CHAPTER ONE
INTRODUCTION

The spiny boll worm (*Earias insulana* Boisduval, 1833) (Lepidoptera: Noctuidae) is a very serious and polyphagous pest attacking many plants of Malvaceae family. Cotton and okra are economically the most important crops attacked by this pest. Two species, that is, *Earias insulana* and *Earias vittella* are widely distributed in North Africa (Johnson, 1999), Indo-Pakistan (Mirmoayedi, 2006), sub-continent and other countries of the world (Arain, 1974).

The pest is active almost throughout the year on the different host plants under field conditions (Abdul-Nasr *et al.,* 1973). In cotton crop, its initial attack is noticed in June and July and the attack on the bolls is generally higher than buds. Generally the maximum infestation is recorded during August and September (Qureshi and Ahmed, 1991).

As a result of attack the quality and quantity of the cotton is reduced a single larva can destroy several buds and bolls in its life among boll worms, *Earias* spp. are more abundant on cotton in Sindh as compared to other bollworm species (Leghari and Karlo, 2002). Chang *et al.,* (2002) were reported 3, 8 to12, 6 damage. Whereas, (Abro *et al.,* 2003) have reported 1, 79 to 2, 38% infestation of cotton bolls due to *Earias* spp. *Earias* spp. are also serious pest of okra they attack growing points, but when fruiting bodies start to appear, they feed mostly inside aquaria, flowers and fruits, a larva damages several fruiting bodies in its life span.

The economic injury level of *Earias* spp. on okra is reported to be 5.3% damage (Krishnaiah *et al*.,1978) and about 36% of harvestable fruits are damage by *Earias* spp. (Krishnaiah, 1980).

Various authors have reported the damage caused by *Earias* spp. to okra crop for example, (Srinivasan and Krishnakumar, 1983) reported 9.3% infestation of *E. vittella*.*Earias* spp. is more severe in Indo- Pakistan sub-continent than other countries of the world (Arain, 1974).The most common host plants of *Earias* spp. in Pakistan are okra, cotton, Gul-e-khera, *Althea rosea* and a weed, *Abutilon indicum*.

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).

Objectives of the study

1- To evaluate the lethal effect of hexane extracts of the leaves of Rehan (*Ocimum basilicum*) and Lupine seeds (*Lupinus termis*) against the 3rd larval instars of spiny bollworm (*Earias insulana* Boisduval, 1833).

2-To investigate the synergistic effect of sesame oil on the toxicity ofthe leaves of Rehan (*Ocimum basilicum*) and Lupine seeds (*Lupinus termis*).

CHAPTER TWO

LITERATURE REVIEW

2.1. The Spiny Boll Worm (SBW), (*Earias insulana* Boisduval 1833)

2.1.1. Synonymous

Spiny boll worms, Egyptian boll worm, rouge Espinosa in Africa, *E. insulana* is often found in mixed populations with *E. biplaga* on cotton. *E. insulana* can be distinguished by its less (spiny) appearance and by the dorsal tubercles on the 8^{th} abdominal segment being white, instead of brown (Schumutterer, 1969).

Spiny bollworm (*Earias insulana* Boisduval 1833) belongs to the Noctuidae family and the larvae of which are considered one of the important pests of cotton and okra in many parts of the world, the pest attains 6 generations (Mirmoayedi, 2006).

2.1.2. Geographical Distribution

Widespread includes Africa, including adjacent islands, Southern Europe, Asia Minor, Pakistan, India, Southeast Asia, Japan, Taiwan, Philippines, and Australia. (Schumutterer, 1969).In the Sudan, the Spiny bollworm is found mainly in the drier, irrigated areas in the North, East and West. The pest has also been observed in the wetter parts of the Central and Southern Sudan where it is outnumbered by *E .biplaga* (Yathom, 1965).

2.1.3. Taxonomy of SBW

Class:	Insecta
Order:	Lepidoptera
Family:	Noctuidae
Genus:	*Earias*
Species:	*insulana*
S.N:	*Earias insulana*
C.N:	Spiny bollworm

2.1.4. Ecology

This pest appears to survive better than other *Earias* spp. In the drier areas, perhaps because it generally an internal fruit feeder (Reed and Choyce, 1961).

2.1.5. Host range

Cotton (*Gossypium* spp.), Okra (*Abelmoschus esculentus*), and many other Malvales, particularly Indian mallow (*Abutilon* Spp), rose mallows (*Hibiscus*sp.), Tomato (*Solanum lycopersicum*), and sometimes also found on (Zee mays), for instance in Egypt. (Pearson, 1958 and Yathom, 1965).

2.1.6. Life cycle and bionomics and history

When well-fed with nectar, the female lays several hundred eggs singly on the shoots, leaves, flower buds and flowers of the host plant. The incubation period lasts for about three to four days under the conditions of the rainy season in the Central Sudan and prolonged in winter. The larval and pupal period are about 2-2$^{1/2}$weeks each or somewhat longer. Pupation takes place either on the plant or in soil debris in a dirty-white or grayish cocoon which is shaped like an inverted boat. There are several generations per year in the irrigated areas of the Sudan as the pest seems to have no resting stage under these environmental conditions. Life cycle at 28c, egg incubation takes 3days, larvae take 9 days, and pupa 9days.The moths lay up to 300eggs. No diapauses have been recorded (Yathom, 1965).

2.1.7. Incubation of SBW

The lowest incubation period 3 days, was observed on high temperature 35±1 °C and highest was 4.5 days, on 27±1 °C. Other researchers also have been reported same findings like (Kiray, 1964) recorded 3.5 days incubation period, (Wilcocks and Baghat ,1973) 4-8 days and (Unlu, 2001), 4 days incubation period.

2.1.8. Larvae of SBW

Like other lepidopteron pests the most injurious stage of this insect are larvae (Plate No.1) (because of their chewing and biting type of mouth parts), their growing and development rate is very important, which directly affects the cotton production. Like *E. vittella*, *E. insulana* also possess four larval instars and their mode of feeding also same so it is difficult to Temperature °C Incubation period (3days) Larval period (9days) Pupal period (9days) Adult life Span (9days) Complete life cycle (30days). After emergence from the egg, first instars larvae start feeding on buds or okra, squares and flowers. The development of larvae was faster on 35±1 °C while slow on 27±1 °C. The shortest larval period was recorded 9 days and longest 17 days at 35±1 °C and 27±1 °C respectively. These results are partially in agreement with Kiray (1964) reported larval period was 8 days, (Kelin *et al.,*1981), 14-22 days at 25±2 °C, larval period 14.1 days (Unlu,2001) recorded 13.56 days at 29±2°C.

2.1.9. Pupae of SBW

Fully grown larvae after last moult converted in to pupal stage. Pupal stage is resting stage of the insect in which development of moth takes place. Before converting in to pupae, the full grown larvae stopped feeding and went in to the resting position and get rest at bottom of jar and Petri- dishes and covered body with leaves (as in the field they pupate on the ground under the debris) . Overall the shortest developmental time was 8 days at 35±1 °C temperature but longest period was 16 days at 27±1 °C. These results are partially in agreement with (Wilcocks, 1937) reported 10-14 days, (Kiray,1964)12 days at 26.9°C and (Unlu,2001) reported 7.90 days at 29±2 °C

2.1.10. Adult life span

Life span of *Earias insulana* was almost same like *Earis vittella*. Life span of male moth was recorded 9 and 14 days while female life span was 16 and 10 days respectively on 35±1°C and at 27±1 °C. The life span of adult female was little bit longer than male (Unlu, 2001).

2.1.11. Duration of life cycle

Duration of life cycle was directly co- related with temperature. Minimum duration of life cycle was 30- 31 day's at 35±1°C and maximum time period of life cycle was 51.5-53.5 days at 27±1 °C, (Wilcocks, 1937).

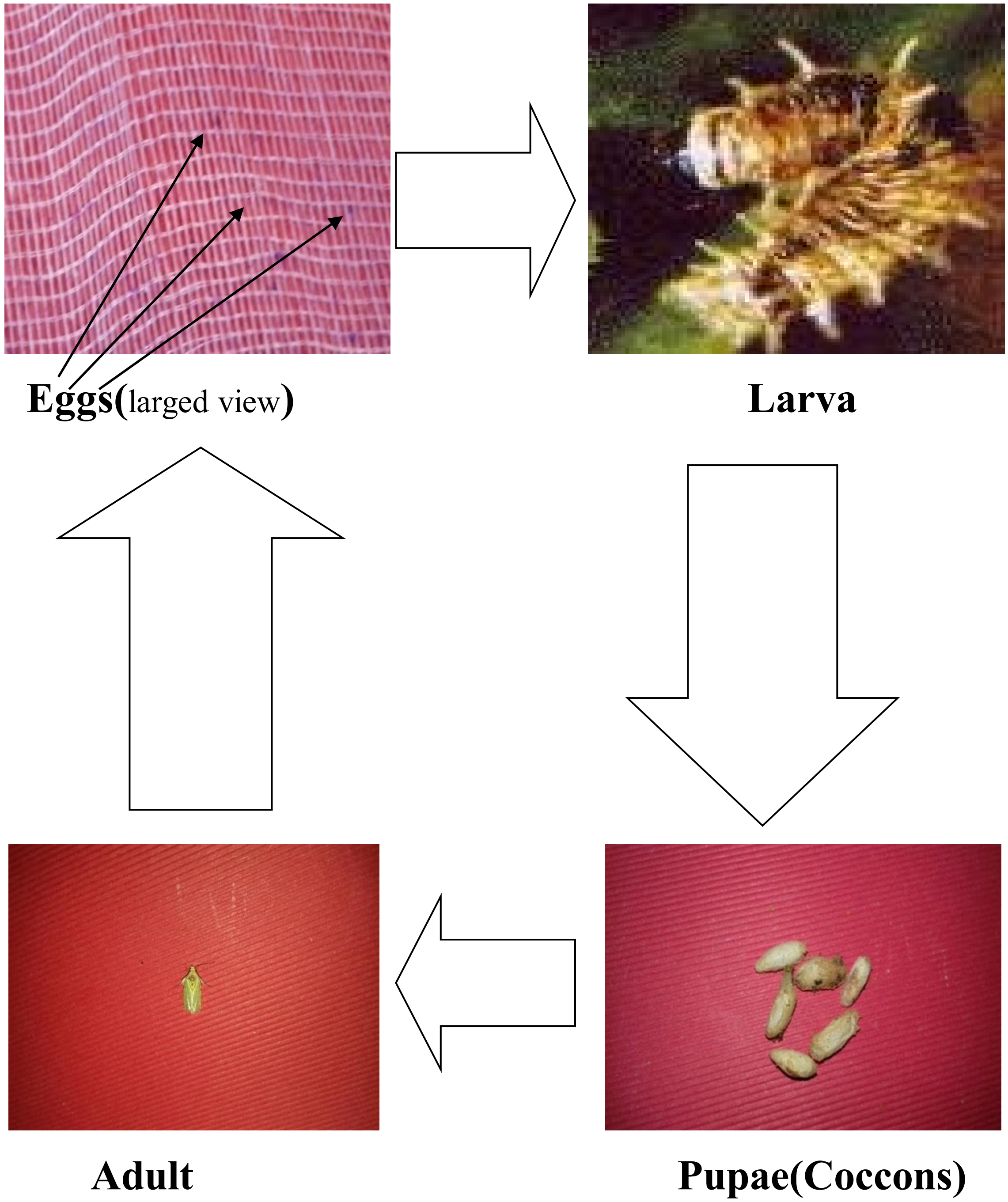


Plate No. (1): Life cycle of Spiny bollworm

2.1.12. Mating and Oviposition

After 2-3 days of emergence, adult moth started mating, this mating process took place at night time egg lying process took 6-8 days. Female laid eggs singly beneath the cloth placed on the top of the cage. Each female laid about 75-150 eggs during life span. Past researchers had different findings about the number of eggs such as, (Wilcocks, 1937) reported that each female laid 200 eggs while (Hiremath, 1987) reported 112 eggs.

2.1.13. Symptoms

The symptoms of attack are similar for all *Earias* species and are often described in general terms rather than for individual species in much of the literature. Attack moves to buds and flowers as they appear which wither and are shed. There is usually a conspicuous hole. Pearson (1958) commented that the shedding of minute buds, often blamed on mirids, might be due to very young *Earias* larvae, although he was not specifically referring to *E. vittella*.

The bolls are also attacked, but only when unripe. The larvae usually bore deeply, filling the tunnel opening with excrement. The tunnel often enters bolls from below, entering at a slight angle to the peduncle (Pearson, 1958). Small bolls up to 1 week old turn brown rot and drop, whereas bigger bolls of 2-4 weeks may not drop but open prematurely and be so badly damaged as to be unharvest able. Bolls of up to 6 weeks of age are vulnerable (Butani, 1976., Sidhu and Sandhu, 1977). The larvae tend to move from boll to boll and damage may be disproportionate to their numbers.

2.1.14. Damage and Economic importance

Faseli (1977) reported that in the south Khorrasan region of Iran, *E. insulana* caused about 80% of the damage to cotton. Stam and Elmosa (1990) report that *E. insulana* was the most damaging pest in the Syrian cotton agro-ecosystem from 1980 to 1983.

Cotton spiny bollworm is the larva of *Earias insulana*, belonging to the Noctuidae family. It is one of the most important pests of cotton, in all over the world and in Iran, the pest, is recorded to be spread in cotton farming areas (Esmaili *et al.,* 1995 and Mirmoayedi, 2009).

2.1.15. Control measure

2.1.15.1. Cultural Control

Much of the literature investigating cultural control of bollworms looks at mixed populations such as *Pectinophora gossypiella*. *Earias* spp is often a secondary component and there is rarely any indication of the damage caused by each pest in isolation. In India, long duration cultivars of cotton supply a host for *Earias* from June or July until February, with okra providing an important carryover crop from one cotton season to the next. Cotton plants not removed after the harvest also assist carryover because they sprout from the stump and continue to provide food for *Earias*. Irrigated cotton in summer also provides extra food for the *Earias* population. Legislation in some countries requires farmers to uproot and destroy harvested plants to ensure an adequate close season, but this is seldom enforced. However, in Cyprus, legislation requiring growers to destroy all okra plants before a fixed date was apparently ineffective in reducing damage, mainly because of the presence of wild malvaceous plants in the vicinity of the crop fields (Kashyap and Verma, 1987).

Eradication of alternative host plants has also been attempted, but is of doubtful benefit because many are valuable sources of food, feed or fiber and their removal may reduce the pool of natural enemies.

Kashyap andVerma (1987) suggested that cotton should be inspected regularly and all wilted shoots removed, thus removing the larvae of *Earias*. Some farmers allow livestock to graze cotton during the vegetative stage with the same effect. Also emphasize the importance of removing infested shoots and suggest that it be done when the egg-masses of *Spodoptera littoralis* are being collected and destroyed. The benefits of topping are controversial, claimed that the removal of the topmost few centimeters of the cotton plant at the beginning of the season reduced infestation and encouraged lateral branches, increasing the yield, without affecting the quality of the fiber.

Other suggested cultural practices include deep ploughing and close spacing of plants (Abdel Fatah *et al.,* 1980). Singh (1997) suggested that missing out rows when sowing cotton, to provide a path for spray operations, lowers the incidence of *E. insulana* and other bollworms, as well as improving yields. Other studies have found that earlier sowings help reduce bollworm infestation.

2.1.15.2. Sanitary methods

If cotton plants are not removed after the harvest, they sprout from the stump. Lower pest incidence was found in fields where stumps were removed after harvest (Sawhney and Nadkarny, 1942). Also claimed good control of *Earias* by completely removing cotton stumps (Patel, 1949).

Pearson(1958) reported damage was reduced by 18% and yield of raw cotton increased by 19% with such a practice. Legislation in some countries requires farmers to uproot and destroy harvested plants, but this is apparently poorly enforced. Removal of possible alternative host plants was recommended by (Patel, 1949).

2.1.15.3. Natural enemies

Several Hymenopterans insects have been recorded as parasites of the spiny bollworm in the Sudan namely including –*Agathus* sp., *Apanteles* spp.,*Bracon brevicornis*, *Chelonus* sp. And *Elasmus* sp. (Reed and Choyce, 1961).

2.1.15.4. Biological control

Sangwan *et al*., (1972) reported an initial 90% parasitization ofcotton bollworms following releases of the introduced *Trichogramma brasiliensis* [*Trichogramma brasiliense*], but it is not known whether effective parasitism continued. Some years later, *T. brasiliense, T. pretiosum*,*T.achaeae*, and the braconids[*Microchelons blackburni*] and *Bracon kirkpatricki* were released in cotton fields in India from imported cultures.

Surulivelu (1989) also reported reduced numbers of *E. vittella* after release of *C.blackburni*. Sangwan *et al*., (1972) reported that *B. kirkpatricki* had been released to control *E. insulana* on okra in Fiji.

Kashyap and Verma (1987) compiled a list of natural enemies for the Indian subcontinent. Far fewer predators have been recorded, probably due to the inherent difficulties in observation.

2.1.15.5. Host plant Resistance

Kashyap and Verma (1987) suggested that cotton should be inspected regularly and all wilted shoots removed, thus removing larvae. Some farmers allow livestock to graze cotton during the vegetative stage with much the same effect. The benefits of such 'topping' are apparently controversial.

Dash *et al*., (1987) found that inter-growing cotton with crops such as rice and groundnuts helped reduce damage. Numerous trials have tested the resistance of various cultivars and reduced susceptibility has been found in many of them. Those with high levels of tannin and gossypol (Sharma and Agarwal, 1984) forego-bract and okra-leaf characters and red pigmentation (Duhoon and Singh, 1980) have been found to be less susceptible than many commercial cultivars and investigated okra varieties and found some had a certain amount of resistance, observed that sticky secretions released by okra when bitten by larvae could act as traps, killing *E. vittella* larvae. Duhoon (1980) discusses the possibility that resistance might be found in wild relatives *(Abelmoschus* spp.) of okra.

2.1.15.6. Pheromone use

The components of the female sex pheromones of *E. insulana* have been identified (Cork *et al.,* 1985). Subsequent synthetic formulations have shown promise in Pakistan, disrupting mating and achieving season-long control.

2.1.15.7. Chemical control

In history; it is necessary to apply the sprays while the caterpillars are still small (Hill, 1981).The recommended insecticides are Endosolphane (2ml/l) or carbaryle 3g/l (Ikisan, 2000). Now in Sudan, we use the chemical to control spiny bollworm within cotton pests' especially American bollworm when the pest reaching the Economic Threshold Level (ETL).this is the most common usage (Abdelrahman, 2014).

1- Engeo 247 SC at 0.187 L/fed (46.2g *a.i.* / fed.), One of chemical that is recommended to control the African bollworm and spiny bollworm. (Engeo 247 SC) is manufactured by Syngenta Agro AG, Switzerland and introduced in the Sudan by Agways Company (Elameen, 2016).

2-Nomolt 50 UL at 0.213L/fed (8.5 g *a.i.* / fed), for the control of spiny bollworm, Nomolt is manufactured by BASF-SE, Germany and introduced in the Sudan by the Central Trading Company (CTC).

2.2. Rehan, Holy basil (*Ocimum basilicum L*).

Sweet basil is native to western and tropical Asia .It belongs to the family Lamiaceae. By the 16th century it was also cultivated in England (Stoll, 2000).

2.2.1. Distribution of Rehan

Today, it is widespread in Europe, Asia, Africa and the western Hemisphere as condiment for medicinal purposes and as scent; sixty different varieties of this plant have been identified in the tropics. Holy basil is found throughout India and Nepal up to elevations of 1800 m in the Himalayas and on the Andaman Nicobar Islands. It is regarded by the Hindus as their most sacred plant (Stoll, 2000).

Synthetic insecticides are expensive for subsistence farmers and they may pose potential risks owing to the lack of adequate technical knowledge related to their safe use. One alternative to Synthetic insecticides is insecticidal plants; African farmers are traditionally familiar with them (Thiam and Ducommun, 1993).Oils extracted from plants have been extensively used in tropical countries for crop protection (Rajapakse and Van Emlen ,1997).The tropical flora is a major source of plant –based insecticides (Aranason *et al.*,1989) Aromatic species, particularly those in the family Labiatae or (Lamiaceae) are among the most widely used plants in insect pest control (Morton, 1981 and Shaaya *et al.,*1997).

Among the most common species of *Ocimum* (Labiatae) in West Africa are *Ocimuum basilicum* L. (Sweet basil) and *Ocimuum canum Sims* (White basil) (Berhaut, 1975).

Essential oils are volatile secondary metabolites that plants produce for their own needs other than nutrition i.e. Protectant or attractant. In general, they are complex mixtures of organic compounds that give characteristic odor and flavor to the plants. The essential oil composition of *O.basilicum* varies depending on the environment and the chemo type (Brophy and Jogia, 1986). It has an essential oil content of 0.2%,with 32chemical components, including linalool (22.3%),methyl Eugenia (24.7%)and (E)-methyl inanimate (23.6%) found that samples from Cuba have an essential oil content of 1.9-2.5% and 30chemical components, including methyl chavicol (66.8%),1.8-cineol (5.4%)and linalool (5%),whereas samples from Burkina Faso (Belanger *et al.*,1995) have an essential oil content of 0.7-1.8 % with 25 chemical components, predominantly 1.8cincol (60.2%) .Terpincol(6.5%) and Bpinene (5.7%).*Ocimuum basilicum* and *Ocimum gratissimum* were cultivated in order to determine the essential oil content and its chemical composition at different harvesting dates after planting (Brophy, and Jogia, 1986). In fresh *O.basilicum* oil content was 0.26% at three and four months after establishment and it drastically decreased to 0.14% at five months after planting; Estragole was the main constituent (81-83%) of *O.basilicum* oil at all harvesting dates (Sanda *et al.*, 2001).

2.2.2. Taxonomy of Sweet basil

Rehan plant can be classified as follows:

Order	Lamiales
Family	Lamiaceae
Genus	*Ocimum*
Species	*basilicum*
S.N	*Ocimum basilicum*
C.N	Rehan or Sweet basil

2.2.3. Medicinal uses of *Ocimum basilicum*

The medical uses of *O. basilicum* were reported by many workers, since long time ago(Dalzeil,1937) reported that *O. americanum* was used for relieving fever, dysentery, as an eye remedy, and as mouth wash to relief toothache. (Watt *et al.,* 1962) reported that *O. basilicum* l. was used in Angola as a hair dresser.

In Kenya, two species of rehan namely *O. basilicum* and O. suave were collected from the central and the rift valley areas and used against fever and livestock diseases (Githinji and Kokwaro, 1993).

2.2.4. Insecticidal Properties of *Ocimum spp*.

Guenther (1961) reported that *Ocimum vividae* has been named the mosquito plant and it was used to repel mosquito in West Africa. *O.vividae* any disease, was used a mosquito repellent. The herbivorous animals refused to eat *O.canumsims*, a perennial which had not suffered from any disease. The sacred tubi-plant *O.santum* according to (Guenther, 1961) has been used in India, beside, medicinal and antibiotic effects, as an insect repellent.

Malaka (1972) stated that a preparation of the leaves of *O. basilicum* L. has been used among many methods to protect yams before planting against termites. (Shah and Patel, 1976) found that *O.sanctum* contained methyl eugenol and had been used to attract the mango fruit fly, *Dacus correctus* Bezz.and observation on the population counts revealed that all the fruit flies attracted to tulsi plant were male only.

Deshpande and Tiphis(1977) obtained by TLC eight fractions from the essential oil of *Ocimum basilicum* .They tested the activity of each fraction against stored grains insect pests, namely *Tribolium castaneum* ,*Sitophilus oryzae*, *Stagobium paniceum* and *Bruchidiuus chinensis.* From bioassay tests methylcinnamate and methylcharicol were found to be the components mainly responsible for the insecticidal activity.

Rajendran and Copalan (1978) found that, *Opium sanctum* showed no clear juvenile– hormone like activity. The same authors (Rajendran and Copalan, 1979) reported that, the extracts of four plant species, including *Ocimum sanctum* Linn caused mortality in *Dysedercus cinqulatus* Fabr., *Spodopter liturales* Fabr and *Pericalla rici*ni. They concluded that, these plants have not only juvenile–hormone mimicking substance but also insecticidal properties. Bowers and Nishida (1980) isolated two compounds with highly potent juvenile hormone activity from the oil of sweet basil, *Ocimum basilicum L*. They named them Juvocimene 1and Juvocimene 2.

Juvocimene 2 induces the formation of nymphal adults' intermediates in the milk weed bug at treatment levels as low as 10 pg. Juvocimene 1 is approximately 10 times less active than Juvocimene 2. Both plant derived hormones are several orders of magnitude more active than the natural juvenile hormone. They suggested that the sweet basil may have developed an additional and far more sophisticated chemical defense against insect morphogenetic development. Pandey *et al*., (1983) investigated the efficacy of certain plant extracts against brinjal *Aphis gossypii* Glov. At different concentrations (0.1, 0.5 and 1.0%) and depending on concentration, an extract of mature seed of *O.basilicum* L.was shown to give 45.40-56.22 % mortality.

Mansour *et al*., (1986) investigated the effect of essential oils isolated from 4 species of the family Labiatae on adult females of *Tetranychus* *cinnabrinus* in the laboratory .They showed that concentrations of the acetone solutions of the oils from 0.1 to 2% cause mortality and induce repellency within 48 hours of introducing adult females, and consequently egg-laying was found to be reduced, and seven day old residues still had some activity. The most effective oils (their EC50 in brackets) were *Lavandula angustifolia*, *L.latifolia* (EC50 0.094), *L. angustifolia* (0.1%), *Ocimum basilicum* (EC50 1.4%), *Salvia fructicosa* (EC50 1.4%) and rosemary (EC50 2.2%).

2.3. Lupine

2.3.1. History and distribution

The earliest archaeological reports on lupins are referred to the Twelfth Dynasty of Egyptian Pharaohs (over 2 thousand years BCE). In their tombs, seeds of *Lupinus digitatus* Forsk. Already domesticated in those times, were discovered. Seven seeds of this species were also retrieved in the tombs of this dynasty dated back to the 22nd century BCE. They are the most ancient evidence of lupine in the Mediterranean (Boguslav and Kurlovich, 2002).

They are traditionally eaten as a pickled snack food, primarily in the Mediterranean and South America. The bitter variety of the beans are high in alkaloids and are extremely bitter unless rinsed for a long time (Boguslav and Kurlovich, 2002).

Lupinus, commonly known as lupin or lupine (North America), is a genus of flowering plants in the legume family, Fabaceae. The genus includes over 200 species, with centers of diversity in North and South America (Drummond, 2012).Smaller centers occur in North Africa and the Mediterranean (Aïnouche and Bayer, 1999). Seeds of various species of lupins have been used as a food for over 3000 years around the Mediterranean (Gladstones 1970) and for as much as 6000 years in the Andean highlands, but they have never been accorded the same status as soybeans or dry peas and other pulse crops. The pearl lupin of the Andean highlands of South America, *Lupinus mutabilis*, known locally as *tarwi* or *chocho*, was extensively cultivated, but there seems to have been no conscious genetic improvement other than to select for larger and water-permeable seeds. Users soaked the seed in running water to remove most of the bitter alkaloids and then cooked or toasted the seeds to make them edible (Gladstones, 1970) or else boiled and dried them to make *kirku* . Spanish domination led to a change in the eating habits of the indigenous peoples, and only recently has interest in using lupine as a food been renewed (Gladstones, 1970).

2.3.2. Description

The species are mostly herbaceous perennial plants 0.3–1.5 m (0.98–4.92 ft) tall, but some are annual plants and a few are shrubs up to 3 m (9.8 ft) tall. An exception is the *chamis de monte* (*Lupinus jaimehintoniana*) of Oaxaca in Mexico, which is a tree up to 8 m (26 ft) tall (Villa-Ruano, 2012).Lupine have soft green to grey-green leaves which may be coated in silvery hairs, often densely so. The leaf blades are usually palmately divided into five to 28 leaflets, or reduced to a single leaflet in a few species of the southeastern United States.

2.3.3. Taxonomy of Lupine

Order	Fabales
Family	Fabaceae
Subfamily	Faboideae
Tribe	Genisteae
Sub tribe	Lupinine
Genus	*Lupinus*
Species	*termis*
S.N	*Lupinus termis*
C.N	Lupine

2.3.4. Culinary

The legume seeds of lupin, commonly called lupin beans, were popular with the Romans, who cultivated the plants throughout the Roman Empire; hence, common names like *lupini* in Romance languages.

The European white lupine (*Lupinus albus*) beans are commonly sold in a salty solution in jars (like olives and pickles) and can be eaten with or without the skin. *Lupini* dishes are most commonly found in Europe, especially in Portugal, Spain, Greece, and Italy. They are also common in Brazil and Egypt. In Egypt lupine is known in Arabic as*termes*, and is a popular street snack in after being treated with several soakings of water, and then brined. In Portugal, Spain, and Spanish Harlem, they are popularly consumed with beer. In Lebanon, Jordan, Syria, Israel and Palestine, salty and chilled lupini beans are called *termos* and are served as part of an apéritif or a snack. The Andean lupine or *tarwi* (*L. mutabilis*) was a widespread food in the Incan Empire. Other species, such as *L. albus* (white lupine), *L. angustifolius* (narrow-leafed lupin) (Murciaand Hoyos, 1998)and*L. hirsutus* (blue lupin) (Hedrick,1919) also have edible seeds. Lupins were also used by many Native American peoples such as the Yavapai in North America. Lupins are known as *altramuz* in Spain, from Arabic termes. The seeds are used for different foods, from vegan sausages to lupine-tofu or baking-enhancing lupine flour.

2.3.5. Agriculture

Whilst originally cultivated as a green manure or forage, lupines are increasingly grown for their seeds, which can be used as an alternative to soybeans. Sweet (low alkaloid) lupines are highly regarded as a stock feed, particularly for ruminants but also for pigs and poultry and more recently as an ingredient in aqua-feeds. The market for lupine seeds for human food is currently small, but researchers believe it has great potential. Lupine seeds are considered "superior" to soybeans in certain applications and there is increasing evidence for their potential health benefits. They contain similar protein to soybean but less fat. As a food source, they are gluten-free and high in dietary fiber, amino acids, and antioxidants, and they are considered to be prebiotic. About 85% of the world's lupine seeds are grown in Western Australia (Ross, 2011).

Three Mediterranean species of lupine, blue (narrow-leafed) lupine, white lupine, and yellow lupine, are widely cultivated for livestock and poultry feed.

Like other legumes, they can fix nitrogen from the atmosphere into ammonia via a rhizobium–root nodule symbiosis, fertilizing the soil for other plants. This adaption allows lupine to be tolerant of infertile soils and capable of pioneering change in barren and poor-quality soils. The genus *Lupinus* is nodulated by *Bradyrhizobium* soil bacteria (Kurlovich and Stankevich, 2002).

2.3.6. Ecology

Certain species, such as the yellow bush lupin (*L. arboreus*), are considered invasive weeds when they appear outside their native ranges. In New Zealand, *L. polyphyllus* has escaped into the wild and grows in large numbers along main roads and streams on the South Island. A similar spread of the species has occurred in Finland after the non-native species was first deliberately planted in the landscaping along the main roads. Lupine have been planted in some parts of Australia with a considerably cooler climate, particularly in rural Victoria and New South Wales (Ross, 2011).

2.3.7. Varieties

Some varieties are referred to as "sweet lupine" because they contain much smaller amounts of toxic alkaloids than the "bitter lupin" varieties.Three Mediterranean species of lupine (blue lupine, white lupine and yellow lupine) are widely cultivated for livestock and poultry feed. Bitter lupins in feed can cause livestock poisoning (Kurlovich and Stankevich, 2002).

2.3.8. Cuisine

The Andean lupin *L. mutabilis*, the Mediterranean *Lupinus albus* (white lupin), and*Lupinus hirsute*are only edible after soaking the seeds for some days in salted water (Azcoytia and Carlos,1980*)*. Lupini beans are commonly sold in brine in jars (like olives and pickles). They can be eaten by making a small tear in the skin with one's teeth and "popping" the seed directly into one's mouth, but can also be eaten with the skin on. Highly skilled lupini eaters learn to fissure the skin by rubbing the bean between forefinger and thumb.

2.3.9. Toxicity and allergenicity

Lupine poisoning is a nervous syndrome caused by alkaloids in bitter lupine, similar to neurolathyrism, affects people that eat incorrectly prepared lupine beans. Mediterranean cultures prefer the historic bitter lupine beans with the required toxin-removal by traditional leaching in water preparation methods due to the better flavor that results. Improper preparation of bitter lupines with insufficient soaking allows pharmacologically significant amounts of the anti-cholinergic alkaloids to remain in the beans, and poisoning symptoms result. While the alkaloids found in raw and dried beans are bitter and unpalatable to many, with merely insufficient soaking the level is reduced (Grande *et al.,* 2004).

There are several references in medical literature to poisoning caused by errors in lupini preparation (Grande *et al.,* (2004).

Symptoms of lupin bean poisoning (from excess alkaloid in cooked food) include dilated unresponsive pupils, confusion, slowed thought and disorientation, flushed face and/or fever, high heart rate and blood pressure, tremors, difficulty with or slurred speech, in-coordination, dizziness, burning dry mouth, stomach pain, and anxiety or"malaise (Kurzbaum *et al.,*2008).

Current media describes the symptoms when referring to recent Australian Medical Journal reports of poisoning from overly bitter lupin flour used in foods reported in the media(Pingault *et al.,* 2009).

Mycotoxic lupinosis is a disease caused by lupin material that is infected with the fungus *Diaporthe toxica* (Williamson, 1994) the fungus produces mycotoxins called phomopsins, which cause liver damage.

2.4. Insecticides synergism

Insecticide synergism is the type of interaction of insecticide with another chemical, usually of low toxicity, which results in an un-expectedly high mortality. Piperonyl butoxide has been used as a synergist to the pyrethroids for the control of some insects such as houseflies. There are reports of its residues on some commodities. Residues of pyrethrin-I (Py-I) and pyrethrin-II (Py-II), the major insecticidal components of the pyrethrum daisy [*Tanacetum cinerariifolium* (Trev.) Schultz-Bip.] and residues of piperonyl butoxide (PBO, a pyrethrum synergist) were found in soil and on potato foliage grown under field conditions (Antonious *et al.*,2001). PBO is not effective in all situations as it failed to synergize a number of insecticides (Scott, 1999., Scott *et al.,* 2005 and Proudfoot *et al.*, 2005).

By the systematic entomological examination of chromatographic fractions of sesame oil two pyrethrum synergists, sesamin and sesamolin, were found to account for practically all the synergistic activity of the oil. Sesamolin, which had not been known to be synergistic, is about five times as active as sesamin and, even though usually present in smaller amount than sesamin, it is believed to account for most of the synergistic activity in sesame oil. Because of sesamolin's marked synergistic activity, studies on its chemistry were made, and some of its chemical properties are reported. The studies have indicated a close similarity between sesamin and sesamolin, and a tentative chemical formula for sesamolin has been proposed as a basis for future work. Infrared absorption spectra of sesamin and sesamolin are reported(Journal of the American Oil Chemists' Society 31(7):302-305 · June 1954 with 25 Reads).

Sesamin increases the detoxification capability of liver, reduces the incidence of chemically induced tumors and protects neuronal cells against oxidative stress and exhibits anti-hypertensive, anti-inflammatory and anti-allergic effect (Hirose *et al.*,1992.,Hou *et al.,* 2003.,Jeng and Hou, 2005).

2.5. Piperonyl butoxide

The synergists that have a methylene dioxyphenyl group were firstly introduced in 1940 to increase the effectiveness of pyrethrum. Since, then many compounds have appeared, but only a few are still marketed. These synergists are piperonyl butoxide, sesamin, sesamolin and sesame Piperonyl butoxide, a semisynthetic derivative of safrole, is the most widely used synthetic pyrethrin synergist and there are no reports available on toxic effects on humans resulting from the exposure to it (Breathnach, 1998). Piperonyl butoxide does not have any pesticidal activity. However, when combined with insecticides, such as pyrethrin, pyrethroid and carbamate insecticides, their potency is increased considerably (Maklakov *et al*., 2001). It was clearly shown that PB interacts (induce or inhibit) with some P450 isozymes both in insects and mammalian species (Hodgson and Philpot 1974 and Adams *et al.,* 1993).

2.6. Uses of sesame oil

Sesame, *Sesamum indicum* L., is an annual herb native to the tropics and grown primarily for its oil-rich seeds. This highly aromatic oil ranges in hue from golden to brown and is extensively used in Asian cuisine. The oil is sometimes used as cooking oil, but most often is used as a seasoning accent in stir-fries, dressings, sauces and marinades. In addition, sesame oil is also used in the some injectable drug formulations in human and veterinary medicine (Hirose *et al*., 1992.,Hou *et al.,*2003.,Jeng and Hou, 2005).

Sesamin increases the detoxification capability of liver, reduces the incidence of chemically induced tumors and protects neuronal cells against oxidative stress and exhibits anti-hypertensive, anti-inflammatory and anti-allergic effect(Hirose *et al.,* 1992.,Hou *et al*.,2003.,Jeng and Hou, 2005).

Sesame lignans (a non-fat constituent) or antioxidants such as sesamin, episesamin, sesaminol and sesamolin are major constituents of sesame oil and all have chemically methylene dioxyphenyl group (Kamal-Eldin *et al.,*1994 and Kamal-Eldin and Appelqvist 1994).

CHAPTER THREE

3- MATERIALS AND METHODS

3.1. Area of study

This study was conducted at the Entomology laboratory, Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology (SUST), during the period of March– July, 2016.

The materials used in this study are listed below:

3.2. Equipments

1- Petri-dishes	12- GPS
2-Brush	13-Micropipette
3-Hand lens	14- plastic cages
4-Rotary evaporator	15-pipette
5-Sensetive balance	16 -Registration form
6-Electronic blender	17-Pencil
7- Digital Camera	18 –Gloves
8-Soxhlet extractor Apparatus	19 –Masks
9- Plastic containers	20 - Conical flasks
10- Surgical blades	21- Max-Min Thermo Hygro and Clock Apparatus
11-Shaker (220 RPM – 16 Hrs.)	22- Filtrated under reducing pressure(Pump)

3.3. Materials

1- Fresh Okra	7- Soap
2- Rehan leaves	8- UHU
3- Lupine seeds	9-Muslin cloths
4- Hexane 99.7%	10- Sand
5- Engeo 247 SC	11- Sucrose (10%)
6- Sesame oil	12-Distilled water

3.4. Collection of target insect

The larvae of spiny boll worm (SBW) (*Earias insulana*) were obtained from a farm located west of Halfaia Bridge, Khartoum State during March 2016 and brought to laboratory.

3.5. Rearing of Spiny Boll Worm

The okra used to feed the larvae of spiny boll worm (SBW) *(Earias insulana)* were obtained from the above mentioned farm during the rearing and bioassay experiment. The larvae were reared in Petri-dishes 9 cm in diameters (Plate 2). Within 10 days the larvae pupated and transferred to cages (37cm x 27cm x 20cm) until emergence of adults.

Adults were then fed on sucrose solution (10%) which was kept in a container covered by a piece of cotton; the sucrose solution was changed daily. Adults were sexed and each twenty pairs were kept in separate cage, the cages were kept in a room of 26-29°C, and 70-80 RH%.

Females laid eggs in a piece of cloth which was later transferred to another cage where food was available (fresh okra). The 3rd instars larval used in the bioassay experiment for evaluating hexane extracts of both Rehan leaves and lupine seeds.

3.6. Collection of Rehan leaves and preparation of the powder

Rehan leaves (*Ocimum basilicum*) were collected from Elselet Agricultural Scheme during March 2016. The leaves were washed and left for 2-3 days to dry under shade. The dry leaves were then ground into fine powder using an electric blender and stored in a tightly covered glass container until being extracted.

3.7. Collection of lupine seeds and preparation of the powder

Lupine seeds (500 grams) were brought from Soqu sita, Khartoum state. The seeds were crushed manually into small parts before grinding them in a mill to become fine powder which were stored in a tightly covered glass container until being extracted.

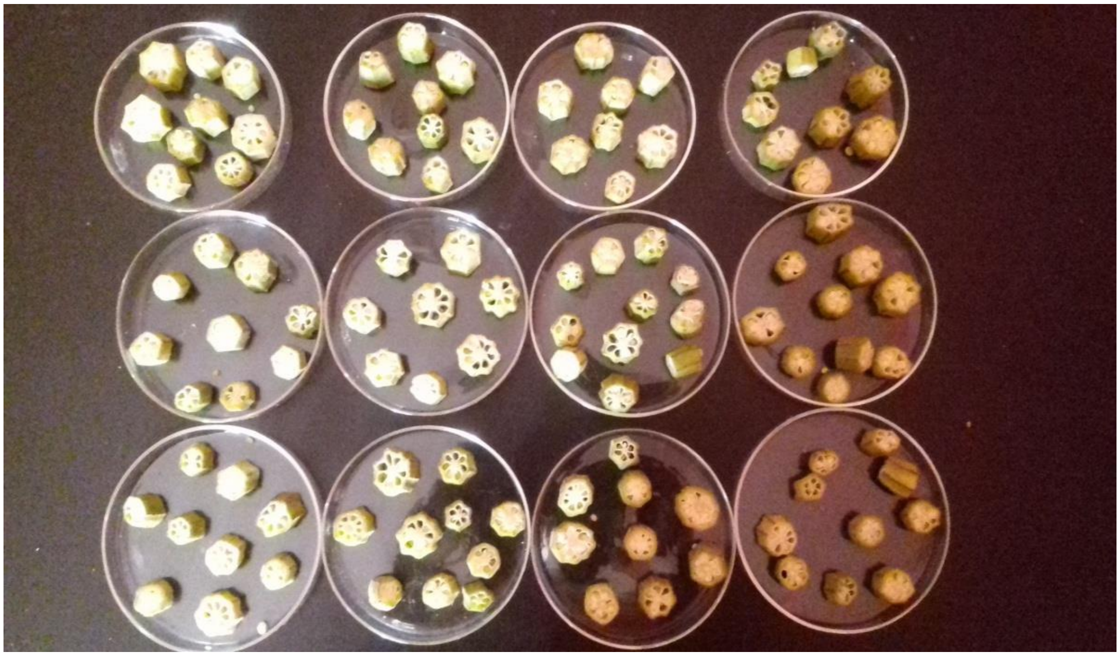


Plate No. (2): Rearing of Spiny boll worm larvae

Plate No. (3): Cages of Adult Rearing

3.8. Extraction method

Extraction processes were conducted at the Chemistry laboratory (JICA laboratory), College of Agricultural Studies, Sudan University of Science and Technology. Hundred grams (100) of each of the previously prepared powders of Rehan leaves and Lupine seeds were divided equally into three parts and then extracted with 200 ml hexane (99.7 %) were added for each sample and put it on the shaker (plate 5) for 16 hours at 220 RPM. Each part was placed separately in a thimble and it was placed in an extraction chamber of a Soxhlet extractor apparatus (Plate4), the crude mixtures were filtrated under reducing pressure (plate 6) and the hexane solvent was removed off the crude extract by Rotary evaporator (Plate 7). The obtained crude materials of each Rehan leaves and Lupine seeds (plate 8) were weighted and carefully stored for the experiments.

3.9. The standard insecticide (Engeo 247 SC)

Engeo 247 SC (Pyrethroid group) at 0.187 L/fed (46.2 g *a.i.* / fed.) it's one of chemicals that is recommended to control the African bollworm and spiny bollworm. Engeo 247 SC is manufactured by Syngenta Agro AG, Switzerland and introduced in the Sudan by Agways Company.

3.10. Preparation of the concentration

According to volumetric law (1.25%, 2.5 %, 5% and 10%) concentrations were prepared for each extract by dilution from crude extracts. On the other hand sesame oil was added to the mentioned concentrations of the two extracts (1:10, oil: extracts) as a Synergist.

3.11. The Bioassay procedure

Third larval instars of the spiny boll worm 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 hexane extracts of Rehan leaves and Lupine seeds, and each treatment was replicated three times. Thirty larvae (three replicates) were treated with Rehan leaves and thirty larvae treated with Lupine seeds plus sesame oil at ratio of (10 : 1) and thirty larvae were treated with the recommended dose of Engeo 247 SC at 0.187 L/fed (46.2 g *a.i.* / fed.) 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 26-29 °c. During treatment period the larvae were fed on fresh okra.

The number of dead larvae and other observations were recorded at the end of the 1st, 2nd and 3rd days (24hrs, 48hrs and 72 hrs.). Average of room temperatures and relative humidity (RH) were recorded during the experiments periods.

3.12. Experiment design

The experiments were assigned in Completely Randomized Design (CRD).

3.13. Statistical analysis

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

3.14. Probit analysis

Environmental Protection Agency (EPA) probit analysis program used for calculating LC values version 1.5 software was adopted to compute LC_{50}values for each plant extract used in these experiments (Matsumura, F.(1976).

Plate No.(4) Soxhlet and Rotary evaporator

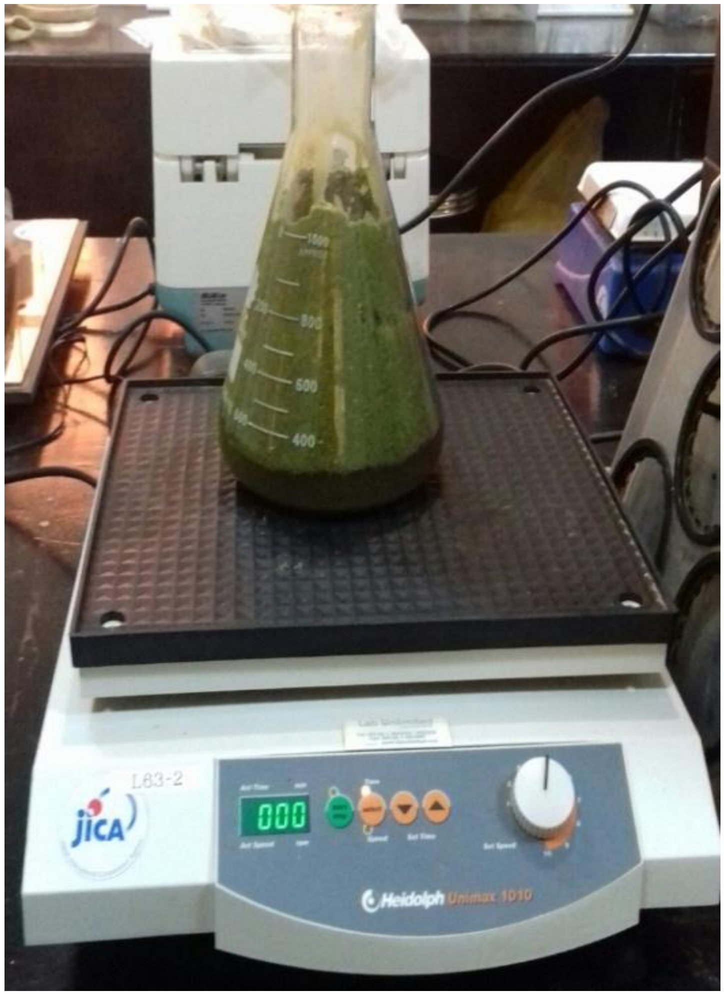
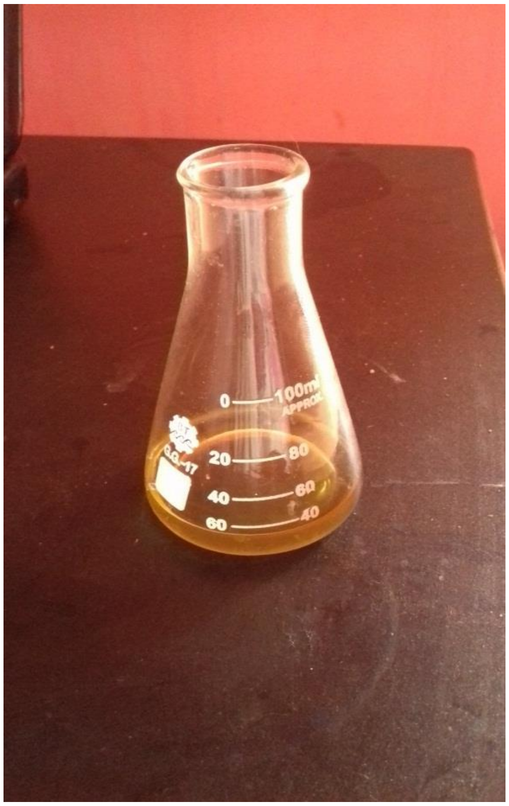
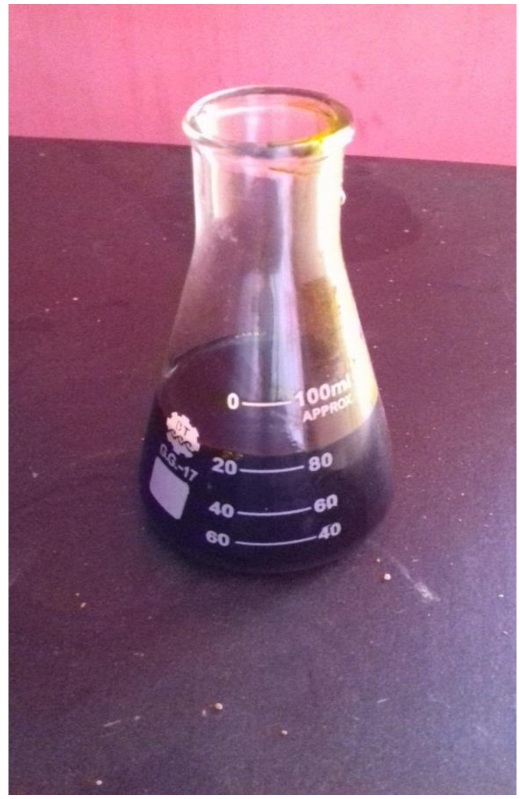


Plate No. (5) Shaker

Plate No.(6): Filtrated under reducing pressure (Pump)

Plate No. (7): Rotary evaporation (Evaporator)





(A) **(B)**

Plate No. (8): (A) =hexane extract of Lupine seeds, **(B)** = hexane extract of Rehan leaves.

CHAPTER FOUR

RESULTS

As seen in table(1) and figure (1), all tested concentration of Rehan leaves extracts gave significantly higher mortality percentage than control, there was no significant difference in mortality percentage caused by the highest concentration and the standard after 24hrs, 48hrs and 72 hrs. of exposure. However; there was a significant difference in the mortality percentage caused by the highest concentration (10%) and (5%) concentration after 24 hrs. and 48 hrs. of exposure, but there was no significant difference between two concentrations after 72 hrs. of exposure.

Table No. (1): Effect of hexane extract of Rehan leaves on the mortality of 3rd larval instars of the spiny bollworm.

Concs. (%)	**Mortality (%)**		
	Exposure time (hrs.)		
	24	**48**	**72**
1,25%	36.667 (6.1) **D**	40 (6.4) **D**	46.667(6.9) **B**
2,5%	50 (7.1) **C**	56.667(7.5)**C**	66.667(8.2)**B**
5%	63.333(8)**B**	76.667 (8.8)**B**	80 (9) **A**
10%	93.333(9.7)**A**	96.667(9.8)**A**	100 (10) **A**
Engeo 247 SC	100 (10) **A**	100 (10) **A**	100 (10)**A**
Control	0.0 (0.7) **E**	0.0 (0.7) **E**	0.0 (0.7) **C**
SE±	**3.3333**	**5.7735**	**5.4433**
C.V. %	**7.13**	**11.47**	**10.17**
LSD	**7.2627**	**12.579**	**11.860**

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

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

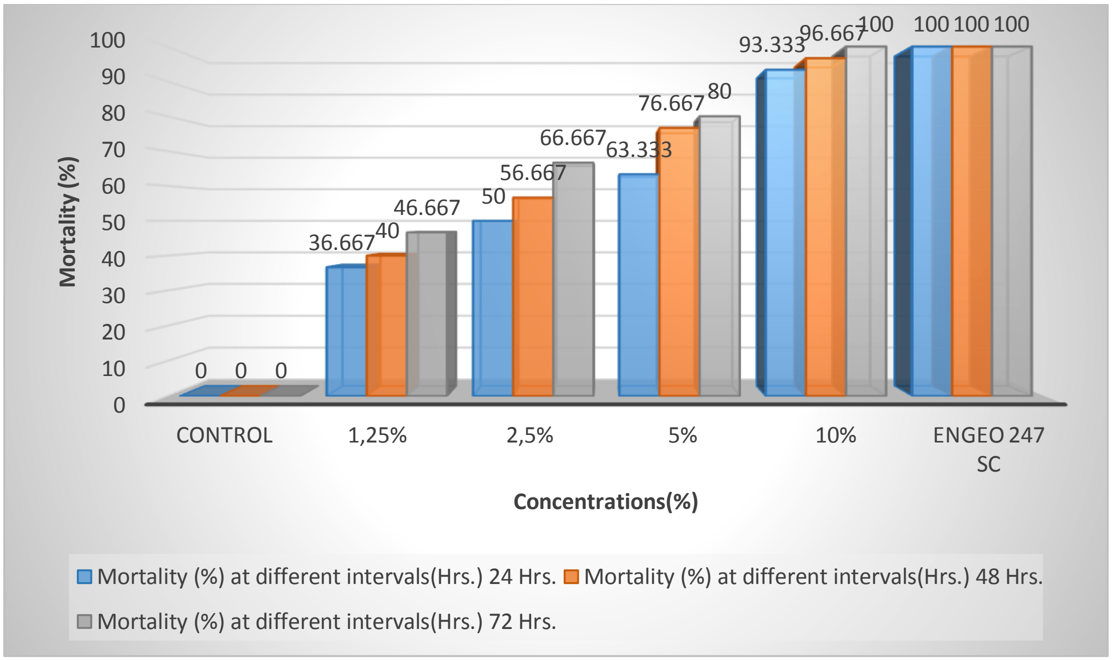


Figure No. (1): Effect of hexane extract of Rehan leaves on the mortality of 3rd larval instars of the spiny bollworm.

As seen in table (2) and figure (2), all tested concentration of Rehan leaves mixed with sesame oil gave a significantly higher mortality percentage than the control after 24 hrs, 48 hrs and 72 hrs. of exposure. It is interesting to note that there was no significant difference in mortality percentage result obtained by the standard compared to synergized (5%) concentration, synergize (10%) concentration. However, there are significant different in the mortality percentage among the three tested lowest synergized concentration (1.25%, 2.5 % and 5%) after 24 hrs, 48hrs, and 72 hrs. of exposure. There was no significant different in mortality generated by the (5%) concentration and (10%) concentration and the standard.

Table No. (2): Effect of hexane extract of Rehan leaves mixed with sesame oil on the mortality of 3rd larval instars of the spiny bollworm.

Concs. (%)	**Mortality (%)**		
	Exposure time (hrs.)		
	24	**48**	**72**
1,25%	60 (7.8) **C**	70 (8.4) **C**	76.667(8.8) **C**
2,5%	76.667 (8.8) **B**	86.667 (9.3) **B**	93.333(9.7) **B**
5%	93.333(9.7) **A**	100 (10) **A**	100 (10) **A**
10%	96.667 (9.8) **A**	100 (10) **A**	100 (10) **A**
Sesame oil	26.667 (5.2) **D**	30 (5.5) D	33.333(5.8) **D**
Engeo 247 SC	100 (10) **A**	100 (10) **A**	100 (10) **A**
Control	0.0 (0.7) **E**	0.0 (0.7) **E**	0.0 (0.7) **E**
SE±	**4.7140**	**3.5635**	**3.0861**
C.V. %	**8.91**	**6.28**	**5.26**
LSD	**10.111**	**7.6429**	**6.6190**

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

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

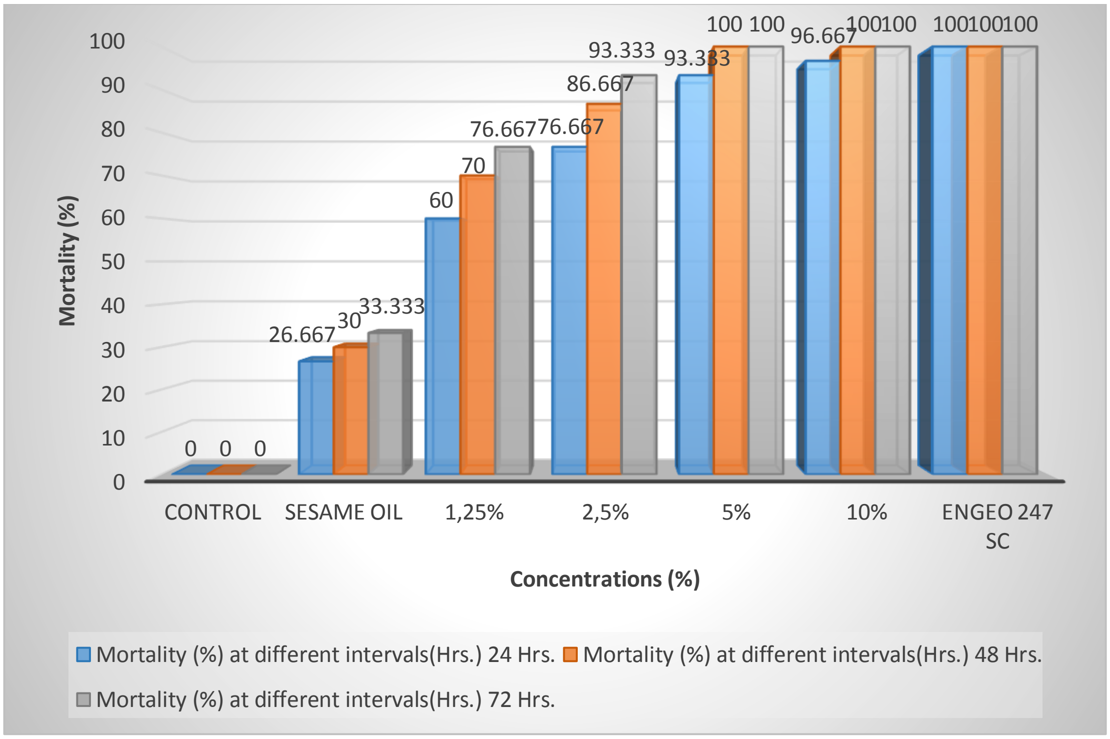


Figure No. (2): Effect of hexane extract of Rehan leaves mixed with sesame oil on the mortality of 3rd larval instars of the spiny bollworm.

As seen in table(3) and figure (3), all tested concentration of Lupine seeds extracts gave a significantly higher mortality percentage than the control, there was a significant difference mortality percentage obtained by the (5%) and (10%) concentration after 24 hrs, 48hrs, and 72 hrs. of exposure. Even the highest concentration gave significantly lower mortality percentage than the standard after 24 hrs, 48hrs, and 72 hrs. of exposure. There was a significant difference in mortality percentage between the two lowest concentrations after 24 hrs, 48hrs, and 72 hrs. of exposure; there was a significant difference in mortality between (2, 5%) and (5%) concentration only after 24 hrs. of exposure, but the difference became insignificant after 48 hrs. and 72 hrs. of exposure.

Table No. (3): Effect of hexane extract of lupine seeds on the mortality of 3rd larval instars of the spiny bollworm.

Concs. (%)	**Mortality (%)**		
	Exposure time (hrs.)		
	24	**48**	**72**
1,25%	20 (4.5) **D**	23.333 (4.9) **D**	26.667(5.2) **D**
2,5%	40 (6.4) **C**	46.667 (6.9) **C**	46.667(6.9) **C**
5%	50 (7.1) **B**	56.667 (7.6) **BC**	56.667(7.6) **BC**
10%	56.667 (7.6) **B**	60 (7.8) **B**	63.333 (8) **B**
Engeo 247 SC	100 (10) **A**	100 (10) **A**	100 (10) **A**
Control	0.0 (0.7) **E**	0.0 (0.7) **E**	0.0 (0.7) **E**
SE±	**3.8490**	**4.714**	**5.0918**
C.V. %	**10.61**	**12.08**	**12.76**
LSD	**8.3863**	**10.271**	**11.094**

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

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

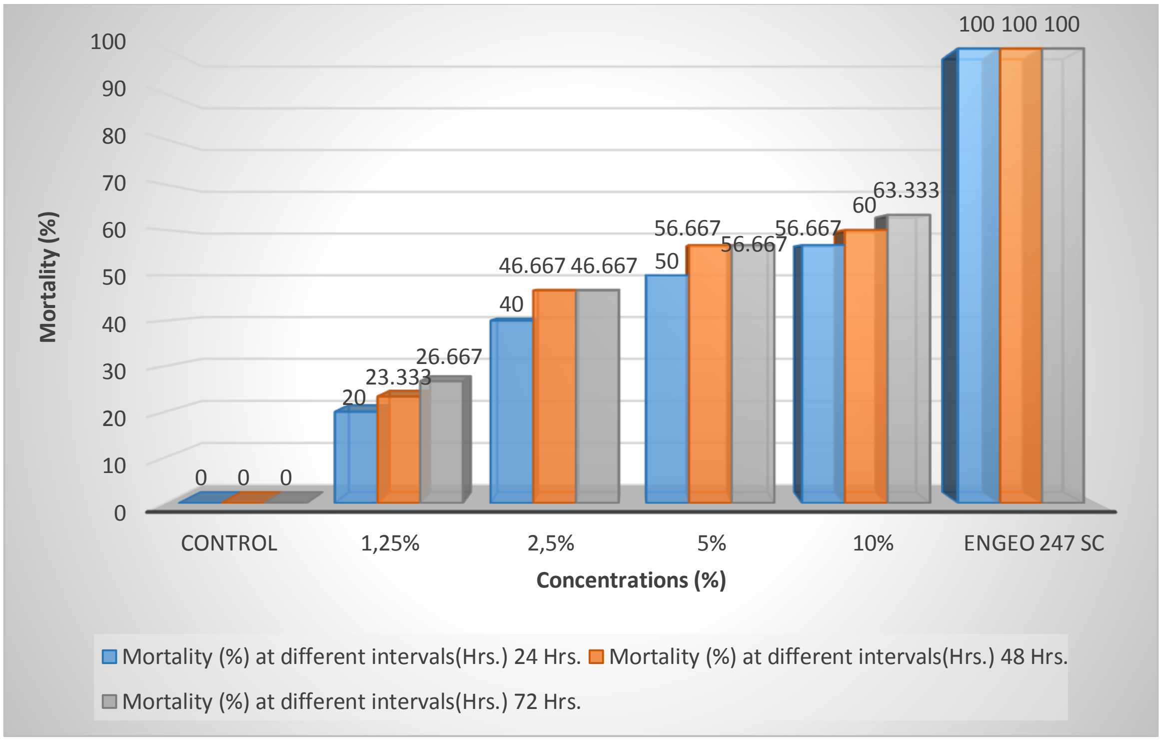


Figure No. (3): Effect of hexane extract of lupine seeds on the mortality of 3rd larval instars of the spiny bollworm.

As seen in table (4) and figure (4), all tested concentration of Lupine seeds extracts mixed with sesame oil gave significantly higher mortality percentage than the control throughout the test period. The (10%) concentration gave significantly lower mortality percentage than the standard after 24 hrs. and 48hrs of exposure but insignificantly different after 72 hrs. of exposure. The results revealed that there was no significant different in mortality between the (5%) and (10%) concentration; However, there was a significant different mortality generated by the (2.5%) and (5%) concentration after 24 hrs, 48hrs, and 72 hrs. of exposure.

Table No. (4): Effect of hexane extract of lupine seeds mixed with sesame oil on the mortality of 3rd larval instars of the spiny bollworm.

Concs. (%)	**Mortality (%)**		
	Exposure time (hrs.)		
	24	**48**	**72**
1,25%	50 (7.1) **D**	56.667 (7.6) **D**	60 (7.8) **C**
2,5%	60 (7.8) **C**	76.667 (8.8) **C**	80 (9) **B**
5%	76.667 (8.9) **B**	90 (9.5) **B**	96.667(9.8) **A**
10%	80 (9) **B**	93.333 (9.7) **B**	100 (10) **A**
Sesame oil	26.667 (5.2) **E**	30 (5.5) **E**	33.333(5.8) D
Engeo 247 SC	100 (10) **A**	100 (10) **A**	100 (10) **A**
Control	0.0 (0.7) **F**	0.0 (0.7) **F**	0.0 (0.7) **E**
SE±	**3.9841**	**3.0861**	**2.5198**
C.V. %	**8.68**	**5.92**	**4.60**
LSD	**8.5450**	**6.6190**	**5.4044**

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

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

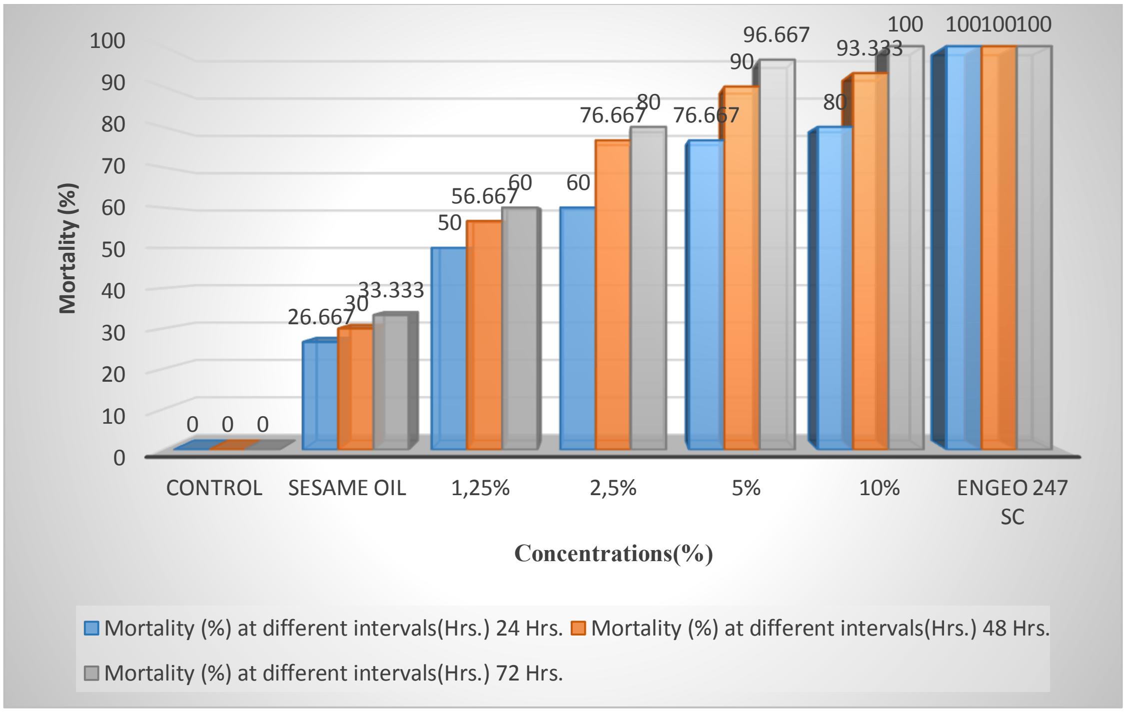


Figure No. (4): Effect of hexane extract of lupine seeds mixed with sesame oil on the mortality of 3rd larval instars of the spiny bollworm.

Table No. (5): LC_{50}values of hexane extract of Rehan leaves and Lupine seeds with and without sesame oil on the mortality of the 3rdinstar larval of spiny bollworm after 24 hours.

Botanical extract	Slope	Intercept	Chi-square	LC_{50} (%)
Rehan leaves	1.775497	4.357391	2.691	2.301
Rehan leaves + Sesame oil	2.084041	4.905483	0.290	1.110
Lupine seeds	1.064620	4.190669	0.822	5.757
Lupine seeds + Sesame oil	1.005870	4.903638	0.343	1.247

Table(5) ,Clearly shows that the Rehan leaves mixed with sesame oil gave the best mortality results after 24 hrs of exposure by scoring the lowest LC_{50} (1.1%);whereas, Lupine seeds were the least effective scoring the highest LC_{50} (5.7%).

Table No. (6): LC_{50}values of hexane extract of Rehan leaves and Lupine seeds with and without sesame oil on the mortality of the 3rdinstar larval of spiny bollworm after48hours.

Botanical extract	Slope	Intercept	Chi-square	LC_{50} (%)
Rehan leaves	2.052567	4.443691	1.287	1.867
Rehan leaves + Sesame oil	2.912925	5.168952	1.151	0.875
Lupine seeds	1.039837	4.339065	1.442	4.321
Lupine seeds + Sesame oil	1.580519	5.059199	0.363	0.917

As seen in table (6), Rehan leaves mixed with sesame oil gave the best mortality at concentration percentage of (0.87%) after 48 Hrs. of exposure. Followed by Lupine seeds mixed with sesame oil at concentration (0.92%).

Table No. (7):LC_{50}values of hexane extract of Rehan leaves and Lupine seeds with and without sesame oil on the mortality of the 3rdinstar larval of spiny bollworm after72 hours.

Botanical extract	Slope	Intercept	Chi-square	LC_{50} (%)
Rehan leaves	2.128634	4.621488	2.568	1.506
Rehan leaves + Sesame oil	3.101917	5.395488	0.319	0.746
Lupine seeds	1.034760	4.386948	0.648	3.913
Lupine seeds + Sesame oil	2.632661	4.933255	0.547	1.060

As seen in table (7), clearly shows that again Rehan leaves mixed with sesame oil gave the lowest LC_{50} (0.75%) being the best of the tested botanicals.

CHAPTER FIVE

DISCUSSION

The spiny boll worm (SBW) *Earias insulana* Boisduval, 1833. (Lepidoptera: Noctuidae), one of the major pests of cotton and okra in oriental tropics, received early and recent investigations by many research workers, particularly in Pakistan (Esmaili *et al*.,1995) , Africa (Johnson,1999) and India (Mirmoayedi,2006).

Use of chemical pesticides has many problems such as negative impacts on non-target organisms including man as well as environmental pollution (Isman, 2006). Additionally, high toxicity, non-biodegradable residues in soil, water resources and crops adversely affect human health (Koul *et al*., 2008). These problems have received great concern from both scientists and public. Among the natural products, plants derived pesticides are more acceptable due to their abundance, nature friendly and short persistence (Isman, 2006).

This study showed that, all tested concentrations of Rehan leaves extracts gave significantly higher mortality percentage than the control. Also all tested concentration of Rehan leaves mixed with sesame oil gave a significantly higher mortality percentage than the control after 24 hrs, 48 hrs. and 72 hrs. of exposure and there was no significant difference in mortality percentage result obtained by the standard, synergized (5%) concentration, and synergized (10%).

The good results obtained by the addition of sesame oil, which is known to suppress mixed function oxidases, to the hexane extract of Rehan leaves and Lupine indicates that the mixed function oxidases (MFO) are responsible for the detoxification of Rehan and Lupine extract.

When sesame oil was added to each concentration of organic hexane extracts of Rehan and Lupine seeds, it gave a synergistic effect. The lowest concentrations (1.25%) of hexane extract of Rehan leaves gave only (76%) mortality after 72 hrs. of exposure .while lupine seeds gave (60%) mortality after 72hrs of exposure. This may indicate that the detoxification mechanism in this insect involves mixed function oxidases (MFO) which are known to be inhibited by sesame oil.

Gewaily (2009) reported that *Hippodamia variegate* mortality increased with increase of time of exposure and dose concentration of Rehan extracts. He also mentioned that Rehan leaves extract had noticeable adverse effect on the development of the African melon ladybird when it was topically applied. He also mentioned increase in the concentration of Rehan resulted in a decrease of the aphids consumed by *H. variegate.* This indicates that Rehan repels the insects *i.e.* has antifeedant effect. Similar results were obtained by Guenther, (1961) who reported that Rehanhad been used in India as an insect repellent besides its medicinal and antibiotic uses.

ALamen (2015) reported that, the egg hatchability of E. *insulana* was reduced by aqueous extracts of the two plants (Jatropha and argel). Mogahed and Abbas (1997) studied the influence of petroleum ether extract of Lupine on some biological aspects of *spodoptera littoralis* under laboratory conditions and found that most concentrations affected the different stages of *spodoptera littoralis*. Also all concentrations affected the egg viability and that hatchability percentage was negatively correlated with the extract concentration. All treated eggs gave significantly lower hatchability than the control. These may be due to the lipophilic properties of acetone extract of lupine which seems to facilitate the penetration of the egg chorine and consequently inhibited the embryonic development. Barakat *et al.,* (1984) found that acetone extract of Lupine is effective against *D. melanogastra* adults.

Mogahed and Abbas (1997) stated that the treatment of the 1st, 2nd and 3rd instars of African boll worm with Lupine extract decreased the mean period of larval duration. He also reported that the mean period of larval duration decreased from 17.1 days in case of untreated larvae to 8.7, 8.8, 10 and 13.1 days for those treated with 1, 2, 3 and 4 % of Lupine extract respectively.

Sathyaseelan and Bhaskaran (2010) found that leaves extract of Rehan gave a very high repellency by (90%) after 48 hrs of release against *Maconellicocus hirsutus* (Green) which is a major pest of mulberry crop. A similar result was obtained by Singh *et al.,* (2012) who reported that Rehan gave (91%) against *Aphis gossypii*. In Nigeria, Inyang and Emosairue (2005) reported that an aqueous solution of Rehan leaves acted as a good repellent and antifeedant against banana weevil *Cosmopolites sordidus* Germar. It is interesting to know that Rehan leaves extract is not phytotoxic according to Oladimeji andKannik (2010).

Zein (2014) reported that, Rehan and Lupine extract were tested as antifeedant agent for *Helicoverpa armigera*. The results of this test showed that all extracts were significantly different and the effects were dose dependent compared to the control. Generally Rehan leaf was very potent as shown by its low feeding ratio. The feeding ratio decreased with increase of concentration and the food deterrence coefficient increased with increase of concentration. Generally Rehan leaves was most effective as shown by its high repulsion percentage .These results are in line with those reported by Sathyaseelan and Bhaskaran (2010) who found the Rehan gave a very high repellency (90.1%) against *Maconellicocus hirsutus* and Singh *et al.,*

(2012) who found that *Ocimum basilicum* have repellency of (91%)against *Aphis gossypii*. This may be due to the fact that Rehan leaves contain phenolic compounds in its constituents like linalool, charvicol, eugenol and esragol. In other studies, its oil was reported to be repellent to garden pests (Quarles, 1999).

The powder of Rehan leaves proved to be effective against some store pest. In fact the treatment of faba bean seeds beetle with Rehan powder and crude extracts significantly reduced the fecundity and also the daily oviposition rate when compared with the control (Ibrahim, 2007).

CONCLUSION AND RECOMMENDATIONS

Cost of pests control and environmental hazards have been among the major problems constraining the use of chemical insecticides. Many botanical formulations have proven to be as 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.

The insecticidal effect of hexane extracts of Rehan leaves and lupine seeds was found to be very toxic against 3rdlarval instars of spiny boll worm. It gave a higher mortality percentage of 3rdlarval instars of spiny boll worm when extracts was mixed with sesame oil as a synergist than when it was used alone.

The lowest concentrations (1.25%) of hexane extracts of Rehan leaves gave (46.67%) mortality percentage after 72 hrs. of exposure, but when the extract was mixed with sesame oil the mortality increased to (76.67%). Similarly, the lowest concentration of hexane extract of Lupine seeds gave (26.67%), but when it was synergized with sesame oil the mortality percentage increased to (60%), indicating that the mixed function oxidases (MFO) are involved in the detoxification of Rehan and Lupine extracts.

Based on the result of this study we may suggest the following recommendations:

1- Sesame oil, which is available in Sudan, could be used as a synergist to improve the insecticidal property of plant extracts.

2- The use of hexane extracts of Rehan leaves and Lupine seeds synergized with sesame oil need further investigation to study their insecticidal properties under field condition against spiny boll worm and other insect pest.
3- Rehan leaves and lupine seeds hexane extracts could be used for combating the spiny boll worm practically by small scale.
4- Further sophisticated studies including fractionation and isolation of active ingredients responsible for the insecticidal properties in Rehan and Lupine need to be conducted. Hence, the isolation and identification of active ingredient was considered as pre-request steps for botanical insecticides formulation.

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APPENDICES

Appendix No. (1): Effect of hexane extract of Rehan leaves on the mortality of 3rd larval instars of the spiny bollworm after 24 hrs.

Concs. (%)	Mortality (%)			
	R1	R2	R3	Mean
1,25%	30 (5.5)	40 (6.4)	40 (6.4)	36.667 (6.1)
2,5%	50 (7.1)	50 (7.1)	50 (7.1)	50.0 (7.1)
5%	70 (8.4)	60 (7.8)	60 (7.8)	63.333 (8)
10%	100 (10)	90 (9.5)	90 (9.5)	93.333 (9.7)
Engeo 247 SC	100 (10)	100 (10)	100 (10)	100 (10)
Control	0.0 (0.7)	0.0 (0.7)	0,0 (0.7)	0.0 (0.7)

Appendix No. (2): Effect of hexane extract of Rehan leaves on the mortality of 3rd larval instars of the spiny bollworm after 48 hrs.

Concs.(%)	Mortality (%)			
	R1	R2	R3	Mean
1,25%	30 (5.5)	40 (6.4)	50 (7.1)	40 (6.4)
2,5%	50 (7.1)	70 (8.4)	50 (7.1)	56.667 (7.5)
5%	80 (9)	80 (9)	70 (8.4)	76.667 (8.8)
10%	100 (10)	100 (10)	90 (9.5)	96.667(9.8)
Engeo 247 SC	100 (10)	100 (10)	100 (10)	100 (10)
Control	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)

Appendix No. (3): Effect of hexane extract of Rehan leaves on the mortality of 3rd larval instars of the spiny bollworm after 72 hrs.

Concs. (%)	Mortality (%)			
	R1	R2	R3	Mean
1,25%	30 (5.5)	50 (7.1)	60 (7.8)	46.667 (6.9)
2,5%	60 (7.8)	70 (8.4)	70 (8.4)	66.667 (8.2)
5%	80 (9)	80 (9)	80 (9)	80 (9)
10%	100 (10)	100 (10)	100 (10)	100 (10)
Engeo 247 SC	100 (10)	100 (10)	100 (10)	100 (10)
Control	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)

Appendix No. (4): Effect of hexane extract of Rehan leaves mixed with sesame oil on the mortality of 3rd larval instars of the spiny bollworm after 24 hrs.

Concs. (%)	**Mortality (%)**			
	R1	**R2**	**R3**	**Mean**
1,25%	60 (7.8)	60 (7.8)	60 (7.8)	60 (7.8)
2,5%	70 (8.4)	80 (9)	80 (9)	76.667 (8.8)
5%	100 (10)	80 (9)	100 (10)	93.333(9.7)
10%	100 (10)	90 (9.5)	100 (10)	96.667 (9.8)
Sesame oil	30 (5.5)	20 (4.5)	30 (5.5)	26.667 (5.2)
Engeo 247 SC	100 (10)	100 (10)	100 (10)	100 (10)
Control	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)

Appendix No. (5): Effect of hexane extract of Rehan leaves mixed with sesame oil on the mortality of 3rd larval instars of the spiny bollworm after 48 hrs

Concs.(%)	**Mortality (%)**			
	R1	**R2**	**R3**	**Mean**
1,25%	70 (8.4)	80 (9)	60 (7.8)	70 (8.4)
2,5%	80 (9)	90 (9.5)	90 (9.5)	86.667 (9.3)
5%	100 (10)	100 (10)	100 (10)	100 (10)
10%	100 (10)	100 (10)	100 (10)	100 (10)
Sesame oil	30 (5.5)	30 (5.5)	30 (5.5)	30 (5.5)
Engeo 247 SC	100 (10)	100 (10)	100 (10)	100 (10)
Control	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)

Appendix No. (6): Effect of hexane extract of Rehan leaves mixed with sesame oil on the mortality of 3rd larval instars of the spiny bollworm after 72 hrs

Concs. (%)	**Mortality (%)**			
	R1	**R2**	**R3**	**Mean**
1,25%	70 (8.4)	80 (9)	80 (9)	76.667 (8.8)
2,5%	90 (9.5)	90 (9.5)	100 (10)	93.333 (9.7)
5%	100 (10)	100 (10)	100 (10)	100 (10)
10%	100 (10)	100 (10)	100 (10)	100 (10)
Sesame oil	30 (5.5)	30 (5.5)	406.4)	33.333 (5.8)
Engeo 247 SC	100 (10)	100 (10)	100 (10)	100 (10)
Control	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)

Appendix No. (7): Effect of hexane extract of lupine seeds on the mortality of 3rd larval instars of the spiny boll worm after 24 hrs.

Concs. (%)	Mortality (%)			
	R1	R2	R3	Mean
1,25%	10 (3.2)	20 (4.5)	30 (5.5)	20 (4.5)
2,5%	40 (6.4)	40 (6.4)	40 (6.4)	40 (6.4)
5%	50 (7.1)	50 (7.1)	50 (7.1)	50 (7.1)
10%	50 (7.1)	60 (7.8)	60 (7.8)	56.667 (7.6)
Engeo 247 SC	100 (10)	100 (10)	100 (10)	100 (10)
Control	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)

Appendix No. (8): Effect of hexane extract of lupine seeds on the mortality of 3rd larval instars of the spiny boll worm after 48 hrs.

Concs.(%)	Mortality (%)			
	R1	R2	R3	Mean
1,25%	20 (4.5)	20 (4.5)	30 (5.5)	23.333 (4.9)
2,5%	40 (6.4)	40 (6.4)	60 (7.8)	46.667 (6.9)
5%	50 (7.1)	60 (7.8)	60 (7.8)	56.667 (7.6)
10%	60 (7.8)	60 (7.8)	60 (7.8)	60 (7.8)
Engeo 247 SC	100 (10)	100 (10)	100 (10)	100 (10)
Control	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)

Appendix No.(9): Effect of hexane extract of lupine seeds on the mortality of 3rd larval instars of the spiny boll worm after 72 hrs.

Concs. (%)	Mortality (%)			
	R1	R2	R3	Mean
1,25%	30 (5.5)	20 (4.5)	30 (5.5)	26.667 (5.2)
2,5%	40 (6.4)	40 (6.4)	60 (7.8)	46.667 (6.9)
5%	50 (7.1)	60 (7.8)	60 (7.8)	56.667 (7.6)
10%	60 (7.8)	60 (7.8)	70 (8.4)	63.333 (8)
Engeo 247 SC	100 (10)	100 (10)	100 (10)	100 (10)
Control	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)

Appendix No.(10): Effect of hexane extract of lupine seeds mixed with sesame oil on the mortality of 3rd larval instars of the spiny bollworm after 24 hrs.

Concs. (%)	Mortality (%)			
	R1	R2	R3	Mean
1,25%	60 (7.8)	50 (7.1)	40 (6.4)	50 (7.1)
2,5%	60 (7.8)	60 (7.8)	60 (7.8)	60 (7.8)
5%	80 (9)	80 (9)	70 (8.4)	76.667 (8.9)
10%	80 (9)	80 (9)	80 (9)	80 (9)
Sesame oil	30 (5.5)	20 (4.5)	30 (5.5)	26.667 (5.2)
Engeo 247 SC	100 (10)	100 (10)	100 (10)	100 (10)
Control	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)

Appendix No. (11): Effect of hexane extract of lupine seeds mixed with sesame oil on the mortality of 3rd larval instars of the spiny bollworm after 48 hrs.

Concs.(%)	Mortality (%)			
	R1	R2	R3	Mean
1,25%	60 (7.8)	50 (7.1)	60 (7.8)	56.667 (7.6)
2,5%	70 (8.4)	80 (9)	80 (9)	76.667 (8.8)
5%	90 (9.5)	90 (9.5)	90 (9.5)	90 (9.5)
10%	100 (10)	90 (9.5)	90 (9.5)	93.333 (9.7)
Sesame oil	30 (5.5)	30 (5.5)	30 (5.5)	30 (5.5)
Engeo 247 SC	100 (10)	100 (10)	100 (10)	100 (10)
Control	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)

Appendix No. (12): Effect of hexane extract of lupine seeds mixed with sesame oil on the mortality of 3rd larval instars of the spiny bollworm after 72 hrs.

Concs. (%)	Mortality (%)			
	R1	R2	R3	Mean
1,25%	60 (7.8)	60 (7.8)	60 (7.8)	60 (7.8)
2,5%	80 (9)	80 (9)	80 (9)	80 (9)
5%	100 (10)	90 (9.5)	100 (10)	96.667 (9.8)
10%	100 (10)	100 (10)	100 (10)	100 (10)
Sesame oil	30 (5.5)	30 (5.5)	40 (6.4)	33.333 (5.8)
Engeo 247 SC	100 (10)	100 (10)	100 (10)	100 (10)
Control	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)

Appendix No. (13): Mortality of spiny boll worm larvae treated with hexane extract of Rehan leaves after 24 hrs.

Concs. (%)	Mortality (%)			
	R1	R2	R3	Tot.
1,25%	3	4	4	11
2,5%	5	5	5	15
5%	7	6	6	19
10%	10	9	9	28
Engeo 247 SC	10	10	10	30
Control	0	0	0	0

Appendix No. (14): Mortality of spiny boll worm larvae treated with hexane extract of Rehan leaves after 48hrs.

Concs.(%)	Mortality (%)			
	R1	R2	R3	Tot.
1,25%	3	4	5	12
2,5%	5	7	5	17
5%	8	8	7	23
10%	10	10	9	29
Engeo 247 SC	10	10	10	30
Control	0	0	0	0

Appendix No. (15): Mortality of spiny boll worm larvae treated with hexane extract of Rehan leaves after 72 hrs.

Concs. (%)	Mortality (%)			
	R1	R2	R3	Tot.
1,25%	3	5	6	14
2,5%	6	7	7	20
5%	8	8	8	24
10%	10	10	10	30
Engeo 247 SC	10	10	10	30
Control	0	0	0	0

Appendix No. (16): Mortality of spiny boll worm larvae treated with hexane extract of Rehan leaves mixed with sesame oil after 24 hrs.

Concs. (%)	**Mortality (%)**			
	R1	**R2**	**R3**	**Tot.**
1,25%	6	6	6	18
2,5%	7	8	8	23
5%	10	8	10	28
10%	10	9	10	29
Sesame oil	3	2	3	8
Engeo 247 SC	10	10	10	30
Control	0	0	0	0

Appendix No. (17): Mortality of spiny boll worm larvae treated with hexane extract of Rehan leaves mixed with sesame oil after 48hrs.

Concs.(%)	**Mortality (%)**			
	R1	**R2**	**R3**	**Tot.**
1,25%	7	8	6	21
2,5%	8	9	9	26
5%	10	10	10	30
10%	10	10	10	30
Sesame oil	3	3	3	9
Engeo 247 SC	10	10	10	30
Control	0	0	0	0

Appendix No. (18): Mortality of spiny boll worm larvae treated with hexane extract of Rehan leaves mixed with sesame oil after 72hrs.

Concs. (%)	**Mortality (%)**			
	R1	**R2**	**R3**	**Tot.**
1,25%	7	8	8	23
2,5%	9	9	10	28
5%	10	10	10	30
10%	10	10	10	30
Sesame oil	3	3	4	10
Engeo 247 SC	10	10	10	30
Control	0	0	0	0

Appendix No.(19): Mortality of spiny boll worm larvae treated with hexane extract of lupine seeds after 24 hrs.

Concs. (%)	**Mortality (%)**			
	R1	**R2**	**R3**	**Tot.**
1,25%	1	2	3	6
2,5%	4	4	4	12
5%	5	5	5	15
10%	5	6	6	17
Engeo 247 SC	10	10	10	30
Control	0	0	0	0

Appendix No.(20): Mortality of spiny boll worm larvae treated with hexane extract of lupine after 48 hrs.

Concs.(%)	**Mortality (%)**			
	R1	**R2**	**R3**	**Tot.**
1,25%	2	2	3	7
2,5%	4	4	6	14
5%	5	6	6	17
10%	6	6	6	18
Engeo 247 SC	10	10	10	30
Control	0	0	0	0

Appendix No.(21): Mortality of spiny boll worm larvae treated with hexane extract of lupine seeds after 72 hrs.

Concs. (%)	**Mortality (%)**			
	R1	**R2**	**R3**	**Tot.**
1,25%	3	2	3	8
2,5%	4	4	6	14
5%	5	6	6	17
10%	6	6	7	19
Engeo 247 SC	10	10	10	30
Control	0	0	0	0

Appendix No.(22): Mortality of spiny boll worm larvae treated with hexane extract of lupine seeds mixed with sesame oil after 24hrs.

Concs. (%)	**Mortality (%)**			
	R1	**R2**	**R3**	**Tot.**
1,25%	6	5	4	15
2,5%	6	6	6	18
5%	8	8	7	23
10%	8	8	8	24
Sesame oil	3	2	3	8
Engeo 247 SC	10	10	10	30
Control	0	0	0	0

Appendix No.(23): Mortality of spiny boll worm larvae treated with hexane extract of lupine seeds mixed with sesame oil after 48hrs.

Concs.(%)	**Mortality (%)**			
	R1	**R2**	**R3**	**Tot.**
1,25%	6	5	6	17
2,5%	7	8	8	23
5%	9	9	9	27
10%	10	9	9	28
Sesame oil	3	3	3	9
Engeo 247 SC	10	10	10	30
Control	0	0	0	0

Appendix No.(24): Mortality of spiny boll worm larvae treated with hexane extract of lupine seeds mixed with sesame oil after 72hrs.

Concs. (%)	**Mortality (%)**			
	R1	**R2**	**R3**	**Tot.**
1,25%	6	6	6	18
2,5%	8	8	8	24
5%	10	9	10	29
10%	10	10	10	30
Sesame oil	3	3	4	10
Engeo 247 SC	10	10	10	30
Control	0	0	0	0