Cairo University
Institute of African Research and Studies
Natural Resources Department

EFFECT OF SPINOSAD AND CONSULT ON FIFTH NYMPHAL INSTAR OF DESERT LOCUST *SCHISTOCERCA GREGARIA* (FORSKAL)

By

SAID MOHAMAD SAID AHMAD
B. Sc. Agric. Sci. (Pesticides), Fac. Agric., Zagazig Univ., Egypt, 2004.
M. Sc. Agric. Sci. (Entomology), Fac. Agric., Cairo Univ., Egypt, 2009.

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SUPERVISION SHEET

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Ph. D. Thesis

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Title of Thesis: Effect of Spinosad and Consult on Fifth Nymphal Instar of Desert Locust *Schistocerca gregaria* (Forskal)
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ABSTRACT

Laboratory studies were carried out in an attempt to disclose the effect of a bio-insecticide (Spinosad) and an IGR (Consult) at different concentrations (15, 36, 75 and 150ppm), (12, 30, 60 and 120ppm), respectively on one day old of the 5th nymphal instar of the desert locust, *Schistocerca gregaria* (Forskal) by feeding technique.

The results indicate some biological aspects as nymphal and adult mortality, reached to 45.1, 69.8, 85.4 and 100% in one day old nymph treatment with Spinosad 3 days after application and to (6.9, 12.3, 27.8 and 51%) 10 days after treatment with consult. Failure of ecdysis to adults and the resulted adults had twisted and cured wings in adults by consult.

Total proteins, carbohydrates, lipids and cholesterol contents and the insect enzymes acid phosphatase, Phenoloxidase and Peroxidase activities were affected fluctuated between increasing and decreasing 2, 4 and 6 days after treatment with Spinosad (at LC_{50} value), consult (at LC_{50} value) and their mixture(at LC_{25} values). Total proteins, total lipids, total cholesterol, acid phosphatase activity, phenoloxidase activity and Peroxidase activity were dramatically declined in all treatments comparing with untreated nymphs. Highly significant decline were recorded in them by mixture.

Consult caused more increase in the total carbohydrates but Spinosad and the mixture caused decrease in the total carbohydrates comparing with untreated nymphs. Highly significant decline were recorded in carbohydrate content by Spinosad.

Key words: *Schistocerca gregaria*, haemolymph components, biochemical changes, activity enzymes, phenoloxidase, acid phosphatase,Peroxidase spinosad, consult

DEDICATION

To Dr/ Mohamad Ahmad Ahmad Eid soul, for his valuable guide in my life

&

To my Father and my Mother and my wife for their patience and help, as well as to my son and my brothers and my friends for all the support they lovely offered

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INTRODUCTION

Crop loss from locusts was noted in the Bible and Qur'an. **(Ceccato *et al.*, 2007)**. The significant crop loss caused by swarming desert locusts exacerbates problems of food shortage, where a single swarm can cover up to 1200 square kilometers and can contain between 40 and 80 million locusts **(Tony, 1979)**.

The normal method for controlling these pests is with chemical pesticides, using ultra-low volume (ULV) application methods. When protecting crops a quick "knock-down" for insects is required by fast acting insecticides, such as pyrethroids. However, many of these chemicals cause environmental damage and some are hazardous to humans and domestic animals.

In response to concerns over pesticide use, so, attention very rapidly focused on some agents of biological control **(Lomer *et al.*, 2001).**

The mechanisms of action of the examined compound, Spinosad causes persistent activation of nicotinic acetylcholine (ACT) receptors. Because ACT and spinosad act on receptors simultaneously, they likely operate at different target sites; this apparently is unique

nicotinic agonists, which normally compete directly with ACT for binding sites **(Salgado *et al*., 1997**) .

IGRs are diverse groups of chemical compounds that are highly active against immature stage of insects and have a good margin of safety to most non-target biota including invertebrates, fishes, birds and other wild life, they are also safe to man and domestic animals, they will play an important role in control programs in the future **(Mulla, 1995)**. The main types of insect growth regulators used commercially are juvenile hormone analogues and chitin synthesis inhibitors **(Parrella and Murphy, 1998)**.

Chitin synthesis inhibitor (Consult) prevent the formation of chitin, which is a carbohydrate an important structural component of the insect exoskeleton. When insects were treated with one of these compounds, the insect grows normally until the time of molting. When the insect molts, the exoskeleton is not properly formed and the insect dies. Death may be quick, but in some insects it may take several days. As well as disrupting molting **(Abdel-Aal, 2012).**

The purpose of this study was evaluating the effect of the Bio-chemical agent spinosad, IGR consult and the mixture between them on the desert locust, *Schistocerca gregaria* (Forskal) through the following steps:

1. Determine the susceptibility of 5^{th} nymphal instar of the desert locust *S. gregaria* to spinosad, consult and their mixture.
2. Effect of Spinosad and consult and their mixture on insect morphogenesis.
3. Study the co-toxicity between spinosad and consult on desert locust
4. Study the effect of spinosad, consult and their mixture on the haemolymph components, i e, total carbohydrates, total lipids, total protein and total cholesterol.
5. Study the effect of spinosad, consult and their mixture on the activity of some important enzymes, I e, phenoloxidase, Peroxidase and acid phosphatase.

REVIEW OF LITERATURE

1. Danger of locusts to agriculture

The desert locust (*Schistocerca gregaria*) has been an important agricultural pest at least since biblical times. Although the ecology, physiology and behavior of this insect species have been well characterized, its bio-geographical origins and evolutionary history are more obscure. **(Lovejoy, *et al.*, 2006).** The desert locust *Schistocerca gregaria* is an economically dangerous pest invading several countries in Africa and Asia (**Hamadah, *et al*., 2013).**

Locust and grasshoppers have been some of the greatest agricultural pests since the beginning of civilization. They are the most voracious pests known capable of eating their own weight (2-3g) of vegetation daily **(Alomenu, 1998)**.

Normally, the desert locust is at low density, that occurs in desert and scrub regions of northern Africa, the Sahel (regions including the countries of Burkina Faso , Chad , Mali , Mauritania, and Niger) and Arabian peninsula(e.g., Saudi Arabia, Yemen, Oman) and parts of Asia to western India **(Steedman, 1988)**.

Grasshoppers and locusts generally have very high reproductive rates and are able to respond to favorable

climatic conditions with rapid population increase **(Bateman *et al*., 1993).** During the low density phase locust populations are low and represent no economic threat. After periods of drought, when vegetation flushes occur in major desert locust breeding areas (e.g. , Indian/Pakistan border), rapid population build up and competition for food occasionally result in a transformation from low density to high density on a regional scale **(Showler, 1995a)**. Following this transformation, which can occur over two or three generations (durations of locust life cycles are variable, depending on species and environmental conditions). Locusts often form dense bands of flightiless nymphs and swarms of winged adults that can devastate agricultural areas **(Showler, 1995b)**. A swarm of locusts can fly 100 km in the general direction of prevailing winds. Bands of nymphs can march bout 1-5 km per day. Plagues may involve hundreds of swarms. Desert locust can consume the approximate equivalent of their body mass each day **(Showler, 1996)**.

When pests cross national borders, internationally coordinated operations are necessary. Plagues develop only when control efforts break down, or political or natural disasters prevent access to breeding areas, and interventions do not start early enough control failures and plague

development have occurred with the desert locust in the red sea basin in 1986 and 1992 **(Showler , 1993)**.

Desert locust *Schistocerca gregaria* is generally recognized as a polyphytophagous acridid that commonly causes substantial damage to pastures and crops (**Tarai and Doumandji, 2009)**.

Plagues of the *S. gregaria* have threatened agricultural production in Africa, and Asia for centuries. The livelihood of at least one-tenth of the world's human population can be affected by this voracious insect. The desert locust is potentially the most dangerous of the deserts pests because of the ability of swarms to fly rapidly across great distances. It has two to five generations per year. The northern highlands of Ethiopia (Tigray) and Eritrea slow the movements of locusts to the breeding areas of the Red Sea coast. Potential plagues originating in east Africa can be prevented if action is taken during or before localized outbreaks in Eritrea and Sudan **(Huis, 1995)**. The locust can live between three to six months, and there is a ten to sixteen fold increase in locust numbers from one generation to the next. From October 2003 to May 2005, West Africa faced the largest desert locust outbreak in 15 years. The upsurge started as small independent outbreaks that developed in Mauritania, Mali, Niger and Sudan in the

autumn of 2003. Two days of unusually heavy rains that stretched from Dakar, Senegal to the Morocco in October allowed breeding conditions to remain favorable for the next six months and the desert locusts rapidly increased **(Huis *et al.*, 2007)**. Lack of rain and cold temperatures in the winter breeding area of Northwest Africa in early 2005 slowed down the development of the locusts and allowed the locust control agencies to stop the cycle. During the upsurge, nearly 130,000 km² were treated by ground and aerial operations in more than 20 countries. The costs of fighting this upsurge have been estimated by the FAO to have exceeded US$400 million and harvest losses were valued at up to US$2.5 billion which had disastrous effects on the Food security situation in West Africa. The countries affected by the 2004 outbreak were Algeria, Burkina Faso, the Canary Islands, Cape Verde, Chad, Egypt, Ethiopia, Gambia, Greece, Guinea, Guinea Bissau, Israel, Jordan, Lebanon, Libyan Arab Jamahiriya, Mali, Mauritania, Morocco, Niger, Saudi Arabia, Senegal, Sudan, Syria and Tunisia. **(Ceccato *et al.*, 2007)**.

The red Sea coastal plains of Africa and the Arabian Peninsula are an important breeding area for desert locust, *Schistocerca gregaria*. This area has been implicated as a source or transit area for locust swarms that threaten

agriculture. The spatial distribution of the desert locust on the southern part of the Red Sea coastal plain of Sudan, between Port Sudan and Tokar, was investigated to determine habitat associations of the desert locust and collect information that might help in planning survey and control operations **(Woldewahid, 2003)**.

2. Control of desert locust

2.1. Chemical control of desert locust

The anti-locust Research Center, established in 1945 developed the use of chemical pesticides against locusts, selecting Dieldrin as the most effective and economical control agent because of its long persistence **(Bennett and Symmons, 1972)**. An increasing awareness of the negative environmental impact of Organic Chlorine pesticides has led to the restriction of their use to certain limited public health applications. Organophosphate, Carbamate and Pyrethroids pesticides replaced Dieldrin for locusts and grasshoppers control **(Allan, 2002)**. The use of chemical pesticides has been the main insect controlling approach during recent decades, but the widespread use of such chemicals has significant drawbacks, such as the development of strain resistance to insecticides **(Garriga and Caballero, 2011)**.

The toxicity of commonly used Pesticides were reviewed and it was found that in 45% - 55% of the

records, Chemicals gave mortality rates > 90% in non-target Species **(Prior and Greathead, 1989)**. Environmental issues arising from the standard use of chemical pesticides against locusts and grasshoppers include the impact on operators, other people, livestock and birds **(Matteson, 1992)**. Other terrestrial vertebrates, aquatic organisms (e.g. fish) and terrestrial arthropods including the natural enemies of locusts and grasshoppers, as well as pollution issues, contamination of groundwater and wells and disposal of surplus pesticides stocks **(Ritchie and Dobson, 1995)**. Ecologically sensitive areas (ESAs) including the mangrove along the Red Sea Coast of Sudan may be subjected to adverse impacts of insecticides for controlling desert locust, *Schistocerca gregaria* (Acrididae). **(Eriksson and Wiktelius, 2011)**.

About $275 million was spent on application of 15 million litres of pesticides in the locust plague of 1986-1989, the first in many years in the Sahel covering over 25.9 million hectares of land **(Showler, 2002)**. Often huge quantities of such pesticides are used. For example, in July 2004, Algeria used 80 000, Morocco 50 000 and Libya 10 000 liters of pesticides **(Ammar and Ben Hamouda 2005).** During the 2004 invasion of South Tunisia by the desert locust, 47% of the captured locusts had unusual

pigment spots on their head and/or sternum. Upon analysis of dead locusts, the spotted ones contained residual chlorpyriphos-ethyl up to 0.2 mg/kg while the nonspotted ones had much lower insecticide concentrations (0.02mg/kg). Spotted locusts have a lower mortality rate than the non spotted ones, indicating that some resistance mechanism may be operating Frontal spots are more frequent in males than in females (**Ammar, *et al*., 2006).**

Biological control involves the use of naturally occur ring enemies-parasites, predators, and disease agents (pathogens). It also includes methods by which the pest is biologically altered, as in the production of sterile males and the use of pheromones or juvenile hormones. Most kinds of biological control agents occur naturally. Releasing more of a pest's enemies or predators into the target area can supplement this natural control. Biological control can be a low-cost control method particularly suited to low-value crops (pastureland, clover, and hay crops) or in areas where some injury can be tolerated (golf course fairways or forest areas) **(CRC, 1998).** Modern agriculture and it's associated large, monoculture plantations have created conditions favoring the rapid establishment and spread of a diverse variety of insects. This has promoted a heavy reliance on synthetic pesticides to control, limit, and

contain the spread of these arthropods. Concerns over environmental pollution, human health risks, and resistance have stimulated the search for alternative control strategies and their use within integrated crop management programmes. Biological control is considered a major component of IPM, but is frequently underutilized. Microbial biocontrol agents, and fungi in particular, can play a significant role in IPM; Compatibility of the fungi with other IPM components and non target organisms must also be ensured. Field efficacy has to be demonstrated through scale-up trials, and the technology refined into a form that can be readily implemented and (with appropriate support and guidance) transferred to the farming community (**Brownbridge, 2006).** Acrididae pests are serious agricultural pests that cause considerable damage to food crops and pasture grasses, particularly during outbreaks. New control strategies aim to use of relatively safe materials such as pathogens. In this study, *Metarhizium anisopliae* var. *acridum* (Green Muscle) was applied against some Acrididae pests at different periods in some places in Egypt considered favorable breeding sites to test its efficacy on the target pests under the Egyptian ecological conditions. Results showed that the efficacy of *M. anisopliae* var. *acridum*, in all treatments, indicated that

it was a specific bio-pesticide for controlling (**Hosny, et al., 2009).**

Also Plant extracts were used during the last plague of the desert locust, *Schistocerca gregaria* in the winter season of 2004-2005 to Egypt, it was observed that Bermuda grass, Cynodon dactylon (L.) argued to resist the infestations of this insect. To explore this observation, in the laboratory by feeding the 4th and 5th instar nymphs, *S. gregaria* on treated maize leaves with different extracts from leaves and roots of Bermuda grass in the solvents, methanol, acetone and etheoxyethanol. Results revealed that growth rate and metamorphosis of the treated nymphs were inhibited. Feeding of the 4th instar nymphs on the treated leaves of maize by methanol leaves extracted from grass leaves resulted in 8.3% mortality and 91.7% malformation of 5th instar nymphs which died after ecdysis. Also, treatment with methanol grass leaves extracts induced 40% mortality in the treated 4th instar nymphs, while the other 60.0% were molted to a weak and small 5th instar nymphs. Feeding of 5th instar nymphs on the treated leaves of maize with methanol, acetone and ethoxyethanol leaves and roots of Bermuda grass resulted in different percentages of mortality ranged from 11.1% to 90.0%. The high mortality percent was obtained by

ethoxyethanol leaves extract (90.0%). e (**El-Gammal, *et al*., 2008).** Three extracts were prepared from the wild plant *Fagonia bruguieri*: methanolic extract, petroleum ether extract and n-butanolic extract. These extracts were assessed against the penultimate and last instar nymphs of *Schistocerca gregaria*. After treatment of the penultimate instar nymphs, a dose-dependent trend of mortality could be observed for the methanolic extract. To some extent, a lesser toxic action was exerted on the nymphs by petroleum ether extract or n-butanolic extract. After treatment of the last instar nymphs, an ascending mortality % was estimated as the concentration level of methanolic extract was increased. After treatment of the penultimate instar nymphs, the growth of the same treated nymphs was affected to some extent by the methanolic extract, irrespective of the concentration level. The remarkably influenced nymphal growth was detected only at the highest concentration level of petroleum ether extract and the higher two concentration levels of n-butanolic extract (**Ghoneim, *et al*., 2009).**

Using anti-feedant as alternative to pesticides where, various neem products were tested against resting and flying *S. gregaria*. Two of the products namely neem Azal-F and the unclarified neem oil were obtained from the

Company Trifolio, whereas neem oil enriched and pure neem oil were a gift of Prof. Schmutterer (University of Giessen). The treatment during flight activity caused, for all products applied an increase of the mortality rate, except the neem oil enriched and pure neem oil of Giessen, up to 70 and 90% respectively. The same products, however, sprayed on resting locusts did not show any remarkable mortality. But this treatment reduced the fitness of the locusts in terms of their flight performance, as well as their adipokinetic potency. In consequence of this, it is to expect that neem treated locusts will not be able to cover long distances. That means the lipid mobilizing system necessary to provide the flight muscles with "fuel" (lipids) is disturbed severely (**Al-Fifi, 2009).** From this point of view, it is necessary to minimize the application of pesticides that considered as a main source of environmental pollution and use other compounds may proof as good alternative of insecticides. Among these compounds are the uses of Spinosad (**El- Sheikh, 2012)**.

2.2. Spinosad

Integrated pest management (IPM) includes a combination of chemical, biological and cultural control strategies **(Sarfraz*, et al.* 2005).** although insecticides will continue to comprise important components of such

programs. Insecticides applied in an agroecosystem not only control the target insect pests but may adversely affect the non-target organisms including biocontrol agents (**Anjum, *et al*., 2007)**.

New chemical insecticides are introduced rapidly but very little is known about their effects on predators and parasitoids of herbivorous pests. Insecticides should not only suppress the insect pest population but also be safe to their natural enemies. It is, therefore‹ imperative to screen the insecticides for their selectivity for important biocontrol agents before they could be incorporated into IPM programs **(Sarfraz and Keddie, 2005)**.

Rosenberger and Jones, (1960) suggesting that haemogram is a useful tool for investigation of toxic effects of insecticides on biocontrol agents. Successful screening of insecticides largely depends on a better understanding of their toxicological effects on target pests as well as on their natural enemies. Haematological studies are of crucial importance for insect toxicology. Haemocytes are involved in detoxification (e.g. phagocytosis and encapsulation) of metabolites and biologically active material in addition to transport of nutrients and hormones **(Patton, 1983).**

Due to the environmental pollution. Toxicity to non-target organisms and humans **(Pretty, 1996)**, there is an

increasing interest for the exploitation of biological control agents, either available as commercial products or still under development of environmental friendly alternatives to control agents **(Lange, 2005)**. Recently, the quality of the environment has become a major issue. Many chemicals (pesticides) previously accepted for locust control at national and international levels would not survive rigorous environmental testing. A bio-insecticide TRACER 24SC Spinosad is a new safe product to control the desert locust, *Schistocerca gregaria* Forskal **(Hosny, *et al*., 2010).**

Suitable integration of safe and effective chemicals with other tactics, including biocontrol agents can prove effective strategies for IPM. Spinosad is a member of the Naturalyte class of pesticides that has been classified as bioinsecticide **(Copping and Menn, 2000)**. It comprises primarily two macrocyclic lactones, spinosyn A and D **(Sarfraz, *et al.* 2005).** Which are secondary metabolites produced by the soil bacterium, *Saccharopolyspora spinosa* Mertz & Yao (Actinomycetales: Pseudonocardiaceae), under natural aerobic fermentation conditions **(Mertz and Yao, 1990)**. Spinosad acts as both stomach and contact insecticide, primarily targeting a nicotinic acetylcholine receptor as well as γ-aminobutyric acid (GABA) and causes

a general paralysis of the insect **(Sparks, 2004)**. Spinosad effectively controls pest species of the orders Lepidoptera, Diptera, and Thysanoptera and also shows toxicity to certain species of Coleoptera, Orthoptera, Hymenoptera, Isoptera, Siphonaptera, Dermaptera and Psocoptera **(Blanc *et al.* 2004)**. Spinosad is known to have exceptional safety to non-target organisms compared to synthetic insecticides **(Sarfraz *et al.* 2005)**.

Spinosad-treated aphids fed to coccinellid and chrysopid larvae caused no mortality of these predators **(Schoonover & Larson, 1995).** Additional studies determined that Spinosad was practically non-toxic to insect natural enemies such as lady beetle, minute pirate bug, lacewing, and predatory mites **(Bret *et al.*, 1997).** Spinosad applications were less toxic than lambda cyhalothrin to the majority of predators including chrysopids, coccinellids, syrphids and geochorids in sweet corn **(Musser & Shelton, 2003)**

Nicotinic acetylcholine receptors (nAChR) play a number of essential physiological roles in both vertebrates and insects, including the well characterized mediation of fast excitatory neurotrasmission at cholinergic synapses **(Karlin, 2002)**.

Spinosad is a relatively new insecticide used to control a wide range of insects. The primary target of spinosad appears to be nAChRs **(Salgado & Sparks, 2005)**. Spinosad exertsits toxic action via nicotinic acetylcholine receptor (nAChRs). Spinosad resistance in house flies appears to be due to an altered target site. **(Gao, *et al*., 2007).** Total haemocyte count (THC) increased one minute after treatment with azadirachtin and Spinosad. Azadirachtin and spinosad resulted in an increased overall THC, whereas Spinosad application resulted in a decreased THC 30 minutes after treatment. After 60 minutes, larvae treated with azadirachtin and spinosad had 1.2 and 1.4-fold more THC respectively. The differential haemocyte count indicated that Spinosad application resulted in an increased percentage of Plasmatocytes, Oenocytes and Spherulocytes, and a decreased percentage of Prohaemocyte and Granulocytes (**Suhial, *et al*., 2007).**

2.3. Consult

Although chemical pesticides are invaluable in controlling insect populations both in the field and storage, their indiscriminate use has resulted in the destruction of beneficial insects and has caused environmental hazards. Moreover, insecticide resistance has already developed in many insects which is now a great concern in post-harvest

ecosystems throughout the world (**Arthur, 1996**). These problems have resulted in the search for alternative control agents which are less toxic to non-target animals and the environment. In this regard, the insect growth regulators (IGRs) which regulate the insect population through the disruption of moulting and metamorphosis have captured the interest of entomologists (**Mondal and Parween, 2000**). According (**Thabit, *et al.*, 2010)**. There are three types of IGRs, each of which has a different mode of action:

1- Chitin synthesis inhibitors: These prevent the formation of chitin, a carbohydrate that is an important structural component of the insect's exoskeleton. When treated with one of these compounds, the insect grows normally until the time to molt. When the insect molts, the exoskeleton is not properly formed and it dies. Death may be quick, but in some insects it may take several days. As well as disrupting molting, chitin synthesis inhibitors can kill eggs by disrupting the normal development of the embryo e.g. Chlorofluazuron.

2- Anti jovenoid: This group of compounds induces a premature and lethal larval moult by direct stimulation of ecdysteroid receptors, especially in larval Lepidoptera e.g. (tebofenozoid).

3- Juvenile hormone analogue: When applied to an insect, these abnormalsources of juvenilizing agent can have striking consequences e.g. pyriproxyfen.

Some of the most famous compounds are methoprene, hydroprene, kinoprene, fenoxycarb, pyriproxyfen and precocenes I and II **(Ghasemi *et al*., 2010).** The second category comprises those compounds inhibiting the chitin biosynthesis, such as diflubenzuron, chlorflauzuron, triflumuron, Flufenoxuron, hexaflumuron, lufenuron, diofenolan, teflubenzuronk triflumuron, and novaluron, or interfering with the moulting process in general such as tebufenozide (RH-5992), methoxyfenozide (RH-2485), halofenozide (RH-0345) and chromofenozide (ANS-118) (**Zibaee *et al.*, 2011**).

In the last few years, scientists directed their efforts towards the control of insects by the use of insect growth regulators to avoid the hazards of insecticides **(Bakr *et al*., 1989)**. IGRs are diverse groups of chemical compounds that are highly active against immature stage of insects and have a good margin of safety to most non-target biota including invertebrates, fishes, birds and other wild life, they are also safe to man and domestic animals, they will play an important role in vector control programs in the future **(Mulla, 1995)**. Development and reproduction in

insects are affected by a number of hormones, including juvenile hormone and ecdysone. Insect growth regulators (IGRs) are synthetic analogues, which mimic the naturally occurring hormones for affecting the physiological processes of insects and are generally classified as juvenile hormone analogues and ecdysone agonists **(Mohandass *et al*., 2006)**.

The ecdysone agonists, or ecdysteroids, are synthetic products interfering with the natural insect moulting hormone (20-hydroxyecdysone), which controls several physiological and biochemical processes of growth and development. They have attracted the attention of many researchers all over the world **(Cadogan *et al*., 1997)**.

The benzoylphenyl ureas constitute a class of Insect Growth Regulators (IGRs) that interfere with insect growth and development by inhibiting chitin synthesis in insect **(Post and Vincent, 1973).** Benzoyl-urea IGRs are slow acting agents, with mortality occurring during or just after the next moult of the insect. This would typically occur between 5 and 12 days after treatment, depending mainly on hopper stage and ambient temperature **(FAO, 2005)**.

Hexaflumuron[N-(((3,5-dichloro-4-(1,1,2,2-tetra fluroethoxy) phenyl) -amino) carbonyl) -2,6 diflurobenz-amide] is a slow acting chitin synthesis inhibitor **(Su, *et al*.,**

1987). Tebufenozide (RH-5992) represents a novel class of IGRs, which directly stimulate the ecdysteroid receptors as the molecular level initiating the moulting process by gene regulation especially in larval Lepidoptera **(Wing *et al.*, 1988)**. Tebufenozide exhibited some histopathological effects on the integument of last instar nymphs of desert locust (*Schistocerca gregaria*) such as detachment of the cuticle from epidermis and undistinguishable epicuticle. Tebufenozide treatments affected the ultrastructural configuration of thoracic muscles, such as disrupted organization of A, I and H bands and the degeneration of Z disc varied from one half of the disc to small central or peripheral degenerated areas. Tebufenozide treatments resulted in the loss of normal architecture of the majority of mid-gut epithelial cells, which had dwarf and deformed microvilli. The most important ultrastructural changes in the intracellular organelles by the action of tebufenozide, as shown in the electron micrographs, were elongation of mitochondria, which appeared with prominent cristae, the lysosomes were supposedly autophagic, Golgi bodies had a bullet shape and the cytoplasm contained scattered granules **(Ghonem, et al., 2008)**. Now, Studies have tended to concentrate on morphometric changes and on assessing pesticidal activity. The histopathological effects of

Tebufenozide, as well as the ultrastructural changes in the desert locust (*S. gregaria* Forskal) were investigated aiming to shed some light on its roles at the cellular and sub-cellular levels. Feeding application of different concentrations of Consult on the 5th nymphal instar of the desert locust, *Schistocerca gregaria* (Forskal) showed mortal action on treated nymphs, failure in ecdysis to adults and prolongation in the 5^{th} nymphal age and adults had malformed wings, color changes of body and failure of completely getting rid of the last nymphal exuvia (**Bakr, *et al.*, 2009).** Histopathological changes in the testes were tested in normal adult males and those developed from treated one day old of the fifth nymphal instar of the desert locust with (LC_{50}) of Consult. The testicular follicles of those males developed from treated one day old of the 5th nymphal instar with Consult (LC_{50}) showed damage in zones of reduction and transformation and degeneration and necrosis appeared in many spermatids and spermatozoa **(Bakr, *et al.*, 2010).**

2.4. Insecticides combination

Chemical insecticides combination provides several distinct advantages for Insect Pest Management programs (IPMs), including the potential effect for reducing the amounts of each agent used. Such reduction would mean

potentially lower costs, lower environmental pollution, less damage to beneficial organisms and reduced selection pressure leading to the development of resistance to each agent **(Temerak, 2005).**

The effects of conventional (profenofos) and nonconventional (emamectin benzoate, spinosad and chlorfluazuron) insecticides at their LC_{10}, LC_{25} and LC_{50} and their binary mixtures were evaluated against 2^{nd} instar larvae of cotton leaf worm, *Spodoptera littoralis* (Boisd.) under laboratory conditions. After 3 days of the treatment, spinosad showed the lowest toxic effect (LC_{50}= 19.9 ppm). After 12 days of the treatment, and at the LC_{25} level, spinosad showed the longest residual effect. At the same concentration level, spinosad and chlorfluazuron had the higher effects on pupation, moth emergence, hatchability and sterility. All the tested mixtures increased mortality percentages of larvae. the mixtures of both emamectin benzoate and spinosad with profenofos produced additive effects Also, mixtures of spinosad (at LC_{50}) with profenofos at LC_{10} and LC_{25} gave the highest effect on egg production 393.9 egg/female and hatchability (19.17%), comparing with the control (1151.6 egg/female. The obtained results indicated that mixtures of conventional–nonconventional insecticides had the combined advantages

of quick speed of killing and a high level of safety **(Korrat *et al*., 2012).**

The LC_{10}, LC_{25} and LC_{50} values of Spinosad and consult were determined using the leaf dipping technique. Dry and clean barseem leaves were dipped for 15 Second in concentrations of the tested insecticides, then left to air dry for 1hr. under room temperature and then offered to 5^{th} instar nymph in clean cage, each cage contained 50 nymphs. Five replicates were used for each concentration of each treatment. Leaves dipped in water served as control. The LC_{10}, LC_{25} and LC_{50} values that obtained by regression lines were statistically estimated and the goodness of fit of the regression lines to the observed data was calculated according to **Finney (1971).**

The variations in efficacy of pyridalyl or /and spinosad either alone or combined with three vegetable oils (corn, sunflower and sesame) at mixing ratios of 99/1, 95/5 and 90/10(insecticide /oil) were evaluated on growth development and reproductive performance of cowpea beetle *Callosobruchus maculates* (F.). The results showed that the activity of pyridalyl or/and spinosad either alone or combined with the three vegetable oils was significantly increased, particularly at the highest concentration tested (1000ppm) ,and that spinosad in combination with the three

oils and in particular with sesame oil was significantly more effective in reducing number of deposited eggs, and F1 emerged adults whereas pyridalyl /corn and pyridalyl/ sunflower combinations were more effective than corresponding mixtures of spinosad in suppressing Hatchability percent. On the other hand spinosad/ oil combinations increased remarkably the duration of development period more then pyridalyl oil combinations **(Manal, *et al*., 2013).**

3. Biochemical changes

3.1. Determination of total protein

When incubated without serum to stabilize the cells, monolayers of both locust and cockroach haemocytes released protein into the culture medium. The amount of protein released by cockroach cells was greater than that released by locust haemocytes cultured at the same density 141 000 cells / cm^2 **(Huxham *et al.*, 1989)**.

Shaaban *et al*. (1985) Showed that total protein content in the 6^{th} instar larvae of *S. littoralis* larvae, significantly decreased after treatment with cypermethrin and Spinosad. which reported that total haemolymph protein content of 6th instar larvae of *S. littoralis* decreased after treatment of the 4^{th} larval instar with pyrethroid compounds. The decrease in the protein content of the

haemolymph might be due to inhibition of DNA and RNA synthesis. The protein concentration in the haemolymph of females of adult grasshopper was 54.4 mg / ml compared to 27.1 mg / ml males. Vegetative development of *B. bassiana* in the haemocoel of the beet armyworm, *Spodoptera exigue* did not cause significant alterations in the profile of proteins, pulse labeling tissue explants revealed that vegetative growth of *B. bassiana* hyphal bodies did not impact the biosynthetic **(Gurwattan *et al*., 1991)**. Capability of either the fat body or cuticle epidermis, both major sites of host protein synthesis. Even at the late stage of hyphal body development. When concentrations reached 3×10^5 spores per micro liter of haemolymph, both fat body and cuticle explants were capable of synthesizing and secreting the majority of proteins detected in naive samples replication of hyphal bodies in haemocytes cultures established from infected larvae induced the synthesis and secretion of 3 peptides having weights of 31, 32 and 40Kda. Interestingly, peptides having identical weights were detected in infected haemolymph samples **(Mazet and Boucias, 1996)**. On the other hand **(Gillespie *et al*., 2000)** showed that the protein concentration in the haemolymph of 5^{th} nymphs instar of desert locust, *S. gregaria* remained constant for the first two days after

inoculation with an entomopathogenic fungus, *M. anisopliae* var. *acridum* of concentration 4 x 10^6 spores/ml and then declined significantly than controls, **(Mettaweh *et al.*, 2001)** showed that treatments during all the periods post inoculation (7, 12, 17, 22 days after infection) with fungi, *M. anisopliae, M. flavoviridae, and B. bassiana* with dose 5x10^6 spores/nymph to 5^{th} nymphs instar decreased the protein levels in the haemolymph the protein contents in case of the fungus, *M. flavoviridae* was highly decreased than in case of *M. anisopliae* and *B. bassiana* at all periods. While protein contents in case of *M. anisopliae* was highly decreased than in case of *B. bassiana* moreover, as the post inoculation period increased the protein content decreased. **Abed El-Kerim (2002)** found a decrease in haemolymph protein in male, female and 5th nymphal instar to *S. gregaria* which were treated with Bancol extract where reached to 82.52:% and 78.40% for female and male. In adult desert locust *S. gregaria* 3days after inoculation with the entomopathogenic fungus, *M. anisopliae* var. *acridum* had significantly less protein in the haemolymph than controls. This was not due to reduced food intake as 3 days of complete starvation had no effect on haemolymph titers of energy reserves in controls **(Seyoum *et al.*, 2002)**. The synthesis of new proteins, both in thermoregulating and

non-thermoregulating locusts did not result in a significant increase in total protein concentration. In contrast infection of the desert locust by the fungus *M. anisopliae* var. *acridum* caused a decline in protein content after two days after inoculation **(Robert *et al*., 2002)**

Feeding application of different concentrations of Consult on one day old and six day old of the 5th nymphal instar of the desert locust, *Schistocerca gregaria* (Forskal) showed reduction of total protein of haemolymph in nymphs and adults, also reduction in total ovary and testis protein of adults were observed **(Bakr, *et al*., 2009)**. **Abdel-Aal (2012)** showed that chlorofluazuron , tebufenozoid and pyriproxyfen decreased the bio-synthesis of total protein, contents of the overioles of *S. littoralis* females as compared with normal female. Also **El- Sheikh, (2012)** found that treatment with both spinosad and cypermethrin significantly decreased total protein contents by about 31.5% and 48.4%, respectively**.**

Soltani and Mazouni (1992) appeared decrease total ovarian protein of *S. littoralis* following treatment by diflubenzuron on ovary in *Cydia Pomonella.* Also **Shurab *et al*.,(1999**) showed that chlorofluazuron and flufenoxuron reduced the total protein in ovary in *A. ipsilon* female. The

decreased ovarian protein may be due to decreased larval haemolyph, protein as a result of treatment.

3.2. Determination of total lipids

The study of flight energetics, it was shown that after locusts fly for more than a few minutes there is an increase in the lipid content of the haemolymph. The increased lipid is mainly in the form of diacylglycerol (DG) as a transport form of metabolic lipid. A key point is, this increase in lipid concentration occurs even though there are considerable increases in the rate of lipid metabolism in flight muscles and in the rate of lipid uptake from circulating haemolymph. We understand this to mean that even though lipids are being rapidly removed from the haemolymph by transport into flight muscles there is a net increase in haemolymph lipid concentration. It appeared from these observations that the haemolymph concentration of lipid is regulated. The regulatory mechanism was guessed to be hormonal regulation **(Manju, 2005)**.

The chemical analysis of total crude lipids and fatty acids composition of the aphid, *Brevicoryne brassicae* were carried out to detect the effect of the entomopathogenic fungus *Verticillium lecanii* on these constituents. The infected aphids had the significant lower values of total crude lipids compared with the values of the uninfected

ones. The reduction of total crude lipids among infected aphids may be due to their use and consumption by the germinated spores and conidial of the fungus which required it for nutrition **(Sewify and Moursy, 1993)**. The concentration of haemolymph plasma lipid of mycosed fungus-infected locusts was significantly less than that of uninoculated control locusts. One possible reason is that the reduced food intake by the mycosed insects was insufficient to maintain haemolymph lipid levels however; 72hr. of starvation had no significant effect on the haemolymph lipid concentration. Furthermore, extended starvation (5 days) even led to a significant increase in whole haemolymph lipid **(Gillespie *et al*., 2000)**. Also **(Mettaweh *et al*., 2001)** showed that the haemolymph lipids were decreased compared to their levels in the uninoculated nymphs with fungi *M. anisopliae, M. flavoviridae, and B. bassiana* during all periods (7, 12, 17, 22 days after infection) with dose $5x10^6$ spores/nymph, also there was a positive correlation between the post inoculation period and the reduction in the lipid content. **Abd El-Kerim, (2002)** found that Usage Bancol occurred decrease in haemolymph lipid between male and female of 5^{th} nymphal instar of desert locust, *S. gregaria* where 82.16% for female lipid and 75.15 % for male. Mycosed

locusts have significantly lower haemolymph lipid and carbohydrate concentration than controls this could in part account for the poor flight performance of fungus-infected. When locusts start flying, they initially use carbohydrate as a fuel subsequently they make use of their more substantial stores of lipid **(Seyoum *et al*., 2002)**. In *Locust migratoria*, activation of phenoloxidase in the haemolymph in response to injection of laminarin is age-dependent: being absent in fifth instar nymphs and newly emerged adult and only becoming evident four days after the final moult. This pattern of change in phenoloxidase activation correlates with the pattern of change in the concentration of apolipophorin-III in the haemolymph **(Mullen and Goldsworthy, 2003)**. **Abdel-Aal, (2012)** showed that chlorofluazuron , tebufenozoid and pyriproxyfen decreased the bio-synthesis of total lipid contents of the overioles of *S. littoralis* females as compared with normal female.

Decreased in total lipid in the overioles of *S. littoralis* treated with Chlorofluazuron and Tebofenozoid may be interperted by the damage of both the nurse cells and follicular epithelium as shown in this study, where these tissues were found to contributed in lipid deposition to the devolping oocyte **(Tirpathi and kumar, 1982). Shurab *et al*.,(1999)** recorded that chlorofluazuron and

flufenoxuron reduced the total lipid in ovary in *A. ipsilon* female. Moreover, it is probably that these compounds affected the fat bodies of *S. littoralis* during the period following larval treatment with them which ultimately led to decreased lipid deposition in the devolping oocyte **(Abdel-Aal, 2012).**

3.3. Determination of total carbohydrates

Carbohydrates play an important role for the structure and functions of all tissues during metamorphosis as well as for the normal functioning of the male and female reproductive organs and embryonic development (**Chippendale, 1978**). On the other hand, the carbohydrate content in the haemolymph is an important indicator of the level of metabolism in insects, and a dynamic balance of the absorption, metabolism, and utilization by different tissues **Zhu *et al*. (2012).** Therefore, a study was carried out aiming to evaluate the effects of three IGRs, Pyriproxyfen, tebufenozide and lufenuron, on the carbohydrate content of both the haemolymph and fat body of the economically dangerous locust, *Schistocerca gregaria*.

The haemolymph of lepidopteran hosts of *Metarhizium anisopliae*, including *Manduca Sexta*, has a high concentration of soluble sugars **(Racioppi and**

Dahlman, 1980). prominent among them is the disaccharide and trehalose. The trehalose is also the major sugar used during flight **(Becher *et al.*, 1996)**. Clearly trehalose must be viewed as a potentially important nutrient source for pathogenic fungi like *M. anisopliae* that are confined largely to the haemolymph, prior to death. Fungi can potentially use the trehalose in the haemolymph of insects by direct uptake.

Secretion of trehalose-hydrolysing enzymes may be a prerequisite for successful exploitation of this resource by the pathogen. And the concentration of trehalose in the haemolymph decreased sharply after infection. RT-PCR also revealed the *ATM1* gene's expression in the haemolymph of the infected insects. The results indicated that the acid trehalase may serve as an "energy scavenger" and deplete blood trehalose during fungal pathogenesis **(Zhao *et al.*, 2006)**.

It was found that in the locust, *locusta migratoria*, carbohydrate is the predominant energy source for the first 20-30 min. of flight **(Jutsun and Goldsworthy, 1976)**. The carbohydrate concentration in the haemolymph of females of adult grasshopper was 19.0 mg/ml compared with males was 10.1 mg/ml. Efficient use of available nutrients will be critical for Pathogenicity competetition for carbon between

the pathogen and host **(Gurwattan *et al*., 1991)**. All treatments significantly decreased total carbohydrate contents by about 26.7%, 12.8% and 44.2% for spinosad, *B. thuringiensis* and cypermethrin, respectively, as compared to control. The total carbohydrates content in 6th larval instar treated with spinosad, *B. thuringiensis* and cypermethrin significantly decreased, Spinosad causes persistent activation of nicotinic acetylcholine (ACT) receptors. Because ACT and Spinosad act on receptors simultaneously, they likely operate at different target sites; this apparently is unique nicotinic agonists, which normally compete directly with ACT for binding sites **(Salgado, *et al*., 1997).** Also **Shurab *et al*., (1999)** recorded that chlorofluazuron and flufenoxuron reduced the total carbohydrate in ovary in *A. ipsilon* female. When infection grasshopper, *Eurpepocnemis plorans plorans* with *M. anisopliae*, *M. flavoviridae,and B. bassiana* ($5x10^6$ spores/nymph) conidia suspension indistiled water and 0.05% tween-80 and the control with distilled water and 0.05 tween-80 only taken the haemolymph samples during (7, 12, 17and 22days after infection) found decrease in haemolymph carbohydrate contents in all period after infection than control. Moreover, in most cases as the post inoculation period increased the carbohydrates content

decreased **(Mettaweh *et al.*, 2001)**. In 3 days of infection by fungus, *M. anisopliae* haemolymph carbohydrate declined significantly during tethered flight of control locust but not in mycosed individuals. Reduced flight performance by mycosed locusts may be due to one or more of a number of factors, in particular, the interference with the availability of the metabolic fuels. Concentration of carbohydrate in the haemolymph plasma of starved insects was not significantly different from that of fed insects. The carbohydrate concentration increased substantially in insects of both treatments and was significantly greater in fed than that starved locusts **(Seyoum *et al.*, 2002)**. Also decreased in haemolymph carbohydrate contents to 5^{th} instar nymphs of desert locust, *S. gregaria* were treated with Bancol extract where 69.12% and 93.42% to control **(Abd El-Kerim, 2002)**. Topical application of the *Metarhizium anisopliae* var. *acridum* special strain CQMa102 to the locust *Locusta migratoria manilensis* resulted changes in the concentrations of trehalose and glucose in the haemolymph. Trehalose decreased significantly during mycosis of locusts by *M. anisopliae.* All these results suggested that this fungus may take advantage of competing nutrient utilization against the insect by its trehalose-hydrolyzing enzyme secretion. It

may provide fundamental knowledge for fungal pathogenesis **(Hua *et al*., 2007)**. **Abdel-Aal, (2012)** showed that chlorofluazuron , tebufenozoid and pyriproxyfen decreased the bio-synthesis of total carbohydrate contents of the overioles of *S. littoralis* females as compared with normal female. According to these findings decreased in total carbohydrate in the ovariole of *S. littoralis* treated with Chlorofluazuron and Tebofenozoid may be accounted for the histological damages of both the oocyte and follicular epithelium.

3.4 Determination of total cholesterols

It well established that cholesterol is the biosynthetic precursor of ecdysone hormone in insects. The application of the entomopathogenic fungi, *M. anisopliae, M. flavoviridae,and B. bassiana* ($5x10^6$ spores/nymph) on 5^{th} instar nymph of grasshopper, *E. plorans plorans* showed a decrease in the cholesterol level in haemolymph of the treated nymphs also there was appositive correlation between post-inoculation period and the reduction in cholesterol content **(Mettaweh *et al*., 2001)**.

3.5. Determination of acid phosphates activity

Organic phosphates such as glucose-1-phosphate and trehalose-6-phosphate are found in high concentrations in the haemolymph **(Pannabecker *et al*., 1992; Wyatt, 1991)**.

These compounds could provide an important source of energy and phosphate for development. Efficient utilization of phospharylated organic molecules, however, requires production of phosphatases, which hydrolyse phosphate from orthophosphate monoesters. **Vincent *et al*. (1993)** found that acid phosphates activity increased dramatically in the haemolymph of grasshoppers infected with the fungus *B. bassiana*. Hypersynthesis of this enzyme also occurs after bacterial infection in molluscs **(Cheng and Butler, 1979)**. During mycosis of the desert locust by *M. anisopliae* var. *acridum* host haemolymph acid phosphatase appeared to be suppressed while new acid phosphatase isoforms appeared that had similar PIs to *in vitro* fungal enzymes **(Xia *et al*., 2002)**. Acid phosphatase activity also increased in the haemolymph of the desert locust, *S. gregaria* on the 3rd day after inoculation with the entomopathogenic fungus *M. anisopliae* var. *acridum*. This coincided with decline in the total haemocyte count and a marked reduction in the proportion of plasmatocytes and coagulocytes that stained positive for acid phosphatase. Therefore a priori it seemed unlikely that the extra acid phosphatase in infected insect came from the host a fungal origin for the enzyme was suggested by the identification of acid phosphatase isoforms from haemolymph of

different treatments. Control inoculated (oil only) insects had an acid phosphatase at a PI of 4.3 that was stimulated further by the injection of laminarin. Additional isoforms appeared at around 7.3-7.5 in the laminarin treatment. However, the 4.3 isoforms appeared to be suppressed in the insects infected with *M. anisopliae* var. *acridum*. The band intensity was more like that of the control than the laminarin-injected insects. Two new isoforms appeared later on in infection. These enzymes had PIs that corresponded to some of the acid phosphatase produced *in vitro* by the fungus. The results are discussed in the light of the possible benefits of secreted fungal acid phosphatase to the pathogen **(Xia *et al*., 2000)**.

3.6. Determination of phenolxidase activity

When a foreign invading organism is too large to be phagocytosed, it becomes encapsulated by multi layers of haemocytes and/or melanin coat. Two types of encapsulation are distinguished in insects: cellular encapsulation, mainly described in Lepidoptera, and melanotic (humeral) encapsulation more typical for Diptera **(Gotz, 1986)**. Cellular encapsulation has been described to occur with or without participation of haemocytes in contrast to melanotic encapsulation which is always

associated with phenoloxidase activity **(Christensen and Severson, 1993)**.

Phenoloxidase is present in host cuticle and catalyse the hydroxylation of mono and di-phenols to quinone intermediates which either autopolymerise to melanin or react which proteins to from protein-catechol complexes. This is important in the sclerotization process **(Sugumaran, 1990)**. Those are some evidence to suggest that some phenoloxidase-derived quinines and melanin have fungi static and fungicidal activates *in vitro*. However, they are not always lethal *in vivo* and may have specific importance against opportunistic pathogens **(Sugumaran and Kanost, 1992)**.

Phenoloxidase activity decreased during the course of infection with the entomopathogenic. The decline in total haemocytes count in later stages of infection could be a result of haemocytes aggregation (nodule formation). Significantly larger numbers of nodules were found in infected-insects with a maximum of 775 ml on day one after inoculation. The nodules were black in color probably due to the production of melanin by the enzyme phenoloxidase which was present in greater quantities in the serum than in the haemocytes **(Gillespie and Khachatourians, 1992)**. Phenoloxidase is present in the

haemolymph as an inactive precursor; prophenoloxidse which is actived via a cascade mechanism has important roles in wound healing and the recognition of foreign material in haemolymph. Phenoloxidase is a copper-containing enzyme that catalyses and formed melanin which is often seen on host cuticle after infection and surrounding the capsules and nodules of parasitized hosts **(Gillespie *et al*., 2000b).** When the fifth instar nymphs and adult male locusts were injected with 20µg of Laminarin or with 20µg of Laminarin plus 20Pmol of AKH-I showed that injection of Laminarin alone into adult Locusts > 5 days after emergence resulted in activation of haemolymph prophenoloxidse, which remained elevated 3h after injection. Co-injection of 20mol of AKH-I resulted in the maintenance of even a higher level of phenoloxidase activity 3h after injection but a response was not present in fifth instar nymphs and newly emerged adults, nor was there any phenoloxidase activation in such locusts earlier than 3h after injection a response was present on day 4 of adult life and remained up to day 5 **(Mullen and Goldsworthy, 2003)**. In adult locusts, starvation for 48h resulted in significantly greater increase in the phenoloxidase activity in the haemolymph in response to injection of Laminarin compared with the response to

injection of Laminarin into fed animals. Phenoloxidase activation in starved adult in response to Laminarin was of the same magnitude as that measured in response to injection of Laminarin and AKH-I in fed adults. Starvation did not significantly affect the phenoloxidase response to co-injection of Laminarin and AKH-I in adults. Starvation had no statistical significance on phenoloxidase activation in fifth instar nymphs in response to Laminarin with or without co-injection of AKH-I **(Goldsworthy *et al.*, 2003)**. Phenoloxidase which catalyses the production of cytotoxic quinones that can undergo polymerization to melanin. It has been shown that injection of laminarin, (β-1, 3 glucan typical of these present cell walls) induces immune responses in both adult and larval locust but applying isolates of *Metarhizium* (Met 728) had no effect on the phenoloxidase activity in the haemolymph or on mortality due to failure to penetrate the insect cuticle **(Mullen and Goldsworthy, 2006).**

3.7. Determination of Peroxidase activity

To test the effect of *M. anisopliae* fungi on the Locusts biochemistry and physiology, fungi at doses 10^3, 10^6 and 10^{12} spores/ml were applied with two different treatment methods; fungi spray and fungi soil application. The insects were treated under either non-

starvation and/or starvation conditions. The insect enzymes Phenoloxidase, Peroxidase activities were affected, fluctuated between increasing and decreasing by fungi infection. The results revealed that there were significant differences ($P \leq 0.001$) in Peroxidase activity at two different treatments and all three doses. The highest elevation in Peroxidase activity was recorded at highest dose of fungus by spray application with ($F_{(3, 8)} = 693.36$, $p \leq 0.001$). At soil treatment slightly prohibit in peroxidase activities at all doses with ($F_{(3, 8)} = 33.59$, $P \leq 0.001$) (**Elbanna, *et al*., 2012).**

MATERIALS AND METHODS

1. Rearing of test insects

Nymphal instars of the desert locust, *Schistocerca gregaria* Forskal (Orthoptera: Acrididae) 2 days after ecdysis were used in all experiments. The individuals were taken from the sock culture maintained for several generations at the Locust Research Section, Plant Protection Research Institute (PPRI), ARC, Dokki, Giza. The insects were reared in the laboratory according to **(Robert *et al.*, 2002)** in wooden framed cages measuring (60 x 60 x 70cm). The cages sides were made of wire gauze with glass top and a small door in the front side, for daily routine feeding, cleaning and handling. A sand layer of 20 cm deep was spread in the bottom of each cage for egg lying, and kept until hatching. Each cage was illuminated and heated by a 100 watt electric bulb. Nymphs were transferred to other cages measured (100 x 100 x 120cm) without a sand layer for rearing the progeny. All cages were placed on a large table and a suitable container was filled with water and placed under every table leg. The electric wires were painted with grease to protect nymphs from ants attack. The daily routine work includes removing the previous uneaten food, faeces and dead nymphs before introducing the fresh food. Both hoppers and adults were

fed on branches of Berseem, *Trifolium alexandrinum* and dry wheat bran fortified with 5% yeast powder as a source of vitamin B_1. Fortified bran was introduced in Petri dishes. The locusts' cages were kept at 30 ± 2 °C and 30-50 % R.H.). When the cages were empty due to the termination of the life cycle for each generation or when the locusts were removed for experimental purposes, they were disinfected by a diluted solution of an antiseptic agent to avoid contamination with harmful microorganisms.

2. Pesticides

2.1. Spinosad

Common Name: Spinosad 22.٤% SC (Suspension Concentrate)

Trade Names: Tracer®

Chemical Name:

spinosyn A

2-((6-deoxy-2,3,4-tri-*O*-methyl-a-Lmannopyranosyl) oxy)-13-(((5- dimethylamino)tetrahydro-6-methyl- 2*H*-pyran-2-yl)oxy)-9-ethyl- 2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16 b-tetradecahydro-14-methyl-1*H*-asindaceno(3,2-d)oxacyclo-dodecin-7,15- dione

spinosyn D

2-((6-deoxy-2,3,4-tri-*O*-methyl-a-Lmannopyranosyl) oxy)-13-(((5- dimethylamino)tetrahydro-6-methyl- 2*H*-pyran-2

yl)oxy)-9-ethyl- 2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16 b-tetradecahydro-4,14-dimethyl-1*H*-asindaceno(3,2 d) oxacyclododecin-7,15- dione **(Wynetta, 2003)**.

Source: Dow Agroscience Co.

Concentration: 65ml/100L

2.2. Chitin-synthesis inhibitor

Common name: Hexaflumuron 10% EC (Emulsifiable Concentrate)

Trade name: Consult®

Chemical name: [N 3, 5-dichloro-4- (1,1,2,2-tetrafluroethoxy) (phenyl-amino) carbony l-2,6- difluroben- zamide] **(Bakr, *et al*., 2009)**

Concentration: 60ml/100L

3. Bioassay of fifth nymphal instar of desert locust, *S. gregaria*

Nymphs of 1-2days old of the 5th nymphal instars of *S. gregaria* during synthesis and deposition of the newly cuticle **(Bakr *et al*. 2008)** were treated by feeding technique with Consult and Spinosad as the following: Leaves of *Trifolium alexandrinum* were dipped in 150ppm, 75ppm, 36ppm and 15ppm from Spinosad and ١٢٠ppm, 60ppm, 30ppm and ١٢ppm from consult for two minutes. Then leaves were air dried before being offered to the nymphs for feeding on it. Five replicates of 100 nymphs

were subjected to each of the treated leaves. After feeding for 24 hours on the treated leaves after that mortality counts or malformed individuals were recorded. Mortality data were summarized as estimates of the Median Lethal Concentration (MLC). LC_{25}, LC_{50} and LC_{90} values and slope of regression lines were calculated by using (Lpd line) software for calculating and drawing the mortality curve according to **Finney Method (1971)**.

4. Insecticides combination:

Nymphs of 1-2days old of the 5^{th} nymphal instars of *S. gregaria* during synthesis and deposition of the newly cuticle **(Bakr *et al*. 2008)** were treated by feeding technique with Consult, Spinosad and their mixture as the following: Leaves of *Trifolium alexandrinum* were dipped in ١٨ppm from spinosad (at LC_{50} value) alone , ١٢٣ppm from consult (at LC_{50} value) and 9ppm from Spinosad (at LC_{25} value) and ٥٠ppm from consult (at LC_{25} value) as mixture for two minutes. Then leaves were air dried before being offered to the nymphs for feeding on it. Five replicates of 100 nymphs, each of 20 nymphs, were subjected to each of the treated leaves, after that mortality counts or malformed individuals were recorded.

The joint action between Spinosad and consult was determined according to the equation of the co-toxicity factors (C.Fs) given by **Mansour *et al*. (1966)** as follows:

C:F= (Observed mortality %

_ Expected mortality % / Expected mortality %) X 100

Where:

Observed % mortality: the mortality percentage among treated insects with Spinosad and consult combination.

Expected % mortality: the sum of mortality percentage among treated insects with each spinosad alone and consult alone.

This factor was used to differentiate between the results into three categories:

1- Potentiation (a positive factor of +20 or more).
2- Antagonism (a negative factor of -20 or more).
3- Additive (an intermediate value between -20 and +20).

5. Characterization of the haemolymph of the 5th nymphal instar after treatment with Spinosad and consult

5.1. Samples collection and preparation

Fifth instar nymphs were taken for experiments where, the biochemical effects of Spinosad (at LC_{50}=19ppm) and consult (at LC_{50}=123ppm) and their mixture (at Spinosad LC_{25}=9ppm + consult LC_{25}=50ppm) on haemolymph components were evaluated. The experiments were carried out by treatment of 1-day old nymphs under laboratory

conditions. One hundred and Fifty treated nymphs were divided into five replications. Nymphs were kept in cages (25 x 25 x 60cm) with a fluorescent lamp as a light source. The control insects were placed in other cages under the same conditions in 16h light and 8h dark **(Robert *et al.*, 2002)**. Samples of the haemolymph were taken at different intervals of 2, 4, and 6 days after treatment.

The haemolymph was collected through a fine puncture in the hind leg membrane and transferred into clean dry centrifuge tubes. Few crystals of phenylthiourea were added to prevent melanization before analysis. A known volume of the collected haemolymph was centrifuged on 13000 rpm to 15 min. to remove blood cells and pigments. Then the supernatant collected for analyses **(El Gawhary, 1997)**.

5.1.1. Determination of the total proteins

Total proteins were determined by the method of **Bradford (1976)**. Preparation of protein reagent as Coomassic Brilliant Blue G-250 (100 mg) was dissolved in 50 ml ethanol 95%. To this solution 100 ml 85% (w / v) phosphoric acid were added. The resulting solution was diluted to a final volume of 1 liter. The standard solution was prepared by dissolving 6 mg Albumin protein in 100 ml distilled water.

5.1.1.1. Protein assay

Sample solution (haemolymph) 50 µg were pipetted into test tube and the same volume of standard solution pipetted into anther tube. The volume in the test tubes was adjusted to 0.1 ml with phosphate buffer (pH 6.6). 5 ml of protein reagent were added to the test tubes and the contents were mixed either by inversion or vortexing. The absorbance at 595 nm was measured after 2 min. and before 1hr. Calculation was measured as mg proteins = (Absorbance of test / Absorbance of standard) x 6

5.1.2. Determination of total lipids

Total lipids were estimated by the method of **Knight *et al*. (1972)**

5.1.2.1. Phosphovanillin reagent

Pure vanillin (0.6 gm) was dissolved in 10 ml ethylalchohol and completed to 100 ml with distilled water. 400 ml of concentrated phosphoric acid were added, and the solution was stored in dark glass bottle at room temperature. The standard solution was a known concentration of lipid (5mg / ml) consists of, Oleic acid and Palmitic acid in a ratio of 3: 7 was prepared by dissolving Oleic acid (350 mg) and Palmitic acid (150 mg) in absolute ethanol (100 ml). Procedure was 250 µl of sample solution was added to conc. Sulfuric acid (5 ml) in a test tube and

heated in a boiling water bath for 10 min. after cooling to room temperature, the digest (500 µl) were added to Phosphovanillin reagent (6.0 ml). After 45 min. the developed color was measured at 525 nm. The standard solution (250 µl) was used and treated in the same manner as the sample solution. Calculation was measured as mg lipids = (Absorbance of test / Absorbance of standard) x 5

5.1.3. Determination of total carbohydrates

Total carbohydrates were determined in acid extracts by the phenol sulfuric acid reaction **(Dubois *et al.*, 1956)**.

5.1.3.1. Procedure

One hundred microlitres sample or 100µl of standard solution were added into a colorimetric tube to 0.5 ml of phenol (20 percent w/v). Then 5 ml of concentrated sulfuric acid were added rapidly with shaking. The tubes were allowed to stand 10 min. then they were shaken and placed for 10-20 min. in water bath at 25 to 30 ºC before readings. Blanks were prepared by substituting distilled water for the sugar solution. The absorbance of characteristic yellow-orange color is measured at 490 nm against blank. Carbohydrates are expressed as mg glucose/100ml haemolymph. The standard solution has a concentration of 100mg glucose/100ml, calculation was measured as mg

carbohydrate = (Absorbance of test / Absorbance of standard) x 100

5.1.4. Determination of total cholesterol

Total cholesterol was determined by the enzymatic colourimetric method of **Richmond (1973)**. All reagents used in this determination were supplied by Ames Division Miles Lab. Inc, England. Reagents was

Solution (1) contains buffer-enzyme-chromogents.

Solution (2) contains Phenol.

Solution (3) one volume of solution (1) + one volume of solution (2). Where one liter of solution (3) contains the following, phosphate buffer 10 mmol pH = 7.7 Cholesterol ester hydrolase ≥ 140µl. cholesterol oxidase ≥ 80 µ .peroxides≥ 500µ. Phenol 10 mmol, 4 amino-phenazone 0.5 mmol potassium ferrocyanide 2µ mol and Sodium cholate 3 mmol.

Solution (4): is the standard solution

The reagents were kept at 5 °C for one month.

Solution (1) and (2) were mixed instantly before use.

5.1.4.1. Procedure

Volume (2-2.5 ml) of solution was pipetted into a test tube to form the blank. The standard test tube was made by placing the standard solution (3) (2.5 ml) and solution (4) (0.02 ml) into a test tube. The standard solution

was added to this tube and 0.2 ml of the unknown sample. After well mixing the test tubes were incubated at 37 °C for 10 minutes or at room temperature for at least 30 minutes and measured at wave length of 410 nm cuvette. Calculation was measured as mg cholesterol / 100 ml filtrate haemolymph = (Absorbance of test / Absorbance of standard) x 200

5.1.5. Determination of acid phosphates in haemolymph

Acid phosphatase (Ac-pase) was determined according to the method described by **Powell and Smith (1954)**. In this method, the phenol released by enzymatic hydrolysis of disodium phenylphosphate, reacts with 4-aminoantipyrine, and by the addition of potassium ferrricyanide, the characteristic brown color is produced.

The reaction mixture consist 1 ml citric buffer (pH 4.9), 1 ml of 0.01 M disodium phenyl phosphate (substrate), and 0.1 ml nymphal haemolymph. Mix gently and incubate for exactly 30 minutes at 37 ºC. At the end of incubation period, 0.8 ml of NaOH was added to stop the reaction. Then added 1.2 ml of $NaHCO_3$, followed by 1 ml of 4-aminoantipyrine solution and 1 ml of potassium ferrricyanide. The produced color was measured, immediately, by spectrophotometer at 510 nm. The enzymatic activity is expresses as mg phenol released/ ml

haemolymph. Phenol standard curve was prepared as a stock of phenol was prepared by dissolving 1 gm pure crystalline phenol in 1 liter HCl. 10 ml of the stock solution (containing 10 mg) was diluted to 100 ml with distilled water. Aliquots of 0.05, 0.1, 0.2, 0.3 and 0.4 ml of the diluted phenol (equal to 5, 10, 20, 30, and 40 mg phenol) were pipetted into test tubes and the volume was completed to 1 ml with distilled water. 1.1 ml of buffer was added followed by 0.8 ml of NaOH, 1.2 ml of NaHCO3, 1ml of aminoantipyrine and 1ml of potassium ferrricyanide. Each tube was mixed well after each addition. The developed color was measured at 510 nm.

5.1.6. Determination of phenoloxidase in haemolymph

Determination of phenoloxidase activity was based on a method described by **Ishaaya (1972)** with some modification. The reaction mixture consisted of 200 µl enzyme solution, 2ml phosphate buffer (0.2 M, pH 7) and 0.5 µl 2 % Catechol. The reaction mixture was incubated at 25 ˚C. The activity was then recorded after 2 min from the beginning of the reaction at absorbency 470 nm.

5.1.7. Determination of Peroxidase (POD) in haemolymph

Peroxidase activity was determined according to **Vetter *et al*. (1958)**. To the sample (200µl), in which the color is to be formed, the following reagents are added: 1ml of

1% o-phenylenediamine (in 95% ethyl alcohol; fresh every 4 hours) and 1ml of 0.3% hydrogen peroxide (in distilled water). The reaction is allowed to proceed for 5 minutes at which time it is stopped by adding 2 ml of saturated sodium bisulfite. The reagent blank for each sample is prepared by adding a dye, followed by the sulfite, and then by hydrogen peroxide. The enzyme is inhibited by the sulfite so that it is inactive when the hydrogen peroxide is added. The samples are centrifuged at approximately 3000 RPM for 5 minutes. The clear supernatant is decanted into a colorimeter tube and its absorbance recorded at 340µm. the colorimeter is set at 100% transmittance with the corresponding blank for each sample. The enzyme activity was expressed as the change in absorbency at (ΔOD340)/min/gm.

6. Statistical analysis

The percentage of nymphal mortality was corrected according to Abbotts formula **(Abbott. 1925)**

Corrected % = {1 – (n in T after treatment/ n in Co after treatment)**}*100**

Where: n = Insect population, T = treated, Co = control

Lc_{25}, Lc_{50}, Lc_{90} values and slope of regression lines were calculated by using (Lpd line) software for calculating

and drawing toxicity lines according to **Finney Method (1971)**.

Other Data were analyzed by analysis of variance (ANOVA) means, within row, bearing different subscripts are significantly different ($P<0.05$)**.**

Results

1. Effectiveness of Spinosad against 5th nymphal instar of the desert locust, *Schistocerca gregaria*

Usage of Spinosad in different concentrations on one-day old 5th nymphal instar of the desert locust, *S. gregaria* caused different mortality percentages. Difference in the response percentage depended on concentration of Spinosad, and periods after application so, these results were different. This is appearing in data in **Table (1)**.

Data cleared that the mortality percentages of the 5th nymphal instar of *S. gregaria* were 45.1, 69.8, 85.4 and 100% 3 days after treatment with 15, 37, 75 and 150 ppm of spinosad, respectively comparing with control (0.0 %).

Table (1) Mortality % of 5th nymphal instar of the desert locust, *S. gregaria* after treated with different concentrations of Spinosad.

Conc. / Time	15ppm	37ppm	75ppm	150ppm	Control
1st day	21	30.9	42.1	51.3	0
2nd day	38.2	54.6	70.5	83.6	0
3rd day	45.1	69.8	85.4	100	0

The probit regression line for the tested concentrations was illustrated graphically in **Figure (1)**. Data in **Table (2)** show $LC_{25,}$ LC_{50} and LC_{90} values for the tested Concentrations of Spinosad against 5^{th} nymphal instar of desert locust, *S. gregaria*. Where $LC_{25}$was 8.8ppm, LC_{50} was 18.7ppm and LC_{90} was77.9ppm. Also, Slope value was 2.0690 ± 0.2303.

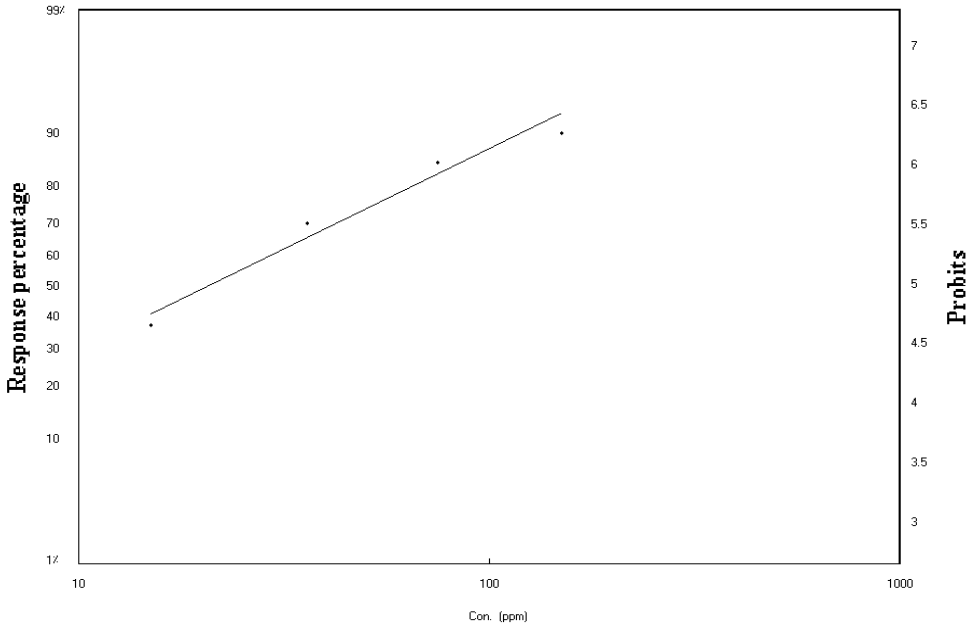

Figure (1). Toxicity line of Spinosad on the 5^{th} nymphal instar of the desert locust.

Table (2) The LC_{25}, LC_{50} and LC_{90} values of the 5^{th} nymphal instar of the desert locust, *S. gregaria* treated by Spinosad.

LC	Conc. ppm	Lower limit ppm	Upper limit ppm
25	8.8365	5.7709	11.7986
50	18.7192	14.5291	22.6689
90	77.9332	63.0724	104.3782

2- Effectiveness of consult against 5th nymphal instar of the desert locust, *S. gregaria*

Results in **Table (3)** show the effectiveness of consult (Chitin synthesis inhibitor) on the 5th nymphal instar of *S. gregaria* during one-day old by feeding technique. Data cleared that the percentages of nymphal mortality were 6.9, 12.3, 27.8 and 51% after 10 days of treatment with 12, 30, 60 and 120ppm of consult, respectively comparing with control (0.0%).

The probit regression line for the tested concentrations of consult was illustrated graphically in **Figure (2)**. Data in **Table (4)** show the $LC_{25,}$ LC_{50} and LC_{90} values for the tested Chitin synthesis inhibitor (Consult), when applied against 5th nymphal instar of the desert locust, *S. gregaria*. The LC_{25}, LC_{50} and LC_{90} of the consult recorded 50ppm, 123ppm and 662ppm, respectively. Also Slope was 1.7528+/- 0.2370 for the tested Concentrations of consult against 5th nymphal instar of the desert locust, *S. gregaria*.

Table (3) Mortality % of 5th nymphal instar of the desert locust, *S. gregaria* treated with different concentrations of consult.

Conc. / days	15ppm	30ppm	60ppm	120ppm	Control
1st	0	0	0	0	0
2nd	0	0	2	2	0
3rd	0	2	4	5	0
4th	2	4	6	9	0
5th	2	4	8.6	18	0
6th	4	6	10.4	23	0
7th	5	6	16.2	30	0
8th	5	8.6	18.2	36	0
9th	5	10.8	22	42	0
10th	6.9	12.3	27.8	51	0

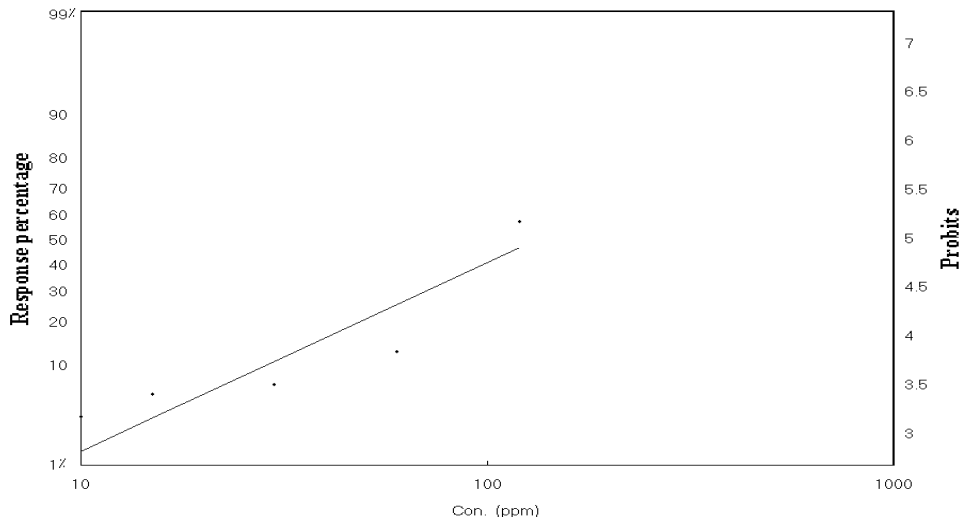

Figure (2). Toxicity line of consult on the 5th nymphal instar of the desert locust.

Table (4) The LC_{25}, LC_{50} and LC_{90} values of the 5th nymphal instar of the desert locust, *S. gregaria* treated by consult.

LC	Conc. ppm	Lower limit ppm	Upper limit ppm
25	50.6501	41.354	61.3337
50	122.8542	96.4766	176.8222
90	661.5427	382.0608	1669.5435

3- Effect of spinosad, consult and mixture of spinosad and consult on mortality percentages in 5th nymphal instar of desert locust.

Data in **Table (5)** show the effect of spinosad at (LC_{50}=19ppm), consult at (LC_{50}=123ppm) and the mixture of them at LC_{25} (spinosad=9ppm) and (consult=50ppm) on the 5th nymphal instar of desert locust, *S. gregaria*. Also, show the co-toxicity factor of mixture of spinosad and consult against desert locust.

The mortality percentages at the LC_{50} values of the tested compounds after 7 days of the experiment ranged from 30% to 64% for both consult and spinosad, respectively.

The joint action of mixing Spinosad at concentration (LC_{25}) with consult at concentration (LC_{25}) increased mortality percentages from 23, 41.2, 49.9, 60.2, 74.9, 85 and 94 to 24, 41.6,

48, 58.9, 754, 86 and 95%, with co-toxicity factor of +4, +1, -4, -2, +1, +1 and +1 after 7 days of application (**Additive effect)**.

Table (5) Effect of mixture of spinosad and consult and their mixture on mortality percentages in 5th nymphal instar of desert locust .

Days After treatment	Spinosad[a] alone	Consult[b] alone	Expected	Observed	Co-toxcicity factor
1st	23.0	0	23	24	+4
2nd	39.2	2	41.2	41.6	+1
3rd	45.9	4	49.9	48	-4
4th	50.2	10	60.2	58.9	-2
5th	56.9	18	74.9	75	+1
6th	61.9	24	85.9	86	+1
7th	64.0	30	94	95	+1

a at concentration 19ppm b at concentration 123ppm

4. Effect of consult and the mixture on morphogenesis:

The treatment of one-day old nymphs of *S. gregaria* with tested compound; Consult induced malformations in emerged adults as showed in **Plate (1) and Table (6)** .When the 5th nymphal instar of *S. gregaria* were treated, some nymphs were unable to moult into adult stage and died without completing the moulting process. Different deformities were observed, some were able to split the old cuticle but unable to complete the moulting process but the old cuticle connected with the resulting

adults in different positions as legs or wings and some were able to complete the moulting process without any deformity in the resulting adults in low concentration of consult.

Treatment of the 5th nymphal instar of *S. gregaria* with different concentrations of consult resulted in molting disturbances which increased with the increase of consult concentrations.

Most of adult emerged were unable to fly and sluggish in walking, jumping and climbing, also they have curled wings. All adults, which resulted of treatment, showed the following morphological changes, hypertrophied and twisted wings, absence of the most wing patches, colour changes (pink with grey, pink with yellow and pale yellow.

Table (6) Effect of consult and on mortality percentages and mlaformes in 5th nymphal instar of desert locust

Effect / Treat.	mortality	malformed	mortality through moulting	total
consult 15ppm	6.9	65.8	27.3	100
consult 30ppm	12.3	65.5	22.2	100
consult 60ppm	27.8	59.2	13	100
consult 120ppm	51	40	9	100
Spinosad 9ppm+consult50ppm	100	0	0	100

A. Normal adult locust

B. Curled wings

C.Old cuticle connected with the result adult Adults in different positions

D. Nymphs were unable to moult into stage and died without completing the moulting process

E. Twisted wings

Plate (1) Abnormal forms of 5th nymphal instar of the desert locust treated with consult.

5. Characterization of the haemolymph of the fifth nymphal instar of desert locust, *S. gregaria* after treated with consult, spinosad and their mixture.

5.1. Total protein:

In this study, the biochemical effects of Spinosad at 19ppm and consult at123ppm and their mixture on haemolymph protein contents were evaluated.

Data in **Table (7)** and **Figure (3)** show that, the levels of haemolymph protein content were significantly and negatively deceased after 2, 4 and 6 days when the 5th nymphal instar were treated during one day old with different concentrations of Consult, Spinosad and their mixture compounds comparing by control nymphs.

Table (7). Total protein (mg /ml haemolymph) of the 5th nymphal instar of desert locust, *S. gregaria* after treated with spinosad, consult and their mixture

Time / treatment	2nd day	4th day	6th day
Control	**53.3±0.907^{a}**	**58.1±1.952^{a}**	**55.6±1.277^{a}**
Consult	**51±1.212^{b}**	**50.3±1.537^{b}**	**48.7±0.971^{b}**
Spinosad	**47.1±1.053^{c}**	**47.4±0.458^{c}**	**45.7±0.306^{c}**
Consult+Spinosad	**44±0.379^{d}**	**42.1±.058^{d}**	**40.7±0.643^{d}**
LSD	**1.9971**	**2.6958**	**2.1646**

Values represent mean±SD, The same letters are not significant

The haemolymph protein levels significantly decreased in treated nymphs 2days after treatment with consult, Spinosad and their mixture, recorded 51, 47.1 and 44 mg/ml respectively compared with 53.3mg/ml of untreated nymphs. Also, haemolymph protein levels decreased in treated nymphs after 4 days of treatment, these levels were 50.3, 47.4 and 42.1 mg/ml at different treatments; consult, Spinosad and their mixture respectively compared with 58.1 mg/ml of normal nymphs, also these levels were 48.7, 45.7 and 40.7 mg/ml haemolymph compared with 55.6 mg/ml of normal nymphs after 6 days.

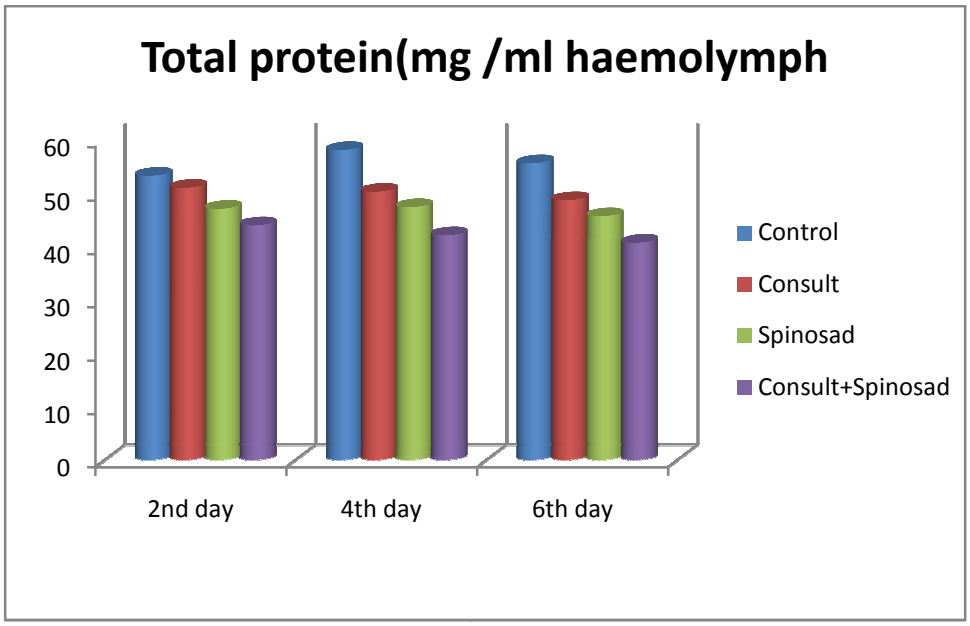

Figure (3). Total protein (mg / 100ml haemolymph) of the 5th nymphal instar of desert locust, *S. gregaria* after treated with spinosad, consult and their mixture.

5.2. Total lipids:

The effects of bio-insecticide, spinosad and IGR, consult 19ppm and 123 ppm, respectively and their mixture at 9ppm of Spinosad and 50 ppm of consult, after 2, 4 and 6 days of treatment are summarized in **Table (8)** and **Figure (4)**.

There were significant differences of total lipid contents at all treatments comparing with control. The mixture show highly significant decrease in the total lipid content, 4.57, 4.04 and 3.57mg/ml haemolymph after 2, 4 and 6 days of treatment respectively, compared to control 7.3, 7.4 and 6.95mg/ml haemolymph.

Table (8). Total lipids (mg/ml haemolymph) of the 5th nymphal instar of desert locust, *S. gregaria* after treated with spinosad, consult and their mixture

Time / Treatment	2nd day	4th day	6th day
Control	**7.3±0.227^{a}**	**7.4±0.404^{a}**	**6.95±0.064^{a}**
Consult	**6.61±0.079^{b}**	**6.44±0.061^{b}**	**6.4±0.201^{b}**
Spinosad	**5.12±0.081^{c}**	**5.06±0.068^{c}**	**4.95±0.051^{c}**
Cons+Spion	**4.57±0.097^{d}**	**4.04±0.055^{d}**	**3.57±0.427^{d}**
LSD	**0.321**	**0.9542**	**0.5259**

Values represent mean±SD, The same letters are not significant

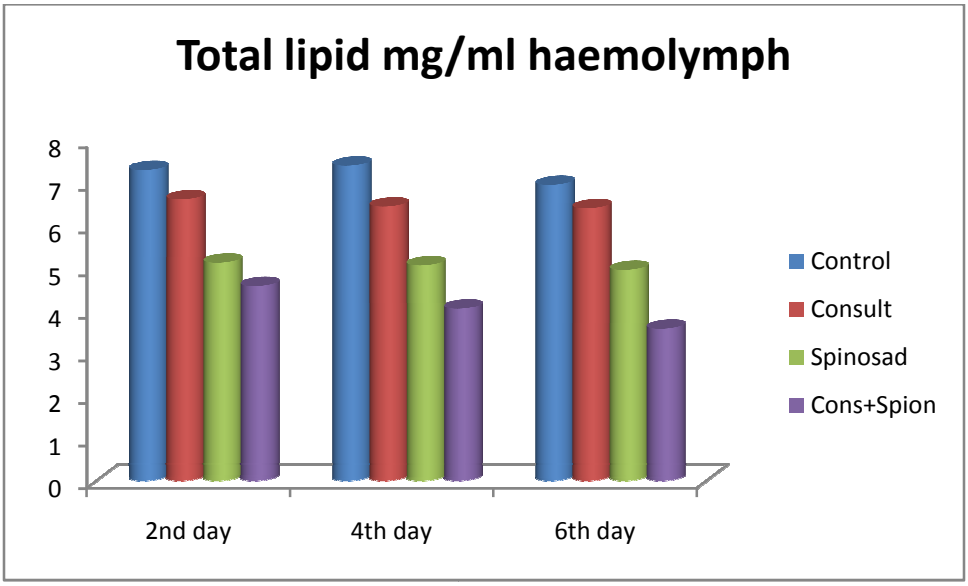

Figure (4). Total lipid (mg/100ml haemolymph) of the 5th nymphal instar of desert locust, *S. gregaria* after treated with spinosad, consult and their mixture.

5.3. Total carbohydrates:

The total carbohydrates contents of 5th nymphal instar of *S. gregaria* treatment with 19ppm, 123ppm and (9ppm+50ppm) of Spinosad, consult and their mixture was determined after 2, 4 and 6 days. The effect of Spinosad, Consult and their mixture on total carbohydrate content of treated 5th nymphal instar are summarized in **Table (9) and Figure (5)**.

There were significant differences of total carbohydrates contents at all treatments comparing with control.

Carbohydrates contents were increased significantly in treated nymphs with consult. However, Carbohydrates contents were dramatically declined in treated nymphs with Spinosad and

the mixture. Highly significant decline were recorded in carbohydrate content at Spinosad.

Table (9). Total carbohydrates (mg/ml haemolymph) of the 5th nymphal instar of desert locust, *S. gregaria* after treated with spinosad, consult and their mixture

Time / Treat.	2nd day	4th day	6th day
Control	4.48±0.434^{c}	4.58±0.135^{b}	4.72±0.040^{b}
Consult	4.81±0.078^{a}	5.44±0.067^{a}	5.73±0.075^{a}
Spinosad	3.8±0.131^{d}	3.46±0.085^{d}	3.27±0.386^{d}
Cons+Spion	4.6±0.100^{b}	4.16±0.053^{c}	4.33±0.095^{c}
L.S.D.	0.2637	0.2295	0.3848

Values represent mean±SD, The same letters are not significant

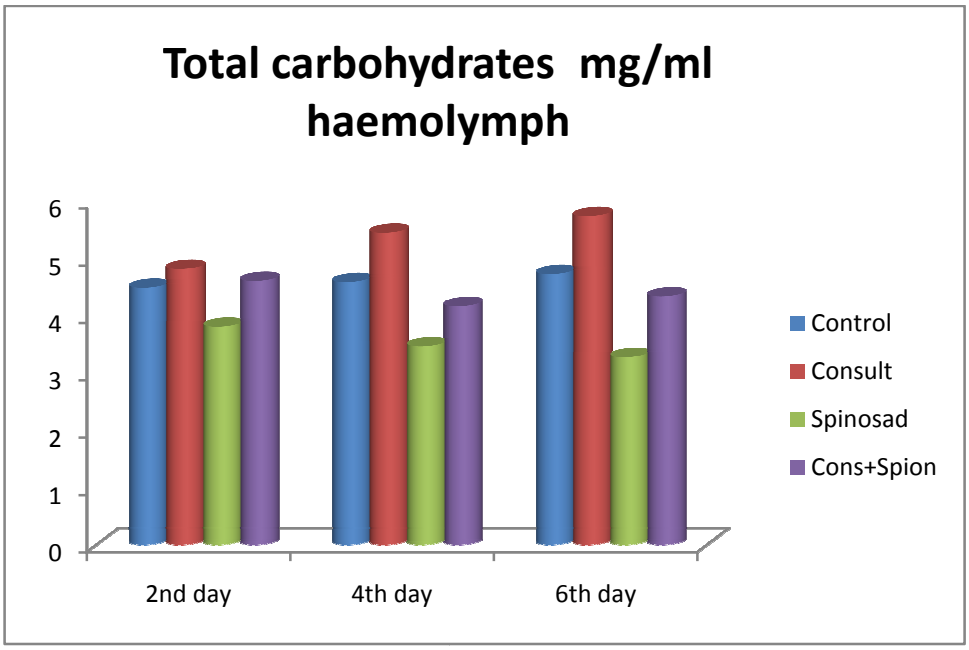

Figure (5). Total carbohydrates (mg/100ml haemolymph) of the 5th nymphal instar of desert locust, *S. gregaria* after treated with spinosad, consult and their mixture.

5.4. Total cholesterol:

Data **in Table (10) and Figure (6)** show that Treatment nymphs of the desert locust, *S. gregaria* with19ppm, 123ppm and (9ppm+50ppm) of Spinosad, consult and their mixture respectively, caused significant differences between treated and untreated nymphs during 2^{nd} , 4^{th} and 6^{th} days of application. Cholesterol contents were declined in all treatments. Highly significant decline were recorded in cholesterol content at nymphs treated with mixture.

Table (10). Total cholesterol (mg/ml haemolymph) of the 5^{th} nymphal instar of desert locust, *S. gregaria* after treated with spinosad, consult and their mixture.

Time / Treatment	2^{nd} day	4^{th} day	6^{th} day
Control	**414±9.644^{a}**	**383.3±20.11^{a}**	**403.7±7.371^{a}**
Consult	**316.3±5.686^{b}**	**323±8.888^{b}**	**290.7±6.658^{b}**
Spinosad	**250.3±0.577^{c}**	**242.6±2.517^{c}**	**234.7±4.509^{c}**
Cons+Spion	**207.3±8.737^{d}**	**207±6.245^{d}**	**195.7±5.033^{d}**
LSD	**18.4960**	**22.3707**	**13.6533**

Values represent mean±SD, The same letters are not significant

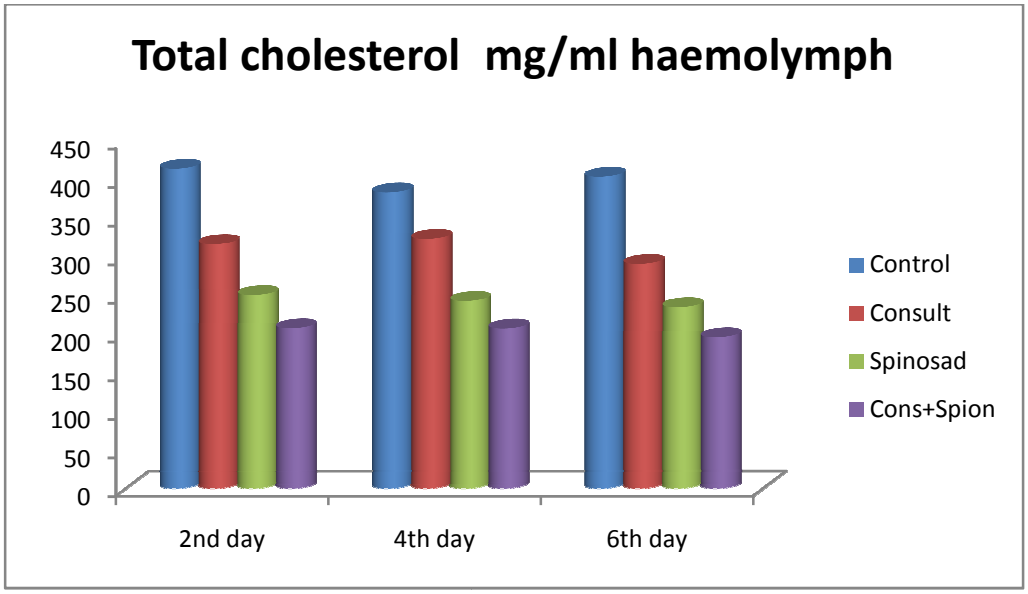

Figure (6). Total cholesterol (mg / ml haemolymph) of the 5th nymphal instar of desert locust, *S. gregaria* after treated with spinosad, consult and their mixture.

5.5. Effect of spinosad, consult and their mixture on activity of acid phosphatase (IU /ml haemolymph) in 5th nymphal instar.

Results presented in **Table (11)** and **Figure (7)** show the activity of acid phosphatase (AcP) of 5th nymphal instar of *S. gregaria* treated with 19ppm, 123ppm and (9ppm+50ppm) of Spinosad, consult and their mixture. The tested compounds significantly decreased the activity of acid phosphatase as compared to the control. The activity of acid phosphatase decreased in treated nymphs with consult, Spinosad and their mixture after 2, 4, 6 days. All treatments caused decrease in Acid phosphatase activity but, the mixture gave the highest decrease in acid phosphatase activity followed by Spinosad and consult,

where these values after 6days were 6.3, 7.76 and 8.5 IU/l respectively as compared with 10.2 IU/l in the control.

Table (11). Acid phosphatase activity (IU /ml haemolymph) of the 5th nymphal instar of desert locust, *S. gregaria* after treated with spinosad, consult and their mixture

Time / Treatment	2nd day	4th day	6th day
Control	9.58±0.366[a]	8.75±0.257[a]	10.2±0.306[a]
Consult	8.62±0.234[b]	8.13±0.154[b]	8.5±0.265[b]
Spinosad	8.08±0.100[c]	7.4±0.153[c]	7.76±0.078[c]
Cons+Spion	7.17±0.207[d]	6.58±0.126[d]	6.3±0.950[d]
LSD	0.299	0.287	0.4616

Values represent= mean±SD, The same letters are not significant **IU/ml:** International unit (the amount of enzyme which under defined assay conditions will catalyze the conversion of one micromole of substrate per minute).

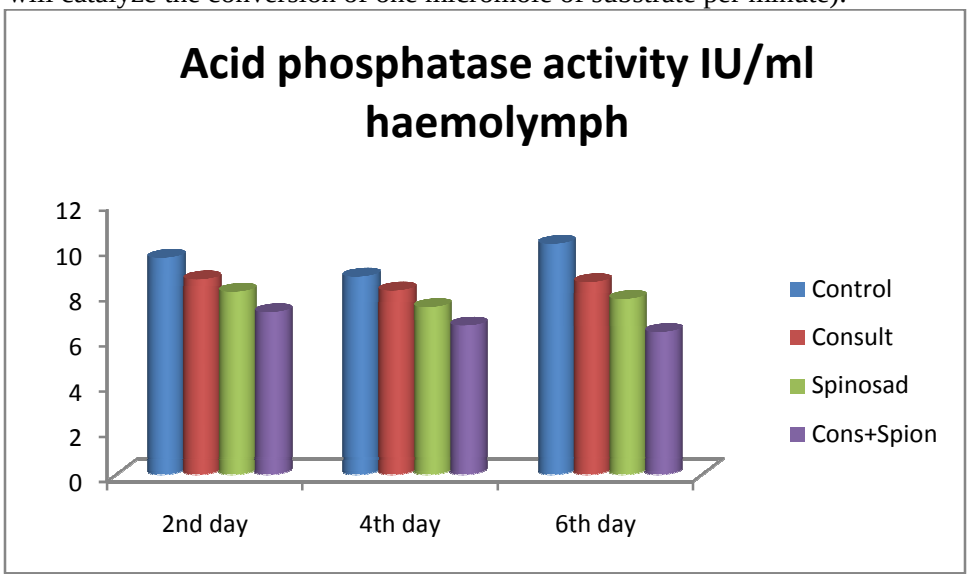

Figure (7) Effect of spinosad, consult and their mixture on activity of acid phosphatase in 5th nymphal instar

5.6. Effect of Spinosad, Consult and their mixture on activity of phenoloxidase (PO) (O.D. unit x 10^3/min./ml haemolymph) in 5th nymphal instar:

Data represented in **Table (12)** and **Figure (7)** Reveal that Spinosad, consult and their mixture caused decrease in PO activity at all treatments. The enzyme activity showed sharply decline at all treatments. The mixture gave the highest decrease in PO activity followed by Spinosad and consult, There was a significant difference in PO activities by nymphs treatment at concentrations 19ppm, 123ppm and (9ppm+50ppm) of Spinosad, consult and their mixture, respectively. Two day after application there were significant differences in phenoloxidase activity between treatments and control. However, there was a large significant decrease in phenoloxidase activity in treated insects on 6th day after application.

Treated insects with Spinosad, consult and their mixture happened continuous reduction in phenoloxidase activity by increase the time.

Table (12). Phenoloxidase activity (O.D. unit / min. / ml haemolymph) of the 5th nymphal instar of desert locust, *S. gregaria* after treated with spinosad, consult and their mixture.

Time / Treat.	2nd day	4th day	6th day
Control	3180±177[a]	3423±68[a]	3313±115[a]
Consult	2980±10[b]	2957±40[b]	2870±20[b]
Spinosad	2720±44[c]	2670±26[c]	2460±43[c]
Cons+Spion	2427±152[d]	2283±49[d]	2110±80[d]
LSD	125.8	131.6	168.9

O.D.: Optical Density

Values represent mean±SD, The same letters are not significan

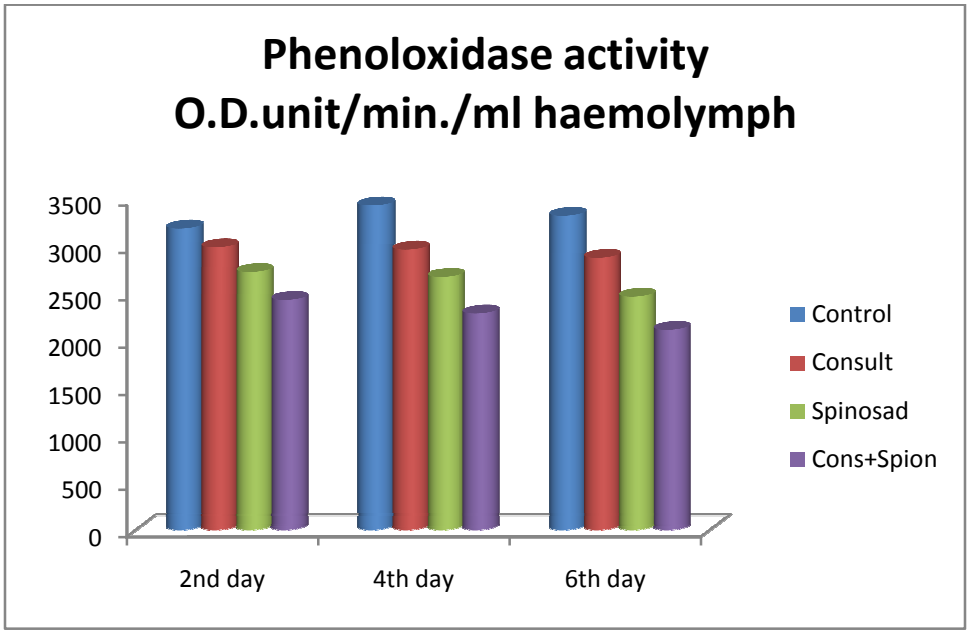

Figure (8) Effect of spinosad, consult and their mixture on activity of phenoloxidase n 5th nymphal instar

5.7. Effect of Spinosad, consult and their mixture on activity of Peroxidase (O.D. unit/min./ml haemolymph) in 5th nymphal instar:

Data in **Table (13)** and **Figure (9)** show that Treatment nymphs of the desert locust, *S. gregaria* with19ppm, 123ppm and (9ppm+50ppm) of Spinosad, consult and their mixture, respectively caused significant differences in Peroxidase activity (POD) at all treatments.

Two days after application there was significant decrease in Peroxidase activity between treatments and control. However, there was a large significant decrease in Peroxidase activity in treated insects on 6th day after application.

All treatments caused decrease in Peroxidase activity but, the highest decrease in Peroxidase activity was recorded at the mixture of Spinosad and consult.

Table (13). Peroxidase activity (O.D. unit/min./ml haemolymph) of the 5th nymphal instar of desert locust, *S. gregaria* after treated with spinosad, consult and their mixture

Time / Treatment	2nd day	4th day	6th day
Control	**9.67±0.147[a]**	**9.93±0.153[a]**	**10.97±0.208[a]**
Consult	**8.87±0.049[b]**	**8.66±0.062[b]**	**8.16±0.055[b]**
Spinosad	**7.84±0.055[c]**	**7.7±0.1[c]**	**7.76±0.151[c]**
Cons+Spion	**7.14±0.055[d]**	**7±0.1[d]**	**6.9±0.1[d]**
LSD	**0.3211**	**0.5227**	**0.9542**

O.D.: Optical Density

Values represent mean±SD, The same letters are not significant

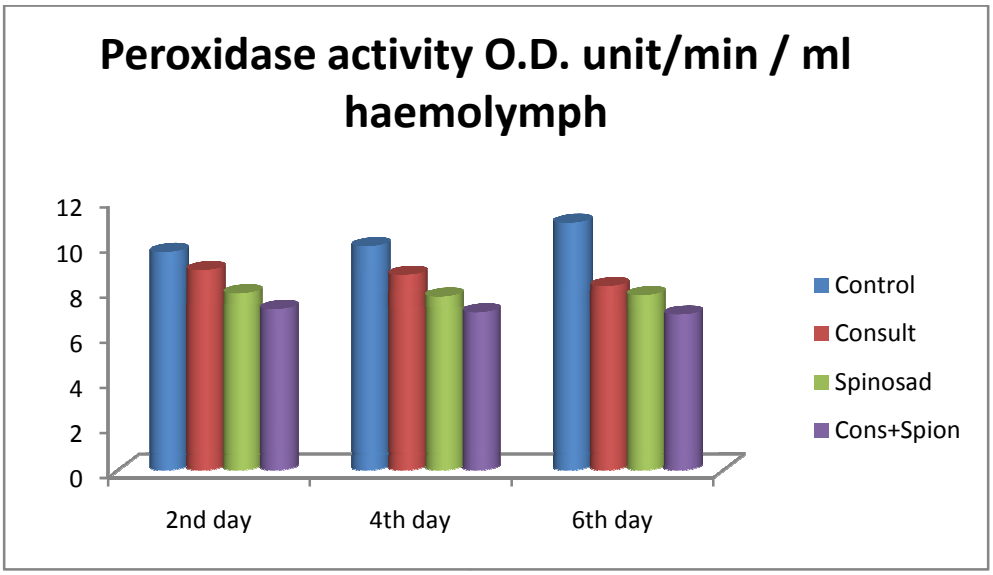

Figure (9) Effect of spinosad, consult and their mixture on activity of peroxidase n 5th nymphal instar

Discussion

1. Effectiveness of Spinosad against 5th nymphal instar of the desert locust, *Schistocerca gregaria*

Spinosad is an acetylcholine receptor agonist. The exact mechanism of Spinosad is somewhat different than that of the neonicotinoid class **(Brown, 2006)**. Recently, the quality of the environment has become a major issue. Many chemicals (pesticides) previously accepted for locust control at national and international levels would not survive rigorous environmental testing. A bio-insecticide Tracer 24SC Spinosad is a new product to control the desert locust, *Schistocerca gregaria* Forskal **(Hosny, *et al*., 2010).**

Our study clarify that the mortality percentages of the 5th nymphal instar of *S. gregaria* were 45.1, 69.8, 85.4 and 100% after 3 days of treatment with 15, 37, 75 and 150 ppm of spinosad, respectively comparing with control (0.0 %). and $LC_{25,}$ LC_{50} and LC_{90} values for the tested Concentrations of Spinosad were 8.8ppm, 18.7ppm 77.9ppm respectively.

These results are in agreement with **Amarasekare and Edelson (2004)** who mentioned that in the field Spinosad provided similar levels of mortality when nymphs of *Melanoplus sanguinipes* were exposed to 24hrs.

Demiral and Cranshaw (2006) reported that Spinosad was an effective insecticide for reducing the population density of the migratory grasshopper *Melanoplus sanguinipes*. Also **Halawa *et al.*, (2007)** found that LC_{50} value of the tested compound (Spinosad) against the newly molted 4^{th} instar larvae of *S. gregaria* recording 47.44ppm after 72 hrs and LC_{25} was 16.03ppm. **El-Gammal and Mohamed (2008)** demonstrated that two spinosyn products (Spinosad and spinetoram) proved good alternatives to the conventional insecticides in controlling the grasshopper *Catantops axillaries*. **Hosny, *et al.*, (2010)** reported that in laboratory spinosad was effective against 2nd and 4th nymphal instars of *S. gregaria* where LC_{50}S were 23.82 and 30.48 ppm, respectively. In the field, spinosad at the concentration of 65 ml/100 L caused 75% mortality among *S. gregaria* nymphs after 24 hr and reached 100% after 48 hr. also when spinosad was applied against common grasshoppers at Baharia Oasis at 50 ml/100 L concentration; it caused 83.3 and 100% mortality after 24 and 48 hr, respectively.

Also, **Ahmed (2004)** reported that Spinosad was the most effective compound against the newly hatched larvae of both pink and spiny bollworms after 12 days for laboratory and field strain, respectively. Finally**, Korrat, *et al.*, (2012)** who found that The effects of conventional

(profenofos) and nonconventional (emamectin benzoate، Spinosad and chlorfluazuron) insecticides at their LC_{10}, LC_{25} and LC_{50} and their binary mixtures were evaluated against 2nd instar larvae of cotton leaf worm, *Spodoptera littoralis* (Boisd.) under laboratory conditions. After 3 days of the treatment, LC_{50} of Spinosad was 19.9 ppm.

2. Effectiveness of consult (hexaflumuron) against 5th nymphal instar of the desert locust, *S. gregaria*

Chitin is an important component of the insect cuticle. Some insecticides, called chitin synthesis inhibitors (CSI), block the production of chitin. An insect poisoned with a CSI cannot make chitin and so cannot molt. Because molting must take place for the insect to reach the adult stage, a CSI-poisoned insect also cannot reproduce. Eventually, the insect dies. Because humans do not make chitin, CSIs are not considered toxic to humans **(Brown, 2006).**

Our results clear that the percentages of nymphal mortality were 6.9, 12.3, 27.8 and 51% after 10 days of treatment with 12, 30, 60 and 120ppm of consult, respectively comparing with control (0.0%). Also that $LC_{25,}$ LC_{50} and LC_{90} values for the tested Chitin synthesis inhibitor (Consult) recorded 50ppm, 123ppm and 662ppm respectively.

These findings are in agreement with **Taha and El-Gammal (1985)** who reported that Laboratory evaluation of diflubenzuron against the 4th nymphal instar of *Schistocerca gregaria*, during the nymphal ecdysis to the last nymphal instar of desert locust, *S. gregaria* causing some mortality. Also diflubenzuron when injected to the instar 5th nymphs of *S. gregaria* resulted in failure of some treated insects to molt and its death **(Roa and Mehrotra, 1986)**. Diflubenzuron caused various mortality %s after 14 days of the treatment of the second instar nymphs of *S. gregaria* **(Azam and Al-Seegh, 1993)**. CSI, IKI-7899 caused increasing mortality during the 4th and 5th instars (**Abdel-Magid, 1993)**. The greatest mortality was recorded during ecdysis of the early 4th nymphal instar to the 5th nymphal instar of *S. gregaria* when treated with chlorfluazuron **(Abo El-Ela *et al*., 1993).** When **Coppen and Jepson (1996)** treated the 2nd nymphal instar of *S. gregaria* with diflubenzuron, hexaflumuron and teflubenzuron they recorded mortality after all treatments. Triflumuron caused different mortality rates against the 5th nymphal instar of the desert locust, *S. gregaria* **(Wilps and Diop, 1997)**. Chlorfluazuron affected the survival potential in a dose-dependent manner **(Tiwari, 2000)**. During few days after feeding the newly molted 4th instar nymphs of *S.*

gregaria on Flufenoxuron treated food, the mortality increased (40%) at the highest concentration level but decreased (10%) at the lowest concentration level. The lethal action of Flufenoxuron appeared also along the later days of penultimate instar at the lower concentration levels **(Bakr *et al*., 2008). Mahdy (2010)** recorded some biological aspects as nymphal and adult mortality and failure of ecdysis to adults reached to 93.33% and 100 in six day old of 5th nymphal instar of *S. gregaria* treatment with consult and lufox, respectively. When **Abdel-Fattah *et al*., (2012)** treated nymphs of the barseem grasshopper, *Euprepocnemis plorans plorans* with chlorfluamzurone, mortality percentages were 20, 45, 65, 80 after 5, 10, 15 and 20 days, respectively. Also Longevity of the last nymphal instar treated with hexaflumuron and lufenuron (about 10 days) was significantly longer than the control larvae (7 days). Hexaflumuron and lufenuron as chitin synthesis inhibitors induce morphological disruptions at molt **(Izadi *et al*. 2012).**

Further studies should focus on the impact of IGRs on physiological activities supporting locust growth, to gain a comprehensive evaluation of how these compounds might contribute to the control of locusts. From the results of the experiments reported, it is clear that

the impacts of these IGRs on locusts vary significantly because they have different mechanisms of action. Further studies should build on this understanding of how these products affect locust physiology and incorporate that understanding into assessments of how they affect locust populations in control projects. This would promote the sustainable control of locusts **(Sun *et al*., 2011)**.

3. Effect of mixture of spinosad and consult on mortality percentages in 5th nymphal instar of desert locust.

The mortality percentages at the LC_{50} values of the tested compounds after 7 days of the experiment ranged from 30% to 64% for both consult and spinosad, respectively.

The joint action of mixing Spinosad at concentration (LC_{25}) with consult at concentration (LC_{25}) increased mortality percentages from 23, 41.2, 49.9, 60.2, 74.9, 85 and 94 to 24, 41.6, 48, 58.9, 754, 86 and 95%, with co-toxicity factor of +4, +1, -4, -2, +1, +1 and +1 after 7 days of application (**Additive effect)**.

These results are in line with **Abdel-Fattah *et al*., (2003)** who reported that the mortality percentages are caused by *Nosema locustae* with 25% of the recommended dose of chlorfluazuron were 10, 40, 85 and 92% after 5, 10, 15 and 20 days of application, respectively. Also **El-Gammal *et al*., (2004)** investigated the integral action of

Metarhizium flavoviride, anti-molting (consult) and the anti-feeding agent (Azadirachtin) in the field of Shark El-Uwainat area against the last instar nymphs of *locusta migratoria migratorioides*. They found that the integration between *M. flavoviride* and the recommended dose of consult was the most effective inducing 62.7% population reduction after 5days of application and 96.8% after 15days. **Abdel-Fattah *et al*., (2012)** found that treatment nymphs of the barseem grasshopper, *Euprepocnemis plorans plorans* with *Metarhizium anisopliae, nosema locustae* and 25% of the recommended dose of chlorfluamzurone) The mortality percentages of grasshopper nymphs caused by the fungus with 25% of recommended dose of chlorfluuazurone were 20, 60, 90 and 100% after 5, 10, 15 and 19days of application. **Abdel-Fattah *et al*., (2013)** reported that use of mixture of (entomopathogenic, *Metarhizium anisopliae* and growth inhibitor, Consult) was more effective than of the entomopathogenic alone except in the case of the 5^{th} nymphal instar.

Also, the results are in agreement with some authors who applicated Spinosad on other insects such as, **Korrat, *et al*., (2012)** found that After 3 days of treatment, the mixtures of emamectin benzoate and Spinosad at different

concentrations with profenofos produced additive effects with co-toxicity factors ranging from 17.54 (LC_{50} of Spinosad) to 18.82 (LC_{25} of emamectin benzo-ate). The mixtures of both emamectin benzoate and Spinosad with profenofos produced additive effects. Also، reported that mixtures of Spinosad (at LC_{50}) with profenofos at $LC_{١٠}$ and $LC_{٢٥}$ gave the highest effect on egg production 393.9 egg/female and hatchability (19.17%), comparing with the control 1151.6 egg/female.

the present study suggests that the use of IGR (Consult) and nonconventional (Spinosad) insecticide combinations would minimize the amount of insecticides applied, reduce the cost of insecticides for controlling process and reduce the level of environmental pollutions.

4. Effect of consult and the mixture on morphogenesis:

Insect Growth Regulators, or IGRs, attack the insect endocrine system, which produces the hormones needed for growth and for development into an adult form **(Brown, 2006).** Insect growth regulators can kill or debilitate treated insects by affecting physiological processes, inhibiting molting, preventing the formation of new integument, or reducing feeding **(Bi *et al.*, 2008).** With IGRs, molting was delayed indicating that it could be an effective control product. IGRs caused growth inhibition,

malformation, and emergence inhibition in the adult of desert locust **(Hu, *et al.* 2012).**

In this study when the 5th nymphal instar of *S. gregaria* was treated with consult some nymphs were unable to moult into adult stage and died without completing the moulting process. Different deformities were observed, some were able to split the old cuticle but unable to complete the moulting process but the old cuticle connected with the resulting adults in different positions as legs or wings and some were able to complete the moulting process without any deformity in the resulting adults in low concentration of consult.

Treatment of the 5th nymphal instar of *S. gregaria* with different concentrations of consult resulted in molting disturbances which increased with the increase of consult concentrations.

Most of adult emerged were unable to fly and sluggish in walking, jumping and climbing, also they have curled wings. All adults, which resulted of treatment, showed the following morphological changes, hypertrophied and twisted wings,

Application of IGR generally disturbs hormone balance inside the insect body.

The present results are agreed with those finding by **El-Guindy *et al*., (1980)** who observed unusual pigmentation in the 5th nymphal instar when treated the 4th nymphal instar by different concentrations of the juvenile hormone analogues. And all color categories were produced from treatment with hydroprne (yellow-green, Pale-green and bright-green). When nymphs were treated during the final instar, at 5 days old they gave rise to brown adults. The morphological abnormalities of nymphs of *S. gregaria* were enhanced as the dose of diflubenzuron increased **(Marity *et al*., 1981)**. **Rao and Mehrotra (1987)** injected diflubenzuron into 2 to 3day old 5th nymphal instar of *S. gregaria*. They recorded a significant reduction in the chitin content of the wings. Also **Kort *et al*. (1991)** observed malformation of the wings and green pigmentation by injecting the last nymphal instar of locusta migratoria with juvenile hormone mimic (pyriproxyfen). IKI induced appreciable failure in molting to adult stage when treated one-day old of the nymphal instar **(El-Gammal *et al*., 1993)**. The epidermis was destroyed and the number of cerebral neurosecretory cells reduced significantly, when treated *Locusta migratoria* manilensis by Cascade (flufenoxuron) **(Wang *et al*., 1997)**. Both diflubenzuron and teflubenzuron caused abortive molt on

the 5th nymphal instar of *S. gregaria* and most survivors developed twisted or mis-shape wings **(Wakgari, 1997)**. While, **Chowdhury *et al*. (1998)** recorded 40, 50 and 100% abnormalities when treated the 5th nymphal instar of *S. gregaria* with some insect growth regulators. Finally **Mahdy (2010)** reported that the morphologenetic aberrations induced by used of IGRs (Consult and Lufox) introducing in food to one day and six day old 5th nymphal instar of *S. gregaria* for 24 hours dietary period were concentrations dependent. The application of consult and lufox resulted in molting disturbance and different morphological defects in treated adult locusts appeared as twisted wings, color changes and the failure of getting rid of the last nymphal exuvia.

Reduced growth, abnormal moult and delayed moults. are related to disruption of endocrine system controlling growth and moulting. The moulting effects are due to disruption in the synthesis and release of ecdysteroids (moulting hormone) and other classes of hormones **(Jennifer-Mordue and Alasdair, 2000)**.

5. Characterization of the haemolymph of the fifth nymphal instar of desert locust, *S. gregaria* after treated with consult, spinosad and their mixture

5.1. Total protein:

The daily activities of an insect require a constant supply of energy. Most adults need food intake to support their activities (dispersal, reproduction). Flight in particular, is a very energy-intensive activity, requiring rapid mobilization of energy sources, transport, and transformation of food energy into ATP. Those metabolic reactions are directly involved in mobilizing stored energy reserves and in releasing that energy for flight **(Chapman, 1971)**. Adults require a nitrogen source as Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, and Valline for maturation of their ovaries and eggs. Those are essential amino acids, and protein deprivation may manifest itself in the failure to secrete Juvenile hormone (JH) which is needed for ovary and egg development in adult females. If JH or an analog such as methoprene is administered to protein-starved insects, they do not produce the normal complement of eggs simply because they do not have sufficient protein reserves in the body. A few insects use Proline as flight fuel. Proline complete metabolism yields

much less energy per unit weight metabolized and only a few insects has evolved to depend on it as a major flight fuel **(Nation, 2002)**. Quantitative assays of protein in the haemolymph and reproductive organs are of considerable importance for the understanding of the different physiological process associated with reproduction. In insects, changes in proteins are prominent during stages undergoing marked development and tissues differentiation such as during metamorphosis **(Bakr *et al*. 2007).**

The reduction of the protein levels may be due to the destructive effect of the tested compounds on some of the cerebral neurosecretory cells of the brain of the treated nymphs **(Engelmann, 1970)**.

In this study, the biochemical effects of Spinosad (at LC_{50}=19ppm) and consult (at LC_{50}=123ppm) and their mixture (at Spinosad LC_{25}=9ppm + consult LC_{25}=50ppm) on haemolymph protein contents were evaluated. The experiments were carried out by treatment of 1-day old of the nymphal instar of S. *gregaria*. The levels of haemolymph protein content were significantly decreased after 2, 4 and 6 days comparing with control nymphs.

The findings are in agreement with the reduction of protein in the haemolymph of the last two nymphal instar of *Locusta migratoria* when studied the effect of juvenile

hormone and ecdysteroids on protein **(Baehr, *et al.* 1979)**. While, **Eid, *et al.* (1982)** reported that, when newly moulted 4th instar nymphs of *Schistocerca gregaria* were treated in laboratory topically with precocoene II at 20 µg/ locust or cycloheximide at 10µg/locust The effects of precocoene II might be attributed to the lack of releasing of juvenile hormone, whereas those of cycloheximide suggested the inhibition of protein. Also **Steel and Hall (1985)** found that the protein content levels in the haemolymph of the treated nymphs were less than in the control nymphs, when treated the last nymphal instar of *Schistocerca gregaria* by benzoyl-phenyl urea (S-71624). The haemolymph protein content in late-aged last instar nymphs of *S. gregaria* was suppressed by the juvenoids (fenoxycarb) **(El-Gammal *et al.* 1989)**. Also, the reduction of protein level in the haemolymph of the treated females and males of the last nymphal instars of *S. gregaria* was recorded by **Badawy and El-Gammal (2000)** when treated the 5th female and male nymphal instars by benzoyal-phenyl urea (S-71624). **Bakr, *et al.* (2009)** found that when haemolymph samples were collected from untreated and treated nymphs, showed quantitative differences in protein content. During one day old treatment, the haemolymph protein levels significantly decreased in treated female

nymphs after 24 hrs of treatment, at different concentrations; 50, 75 and 100 ppm, of consult compared with untreated females. Also, haemolymph protein levels decreased in treated females after 168 hrs of treatment. Also showed that, the protein content of ovaries and testes were significantly decreased in treated adult females and males of the treated 5th nymphal instar during one and six day old when increasing Consult concentrations. **Mahdy, (2010)** revealed that Consult caused inhibition effect on total protein in nymphs and adults**.** Estimated depleted protein content for the last instar nymphs after treatment with chlorfluazuron (IKI- 7899); pyriproxyfen prevent the mid and late-aged nymphs (5th) of S. gregaria to gain the normal hemolymph protein content **(Ghoneim *et al*., 2012). Abdel-Fattah *et al*., (2013)** reported that treatment 5th nymphal instar of *S. gregaria* with mixture of (entomopathogenic, *Metarhizium anisopliae* and growth inhibitor, Consult) resulted in decrease in the protein content than that of control. Also the effect of flufenoxuron on the haemolymph protein content of early- and mid-aged nymphs depended upon the concentration level because it exhibited a reducing effect at the high concentration level but an increasing one at the lower concentration level (**Hamadah, 2014)**.

On the other hand, **El-Gammal, *et al*. (1994)** who found that, IKI increased haemolymph protein concentration and protein bands in 8-day after application of IKI against one day old of 5^{th} nymphal instar of S. *gregaria*. Also, when treatment desert locust with two compounds of the egg pod (forth and egg) were extracted separately in hexane and ethanol. Each extract was added to the sterilized sand in the treated cups as ovipositing sites for solitary and gregary phases. The ovipositing 8days adult female and male of the same age was exposed to these cups till egg laying the haemolymph was sampled in the 10^{th}, 17^{th}, 19^{th} and 21^{st} day. The total haemolymph protein was higher in solitary phase, in all days of sampling, than gregary phase. In both phase the content of 21^{st} day was highest. The treatment with egg pod extracts caused remarkable reduction in solitary female haemolymph protein content **(Eid, *et al*., 2004)**.

On the contrary, the protein content was unaffected by diflubenzuron and triflumuron **(El-Kordy, 1985)**.

The decline in protein content obtained by consult can be explained according to **Mitilin *et al*. (1977)** who said that the inhibition of protein synthesis as a result of inhibition of DNA and RNA synthesis as the first sign of cell death. Also attributed this reduction to the inhibitory

role of the tested IGRs on tissue protein synthesis. Also **Bakr *et al.*, (2007)** reported that the reduction of protein level might be due to the destructive effect of consult on some of the cerebral neurosecrotory cells of the brain responsible for secretion of the proteins of the treated nymphs of *S. gregaria*. It is worthy to know that protein presented in all viable cells is essential to the process of cell division control of many chemical reactions in the metabolism of cells. Also **Bakr, *et al.*, (2010)** found that application of different IGR's (Consult and Lufox) caused a disturbance in the chemical reactions of cell metabolism, which in turn resulted an inhibitory action on the biological characters that appears as morphological and histological malformation in all different treated locust tissues.

5.2. Total lipids:

Immature stages of some insect groups need poly unsaturated fatty acids for normal development. Some groups (Lepidoptera, Orthoptera, and some others) use burn lipids (fatty acids) as flight fuels, which release large amounts of energy per unit weight of the substrate metabolized. The ability to rapidly mobilize and transport lipids from the fat body and the availability of oxygen from the tracheal system are major adaptations in those insects to burn fatty acids for flight. Some insects that metabolize

lipids are able to fly continuously for hours and undertake long distance migration **(Chapman, 1971)**. Several types of lipid molecules (e.g. phospholipids and sphingolipids) are considered important structural components in cell membrane while other types used as energy reservoirs. Other types of lipids molecules serve as chemical signals, vitamins, or pigments. Finally some lipid molecules which occur in the outer coatings of various organisms have protective or waterproofing functions. Still other lipid molecules act as hormones, antioxidants, vital growth factors **(Nation, 2002)**.

In our results the effects of bio-insecticide, spinosad and IGR, consult with their LC_{50} values 19ppm and 123 ppm, respectively and compared with their mixture at LC_{25} values 9ppm and 50 ppm, respectively on total lipid after 2, 4 and 6 days of treatment are found. There were significant differences of total lipid contents at all treatments comparing with control. The mixture show highly significant decrease in the total lipid content, 4.57, 4.04 and 3.57mg/ml haemolymph after 48, 96 and 144hrs of treatment respectively, compared to control 7.3, 7.4 and 6.95mg/ml haemolymph.

These results are in line with **Hamadah *et al*., (2012)** who found that the metabolic effects of pyriproxyfen,

tebufenozide or lufenuron on the lipid content in two different tissues: hemolymph and fat body of the early-, mid- and late-aged old nymphs as well as 1- and 4-day old adult females. Hemolymph lipid content of the early-aged nymphs had been subjected to a reducing effect after treatment with high concentration of insect growth regulators (IGRs). With the age of nymphs, all IGRs could significantly or non-significantly reduce the lipid content of hemolymph. Concerning the lipid content in fat bodies of nymphs, a predominant inhibitory effect of all IGRs was detected. With regard to the adults, nymph treatments led to remarkable or slight decrease lipid content in the haemolymph. Also **Abdel- Fattah *et al*. (2013)** showed that treatment 5^{th} nymphal instar of *S. gregaria* with mixture of (entomopathogenic, *Metarhizium anisopliae* and growth inhibitor, Consult) causing decrease in lipid contents than that of control.

Injection of 0.1 µmol of the synthetic adipokinetic hormone (Peram-AKH II) onto the American cockroach *Periplaneta Americana* led to a significant reduction of the levels of neutral lipids and phospholipids in Haemolymph **(Michitsch and Steele, 2008)**.

Abdel-Aal, (2012) reported that chlorofluazuron , tebufenozoid and pyriproxyfen decreased total lipid

contents of the overioles of *S. littoralis* females as compared with normal females.

On the other hand, **Eid, *et al*., (2004)** who reported that when, two compounds of the egg pod (forth and egg) were extracted separately in hexane and ethanol. Each extract was added to the sterilized sand in the treated cups as ovipositing sites for solitary and gregary phases. The ovipositing 8days adult female and male of the same age was exposed to these cups till egg laying the haemolymph was sampled in the 10^{th}, 17^{th}, 19^{th} and 21^{st} day. The total haemolymph lipid was higher in gregary phase. In both phase the content of 19^{th} day was highest. The treatment with egg pod extracts caused remarkable increased in solitary female haemolymph lipid content. The gregary extracts was more increasing the lipid content.

5.3. Total carbohydrates

Carbohydrates are not just an important source of rapid energy production for development and growth living cells, but they also serve as structural building blocks of cells and components of numerous metabolic intermediates. A broad range of cellular phenomena, such as cell recognition and cell binding (e.g. by other cells, hormones, and viruses) are also dependent on carbohydrate **(Wang, *et al*., 2007)**.

The two most common carbohydrate stored reserves in insects are the disaccharide trehalose and the polysaccharide glycogen. The haemolymph, fat bodies, and gut tissues are major sources of stored carbohydrates. Trehalose is usually present in large quantities in the haemolymph; and it is considered as the principal storage sugar for insects in the haemolymph. It is rapidly hydrolyzed to two glucose molecules for tissues to use. Glycogen is another form of stored energy. Insect flight muscles contain glycogen, but in small amounts which is only sufficient for a few minutes of flying. Glucose can be released from glycogen which is stored in the fatty bodies and the gut cells by hydrolysis. Some insects use carbohydrates in the haemolymph as only source of fuel to fly for 30 minutes then they switch to another source like proline or fatty acids **(Nation, 2002)**. The content of carbohydrates and lipids in insect haemolymph is regulated by the adipokinetic hormone secreted by corpus cardiacum, and this hormone could increase the level of trehalose and reduce the level of lipid in haemolymph **(Michitsch and Steel. 2008**).

In this study was found significant differences of total carbohydrates contents at all treatments comparing with control.

Carbohydrates contents were dramatically declined in treated nymphs with Spinosad and the mixture during 2, 4 and 6 days after application. But, carbohydrates contents increased in treated nymphs with consult comparing with untreated nymphs. Highly significant decline were recorded in carbohydrates content at the Spinosad.

The increase in total carbohydrates content by consult in our study are in agreement with the increase in glucose (or chitin or total carbohydrates) content was noticed by **El-Gammal, *et al*., (1993)** who found increasing carbohydrate content of *S. gregaria* was triggered by chlorfluazuron. Also, **Tanani *et al*., (2012)** reported that newly molted last (5th) instar of the desert locust, *Schistocerca gregaria* (Forsk.), was treated through fresh plant food with 2 concentrations: high (1000.0 ppm) or low (62.5 ppm) of 3 IGR, tebufenozide. Carbohydrate content was determined in the hemolymph and in the fat body of the early-aged, mid-aged, and late-aged 5th instar, as well as of 1- and 4-day old adult females. Tebufenozide induced the nymphs to gain more carbohydrates. While, **Hamadah, (2014)** showed that all nymphs and adults had been enhanced to gain excess carbohydrates in their fat bodies. For some details, the greatest inducing effect of

flufenoxuron appeared as increased fat body carbohydrates of mid-aged nymphs

On the other hand various juvenoids (JHAs), and IGRs in general, suppressed carbohydrate content in some insects, e.g., *S. gregaria* by fenoxycarb **(El-Gammal *et al*. 1989)**. Newly molted last (5^{th}) instar of the desert locust, *Schistocerca gregaria* (Forsk.), was treated through fresh plant food with 2 concentrations: high (1000 ppm) or low (62.5 ppm) of 3 IGRs: pyriproxyfen and lufenuron. Carbohydrate content was determined in the hemolymph and in the fat body of the early-aged, mid-aged, and late-aged 5th instar, as well as of 1- and 4-day old adult females. Pyriproxyfen prevented the nymphs to gain normal carbohydrate content in the haemolymph. Also, Lufenuron caused hemolymph carbohydrate content to decrease slightly in the early-aged nymphs **(Tanani *et al*., 2012)**.

Reduction in carbohydrates content of treated nymphs with Spinosad in our results are in line with **El-Leithy, *et al*, (2004)** who found that total carbohydrates content in 6^{th} larval instar of *Spodoptera littoralis* treated with Spinosad, *B. thuringiensis* and cypermethrin significantly decreased. **El- Sheikh, (2012)** showed that the treatments with both Spinosad, cypermethrin and *Bacillus*

thuringiensis significantly decreased total carbohydrate contents to 4th larvae instar, *Spodoptera littoralis* instars by about 26.7%, 12.8% and 44.2% for Spinosad, *B. thuringiensis* and cypermethrin, respectively, as compared to control.

The general disturbance in carbohydrate metabolism by IGR as expressed by reduction of carbohydrate hydrolyzing enzymes activities could be result from a chain effect originating primarily from inhibition of chitin synthesis **(Salem *et al.*, 1995).** The disturbance of trehalase activity might hamper the supply of glucose needed for chitin build up **(Kandy and Killy, 1962).**

Decreasing content of carbohydrates after treatment with spinosad may be due to a decrease in the trehalase activity (**El-Shiekh, 2002**), or to their effects on the carboxylase activity (**Mukherjee and Sharma, 1996**).

5.4. Total cholesterol:

Insects can not synthesize sterols and thus immature insects need sterols as precursor that can be transformed into the molting hormone, which has a sterol structure. Eggs also contain sterols and the first instar may be able to molt without a dietary source, but subsequent molts may be impossible if dietary sterol is not present. Some adult insects need sterol to produce the normal number and/or

hatching of eggs. The cholesterol is the biosynthesis precursor of ecdysone hormone in insects so, the estimation of the Juvenile hormone mimic role on this metabolite deemed necessary to evaluate its action upon the precursor of molting hormone (ecdysone) in *S. gregaria* **(Rees, 1985)**.

Our results show that Treatment nymphs of the desert locust, *S. gregaria* with19ppm, 123ppm and (9ppm+50ppm) of Spinosad, consult and their mixture respectively, caused significant differences between treated and untreated nymphs during 2^{nd} , 4^{th} and 6^{th} days of application. Cholesterol contents were declined in all treatments. Highly significant decline were recorded in cholesterol content at nymphs treated with mixture.

These results are in agreement with **Mettaweh *et al.* (2001)** who found that total cholesterols in the haemolymph of treated-grasshopper, *Eurpepocnemis plorans plorans* (5^{th} instar nymphs) with the entomopathogenic fungus, *M. flavovride* (5 $x10^6$spores/ml) decreased than the untreated ones. Also, **Abdel- Fattah *et al*. (2013),** showed that treatment 5^{th} nymphal instar of *S. gregaria* with mixture of (entomopathogenic, *Metarhizium anisopliae* and growth inhibitor, Consult) causing decrease in contents than that of control.

On the other hand **Eid, *et al*., (2004**) who found that usage two compounds of the egg pod (forth and egg) were extracted separately in hexane and ethanol. Each extract was added to the sterilized sand in the treated cups as ovipositing sites for solitary and gregary phases. The ovipositing 8days adult female and male of the same age was exposed to these cups till egg laying the haemolymph was sampled in the 10^{th}, 17^{th}, 19^{th} and 21^{st} day. The total haemolymph cholesterol was higher in gregary phase. Ethanol extracts caused remarkable increased in both phases.

5.5. Effect of Spinosad, consult and their mixture on activity of acid phosphatase in 5^{th} nymphal instar

Detoxification enzymes in insects are generally demonstrated as the enzymatic defense against foreign compounds and play a significant roles in maintaining their normal physiological functions **(Li and Liu, 2007)**. Also acid phosphatase (ACP) plays an important role in the detoxification process of toxic compounds entering the body **(Zheng, *et al*., 2007)**. The suppression of detoxification enzymes indicated that these enzymes play no role in the detoxification of tested compounds and may be increase the susceptibility of insect pest to these insecticides **(Abd-Elaziz and El-Sayed, 2009)**.

Our study showed that the activity of acid phosphatase (AcP) of 5th nymphal instar of *S. gregaria* treated with 19ppm, 123ppm and (9ppm+50ppm) of Spinosad, consult and their mixture was determined. The tested compounds significantly decreased the activity of acid phosphatase as compared to the control. The activity of acid phosphatase decreased in treated nymphs with consult, Spinosad and their mixture after 2, 4, 6 days. All treatments caused decrease in Acid phosphatase activity but, the mixture gave the highest decrease in acid phosphatase activity followed by Spinosad and consult, where these values after 6days were 6.3, 7.76 and 8.5 IU/l respectively as compared with 10.2 IU/l in the control.

These results are not in agreement with **Gillespie *et al*., (2000) and Said (2009)** who found that Acp Activity in the haemolymph of treated-desert locust, *S. gregaria* (5th instar nymphs) with the entomopathogenic fungus, *M. anisopliae var. acridium* (5 x10^{6}spores/ml) increased than the untreated ones.

But, these results are in agreement with some authors who applicated IGR on other insects such as **Bakr, *et al*., (2010b)** who studied the effect of the sub-lethal doses LC_{25}, $LC_{50}$and LC_{90} of flufenoxuron (Cascade) on the activity of detoxification enzymes, acid phosphatase and

the non- specific esterases (α, β esterases), of 2nd and 4th larval instars of *S. littoralis.* results showed that the activity of all enzymes decreased significantly in treated larvae at different times intervals post treatments. **Also, Bakr, *et al*., (2013),** showed that tebufenozide and lufenuron exhibited a severe reduction in the activities of the detoxification enzymes, acid phosphatase and esterases (αand β), as compared to the control. The enzymatic activities were inhibited with the increase in the time post-treatment and also with the increase in dose. Therefore, the tested IGRs, tebufenozide and lufenuron , may be not detoxify by these enzymes.

On the other hand **El-Sheikh, *et al*., (2009)** reported that the activity of haemolymph acid and alkaline phosphatase activity was significantly increased after treatment of *S. Littoralis* with Spinosad and tebufenozide. A significant increase in phosphatasis activity was obtained by **El-Sheikh, (2012)** who Showed that the acid phosphatase activity was significantly increased by about 42.1%, 48.3% and 52.8 % after treatment with Spinosad, *B. thuringiensi*s and cypermethrin, respectively, as compared to control.

Acid phosphatases have been shown to be associated with insect development, especially in relation to nutrition and egg maturation **(Tsumuki and Kanehisa, 1984).**

Acid phosphatase has received considerable attention in developmental studies because of its association with histolysis. It is known that acid phosphatase hydrolyzes a variety of orthophosphorylation reactions **(Hollander, 1971)**. Ecdysone is responsible for increase in the number of lysosomes **(Radford and Misch, 1971)** and of the activity of acid phosphatase **(van Pelt-Verkuil, 1979)**. This indicates that the decreased activity of acid phosphatase in this study may be due to decreased number of lysosomes. **Sridhara and Bhat (1963)** stated that the increase or decrease of phosphatases enzyme during development is reflected an increase or decrease in the acid-soluble phosphorus content.

5.6. Effect of Spinosad, consult and their mixture on activity of phenoloxidase (PO) in 5th nymphal instar

Phenoloxidase is important component of insect immuno system. In addition, phenoloxidase correlate with resistance to some parasites and pathogens across species **(Nigm *et al.* 1997)**

Our results reveal that Spinosad, consult and their mixture caused decrease in PO activity at all treatments.

The enzyme activity showed sharply decline at all treatments. The mixture gave the highest decrease in PO activity followed by Spinosad and consult, There was a significant difference in PO activities by nymphs treatment at concentrations 19ppm, 123ppm and (9ppm+50ppm) of Spinosad, consult and their mixture, respectively. Two day after application there were significant differences in phenoloxidase activity between treatments and control. However, there was a large significant decrease in phenoloxidase activity in treated insects on 6^{th} day after application.

Treated insects with Spinosad, consult and their mixture caused continuous reduction in phenoloxidase activity by increase the time.

These results are in agreement with **Assar *et al*., (2012)** who showed that the activity of phenoloxidase of 4^{th} instar larvae of *Culex pipiens* treated as 2^{nd} instar larvae with 0.1 and 1 ppm of cyromazine. The tested IGR significantly decreased the activity of phenoloxidase.

On the other hand **Said (2009)** reported that (Po) Activity in the haemolymph of treated-desert locust, *S. gregaria* (5^{th} instar nymphs) with the entomopathogenic fungus, *M. anisopliae var. acridium* (5×10^6spores/ml) increased than the untreated ones.

Although **Hung and Boucus (1992)** reported that there were no significant differences in the phenoloxidase activity in the haemolymph of the sixth instar larvae of *S. exigue* during (24 hrs) post challenge with *B. bassiana* but after 48-60hrs, phenoloxidase titers in the haemolymph sampled from infected larvae decreased seven-folds.

The effects of CSIs on insects vary according to species, the developmental stage at the time of application, the kind of compound and the administered dose **(Mulla *et al.* 2003).**

Prevent phenoloxidase production by locust haemocytes may be as a result of destruction of the cells that produce prophenoloxidse **(Cerenius *et al*., 1990).**

5.7. Effect of spinosad, consult and their mixture on activity of Peroxidase in 5th nymphal instar:

Pyroxidases is the primary enzymes in insects that dedicated to removal of damaging reactive oxygen species (**Ahmed, 1995**).

Peroxidase acts as catalysts to facilitate a variety of biological processes. Specifically, peroxidase activity involves donating electrons to bind to other substrate substances, such as ferro-cyanide and ascorbate, in order to break them down into harmless components. Most notably, peroxidase enzymes degrade hydrogen peroxide, a naturally occurring by product of oxygen metabolism in the

body. As a result, this substance is converted into water and oxygen. The functions of insect peroxidases include detoxification, stabilization of extracellular matrices, and possible involvement in insect immunity **(Zhao *et al.*, 2001)**.

In addition, A lot of types of enzymes are located in the insect cuticle having different functions during cuticular events, such as molting and sclerotization. Several of the enzymes involved in sclerotization, such as prophenoloxidase, diphenoloxidase, laccases, peroxidases, isomerases, and tautomerases, have been identified in insect cuticle **(Andersen, 1979)**.

Our results show that Treatment nymphs of the desert locust, *S. gregaria* with19ppm, 123ppm and (9ppm+50ppm) of Spinosad, consult and their mixture, respectively caused significant differences in Peroxidase activity (POD) at all treatments.

Two days after application there was significant decrease in Peroxidase activity between treatments and control. However, there was a large significant decrease in Peroxidase activity in treated insects on 6^{th} day after application.

All treatments caused decrease in Peroxidase activity but, the highest decrease in Peroxidase activity was recorded at the mixture of Spinosad and consult.

Our results are in agreement with **Gillespie *et al*., (2000)** who reported that peroxidase activity of *S. gregaria* treated with *Metarhizium anisopliae* was declined. or remains unchanged as *Spodoptera exigua* **(Boucias *et al*., 1994).** On the other hand, **Srour (2014),** showed that methanolic extract of Alphonso leaves exhibited concentration-dependant contact toxicity against larvae of *S. littoralis* with LC50 of 325 µg/ cm^2 peroxidase activity increased.

SUMMARY

Biological control products have been under development since the late nineties. IGRs are from the most promising agents for immature stages control because they (IGRs) owing to their effects on certain physiological regulatory processes essential to the normal development of insects. The insect grows normally until the time to molt. When the insect molts, the exoskeleton is not properly formed and it dies. Death may be quick, but in some insects it may take several days. Such biological pesticides would be environmentally safe and it is harmless to humans and other animals.

Recently, the quality of the environment has become a major issue. Many chemicals (pesticides) previously accepted for locust control at national and international levels would not survive rigorous environmental testing. A bio-insecticide Tracer 24SC Spinosad is a new safe product to control the desert locust, *Schistocerca gregaria* Forskal.

Chemical–bioinsecticides combination provides several distinct advantages for Insect Pest Management programs (IPMs), including the potential effect for reducing the amounts of each agent used. Such reduction would mean potentially lower costs, lower environmental pollution and less damage to beneficial organisms.

This work is carried out to study the effect of spinosad , consult and the mixture between them on the 5th nymphal instar of the desert locust, *Schistocerca gregaria* through calculating the mortality percentages.

The work was extended to study effect of spinosad, consult and their mixture on haemolymph components of the fifth nymphal instar during different periods after infection. Also, it cleared their effects on activity of phenoloxidase, Peroxidase and acid phosphatase enzymes in the haemolymph of the fifth nymphal instar.

Data obtained can be summarized as follows:

1. **Effects of Spinosad, consult and their mixture on mortality percentages of 5th nymphal instar of *S. gregaria*:**

The mortality percentages of the 5th nymphal instar of *S. gregaria* were 45.1, 69.8, 85.4 and 100% after 3 days of treatment with 15, 37, 75 and 150 ppm of spinosad, respectively comparing with control (0.0 %). also LC_{25} was 8.8ppm, LC_{50} was 18.7ppm and LC_{90} was77.9ppm.

Nymphal mortality percentages were 6.9, 12.3, 27.8 and 51% after 10 days of treatment with 12, 30, 60 and 120ppm of consult, respectively comparing with control (0.0%). The LC_{25}, LC_{50} and LC_{90} of consult recorded 50ppm, 123ppm and 662ppm, respectively.

The mortality percentages at the LC_{50} values of the tested compounds after 7 days of the experiment ranged from 30% to 64% for both consult and spinosad, respectively.

The joint action of Spinosad at concentration (LC_{25}) with consult at concentration (LC_{25}) increased mortality percentages from 23, 41.2, 49.9, 60.2, 74.9, 85 and 94 to 24, 41.6, 48, 58.9, 75.4, 86 and 95%,

The treatment of the nymphs with tested compound; consult induced malformations in emerged adults while Spinosad didn't cause malformations.

2. Characterization of the haemolymph of the fifth nymphal instar of desert locust, *S. gregaria* after treated with consult, spinosad and their mixture

The samples of the haemolymph were taken at different intervals of 2, 4and 6 days after treatment.

The treatment of Spinosad, consult and their mixture recorded reduction of total protein, total lipid and total cholesterol in treated-nymphs than the untreated-nymphs in all treatments during periods of the experiment. Highly significant decline were recorded in treated-nymphs with mixture.

The treatment of spinosad and mixture indicated decrease in carbohydrate level in the haemolymph of treated-

nymphs than the untreated-nymphs during period of the experiment but the treatment of consult caused increase in carbohydrates contents than untreated ones.

Acid phosphatase, phenoloxidase and Peroxidase activity decreased in all treatments. Highly significant decline were recorded in treated-nymphs with mixture.

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الملخص العربى

يعتبر الجراد الصحراوى حشرة من أشد الأفات ضررا حيث يتغذى على كل أنواع النباتات حتى لحاء الأشجار والأخشاب الجافة ولا تترك شئ الا اتت عليه تماما تتغذى الحشرات الكاملة المكونه للأسراب يوميا مقدار وزنها طعام اى من ١-٢ جرام يوميا فالكيلو متر الواحد من سرب الجراد الصحراوى يتغذى على ١٠٠-١٥٠ طن مادة خضراء يومي، وفى حصر تقديرى عن الأضرار خلال عشرة سنوات من ١٩٢٥-١٩٣٤م تبين بأن قيمة الأضرار تبلغ نحو ١٠٠ مليون دولارا سنويا، أما فى سنة ١٩٥٤-١٩٥٥م خسرت بساتين الفاكهة فى مراكش ١٥ مليون دولار. وفي كينيا عام ١٩٥٤م قدر عدد الأسراب التي أغارت عليها بحوالي خمسين سرباً قدر وزنها بحوالي ١٠.٠٠٠ طن أي أنها كانت تأكل حوالي ١٠٠ ألف طن يومياً من النباتات فإذا ما تركت هذه الأسراب وشأنها لعدة أسابيع ولم تقاوم فإن مقدار ما كانت تأكله يعادل ما ينتج محصولاً قدره ٢٥٠ ألف طن من الذرة.

يستخدم فى المكافحة وسائل مختلفة واهم على الاطلاق المبيدات الحشرية مثل مبيدات الفوسفات العضوية ومبيدات الكربامات ومبيدات البيروثرويد وخاصة اثناء هجوم الأسراب وحدوث الفورانات.

أصبحت في الآونة الأخيرة جودة البيئة قضية أساسية. فى الماضى قبلت العديد من المواد الكيميائية (المبيدات) لمكافحة الجراد على الصعيدين الوطني و الدولي ولم تحظى باختبار بيئى صارم. ومن هذا المنطلق يعتبرالمبيد الحيوى للحشرات Spinosad SC24 هو منتج جديد أمن بيئيا لمكافحة الجراد الصحراوي ، *Schistocerca gregaria*. مقارنة بالمبيدات التقليدية.

ومنذ أواخر التسعينات ومنتجات المكافحة الحيوية قيد التطوير. حيث تعتبر منظمات النمو الحشرية من عوامل المكافحة الحيوية الواعدة لمكافحة الأطوار غيرالكاملة نظرا لتأثيرها على بعض العمليات الفسيولوجية الأساسية المنظمة للتطور الطبيعي للحشرات. حيث تنمو الحشرات بشكل طبيعي حتى وقت الإنسلاخ وعندما

تنسلخ لا يتكون جدار الجسم الخارجى بشكل صحيح وقد تموت. قد يكون الموت سريع ، ولكن في بعض الحشرات قد يستغرق عدة أيام. إن مثل هذه المركبات الحيوية تكون آمنة بيئيا وغير مؤثره على الإنسان و الحيوانات الأخى. يزودنا خلط المبيدات الحيوية والكيميائية بعدة مزايا واضحة لبرامج المكافحة المتكاملة للآفات الحشرية ، بما في ذلك خفض كمية المواد المستخدمه. بما يعني إنخفاض التكاليف، وإنخفاض التلوث البيئي. وكذلك إنخفاض الأضرار التي قد تلحق بالكائنات النافعة.

اجريت هذه الدراسة لمعرفة تأثير سبينوساد والكونصلت والمخلوط بينهما على حوريات العمر الخامس للجراد الصحراوي، من خلال تقدير النسب المئوية للموت وذلك باستخدام اربع تركيزات من كل مركب ١٥، ٣٧، ٧٥ و ١٥٠ جزء في المليون للسبينوساد و، ٣٠، ٦٠ و ١٢٠ جزء من المليون من الكونصلت وذلك بغمر الغذائ المقدم للحشرات المعاملة فى كل تركيز ثم ترك الغذاء ليجف لمدة ساعة وتقديمه للحشرات للتغذية علية لمدة ٢٤ ساعة ثم التخلص مما تبقى من الغذاء المعامل وتقدير غذاء اخر نظيف وتقدير نسب الموت.

شملت الدراسة ايضا تأثير الكونصلت بتركيز ١٢٣ جزء من المليون والسبينوساد بتركيز ١٩ جزء من المليون والملخوط بينهما بتركيز ٩ جزء من المليون من السبينوساد و٥٠ جزء من المليون من الكونصلت على محتوى هيموليمف حوريات العمر الخامس من الكربوهيدرات والبروتينات والدهون الكولستيرول بعد فترات مختلفة من المعاملة. وأيضا دراسة تأثير هذه التركيزات على نشاط إنزيمات الفينولوكسيداز ، البيروكسيداز والأسيد فوسفاتيز.

ويمكن تلخيص النتائج المتحصل عليها على النحو التالي :

١. تأثيرات السبينوساد، الكونصلت والمخلوط بينهم على نسب الموت لحوريات العمر الخامس للجراد الصحراوى:

سجلت الحوريات بعد ٣أيام من المعاملة بالسبينوساد بالتركيزات ١٥، ٣٧، ٧٥ و ١٥٠ جزء في المليون نسب موت ٤٥.١ ، ٦٩.٨ ، ٨٥.٤ و ١٠٠ ٪ على

التوالى ، مقارنة بالحوريات غير المعاملة والتى سجلت موت بنسبة (٠.٠ ٪) . كما قدرت قيم LC_{25}, LC_{50} و LC_{90} وكانت ٩ جزء من المليون، ١٩ جزء من المليون و ٧٧.٩ جزء من المليون على التوالى .

قدرت نسب الموت للحوريات المعاملة بالتركيزات ١٢، ٣٠، ٦٠ و ١٢٠ جزء من المليون من الكونصلت وكانت ٦.٩ ، ١٢.٣ ، ٢٧.٨ و ٥١ ٪ على التوالى، بعد ١٠ يوما من المعاملة مقارنة بالحشرات الغير معاملة (٠.٠ ٪) . وكانت قيم LC_{25}، LC_{50} وLC_{90} للكونصلت ٥٠ ، ١٢٣ و ٦٦٢ جزء من المليون على التوالي.

وتراوحت نسب الموت لتركيزات الممثله لقيم LC50 للمركبات المستخدمة (سبينوساد و كونصلت) بعد ٧ أيام من المعاملة من ٣٠٪ إلى ٦٤ ٪ على التوالي. أحدث المخلوط بين السبينوساد والكونصلت بتركيز ممثل للقيمة (LC_{25}) من المركبين زيادة نسب الموت من ٢٣، ٤١.٢ ، ٤٩.٩ ، ٦٠.٢ ، ٧٤.٩ ، ٨٥ و ٩٤ الى ٢٤ ، ٤١.٦ ، ٤٨ ، ٥٨.٩ ، ٧٥٤ ، ٨٦ و ٩٥ ٪.

سببت معاملة الحوريات الجراد الصحراوى بالكونصلت تشوهات فى الحشرات الكاملة في حين لم يلاحظ تشوهات فى الحوريات المعاملة بالسبينوساد.

٢. وصف هيموليمف حوريات العمر الحورى الخامس للجراد الصحراوي بعد المعاملة بالسبينوساد، الكونصلت والمخلوط بينهم:

أخذت عينات الهيموليمف على فترات مختلفة بعد ٢، ٤ و٦ أيام من المعاملة بالسبينوساد والكونصلت والمخلوط بينهما. أحدثت كل المعاملات إنخفاض فى محتوى الهيموليمف من البروتين و الدهون و الكوليسترول الكلى مقارنة بالحوريات غير المعاملة. وكذلك فقد سجل المخلوط من الكونصلت والسبينوساد اكبر انخفاض فيهم.

اوضحت التجارب ان معاملة الحوريات بالسبنوساد والمخلوط ادى لإنخفاض في محتوى الهيموليمف من الكربوهيدرات عنه فى الحوريات غير المعاملة خلال فترة

التجربة. وعلى العكس تماما فقد سبب الكونصلت زيادة في الكربوهيدرات فى الحشرات المعاملة عن الحشرات غير المعاملة.

بينت التجارب إنخفاض نشاط إنزيمات الاسيد فوسفاتيز والفينولواكسيداز والبيرواكسيداز في جميع المعاملات وكانت المعاملة بالمخلوط هى الاكبر تأثيرا على نشاط الإنزيمات.

ومما سبق يمكن القول ان مركب السبينوساد ومركب الكونصلت بكونهما اكثر امانا على البيئة يمكن استخدامهما ضمن منظومة المكافحة المتكاملة للجراد الصحراوى

كما ان المخلوط بينهما ادى لزيادة نسب الموت عن استخدام كل مركب على حده وذلك يمكن استخدامهما معا لمكافحة الجراد الصحراوى فى اوقات الانحسارات واماكن تواجد الحوريات وذلك بغرض تقليل التلوث الحادث نتيجة استخدام المبيدات التقليدية .

اسم الطالب: سعيد محمد سعيد أحمد **الدرجة:** الدكتوراة

عنوان الرسالة: تأثير مركب السبينوساد ومركب الكونصلت على العمر الحورى الخامس للجراد الصحراوى

المشرفون : الأستاذ الدكتور : حسن محمد صبحى
الأستاذ الدكتور : وفائى زكى عازر
الأستاذ الدكتور : ثروت عبد المنعم عبد الفتاح
قسم: الموارد الطبيعية **فرع:** موارد حيوانية

تاريخ منح الدرجة: / /

المستخلص العربي

أجريت الدراسات المعملية في محاولة لمعرفة فاعلية المبيد الحيوى، سبينوساد ومنظم النمو الحشرى كونصلت بتركيزات مختلفة (١٥، ٣٦، ٧٥ و ١٥٠جزء من المليون) و (١٢، ٣٠، ٦٠ و ١٢٠جزء من المليون) على التوالي على العمر الخامس الحورى للجراد الصحراوي بعد الخروج من الإنسلاخ بيوم واحد بتغذية الحشرات على الغذاء المعامل بالمركبات سابقة الذكر.

أشارت النتائج إلى بعض التأثيرات البيولوجية من خلال حدوث موت للحوريات ، وصلت إلى (٤٥.١ ، ٦٩.٨ ، ٨٥.٤ و١٠٠ ٪) بعد ٣ ايام من تغذية الحوريات على الغذاء المعامل بالسبينوساد و (٦.٩ ، ١٢.٣ ، ٢٧.٨ و ٥١ ٪) بعد ١٠ أيام من تغذية الحوريات على الغذاء المعامل بالكونصلت، ولم تستطع الحوريات الإنسلاخ والوصول للحشرات كاملة، كما حدث التواء وتعرج في أجنحة الحشرات الكاملة الناتجة من الإنسلاخ نتيجة المعاملة بالكونصلت.

تأثر المحتوى البروتينى والكربوهيدراتى والدهونى والكوليسترولى الكلي ونشاط الإنزيمات أسيد فوسفاتيز، فينولواكسيداز وبيروكسيداز بشكل متأرجح بين الزيادة والإنخفاض بعد ٢ و ٤ و ٦ أيام من المعاملة بتركيز المساوى لقيمة (LC_{50}) من السبينوساد والكونصلت والمخلوط بينهما بتركيز مكافأ لقيم (LC_{25}) من المركبين. إنخفضت قيم البروتينات الكلية و الدهون الكلية و الكوليسترول الكلي و نشاط الأسيد فوسفاتيز و نشاط الفينولواكسيداز و نشاط البيروكسيداز بشكل كبير في كل المعاملات مقارنة بالحوريات غير المعاملة. وسجل أكبر إنخفاض لهم فى الحوريات المعاملة بالمخلوط.

أحدث الكونصلت زيادة كبيرة في الكربوهيدرات الكلية بخلاف السبينوساد والمخلوط اللذان أحدثا إنخفاض في الكربوهيدرات الكلية مقارنة بالحوريات غير المعاملة. وفى نفس السياق فقد أحدث السبينوساد أكبر إنخفاض فى محتوى الكربوهيدرات الكلية.

الكلمات المفتاحية: الجراد الصحراوي ، الهيموليمف، التغيرات البيوكيميائية، نشاط الإنزيمات، السبينوساد، الكونصلت.

جامعة القاهرة
معهد البحوث والدراسات الأفريقية
قسم الموارد الطبيعية

تأثير مركب السبينوساد ومركب الكونصلت على العمر الحورى الخامس للجراد الصحراوى

رسالة دكتوراة الفلسفة
في الدراسات الأفريقية
قسم الموارد الطبيعية
(موارد حيوانية)

مقدمه من

سعيد محمد سعيد أحمد

بكالوريوس في العلوم الزراعية (مبيدات)- كلية الزراعة - جامعة الزقازيق ، ٢٠٠٤
ماجستير في العلوم الزراعية (حشرات)- كلية الزراعة - جامعة القاهرة ، ٢٠٠٩

لجنة الإشراف

الدكتور/ حسن محمد صبحى .....
أستاذ ورئيس قسم الموارد الطبيعية - معهد البحوث والدراسات الأفريقية- جامعة القاهرة

الدكتور/ وفائى زكى عازر.....
أستاذ الموارد الطبيعية - معهد البحوث والدراسات الأفريقية- جامعة القاهرة

الدكتور/ ثروت عبد المنعم عبد الفتاح.....
أستاذ ورئيس قسم الجراد والنطاط- معهد بحوث وقاية النباتات- مركز البحوث الزراعيه

جامعة القاهرة
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رسالة مقدمه من

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ماجستير في العلوم الزراعية (حشرات)- كلية الزراعة - جامعة القاهرة ، ٢٠٠٩

للحصول على

دكتوراة الفلسفة

في

الدراسات الأفريقية
قسم الموارد الطبيعية
(موارد حيوانية)

٢٠١٤