over an area of 361,000 ha with a production figure of 347,000 tonnes (as seeds) in 2002. It is used both as condiment and thickener in various Nigerian local soups.

Watermelon is thought to have originated in southern Africa, where it is found growing wild. It reaches maximum genetic diversity there, with sweet, bland and bitter forms. In the 19th century, Alphonse de Candolle claimed the watermelon was indigenous to tropical Africa. Though *Citrullus colocynthis* is often considered to be a wild ancestor of watermelon and is now found native in north and West Africa, it has been suggested on the basis of chloroplast DNA investigations that the cultivated and wild watermelon diverged independently from a common ancestor, possibly*C. ecirrhosus* from Namibia(http://en.wikipedia.org/wiki/Citron_melon).Watermelon is mildly diuretic and contains large amounts of beta carotene. Watermelon with red flesh is a significant source of lycopene. Preliminary research indicates the consumption of watermelon may have antihypertensive effects (http://en.wikipedia.org/wiki/Watermelon).

2.1.2 *Albizia lebbeck*

Albizia lebbeck is a species of *Albizia* native to tropical southern Asia, and widely cultivated and naturalized in other tropical and subtropical regions. English names for it include Lebbeck, Lebbek tree, Flea tree, Frywood, Koko and Woman's tongues tree. The later is a play on the sound the seeds make as they rattle inside the pods. Being one of the most widespread and common species of *Albizia* worldwide, it is often simply called "siris" though this name may refer to any locally common member of the genus (USDA, 1994). The fruit is a pod 15–30 cm long and 2.5-5.0 cm broad, containing six to twelve seeds (Ali, 1973). Lebbeck is an astringent, also used by some cultures to treat boils, cough, to treat the eye, flu, gingivitis, lung problems, and pectoral problems, is used as a tonic, and is used to treat abdominal tumors (Duke, 2008). The bark is used medicinally to treat inflammation (Lowry *et al*., 1994).

2.1.3 *Khaya senegalensis*

Khaya senegalensis (also called African Mahogany, Benin Mahogany, Dry Zone Mahogany, Senegal Mahogany or Cailcedrat) is a species of plant in the Meliaceae family. It is found in Benin, Burkina Faso, Cameroun, Central African Republic, Chad, Ivory Coast, Gabon, Gambia, Ghana, Guinea, Guinea – Bissau, Mali, Niger, Nigeria, Northern Australia, Senegal, Sierra Leone, Sudan, Togo, and Uganda. The bark is often harvested as a traditional medicine (Joffe, 2007). It is recognized by its round evergreen crown of dark shiny foliage, pinnate leaves and characteristic round capsules (Irvine, 1961; Keay *et al*., 1989). The bark of the tree is very bitter and is extensively used for the treatment of fever and dressing ulcers on the backs of sheep, carmels and horses. The flowers are used for treating stomach diseases, and as an ingredient in antisyphlytic prescriptions. The oil from the seeds is used as emmenegogue (Daziel, 1956; Watt and Breyer-Brandwijk, 1962).

Some limnoids have been isolated from the stems, barks, leaves and flowers of *K. senegalensis*. They include phragmalin limnoids named khayanolides D and E, khayanosides, 2,6-dihydrofissinolide and two mexicanolides named khayanone and 2-hydroxyseneganolide (Olmo *et al*., 1997; Khalid *et al*., 1998; Nakatani *et al*., 2002).

Abdelgaleil *et al*. (2004) also reported the isolation of three other mexicanolide limnoids named seneganolide A, 2-hydroxyseneganolide A and 2-acetoxyseneganolide A. these limnoids have a wide range of biological activities, including insect antifeeding and growth-regulating properties, and medicinal activities in humans and animals. They also possess antiviral, antifungal and bactericidal properties (Abdelgaleil *et al*., 2001; Abdelgaleil and Nakatani, 2003; Ademola *et al*., 2004).Traditional healers in some parts of northern Nigeria use the seed oil to treat diabetes mellitus.

2.1.4 *Anacardium occidentale*

The cashew, *Anacardium occidentale*, is a tree in the family Anacardiaceae. Its English name derives from the Portuguese name for the fruit of the cashew tree, caju, which in turn derived from the indigenous Tupi name, acajú which means "nut that produces itself". Originally native to Northeastern Brazil, it is now widely grown in tropical climates for its cashew apples and nuts. The name *Anacardium* actually refers to the shape of the fruit, which looks like an inverted heart (ana means "upwards" and -cardium means "heart"). The fruit of the cashew tree is an accessory fruit (sometimes called a pseudocarp or false fruit). What appears to be the fruit is an oval or pear-shaped structure, a hypocarpium that develops from the pedicel and the receptacle of the cashew flower. Called the cashew apple, better known in Central America as "marañón", it ripens into a yellow and/or red structure about 5–11 cm long. It is edible, and has a strong sweet smell and a sweet taste (http://en.wikipedia.org/wiki/Cashew).

Within the true fruit is a single seed, the cashew nut. Although a nut in the culinary sense, in the botanical sense the nut of the cashew is a seed. The seed is surrounded by a double shell containing an allergenic phenolic resin, anacardic acid, a potent skin irritant chemically related to the better-known allergenic oil urushiol which is also a toxin found in the related poison ivy (Azam-Ali and Judge 2004). The cashew nutshell liquid (CNSL), a byproduct of processing cashew, is mostly composed of anacardic acids (70%), cardol (18%) and cardanol (5%). These acids have been used effectively against tooth abscesses due to their lethality to a wide range of Gram-positive bacteria. Seeds are ground into powders used for antivenom for snake bites. The nut oil is used topically as an antifungal and for healing cracked heels (Akash *et al*., 2009).

2.2 Distribution of *Dermestes maculatus*

Dermestes maculatus is native throughout the continental United States and Canada, and also Hawaii. It is also known to occur in Oceania, Southeast Asia, and Italy (Integrated Taxonomic Information System, 2009, Veer *et al.*, 1996). Several different species of *Dermestes* have been recorded infesting cured fish or fish meal of which *Dermestes maculatus*, *D. frischii* and *D. ater* are the common species in developing tropics (Sub-sahara Africa) (Haines and Rees, 1989).

2.3 Description of *Dermestes maculatus*

The bodies of the larvae are covered in rows of hairs of different lengths, called setae. The underside of the abdomen is typically yellowish-brown while the dorsal surface is typically dark brown, usually with a central yellow line. Two long horn-like protrusions are located on the upper surface of the last segment, partially hidden by surrounding hairs (Haines and Rees 1989). The protrusions, called urogomphi, curve upward and away from the tip of the abdomen. This distinguishes the larvae from larvae of *Dermestes lardarius*, which has the urogomphi curving downward toward the tip of the abdomen (Hedges and Lacey, 1996).

The pupae are an oval shape, usually smaller than the larvae, and do not have the many long hair-like projections (Kulshrestha and Satpathy, 2001). *Dermestes maculatus* adults range in size from 5.5 to 10.0 mm. Each side of the thorax has a band of white hairs. The underside of the abdomen is primarily white with black spots at the sides, and a large black patch on the last segment. The elytra are dark brown or black, with hairs that are mostly black, yellow, or white. The antennae are short and segmented with a club at the tip. The edges of the abdominal end of the elytra are serrated and end in a small spine projecting straight out (Haines and Rees 1989, Hinton 1945). According to Schroeder *et al*. (2002), frass or feaces from *D. maculatus* appear dark brown, fibrous and resemble horse hair and the evidence of it can indicate the past presence of beetles.

2.4 Life Cycle of *Dermestes maculatus*

Pheromones secreted by males are used to attract females. Males and females will mate multiple times and the female will lay eggs within 24 hours of the first mating (Jones *et al*., 2006). Eggs are laid in cracks of the matter on which they are feeding (Haines and Rees, 1989). Larvae will pass through five to eleven instars, the number of instars increasing with unfavourable conditions (Haines and Rees, 1989; Hinton, 1945). During the last 10 days of the final instar, the larvae will seek out a place to pupate, typically within the meat or a non – food substance such as wood. Larvae without a suitable place to pupate can delay pupation by over 20 days, but at the cost of lower adult body mass and increased risk to fatal disease (Archer and Elgar, 1998). Once adults, the beetles can disperse to other food sources by flying (Haines and Rees, 1989). Adult beetles typically live between four to six months.

The development of *D. maculatus* in dried fish was studied under uncontrolled laboratory conditions in Nigeria (Osuji, 1975d). Females laid eggs within 12 h of copulation (Fig. I) and oviposition was improved by the presence of free water. Hatching occurred about 48 h after oviposition. Larval development was completed in 33.5 days, during which seven moults occurred and a body length of 14mm was attained. It was also observed that crowding prolonged larval life. When intact pieces of fish were available, the last-instar larva bored into one of them and pupated within the hardened larval skin, but when ground fish was provided, a quiescent pre-pupal stage was observed. The adult emerged about 11 days after the end of the last larval instar, irrespective of the mode of pupation. A comparative assessment of the biological performance of *D. maculatus* in various dietary media namely dried fish, fish meal, bone meal, palm kernel meal, blood meal, and whole meal has been made (Osuji, 1978) and the dried fish followed by fish meal was found to be significantly superior to the commercial feeds. He suggested that the greater suitability of the dried fish diet for the development and biological performance of the beetle might be attributable to its superior nutritional composition in respect of crude proteins, total lipids, and water content, among other factors. Some aspects of the life patterns of *D. maculatus* and *Dermestes lardarius*, had been described by Schmidt (1974).

He observed that the beetles and larvae consumed mainly pure fats or food substances with fatty components. The hair like sense organs in the antennae was used for the reception of smell. Bhuiyan and Saifullah (1997) studied some aspects of the biology of *N. rufipes* using a mixture of dried fish and copra as food. The average longevity of adults was found to be 60.64 ±39.46 days for females and 49.42 ±18.2 days for males. The mean fecundity recorded was 89.7±17.8 eggs with arrangement of 0-350 eggs per female. The hatchability was 89.59±7.27 percentages. Egg laying continued until the death of the female.Azab *et al*. (1973 a) reported from Egypt that*D. maculatus* when reared in the laboratory on dried meat, 5-6 overlapping generations developed in a year. Both males and females paired several times, and the females laid their eggs singly or in groups of up to 25. Adult males and females lived for up to 189 and 178 days, respectively in autumn (21.5˚C). The sex ratio was 1:1.

Nath and Pande (1996) examined the biology of *D. maculatus* under uncontrolled laboratory conditions on dried fish in north –east India and reported that pre-oviposition, oviposition and post-oviposition periods lasted for 5 to 6 days, 40 to 47 days, and 8 to 10 days, respectively. The fecundity varied from 29 to 95 and spectacularly improved by the addition of wet cotton. The egg stage lasted for 36 to 48 hours. Larvae underwent 7 instars and the development was completed in 25 to 30 days. Larval duration was prolonged by crowding. Pupation took place in the crevices of the fish body and lasted for 5 to 7days. Adults were long-lived and polygamous.

Roesli and Subramanyam (2002) noted the red-legged ham beetle (RLHB; *Necrobia rufipes*), a pest of stored products such as copra, ham, cheese, dried fish, and other protein-rich foods noting that *N. rufipes* infests various commercially important stored

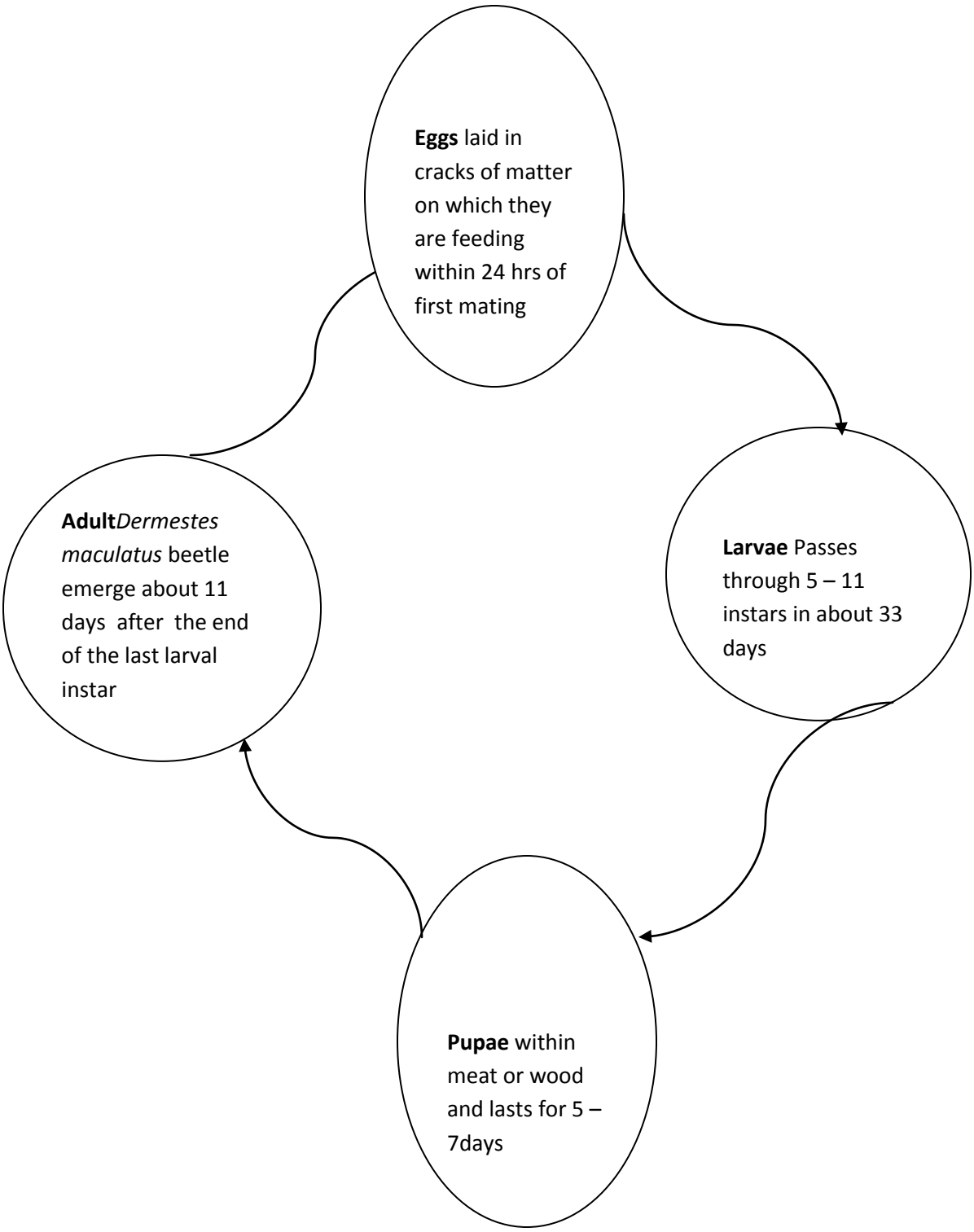


Figure I: Life cycle of *Dermestes maculatus* Degeer

commodities in Nigeria. Development was completed in dried fish (where it was most rapid), palm kernels, groundnuts, and copra but not in cacao beans or maize. A survey in the market in Ibadan, Nigeria in the period from January 1971 to July 1972 showed that Coleoptera, especially *Dermestes maculatus* (which accounted for 71.5% of the observed infestation) and *N. rufipes* (28.0%), infested a high proportion of the dried fish sold. Although both species were abundant throughout the year, infestation was highest in the hot dry months and lowest in the rainy ones. *Tribolium castaneum* and *Trogoderma granarium* were found in considerable numbers. (Osuji, 1974a).

When samples of dried fish of the genera viz. *Citharinus*, *Clarias*, *Heterotis* and *Synodontis* on sale in the market in Ibadan, were examined the numbers of examples of *D. maculatus* and *N. rufipes* found per 100 g fish averaged 6.4 (1.53), 59.5 (24.5), 33.7 (19.2) and 29.5 (9.65), respectively . The lipid contents of the samples averaged 12.29, 16.64, 12.87, and 13.42%, for the four genera respectively (Osuji, 1974 b).

Occurrence of *N. rufipes* damaging cashew nut was recorded by Pratissoli (1997). In a survey of godowns in Kerala, India, *N. rufipes* and *O. surinamensis* were found to be the most important pests (Kumari *et al*., 1992). Investigations on insect pests of stored oil-palm kernels (Allotey and Kumar, 1989) established the value of *N. rufipes* as a predator. *N. rufipes* was also found in a museum infesting the crevices and spongy parts of the bones of a recently prepared skeleton of a whale at Kozhikode, Kerala. (Adolph and Soans, 1969). The insect fauna on stored palm produce was studied in Nigeria in a transit shed (Allotey, 1988). The pooter search method revealed individuals of the clerid *N. rufipes* throughout the year, despite fumigation with phostoxin (aluminium phosphide).

2.5 Effect of Temperature and Humidity on Development andFecundity of *Dermestes maculatus*

Toye (1970) studied the humidity and temperature reactions of adult *D. maculatus* Deg. with reference to infestation on dried fish and found the adult to have stronger

preference than larvae for the higher humidity. In an investigation on the development, fecundity and longevity of *Demestes ater* at various combinations of temperature and humidity, the last larvae and adults were found to avoid a temperature range of 30-45ºC. Longevity recorded a maximum of 100 days at 25ºC and the fecundity was very variable (0-135) as was the percentage hatch (0-69 %) (Coombs, 1981). Both *Dermestes haemorrhoidalis* and *D. peruvinus* (Coombs, 1979) developed at all humidities tested at 25ºC and the rate of oviposition found to be diminishing with age of female. Howe (1953) observed that relative humidity has a marked effect than temperature on the development of *Dermestes frischii*. Pupal period was shortest at 37ºC.

Life cycle of *Dermestes lardarius* was completed in 223 days at 17.5 and 20ºC, but at 30ºC it took only 55 days. Temperature had a great influence on the number of eggs laid. The total yield of eggs reached a peak of 1261 at 20ºC and then declined to 52 at 27.5. No eggs were laid at 30ºC. Optimal temperature for egg laying in the species was found to be 20ºC (Jacob and Fleming, 1984a). An investigation into the effect of illumination intensity on the response of the hide beetle, *D. maculatus*, to aggregation pheromone (Rakowski, 1988) revealed that the intensity of light do play an important role in the behaviour of *D. maculatus*. Larval and pupal development at different moisture levels and on various media were examined (Scoggin and Tauber, 1951) and the results established that when the water content was maintained between 10-15 percentages, larval mortality was lowered and the number of larval instars and duration of larval development decreased, and larger adults emerged. An inquiry was made into the effect of temperature and relative humidity upon the development and fecundity of *D. lardarius* (Coombs, 1978) suggested that development of the female *D. lardarius* took longer than that of the males. Shortening of larval period was observed at high humidities. Development was more rapid at higher temperature Unsuccessful pupation was the rule at 15ºC and at 30ºC none of the adult pairs laid eggs.The fertility of the eggs was very variable and only of the order of 50 percentages.

The duration and viability of the egg stage of *D. maculatus* were determined using a wide range of constant temperatures and humidities (Jacob and Fleming, 1985) Temperatures over which the eggs hatched greatly influenced the duration of the egg stage but had little effect on viability, except at the lowest temperatures studied (15 and

17.5°C). The mean duration of the egg stage varied from about 2 days at 37.5 °C to up to 17 days at 15°C. Humidity had little effect on the incubation period except at the lowest temperatures, but had a marked effect on viability. Viability was highest at 90% relative humidity and lowest at 20% relative humidity. The duration of development, pattern of egg laying, and fecundity of *Dermestes haemorrhoidalis* were investigated on a fishmeal diet at various temperatures and relative humidities (Jacob and Fleming, 1984 b). At 65% R.H., eggs failed to hatch at temperatures below 15 or above 32.5°C; the mean duration of the egg stage varied from 2.6 days at 32.5°C to 11.6 days at 15°C. At 25°C, neither the duration of the egg nor egg viability appeared to be affected by humidities in the range 20-90%. At 65% R.H., there were 6-8 larval instars at 20 and 25°C and 7-9 at 30°C. Humidity also affected the number of instars. The shortest mean larval developmental period was 37.3 days at 30°C, 65% R.H, and the longest was 76.0 days at 20°C, 65% R.H. Adults were comparatively long-lived and laid higher numbers of eggs for longer periods at 20°C than at other temperatures. Below 20°C, fewer eggs were laid, and above this temperature adult lifespan and fecundity declined. The life-span and fecundity of mated pairs of adults of *Dermestes lardarius* were compared at 25°C and 65% R.H. when water was not provided, given once a week, once a day, or supplied continuously. It was found that the moisture content of the food alone did not support adult life for long. Drinking water was required at least once a week to achieve the maximum life span, and was needed more often if females were to be more productive. The longest oviposition periods were obtained when water was provided most often (Jacob and Fleming, 1982).

Thornton (1981) evaluated the effect of temperature on the growth and development of a South African strain of *D. maculatus*. At constant temperatures of 20-35°C, the incubation period averaged 6.7-2.1 days, and the larval, pre-pupal and pupal periods averaged 43.9-20.8, over 10 to 4, and 11.1-6.0 days, respectively. No pupae formed at 20°C. Females kept at 35°C laid an average of 605 eggs, as compared with 1129 – 1464 at lower temperatures. Egg mass was inversely related to temperature, averaging 0.33 mg at 20°C and 0.18 mg at 35°C. Adult females of *D. lardarius* maintained at 27.5°C and 80% R.H. began to lay eggs when 10-21 days old, continued laying for 7-21 days, and laid up to 17 eggs, of which less than 50% hatched. At 20°C and 80% R.H., most

females began to lay when 57-101 days old, and produced 14-58 eggs in 14-119 days, about 50% of them fertile. At 25°C and 80% R.H., most females laid some eggs when a few days old; there was then a gap of 14 weeks or more before the rest of the eggs were laid. Lower humidity (65% R.H.) appeared to eliminate early oviposition (Jacob and Fleming, 1981). Fleming and Jacob (1986) studied the influence of temperature and relative humidity upon the number and duration of larval instars in *D. lardarius*.

Under most conditions and, contrary to the findings of other workers, number of instars varied. At 65% R.H., the number of instars increased from the usual 6 at 20°C to 8 at 27.5°C. At 25°C, the number of instars decreased from 8 at 50% R.H. to 6 (for most larvae) at 80% R.H. At 65% R.H., the shortest mean larval development period was 51.5 days (at 27.5°C) and the longest was 109.7 days (at 17.5°C). Mortality was high at 17.5 and above 22.5°C. It was concluded that the optimal conditions for development were 20-22.5°C and 65% R.H. Larvae of *D. lardarius* were bred at 80% R.H. and 15 or 30°C on a diet of fishmeal, yeast and cholesterol. Adults obtained at 15°C were morphologically normal and lived up to 211 days; most females failed to lay eggs but 2 laid a small number (4 and 12) of infertile eggs after 89 days. At 30°C, adults lived only up to 36 days and none of the females laid eggs. Larvae were also reared at 65% R.H. and 15 or 30°C until they pupated and then they were transferred to 20 and 25°C, respectively. Adults at 25°C lived up to 61 days but laid no eggs. Adults obtained at 20°C were morphologically normal but very small in size and lived up to 165 days; mating between them was infertile (Jacob and Fleming, 1980a). Duration of the egg stage of *D. lardarius* and percentage hatch at various combinations of temperature and relative humidity were examined (Jacob and Fleming, 1980 b). As the temperature increased from 15 to 32.5°C, the duration decreased from 12.9 to 3.0 days and percentage hatch from 50.8 to 12.5. Relative humidity had little effect on either duration or percentage hatch. When eggs were kept only at 25 and 30 °C and 65 and 90% R.H., percentage hatch ranged from 34 to 44. Study on the biology of the beetle, *D. maculatus* was conducted (Pisfil and Korytkowski, 1974) at 24°C and 70% R.H, fishmeal in the form of powder or pellets being provided as food. The egg stage lasted for an average of 3.4 days, the six larval instars together averaged 21.05 days, the pre-pupal and pupal stages averaged 4.8 and 8.3 days, respectively, and the adult males and females lived for

averages of 116.15 and 114.35 days, respectively. A study was conducted to determine the effects of temperature and humidity on the development of stored products pest, *D. maculatus* and development of adults was more adversely affected by temperature compared to relative humidity. The longest duration of maturation (6.70±0.36 days) was obtained at 28±2°C and 60±5% RH (Majeed, 2002). The shortest duration 4.90±0.97, 23.1±0.72 and 3.70±0.31 days for maturation, oviposition and post-oviposition, respectively, was recorded at 38±2°C and 40±5% RH. An increase in temperature at constant relative humidity affected the development and decreased the maturation, oviposition, post-oviposition and longevity of adults. An increase in relative humidity decreased the rate of maturation, oviposition, post-oviposition and longevity at a particular temperature. The larval survival percentage decreased with temperature increase. Temperature and relative humidity affected the larval duration and developmental index. However, the larvae developed faster at higher temperatures and lower humidities. Temperature and humidity affected the pupal period and survival. The pupae developed faster at higher temperature and lower humidity.

Azab *et al*. (1973b) reported that at 75% R.H. and 21, 27 or 35˚C, the egg stage*D. maculatus* averaged 5.91, 3.02 and 1.87 days, respectively. At 55˚C R.H., the larval stage averaged between 18 days at 35˚Cand 64 days at 21˚C; at 27˚C; it averaged 22 days at 75% R.H. and 46 days at 55% R.H., the pupal stage averaged between 4 days at 35˚C and 13 days at 21˚C; relative humidity appeared to have little effect on the duration of this stage. At 75% R.H., the adults lived for a mean of 169-173 days at 21˚C and 49.1-51.9 at 35˚C. Adults of both sexes lived longer at 55% than at 75% R.H. Survey on factors influencing the rate of oviposition in *D. maculatus* (Azab *et al*., 1973c) revealed that when the relative humidity was 75%, females kept at 21, 27 or 35˚C laid average totals of about 214, 362 and 83 eggs each, respectively, and the oviposition period averaged about 130, 92 and 28 days, respectively. At 27˚C and 55% relative humidity, the average total number of eggs laid per female was about 333 and the oviposition period averaged about 86 days. Females oviposited normally only when they had been provided with water in addition to suitable food. To obtain maximum numbers of eggs it was necessary to keep males and females together continuously. The reactions to temperature of the larvae of *D. maculatus* were studied using a radial temperature

gradient apparatus (Osuji, 1975c). The larvae consistently avoided the hottest zones of the gradients (39.5 or 44˚C) and were preferentially distributed in the cooler zones (23.5 - 25˚C or 32 - 34˚C).

Nakahira and Arakawa (2005) found that the larval developmental period of the green lacewing, *Chrysopa pallens* was affected by photoperiod. Whittaker and Kirk (2004) reported that the amount of walking, pollen consumption, and ovipositionof adult females of the western flower thrips, *Franklinella occidentalis* (Pergande) (Thysanoptera: Thripidae) at 25˚C all increased with increasing photophase. Nakamura (2003) examined the effect of photoperiod on *Dolycoris baccarum* nymphal development; growth and adult size and found that the developmental period was longer under short rather than a long day photoperiod. Adult size was largest under an intermediate photoperiod of L13: D11, and was smaller under both longer and shorter photoperiods.

Ekesi *et al*. (1999) reported that the highest pre-oviposition period of the legume flower thrips, *Megalurothrips sjostedti* was observed at 29ºC under a photoperiod of L16:D8 and that egg production also ceased at this temperature/photoperiod combination. At 23-25ºC and 80-90 % RH, the oviposition period of *D. maculatus* lasted for up to 73 days and female laid up to 270 eggs each, the larval stage averaged 40 days and the pupal stage 12 days, and the adult life-span 76 days for females and 70 days for males (Shahhosseini, 1980). At 30ºC, the average duration of adult life of *Dermestes frischii*varied from about 12 days (at the lowest relative humidity and salt content) to nearly 60 days (at high relative humidity and no salt content). At 35ºC, the effects of humidity and salt content were similar but less marked and adult life lasted about 12 – 25 days(Amos and Morley, 1971).

2.6 Mating Behaviour

In the oriental beetle, mating and copulation occurred without an obvious complex courtship, but observations of post mating behaviours suggested that mate guarding occurs (Facundo *et al*., 1999). Edvardsson and Arnqvist (2005) examined the effect of

copulatory courtship on differential allocation in the red flour beetle, *Tribolium castaneum*, which indicated an increase in female oviposition rate in response to intensive leg rubbing but failed to find any support for an effect on sex allocation. The overall sex ratio of offspring was slightly male biased but females did not appear to regulate the sex ratio of their offspring. Wang *et al*. (2005) studied the effect of diamond black moth (DBM), *Plutella xylostella* (Lep. Plutellidae) male and female multiple mating on fecundity, fertility, and longevity and found that there were no significant differences in the fecundity, fertility, and longevity between the single and twice mated females. Results suggested that DBM females might be monandrous. Multiple mating did not increase male or female mating fitness.

2.7 Deterrents, Repellents and Antifeedants

Akinwumi *et al*. (2007) revealed significant repellent effect of the ethanol extracts of four tropical plant materials: pepper fruit; *Dennettia tripetala* Baker; Clove, *Eugenia aromatic* Hook; African nut – meg, *Monodora myristica* (Dunal) and black pepper, *Piper guineense,* on *D. maculatus* on smoked African mud Catfish, *Clarias gariepinus* Burchell. This agrees with the report that repellency is a major mechanism by which plant products control insect damage to stored produce (Stoll, 2000). The oil of *Detarium microcarpum*caused high mortality and repellency to the larvae of *D. lardarius* and even at low concentrations for the protection of dried fish against dermestid attack (Adebote *et al*., 2006). An investigation into the effects of secondary compounds from tropical plants (*Artocarpus heterophyllus*, *Anacardium occidentale* and *Mimosa pudica*) on the diamondback moth (*Plutella xylostella*), confirmed the oviposition deterrence and antifeedant effects in all extracts and that the deterrent effect was reduced with time (Qin-Wei Quan *et al*., 2004). *Anacardium occidentale* extracts showed continuous oviposition deterrent effect.

Raja *et al*. (2003) established that the hexane, diethyl-ether, dichloromethane, ethyl acetate, methanol and aqueous extracts collected from leaves and roots of *Artemisia nilagirica,* and from the leaves of *Acorus calamus*, *Anisomeles malabarica*, *Cassia*

auriculata, *Holoptelea integrifolia*, *Lobelia leschenaultiana*, *Tarrena asiatica*, *Pergularia daemia* and *Wedelia calendulacea* showed significant ovicidal, insecticidal and ovipositional deterrent activities. *Eupatorium odoratum* [*Chromolaena odorata*] and *Eucalyptus robusta* were found to have the greatest oviposition deterrent effects against *Conopomorpha sinensis*(JiDong *et al*., 2002). Oil, methanolic seed extract, acetone leaf extract, aqueous seed extract, chloroform seed extract and petroleum ether seed extract of karanj were found to act as oviposition deterrents, antifeedants and larvicides against a wide range of insect pests (Kumar and Singh, 2002). (Ming *et al*., 2002) confirmed that celangulim(from *Celastrus angulatus* extracts) strongly deter the adultsof diamondback moth *Plutella xylostella* from laying eggs and significantly inhibit larval feeding.

Studies on extracts of *Rhododendron molle* as oviposition deterrents and ovicides against *Plutella xylostella* (Lepidoptera: Plutellidae) by Hua (2000) proved that extracts of *Rhododendron molle* is an effective deterrent and ovicide against *Plutella xylostella*(Lepidoptera: Plutellidae). Adedire and Lajide (2000)revealed that the powdered fruits and bark of *Dennettia tripetala* had ovicidal, larvicidal, and insecticidal effects on *C. maculatus* pest of stored cowpea. Aziz and Ismail (2000) reported that the oils of *Nigella* sp. *Nigella sativa*, *Boswellia sacra* and, *Cucurbita maxima* possess repellent, oviposition deterrent and protectant effectson the bean bruchids (*Bruchidius incarnates*).

Elhag *et al*. (1999) found that methanol and diethyl ether extracts of harmal(*Rhazya stricta*), neem seed kernels (*Azadirachta indica*), cloves (*Syzygium aromaticum*), citrus peel and ramram (*Heliotropium bacciferum*) significantly reduced ovipositionby *Callosobruchus maculatus* on treated chickpeas seeds. Aqueous extracts of *Trichilia pallida* leaves and twigs affected oviposition and developmentof *Tuta absoluta*, mainly at the larval stage (Thomazini *et al*., 2000). Methanol extracts of leaves of *Ageratum houstonianum*, *Artemisia brevifolia*, and leaves and drupes of *Melia azedarach*, showed varying degrees of oviposition deterrent effect against *Henosepilachna vigintioctopunctata* (Meena *et al*., 1998).Kumari *et al*. (1998) reported that Citronella oil and palmarosa oil gave total protection to groundnut pods by inhibiting oviposition by the bruchid, *Caryedon serratus*, and among the plant powders(neem seed kernel powder,

neem leaf powder and *Lantana camara* leaf powder), *L. camara* had a good oviposition deterrent activity, but lost effectiveness gradually after one month.Dwivedi and Kumar (1999) revealed that extracts of *Cassia occidentalis* and *Withania somnifera* were effective oviposition deterrentsagainst khapra beetle, *Trogoderma granarium*in comparison to all the other extracts evaluated.

A methanol extract of *Momordica charantia* leaves strongly deterred Cucurbitaceous feeding beetle species(*Aulacophora femoralis*, *A. nigripennis*, *Epilachna admirabilis*, and *E. boisduvali*)from feeding (Abe and Matsuda, 2000). The plant extracts of neem (*Azadirachta indica*), arandi (*Ricinus communis*), karanj (*Derris indica*) [*Pongamia pinnata*], pilu (*Salvadora oleoides*), marva (*Ocimum basilicum*), amaltas (*Cassia fistula*), bluegum (*Eucalyptus globulus*), guava (*Psidium pyriferum*), dhatura (*Datura metel*), and bougainvillea (*Bougainvillea* sp.) applied to sorghum significantly reduced the oviposition of *Tribolium castaneum* on jowar seeds (Lohra *et al*., 2001). Volatile oil of air-dried leaves of *Ageratum conyzoides* (Asteraceae) resulted in acute toxicity to adults of the cowpea weevil, *Callosobruchus maculatus*(Gbolade *et al*., 1999). Sharma *et al*.(1998) revealed that 2 formulations containing Neemrich I + oil of *Salvadora oleoides* and Neemrich I + neem extract (Plantmix I and Plantmix II, respectively) showed better oviposition deterrent and antifeedent activity than their individual constituents against *Phthorimaea operculella*.

Acetone extracts of *Cassia occidentalis* and *Croton bonplandianus* and pet-ether extracts of *Verbesina encelioides* and *Cassia occidentalis* were effective in deterring oviposition in *Callosobruchus chinensis* (Maheshwari and Dwivedi, 1997). Sharma *et al*. (1997) reported that alcohol extracts was superior to that of acetone, benzene, petroleum ether and distilled water in reducing egg hatch and oviposition of *Phthorimaea operculella*.(Weissling *et al*., 1997) indicated that various materials such as horticultural oils, an insecticidal soap, neem, garlic extract, a sugar ester, and a synthetic insect growth regulator (fenoxycarb) could be successfully used as oviposition and feeding deterrents of *Cacopsylla pyricola*. Spraying the bhendi (Okro) crop with various neem products resulted in an oviposition – deterrent effect on females of the pest *Amrasca biguttula biguttula* (Patel and Patel, 1996).

Babu *et al.*(1996) showed high feeding deterrence with hexane extract of leaf and chloroform extract of seed of *Annona squamosa* on adult *Longitarsus nigripennis* (Coleoptera: Chrysomelidae). Application of three(3) concentrations of oil – free neem seed extracts (*Azadirachta indica)* to cabbage plants in cages did not deter oviposition by individuals of 3 species of noctuid moths, *Trichoplusia ni*, *Peridroma saucia* and *Spodoptera litura*. 1% crude oil emulsion significantly reduced the proportion of eggs laid by *S. litura* on treated plants. Sprays consisting of highly processed neem seed extracts, used at concentration that provide larval control are unlikely to be generally effective as oviposition deterrents to noctuid pests (Naumann and Isman, 1995).

Dilawari *et al*. (1994) indicated that the methanolic extract of *Melia azedarach* effectively checked the fecundity and contributed to the mortality at various stages of the life cycle of diamond-back moth, *Plutella xylostella.* Extracts from calyxes of an alternate host plant, *Hibiscus sabdariffa* exhibited antifeedant as well as oviposition-deterrent activities against *Earias vittella* (Dongre and Rahalkar, 1992). Cobbinah and Kwarteng (1989) reported that although copra and palm kernel oil reduced attack by *S. zeamais*, these edible oils were not as effective as neem oil. Ethanolic extracts of seeds of neem (*Azadirachta indica*), *Caropa procera*, *Lansium domesticum and Swietenia macrophylla* were highly active feeding deterrents against the southern corn rootworm, while hexane extracts were ineffective as deterrents. (Landis and Gould, 1989).

Vegetable oils, particularly groundnut and palm oils, are known 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). In Senegal, traders in Dakar retail market coat dried fish with vegetable oil to protect it from insect infestation (Wood 1982). However, results from an experiment in the Lake Turkana region of Kenya, in which bottled cod-liver was applied to dried tilapia (*Oreochromis* sp.) at a treatment level of 44 ml/kg, showed that after 45 days storage there was little difference in insect infestation between treated and untreated samples (Walker and Wood, 1986).

Leatemia and Isman (2004) reported that crude aqueous extractsof *Annona squamosa* deterred feeding of 4th instar of diamond-back moth, *Plutella xylostella*in a leaf disc

choice bioassay. Aqueous seed extracts and aqueous emulsion of ethnolic seed extracts were equally toxic to both cabbage looper, *Trichoplusia ni* and diamond-back moth, *Plutella xylostella*. Application of oils (ground nut, traditional coconut, industrial coconut, palm, and shark liver oil) against *Dermestes maculatus* on dried trout (*Salmo gairadnerii*) significantly reduced the development of progeny of *D. maculatus* only at dosages of 56ml/ kg(Don-Pedro, 1989). When *D. 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/ 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). Using the dry fish weight loss, number of live larvae, number of pupae formed and number of live adults as indices of activity, Adedire and Lajide (2000), suggested that *Piper guineense* and *Dennettia tripetala* possess contact toxicity, fumigant, ovipositioninhibition, ovicidal and larvicidal activities against *D. maculatus*.

Treatment of dried Tilapia with 0.25, 0.50, 1, and 2 g of neemseed powder per 25 gm of fish affected the oviposition and the hatchability of different age group of eggs of *D. maculatus* and also killed the adults. Incubation period was prolonged. Larvae did not develop beyond the 2^{nd} instar and 93% of the larvae died by day 30(Okorie *et al*.,1990). However, some adverse features of using neem have been observed. Neem powder produces bitterness in taste, which was removed by boiling, and neem oil was observed by Mathan *et al*.(1992) to have nauseating, objectionable odour that was picked up by both the packaging and the fish.Chen *et al*.(1995) demonstrated that toosendanin, a limnoid allelochemical from the bark of the trees *Melia toosendan* and *M. azedarach* significantly deterred feeding of 2^{nd} and 4^{th} instar larvae of variegated cutworm, *Peridroma saucia* in diet choice and leaf disc bioassays, respectively. They were also able to prove that toosendanin was a reasonably effective antifeedant against *P. saucia* with a DC_{50} of 8.0gcm^{-2} in the leaf disc choice test.Pacheco *et al*.(1995) proved refined castor oil as an effective protectant than refined soybean oil for the control of infestations of *Callosobruchus maculatus* and *C. phascoli* in stored chick-pea, *Cicer arietinum*. No harmful effect was observed on the germination of oil treated seeds.

Rajapakse and Van Emden (1997) reported that all four oils tested (corn, ground nut, sunflower and sesame oils) significantly reduced the oviposition of all three bruchid species (*Callosobruchus chinensis, C.maculatus, C. rhodesianus*) at 10ml/kg and also significantly reduced the longevity of adults of *C. maculatus* and *C. chinensis* at this dose. Only corn and sunflower oil caused a significant reduction of longevity of *C. rhodensianus* at 10ml /kg. Makanjuola (1989) showed that the effectiveness of neem is affected by differences in insect behaviour. The extracts were more active as suppressants *of C. maculatus* than *Sitophilus* spp. and there was no effect on *C. puncticollis*. All of the extracts tested resulted in a significant reduction in oviposition, percentage egg hatch and percentage adult emergence in *C. maculatus* and in adult emergence of *Sitophilu*s spp. Two commercially available repellents (oil of clove and citronellol) were found to be effective (Plarre *et al*., 1997) against the webbing clothes moth, *Tineola bisselliella* (Lepidoptera: Tineidae). Inhibition of larval growth was directly related to concentration of the respective extracts (Villani and Gould, 1985).

Out of 78 plant species (24 families) screened for antifeedant activity against the corn wireworm, *Melanotus communis,* five extracts from four families significantly reduced wireworm-feeding damage in a series of choice feeding tests. Two extracts, *Asclepias tuberosa* and *Hedera helix*, exhibited exceptional levels of feeding deterrency.(Osuji, 1975b) reported prolonged larval development of*D. maculatus* and *Necrobia rufipes* in salted fish and larval mortality was total in *D. maculatus* at salt concentrations of 9.20% and 10.20%. Topical application of different doses of acetone extracts of *Anthocephalus cadamba, Lantana camara, Tectona grandis, Calophyllum* sp. and *Phyllanthus emblica* to the newly moulted last nymphal instar of *Dysdecus cingulatus* resulted in the 6th instars retaining varying degrees of nymphal characters (Prabhu and John, 1975). Four plants(*Piper gunieense*, *Cyperus rotundus*, *Dennettia tripetala* and *Capsicum frutescens*)were found to be effective in controlling *Dermestes maculatus* on stored, smoked catfish, *Clarias gariepinus* (Adedire *et al*., 1999). Piper fruit oil at dosages of 0.125ml/25g fish and 0.150ml/25g, fish were found to be efficient in the control of the development of *D. maculatus* adults and larval stages on tested dried fish (*Clarias* spp.) and was therefore recommended as appropriate dosages for prevention of insect infestation on dried fish (Amusan and Okorie, 2002).

(Don-Pedro, 1999) revealed that citrus peel oils are fast-acting fumigant insecticides with possible neurotoxic or anti-respiratory properties. The rapid action of citrus oil fumes was demonstrated by LT_{50} values of 40.7, 106 h for lime peel oil and 4.6, 14.8 h for d-limonene against *C. maculatus* and *D. maculatus*, respectively. *D. tripetala* seed powder showed higher repellency than pyrethrins. Acetone and ethanol extracts were good repellents to *D. maculatus*. Water extracts did not meet the minimum requirement for good repellents (Egwunyenga *et al*., 1998). The biological action of citrus peel oils was shown to depend on a strong fumigant action (Don-Pedro, 1996a). Bioassays conducted showed that all the 6 citrus oils tested had vapour toxicity to adults of *C. maculatus*, *Sitophilus zeamais*, and *D. maculatus*. The 24-h LC_{50} value of lime peel oil,a typical citrus oil vapour against *C. maculatus* was 7.99µl/litre which made it 1.5 and 1.6 times less toxic to the smaller *S. zeamais* and the larger *D. maculatus* adults, respectively. When immature stages were fumigated, lime peel oil vapour had 24-h LC_{50s} of 7.8 and 21.5µl/litre against eggs of *C. maculatus* and *D. maculatus*, respectively and 9.1, 17.8, 23.1, 23.9µlitre/litre against early larvae and pupae of *C. maculatus* and late larvae and pupae of *D. maculatus*, respectively.

Treatment (> 10 ml/mg) against *C. maculatus* or *Sitophilus zeamais*;(>20 ml/kg against *D. maculatus*) with citrus peel oils (lime, tangerine [mandarin] and grapefruit) reduced oviposition or larval emergence through parental adult mortality, but had no residual activity on the eggs or larvae produced by survivors (Don-Pedro, 1996b). Oil-treated cowpeas (7 ml/kg against *C. maculatus*) or dried fish (28 ml/kg against *D. maculatus*), which caused 100% mortality 1 h after application lost all activity within 24 h, thus confirming the non-residual nature of the effects. Don Pedro (1985) found that orange peel (*Citrus sinensis*) powder had greater insecticidal and repellency effects than grapefruit peel (*C. paradisi*) powder using chips of dried fish. Treatment of fish with 14.1% by weight of orange peel powder killed 50% of adult *D. maculatus* after 7 days: a 21.3% treatment killed 99% in the same period. At applications of 15.0 and 18.0% by weight orange peel powder reduced progeny development and slowed larval development. At 18% the number of emerging larvae was reduced by 60% compared with the untreated control. Of the larvae that did emerge only 32.7% and 37.1% of the 18.0% and 15.0% treatments, respectively, developed into F1 adults compared with

87.8% of the larvae from the untreated controls. Subsequent work by Don Pedro (1996a; 1996b) demonstrated that topical toxicity of citrus peel oils was relatively ineffective when compared with activity in the vapour phase. The volatile components possessed activity against *D.maculatus* life stages; eggs being most susceptible and last instar larvae and pupae being least susceptible, though the differences were slight. In the presence of dried fish pieces the activity was greatly reduced as a result of sorption of the volatile components.

Population suppression and toxicity tests were carried out on dried fish pests using the African locust bean plant, *Parkia clappertoniana* (Odeyemi *et al*., 2000). The pod and pulp of *P. clappertoniana* at the rate of 1g, 1.5 g, and 2.0 g for dry powder treatment per 100 g dried fish samples, were toxic to adults and larvae of *D. maculatus* and *N. rufipes*. The population of adults decreased significantly ($P<0.05$) on treated samples. The powder-in-oil treatment had more insecticidal effect on the beetles than the pulp. Saha and Shajahan (1998) tested the effect of alcoholic extracts of neem and gamma radiation on the 6th instar larvae of *D. maculatus* to investigate their effect on larval mortality, pupation, adult emergence and longevity. 80% larval mortality was obtained at a dose of 19700 ppm of the crude neem extract. Though this dose could not prevent adult emergence, it resulted in deformed adults with reduced life span.

Gakuru and Faua-Bi (1996) compared the effects of essential oils of four plants against *C. maculatus* and rice weevil, *S. oryzae*. Results proved that essential oils had no effect on *S. oryzae*, however, the essential oils of *Eucalyptus citriodora* and *O. basilicum* were more potent against *C. maculatus*. The study of Lale and Ajay (2000) revealed that clove oil was significantly more toxic to adults and larvae of *Tribolium castaneum* than other oils tested. The period of exposure appeared to be the most important factor to determine the efficiency of extracts rather than dosage (El- Nahal *et al*., 1989). Ali *et al*., (1983) reported that seeds treated with neem, coconut, mahua, sesame, and palm oil did not permit adult beetles (*C. maculatus*) to lay eggs and thus inhibited the development of subsequent population. Seed oils of *Cassia occidentalis* induced high mortality of bruchid eggs and first instar larvae than the fresh and dry leaves or ground seeds

(Lienard *et al.*, 1993). Several fatty acids (linoleic, oleic, and stearic) present in the oil were responsible for this toxicity.

Gupta *et al*. (2000) evaluated the efficacy of different vegetable oils such as castor, mustard, linseed, soybean, coconut, groundnut, and sesame against *S. oryzae*. It was observed that all the oils afforded protection over a period of around 120 days. Among these, mustard and linseed oils were significantly superior in comparison to other oils. According to Shaaya *et al*. (1997), edible oils are potential control agents against stored grain pests like *C. maculatus*, *S. zeamais*, *S. oryzae* and *S. cerealella* on the farm level itself. Richa *et al*. (1995) reported that essential oils of basil, geranium, rue, lemon grass, citronella, eucalyptus, and lemon were effective in protecting faba beans from *C. chinensis*. According to their findings, essential oils of basil and geranium had the greatest insecticidal effect, while oils of lemon grass and eucalyptus were not toxic to adults but showed some effect on oviposition. Insecticidal effect of volatile oils of *Lippia adoensis*, *Cymbopogon citrates*, *Lantana camara* and *Chromolaena odorata* against *C. maculatus* was studied by Gbolade and Adebaye (1995).

Plant oils obtained from cottonseeds, soybean, maize and peanuts act as very good repellents against *S. granaries* in stored wheat (Yun and Burkholder, 1981). Jilani and Su (1983) have demonstrated the repellent effects of turmeric (*Curcuma longa*), neem (*A. indica*) and fenugreek (*Trigonella foenumgracum*) against three species of stored product insects viz. *T. castaneum*, *R. dominica* and *S. granaries*. Results showed that the turmeric powder was the most effective against *S. granaries* and *R. dominica*while only solvent extract was effective against *T.castaneum*. Jilani *et al*. (1988) reported that repellency increased with increasing concentration of turmeric oil, sweetflag oil, neem oil, and Margosan on red flour beetle, *Triboleum castaneum*. According to Singh and Singh (1991)the essential oils obtained from *Ocimum gratissimum*, *Thymus serpyllum*, *Illicium verum*, *Myristica fragrans*, and *Curcuma amada* showed 100% repellent activity, and *A.calamus* showed 40% activityagainst house fly, *Musca domestica*. Malik and Naqvi (1984) have screened seven plant species for their repellent activity against *T. castaneum* and antifeedant activity against *R. dominica*. The best repellent activity was

for the rhizomes of *Saussurea lappa* and antifeedant activity for the leaves of *Chenopodium ambrosioides* and for azadirachtin isolated from neem kernel.

Behal (1998) showed that there was a complete repellency of the larvae of the rice moth, *C. cephalonica* with sweetflag, (*A. calamus*) oil irrespective of its concentration, while with other oils, a concentration dependent repellency was noticed. Petroleum ether extracts of *Cassia tora*, *C. fistula*, and *C. articulata* seeds exhibited more than 80% repellency against *T. castaneum* (Pradeep and Radhakrishnan, 1999). Sahayraj and Paulraj (2000) observed that *Spodoptera litura* larva was repelled by groundnut leaves treated with *Tridax procumbens* leaf extract and the repellency increased as the concentration of leaf extract increased. The percent repellency was found to decrease over a long interval of time (Urs and Srilatha, 1990).

When essential oil of eucalyptus was used against the rice weevil, *S. oryzae*, 80% repellency was shown after 10 min, 50% after 30 min and after 60 min it decreased to 20% (Ahmed and Eapen, 1986). Malik and Naqvi (1984) also reported a similar time-dependent decrease of repellent property in the case of *T. castaneum*. Dormusoglu *et al*. (2003) observed that Neem Azal T/S and neem oil had no significant effect on adults and newly laid eggsof *Nezara viridula* (L.) (Heteroptera: Pentatomidae). However neem oil was found to be more effective than Neem Azal T/S on nymphs and old laid eggs after 7 and 14 days respectively. Zabel *et al*. (2002) proved high antifeedency and low toxicity of the neem extract on *Lymantria dispar* and on 3rd instar larvae of *Leptinotarsa decemlineata*. Ahmad *et al.*(2003) reported that only neem oil was found to exert a negative impact on the hatching rate of *Cocinella septempunctata* and *Chrysoperla carnea* and the 1st instar larvae *Episyrphus balteatus* proved to be highly susceptible, when feeding 24h. on aphid sprayed with neem kernal water extract. Ma *et al*. (2000b) observed high mortality of larvae of *Helicoverpa armigera* fed on potted cotton plants (*Gossypium hirtum* L) sprayed with formulated neem extract (3% azadirachtin emulsifiable concentrate). He also found physiological effects such as difficulty in moulting between various instars and abnormal pupae in larvae fed on the artificial diet.

Bruce *et al*. (2004) observed low oviposition rates, immature survival, fecundity, and egg viability in the neem treatments and a relatively high persistence of neem oil on *Sesamia calamistis* and *Eldana saccharina*. Mathen *et al*. (1992) reported that using dried silver belly (*Leiognathus* sp.),mustard oil was observed to be the best insect repellent, the treated sample remaining insect free for 40 days, followed by hydnocarpus, sunflower and cashew. Peppers (*Capsicum* sp.) are used traditionally in Africa as a means of repelling blowflies but there is no evidence to suggest a use on dried fish. Pepper is used domestically as a preservative for prawns in Kerala, India. When cured prawns were sprinkled with 1.5% by weight of pepper powder and stored in a mite-infested godown (store) they remained insect – free for as long as the pepper smell persisted, about 7 weeks. (Pillai, 1957).

Wood *et al*. (1987) reported that the maximum weight losses in unsalted fish (dried whiting) varied between trials and ranged from 15 to 41%. Increases in salt content above the natural 2% level to 5% did not give any marked protection against insect infestation. A salt content of greater than 9% largely protected the fish from damage, reduced weight losses to less than 10% and greatly inhibited insect development. Experiments with salted dried fish (the freshwater fish, *Roccus chrysops* being used) showed that a salt content of 13% or more prevented the development of infestation by *D. maculatus* from eggs. The adverse effects were reduction of larval survival and retardation of larval development rather than reduction of egg viability. The results suggested that eggs laid in the crevices of salted fish will hatch but that the larvae will mostly perish (Mushi and Chiang, 1974). 1 ml of 1 percent *Allium sativum* L. (Garlic) oil applied to filter paper and stored for four months produced 88 percent repellency against adult *T. castaneum* in a choice-chamber experiment, when assessed after five days of exposure. The level of repellency had declined to 48 percent after eight weeks (Mohiuddin, *et al*., 1987). 2 ml/kg cowpea seed of *Piper guineense*, African black pepper, caused 100 percent mortality in adult *C. maculatus* within 24 hours and prevented the emergence of F1 adults (Ivbijaro, 1990).

2.8 Chemical Constituents of Essential Oils

Hydrodistillation of dried leaves of *B. balsamifera* yielded 0.88% essential oil (v/w), and the density of the essential oil was determined as 0.87%. A total of 27 components were identified in the essential oil of *B. balsamifera*, accounting for 99.23% of the total oil. The main components are 1,8-cineole (20.98%), borneol (11.99%), β-caryophyllene (10.38%), camphor (8.06%), 4-terpineol (6.49%), α-terpineol (5.91%), and caryophyllene oxide (5.35%). Monoterpenoids represented 12 of the 27 compounds, corresponding to 70.47% of the whole oil while 13 of the 27 constituents were sesquiterpenoids (27.42% of the crude essential oil). The chemical composition of *B. balsamifera* essential oil was different from that reported in other studies. For example, borneol (33.2%), caryophyllene (8.2%), ledol (7.1%), tetracyclo[6,3,2,0,(2.5).0(1,8)] tridecan-9-ol, 4,4-dimethyl (5.2%), phytol (4.6%), caryophyllene oxide (4.1%), guaiol (3.4%), thujopsene-13 (4.4%), dimethoxydurene (3.6%), and γ-eudesmol (3.2%) were the dominant components in the essential oil of *B. balsamifera* leaves collected from Bangladesh (Bhuiyan *et al*., 2009). However, borneol (57.7%), caryophyllene (7.6%), and camphor (5.0%) were the three main components of the essential oil of *B. balsamifera* from Guizhou, China, while bornel (52.4%) and camphor (17.7%) were the two components of the essential oil from Yunnan, China(Du *et al*., 2009). The major components of the essential oils were borneol (57.8%), caryophyllene (8.3%), δ-cadinol (8.0%), and caryophyllene oxide (3.1%) (Ha Giang, 22.80°N, 104.98°E); borneol (50.6%), camphor (18.7%), caryophyllene (10.1%), δ-cadinol (3.1%), patchoulene (3.0%), and veridiflorol (2.0%) (Hanoi, 21.02°N, 105.51°E); camphor (70.1%), caryophyllene (10.5%), borneol (5.7%), and carvacrol (5.7%) (Dak Lak, 22.80°N, 104.98°E).

1,8-Cineole (1) (LC_{50} = 2.96 mg/L air), 4-terpineol (4) (LC_{50} = 4.79 mg/L air), and α-terpineol (5) (LC_{50} = 7.45 mg/L air) showed stronger fumigant toxicity than the crude essential oil (LC_{50} = 10.71 mg/L air) against *S. zeamais*. It indicates that fumigant toxicity of the essential oil may be attributed to the three constituent compounds. In the previous studies, 1,8-cineole was found to exhibit fumigant toxicity against red flour beetles, *Tribolium castaneum* adult with LC_{50} = 41μL/L air (Suthisut *et al*., 2011),

15.3μL/L air (Lee *et al*., 2004) and 1.52mg/L air (Tripathi *et al*., 2001). It also possesses fumigant toxicity against several other stored product insects and cockroaches as well as mosquitoes, for example, the rice weevil (*S. oryzae*) (LC_{50} = 22.8μL/L air (Lee *et al*., 2004)), the lesser grain borer (*Rhyzopertha dominica*) (LC_{50} = 9.5μL/L air (Lee *et al*., 2004)). However, compared with the current used fumigant (MeBr, LC_{50} = 0.67mg/L air), the three compounds and the crude essential oil exhibited only 4–16 times less toxic to the maize weevils. The three constituent compounds and the crude essential oil were more toxic to *S. zeamais* than another two isolated compounds, camphor (LC_{50} = 21.67mg/L air) and borneol (LC_{50} = 29.64mg/L air).

Compared with the other essential oils in the previous studies that were tested using a similar bioassay, *B. balsamifera* essential oil exhibited stronger fumigant toxicity against *S. zeamais* adults, for example, essential oils of *Schizonepeta multifida* (Liu *et al*., 2011), *Murraya exotica* (Li *et al*., 2010) and several essential oils from Genus Artemisia (Chu *et al*., 2010; Liu *et al*., 2010a; Liu *et al*., 2010b). Moreover, the two other active constituent compounds, 4-terpineol and α-terpineol, have been found to possess strong fumigant toxicity against several grain storage insects, such as *S. granarius*, *S. oryzae*, *T. castaneum*, *T. confusum*, and *R. dominica* (Lee *et al*., 2004; Kordali *et al*., 2006). The two other active constituents, camphor and borneol, were also demonstrated fumigant toxicity against grain storage insects (Suthisut, 2011; Karaborklu *et al*., 2011). Oliver-Bever (1986) reported that the constituents of curcubit seed oil include charantin, momordicin and foetidin; 5-hydroxytryptamine, diosgenin and *p*-sitosterol.

Cashew, *A. occidentale*, seed powder and oil completely killed adult *C. maculatus* on stored cowpea seeds. This insecticidal effect was in agreement with Dungum *et al*. (2005), Oparaeke and Bunmi (2006) and Adedire *et al*. (2011). The mode of action of this plant powder oil could be as a result of the powder and oil coating the treated cowpea seeds which prevented contact between the seeds and the bruchid resulting into starvation. They also blocked the spiracles which may lead to suffocation as suggested by (Hall and Harman, 1991; Adedire *et al*., 2011). The insecticidal activity of *A. occidentale* oil extract could be linked to the presence of anacardic acid, cardol and cardanol (Rehm and Espig, 1991). Others include quercetin and kaempferol glycosides

(Oliver-Bever, 1986). Garlic powder and oil may have been very toxic to *C. maculatus* as a result of the strong choky odours it produce which may disrupt normal respiratory activity of insect, thereby leading into asphyxiation and subsequent death (Adedire and Ajayi, 1996). This result agrees with the finding of Bhatnager-Thomas and Pal (1974) who reported the effectiveness of garlic oil against *Musca dominica* and *Trogoderma granarium*. Nasseh (1990) reported the insecticidal activities of *A. sativum* for the control of *Epilachna verivestis*.

Arannilewa *et al*. (2006) reported 85% adult mortality of *Sitophilus zeamais* and 9 emerged adult maize weevil on treated maize grains with methanol extract of *A. sativum*. The reduced oviposition and no adult emergence observed in all the treated seeds with oil extract could be as a result of high adult mortality of *C. maculatus*. The oil inhibits locomotion which affect mating activities an effect that had been reported by many authors (Okonkwo and Okoye, 1996; Adedire, 2002; Akinkurolere *et al*., 2006; Oni and Ileke, 2008; Adedire *et al*., 2011; Ileke and Oni, 2011). The few eggs that were laid are unable to stick to the surface of the seeds as a result of oil presence also prevents progeny production. The lower F1 adult bruchid in cowpea seeds treated with *A. sativum* powder at rate of 1g may be due to concentration used. The ovicidal and larvicidal of the plants powders and oils may be due to the present of lipophlic compound (Richards, 1978). Don-Pedro (1990) reported that eggs mortality may be as a result of physical properties of the oils resulting into blockage of respiratory pores (spiracle) while the larvicidal properties of the plants oils could be as a result of both physical and chemical toxicity of oils (Ivbijaro, 1984).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The experiment was conducted in the Fisheries Research Laboratory of the Department of Biological Sciences, Ahmadu Bello University, Samaru, Zaria, Kaduna State, Nigeria at ambient temperature of between 25 - 30°C.

3.2 Sources of Plant Materials

The seeds of *A. occidentale, K. senegalensis* and *A. lebbeck* used for this study were collected from fruiting trees within the Main Campus of Ahmadu Bello University, Samaru, Zaria, while *C. colocynthis* and *C. vulgaris* was purchased at Sabon – Gari market, Zaria. All seeds were properly identified and authenticated at the herbarium unit of Biological Sciences Department, AhmaduBelloUniversity, Zaria.

3.2.1 Processing of plant materials

Seeds of *A. occidentale, K. senegalensis,A. lebbeck, C. colocynthis* and *C. vulgaris* were removed from their respective pods then sun – dried for 17 days after which they were pulverized using a mortar and pestle. The pulverized seeds were sieved and stored in pre – labeled new cellophane bags.

3.2.2 Extraction of oil by soxhlet extraction method

40g of each seed powder was weighed into separate muslin cloth and introduced separately into the soxhlet chamber for the oil extraction. In the round bottom flask, 350ml of n – hexane was introduced as extraction solvent. The extraction was done at 60 - 80°C until the solvent in the soxhlet chamber became transparent. The soxhlet apparatus was dismantled and the content of the round bottom flask were transferred to a rotary evaporator which was used to evaporate excess solvent from the oil. The extracted seed oils were stored in separate labeled bottles in a cool place until used for bioassay.

3.3 Collection and Treatment of Insects

Insects belonging to *D. maculatus* taxon were obtained from infested fish pieces purchased from Sabon – Gari market (11°13'N and 07°52'E), Zaria. Their larvae were properly identified under X40 magnification of a stereo microscope using the pictorial keys of Haines (1991). The larvae were acclimatized for 24 hours in the laboratory before being subjected to bioassay.

3.4 Collection of Smoke – Dried Fish

Smoked catfish, *Clarias gariepinus* specimenweighing between 17 – 20g, 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 0.234mlg^{-1} that is, preceeding concentration multiplied by 3 gives the next concentration) of the oil to the heat sterilized fishes in triplicate and placing them singly in Kilner jars. Thirty (30) late instar larvae of *D. maculatus* were then introduced in triplicate into each jar. The set up were observed at 24, 48, 72 and 96 hours to establish total and zero mortality/minimal mortality range. Five concentrations between total and zero mortality observed in this study were chosen for the bioassay test.

3.6 Bioassay

The smoke – dried fishes were heat sterilized in the oven set at 60±2°C for one hour and then allowed to cool. After cooling to room temperature, each fish was weighed and tagged. From the pilot study conducted, the following application levels 0.003mlg^{-1}, 0.009mlg^{-1}, 0.027mlg^{-1}, 0.081mlg^{-1}, and 0.234mlg^{-1}of the oils were determined. Oil treated fishes were introduced into separate Kilner jars for the larvicidal test. Thirty (30) late instar larvae of *D. maculatus* were introduced into the jars and the entire set up were replicated. Observations were recorded at 24, 48, 72 and 96hours exposure time. The repellent effects of the oils were tested in triplicates using rectangular glass containers of 18cmx12cmx10cm dimension. A treated and an untreated fish were placed at opposite extremes in the container and 30 late instar larvae were introduced at the mid-point of it. The setup were left for 24 hours at which time larvae found within 1cm radius of the treated fish are considered repelled.

3.7 Determination of the Physico-Chemical Properties of the Seed Oils

The physico-chemical properties of the seed oils of *A. lebbeck, A. occidentale,C. colocynthisC. vulgaris* and *K. senegalensis* were determined using AOAC Standard Methods (Official Methods of Analysis, 1998; Oils and Fats, 2012).

3.7.1 Determination of acid value

A solvent mixture of 25ml Diethyl ether was neutralized with 0.1M ethanolic KOH using phenolphthalein as indicator. About 4g of oil was dissolved in the neutralized solvent mixture and titrated with 0.1N KOH.

$$\text{Acid Value} = \frac{56.10 \times T \times (V_2 - V_1)}{\text{Mass of test oil (g)}}$$

Where:

T = Molar concentration of ethanolic KOH used

V_1 = Volume of KOH solution used for the blank test

V_2 = Volume of KOH solution used for the test oil

3.7.2 Determination of iodine value

Exactly 0.2g of each oil sample was weighed into quick-fit conical flasks; 15ml of CCL_4 and 25ml Wij's reagent were added into each flask and placed in the dark for 1 hour. After the set time, 20ml of 10% KI and 150ml distilled water were added. The resulting mixture was titrated against 0.1M $Na_2S_2O_3$ using 1% starch solution as indicator. A blank test was also carried out simultaneously under the same conditions.

$$\text{Iodine Value} = \frac{12.69 \times T \times (V_1 - V_2)}{\text{Mass of test oil (g)}}$$

Where:

T = Molar concentration of $Na_2S_2O_3$

V_1 = Volume of $Na_2S_2O_3$ solution used for the blank test

V_2 = Volume of $Na_2S_2O_3$ solution used for the test oil

3.7.3 Determination of peroxide value

Exactly 4g of test oils were weighed into quick – fit conical flasks and 10ml chloroform was added to each flask to dissolve the oil samples. Then 15ml acetic acid and 1ml 5% KI solution were added. The mixture was kept in the dark for 5 minutes after which 75ml of water was added and titrated with 0.002N $Na_2S_2O_3$.

$$\text{Peroxide Value} = \frac{V \times T}{m}$$

3.7.4 Determination of saponification value

About 2g of each test oil was weighed into a quick – fit conical flasks and 25ml of 0.5M ethanolic KOH was added. Boiling chips were also added, then a reflux condenser was fitted and the mixture refluxed for one hour. After 1 hour, 0.5ml of phenolphthalein was added and titrated with 0.5M HCL solution. A blank test was carried out simultaneously.

$$\text{Saponification Value} = \frac{56.10 \times T \times (V_2 - V_1)}{\text{Mass of test oil (g)}}$$

Where,

V_2 =Volume in ml of standard hydrochloric acid required for the blank

V_1 =Volume in ml of standard hydrochloric acid required for the sample

T = Molar concentration of the standard hydrochloric acid

3.7.5 Determination of specific gravity

The specific gravity of the oils was determined using the specific gravity bottle at 25°C. The specific gravity bottle was weighed empty (W_1) after which the oil sample was carefully poured into the specific gravity bottle and weighed (W_2). The same step was repeated with distilled water.

Weight = $W_2 - W_1$

Where W_2 = Weight of oil sample + weight of specific gravity bottle

W_1 = Weight of specific gravity bottle

$$\text{Specific gravity of oil} = \frac{\text{Weight of oil held in specific gravity bottle}}{\text{Weight of water held in specific gravity bottle}}$$

3.7.6 Determination of refractive index

The refractive indices of the oils were determined using an Abbe Refractometer with temperature control set at 25°C. The double prism is opened with the help of the screw head and a drop of oil is placed on the prism. Close prism firmly by tightening screw heads. Circulate water through the instrument. Let instrument stand for few minutes before taking reading so that the temperature of the test sample and instrument are the same. Clean the prism between readings by wiping off oil with cotton pad moistened with ethyl alcohol/toluene/petroleum ether and let dry. If temperature correction is necessary use the following formula:

$R = R^1 + K^1(T^1 - T)$

Where,

R = Reading of the refractometer reduced to the specified temperature T˚C

R^1 = Reading at T^1C

K = constant 0.000365 for fats and 0.000385 for oils

T^1 = temperature at which the reading R^1 is taken and

T = specified temperature (generally 40˚C)

3.7.7 Gas chromatography/mass spectroscopy analysis

The essential oil was analyzed on a Hewlett-Packard model: 6890NGC-MS system (Agilent Technologies) coupled to a mass-spectrometer model: HP5973. Separations were carried out using a DB-5 capillary column (30m × 0.25mm i.d., 0.25µm film thickness). The injector and detector temperatures were maintained at 220 and 280˚C, respectively. Column temperature was initially kept at 60˚C for 2 minutes and then gradually increased to 240˚C at the rate of 50C min^{-1}. Helium was used as carrier gas at a constant flow of 1.0mL min^{-1} and an injection volume of 1 µL was employed. The MS

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 splittless mode. The identification of the components was based on comparison of their mass spectra with those of NIST3.0 Libraries provided with the computer-controlling GC-MS system.

3.8 Statistical Analysis

All repellent and mortality data were subjected to Analysis of Variance (ANOVA) at $p< 0.05$ to determine significant difference between treatments. Least Significant Difference (LSD) was employed to separate the means. Mortality data were subjected to Probit Analysis to determine their median lethal concentration (LC_{50}) of all the seed oils.

CHAPTER FOUR

4.0 RESULT

4.1 Physico-Chemical Properties of the Seed Oils

The physicochemical properties of the five seed oils are shown in Table 1. *A. occidentale*(cashew) nut oil had the highest (0.96) relative density, while *K. senegalensis* seed and *C. vulgaris*were 0.90 and 0.85 respectively. The refractive indices of the oils are similar (1.47) except for *A. occidentale* that had a 1.46 refractive index. The saponification value ($mgKOHg^{-1}$) ranged from 115.94 in *C. vulgaris* to 196.47 in *C. colocynthis.* The peroxide value was highest (10.75) in *C. vulgaris* and lowest (2.94) in *C. colocynthis*, while*K. senegalensis*, *A. lebbeck* and *A. occidentale* had 4.75, 4.49 and 4.30 respectively. The iodine value ($mgiodineg^{-1}$) range from 42.40 in *A. occidentale* to 114 in *A. lebbeck*. The acid values of all the oils were below $10mgKOHg^{-1}$ with *K. senegalensis* having the least (2.71) while *C. vulgaris* had the highest value of 8.89 but *C. colocynthis*, *A. lebbeck* and *A. occidentale* had 4.13, 5.14 and 7.04 acid values respectively.

The fatty acid composition of the five (5) seed oils are shown in Table 2. Behenic acid, linoleic acid, lignoceric acid and palmitoleic acidwere detected only in *A. occidentale* nut oil in the following amounts; 6.89%, 0.06%, 0.17% and 1.24% respectively. *A. occidentale* had 4.07% while *A. lebbeck* had 1.54% Arachidic acid. Also, *A. occidentale* had the highest Linoleic acid content (59.67%) while *K. Seneganlesis*had the least (22.35%). *C. colocynthis*, *A. lebbeck*and *C. vulgaris*had 59.64%, 40.95% and 33.38% Linoleic acid respectively. Oleic acid was also detected in *A. occidentale*(12.90%) and *K. Seneganlesis* (43.98%). *A. occidentale*had the highest (18.33%) palmitic acid content while *C. vulgaris*had the least (5.27%). *A. lebbeck*, *C. colocynthis* and *K. Seneganlesis* had 13.40%, 10.48% and 10.20% palmitic acid content respectively. Stearic acid was not detected in *C. vulgaris*but was detected in all the other oils. *A. lebbeck* had the highest value (4.59%) followed by *K. Seneganlesis*(3.14%). *A. occidentale*and *C.*

*colocynthis*had the same amount (1.49%) of Stearic acid in their oils. Only *A. occidentale*had Myristic acid (0.27%) in its nut oil.

Oxirane content of *C. vulgaris* (2.75%) was highest followed by *C. colocynthis* (1.31%). *A. lebbeck* had 0.45% while *K. senegalensis* had 0.26%. *A. occidentale* was least with 0.20%. Linolein was also detected in all the oils with *A. lebbeck* having the highest (15.38%) and *C. colocynthis* having the least (3.02%). Linolein present in *A. occidentale*, *C. vulgaris* and *K. senegalensis* were 4.01, 6.82 and 6.10% respectively. Tetrahydrofuran was only detected in *A. occidentale* (0.30%) and *C. vulgaris* (1.66%) while lupeol and squalene were detected in *C.colocynthis* (12.83%, 1.20%) and *C. vulgaris* (27.01%, 1.71%) respectively. Quercetin and kaempferol glycoside were also detected in *A. occidentale* (1.17%, 2.03%) and *K. senegalensis* (1.22%, 12.75%) respectively. Charantin (0.91%), 5-hydroxytryptamine (1.68) and p-sitosterol (7.37%) were only detected in *C. colocynthis* seed oil. Olean-12-en-3-yl acetate (21.40%) was only detected in *C.vulgaris* while ethanone (1.27%), 2,2-dimethylvaleraldehyde (0.33%), linoleic acid chloride (12.40%), pentacosane (6.08%) and tetracosane (3.62%) were all detected in *A. lebbeck* seed oil as shown in Table 3 below.

4.2 Toxicity of the Seed Oils

4.2.1 Repellent effect of the seed oils

Data in (Table 4) showed that at highest concentration 0.234mlg-1, the seed oil of *Albizialebbeck* had strongest repellent action (100%) against the larvae of *Dermestes maculatus*. Similarly, *Anacardium occidentale* (Table 5), *Citrullus colocynthis* (Table 6), *Citrullus vulgaris* (Table 7), and *Khaya senegalensis* (Table 8) had strongest repellent effect on the larvae of *D. maculatus*. The repellent activity of all the five seed oils increased progressively with increase in concentration against the larvae of *D. maculatus*. Analysis of Variance showed significant ($p<0.05$) repellent effect between the different concentrations of all the seed oils but *A. occidentale* had the best result even at the lowest concentration (26.67%) in comparison to all the other oils.

Table 1: Physical properties of seed oils of *Albizia lebbeck, Anacardium occidentale, Citrullus colocynthis, Citrullus vulgaris, Khaya senegalensis*used for the experiment.

Seed oils	Relative Density	Refractive Index	Saponification value	Peroxide value	Iodine value	Acid value
A. lebbeck	0.92 ± 0.01^{b}	1.47 ± 0.01^{a}	196.30 ± 0.48^{a}	4.49 ± 0.09^{b}	114.00 ± 1.32^{a}	5.14 ± 0.10^{c}
A. occidentale	0.96 ± 0.01^{a}	1.46 ± 0.01^{a}	166.00 ± 0.84^{c}	4.30 ± 0.12^{b}	42.40 ± 0.10^{d}	7.04 ± 0.09^{b}
C. colocynthis	0.90 ± 0.01^{b}	1.47 ± 0.00^{a}	196.47 ± 0.29^{a}	2.94 ± 0.01^{c}	110.00 ± 0.22^{b}	4.13 ± 0.05^{d}
C. vulgaris	0.85 ± 0.01^{c}	1.47 ± 0.01^{a}	115.94 ± 0.64^{d}	10.75 ± 0.52^{a}	89.71 ± 0.55^{c}	8.98 ± 0.18^{a}
K. senegalensis	0.96 ± 0.01^{a}	1.47 ± 0.01^{a}	192.26 ± 0.54^{b}	4.75 ± 0.04^{b}	88.43 ± 0.11^{c}	2.71 ± 0.03^{e}
P-Value	**0.00**	**0.79**	**0.00**	**0.00**	**0.00**	**0.00**

Mean ± SEM with the same superscript along columns are not significantly different at $P < 0.05$

Table 2: Fatty acid composition (%) of seed oils of *Albizia lebbeck*, *Anacardium occidentale*, *Citrullus colocynthis*, *Citrullus vulgaris* and *Khaya senegalensis* used for the experiment.

Fatty acid	*A. lebbeck*	*A. occidentale*	*C. colocynthis*	*C. vulgaris*	*K. senegalensis*	P-Value
Arachidic acid	1.54 ± 0.02^{b}	4.07 ± 0.04^{a}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	**0.00**
Behenic acid	0.00 ± 0.00^{b}	6.89 ± 0.06^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	**0.00**
Linoleic acid	40.95 ± 0.20^{b}	59.67 ± 0.50^{a}	59.64 ± 0.07^{a}	33.38 ± 0.15^{c}	22.35 ± 0.05^{d}	**0.00**
Linolenic acid	0.00 ± 0.00^{b}	0.06 ± 0.01^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	**0.00**
Lignoceric acid	0.00 ± 0.00^{b}	0.17 ± 0.01^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	**0.00**
Myristic acid	0.00 ± 0.00^{b}	0.27 ± 0.01^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	**0.00**
Oleic acid	0.00 ± 0.00^{c}	12.90 ± 0.06^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	43.98 ± 0.30^{a}	**0.00**
Palmitic acid	13.40 ± 0.18^{a}	10.33 ± 0.02^{b}	10.48 ± 0.03^{b}	5.27 ± 0.05^{c}	10.20 ± 0.10^{b}	**0.00**
Palmitoleic acid	0.00 ± 0.00^{b}	1.24 ± 0.01^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	**0.00**
Stearic acid	4.59 ± 0.06^{a}	1.49 ± 0.01^{c}	1.49 ± 0.01^{c}	0.00 ± 0.00^{d}	3.14 ± 0.02^{b}	**0.00**

Mean ± SEM with the same superscript along rows are not significantly different at $P < 0.05$

Table 3: Detected Metabolites compositions (%) of seed oils of *Albizia lebbeck*, *Anacardium occidentale*, *Citrullus colocynthis*, *Citrullus vulgaris* and *Khaya senegalensis* used for the experiment.

Metabolites	*A. lebbeck*	*A. occidentale*	*C. colocynthis*	*C. vulgaris*	*K. senegalensis*	P-Value
Charantin	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.91 ± 0.01^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	**0.00**
Mormordicin	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.07 ± 0.01^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	**0.00**
5-hydroxytryptamine (Serotonin)	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	1.68 ± 0.01^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	**0.00**
p-Sitosterol	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	7.37 ± 0.04^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	**0.00**
Ethanone	1.27 ± 0.05^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	**0.00**
2,2-Dimethylvaleraldehyde	0.33 ± 0.02^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	**0.00**
Oxirane	0.45 ± 0.02^{c}	0.20 ± 0.03^{e}	1.31 ± 0.01^{b}	2.75 ± 0.02^{a}	0.26 ± 0.01^{d}	**0.00**
Linoleic acid chloride	12.40 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	**-**
Pentacosane	6.08 ± 0.02^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	**0.00**
Linolein	15.38 ± 0.02^{a}	4.01 ± 0.03^{d}	3.02 ± 0.01^{e}	6.82 ± 0.01^{b}	6.10 ± 0.09^{c}	**0.00**
Tetracosane	3.62 ± 0.04^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	**0.00**
Olean-12-en-3-yl acetate	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	21.40 ± 0.25^{a}	0.00 ± 0.00^{b}	**0.00**
Lupeol	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	12.83 ± 0.03^{b}	27.01 ± 0.20^{a}	0.00 ± 0.00^{c}	**0.00**
Squalene	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	1.20 ± 0.08^{b}	1.71 ± 0.04^{a}	0.00 ± 0.00^{c}	**0.00**
Tetrahydrofuran	0.00 ± 0.00^{c}	0.30 ± 0.02^{b}	0.00 ± 0.00^{c}	1.66 ± 0.04^{a}	0.00 ± 0.00^{c}	**0.00**
Quercetin	0.00 ± 0.00^{c}	1.17 ± 0.01^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	1.22 ± 0.16^{a}	**0.00**
Kaempferol glycosides	0.00 ± 0.00^{c}	2.03 ± 0.02^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	12.75 ± 0.01^{a}	**0.00**

Mean ± SEM with the same superscript along rows are not significantly different at $P < 0.05$

Table 4: Repellent effect of *Albizia lebbeck* seed oil against *Dermestes maculatus* larvae at 24 hours exposure period.

Application(ml) per 17g fish	Mean number of larvae on treated fish	Mean number of larvae on untreated fish	Repellency Rate (%)	Repellency Class (RC)
0.05(0.003mlg^{-1})	29.33	0.67±0.33^{e}	-95.56	**I**
0.15(0.009mlg^{-1})	25.33	4.67±0.33^{d}	-68.89	**I**
0.46(0.027mlg^{-1})	14.33	15.67±0.67^{c}	4.44	**II**
1.38(0.081mlg^{-1})	3.33	27.67±0.33^{b}	77.78	**V**
4.13(0.243mlg^{-1})	0.00	30.00±0.00^{a}	100.00	**VI**

Mean ± SEM with the same superscript along the column are not significantly different at $P < 0.05$

Table 5: Repellent effect of *Anacardium occidentale* seed oil against *Dermestes maculatus* larvae at 24 hours exposure period.

Application(ml) per 17g fish	Mean number of larvae on treated fish	Mean number of larvae on untreated fish	Repellency Rate (%)	Repellency Class (RC)
0.05(0.003mlg^{-1})	22.00	9.33±0.33^{e}	-46.67	**I**
0.15(0.009mlg^{-1})	18.67	11.33±2.33^{d}	-24.44	**I**
0.46(0.027mlg^{-1})	12.67	17.33±3.67^{c}	15.56	**II**
1.38(0.081mlg^{-1})	6.00	24.00±0.58^{b}	60.00	**V**
4.13(0.243mlg^{-1})	0.00	30.00±0.00^{a}	100.00	**VI**

Mean ± SEM with the same superscript along the column are not significantly different at $P < 0.05$

Table 6: Repellent effect of *Citrullus colocynthis* seed oil against *Dermestes maculatus* larvae at 24 hours exposure period.

Application(ml) per 17g fish	Mean number of larvae on treated fish	Mean number of larvae on untreated fish	Repellency Rate (%)	Repellency Class (RC)
0.05(0.003mlg^{-1})	24.33	5.67±1.20^{e}	-62.22	**I**
0.15(0.009mlg^{-1})	23.67	6.33±0.88^{d}	-57.78	**I**
0.46(0.027mlg^{-1})	21.33	8.67±0.88^{c}	-42.22	**I**
1.38(0.081mlg^{-1})	8.33	21.67±3.84^{b}	44.44	**IV**
4.13(0.243mlg^{-1})	0.00	30.00±0.00^{a}	100.00	**VI**

Mean ± SEM with the same superscript along the column are not significantly different at $P < 0.05$

Table 7: Repellent effect of *Citrullus vulgaris* seed oil against *Dermestes maculatus* larvae at 24 hours exposure period.

Application(ml) per 17g fish	Mean number of larvae on treated fish	Mean number of larvae on untreated fish	Repellency Rate (%)	Repellency Class (RC)
0.05(0.003mlg^{-1})	27.33	2.67±0.88^{d}	-82.22	**I**
0.15(0.009mlg^{-1})	24.00	6.00±1.16^{c}	-60.00	**I**
0.46(0.027mlg^{-1})	23.33	6.67±0.67^{c}	-55.56	**II**
1.38(0.081mlg^{-1})	4.33	25.67±0.33^{b}	71.11	**V**
4.13(0.243mlg^{-1})	0.00	30.00±0.00^{a}	100.00	**VI**

Mean ± SEM with the same superscript along the column are not significantly different at $P < 0.05$

Table 8: Repellent effect of *Khaya senegalensis* seed oil against *Dermestes maculatus* larvae at 24 hours exposure period.

Application(ml) per 17g fish	Mean number of larvae on treated fish	Mean number of larvae on untreated fish	Repellency Rate (%)	Repellency Class (RC)
0.05(0.003mlg^{-1})	25.67	4.33±1.33^{e}	-60.00	**I**
0.15(0.009mlg^{-1})	18.33	11.67±1.86^{d}	-22.22	**I**
0.46(0.027mlg^{-1})	15.00	15.00±3.06^{c}	0.00	**I**
1.38(0.081mlg^{-1})	4.67	25.33±1.86^{b}	68.89	**V**
4.13(0.243mlg^{-1})	0.00	30.00±0.00^{a}	100.00	**VI**

Mean ± SEM with the same superscript along the column are not significantly different at $P < 0.05$

4.2.2 Mortality Effect of the seed oils

All the tested seed oils significantly ($p<0.05$) killed the larvae of *Dermestes maculatus* exposed to treated smoke – dried fish, *Clarias gariepinus*. All the seed oils showed larval mortality ranging from 1.11 to 100%. Larval mortality increased with length of exposure. There was significant difference ($p<0.05$) in mortality of larvae amongst the treatments. Though total mortality was recorded for all the seed oils tested at $0.243mlg^{-1}$, but *Anacardium occidentale* (Figure 3) and *Khaya senegalensis* (Figure 6) seed oils were most effective even at low concentration ($0.027mlg^{-1}$) against the larvae of *D. maculatus* evoking between 40 to 44.44% mortality after 72 hours of exposure period. This was followed by *Albizia lebbeck* (Figure 2) that recorded 17.78% after 72 hours exposure. *Citrullus colocynthis* (Figure 4) and *Citrullus vulgaris* (Figure 5) were the least toxic at $0.027mlg^{-1}$ causing 2.22 to 3.33% larval mortality after 72 hours exposure period.

4.3 Median Lethal Concentration (Lc_{50}) of the Seed Oils

Anacardium occidentale (Fig 8) seed oil was the most effective against the larvae of *D. maculatus* with LC_{50} value of $0.022mlg^{-1}$ which was the least in comparison to all the other oils. This was followed by *Khaya senegalensis* (Fig 11) and *Albizia lebbeck* (Fig 7) with LC_{50} value of $0.035mlg^{-1}$. The LC_{50} value of *Citrullus colocynthis* (Fig 9) seed oil used against the larvae of *D. maculatus* was found to be $0.045mlg^{-1}$. *Citrullus vulgaris* (Fig 10) was the least effective with LC_{50} value of $0.055mlg^{-1}$. A result summary of this is shown in Table 9 below.

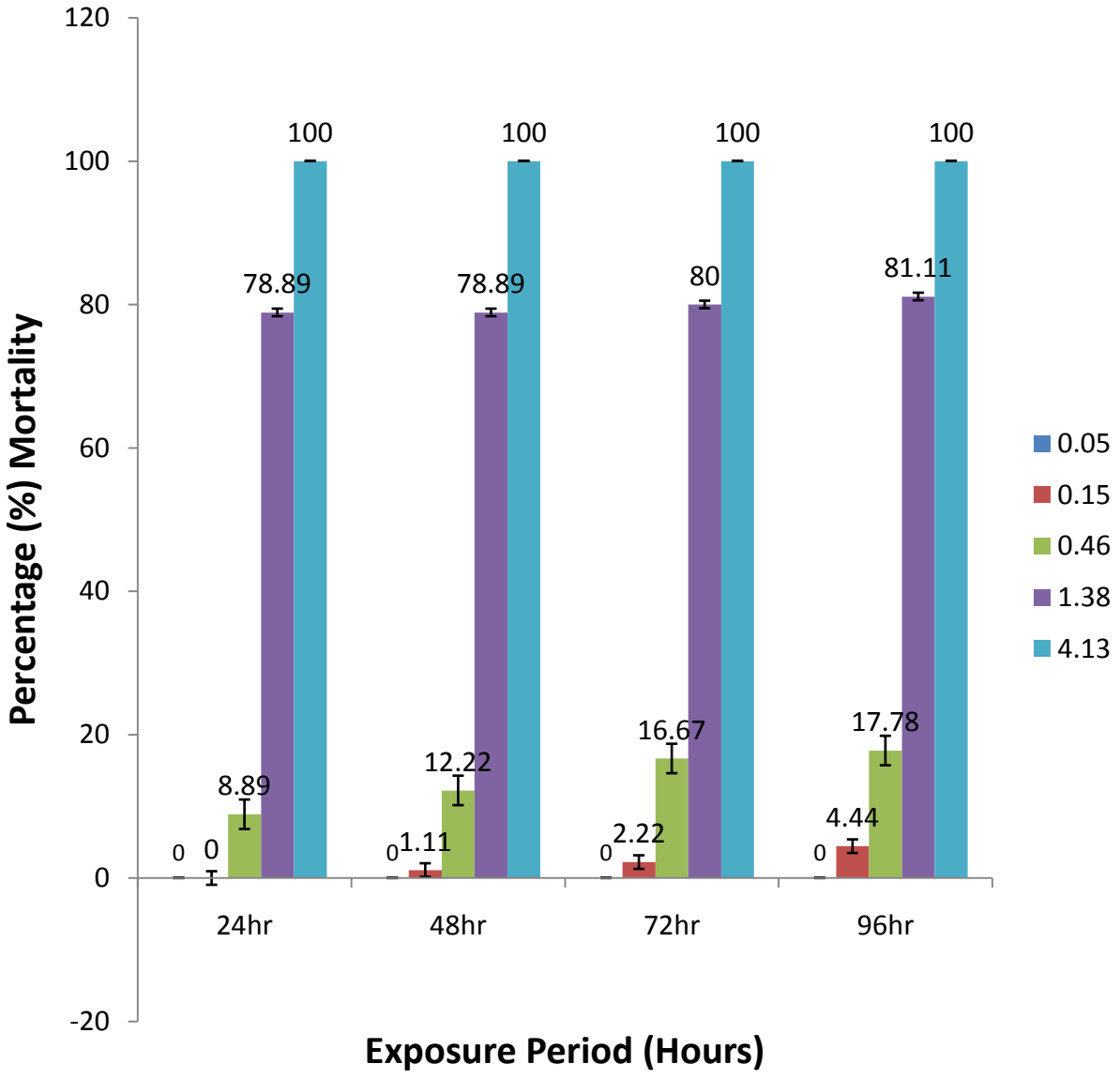


Figure 2: Mortality effect of *Albizia lebbeck* seed oil on *Dermestes maculatus* larvae

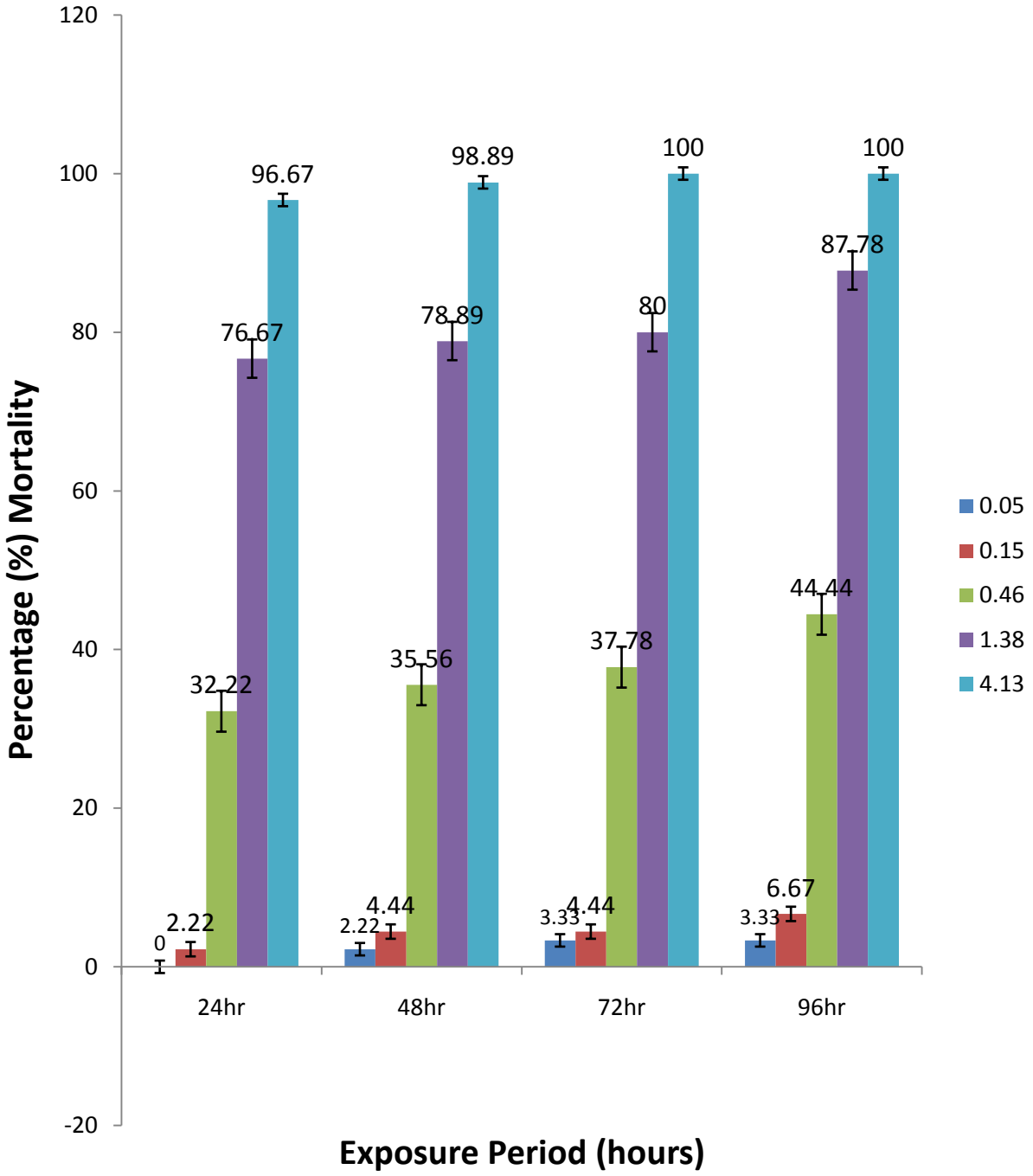


Figure 3: Mortality effect of *Anarcadium occidentale* seed oil on *Dermestes maculatus* larvae

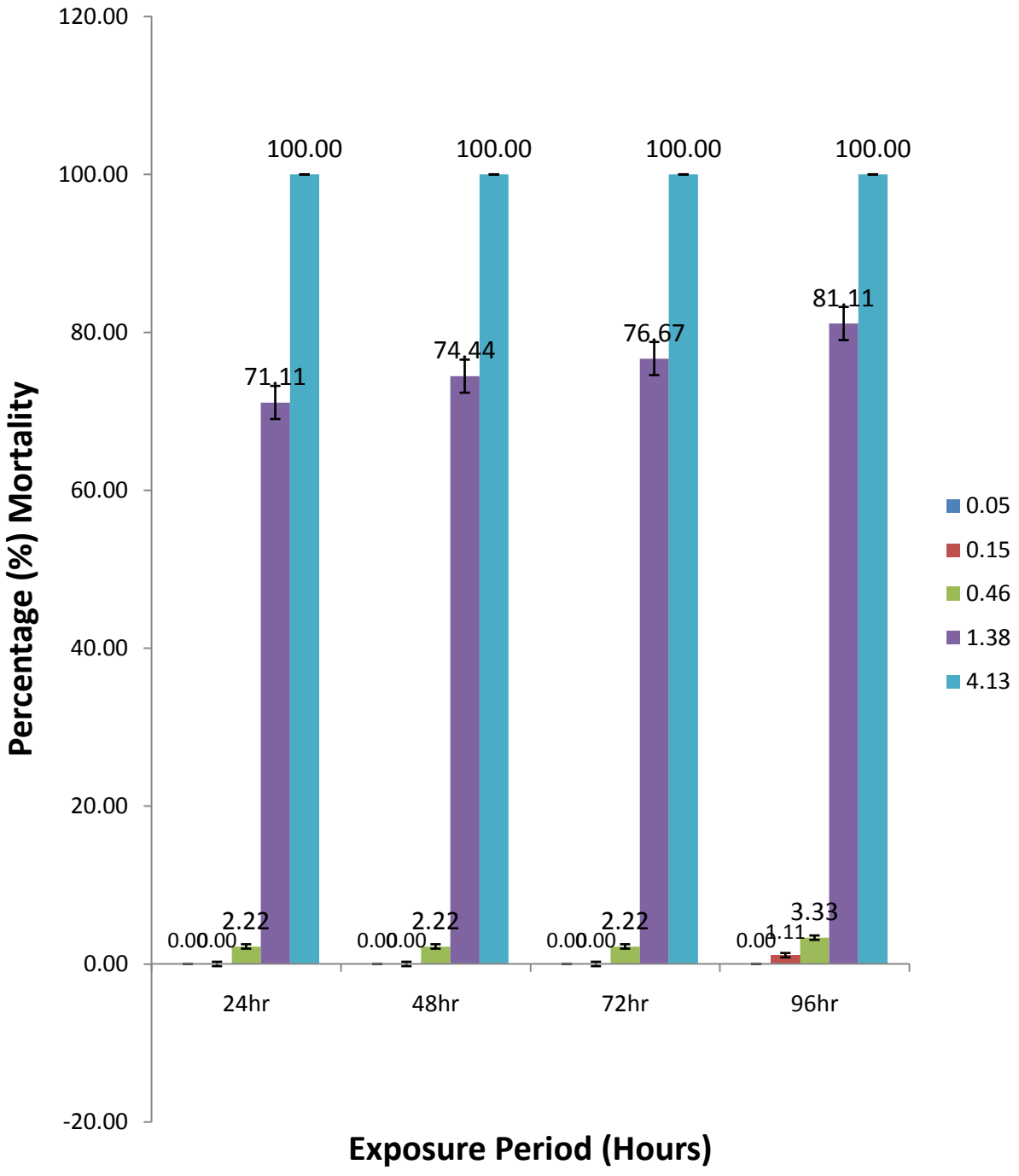


Figure 4: Mortality effect of *Citrullus colocynthis* seed oil on *Dermestes maculatus* larvae

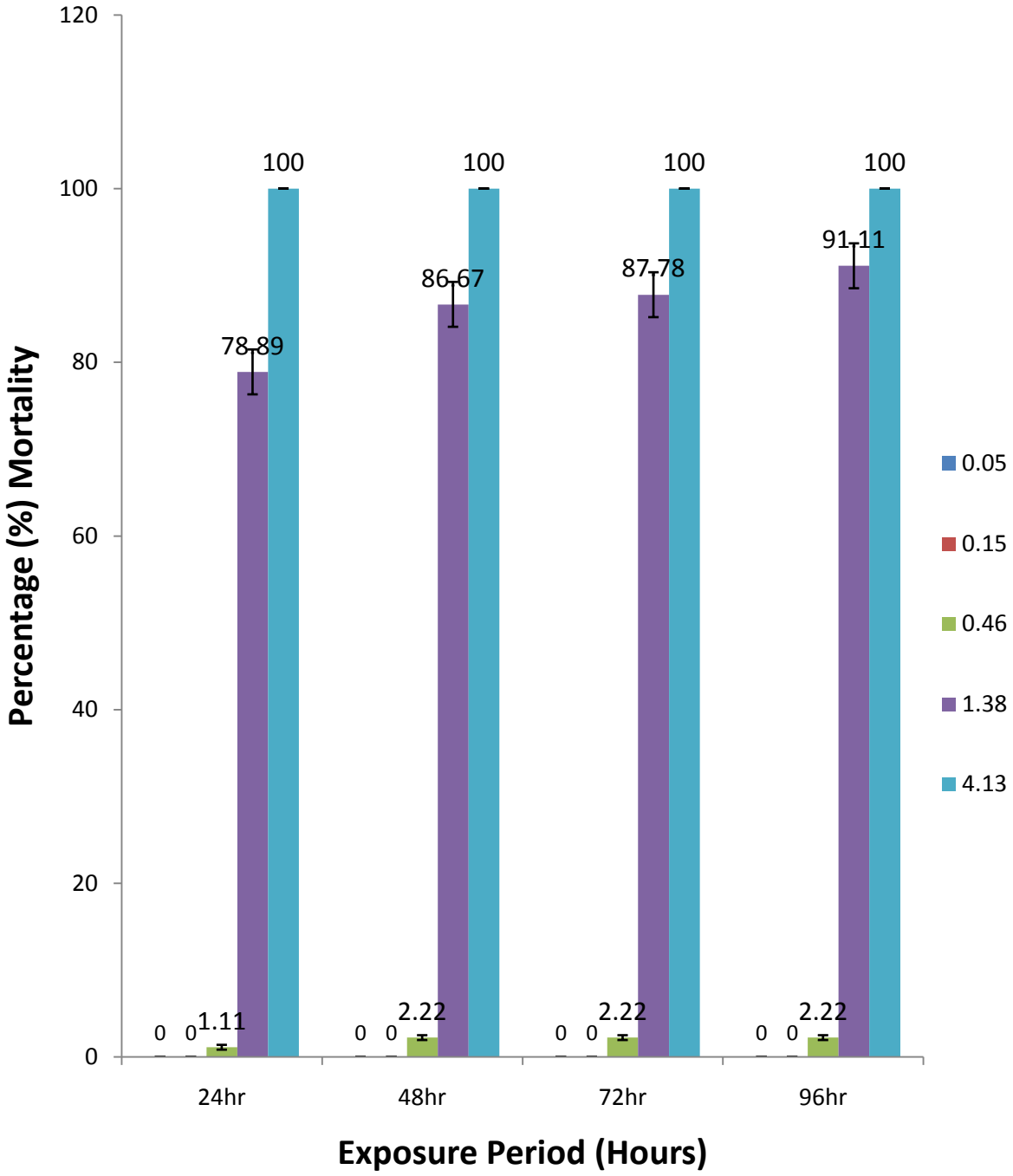


Figure 5: Mortality effect of *Citrullus vulgaris* seed oil on *Dermestes maculatus* larvae

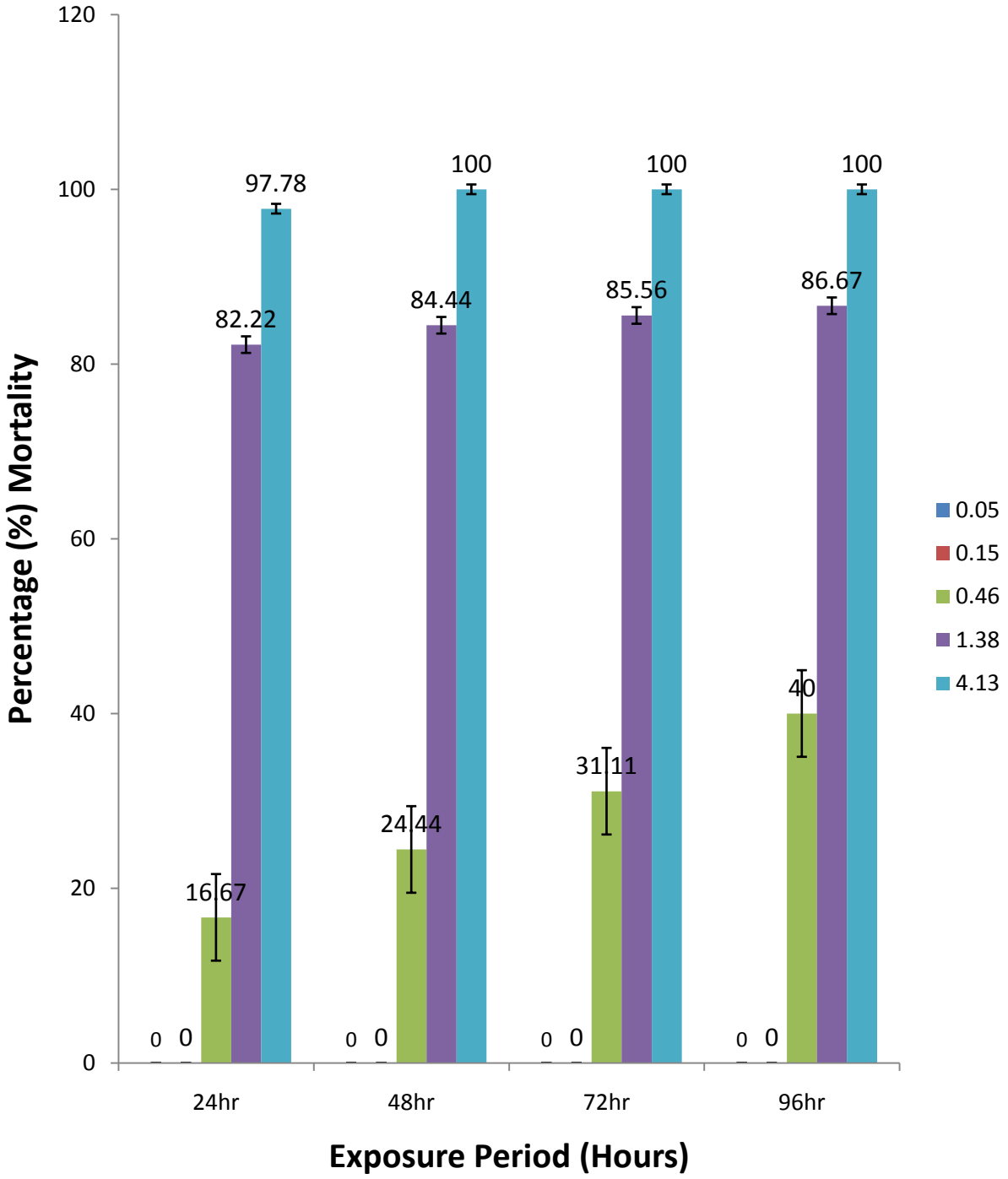


Figure 6: Mortality effect of *Khaya senegalensis* seed oil on *Dermestes maculatus* larvae

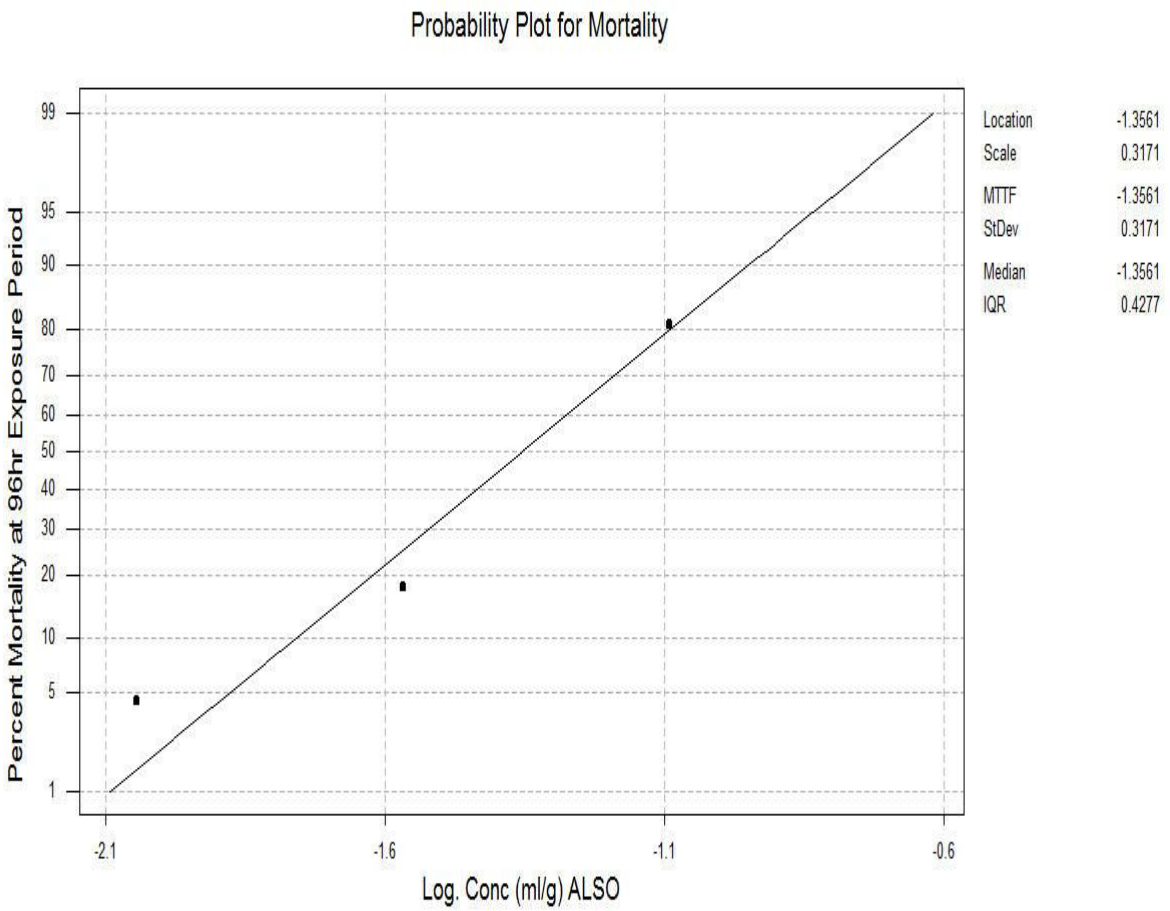


Figure 7: Probit graph use to determine the LC_{50} of *Albizia lebbeck* seed oil against *Dermestes maculatus* larvae

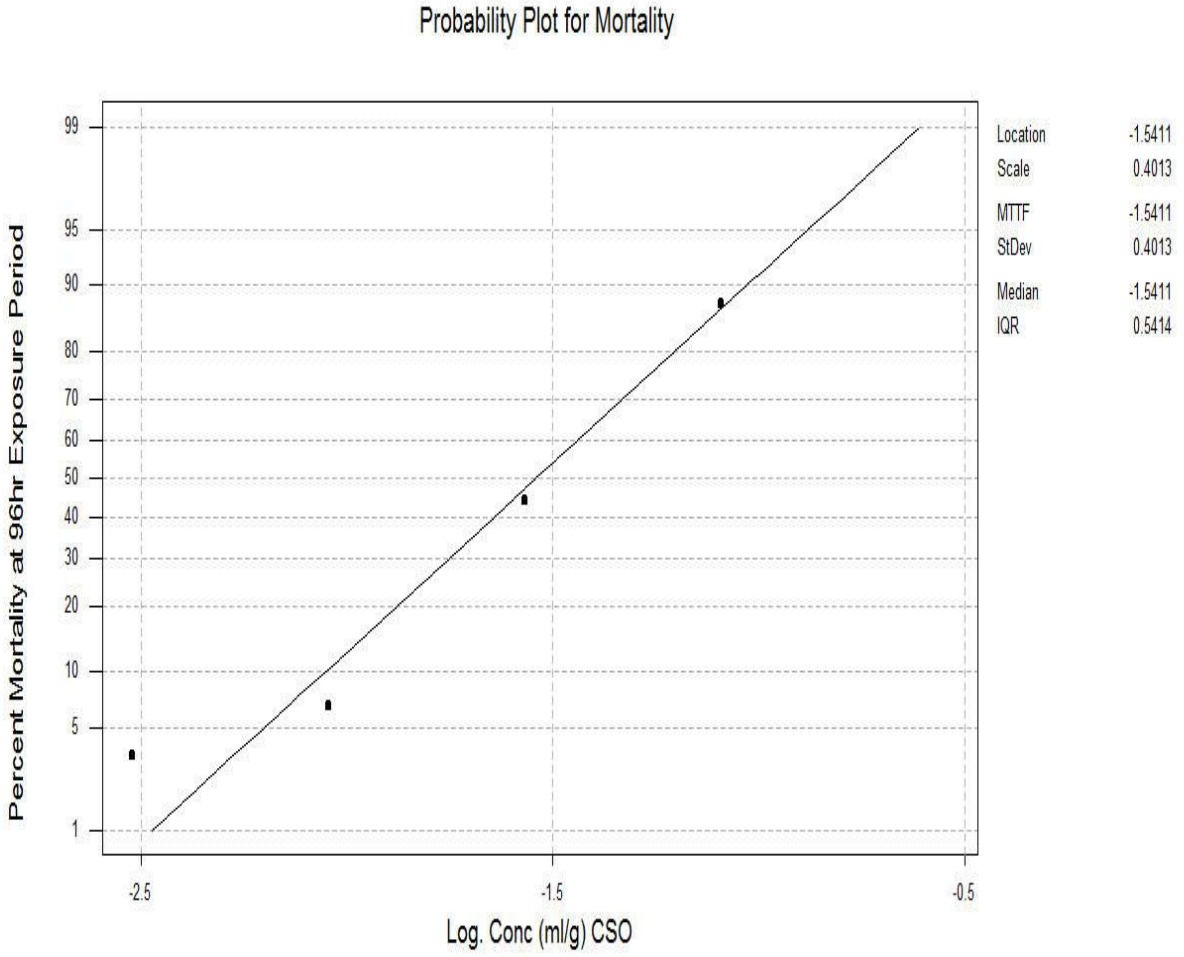


Figure 8: Probit graph use to determine the LC_{50} of *Anacardium occidentale* seed oil against *Dermestes maculatus* larvae

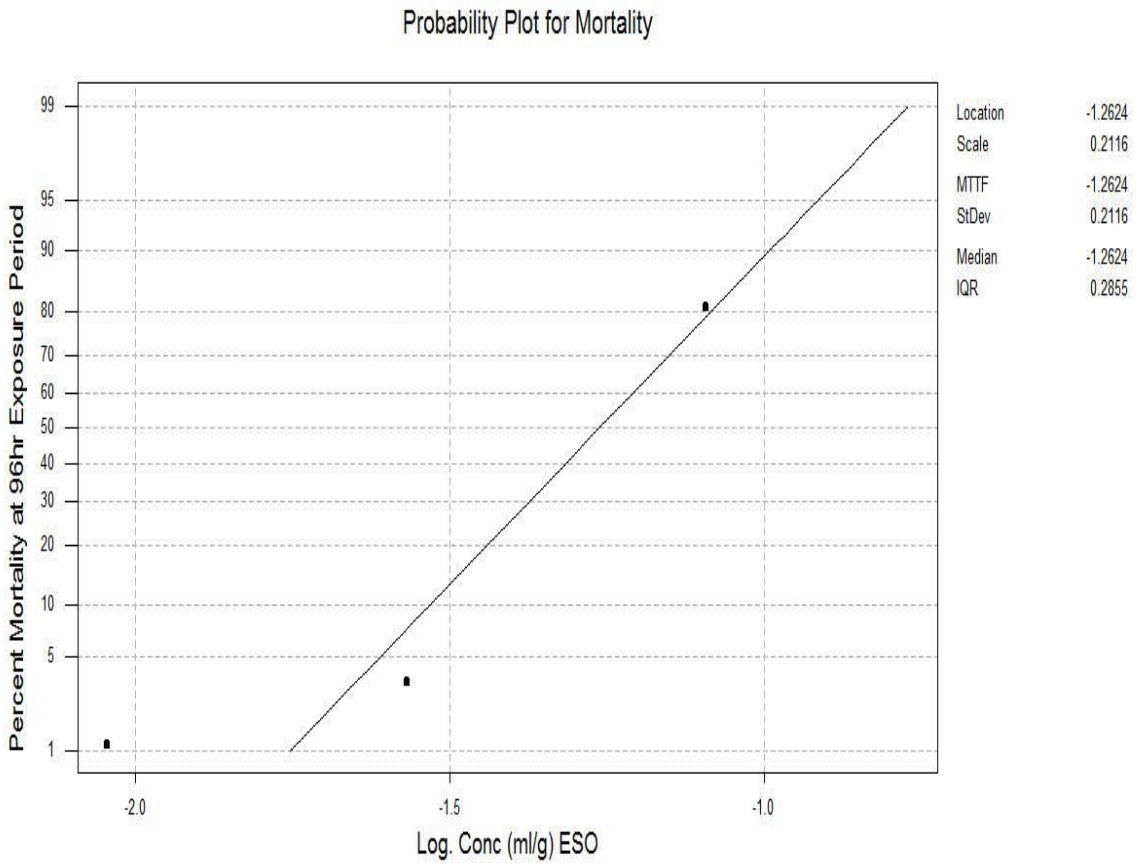


Figure 9: Probit graph use to determine the LC_{50} of *Citrullus colocynthis* seed oil against *Dermestes maculatus* larvae

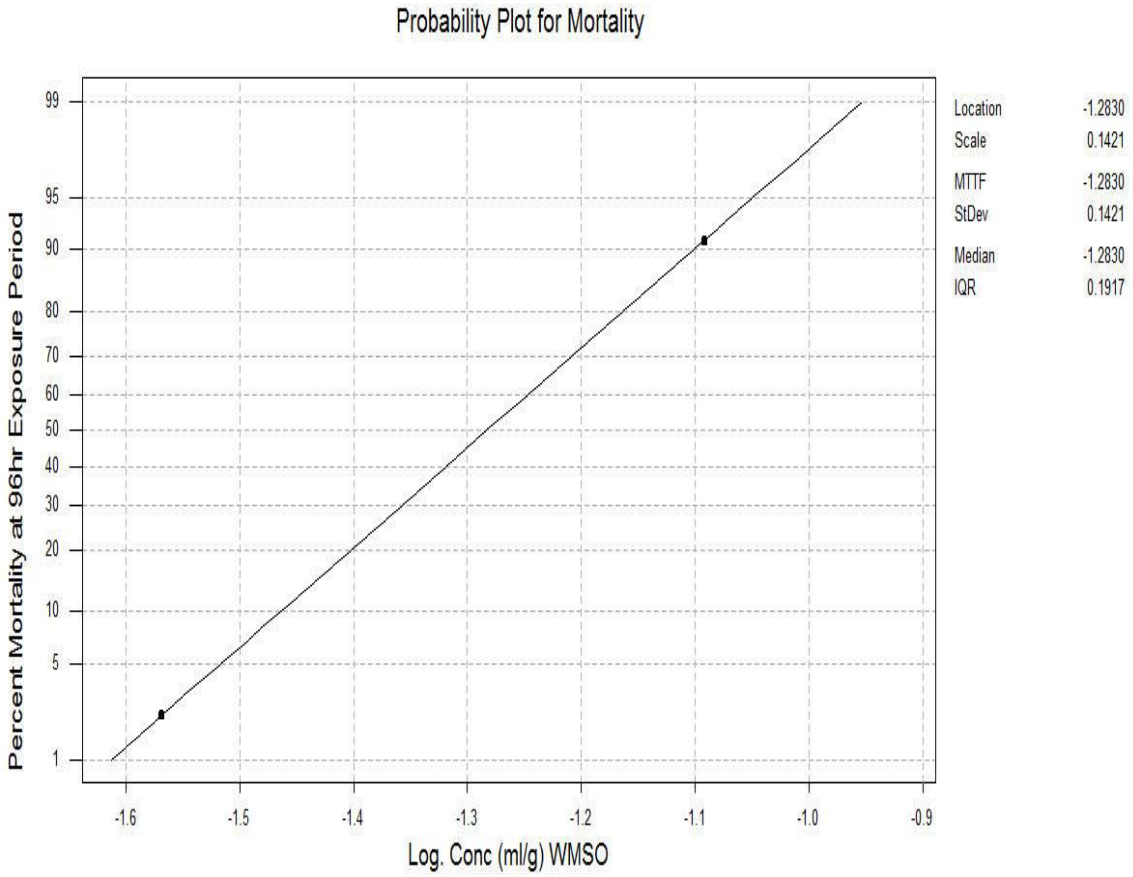


Figure 10: Probit graph use to determine the LC_{50} of *Citrullus vulgaris* seed oil against *Dermestes maculatus* larvae

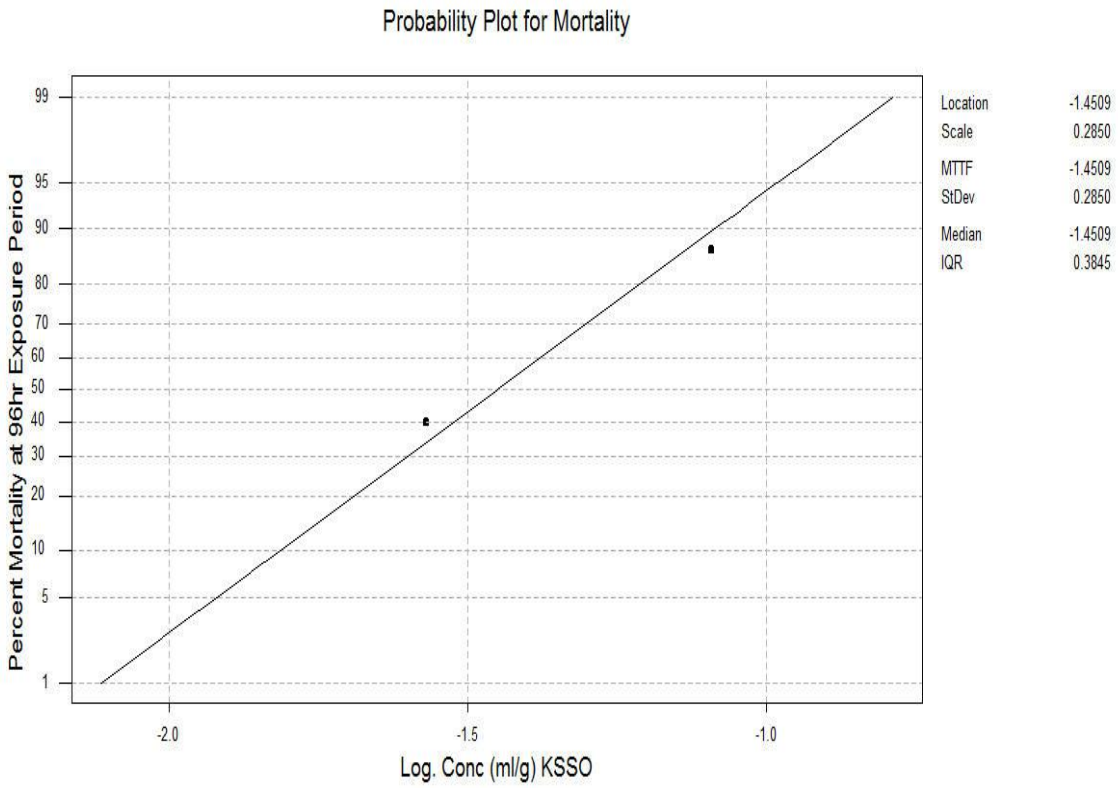


Figure 11: Probit graph use to determine the LC_{50} of *Khaya senegalensis* seed oil against *Dermestes maculatus* larvae

Table 9: 24 – 96hours Median Lethal Concentration (LC_{50}) of seed oils against *D. maculatus* larvae by Probit Analysis

SEED OILS	**LC_{50} VALUES (ml/g of fish)**			
	24hr	**48hr**	**72hr**	**96hr**
Albizia lebbeck	0.0536	0.0504	0.0466	0.0441
Anacardium occidentale	0.0434	0.0370	0.0345	0.0288
Citrullus colocynthis	0.0638	0.0618	0.0605	0.0547
Citrullus vulgaris	0.0609	0.0548	0.0541	0.0521
Khaya senegalensis	0.0492	0.0425	0.0392	0.0354

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physicochemical Properties and Fatty Acid Compositions of the Seed Oils

The physicochemical properties and fatty acid composition of the different seed oils analysed in this study vary and the variation depend on species. According to Ihekoronye and Ngoddy(1985), the variations depend on species, geographical locations and seasons. The iodine values of three oils in this study were lower than 100, the oils were classified as non-drying oils which implies that when they are applied on the surface of the fish, they would stay longer, offering protection to the fish against destructive insect pests. Non drying oil remain as liquid for a long period with little oxidation (Eromosele and Eromosele, 1993).Pa Quart (1979) and Pearson (1981) reported that higher iodine value of oil above 100 is an indication of high degree of unsaturation and therefore higher tendency for the onset of rancidity.

A higher percentage of acid content was found in cashew and watermelon oils in comparison with other oils, indicating that these oils maybe more rancid than other oils and as the rancidity increases, oils achieve a foul smell along with a sour taste (Pearson, 1981), which may repel insects and protect the treated smoke – dried fish from pests. The refractive indices of all the seed oils showed that the values fell within the range for edible oils, suggesting that it may be less toxic to man. A refractive index of 1.47 was also an indication that the oil is lighter than most drying oil (Rossell, 1991). The relative densities of the oils indicates that these oils will float on water, are light oils and has great capacity to spread easily over surfaces applied; this is an advantageous property of the oils on their application to smoke – dried fish.The low peroxide value (<5) in most of the seed oils except for *C. vulgaris* (>10) is an indication that the oils would be susceptible to low oxidative rancidity, which confers on them an advantage, as bioinsecticides, on the oils. The saponification values obtained compare favourably with values reported for some common edible vegetable oils such as palm oil (196-205 mg

K0H/g) and soy beans oil (190-210 mg K0H/g oil)(Pearson, 1981). The value is an indication of average molecular weight of lipids (Otitologbon *et al*., 1996). It indicates that the oils contain mainly fatty acids of fairly high molecular mass and therefore they would serve as important raw materials for the manufacture of low toxic insecticides, cosmetics, lubricants and candles (Anhwange *et al*., 2005).

Oils high in unsaturated fatty acids are liquid when they are cold and since unsaturated fatty acids have the highest percentage in the oils, it implies that the oils may not congeal at room temperature (≤25˚C). Oleic acid, an unsaturated fatty acid, was present only in*A. occidentale* which had the best effect in terms of mortality and in *A. lebbeck*which had the best effect in terms of repellency of *D. maculatus* larvae. This component could have repelled or killed the insect larvae that were exposed to or that fed on the fish treated with the oil.According to Rollo *et al*. (1995), oleic acid and linoleic acid have been implicated in death recognition and death aversion (repellency) in cockroaches. It is not the quantity of these polyunsaturated oils which governs the harm they do, but the relationship between them and the saturated fats. The higher this ratio is, the greater the probability of harm there is. Sauda *et al*. (2011) reported the insecticidal effects of oleic acid, palmitic acid and stearic acid against *Spodoptera frugiperda* and concluded that palmitic acid was the most active agent followed by oleic and stearic acid extracted from*Carica papaya*. These oils were also detected in the present study at varying quantities. Oleic acid was only seen in *A. occidentale* and *K. senegalensis* and could have in synergy with linoleic, palmitic and stearic acid brought about the insecticidal effect of the oils since it was found that linoleic and linolenic acids were particularly toxic to *Haematococcus pluvialis* and it was concluded that the toxicity of the fatty acids in the culture distillate was due mainly to the Poly Unsaturated Fatty Acids (Proctor, 1957). *A. lebbeck* had the highest palmitic and stearic acid content and could have contributed to the efficacy of the oil against *D. maculatus* larvae.

A number of compounds including tocopherols, polyphenols, flavonoids, carotenoids, and other terpenoids which may have been acting in a synergistic way to bring about the repellent effect observed in this study. For instance,Oxirane otherwise known as Ethylene oxide detected in all the oils in the present study could have

contributed to toxicities since it was in common use as a fumigant for grain, and by the 1950's, it was widely used in museums, libraries and archives for insect pest control (Ballard and Baer, 1986). Pentacosane and Tetracosane are alkanes and triterpenes detected only in *A. lebbeck* seed oil and could have contributed to the toxicity to the larvae of *D. maculatus*. These alkanes have been noted to possess insecticidal properties and are used to kill insects that damage crops (Sanford and Satish, 1998). Quercetin, an important component of *A. occidentale* and *K. senegalensis* seed oils, was also extracted from *Kalanchoe pinnata* and it possess potent oral efficacy against cutaneous leishmaniasis (Muzitano, 2009).

This study revealed that *C. colocynthis* and *C. vulgaris* were the only seed oils that contained Lupeol. According to Marie *et al*. (2009) lupeol isolated from branches of *Simarouba versicolor* showed just a slight (20%) inhibitory effect to the symbiotic fungus and ticks (Lan-qing *et al*., 2012). Squalene, a hydrocarbon and a triterpene, detected in *C. colocynthis* and *C. vulgaris*seed oils in this study has been proposed to be an important part of the Mediterranean diet as it may be a chemopreventive substance that protects people from cancer and it is also used in cosmetics, and more recently as an immunologic adjuvant in vaccines (Smith, 2000; Owen *et al.*, 2004). Tetrahydrofuran was present in *A. occidentale* and *C. vulgaris* and could have contributed to the potency of the oils in killing the larvae of *D. maculatus*. It has been implicated to show insecticidal activity against the larvae of *Spodoptera exigua*, reducing their growth and also killing them (Rodriguez-Saona *et al.*, 2000). It can therefore be deduced that these metabolites could have worked in a synergistic way with the fatty acid components of the oils to bring about the repellency and mortality of the larvae of *D. maculatus* observed in this study.

5.2 Repellent Effect of the Seed Oils

The repellent effect of the oils of *Albizia lebbeck, Anacardium occidentale, Citrullus colocynthis, Citrullus vulgaris,* and *Khaya senegalensis* were significant at $p<0.05$. They excellently repelled the larvae of *Dermestes maculatus* from treated smoke – dried fish,

Clarias gariepinus thereby preserving the fishes. The repellency effect increased with increasing concentration of the oils of all the five plants. This view is largely supported by Stoll (2000) who reported repellence as a major mechanism by which plant products control insect damage to stored produce. Akinwumi *et al*. (2007) reported that extracts of *Dennettia tripetala, Eugenia aromatica, Monodora myristica* and *Piper guineense* exhibited repellency against *D. maculatus*. Similarly, Jilani *et al*.(1988) showed that repellency increased with increasing concentration of the oils of Tumeric oil, Sweetflag oil, Neem oil and Margosan on red flour beetle on red flour beetle, *Tribolium castaneum* while Behal (1998) reported that there was a complete repellency of the larvae of the rice moth, *C. cephalonica*, with sweetflag oil irrespective of its concentration, while with other oils, a concentration dependent repellency was noticed.This repellent effect could be as a result of olfactory and gustory sensations which is in agreement with Egwunyenga *et al*. (1998) who also attributed the repellence of *D. maculatus* and *Necrobia rufipes* from admixed fish to olfactory and gustory sensations.

Though the active principle responsible for mortality and repellency was not the main focus of this study but some compounds like monoterpenes, sesquiterpenes, β – caryophyllene, diterpenes alcohol (phytol), triterpenes and volatile terpenoidswhich are repellent to most insects (Nerio *et al*., 2010), make up a good proportion of the oils tested in this study and could likely have been responsible for the repellent effect observed against the larvae of *D. maculatus*. These repellent molecules could have interacted with the olfactory receptors and block the sense of smell.Oils of Osage orange (Hedgeapple) (*Muchia promifera*) contains numerous sesquiterpenoids, many of them were repellent to the German crockroach (Peterson and Coats, 2001). In addition, cineole, geraniol, and piperidine found in bay leaves (*Laurus nobilis*) possess repellent properties towards crockroaches (Verma and Meloan, 1981). This is a pointer to the fact that the chemical constituent of the oils used in this present study contains repellent properties which is largely responsible for the repellent effects observed.

5.3 Mortality Effect of the Seed Oils

All the five seed oils tested in this study showed significant mortality ($p<0.05$) of *D. maculatus* larvae exposing these oils as valuable products for the control of insect pests of smoke – dried African catfish. The seed oils are suspected to have a contact mode of action on the larvae since they were killed as a result of their transient contact with the treated fish. Lale (1995) reported that plant extracts are highly lipophilic and could penetrate the cuticle of insects. The toxicity effect observed in this study can also be attributed to the various chemical ingredients like terpenoids and triterpenes present along side the fatty acid compositions in the oils. This is in agreement with Butterwoth and Morgan (1968) who reported that toxicity of essential oils is associated with the presence of many chemical ingredients such as triterpenoids. The present study also collaborates Kilonzo (1995) who reported that neem kernel powder to have a high toxic effect on feeding and survival of different pest species including keratinophagus insects under laboratory condition demonstrating that neem contains azadiractins which is toxic to stored product insect pests.Lu and Wu(2010) also suggested that various essential oils or their constituents cause symptoms that suggest a neurotoxic mode of action. According to Lienard *et al*. (1993), seed oil of *Cassia occidentalis* induced high mortality of bruchid eggs and first instar larvae than the fresh and dry leaves or ground seeds and that several fatty acids (linoleic, oleic and stearic acid) present in the oil were responsible for this toxicity. Odeyemi *et al.* (2000) observed that cases of high mortality occur in larvae partly because of their inability to detoxify plant toxins when feeding actively, especially at the 1^{st} to 4^{th} instar larval stages. Okonkwo and Okoye (2001) reported 100 percent kill of larvae of *D. maculatus* when treated with extracts of *D. tripetala* and *P. guineense* at dosages lower than the powders which was similarly the case reported for all the seed oils in this study ($0.243 mlg^{-1}$).

The mortality effect of the oils in this present study could also be attributed to the oils coating the surface of the fish thereby causing the larvae to avoid the treated fish resulting in starvation and death. This insecticidal effect is in agreement with Dungum *et al*. (2005), Oparaeke and Bunmi (2006). Adedire *et al*. (2011) suggested that the mode of action of the plant powder and oil could be as a result of the powder and oil coating

the treated cowpea seeds which prevented contact between the seeds and the bruchid resulting in starvation. Again, the oils could have blocked the spiracles of the larvae in contact with the treated fish thereby resulting in their suffocation and eventual death(Hall and Harman, 1991; Adedire *et al*., 2011). Exposing of larvae of *D. maculatus* to smoke – dried *Clarias gariepinus* fish (weighing 17g) treated with 0.4896ml of the oil resulted in 50 percent kill. Exposing the larvae to the same size of fishes treated with the oils of *Albizia lebbeck, Citrullus colocynthis, Citrullus vulgaris* and *Khaya senegalensis* at 0.7497ml, 0.9299ml, 0.8857ml and 0.6018ml respectively resulted in 50 percent mortality of larvae suggesting that *Anacardium occidentale* is most effective in the control of *D. maculatus* larvae. The significant difference observed between the periods of exposure of the larvae and also between the different treatments is a clear pointer to the fact that *Anacardium occidentale* oil is the most effective of all the oils tested.

This insecticidal activity of *Anacardium occidentale* oil could be linked to the presence of cardinal, quercetin and kaempferol glycosides which was also suggested by Rehm and Espig (1991); Oliver – Bever (1986). Linolein, Olean – 12 –en – 3 – yl acetate, squalene or supraene, fagarsterol or lupeol, tetrahydrofuran were present in *Citrullus vulgaris* seed oil. Charantin, momordicin, 5 – hydroxytryptamine, p – sitosterol were identified as constituents of *Citrullus colocynthis* seed oil suggesting that these constituents could be responsible for the lethal effect shown by these curcubits. This compares favourably with Basalah *et al*. (1985) who attributed the toxicity of the testa powder to colocynthin, a toxic poisonous substance known to be present in the white fleshy pulp of the melon fruit. According to Nadeem *et al*. (2012), ethanol extract of *C. colocynthis* significantly killed more *T. castaneum* at all levels of concentration as compared to the control. Oxirane, nonacosane, linolein, ethanone and other terpenoids are the constituents that make up 39.53 percent of *Albizia lebbeck* seed oil used in this study and contribute largely to the mortality observed in the larvae of *D. maculatus*. *Khaya senegalensis* seed oil killed the larvae of *D. maculatus* even at low concentration, making it reliable as a protectant. This result is comparable with that of Bamaiyi *et al*. (2006;2007) who reported that *K. senegalensis* seed oil killed nearly all adults of *C. maculatus* within 24hours.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary and Conclusion

The seed oils of *Albizia lebbeck*, *Anacardium occidentale*, *Citrullus colocynthis*, *Citrullus vulgaris* and *Khaya senegalensis* differ in their physico-chemical properties and all the seed oils are stable, non-drying and consumable. The fatty acid components and the secondary metabolites of the oils play an important role in contributing to the mortality and repellency of *D. maculatus* larvae. The seed oils of *Albizia lebbeck, Anacardium occidentale, Citrullus colocynthis*, *Citrullus vulgaris* and *Khaya senegalensis* is toxic to the larvae of *D. maculatus* and also repelled the larvae. Comparison of the performance of the seed oils of *Albizia lebbeck, Anacardium occidentale, Citrullus colocynthis, Citrullus vulgaris* and *Khaya senegalensis* at 0.15ml/17g, 0.46ml/17g and 1.38ml/17g concentrations showed that all five oils performed significantly different ($p<0.05$) from one another with *Anacardium occidentale* having the best mortality effect and *Citrullus colocynthis* the least. Though the performance of *Khaya senegalensis* and *Albizia lebbeck* did not differ significantly, they performed better than *Citrullus vulgaris* seed oil.

In conclusion, oils of *Albizia lebbeck, Anacardium occidentale*, *Citrullus colocynthis*, *Citrullus vulgaris* and *Khaya senegalensis* show repellent and larvicidal activities on the larvae of *D. maculatus* and retain activity even at 96h exposure period, confirming the residual nature of the effects of these oils. Therefore, the oils of *Albizia lebbeck, Anacardium occidentale, Citrullus colocynthis*, *Citrullus vulgaris* and *Khaya senegalensis* could form the basis for a successful formulation and commercialization of biopesticides against *D. maculatus*. They could provide valuable alternatives to the synthetic insecticides in the management of storage insect pests of smoke-dried fishes.

6.2 Recommendations

4.13ml of the oils from seeds of *Albizia lebbeck*, *Anacardium occidentale*, *Citrullus colocynthis*, *Citrullus vulgaris* and *Khaya senegalensis* are recommendedto fish processors for maximum repellency of *D. maculatus*larvae, pest of smoke-dried fish and serve as good alternatives to synthetic insecticides in food fish protection. The degradation of the oils overtime and its likely persistence in smoke – dried fish needs determination.

Further studies on the effect of other unexploited seeds on insect pests particularly *Dermestes maculatus*, *Dermesteslardarius* and *Necrobia rufipes* are recommended. The persistency of the seed oils in smoke – dried fish should be determined in order to elucidate its potential danger (if any) to humans that may eventually consume the treated fish.

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APPENDIX



PLATE I: Adult *Dermestes maculatus* beetle



PLATE II: *Dermestes maculatus* larvae

Appendix I: Repellent effect of *Albizia lebbeck, Anacardium occidentale, Citrullus colocynthis, Citrullus vulgaris* and *Khaya senegalensis* seed oils against the larvae of *Dermestes maculatus*

Anova: Two-Factor With Replications

SUMMARY	0	0.003	0.009	0.027	0.081	0.243	Total
A. occidentale							
Count	3	3	3	3	3	3	18
Sum	0	28	34	52	72	90	276
Average	0	9.333333	11.33333	17.33333	24	30	15.33333
Variance	0	0.333333	16.33333	40.33333	1	0	109.4118
A.lebbeck							
Count	3	3	3	3	3	3	18
Sum	0	2	14	47	50	90	203
Average	0	0.666667	4.666667	15.66667	16.66667	30	11.27778
Variance	0	0.333333	0.333333	1.333333	0.333333	0	120.683
C. colocynthis							
Count	3	3	3	3	3	3	18
Sum	0	17	19	26	65	90	217
Average	0	5.666667	6.333333	8.666667	21.66667	30	12.05556
Variance	0	4.333333	2.333333	2.333333	44.33333	0	120.0556
C. vulgaris							
Count	3	3	3	3	3	3	18
Sum	0	8	18	20	77	90	213
Average	0	2.666667	6	6.666667	25.66667	30	11.83333
Variance	0	2.333333	4	1.333333	0.333333	0	143.2059

Appendix I contd.

K.senegalensis

Count	3	3	3	3	3	3	18
Sum	0	13	35	45	76	90	259
Average	0	4.333333	11.66667	15	25.33333	30	14.38889
Variance	0	5.333333	10.33333	28	10.33333	0	126.2516

Total

Count	15	15	15	15	15	15
Sum	0	68	120	190	340	450
Average	0	4.533333	8	12.66667	22.66667	30
Variance	0	10.98095	13.85714	29.38095	19.80952	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	226.6222	4	56.65556	9.675522	4.18E-06	2.525215
Columns	9723.556	5	1944.711	332.1139	2.35E-42	2.36827
Interaction	458.4444	20	22.92222	3.914611	2.13E-05	1.747984
Within	351.3333	60	5.855556			
Total	10759.96	89				

Appendix II: Mortality effect of *Albizia lebbeck* seed oil against *D. maculatus* larvae

Anova: Two-Factor With Replication

SUMMARY	24h	48h	72h	96h	Total
0					
Count	3	3	3	3	12
Sum	0	0	0	0	0
Average	0	0	0	0	0
Variance	0	0	0	0	0
0.003					
Count	3	3	3	3	12
Sum	0	0	0	0	0
Average	0	0	0	0	0
Variance	0	0	0	0	0
0.009					
Count	3	3	3	3	12
Sum	0	1	2	4	7
Average	0	0.333333	0.666667	1.333333	0.583333
Variance	0	0.333333	0.333333	0.333333	0.44697
0.027					
Count	3	3	3	3	12
Sum	8	11	15	16	50
Average	2.666667	3.666667	5	5.333333	4.166667
Variance	0.333333	0.333333	0	0.333333	1.424242
0.081					
Count	3	3	3	3	12
Sum	71	71	72	73	287
Average	23.66667	23.66667	24	24.33333	23.91667
Variance	0.333333	0.333333	0	0.333333	0.265152

Appendix II contd.

0.243

Count	3	3	3	3	12
Sum	90	90	90	90	360
Average	30	30	30	30	30
Variance	0	0	0	0	0

Total

Count	18	18	18	18
Sum	169	173	179	183
Average	9.388889	9.611111	9.944444	10.16667
Variance	165.6634	162.3693	160.2908	159.3235

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	10992.94	5	2198.589	17588.71	4.6E-77	2.408514
Columns	6.444444	3	2.148148	17.18519	1.02E-07	2.798061
Interaction	11.05556	15	0.737037	5.896296	1.13E-06	1.880175
Within	6	48	0.125			
Total	11016.44	71				

Appendix III: Mortality effect of *Anacardium occidentale* seed oil against *D. maculatus* larvae

Anova: Two-Factor With Replication

SUMMARY	24h	48h	72h	96h	Total
0					
Count	3	3	3	3	12
Sum	0	0	0	0	0
Average	0	0	0	0	0
Variance	0	0	0	0	0
0.003					
Count	3	3	3	3	12
Sum	0	2	3	3	8
Average	0	0.666667	1	1	0.666667
Variance	0	1.333333	1	1	0.787879
0.009					
Count	3	3	3	3	12
Sum	2	4	4	6	16
Average	0.666667	1.333333	1.333333	2	1.333333
Variance	0.333333	1.333333	1.333333	1	0.969697
0.027					
Count	3	3	3	3	12
Sum	29	32	34	40	135
Average	9.666667	10.66667	11.33333	13.33333	11.25
Variance	66.33333	66.33333	57.33333	60.33333	47.47727
0.081					
Count	3	3	3	3	12
Sum	69	71	72	79	291
Average	23	23.66667	24	26.33333	24.25
Variance	1	0.333333	0	1.333333	2.204545

Appendix III contd.

0.243

Count	3	3	3	3	12
Sum	87	89	90	90	356
Average	29	29.66667	30	30	29.66667
Variance	30.333333	0	0	0.787879	

Total

Count	18	18	18	18
Sum	187	198	203	218
Average	10.38889	11	11.27778	12.11111
Variance	152.3693	154.7059	155.9771	165.634

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	10140.78	5	2028.156	184.6109	4.02E-30	2.408514
Columns	27.61111	3	9.203704	0.837758	0.479848	2.798061
Interaction	19.55556	15	1.303704	0.118668	0.999972	1.880175
Within	527.3333	48	10.98611			
Total	10715.28	71				

Appendix IV: Mortality effect of *Citrullus colocynthis* seed oil against *D. maculatus* larvae

Anova: Two-Factor With Replication

SUMMARY	24h	48h	72h	96h	Total
0					
Count	3	3	3	3	12
Sum	0	0	0	0	0
Average	0	0	0	0	0
Variance	0	0	0	0	0
0.003					
Count	3	3	3	3	12
Sum	0	0	0	0	0
Average	0	0	0	0	0
Variance	0	0	0	0	0
0.009					
Count	3	3	3	3	12
Sum	0	0	0	11	
Average	0	0	0	0.333333	0.083333
Variance	0	0	0	0.333333	0.083333
0.027					
Count	3	3	3	3	12
Sum	2	2	2	39	
Average	0.666667	0.666667	0.666667	10.75	
Variance	1.333333	1.333333	1.333333	1	0.931818
0.081					
Count	3	3	3	3	12
Sum	64	67	69	73	273
Average	21.33333	22.33333	23	24.33333	22.75
Variance	52.33333	66.33333	76	41.33333	44.20455

Appendix IV contd.

0.243					
Count	3	3	3	3	12
Sum	90	90	90	90	360
Average	30	30	30	30	30
Variance	0	0	0	0	0

Total				
Count	18	18	18	18
Sum	156	159	161	167
Average	8.666667	8.833333	8.944444	9.277778
Variance	166	172.2647	176.6438	177.3889

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	11275.24	5	2255.047	224.2588	4.71E-32	2.408514
Columns	3.597222	3	1.199074	0.119245	0.948358	2.798061
Interaction	11.15278	15	0.743519	0.073941	0.999999	1.880175
Within	482.6667	48	10.05556			
Total	11772.65	71				

Appendix V: Mortality effect of *Citrullus vulgaris* seed oil against *D. maculatus* larvae

Anova: Two-Factor With Replication

SUMMARY	24h	48h	72h	96h	Total
0					
Count	3	3	3	3	12
Sum	0	0	0	0	0
Average	0	0	0	0	0
Variance	0	0	0	0	0
0.003					
Count	3	3	3	3	12
Sum	0	0	0	0	0
Average	0	0	0	0	0
Variance	0	0	0	0	0
0.009					
Count	3	3	3	3	12
Sum	0	0	0	0	0
Average	0	0	0	0	0
Variance	0	0	0	0	0
0.027					
Count	3	3	3	3	12
Sum	1	2	2	2	7
Average	0.333333	0.666667	0.666667	0.666667	0.583333
Variance	0.333333	0.333333	0.333333	0.333333	0.265152
0.081					
Count	3	3	3	3	12
Sum	71	78	79	82	310
Average	23.66667	26	26.33333	27.33333	25.83333
Variance	6.333333	1	2.333333	2.333333	4.151515

Appendix V contd.

	0.243				
Count	3	3	3	3	12
Sum	90	90	90	90	360
Average30	30	30	30	30	
Variance	0	0	0	0	0
	Total				
Count	18	18	18	18	
Sum	162170171174				
Average	9	9.444444	9.5	9.666667	
Variance	172.7059	183.9085	186.0294	192.1176	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	12446.74	5	2489.347	4480.825	7.91E-63	2.408514
Columns	4.375	3	1.458333	2.625	0.061095	2.798061
Interaction	17.54167	15	1.169444	2.105	0.02619	1.880175
Within	26.66667	48	0.555556			
Total	12495.32	71				

Appendix VI: Mortality effect of *Khaya senegalensis* seed oil against *D. maculatus* larvae

Anova: Two-Factor With Replication

SUMMARY	24h	48h	72h	96h	Total
0					
Count	3	3	3	3	12
Sum	0	0	0	0	0
Average	0	0	0	0	0
Variance	0	0	0	0	0
0.003					
Count	3	3	3	3	12
Sum	0	0	0	0	0
Average	0	0	0	0	0
Variance	0	0	0	0	0
0.009					
Count	3	3	3	3	12
Sum	0	0	0	0	0
Average	0	0	0	0	0
Variance	0	0	0	0	0
0.027					
Count	3	3	3	3	12
Sum	15	22	28	36	101
Average	5	7.333333	9.333333	12	8.416667
Variance	0	70.33333	72.33333	63	49.53788
0.081					
Count	3	3	3	3	12
Sum	74	76	77	78	305
Average	24.66667	25.33333	25.66667	26	25.41667
Variance	9.333333	5.333333	4.333333	7	4.992424

Appendix VI contd.

0.243

Count	3	3	3	3	12
Sum	88	90	90	90	358
Average	29.33333	30	30	30	29.83333
Variance	0.333333	0	0	0	0.151515

Total

Count	18	18	18	18
Sum	177	188	195	204
Average	9.833333	10.44444	10.83333	11.33333
Variance	165.5588	174.9673	175.2059	175.7647

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	11175.61	5	2235.122	207.1156	2.91E-31	2.408514
Columns	21.66667	3	7.222222	0.669241	0.575086	2.798061
Interaction	61.83333	15	4.122222	0.381982	0.977666	1.880175
Within	518	48	10.79167			
Total	11777.11	71				