



بسم الله الرحمن الرحيم

Sudan University of Science and Technology

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Effect of NeemAzal- T/S against the larvae of the Spiny bollworm,

***Earias insulana* (Boisd.) (Lepidoptera: Noctuidae).**

أثر مبيد النيمازال على يرقات ديدان اللوز الشوكية (المصرية).

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for the degree of B.Sc. in Plant Protection.

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الآية

بسم الله الرحمن الرحيم

(وَاضْرِبْ لَهُمْ مَثَلَ الْحَيَاةِ الدُّنْيَا كَمَاءٍ أَنْزَلْنَاهُ مِنَ السَّمَاءِ فَاخْتَلَطَ بِهِ نَبَاتُ الْأَرْضِ فَأَصْبَحَ هَشِيمًا تَذْرُوهُ الرِّيَّاحُ ۚ وَكَانَ اللَّهُ عَلَى كُلِّ شَيْءٍ مُقْتَدِرًا)

صدق الله العظيم

سورة الكهف الآية (45)

DEDICATION

To all those who encouraged and guided me in my life

To my family

To my dear parents,

To my brothers and sister,

To my teachers,

And to all my dear friends and colleagues

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All my thanks and prays to “Allah” , who gave me strength and Patience to complete this research.

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Abstract

Experiments were conducted in the laboratory of Entomology and Zoology, Department of Plant Protection, College of Agricultural Studies (Shambat), Sudan University of Science and Technology (SUST) in the period of April-June, 2016. The aim of this study was to examine the effectiveness of (NeemAzal-T\S) against the larvae of Spiny boll worms *Earias insulana* (Boisd.).

Four concentrations of NeemAzal- T/S were used (**4, 2, 1 and 0.5 ml/liter**) the results that the exterminator four concentrations negatively affect the mortality ratio in the larvae; the results showed a significant difference in the mortality percentage between different concentrations were as follows (respectively **30%, 23.33%, 16.66%, and 16.66%**) After two hours of treatment and (**86.66%, 73.33% 38.66%, 23.33%**) After 24 hours of treatment and (**90% 83.33%, 73.33%, 63.33%**) After 36 hours of treatment and (**100%, 96.66%, 93.33%, 90%**) After 48 hours of treatment, respectively, compared with the control (**0%**). Additionally note that high repellent to the larvae

ملخص البحث

أجريت التجارب بمعمل الحشرات والحيوان الزراعي بقسم وقاية النبات، كلية الدراسات الزراعية - شمبات، جامعة السودان للعلوم والتكنولوجيا في الفترة من ابريل - مايو 2016م
الهدف من الدراسة هو اختبار أثر وفعالية مبيد (NeemAzal-T\S) على يرقات ديدان اللوز الشوكية

Earias insulana (Boisd.)

حيث تم إستخدام أربعة تراكيزات من المبيد هي (4 ، 2 ، 1 و 0.5 مل /لتر).
أوضحت النتائج ان المبيد بتركيزاته الأربعة يؤثر سلباً على نسبة الموت في طور اليرقي لديدان اللوز الشوكية (المصرية) وأظهرت النتائج إختلافاً واضحاً في نسب الموت بين التركيزات المختلفة وكانت كالتالي (30%، 23.33%، 16.66%، 16.66%) بعد ساعتين من المعاملة و (86.6%، 73.33%، 38.6%، 23.33%) بعد 24 ساعة من المعاملة و (90%، 83.33%، 73.33%، 63.33%) بعد 36 ساعة من المعاملة و (100%، 96.66%، 93.33%، 90%) بعد 48 ساعة من المعاملة على الترتيب مقارنة مع الشاهد (0%). بالإضافة الى ذلك نلاحظ أن التأثير الطارد للمبيد علي اليرقات عالي.

CHAPTER ONE

INTRODUCTION

Earias insulana (Boisd.) is a major insect pest of cotton and okra (Ismail *et al.*, 2005). The genus *Earias* occurs in Africa , two species *E. insulana* (Boised) and *E. biplaga*(WIK) have been identified. Both species are being commonly known as the spiny bollworm, the name being derived from the characteristic bristles or spines which are formed on the larva. *E. insulana* is a very important pest in Egypt and is therefore, also known as the Egyptian bollworm ((Ripper and George, 1965).The mitral damage to the cotton crop by the spiny bollworm occurs in the early stages of the plant growth, as the cotton plant grows and produces flower buds and unripe fruit, in Egypt. Insecticides are being used for controlling bollworms, insecticides can provide economical protection by killing insect pests that otherwise would cause significant loss, Nevertheless, the extensive use of insecticides in cotton field has seriously affected the population densities of the natural enemies (Yang *et al.*, 2002 and Younis *et al.*, 2007). Moreover the wide spread application of pesticides might accelerate some cotton pests, mostly insects and mites to develop resistance to certain pesticides (Elzen and Hardee, 2003).

The large scale use of pesticides in agricultural areas, forests, and human habitations results in environmental and human health hazards by polluting food, water, soil, and air. Pesticides affect non- target organisms such as beneficial insects (parasites and predators), aquatic and soil microorganisms, fish, birds and wild life (Abdalla, 1986). This situation has led to the search for other alternatives methods of control, particularly those of plant origin to reduce the heavy using of chemical insecticides and alleviate their hazards (Fernandez and Montagne ,1990). Natural pesticides are good alternative to synthetic pesticides because they are safe

to environment, natural enemies, humans and other animals, *e.g.* most botanical pesticides have low to moderate mammalian toxicity, therefore it can be applied by farmers and small scale industries with little cost (Hassan, 1992 and Georges *et al.*, 2008).

The use of *Azadirachta indica* as a source of natural insecticide was discovered approximately 30 years ago (Ascher, 1993). Pest control using extracts from the Neem tree is currently practiced in more than 25 countries through the world Neem precuts have been in use in parts of Asia such as Burma and India for over 2,500 years (Stoll, 2000).

The objective of this study is to investigate through laboratory screening the activity of NeemAzal-T/S against the larvae of Spiny bollworm (*Earias insulana* (Boisd.)).

CHAPTER TWO

LITERATURE REVIEW

2.1 The spiny boll worm

2.1.1 Scientific classification

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Lepidoptera
Family: Nocutidae
Genus: *Earias*
Species: *insulana*

Binomial name: *Earias insulana* (Boisduval, 1833)

There are seven *Earias* spp. globally, but only two of these species occur in South Africa, namely *Earias insulana*(Boisduval) and *E. biplaga* (Walker) (Bennett, 2015). The spiny bollworm forms part of the bollworm complex in South Africa that consist of three different species of lepidopteran pests, namely the red bollworm *Diparopsis castanea* (Hampson), African bollworm *Helicoverpa armigera* (Hübner) and spiny bollworm the *Earias* spp. (Bennett, 2015). These pests are common in the cotton growing regions, and the damage caused by the spiny bollworm is often under estimated (Bennett, 2015).

There are very few differences between the larvae of *E. insulana* and *E. biplaga*. Pearson and Darling (1958) speculated that the larvae with an orange-brown appearance are *E. biplaga* and the yellower-green larvae, *E. insulana*. The pattern of the forewing during the adult stage is a better indicator for identification of these species (Bennett, 2015). The colour of the outer fringe of the wing of *E. insulana* is the same as the rest of the wing and may vary from silver green to yellow (Bennett, 2015). The outer fringe of the wings of *E. biplaga* is a darker brown. The colour of the wings may vary from metallic green to yellow (Bennett, 2015). Only the colour of the fringe and not colour variation of the rest of the wings can be used for identification purposes

2.1.2 Geographical distribution

The genus *Earias* is confined to the old world and Australia. *E. insulana* has an extremely wide range, covering most of Africa and including Madagascar, Mauritius and the Canary Isles. It extends northwards to the Mediterranean islands and southern Europe and eastwards through the near and middle East, including Southern Arabia to India and Southeast Asia in Japan, Taiwan, the Philippines, and Hawaii (Pearson, 1958; Schumutterer, 1969 and Hill, 1981). Also found in Syria

(Stam and Al-Mosa, 1990), Israel (Avidou and Harpaz, 1969) and Turkey (Ünlü, 2004). It is a rare immigrant in Great Britain.

In Sudan, the Spiny bollworm *E. insulana* was reported as an important pest of cotton as early as 1908 in Zeidab Scheme Northern Sudan (King, 1908).

2.1.3 Morphology

E. insulana adult moth is characterized by wing span of 16-22 mm and body length of 8-12 mm. head and thorax are green, yellow forewings with pale green, sometimes yellowish or brown, with a diagonal green stripe (Schumutterer, 1969 and Klein, 1988). The hind wings are dull white with a brown sub terminal line.

Egg is bluish-green, globular, 0.5-0.6 mm in diameter. The larva is initially grey, later grey-blue with yellow spots, its dorsum with small tubercles bearing short hairs. The head is dark and shiny and the final length is 15-18 mm. Pupa 12-14 mm long, dark brown and around at both ends (Schumutterer, 1969 and Dahi, 2012)..

2.1.4 Host plants

Earias insulana (Boisduval) (Lepidoptera: Noctuidae) is one of the most common pests that attack okra *Abelmos esculentus* (Malvales: Malvaceae) in Egypt (Kandil, 2013), cotton *Gossypium hirsutum* (L) (Malvales: Malvaceae) in Pakistan (Shah *et al.*, 2012), South Africa (Bennett, 2015) and India (Venilla, 2007) as well as other Malvaceae plant families (Kandil, 2013; Venilla, 2007). *Earias* species were responsible for more than 10% of damage on cotton in India during the 2005 growing season (Venilla, 2007).

The spiny bollworm is oligophagous (Dahi, 2012). The Main hosts are Cotton, okra and alternative species of *Hibiscus* and *Abutilon* and other Malvaceae. It can also feed on cocoa and a few members of the Tiliaceae and Sterculiaceae. In Egypt was found on Maize (Pearson, 1958 and Yathom, 1965). Both *E. insulana* (Boisd.)

and *E. vittella* (F.) is early bollworm pest of cotton and their alternate hosts are members of the malvales (Depury, 1968). In Sudan they are more found to attack crops such as kenaf *Hibiscus cannabinus* (Bedford, 1931), Bamia *H. esculentus* (Joyce, 1953), Karkadi *H.sabdarifa* (Abdel Rahman, 1967) and *Abutilon* spp. (Eltayeb, 1976). Arif and Attique (1990) reported that incidence of *E. vittella* (F.) was higher on cotton, okra and kenaf, while *E. insulana* (Boisd.) was more on ornamental plants China-rose, cotton-rose, weeds and *Abutilon*. Similar results have also been reported by (Khan, 1941). Host preference studies of *E. vittella* showed that it is major and serious pest of cotton *Gossypium* spp. In India it also attacks a few other Malvaceous plants such as *H. esculentus*, *Abuliton indicum*, *Althea rosa*, *Sidacordiflora* and *Urena* spp. (Kranz *et al.*, 1977).

2.1.5 Biology and life cycle

2.1.5.1. Eggs

E. insulana female lays several hundred eggs singly, placed on the flower buds, fruits or in the leaf axils (Dudgen, 1916). The incubation period lasts for about 3-4 days under the conditions of the rainy season in the central Sudan and is somewhat prolonged in winter (Schumutterer, 1969). As for *E. vittella* a single female lays 63-697 eggs during the oviposition period, the incubation period varies from 3-4 days in summer to 5-7 days in winter (Kranz *et al.*, 1977).

Both *E. insulana* and *E.vittella* eggs were found to be laid at night in the breeding cages. Eggs were deposited singly or in small groups between (4-8) on the lower surface of the leaves. The hatching of the eggs occurred during the day. The average of incubation period was 4.00 ± 0.00 days (Mursal, 2005). In the summer the incubation period of the egg is about three days.

2.1.5.2 Larvae

The larvae of the first generation bore into terminal cotton buds, those of later generations into flower buds, flowers and newly-set bolls. Larval development can take 3 weeks during summer, require twice as long at 19°C. The mature larva spins a white cocoon, attaches it to plant parts. The pest develops all year around in the Eastern Mediterranean region, without a winter diapause. During winter it lives on cultivated shrubs like *Hibiscus* spp. It may rise up to six annual generations in the Middle East, one per month in summer; the size of its populations is restricted by the availability of various host plants, especially cotton (Horowitz *et al.*, 1992).

The duration of the larval development of *E. insulana* from egg hatching to pupation lasted from 10 to 13 days with an average of 11.20 ± 1.22 . The duration of the four larval instars was 4-5 days, while other instars need only about 2-3 days (Mursal, 2005).

2.1.5.3 Pupae

Before pupation the fully matured larva stopped feeding and wandered to settle in a place for pupation. During the pupation, old larva began to form a whitish colour of a silk cocoon on the upper side or under the lower side of the filter paper within the Petri-dishes and at the roof of the dish or on food remains surface. The fully weaved cocoon was made in a form of an inverted boat with a weak exit side at its ends. The pupation period of *E. insulana* was ranged between 10 to 12 days with an average of 10.90 ± 0.56 days (Mursal, 2005).

2.1.5.4 Adults

In the laboratory, adults were found outside their pupal cases always in the morning. This indicates their emergence during night or early morning time. Some were seen with wings not yet fully stretched and kept courted over their backs. Feeding, mating and oviposition were not observed at day-time and these activities

were practiced at night time (Mursal, 2005). The per-oviposition period is about the same as the incubation period, under optimum condition, and then a generation lasts about five weeks. At 28°C, egg incubation takes 3days, the moth lay up to 300 eggs no diapauses have been recorded, larvae about 9days the four larval instars were 4-5 day and the pupa 9days, and (Horowitz *et al.*, 1992 and Mursal, 2005).

2.1.6 Ecology

The insect was found to occur in high population during rainy season and its number drop in summer as the temperature increases. The development period of different stages prolonged during winter, the longevity fecundity and coloration of the adult also fluctuate with environmental temperature and humidity (Schumutterer, 1977). The length of the life cycle of *Earias insulana* depends on the temperature and thus varies with the season. There is no diapause, though development is retarded in cold weather. *E. insulana* tolerates a wide variety of climatic conditions, but it does not adapt well to damp conditions. The seasonal colour polymorphism, which appear to be determined by climate acting on the pupal stage. A typical form occurs with optimum temperature and humidity, and colour variations occur when the conditions vary from the optimum (Couilloud, 1983). Field experiments were carried out in India to determine the most favourable climatic conditions for the development of *E. insulana* on cotton. The optimum relative humidity was 65-85%. The most favourable conditions for rapid multiplication were warm, but not excessively hot, weather, cloudiness and frequent light rain (Katiyar, 1982). Field studies in Egypt showed that population fluctuations were apparently not related to the prevailing temperature (Nasr *et al.*, 1980). In contrast, Balasubramanian *et al.* (1981) found significant positive correlations between the incidence of *E. insulana* and the maximum temperature

and hours of sunshine. Significant negative correlations were found between pest incidence and minimum temperature, morning relative humidity, evening relative humidity, intensity of rainfall and number of rainy days. *E. insulana* is more resistant to low temperatures than most other members of the genus. However, Heidari *et al.* (1981) studied *E. insulana* in the field in northern Iran and found that temperatures of 2 or 3°C killed the larvae as well as effectively wiping out their food source. After a very hard winter, the pest virtually disappeared from cotton and other food plants in the following summer.

2.1.7 Damage and economic importance

The larvae feed on okra, cotton and hibiscus, but have also been recorded on rice, sugarcane and corn. Initially, larvae tunnel into the buds of their host plant. Later, the larva feeds on the bolls, which become brown and fall off. Secondary invasion by fungi and bacteria sometimes occurs. Full-grown larvae are 13–18 mm long; wingspan is generally approx. 24–28 mm (Dahi, 2012). The larvae feed on several parts of the infested plants, in the absence of flower and bolls, also they penetrate the young tender shoots of young cotton plant and hollow them out, the attacked shoots wither and die, on Okra the capsules are mainly attacked, the damage caused to cotton and Okra in the Gezira Area of the Sudan is up to now of minor economics (Kandil mervat, 2013). This pest is most damaging on irrigated cotton, the larva moves about considerably, not confining its feeding to a single boll and often not completely eating out the interior of the bud or boll, the damage may thus be disproportionate to the number, only unripe fruit is attacked the delayed crops are often totally destroyed (Pearson, 1958 and Yathom, 1965).

2.1.8 Symptoms

The symptoms of attack are similar for all *Earias spp.* Cotton infestation generally starts with shoot boring in the young crop. *E. insulana* enters the terminal bud of

the vegetative shoot and channels downwards from the growing point, or directly penetrates the internode. Only soft growing tissue is attacked. Extensive tunnelling results in wilting of the top leaves and the collapse of the apex of the main stem. The whole apex turns blackish-brown and dies. The result is bunched growth in young plants and death of the growing point in the mature plant. If only the apical bud is attacked, the damage may not be noticed until the main stem divides (twinning) when the auxiliary buds take over growth (Kashyap and Verma, 1987 and Reed, 1994). The larvae tend to move from a boll to another in the same plant or even in neighbouring plants and usually bore deeply, filling the tunnel opening with excrement. The tunnel often enters the bolls from below, at a slight angle to the peduncle. Small bolls, up to 1 week old, turn brown, rot and drop. Larger bolls 2-4 weeks old may not drop but open prematurely and may be so badly damaged they cannot be harvested (Pearson, 1958).

2.1.9 Control

2.1.9.1 Cultural control

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 (Melifronides *et al.*, 1978). Eradication of alternative host plants has also been attempted, but is of doubtful benefit because many are valuable sources of food, feed or fibre and their removal may reduce the pool of natural enemies. Kashyap and Verma (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. Nasr and Azab (1969) claimed that the removal of the topmost few centimetres 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 fibre. Other suggested cultural practices include deep ploughing (Faseli, 1977) and close spacing of plants (Abdel Fatah *et al.*, 1980). High doses of nitrogen fertilizers have been found to increase infestation (Reed, 1994). Singh *et al.* (1997) suggest 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 (Bishara, 1969; Ilango and Uthamasamy, 1989 and Abdalla, 1991).

2.1.9. 2 Host plant resistance

Host plant resistance should also be considered when choosing a new cultivar for Production. Three different plant resistance concepts can be described.

□ Antibiosis can be described as the resistance mechanism that involved characteristics of a plant that have a negative effect on the insect survival (Manglitz & Danielson, 1992). This can influence mortality in insect pests and may reduce their longevity and reproduction (Manglitz & Danielson, 1992; Teetes, 2009). It can be caused by chemicals produced by the plant, that for example can reduce growth in insect pests or this can be because of structures produced by the plant such as trichomes that prevent an insect to harm the plant (Chadwell *et al.*, 2005).

□ Antixenosis or non-preference is a resistance mechanism that influences an

insect's behaviour (Manglitz & Danielson, 1992).

□ Tolerance describes the plant's response to damage (Teetes, 2009) and represents how the plant grows, its reproduction and repairing ability to damage caused by insects or other herbivores (Manglitz & Danielson, 1992).

Resistance is one of the eco-friendly and hazardless methods of pest control. A number of genetic characters are now available to cotton breeders for insect resistance such as nectar less, high gossypol, hairiness and etc. (Wilson and George, 1982; Jenkins, 1986 and El Zik and Thaxton, 1989).

2.1.9.3 Biological control

Twenty seven species of parasitoids have been reported for the spiny and the spotted bollworm, (Greatehead, 1966). He also reported 44 species of natural enemies for Africa such as *Agathis aciculate* (Brues), *Nelelia parvulus* (Szep.) and *Tachinidae*. Releases of *Trichogramma* spp. can be tried for the control of *E. insulana* and *E. vittella* in their egg stage (Mohyuddin, 1991).

2.1.9.4 Chemical control

The use of chemicals to control bollworms should be done before larvae tunnel into the fruiting bodies of the plant, thus during the first and second larval stage of their development (Hashmi, 1994; Malinga, 2010). A threshold level for chemical control of bollworm on the fruiting parts is a 5-10% infestation in Pakistan (Hashmi, 1994). In South Africa, an economic threshold level for spiny bollworms is two larvae per 24 plants (Malinga, 2010). Scouting is very important, and all insecticide applications should be based on exceeding of the threshold level.

To control *E. insulana* and *E. vittella* effectively, it is necessary to apply the sprays while the caterpillars are still small (Hill, 1981). Cotton bollworms can be controlled using selective and non-selective chemical insecticides. Selective

insecticides are preferable because natural enemies are not badly affected. The use of non-selective insecticides just prior to the bollworm flight should be avoided in both conventional and Bt cottons because their use destroys predacious arthropods and this can result in more crop damage even with more intensive spraying for bollworm control (Turnipseed and Sullivan, 1999). The intensive and injudicious pesticide sprayings enhance the detoxification capabilities of pest populations (Rajendran, 2000). It is therefore preferable to apply a pest management strategy which uses a judicious blend of chemical and biological tactics. In India, synthetic pyrethroid insecticides were employed to fight against ever-increasing number of insect pests in cotton systems from time to time (Rajendran, 2000). However, the early use of synthetic pyrethroid like cypermethrin and decamethrin was found to increase secondary pests such as aphids and whiteflies. Chemical control is still one of the major tools for controlling bollworms. The control of this pest depended on the stages, which are found outside the fruit bodies, mainly egg, newly hatched larvae as well as moths. So, it is important to determine the generations of the pest and the time of insecticidal application with the appearance of the target stage Zaki (2006).

The great efficiency of synthetic pyrethroids compared to other conventional pesticides was confirmed in previous study by (Khanzada, 2002), who reported excellent performance of two formulations of Baythroid against *E.insulana*. Also our data reconfirmed by the finding of (Younis, *et al.* 2007) who mentioned that lambdacyhalothrin exhibited great reduction in bollworm infestation compared to other tested insecticides. In another study, alpha-cypermethrin was less effective than deltamethrin against the spiny bollworm, *Earias insulana* (Scott-Dupree *et al*, 2008). In more recent study by Ibrahim and (Younis, 2012), two formulations of lambda-cyhalothrin and zeta-cypermethrin were tested against bollworms and all were effective without significant difference between them in this respect; they

gave more than 80% reduction in bollworm larval content. Also, another study conducted by (Zidan, *et al.* 2012), revealed that tested pyrethroids (cypermethrin and lambdacyhalothrin) were effective in controlling the field populations of the spiny bollworm.

Younis, *et al.* (2007) found that some of synthetic pyrethroids (lambdacyhalothrin, esfenvalerate and deltamethrin) treatments were associated with great reduction in the population of predators. (Younis and Ibrahim, 2010) confirmed the harmful effect of synthetic pyrethroids. In addition, (Zidan, *et al.* 2012) found that cypermethrin, and lambdacyhalothrin were more toxic against predators than methomyl which induced a moderate effect.

2.2 NeemAzal-T/S

2.2.1 Introduction

NeemAzal is a trademark of Trifolio-M GmbH, Lahnau, Germany, NZ trademark of sustain - Ability Ltd, Motueka. NeemAzal is a broad spectrum botanical insecticide derived from the Neem seed kernel, it contains 10g/litre azadirachtin in the form of an emulsifiable concentrate (EC). Neem tree, is considered to be one of the most promising trees of the 21 century. It has great potential in the field of pest management, environment protection and medicine. Also it has showing great promise as potential fertilizer (Abdalla, 2010). In S Sudan Siddig (1993) reported that Neem seed water extracts at 1Kg/1Liter of water repelled foliage pest of potato including *B. tabaci*, *Aphis gossypii* and *J. lybica* and yield increased to 5 ton/ ha. Mohammed (2002) reported that Neem seed showed good performance against *A. gossypii*, *B. tabaci*, and *J. Lybica* on Okra. The insecticidal activity of Neem tree has been known for many years, people in India and other Asia countries mixed Neem leaves with beds and stored grain to reduce infestation by moth and bedbugs (Dreyer, 1984). Singh (1993) reported that 0.001% concentration of neem seed

kernel extract caused absolute feeding detergency against the desert locust *Schistocerca gregaria*. While 0.05% concentration was needed for some effects on the migratory locust *Locusta migratoria*. The larvicidal activity of different fraction of neem leaves showed that two fractions of 1% petroleum ether extract NPZI and NPZII produced respectively 100% and 80% mortality in 24 hour on the fifth instar larvae of mosquito (Sharmma, 1993). Goudegnonet *et al.*, (2000). studied the comparative effects of deltamethrin and neem kernel solution treatment on Diamond back moth. The moth populations were 10 times larger in deltamethrin plots, than in neem plots after treatment. The neem extracts could influence over 200 species of insects, many of which are resistant to synthetic pesticides; these include insects from order Diptera, Coleoptera, Lepidoptera, Orthoptera (Dhawan and Patniaik, 1993). Soliman (2005), reported remarkable effect of NeemAzal – T/S® on survival of the greater wax moth larvae. Also Elawad (2006) found that NeemAzal T/S® was more effective against 2nd and 4th larvae of African bollworm (*Helicoverpa armigera*).

Neem seed contains a number of chemical compounds the most important of which are Azadirachtin and Salannin in the triterpenoid fraction (Siddig 1991) Other active compounds contained in the seed kernel are Salannin 4- epoxyazaradion, Gedunin, Nimbinen and Dimethyl nimbinen (Jones *et al*, 1989).

Azadirachtin was first isolated in 1968, and is thought to be the most bioactive ingredient found in the neem tree; however, such speculation may be due to it having been investigated more thoroughly than the other compounds. Azadirachtin, one of the more than 70 compounds produced by the neem tree, acts mainly as an insect growth regulator, but also has anti-feedant and oviposition (egg-laying) deterrent properties (Quarles, 1994 and Thacker, 2002). There is evidence that other compounds found in neem have insecticidal attributes (Stark and Walter, 1995)

One of the advantages of Azadirachtin is that it has low mammalian toxicity, and degrades rapidly in the environment and has low side effects on non-target species and beneficial insects.

Many commercial Neem products exist, including Align, AzatinXL, Margosan-O, Neem-Away, Neemix, Salers BioNeem and Trilogy, these products control Leaf miners, Sweet potato white flies, thrips, loppers, caterpillars and mealy bugs (Pedigo, 1999).

2.2.2 The Efficacy of NeemAzal -T/S for controlling insect's pests

The efficacy of NeemAzal-T/S was tested against more than 120 species of insects and mites from Acarina, Coleoptera, Diptera, Heteroptera, Hymenoptera, Lepidoptera, and Thysanoptera. The results show that NeemAzal is effective against free feeding sucking and biting pests, such as caterpillars, beetles, mealy bugs, aphid, whitefly, leaf miners, thrips, and spider mites. (Ahmed, 2001) reported that NeemAzal increased the yield of okra and obtained significant reduction of number of aphids, whitefly, bollworms and flea beetles. On tomato NeemAzal increased the yield by 38% over the control. It reduced the number of leaf miner and whitefly significantly, while it reduced the number of aphids by only 10% under the control. On onion, the product increased the weight of onion by 15% over the control, a reduction by (21%-26%) of the number of thrips was also recorded. Neem formulations with rich azadirachtin contents such as NeemAzal were more effective than low azadirachtin formulations in suppressing Bollworms incidence and also was more effective than the conventional insecticide endosulfan, and increasing yield of seed cotton (Gupta, 2002).

2.2.3 Mode of action and toxicological properties of Neemazal

NeemAzal is a slow acting, naturally based anti-feeding insecticide , it leads to feeding inhibition (after some hours), reduction of molting (after some days) , fecundity and breeding ability reduction (after days, or weeks)Thus, assessments should be done with respect to decrease in damage of the target crop rather than the mortality of the insects . NeemAzal degrade in aqueous systems to half-life within a few days. In addition to the very favorable toxicological properties the rapid degradation of the active ingredient assures the safety of the consumer due to the lack of residues , non - toxic to micro- organisms , aquatic organisms , beneficial , warm blooded animals and etc.(Kleeberg, 2001).

2.2.4 Compatibility of Neemazal -T/S with other bio-pesticides

Cornale (2001) reviewed that NeemAzal-T/S has a limit effect when it was used alone. The efficacy achieved by NeemAzal alone has been found to be from (54% 68%),but when it was tank-mixed with other bio-pesticides such as natural pyrethrum and/or a commercial product based on *Beauveria bassiana*(Naturalis) , the efficacy achieved was 90%and above .These results show that NeemAzal –T/S can be more effective and highly compatible with many bio-pesticides .

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Location

The experiment was conducted in Entomology and Zoology Laboratory, Department of Plant Protection, College of Agricultural Studies (Shambat), Sudan University of Science and Technology (SUST), during April-June, 2016.

3.2 Insects rearing

The spiny boll worm larvae were collected from infested wild plant (Hambuk - *Abutilon* spp (Plate.1) grown in or around the college fields, and then transferred to Petri dishes (9.0 cm in diameter) for rearing (Plate. 2). Each 10 larvae were kept in separate Petri dish and supplied with small pieces of fresh okra fruits. The food was renewed daily until pupation. The pupae were kept in plastic cups covered with muslin clothes until the emergence of the adults. The emerged adults were introduced into glass cages (15 × 25 cm in dia.) covered with muslin clothes for egg laying; about 15 to 20 adults were placed in each cage (Plate .3). The adults were fed on sugar solution 10%, the food was placed in small plastic cups (4 × 3 cm in dia.) containing a piece of medical cotton soaked with sugar solution (Plate. 4). The muslin cloth and small plastic cups were replaced after 2days. The fine cloths containing eggs were daily placed in plastic bottles until egg hatching (after 2-4 days) to give first instar which transferred into Petri dishes (9.0 cm in diameter) for rearing. The rearing temperature was ranging between (28-35C°).

3.3 The Experiment

The experiment was designed to determine the susceptibility of the 3rd instar larvae of Spiny bollworm (*E. insulana*) to different concentration of NeemAzal. T/S[®] a commercial neem preparation (Trademark of Trifolio – M GmbH, Lahnau,

Germany, Nz Trademark of Sustain- Ability Ltd, Motueka). Four concentrations were used in this experiment (0,5ml /liter, 1ml / liter, 2 ml/ liter and 4 ml/ liter).

3.4 The bioassay tests

Small pieces of okra fruits were placed in fine clothes and dipped in each prepared concentration of NeemAzal T/S[®] for about one minute. Treated okra pieces were left to dry for 5-10 minutes under room conditions. Each 10 larvae were placed in separate Petri dish containing treated okra pieces. After 2-3 days the larvae were provided with fresh food until pupation. Mortality data were recorded 2hrs, 24 hrs, 36 hrs and 48 hrs after treatment. The experiment was replicated three times.

3.5 Experimental design and statistical analysis

The experiment was designed in a Completely Randomized Design (CRD) and the data was statistically analyzed according to analysis of variance (ANOVA) using Statistix -8 program. LSD test was used for means separation.



Plate. 1 Infested fruits of Hambuk (*Abutilon* spp.)



Plate. 2 Larvae feeding



Plate. 3 Adult cages



Plate. 4 Adults feeding



Plate.5 Tools and equipment s

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Effect of NeemAzal – T/S against the larvae of spiny bollworm (*E. insulana*)

In this study the results in **Table(1)** showed in concentrations (4, 2, 1 and 0.5 ml/liter) against *E. insulana* larvae, caused a significant mortality percentage compared with untreated control, In the first (2hr) after treatment, the mortality percentage ranged between (16.66%- 30%) in different concentrations compared to untreated control. (24hr) after treatment, the mortality percentage reached (86.6%, 73.33%, 38.66% and 23.33%), in concentrations respectively, and (36hr) after treatment, the mortality percentage reached (90%, 83.33%, 73.33% and 63.33%), in concentrations respectively and (48hr) after treatment, the mortality percentage reached (100%, 96.66%, 93.33% and 90%), in concentrations respectively, In this experiment the larvae were observed far away from the treated place (around the edges of the Petri dishes), From these results it's clear that 4ml were more effective against *E. insulana* larvae at all tested hours compared to other concentrations,

Table .1the mean of mortality (%) caused by NeemAzal – T/S against the larvae of spiny bollworm (*E. insulana*)

Concentration	Mean mortality (%)			
	2Hr	24Hr	36Hr	48Hr
4ml	30 (3 ^a)	86.6 (8.6 ^a)	90 (8.6 ^a)	100 (10 ^a)
2ml	23.33 (2.3 ^a)	73.33 (8.3 ^a)	83.33 (8.3 ^{ab})	96.66 (9.6 ^a)
1ml	16.66 (1.1 ^a)	38.66 (3.6 ^b)	73.33 (7.3 ^{ab})	93.33 (9.3 ^a)
0.5ml	16.66 (1.6 ^a)	23.33 (2.3 ^b)	63.33 (9.3 ^b)	90 (9 ^a)
control	0.0 ^b	0.0 ^c	0.0 ^c	0.0 ^b
SE±	0.91	0.94	0.94	0.52
CV (%)	15.6	20.99	15.06	6.79

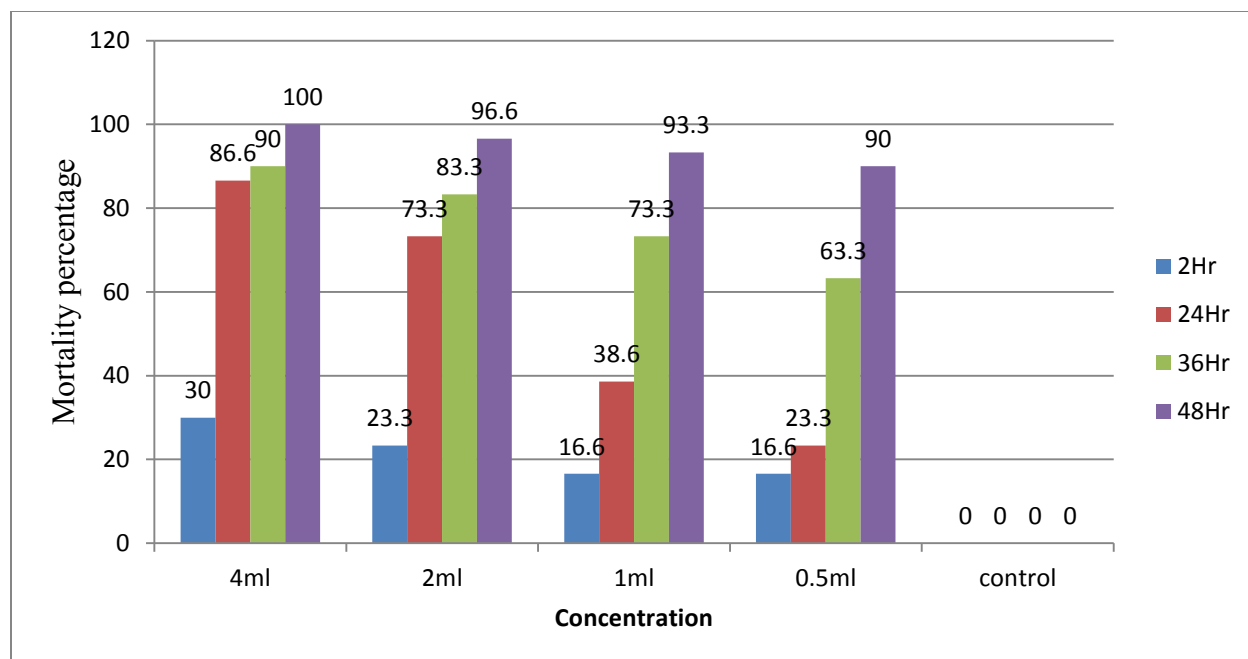


Fig. 1.The effect of the NeemAzal-T/S against larvae of spiny bollworm (*E. insulana*)

DISCUSSION

The synthetic conventional pesticides caused for the time being many problems, some of which are the appearance of highly resistant strains of numerous pests, and deleterious effects on the environment. At the same time such pesticides are very expensive, therefore, it's necessary to start with preparation of more suitable bio-active substances which have satisfactory properties, concerning their effect on the target pests, not expensive to produce and also environmentally safe. On the other hand, bio-active pesticides possess great advantages over synthetic pesticides of being more environmentally friendly to be accepted by the majority of the farmers and decision makers. The efficacy of these bio pesticides is comparable to that of chemicals, and they give consistent results under practical field conditions. (Kelany, 2001).

The present research was undertaken to study the efficacy of NeemAzal as biological control against the *E. insulana* larvae. The results obtained that demonstrably the products were effective against *E. insulana* larvae. Results in Table (1). (Fig. 1), showed that NeemAzal at different tested concentrations, after two days caused a significant mortality percentage against the larvae of *E. insulana* this may be due to the high repellency effect, and feeding inhibition or direct toxicity by inhalation, NeemAzal also reduced insect population growth and affected larval molting. These results agreed with El-Sayed (2001) who reported that tested concentrations NeemAzal – T/S caused highly significant increase in larval period of spiny bollworm as compared with untreated, These results are also similar to those reported by Gupta, (2002), who observed that Neem formulations containing azadirachtin such as NeemAzal were more effective against the bollworms, In general neem derivatives often modify the development of insects by their influence on the hormonal system, specially on ecdysteroids (Schumutterer,

1990), leading to growth regulatory effects , exhibited by growth inhibition , malformation and mortality (Mordue and Blackwell,1993).

These results agreed with the findings of Weinzierl and Henn (1991),who reported that Azadirachtin (The active ingredient of NeemAzal) prevent insect from molting by inhibiting the production of ecdysone hormone , the hormone responsible for triggering molts, and also may cause insect to stop feeding after ingestion due to secondary physiological effects. And agreed also Elawad (2006) found that NeemAzal T/S® was more effective against 2nd and 4th larvae of African bollworm (*Helicoverpa armigera*).

Conclusion

The spiny bollworm *E. insulana* Showed high susceptibility to NeemAzal-T/Sin Larvae .The results showed that NeemAzal-T/S was more effective larvae compared with untreated. NeemAzal-T/S showed high performance, in addition to high repellency effect. Thelarval development was also negatively affected.

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