

**Sudan University of Science and Technology**

**College of Graduate Studies**



**Toxicity of Seeds Ethanolic Extracts of Coffee Senna and Damas  
Co-administered with Sesame Oil Against African Melon  
Ladybird *Henosepilachna elaterii* Rossi**

سمية المستخلصات الإيثانولية لبذور السوريب و الدمس مخلوطة بزيت السمسم ضد خنفساء  
ابو العيد الافريقي.

A thesis submitted in partial fulfillment of the requirements for the M. Sc. degree  
in plant protection

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## الآية

قال تعالى:

وَأَنْبَتْنَا عَلَيْهِ شَجَرَةً مِنْ يَقْطِينٍ (١٤٦) وَأَرْسَلْنَاهُ إِلَى مِائَةِ أَلْفٍ أَوْ يَزِيدُونَ

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صدق الله العظيم

سورة الصافات الآيات (١٤٦ - ١٤٨)

## **DEDICATION**

To my mother, father and brothers

To my extended family

To all my teachers and friends with  
great regard and respect.

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## ABSTRACT

Laboratory experiments were conducted in the Department of Plant protection, College of Agricultural Studies, Sudan University of Science and Technology (SUST) to evaluate the lethal effect of seed ethanolic extracts of Coffee senna *Cassia occidentalis* L. and Damas *Concorpus lancifolius* Engl. against the second larval instars of the African melon ladybird *Henosepilachna elaterii* Rossi. Also further experiments were conducted to investigate the efficacy of sesame oil as a synergist. Five concentrations of seeds ethanolic extracts of each plant were used in this study. The results showed that all tested concentrations of both plants caused higher mortality percentage than the control. The lowest concentration (1%) of seed ethanolic extract of *C. occidentalis* gave only 3.3 % mortality after 24 hrs of exposure; however, when mixed with sesame oil it increased significantly to 90%. Seed ethanolic extract of *C. occidentalis* at 10.5% and 14% concentrations generated 90 % and 96.6% mortality respectively after 24 hrs of exposure and the results were not significantly different from Malathion.

On the other hand, the lowest concentration (1%) of seed ethanolic extract of *C. lancifolius* caused only 6.6% mortality after 24 hours of exposure but when mixed with sesame oil it increased significantly to 53.3% mortality. The highest concentrations of seed ethanolic extract of *C. lancifolius* (14% ) gave only 53.3% whereas the same concentration of seed ethanolic extract of *C. occidentalis* generated 100% motality after 72 hrs of exposure.

The results obtained after 72 hrs of application showed that the LC<sub>50</sub> values were 5.584%, 0.001%, 20.596% and 0.162% for *C. occidentalis*, *C. occidentalis* + sesame oil, *C. lancifolius* and *C. lancifolius* +sesame oil respectively.

## ملخص البحث

اجريت تجارب معملية في قسم وقاية النبات، كلية الدراسات الزراعية، جامعة السودان للعلوم و التكنولوجيا. هدفت هذه الدراسة لتقييم الاثر القاتل للمستخلصات الإيثانولية لبذور السوريب *Cassia occidentalis* L. و الدمس *Concarpus lancifolious* Engl ضد الطور اليرقي الثاني لخنفساء ابو العيد الافريقي *Henosepilachna elaterii* Rossi. كما هدفت ايضا لاختبار فعالية زيت السمسم كمنشط. أستخدمت في هذه الدراسة خمس تركيزات من كل مستخلص نباتي.

اوضحت النتائج ان كل التركيزات المختبرة من كلا النباتين احدثت نسبة موت عالية مقارنة بالشاهد. اعطى اقل تركيز (١%) من المستخلص الإيثانولي لبذور السوريب فقط نسبة موت ٣,٣% ولكن عندما خلط مع زيت السمسم اعطى زيادة معنوية في نسبة الموت وصلت الي ٩٣,٣% بعد ٢٤ ساعة من المعاملة. اعطت التركيزات ١٠,٥% و ١٤% من المستخلص الإيثانولي لبذور السوريب نسبة موت ٩٠% و ٩٦,٦% علي التوالي بدون فرق معنوي مقارنة بمبيد الملاثيون بعد ٢٤ ساعة من المعاملة.

ومن ناحية اخرى اعطى اقل تركيز (١%) من المستخلص الإيثانولي لبذور الدمس فقط نسبة موت (٦,٦%) بعد ٢٤ ساعة من المعاملة ولكن عندما خلط بزيت السمسم اعطى زيادة معنوية في نسبة الموت وصلت الي ٥٣,٣%. اعطى اعلى تركيز (١٤%) من المستخلص الإيثانولي لبذور الدمس فقط نسبة ٥٣,٣% في حين ان نفس التركيز من المستخلص الإيثانولي لبذور السوريب اظهر نسبة موت ١٠٠% بعد ٧٢ ساعة من المعاملة.

كما اوضحت النتائج المتحصل عليها بعد ٧٢ ساعة من المعاملة، ان التركيزات النصفية القاتلة هي ٥,٥٨٤% ، ٠,٠٠١% ، ٢٠,٥٩٦% و ٠,١٦٢% للسوريب، السوريب + زيت السمسم، الدمس والدمس + زيت السمسم علي التوالي.

# CHAPTER ONE

## INTRODUCTION

Cucurbits are medium sized plants primarily found in the warmer regions of the world. They belong to the family cucurbitaceae which is known to contain many economically important species, the fruits of which are used for nutrition and medicinal purposes (Mariod *et al.*, 2007). In the Sudan cucurbits crops constitute a major portion of vegetables and are grown in different regions of the country under irrigation on silt soils along the Nile bank while some cucurbits are grown under rain fed condition. The important cultivated species in the Sudan include water melon, snake melon, cucumber, melon, pumpkin and squash. Water melon *Citrullus lanatus* locally known as "Battikh" are cultivated everywhere particularly in the western parts of the country especially in Darfur and Kordofan States. The melon watery juices are considered in many dry areas as a water substitute. Melon seeds are roasted and consumed locally as a popular snack "Tassali". Also some melon seeds are exported to some Middle Eastern and Arab countries (Beshir *et al.*, 2009).

Snake melon *Cucumis melo var flexuosus* known in the Sudan as "Agoor" are very popular salad plant, the fruits of which contains some amount of carbohydrates, minerals and vitamins. *Cucumis melo var. agrestis* "Tibish" wild melon are widely distributed in the Sudan and used as salad crops instead of cucumber in the western States (Mariod *et al.*, 2007). Cucumber *Cucumis savatis* are a very popular salad crop in the Sudan. The immature fruits are consumed fresh or cooked. Sweet melon *Cucumis melo melo*, pumpkin *Cucurbita maxima* Duch. and Squash *Cucurbita peopo* are promising export crops which can be

readily produced at a low cost during the winter season of the Sudan. Some wild species are also found namely bitter water melon "Handal" *Citrullus colocynthis* L. and white flowered gourd *Lageria sicerar* L. Generally cucurbits are attacked by many diseases and insect pests. The major diseases of cucurbits in the Sudan includes powdery mildews *Leveillula taurica* Lev., *Sphaerotheca fuliginea* schlecht and mosaic virus (Siragelnour, 1986). Red spider mite *Tetranychus urticae* Koch is also considered as one of the most important cucurbits pests. A common nematode disease that attack cucurbits in the Sudan is root-knot nematode *Meloidogyne* spp. Among vertebrate pests is the Nile rat *Arvicanthis niloticus* which is believed to be responsible for a significant reduction in yield. The main insect pests of cucurbits in the Sudan include African melon ladybird beetle *Henosepilachna elaterii* Rossi, Red melon beetle *Aulacophora africana* Weise, Melon bug *Spongopus viduatus* F., Fruit flies *Dacus vertebratus* Bezzi and *Dacus ciliatus* Loew, White fly *Bemisia tabaci* Gen, Aphid *Aphis gossypii* Glover, and Leaf miner *Liriomyza trifolii* Burgess (Schmutterer, 1969).

African melon ladybird is considered as one of the most important insect pests on cucurbits in the Sudan. It attacks the lower surface of leaves and a heavy attack may result in removal of the lower epidermal layer and eventually drying of leaves. Some insecticides are used to control this pest including malathion and diazinon (Organophosphates) and carbaryl (Carbamates). However because of the hazards of synthetic insecticides, recently the pesticidal effects of botanical extracts have been investigated by several researchers worldwide (Obydalla, 2001, Rakesh *et al.*, 2001, Abdel-moniem, 2003, Patil *et al.*, 2003, Elsiddig, 2007, Innocent *et al.*, 2008, Abdullahi, 2011 and Taiwo *et al.*, 2013). Higher plants are extremely abundant with biologically active secondary metabolites. Over 80% of all known alkaloids, phenols and other secondary metabolites were produced by higher plants (Elsiddig, 2007). Many plant extracts or products have proven to be

as potent as many conventional synthetic pesticides and are effective at very low concentrations. On the other hand botanical insecticides possess great advantage over synthetic pesticides in being more environmentally friendly, to be accepted by the majority of farmers, governmental organization and decision makers (Kelany, 2001).

Stoll (2000) demonstrated that the use of plant extracts to control destructive insect is not new, Rotenone, Nicotine and Pyrethrin have been used for a considerable time in small scale subsistence and also commercial agriculture.

Insecticide synergists have been used not only to monitor the insecticide resistance mechanisms but also as an admixture in these insecticides for the control of many insects. They contribute significantly to the improvement of insecticides efficacy, particularly when problems of resistance need to be addressed (Lorini and Galley, 2000).

Piperonyl butoxide which is isolated from sesame oil has been used as a synergist with many organophosphates and pyrethroids insecticides to control various pests (Morris, 2002).

The objectives of this study are:

- 1- To evaluate the lethal effect of seeds ethanolic extracts of coffee senna *Cassia occidentalis* and damas *Concarpus. lancifolius* against the second larval instars of the African melon ladybird *H. elaterii*.
- 2- To investigate the synergistic effect of sesame oil on the toxicity of seeds ethanolic extracts of coffee senna *C. occidentalis* and damas *C. lancifolius*.

# CHAPTER TWO

## 2-LITERATURE REVIEW

### 2-1-African melon ladybird beetle

#### 2-1-1-Back ground:

The family coccinellidae is very important economically, because it includes some highly beneficial insects as well as serious pests. The family comprises 5200 described species worldwide. Numerous species of coccinellids are major biological agents of pests such as aphid, mealy bugs, scale insects, thrips and mites in all parts of the world (Khan *et al.*, 2007).

African melon lady bird beetle *H. elaterii* belongs to this family and its considered as an important insects pests of cucurbits.

#### 2-1-2-Taxonomy:

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Coleoptera

Family: Coccinellidae

S. N: *Henosepilachna elaterii* (Rossi)

C. N: African melon lady bird



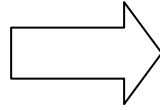
### **2-1-3-Geographical distribution:**

African melon lady bird beetle has a wide distribution in tropical and subtropical Africa (East Africa and West Africa), as well as Mediterranean, Near and Middle East, Southern Europe, Asia, Afghanistan. In the former USSR it is known in the lowlands of Azerbaijan, Turkmenistan, Uzbekistan, and Tajikistan south ward of latitude 42 N (David'yan, 2009). In the Sudan the African melon lady bird is found all over the country where cucurbits are grown and where sufficient wild cucurbits occur (Schmutterer, 1969).

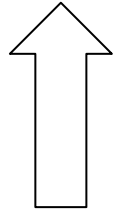
### **2-1-4-Description:**

The eggs are yellow to orange yellow in color, elliptical in shape about 1.7 - 1.9 mm in length. The larvae have four instars and the last larval instars when fully fed measured about 7- 9 mm in length, with six ramified spines. The pupae are yellow in color and are attached to the leaves by its posterior portion. The adult is hemispherical in shape about 6 – 8 mm long, and its pronotum orange to orange red in color. The elytron have an orange to orange red color and twelve rimmed black spots arranged in three transverse rows (Schmutterer, 1969).

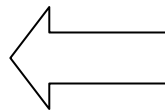
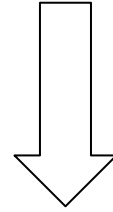
The body surface of adults covered with golden pubescence, the antennae are clavate type (club shaped) rather small and with eleven segments. The mouth parts are of chewing type and the legs are of the walking and running type. The abdomen has ten segments eight clear and two hidden, the compound eyes are kidney-shaped. This species is characterized by the confluence of ventral and sub ventral sterna on the seventh and eight segments and thus can be easily separated from other *Epilachna* species (Kapur,1950).



Egg stage



Larvae stage



Adult stage

Pupa stage

**Plate. 1: African melon ladybird beetle *H. elaterii***

### **2-1-5- Biology:**

The biology of *H. elaterii* was studied by many workers including (Gunna,1926; Kapur,1950; Ghabn,1951 and Elkhidir,1969). They found that the succession of stages through which the species passes in order to complete development is eggs, larvae, pupae and adults. After a pre-oviposition period of several weeks, the female lays small elliptical yellow eggs about 100 - 200 eggs in groups of 5 - 22 on the lower surface of the leaves, although some may be laid on the upper surface of the leaves, stems, flowers and fruits. The oviposition period takes about 3 - 4 weeks, the eggs hatch after 3- 6 days and young larvae feed at first in batches and later on, they disperse on the host plant. The last larval instars is reached after four moults and pupation takes place on the leaves. The adult beetle emerges after 5 - 6 days, the whole life cycle is completed within four weeks (Schmutterer, 1969).

In the Sudan Ali (2009) found that the pre-oviposition period ranged between 3 - 13days with an average of 7.4 days. The oviposition period takes about 11- 22 days with an average of 17.4 days and the post oviposition period ranged between 5 - 13 days with an average of 8 - 7 days. The number of eggs per female are about 87- 289 with an average of 171. The eggs per batch are about 3 - 45 eggs. Pupation takes place on the leaves and the pupal duration takes about 5 – 9 days. The whole life cycle is completed within 15 - 32 days. The life span for the females about 31 days which was shorter than that of the male which is 35days.

### **2-1-6-Ecology:**

African melon ladybird gives 2- 4 generations through it is life span. Adults hibernate under the litter in fields or under rush, where some times hundreds of beetles per one plant are aggregated. Adults survive only a short time at temperature of -14 °c therefore, population mortality during winter diapauses sometimes may reach to 80% (David'yan, 2009). Adults have diurnal activity and

fly very well, the optimum temperature for this pest is 27 - 32 °c. Oviposition begins after a few days of additional feeding and females lay eggs on the lower surface of the leaves (Schmutterer, 1969).

The largest fertility is observed in the first generation whereas beetles of third and fourth generation don't lay eggs. Embryogenesis lasts for about 4 – 5 days. Larvae have four instars develop in 12 - 22 days. Pupation occurs on leaves and pupa develops in 5 – 6 days. Hibernation begins in November, after average daily temperature lowers to 12 – 14 °c. (David'yan, 2009).

### **2-1-7-Behavior:**

The insect can live without food for several days and the activity of the beetle was found to be greater in the cool mornings and evenings. However, during the summer they shelter during the hotter parts of the day (Siragelnour,1986). The larvae were found to develop cannibalistic habits even in presence of food, the newly hatched larvae have cannibalistic tendencies towards the un-hatched eggs (Elkhidir, 1969).

The beetle was found to hibernate in the adult stage during winter by some workers (Willcocks,1922 and Gunna, 1926). The larvae have sluggish habits and a tendency to remain in plants until the leaves have been entirely skeletonized which makes the insects comparatively easy to control (Siragelnour,1986).

### **2-1-8-Host plants:**

The main host plants of the phytophagous African melon ladybird are found among the cucurbitaceae namely cultivated cucurbits such as water melon, melon, cucumber, pumpkin and vegetable marrow, although the beetle may be observed occasionally in sunflower, lettuce and cotton (Schmutterer, 1969).

### **2-1-9-Damage and economic importance:**

In the Sudan African melon ladybird beetle is considered as an important insect pest of cucurbits. Both adults and larvae are strong feeders and may cause considerable damage to the plant. They preferably attack the leaves but sometimes they also attack flowers and fruits. The leaves are skeletonized (fine network of veins) by feeding on their epidermis as well as on their parenchymatic tissues. Badly damaged leaves shrivel and dry up. In cases of heavy infestation the whole plant may be destroyed as often happens in young cucurbits, whereas, seriously damaged older plants don't yield fruits (Schmutterer, 1969). This beetle also known to acts as a vector of squash mosaic virus (Siragelnour 1986).

### **2-1-10-Control measures:**

#### **2-1-10-1-Biological control:**

There are many natural enemies of the African melon lady bird beetle. Among vertebrates the cape wagtail bird *Motacilla capensis* feeds on the larvae of the African melon ladybird beetle. Other natural enemies include *Pleurotopics epilachnae* as a parasitoid of the larvae, *Tetrastichus ovulorum* as an egg parasitoid and *Tetrastichus epilachnae* as a predator of the pre-pupae and pupae (Siragelnour, 1986).

#### **2-1-10-2-Botanical control:**

Spraying of neem *Azadirachta indica* extracts and simple neem-based pesticide have given a good control of the African melon ladybird beetle in Togo. Thus damage by *Epilachna* beetles could be reduced significantly by weekly application of aqueous neem kernel extracts at concentrations of 25, 50 and 100 g/L and neem oil applied with an ultra-low volume (ULV) sprayer at 10 and 20 L/ha (Ostermann and Dreyer, 1995).

Alcoholic extracts of *Citrullus colocynthis*, *Dillenia indica* and *Datura fastusca* at concentration of 50% gave 70 – 80 % mortality of *Epilachna* and various species of caterpillars (Siragelnour, 1986).

### **2-1-10-3- Cultural control:**

Cultural practices to control this pest include removal of plant residues after harvesting, autumn plowing and crop rotation. Additionally early sowing is advisable to escape the period of activity of the pest. Hand picking of adult, larvae, pupae and eggs may be practiced in small plots of cucurbits but is a tedious and time consuming method (Schmutterer, 1969).

### **2-1-10-4- Chemical control:**

Historically chemical control was used and proven to be effective with many classes of synthetic insecticides such as carbaryl (Carbamates), methoxychlor (Chlorinated hydrocarbon) (Schmutterer, 1969), folimat (Organophosphates) (Satti, 1997), dimethoate and malathion (Organophosphates) (Siragelnour, 1983). The insecticide lannate (Carbamates) was found to be more effective than dimethoate and malathion in control of this pest (Zeinalabdin and Siragelnour 1991).

In west Bengal dichlorvos 100 EC (0.03 %) followed by quinolphos 25 E.C. and demeton - methyl 25 E.C. (0.03%) gave an effective control of *Epilachna* (Obydalla, 2001).

### **2-2-Coffee senna (Soreib)**

*Cassia* species (Caesalpinaceae) are annual under shrub grows all over the tropical countries. Traditionally, the leaves of *Cassia* species are popular as pot herb. It is used as natural pesticide in the organic farming also *Cassia* species contain chrysophanic acid-9-anthrone which is an important fungicide (Singh *et al.*, 2013).

The genus *Cassia* comprises more than 40 species amongst which some are economically important in the production of timber, gum, tanning, dying materials and fish poisons. In the Sudan, this genus is represented by at least 13 species (Omer *et al.*, 2012).

### **2-2-1-Taxonomy:**

Family: Caesalpinaceae

Genus: *Cassia*

Species: *occidentalis*

S. N: *Cassia occidentalis* L.

C. N: Coffee senna

### **2-2-2- Botanical description:**

Coffee senna *Cassia occidentalis* ( *C. occidentalis*) is an erect somewhat branched, smooth, half woody herb or shrubby plant, about 0.8 -1.5 meters in height. The flowers are yellow about 2 cm in length and borne on auxiliary and terminal racemes. Seed pods are narrow and semi-flattened about 10 cm long, thickened and containing about 40 or more brown to dark-olive, ovoid seeds about 4 mm long. The species is distinguished by *a fitted odour, absence of spines. Leaves with 3 - 7 leaflets about 2–10cm long and 0.6–4cm wide. Flowers with 10 fertile and sterile stamens, 6 or 7 fertile anthers and cylindrical seeds* (Podsilva, 2003).

### **2-2-3-Geographical distribution:**

Coffee senna grows throughout the tropics and subtropics including the United States from Texas to Iowa eastward, Hawaii, the Pacific Island territories, Puerto

Rico, and the U.S. Virgin Islands. It appears to be of South American or New World origin (Singh *et al.*, 2013). *Cassia occidentalis* are most commonly found in savannah areas of Africa and are utilized for various purposes. This plant is found in many parts of the Sudan and commonly known as Soreib (Mariod and Matthäus, 2008).

#### **2-2-4-Cultivation:**

*C. occidentalis* can flower and fruit throughout the year or only periodically, depending on rainfall and temperature conditions and seasons. In cold or dry climates, the life cycle of *C. occidentalis* is complete in 6 to 9 months. In warm, continually moist areas, however, plants may last a full year. Well-dried seed stored in airtight containers remain viable for more than three years. Seed should be treated to enhance germination. The distal end of each seed should be nipped, or the seed can be immersed in concentrated sulphuric acid for 10 minutes and then rinsed with plenty of water. Seed should germinate between 5 and 36 days after sowing. *C. occidentalis* is planted in hedges and as an ornamental, but has the potential to become a weed in farmland, and is often found in disturbed areas. It should therefore be managed carefully. The species can be controlled with broadleaf herbicides (Dharani *et al.*, 2010).





(a)



(b)

**Plate. 2:** *C. occidentalis* plant (a) and *C. occidentalis* seeds (b).

### **2-2-5-chemistry:**

Chemical constituents isolated from *C. occidentalis* including sennoside, anthraquinone glycoside, fatty oils, flavonoid glycosides, galactomannan, polysaccharides, and tannins (Yadav *et al.*, 2010). The stem, bark and leaf extract of *C. occidentalis* have been found to contain important phytochemicals such as anthraquinones, carbohydrates, glycosides, cardiac glycosides, steroids, flavanoids, saponins, phytosterols, gum and mucilage (Colle *et al.*, 2003). This plant also include wide range of chemical compounds such as achrosin, aloemodin, emodin, anthrones, apigenin, aurantiobtusin, campesterol, cassiollin, chryso-obtusin, chrysophanic acid, chrysarobin, chrysophanol and chrysoeriol (Dave and Ledwani, 2012). Terpenoids, flavonoids and anthraquinone derivatives have been detected in active fractions obtained from the leaf extract. Also in a separate investigation, new C-glycosidic flavonoids (cassiaoccidentalins A, B and C) were isolated from this plant (Dharani *et al.*, 2010).

### **2-2-6-Medicinal uses:**

*C. occidentalis* leaves are used for the treatment of yaws, scabies, itches and ringworm among the Yoruba tribe of southwestern Nigeria. In addition to this, the leaves are also known to be effective against jaundice, headache and toothache. Infusion of *C. occidentalis* leaves is used as an effective treatment for hepatitis among the rural dwellers in northern part of Nigeria (Taiwo *et al.*, 2013). Also *C. occidentalis* leaves have ethno medical importance like wound healing, treatment of sores, cutaneous diseases, bone fracture, fever, and throat infection , it also used as a diuretic and in the treatment of snake-bite (Yadava and Satnami, 2011). Different parts of this plant have been reported to possess anti-inflammatory and antiplasmodial activities (Tona *et al.*, 2004). *C. occidentalis* has long been used as natural medicine in rainforests and other tropical regions for the treatment of

inflammation, liver disorders, constipation, worms, fungal infections, ulcers, and respiratory infections (Dave and Ledwani, 2012). This plant also used to cure sore eyes, hematuria, rheumatism, typhoid, asthma, and disorder of haemoglobin and is also reported to cure leprosy. An infusion of the plant bark is given by the folklore in diabetes (Sini *et al.*, 2011). Aqueous extract of stem and leaves of this plant showed a suppressive effect on *Trypanosoma cruzi* infected rats (Ibrahim *et al.*, 2010). The ethanol extract of this plant has also been found to show significant antihepatotoxic activity against carbon tetrachloride and thioacetamide as hepatotoxins and antidiabetic activity in normal and alloxan induced diabetic rats (Mustapha *et al.*, 20013).

### **2-2-7- Uses in pest control:**

The leaves of this plant are used for various disease treatments as well as in the control of some stored product insects especially in many parts of Africa (Abdullahi, 2011). In Senegal the leaves of *C. occidentalis* are used to protect cowpea seeds against *Callosobruchus maculatus* . Both fresh and dry leaves as well as whole and ground seeds had no contact toxicity on the cowpea beetle, in contrast, seeds oil induced an increase in mortality of *C. maculatus* eggs and first larval instars at the concentration of 10 ml/kg cowpea (Lienard, *et al.*, 1993). In addition, coffee senna has been used to reduce the number of mosquitoes indoors at night (Paisson and Jaenson, 1999) and for the control of a large variety of insects (Dweivedi and Kumar, 1998)

### **2-2-8- Other uses:**

Coffee senna is used as a flowering shrub for landscape purposes .It is also used as a coffee substitute, where it has some medicinal uses as seeds are brewed into the coffee-like beverage which is used for asthma (Nassar *et al.*, 2011).The leaves are widely used as a leaf vegetable and are eaten either raw or mixed with

coconut, chilli, and onion (Nassar *et al.*, 2013). The gum derived from seed endosperm can be potentially utilized in a number of industries to replace the conventional gum (Gupta *et al.*, 2005)

### **2-2-9-Animal toxicity:**

Several animal studies have demonstrated the toxicity of the fresh and / or dried / roasted beans (seeds). Ingestion of large amounts of the seed pods by grazing animals has caused serious illness and death. Cattle, sheep, goats, horses, pigs, rabbits, and chickens have been shown to be susceptible to poisoning by *Cassia* spp. (Rowe *et al.* 1987). Also all parts of the plant are toxic, most poisoning occurs when animals eat the pods and beans, or fed green chop containing *Cassia* plants. The toxic effects are seen on skeletal muscles, liver, kidney and heart in animals. One interesting attribute of *C. occidentalis* poisoning in animals is its propensity to cause different manifestations of toxicity in different animal species. However, the physiologic systems involved in toxicity depend also upon the dose of the beans consumed. When the dose is low the animal develops features of mild liver damage and myodegeneration and at higher doses hepatic degeneration may be rapidly fatal before myodegeneration has time to develop (Vashishta *et. al.*, 2009).

Toxicity studies on the aerial parts, leaves and roots of *C. occidentalis* reported that various leaf and root extracts given to mice (administered orally and injected at up to 500mg/kg) cause mortality (Sadiq *et al.*, 2012). In another recent study, the leaf extract was observed to be potentially toxic to mice with an intraperitoneal LD<sub>50</sub> of 1000mg/kg body weight (Mustapha *et al.*, 2013). Roasting of the beans partially reduces their toxicity such that goats fed on 2.5 g/kg per body weight of roasted beans were unaffected, whereas unroasted beans at this dosage were fatal (Suliman and Shommein, 1986). Apparently all toxic effects are acute and it is believed that the toxins do not accumulate in body tissues. However, when consumed repeatedly over time the ill effects would be seen as

chronic, but in fact it is the result of repeated acute poisoning due to the inclusion of *Cassia* vegetation in fresh green feed install fed animals (Vashishta *et al.*, 2009). *C. occidentalis* was proved to be toxic to heifers with more prominent clinical symptoms depressed muscular tone, weakness, and slow march (Marrero *et al.*, 1998). There are several compounds that bind strongly to cell membranes occur in *Cassia* spp., but the specific toxin(s) responsible for muscle degeneration have not been identified while the exact toxic principles are yet to be defined, various anthraquinones and their derivatives like emodin glycosides, toxalbumins, and other alkaloids are usually blamed for *C. occidentalis* toxicity (Vashishtha *et al.*, 2009).

### **2-3-Damas:**

Family Combretaceae comprises about 20 genera and about 600 species found in tropical and subtropical regions of the world. The family has few genera with great economic value, an useful timber is obtained from some species belong to it and other species have medicinal importance. Damas *Conocarpus lancifolius* Engl is one of the most important species in this family (Pandey and Misra, 2008).

#### **2-3-1-Classification:**

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Myrtales

Family: Combretaceae

S. N : *Conocarpus lancifolius* Engl.

### **2-3-2-Botanical Description:**

Damas is an evergreen tree that grows up to 20 m in height and 60 - 250 cm or more in diameter. However, it is believed that the larger trees have now been almost entirely felled. Whereas it is usually a multi-branched tree in its natural habitat, trees planted in the Sudan formed a single, straight stem (NAS, 1983). Bark is grey-brown, fissured and the leaves are smooth and shiny, about 10 cm long, narrowing towards the base (lanceolate), in dense spirals. Flowers are yellow-green, in round heads on branched stalks, slightly fragrant and its fruit exist in dry, round, greenish heads, cone-like, containing tiny, scale-like hard seeds (Bein *et al.*, 1996).

### **2-3-3-Geographical distribution:**

Natural stand of damas are found beside intermittent watercourses of northern Somalia and in the southwest part of the Arabian peninsula. Some of these streams are salty and some sulphurous. The tree is also cultivated in Somalia, as it is in Djibouti, Sudan, Kenya, north and south Yemen, and Pakistan . A small plantation has been established in the Sudan in Khashm El-girba arboretum and about 10000 trees have been planted successfully in limestone near Mombasa, Kenya (NAS, 1983).



**Plate. 3:***Conocarpus lancijolius* tree.

### **2-3-4-Economic importance:**

*C. lancifolius* is multipurpose, wood which is the main product is used domestically for house construction, firewood and excellent charcoal. Commercially timber was more useful formerly, it was cut and exported from Somalia to Arabia for dhow construction. Other potential uses include wood based board, bark may be a useful source of tannins (Booth and Wickens, 1993).

The tree is evergreen and its foliage makes a good fodder, also it is a good shade and roadside tree. It is used as wind breaks around irrigated agricultural areas and for avenue planting. A drought-resistant species, *C. lancifolius* is one of the more promising trees for trials in arid areas. It is recommended for a variety of soil types including saline soils, and yields excellent charcoal and valuable wood (NAS, 1983).

Information on the importance of *C. lancifolius* in its native distribution areas relative to other species with similar wood, fuel and forage uses is lacking hence it is difficult to assess its importance. However Somali tribe owing the damas (Tugs) dry river valleys (wadis) containing *C. lancifolius* have restricted cutting because of the threat of overexploitation (Booth and Wickens, 1993).

### **2-3-5-Cultivation:**

Damas grows best in areas where the mean annual temperature ranges from 20°C -30°C, but where the maximum summer temperature has reached 50°C. The tree grows from sea level up to about 1000 m. The rainfall in its natural habitat is generally between 50 mm and 400 mm, but the tree grows mainly along seasonal watercourses. It can be grown in plantations in areas with less than about 400 mm but grows well only if irrigated or within reach of groundwater. It withstands drought conditions for several months when irrigation fails. Damas does well on



deep soils ranging from pure sand to clays and loams, but has difficulty on shallow soils. It will tolerate moderately saline soils (NAS, 1983).

Damas trees are often dominant in dry river valleys in Somalia. It is now cultivated as it is one of the fastest growing trees in very dry areas. It tolerates sandy, saline and coral soils (Bein *et al.*, 1996).

#### **2-4-Insecticides synergists:**

Synergists are among the most straightforward tools for overcoming metabolic resistance because they can directly inhibit the resistance mechanism itself. Their effective application against agricultural pests has offered tremendous promise but achieved little utility. This is partly because of difficulties in their use and partly because we have lacked basic understanding of insect detoxification systems (Raffa and Priestler, 1985).

Insecticide synergism can be used to control or study insecticide resistance. It contributes significantly to improve the efficacy of the insecticides, particularly when problems of resistance have arisen. Synergists are an important research tool in the laboratory to determine the mechanisms of resistance involved in a particular pest population. This kind of investigation has generated valuable results in understanding pesticides resistance. Insecticide synergists have been used not only to monitor the insecticide resistance mechanisms but also as an admixture in these insecticides for the control of many insects (Lorini and Galley, 2000).

Piperonyl butoxide has been used as a synergist with organophosphate and pyrethroid insecticides to control many insect pests. This compound was isolated from sesame oil (Morris, 2002). Initial trials in the UK proved that sesame extract works well to combat insecticide resistance in pests of greenhouse crops such as tomatoes and cucumbers. In Australia and South Africa trials were successful in

cotton against the *B* biotype of *Bemisia tabaci* Gen. which is virtually uncontrollable with the use of conventional insecticides as well as against cotton aphid *Aphis gossypii* Glover. Sesamin alignin occurring in sesame seed oil has been reported as synergist insecticide, antiseptic, and bactericide. Sesamolin also has insecticide properties and is used as a synergist for Pyrethrum insecticides (Ahmed and Irfanullah, 2007).

Sesame oil has many uses: as a cosmetic for skin protection, in medicine as an adjuvant for many drug emulsions for ulcer, in cooking as a food additive. Furthermore, the chemical structure of the oil is similar to that of the insecticide synergist piperonyl butoxide, which has played a large role in reducing population of pyrethroid resistance insects (Collins, 1990).

# CHAPTER THREE

## 3-MATERIALS AND METHODS

These experiments were conducted at the laboratory of the Entomology, Department of Plant protection, College of Agricultural Studies, Sudan University of Science and Technology (SUST). to evaluate the lethal effect of seeds ethanolic extracts *C. occidentalis* and *C. lancifolius* against the African melon ladybird *H. elaterii*. The study also investigate the efficacy of sesame oil as synergist. The materials and methods used in this study are mentioned below:

### 3- 1-Equipments:

- |                               |                      |
|-------------------------------|----------------------|
| 1-plastic cages               | 11-Collection sample |
| 2-Brush                       | 12-Micropipette      |
| 3-Hand lens                   | 13-Petri-dishes      |
| 4-Hand sprayer                | 14-Marker pen        |
| 5-Sensetive balance           | 15-Registration form |
| 6-Electronic blender          | 16-Pencil            |
| 7-Thimble                     | 17-Gloves            |
| 8-Soxhlet extractor Apparatus | 18-Masks             |
| 9-Rotary evaporator           | 19-Scalpel           |
| 10-Pipette                    | 20-Camera            |

### 3-2-Materials:

- |                       |                       |
|-----------------------|-----------------------|
| 1-Pumpkin leaves      | 6- Soap               |
| 2- Coffee senna seeds | 7- UHU                |
| 3- Damas seeds        | 8-Muslin cloths       |
| 4- Ethanol 99.7%      | 9- Sand               |
| 5- Distillied water   | 10-Malathion 57% E.C. |

### **3-3-Rearing methods:**

#### **3-3-1-Collection of target insect:**

Adults of the African melon ladybird were collected from unsprayed pumpkin plants grown in Shambat area Khartoum north and brought to the laboratory for rearing.

#### **3-3-2-Adult rearing:**

Previously collected adults of the African melon ladybird were reared in plastic cages 31x20x19 cm covered with muslin cloth using UHU (Plate 4). The bottom of these cages were filled with sand 2 cm in height and moistened daily with water by hand sprayer to make the condition optimum for insects growth and reproduction. Fresh pumpkin leaves were renewed daily for insects feeding and eggs laying, the leaves were examined thoroughly well for eggs before replacement with fresh leaves. Leaves that contain egg batches were cut in triangular shape and transferred to other rearing cages.

#### **3-3-3-Egg preservation:**

Collected egg batches were kept in plastic cages 19 cm in diameter (Plate 5) covered with muslin cloth containing moistened sand 2cm in height. The egg batches were placed on pumpkin leaves. The upper leaf was replaced daily with another fresh leaf. The number of eggs and the date of eggs laying were recorded.

#### **3-3-4-Larvae rearing:**

After two days of hatching, larvae were transferred to other plastic cages 25 X 17 X 16 cm (Plate 6) covered with muslin cloth. The bottom of each cage was filled with sand and moistened daily. Fresh pumpkin leaves were renewed daily for larvae feeding until pupal stage.

### **3- 3-5-Pupae preservation:**

Pupae were transferred to separate plastic cages similar to those used in the larvae rearing. Newly emerged adults were transferred to adult cages.

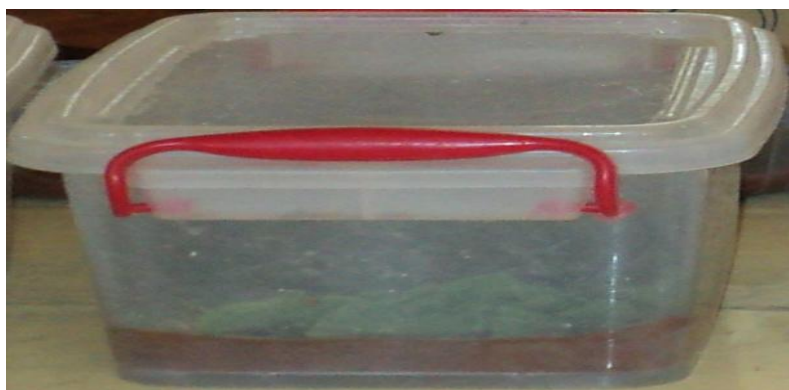
The rearing process continued until sufficient number of larvae and adult were collected for the experiments.



**Plate. 4: Adults cage**



**Plate. 5: Egg cages**



**Plate. 6: Larvae cage**

### **3-4-Collection and preparing of plant materials:**

Damas *C. lancifolius* seeds were collected from damas trees grown in the College of Agricultural Studies, Sudan University of Science and Technology whereas, the seeds of Coffee senna *C. occidentalis* were collected from river bank, Shambat area. Both plant materials were brought to the laboratory of the Entomology where they were shade-dried. After complete dryness the plant samples were crushed separately by an electronic blender to obtain the powder for the extraction processes.

### **3-5-Extraction method:**

Extraction processes were conducted at the Chemistry laboratory, College of Agricultural Studies, Sudan University of Science and Technology. Sixty gram of each of the previously prepared powders of coffee senna and damas seeds were divided equally into three parts. Each part was placed separately in a thimble and it was placed in an extraction chamber of a Soxhlet extractor apparatus (Plate 7), and then extracted with 500 ml ethanol 99.7 % for each sample. The extraction continued for six hours, and the ethanol solvent was removed off the crude extract by Rotary evaporator (Plate 8). The obtained crude materials of each of coffee senna and damas seeds were weighted and carefully stored for the experiments.

### **3-6-Preparation of the concentration:**

According to volumetric law 1% , 3.5% , 7% , 10.5% and 14% concentrations were prepared for each extract by dilution from crude extracts.



**Plate. 7: Soxhlet Extractor Apparatus**



**Plate. 8: Soxhlet and Rotary Evaporator**



### **3-7-Bioassay procedure:**

Second larval instars of the African melon ladybird beetle were used in this study. A micropipette was used to apply one micro liter of a desired concentration on the dorsal thoracic surface of the larva. Ten larvae were used for each treatment of the seeds ethanolic extracts of coffee senna and damas, and each treatment was replicated three times. Thirty larvae (three replicates) were treated with *C. occidentalis* and *C. lancifolius* plus sesame oil at ratio of (10 : 1) and thirty larvae were treated with the recommended dose of malathion 57% E.C. (1.5 liter malathion / 100 liter water / feddan) as standard. Also, thirty larvae were used as a control in which only distilled water was administered, in addition to thirty larvae treated with water and sesame oil.

All treated larvae were kept in Petri-dishes 9 cm in diameter at temperature range between 27-32 °c. During treatment period the larvae were fed on fresh pumpkin leaves. The mortality counts were recorded after 24, 48 and 72 hours after application.

### **3-8-Experiment design:**

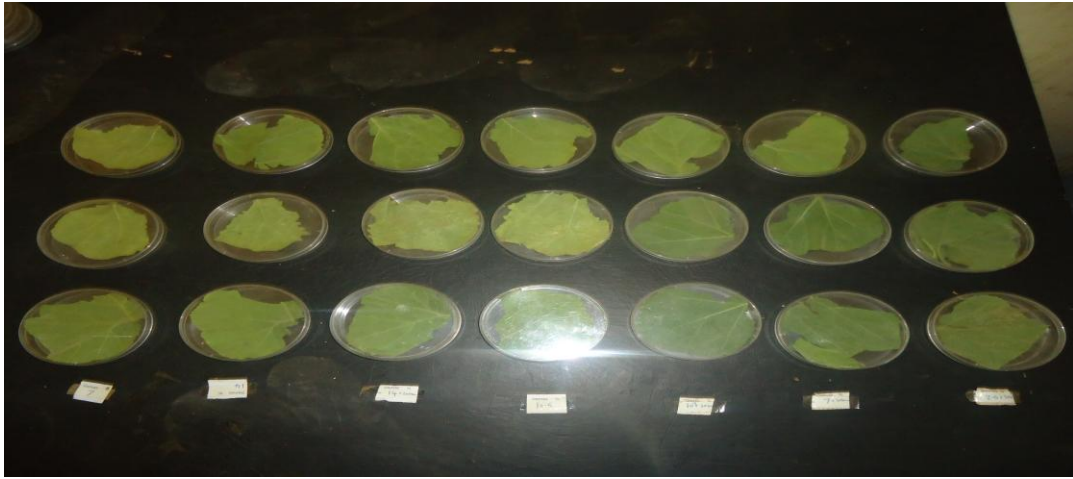
These experiments were designed in a Complete Randomized Design (Plate 9).

### **3- 9-Statistical analysis:**

The obtained data was statistically analyzed according to analysis of variance (ANOVA); Duncan's Multiple Range Test was used for means separation.

### **3-10-Probit analysis:**

Environmental Protection Agency (EPA) probit analysis program used for calculating LC/ EC values version 1.5 software was adopted to compute LC<sub>50</sub> values for each plant extract used in these experiments.



**Plate. 9: Experiment Design**



**Plate. 10: Equipment's used in this study**



**Plate. 11: Rearing cages.**

# CHAPTER FOUR

## RESULTS

As seen in Table (1), Figure (1) and Appendices (1), (2) and (3) all concentrations of the seeds ethanolic extract of coffee senna except 1% concentration gave significantly higher mortality percentage than the control after 24hrs of exposure. Additionally, all the increments in the concentration of extracts were accompanied with an increase in mortality percentage. The mortality caused by the two highest concentrations used in this study (10.5% and 14%) were comparable and not significantly different than the mortality caused by the recommended dose of malathion even after 48 and 72 hrs of exposure.

The mortality results obtained after 24 hrs of exposure to all concentrations of seeds ethanolic extracts of coffee senna remain the same after 48 hrs of exposure. Similarly the mortality percentage scored by the lowest concentration (1 %) remains the same throughout the experimental period.

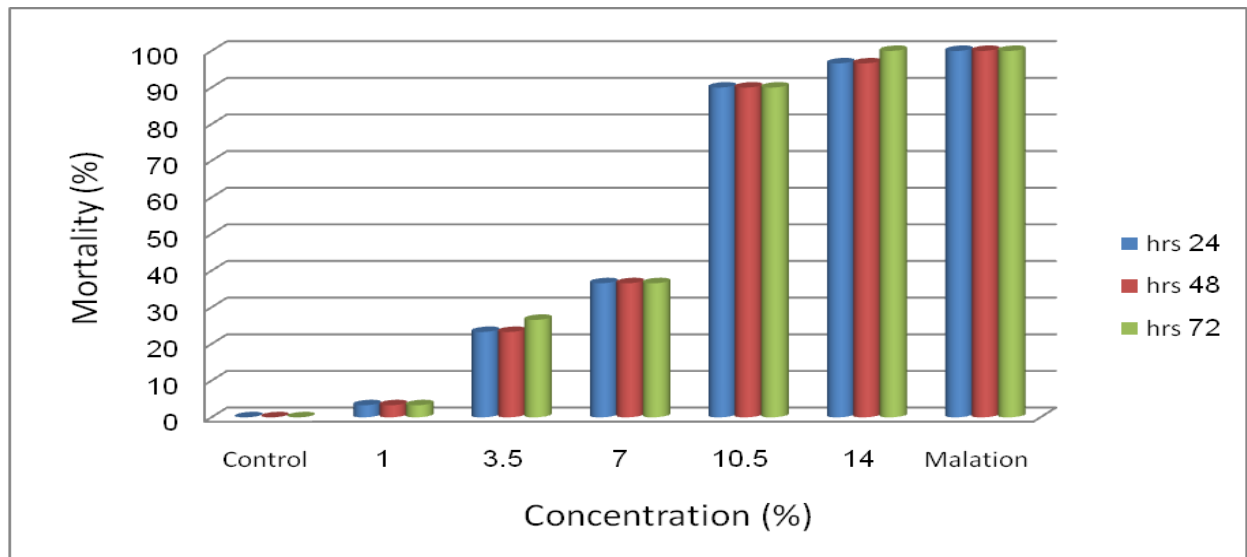
The results exhibited in Table (2), Figure (2) and Appendices (4), (5) and (6) showed that each concentration of the seeds ethanolic extract of coffee senna mixed with sesame oil gave significantly higher mortality percentage after 24hrs of exposure than its counterpart alone. Meanwhile after 72 hrs of application, all concentrations of the seeds ethanolic extract of coffee senna mixed with sesame oil generated high mortality percentages which were not significantly different from that obtained by standard malathion.

**Table. 1: Effect of seeds ethanolic extract of coffee senna on the mortality of second larval instars of the African melon ladybird.**

Concs. (%)	Mortality (%)		
	Exposure time (hrs.)		
	24	48	72
1	3.3 (1.5) d	3.3 (1.5) d	3.3 (1.5) c
3.5	23.3 (4.8) c	23.3 (4.8) c	26.6 (5.1) b
7	36.6 (6.1) b	36.6 (6.1) b	36.6 (6.1) b
10.5	90.0 (9.5) a	90.0 (9.5) a	90.0 (9.5) a
14	96.6 (9.8) a	96.6 (9.8) a	100.0 (10.0) a
Malathion	100.0 (10.0) a	100.0 (10.0) a	100.0 (10.0) a
Control	0 (0.7) d	0 (0.7) d	0 (0.7) c
SE±	0.8	0.8	0.8
C.V. (%)	10.4	10.4	10.1

Means followed by the same letter (s) are not significantly different at ( $P < 0.05$ ).

Means between brackets are transformed according to  $\sqrt{X + 0.5}$



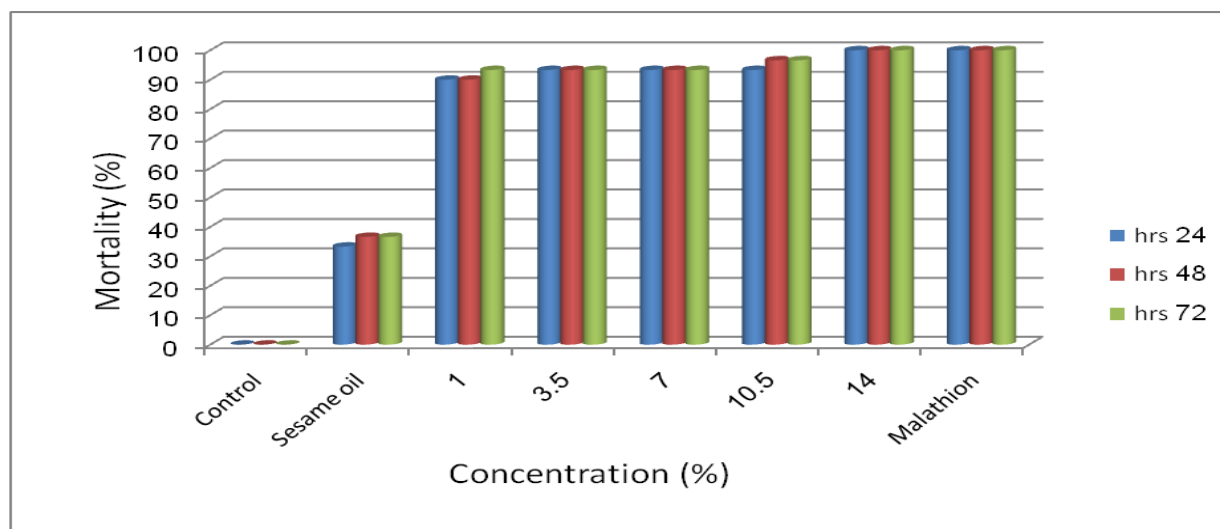
**Fig. 1: Effect of seeds ethanolic extract of coffee senna on the mortality of second larval instars of the African melon ladybird.**

**Table. 2: Effect of seeds ethanolic extract of coffee senna mixed with sesame oil on the mortality of second larval instars of the African melon ladybird.**

Concs. (%)	Mortality (%)		
	Exposure time (hrs.)		
	24	48	72
1	90.0 (9.5) b	90.0 (9.5) b	93.3 (9.7) a
3.5	93.3 (9.7) ab	93.3 (9.7) ab	93.3 (9.7) a
7	93.3 (9.7) ab	93.3 (9.7) ab	93.3 (9.7) a
10.5	93.3 (9.7) ab	96.6 (9.8) ab	96.6 (9.8) a
14	100.0 (10.0) a	100.0 (10.0) a	100.0 (10.0) a
Sesame oil	33.3 (5.8) c	36.6 (6.1) c	36.6 (6.1) b
Malathion	100.0 (10.0) a	100.0 (10.0) a	100.0 (10.0) a
Control	0 (0.7) d	0 (0.7) d	0 (0.7) c
SE±	0.6	0.6	0.6
C.V. (%)	3.1	3.1	3.3

Means followed by the same letter (s) are not significantly different at (P< 0.05).

Means between brackets are transformed according to  $\sqrt{X + 0.5}$



**Fig. 2:Effect of seeds ethanolic extract of coffee senna mixed with sesame oil on the mortality of second larval instars of the African melon ladybird.**

As seen in Table (3), Figure (3) and Appendices (7), (8) and (9) all concentrations of seeds ethanolic extract of damas gave significantly higher mortality percentage than the control after 24hrs of exposure. It can also be noted that all the increments in the concentration of extracts resulted in an increase in mortality percentage.

Results after 72 hours of exposure, showed that malathion generated 100% mortality, whereas the highest concentration (14% ) of seeds ethanolic extract of damas generated only 53.3% mortality. It is interesting to note that the mortality resulted from a 48hrs exposure to 1%, 3.5%, 7%, and 10.5% concentrations of seeds ethanolic extract of damas remains the same after 72hrs of exposure.

The data presented in Table (4), Figure (4) and Appendices (10), (11) and (12) revealed that each concentration of the seeds ethanolic extract of damas mixed with sesame oil gave significantly higher mortality percentage after 24hrs of exposure than its counterpart alone.

Results exhibited in Table (5) revealed that the seeds ethanolic extract of *C. occidentalis* scored only 5.815% LC<sub>50</sub> value after 24 hrs whereas, seeds ethanolic extract of damas gave 47.015% LC<sub>50</sub> value.

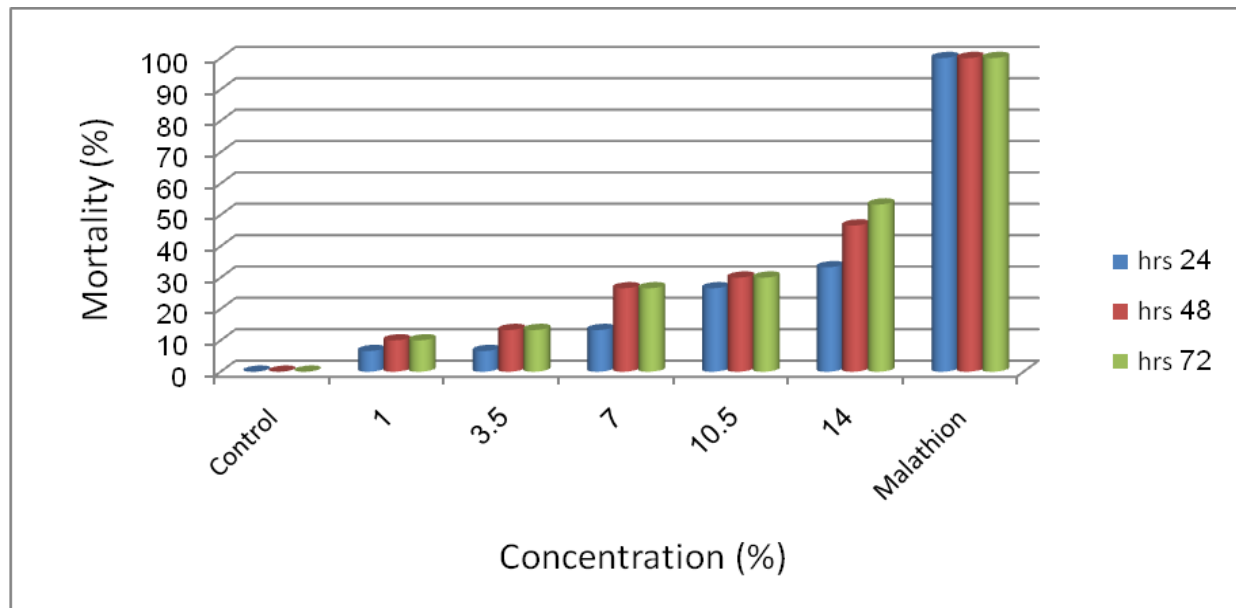
Seeds ethanolic extract of *C. occidentalis* mixed with sesame oil generated lowest LC<sub>50</sub> values (0.003%, 0.014% and 0.001%) throughout the experimental period whereas, seeds ethanolic extract of *C. lancifolius* mixed with sesame oil scored 0.572%, 0.382% and 0.162% LC<sub>50</sub> values as in Tables (5), (6) and (7).

**Table. 3:Effect of seeds ethanolic extract of damas on the mortality of second larval instars of the African melon ladybird.**

Concs.( %)	Mortality (%)		
	Exposure time (hrs.)		
	24	48	72
1	6.6 (2.4) c	10.0 (3.2) d	10.0 (3.2) d
3.5	6.6 (2.4) c	13.3 (3.6) d	13.3 (3.6) d
7	13.3 (3.6) c	26.6 (5.1) c	26.6 (5.1) c
10.5	26.6 (5.1) b	30.0 (5.5) c	30.0 (5.5) c
14	33.3 (5.8) b	46.6 (6.9) b	53.3 (7.3) b
Malathion	100.0 (10.0) a	100.0 (10.0) a	100.0 (10.0) a
Control	0 (0.7) d	0 (0.7) e	0 (0.7) e
SE±	0.6	0.6	0.6
C.V. (%)	20.3	7.8	7.7

Means followed by the same letter (s) are not significantly different at (P< 0.05).

Means between brackets are transformed according to  $\sqrt{X + 0.5}$



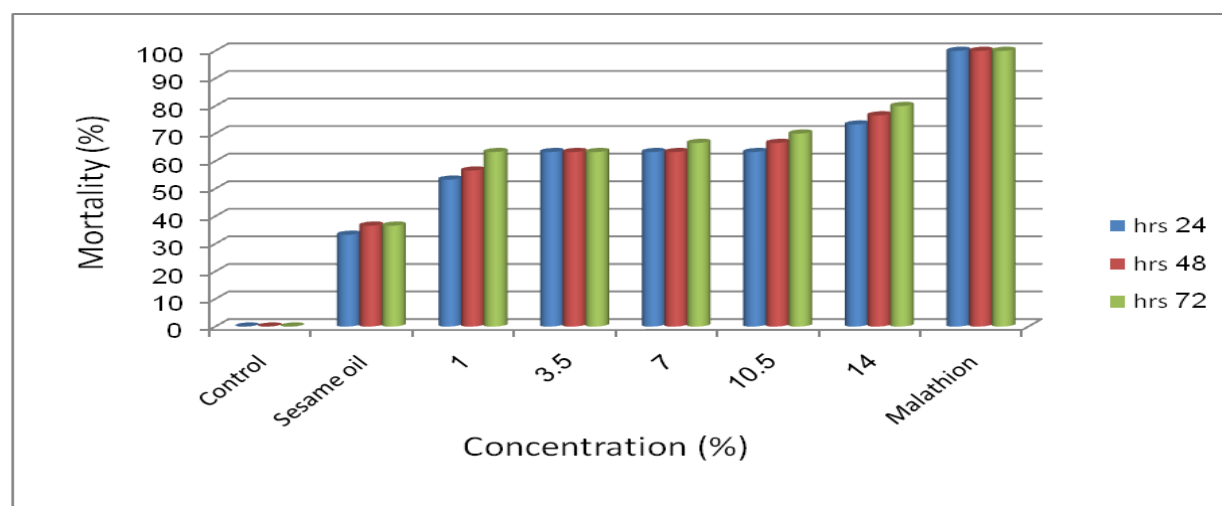
**Fig. 3: Effect of seeds ethanolic extract of damas on the mortality of second larval instars of the African melon ladybird.**

**Table. 4: Effect of seeds ethanolic extract of damas mixed with sesame oil on the mortality of second larval instars of the African melon ladybird**

Concs. (%)	Mortality (%)		
	Exposure time (hrs.)		
	24	48	72
1	53.3 (7.3) d	56.6 (7.6) d	63.3 (8.0) c
3.5	63.3 (8.0) c	63.3 (8.0) cd	63.3 (8.0) c
7	63.3 (8.0) c	63.3 (8.0) cd	66.6 (8.2) c
10.5	63.3 (8.0) c	66.6 (8.2) c	70.0 (8.4) c
14	73.3 (8.6) b	76.6 (8.8) b	80.0 (9.0) b
Sesame oil	33.3 (5.8) e	36.6 (6.1) e	36.6 (6.1) d
Malathion	100.0 (10.0) a	100.0 (10.0) a	100.0 (10.0) a
Control	0 (0.7) f	0 (0.7) f	0 (0.7) e
SE±	0.6	0.6	0.6
C.V. (%)	4.8 %	4.7 %	3.8 %

Means followed by the same letter (s) are not significantly different at ( $P < 0.05$ ).

Means between brackets are transformed according to  $\sqrt{X + 0.5}$



**Fig. 4: Effect of seeds ethanolic extract of damas mixed with sesame oil on the mortality of second larval instars of the African melon ladybird**



**Table. 5:LC<sub>50</sub> values of seeds ethanolic extracts of coffee senna and damas with and without sesame oil on the mortality of second larval instars of the African melon ladybird after 24 hours**

Botanical extract	Slope	Intercept	Chi-square	LC <sub>50</sub> (%)
<i>C. occidentalis</i>	3.338	2.448	14.880	5.815
<i>C. occidentalis</i> +Sesame oil	0.497	6.234	1.862	0.003
<i>C. lancifolius</i>	1.070	3.210	2.415	47.015
<i>C. lancifolius</i> +Sesame oil	0.361	5.088	0.598	0.572

**Table. 6:LC<sub>50</sub> values of seeds ethanolic extracts of coffee senna and damas with and without sesame oil on the mortality of second larval instars of the African melon ladybird after 48hours**

Botanical extract	Slope	Intercept	Chi-square	LC <sub>50</sub> (%)
<i>C. occidentalis</i>	3.338	2.448	14.880	5.815
<i>C. occidentalis</i> +Sesame oil	0.655	6.208	1.477	0.014
<i>C. lancifolius</i>	1.043	3.531	1.858	25.593
<i>C. lancifolius</i> +Sesame oil	0.336	5.141	1.158	0.382

**Table. 7:LC<sub>50</sub> values of seeds ethanolic extracts of coffee senna and damas with and without sesame oil on the mortality of second larval instars of the African melon ladybird after 72 hours**

Botanical extract	Slope	Intercept	Chi-square	LC <sub>50</sub> (%)
<i>C. occidentalis</i>	3.382	2.474	17.100	5.584
<i>C. occidentalis</i> +Sesame oil	0.465	6.379	1.786	0.001
<i>C. lancifolius</i>	1.155	3.483	3.381	20.596
<i>C. lancifolius</i> +Sesame oil	0.327	5.258	1.188	0.162

# CHAPTER FIVE

## DISCUSSION

In the last four decades many botanical formulations have proven to be potent and effective as many as conventional synthetic pesticides even at low concentrations. In fact, botanical insecticides have drawn great attention as major control agents in organic farming. Many plant oils such as sesame oil have been used in specific applications, as additives, for enhancing the efficacy of formulations by synergism.

This study is aimed to evaluate the lethal effect of seed ethanolic extracts of coffee senna *C. occidentalis* L. and damas *C. lancifolius* against the African melon ladybird *H. elaterii* and to investigate the effect of sesame oil as synergist. The two highest concentrations of seed ethanolic extracts of *C. occidentalis* used in this study (10.5% and 14%) induced a high mortality percentage of 90% and 100% respectively, and were not significantly different from malathion after 72 hours of exposure. This clearly demonstrates that the seed ethanolic extract of *C. occidentalis* has a lethal effect against the African melon ladybird. Similar results were obtained by Lienard *et al.* (1993) who found that, seeds oil of *C. occidentalis* increased the mortality of both eggs and first larval instars of cowpea beetle *Callosobruchus maculatus* F.. Paison and Jaenson (1999) reported that *C. occidentalis* can also be used as a repellent as it effectively reduces the number of mosquitoes indoor at night.

The mortality resulted from a 24 hours of exposure to all concentrations of seeds ethanolic extract of coffee senna remain the same even after 48 hrs of exposure. This may be ascribed to an acute action of *C. occidentalis*. Vashishta *et al.* (2009)

reported that all toxic effects of *C. occidentalis* in vertebrates are acute and its toxins do not accumulate in body tissues.

Seed ethanolic extract of *C. lancifolius* when applied at 14 % concentration against second larval instars of *H. elaterii* gave 53.3% mortality after 72 hrs of exposure. Feeny (1970) reported that tannin content in Oak leaves inhibits the growth of winter moth caterpillars and causes death. The result obtained in this study may also be attributed to the tannin content in *C. lancifolius* seeds.

When sesame oil was added to each concentration of seeds ethanolic extract of *C. occidentalis* and *C. lancifolius* it exhibited a synergistic effect. In fact, the lowest concentration (1%) of seeds ethanolic extract of *C. occidentalis* gave only 3.3 % mortality after 24 hrs of exposure; however, when mixed with sesame oil it increased significantly to 93.3%. This may indicate that the detoxification mechanism in this insect involves mixed function oxidases which are known to be inhibited by sesame oil (Matsumura, 1976).

## CONCLUSION AND RECOMMENDATIONS

This study clearly demonstrates that both tested plants have a lethal effect on the larvae of the African melon ladybird. However, seeds ethanolic extract of *C. occidentalis* seems to be much more toxic than the seeds ethanolic extract of *C. lancifolius*. This study also revealed that sesame oil has a synergistic effect when added to the seeds ethanolic extracts of both *C. occidentalis* and *C. lancifolius*.

Based on the above mentioned results, seeds ethanolic extracts of *C. occidentalis* and *C. lancifolius* mixed with sesame oil can be recommended to be used as a control agent for *H. elaterii*. However, further comparative studies should be conducted to evaluate the effects of these seeds with other organic solvents and also in other insect pests. Finally, a comprehensive study should be conducted to specify the active ingredients.

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## APPENDICES

Appendix (1) Effect of seeds ethanolic extract of coffee senna on the mortality of second larval instars of the African melon ladybird after 24 hours.

Concs. (%)	Mortality (%)			
	R1	R2	R3	Mean
1	10.0 (3.2)	0 (0.7)	0 (0.7)	3.3 (1.5)
3.5	20 (4.5)	20 (4.5)	30 (5.5)	23.3 (4.8)
7	40 (6.4)	30 (5.5)	40 (6.4)	36.6 (6.1)
10.5	90 (9.5)	90 (9.5)	90 (9.5)	90.0 (9.5)
14	90 (9.5)	100 (10.0)	100 (10.0)	96.6 (9.8)
Malathion	100 (10.0)	100 (10.0)	100 (10.0)	100.0 (10.0)
Control	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)

Appendix (2) Effect of seeds ethanolic extract of coffee senna on the mortality of second larval instars of the African melon ladybird after 48 hours.

Concs. (%)	Mortality (%)			
	R1	R2	R3	Mean
1	10 (3.2)	0 (0.7)	0 (0.7)	3.3 (1.5)
3.5	20 (4.5)	20 (4.5)	30 (5.5)	23.3 (4.8)
7	40 (6.4)	30 (5.5)	40 (6.4)	36.6 (6.1)
10.5	90 (9.5)	90 (9.5)	90 (9.5)	90.0 (9.5)
14	90 (9.5)	100 (10.0)	100 (10.0)	96.6 (9.8)
Malathion	100 (10.0)	100 (10.0)	100 (10.0)	100.0 (10.0)
Control	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)

Appendix (3) Effect of seeds ethanolic extract of coffee senna on the mortality of second larval instars of the African melon ladybird after 72 hours.

Concs. (%)	Mortality (%)			
	R1	R2	R3	Mean
1	10 (3.2)	0 (0.7)	0 (0.7)	3.3 (1.5)
3.5	20 (4.5)	30 (5.5)	30 (5.5)	26.6 (5.1)
7	40 (6.4)	30 (5.5)	40 (6.4)	36.6 (6.1)
10.5	90 (9.5)	90 (9.5)	90 (9.5)	90.0 (9.5)
14	100 (10.0)	100 (10.0)	100 (10.0)	100.0 (10.0)
Malathion	100 (10.0)	100 (10.0)	100 (10.0)	100.0 (10.0)
Control	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)

Appendix (4) Effect of seeds ethanolic extract of coffee senna mixed with sesame oil on the mortality of second larval instars of the African melon ladybird after 24 hours.

Concs. (%)	Mortality (%)			
	R1	R2	R3	Mean
1	90 (9.5)	90 (9.5)	90 (9.5)	90.0 (9.5)
3.5	90 (9.5)	90 (9.5)	100 (10.0)	93.3 (9.7)
7	90 (9.5)	100 (10.0)	90 (9.5)	93.3 (9.7)
10.5	90 (9.5)	90 (9.5)	100 (10.0)	93.3 (9.7)
14	100 (10.0)	100 (10.0)	100 (10.0)	100.0 (10.0)
Sesame oil	30 (5.5)	40 (6.4)	30 (5.5)	33.3 (5.8)
Malathion	100 (10.0)	100 (10.0)	100 (10.0)	100.0 (10.0)
Control	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)

Appendix (5) Effect of seeds ethanolic extract of coffee senna mixed with sesame oil on the mortality of second larval instars of the African melon ladybird after 48 hours.

Concs. (%)	Mortality (%)			
	R1	R2	R3	Mean
1	90 (9.5)	90 (9.5)	90 (9.5)	90.0 (9.5)
3.5	90 (9.5)	90 (9.5)	100 (10.0)	93.3 (9.7)
7	90 (9.5)	100 (10.0)	90 (9.5)	93.3 (9.7)
10.5	90 (9.5)	100 (10.0)	100 (10.0)	96.6 (9.8)
14	100 (10.0)	100 (10.0)	100 (10.0)	100.0 (10.0)
Sesame oil	40 (6.4)	40 (6.4)	30 (5.5)	36.6 (6.1)
Malathion	100 (10.0)	100 (10.0)	100 (10.0)	100.0 (10.0)
Control	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)

Appendix (6) Effect of seeds ethanolic extract of coffee senna mixed with sesame oil on the mortality of second larval instars of the African melon ladybird after 72 hours.

Concs. (%)	Mortality (%)			
	R1	R2	R3	Mean
1	100 (10.0)	90 (9.5)	90 (9.5)	93.3 (9.7)
3.5	90 (9.5)	90 (9.5)	100 (10.0)	93.3 (9.7)
7	90 (9.5)	100 (10.0)	90 (9.5)	93.3 (9.7)
10.5	90 (9.5)	100 (10.0)	100 (10.0)	96.6 (9.8)
14	100 (10.0)	100 (10.0)	100 (10.0)	100.0 (10.0)
Sesame oil	40 (6.4)	40 (6.4)	30 (5.5)	36.6 (6.1)
Malathion	100 (10.0)	100 (10.0)	100 (10.0)	100.0 (10.0)
Control	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)

Appendix (7) Effect of seeds ethanolic extract of damas on the mortality of second larval instars of the African melon ladybird after 24 hours.

Concs. (%)	Mortality (%)			
	R1	R2	R3	Mean
1	0 (0.7)	10 (3.2)	10 (3.2)	6.6 (2.4)
3.5	10 (3.2)	10 (3.2)	0 (0.7)	6.6 (2.4)
7	20 (4.5)	10 (3.2)	10 (3.2)	13.3 (3.6)
10.5	30 (5.5)	20 (4.5)	30 (5.5)	26.6 (5.1)
14	40 (6.4)	30 (5.5)	30 (5.5)	33.3 (5.8)
Malathion	100 (10.0)	100 (10.0)	100 (10.0)	100.0 (10.0)
Control	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)

Appendix (8) Effect of seeds ethanolic extract of damas on the mortality of second larval instars of the African melon ladybird after 48 hours.

Concs. (%)	Mortality (%)			
	R1	R2	R3	Mean
1	10 (3.2)	10 (3.2)	10 (3.2)	10.0 (3.2)
3.5	10 (3.2)	10 (3.2)	20 (4.5)	13.3 (3.6)
7	30 (5.5)	30 (5.5)	20 (4.5)	26.6 (5.1)
10.5	30 (5.5)	30 (5.5)	30 (5.5)	30.0 (5.5)
14	50 (7.1)	40 (6.4)	50 (7.1)	46.6 (6.9)
Malathion	100 (10.0)	100 (10.0)	100 (10.0)	100.0 (10.0)
Control	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)

Appendix (9) Effect of seeds ethanolic extract of damas on the mortality of second larval instars of the African melon ladybird after 72 hours.

Concs. (%)	Mortality (%)			
	R1	R2	R3	Mean
1	10 (3.2)	10 (3.2)	10 (3.2)	10.0 (3.2)
3.5	10 (3.2)	10 (3.2)	20 (4.5)	13.3 (3.6)
7	30 (5.5)	30 (5.5)	20 (4.5)	26.6 (5.1)
10.5	30 (5.5)	30 (5.5)	30 (5.5)	30.0 (5.5)
14	50 (7.1)	50 (7.1)	60 (7.8)	53.3 (7.3)
Malathion	100 (10.0)	100 (10.0)	100 (10.0)	100.0 (10.0)
Control	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)

Appendix (10) Effect of seeds ethanolic extract of damas mixed with sesame oil on the mortality of second larval instars of the African melon ladybird after 24 hours.

Concs. (%)	Mortality (%)			
	R1	R2	R3	Mean
1	50 (7.1)	50 (7.1)	60 (7.8)	53.3 (7.3)
3.5	60 (7.8)	70 (8.4)	60 (7.8)	63.3 (8.0)
7	70 (8.4)	60 (7.8)	60 (7.8)	63.3 (8.0)
10.5	60 (7.8)	60 (7.8)	70 (8.4)	63.3 (8.0)
14	80 (9.0)	70 (8.4)	70 (8.4)	73.3 (8.6)
Sesame oil	30 (5.5)	40 (6.4)	30 (5.5)	33.3 (5.8)
Malathion	100 (10.0)	100 (10.0)	100 (10.0)	100.0 (10.0)
Control	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)

Appendix (11) Effect of seeds ethanolic extract of damas mixed with sesame oil on the mortality of second larval instars of the African melon ladybird after 48hours.

Concs. (%)	Mortality (%)			
	R1	R2	R3	Mean
1	60 (7.8)	50 (7.1)	60 (7.8)	56.6 (7.6)
3.5	60 (7.8)	70 (8.4)	60 (7.8)	63.3 (8.0)
7	70 (8.4)	60 (7.8)	60 (7.8)	63.3 (8.0)
10.5	70 (8.4)	60 (7.8)	70 (8.4)	66.6 (8.2)
14	80 (9.0)	70 (8.4)	80 (9.0)	76.6 (8.8)
Sesame oil	40 (6.4)	40 (6.4)	30 (5.5)	36.6 (6.1)
Malathion	100 (10.0)	100 (10.0)	100 (10.0)	100.0 (10.0)
Control	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)

Appendix (12) Effect of seeds ethanolic extract of damas mixed with sesame oil on the mortality of second larval instars of the African melon ladybird after 72hours.

Concs. (%)	Mortality (%)			
	R1	R2	R3	Mean
1	60 (7.8)	60 (7.8)	70 (8.4)	63.3 (8.0)
3.5	60 (7.8)	70 (8.4)	60 (7.8)	63.3 (8.0)
7	70 (8.4)	60 (7.8)	70 (8.4)	66.6 (8.2)
10.5	70 (8.4)	70 (8.4)	70 (8.4)	70.0 (8.4)
14	80 (9.0)	80 (9.0)	80 (9.0)	80.0 (9.0)
Sesame oil	40 (6.4)	40 (6.4)	30 (5.5)	36.6 (6.1)
Malathion	100 (10.0)	100 (10.0)	100 (10.0)	100.0 (10.0)
Control	0 (0.7)	0 (0.7)	0 (0.7)	0 (0.7)



