J. Egypt. Soc. Toxicol.
(Vol. 36: 61-66 Jan. 2007)
WWW.estoxicology.org

EFFECT OF SOME INSECTICIDES ON THE HAEMOLYMPH OF DESERT LOCUST *Schistocerca gregaria* Forskal

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ABSTRACT

The toxicity and the physiological effects of insecticides, chlorozan, marshal and deltamethrine and the alternative pesticides spinosad and proclaim on the desert locust *Schistocerca gregaria* were tested. The toxicity index, LC_{50} values after 24 hrs were 171.16, 44.29 and 410.05 ppm. for chlorozan, marshal and deltamethrine, respectively. Also the values of LC_{50} after 48 hrs were 425.58 and 101.58 ppm. for spinosad and proclaim, respectively. The effect of all tested insecticides, on the total and different haemocytes count of the 4th instar nymphs of *Schistocerca gregaria* were evaluated. The obtained results revealed that the total haemocyte counts were clearly affected by insecticides tested at LC_{50} values. Chlorozan and proclaim decreased the total haemocytes count (THCs) 50 % compared to control. Also marshal and deltamethrine decreased the total haemocyte count by 35 and 18.3%, respectively. While spinosad showed a slight decreasing in the total haemocyte by 8.9% compared to control. Six different haemocyte types were identified as prohaemocytes (pr), granulocytes (gr), non-granulocytes (n-gr), plasmatocytes (pl), oenocytes (oe) and spherulocytes (sph) cells were monitored. In general, all the tested insecticides decreased the counts of all the haemocyte types. The application of spinosad increased the pr, pl, oe and sph. While it dercreased both n-gr. and gr. The toxicological profile of the tested pesticides described herein characterizes their effects on *S. gregaria* haemocyte types and the total haemocytes count (THCs) when compared with control. These results could be indicate a new proposed mode of action to those friendly environmental promising compounds.

Key words: ***Schistocerca gregaria*, insecticides, haemocyte, total haemocytes count (THCs).**

INTRODUCTION

Insects are generally short live cycle. They may die from a slight accident or injury. Intense external stress such as mechanical immobilization or enforced activity sometimes triggers autointoxication, culminating in paralysis and death (Matsumoto *et. al.*, 2003 and Gaaboub and Halawa, 2003). The desert locust *Schistocerca gregaria* is distributed in the tropical and subtropical regions of the world and has been recognized as a major crop pest in the world Haas-Stapleton *et al.,* 2005. The control of this pest is still mostly achieved by the conventional insecticides. Therefore, it is important to replace these insecticides by the modern environmental friend insecticide. Cohen and Patina (1982) noted that the exposure to such insecticides has been shown to influence various aspects of haemolymph composition of the beat armyworms. Pyrethrum treatment exhibits dissimilar changes in respect of sodium and potassium ion concentrations in the haemolymph and CNS of the carnivorous, orthopteran insect *Schizodactylus monstrosus* Banerjee and Choudhuri (1987). Kulkarni and Mehrotra, (1974) studied the effects of some insecticides on amino acids, nitrogen and proteins in the haemolymph of *S. gregaria,* they found that initially the protein content of haemolymph depleted and increased significantly at the acute poisoning stage, Further, a possible role of haemolymph proteins as insecticide carriers has been postulated. One aspect that has shown response to this stress is the blood cells of the haemolymph Davenport and (Evans, 1984).

The biochemical basis of buffering in the haemolymph of insects has received little attention, and the chemical complexity of insect haemolymph Peel and Akam (2007) suggested that the relative importance of various compounds to buffering may be quite different from that in other arthropods or vertebrates. Harrison *et. al.* (1990) describe the biochemical basis of haemolymph buffering in the migratory locust *Schistocerca gregaria.* However, this

haemocyte population is not only affected by changes during the development process, but also by various forms of stress such as insecticides, parasitization and starvation Nittono (1960); Essawy (1985 and 1991). Injection of the protein dye Fast Green or the fluid-phase probe fluorescein dextran into the haemolymph of vitellogenic female desert locusts (*Schistocerca gregaria*) resulted in their incorporation into oocytes (Peel and Akam, 2007). Exhaustive flight in male *Rhodnius prolixus* Stal (Hemiptera: Reduviidae) can cause up to a 50% decrease in haemolymph volume. The water percentage in the haemolymph does not appear to change substantially during exhaustive flight (Gringorten and Friend, 1979). The possibility that levels of dietary protein and haemolymph composition affect the response of the maxillary palp gustatory receptors is investigated (Abisgold and Simpson, 1988 and Simpson and Simpson, 1992).

Although pest controlling by chemical pesticides have an important role in mangement insect pest attacking crops, the extensive use of synthetic pesticides has inevitably been followed by target pest resurgence, secondary pest outbreaks, development of insecticide resistance in target pest and disruption of natural enemies (Chao and Allen, 1986 and Kristensen *et. al*., 1998) and increased pesticides in the agriculture ecosystem (Metcalf, 1980). The integration of many method or disciplines under the umbrella of IPM may be useful to control various pests in the near future El-Aswad *et. al.* (2001). Spinosad (Tracer®) and emamectin benzoate (Proclaim®) are naturally derived biorational insecticides with an environmentally favourable toxicity profile Bond *et al.* (2004). They are considered important components in pest-management programmes for controlling field crop pests with low toxicity to non-target organisms and the environment. They are powerful compounds for controlling the cotton leafworm *Spodoptera. littoralis* (Josseph *et. al*., 2002). Spinosad is a mixture of spinosyns A ($C_{41}H_{65}NO_{10}$) and D ($C_{42}H_{67}NO_{10}$) belongs to a new class of polyketide-macrolide insecticides (Wyss *et. al*., 2003). In many countries, spinosad is used in control of lepidopteran pests in cotton, tobacco and other crops. It has no systemic effects on plants (Hilal and Oktay, 2006). The activity of spinosad is based on an aerobic fermentation product of the bacterium *Saccharopolyspora spinosa* on nutrient media, and was discovered during the 1980s (Mertz and Yao, 1990). Emamectin benzoate is a highly potent, unique foliar insecticide that controls lepidopteran caterpillars. This compound effectively controls the larval stages of these pests at low use rates, thereby increasing the crop's value. It is a semi-synthetic second generation of averment insecticide. It can be applied by ground or by air, giving growers the flexibility needed for effective Integrated Pest Management (IPM) programs.

The present investigation was performed to throw more lights on the bioassay of some pesticides and pesticide alternatives against the 4th nymph instar of a laboratory strain of *S. gregaria*. Also, this study focused mainly on the effects of these compounds on some hematological parameters as a new mode of action.

MATERIALS AND METHODS

Tested insect

Experiments were carried out on the 4th instar nymphs of desert locusts, *S. gregaria* (Forskal), approximately 2 days post-moult, were segregated from the gregarious stock of *S. gregaria* which had been maintained under the crowded conditions of Hunter-Jones (1961) for three years in the Department of Plant Protection, Faculty of Agriculture Moshtohor, Benha University. Hoppers were kept in wooden cages with glass sides (50x50x50cm) at a rate of 100 per cage. All cages were incubated at 32 ± 2°C and 65±5% R.H. The leguminous plant *Sesbania aegyptiaca*, was daily provided as feeding material.

Insecticides used

Chlorozan (chlorpyrifos 48% EC), marshal 25% WP, chothrin Elnasr (deltamethrin 5% EC), spinosad (Tracer®) and emamectin benzoate (Proclaim®). Emamectin benzoate (Proclaim®) 5% which is a macrocyclic lactone and is like abamectin, supplied by Syngenta Co. and recommended at rate of 60 gm/Feddan and Tracer® 24% SC (the commercial formulation of spinosad) is produced by Dow Agro Sciences Co. and recommended at rate of 120gm/Feddan. Concentration used 500, 250, 150,100, 50, 40, 30, 25, 20, 10 and 5 ppm.

Procedure

For the determination of the base line (LC-p-lines), lettuce leaves dipped for 15 seconds in the investigated insecticide concentration. Series of concentrations were prepared by dissolving the appropriate amount of insecticide into 1000 ml tap water each. Dipped lettuce leaves were dried in the open air. Ten 4th instar nymphs of desert locusts, *S. gregaria* were fed for 24 hrs on the treated leaves, for each tested insecticide concentration, in a clean plastic cup covered with muslin. Each concentration was replicated three times. Control were fed for 24 hrs on lettuce leaves dipped for 15 seconds in tap water and dried in open air. After feeding for 24 hrs on the treated lettuce leaves, the treated nymph were daily fed on untreated fresh lettuce leaves. Mortality counts were observed and recorded daily for 3 successive days post treatment. Percentages mortality were corrected, according to Abbott formula (Abbott, 1925). The concentration required to kill 95, 50 and 5 % of 4th instar nymphs of desert locusts, *S. gregaria* (LC_{95}, LC_{50} and LC_5) in ppm were then determined for each tested insecticide according to the method of Finney, 1971.

Haemolymph studies

Lettuce leaves were soaked in the determined LC_{50} for each insecticide and used for feeding the 4th instar nymphs *S. gregaria*. One hundred eighteen of the 4th instar nymphs

S. gregaria were used for each insecticide. Three replicates were used (60 each) in this respect. *S. gregaria* nymphs were placed in glass jars and provided with insecticide treated leaves. After 24 hrs, surviving nymphs were transferred to clean jars containing fresh leaves. The haemolymph samples were taken after 24 hrs in chlorozan, marshal and deltamethrin and 48 hrs in proclaim and spinosad. The total haemocyte count (THC) were carried out using the haemocytometer as reported according to method described by Jones, 1962 and Essawy *et al.,* 1999. The differential haemocyte count (DHC) was calculated using method of Akai and Sato, 1973. To calculate the (DHC), 100 cells were identified to their typical haemocyte type after staining a smear of haemolymph with Wright's stain Essawy, 1985 and 1990.

RESULTS AND DISCUSSION

Nymphal toxicity

The LC-p-lines for each tested insecticide against 4th instar nymphs of desert locusts, *S. gregaria* were drawn according to method of Finney (1971). Table (1) show the LC_{95}, LC_{50} and LC_5 values for each tested insecticides against 4th instar nymphs of desert locusts, *S. gregaria* as calculated from the resulted LC-p-lines. The evaluated LC_{50} of chlorozan, organophosorus (OP-insecticide) which evaluated, was 171.16 ppm 24 hrs post treatment. After 48 hrs from treatment, the toxicity indexes decreased to 48.98 ppm. The LC_{50} values of carbamate insecticide, marshal was 44.29 ppm, 24 hrs post treatment, which highly decreased to 20.3 ppm 48 hrs post treatment. Regarding to deltamethrin, pyrethroids, the LC_{50} was 410.05 ppm 24 hrs post treatment, which highly decreased to 33.35 ppm 48 hrs post treatment. Accordingly, the 4th instar nymphs *S. gregaria* were found to be more susceptible to marshal than chlorozan and deltamethrin.

According to LC_{50} values of the bioinsecticide proclaim was effective on the 4th instar nymphs *S. gregaria* were more than spinosad, at both 48 and 72 hrs post treatment. The same trend of results was obtained for LC_{95} and LC_5 values.

Effect of the tested insecticides on total haemocyte counts (THCs)

The effects of insecticides chlorozan, marshal and deltamethrin with their LC_{50} values after 24 hrs from treatment and the alternative insecticides, spinosad and proclaim with their LC_{50} values after 48 hrs from treatment, respectively were evaluated on the haemolymph parameters. The total haemocyte counts were clearly affected by all tested compounds.

Fig (1) show the fluctuation of the calculated mean number of haemocytes in the haemolymph of the 4th instar nymph of *S. gregaria* as a result of insecticide treatments. The total haemocyte counts were clearly affected by the tested insecticides. Chlorozan at 171.15 ppm and proclaim at 101.58 ppm values decreased the total haemocyte count by about 50% compared to control. Also, marshal at 44.29 ppm and deltamethrin at 410.05 ppm values decreased the total haemocyte count by about 35 and 18.3%, respectively. While, spinosad at 425.58 ppm showed a slight decreasing of THC by about 8.9% compared to control.

The total haemocyte counts were clearly affected by the tested insecticides spinosad and proclaim at LC_{50} value. Fig. (1) shows the fluctuation of the calculated mean number of haemocytes in the haemolymph of the 4th instar nymph of *S. gregaria* as a result of proclaim and spinosad treatments. Significant decrease was observed regarding the total haemocyte count estimated by 50% with the treatment of proclaim and 8.9%, after using spinosad, compared with control.

As for the differential haemocyte counts, six haemocyte types were identified, Prohaemocytes, Granulocytes, Non-granulocytes, Plasmatocytes, Oenocytoides, Spherulocytes. As shown in (Fig., 2), marshal, chlorozan and deltamethrin treatments reduced the number of (pr) to 6±4.3, 6±3.1 and 8±3.5 cells, respectively comparing to 12±1.2 in the control. The same trend was observed in the number of (Gr) which reduced to 37.3±4.2 in the control versus 14±1.3, 10±1.2 and 13±1.1 cells and also in the number of (N-Gr) 25±3.5 cells in the control, 8.7±1.5, 9.2±2.2 and 10.5±3.1 cells. The number of (Pl) were 27±3.4 in the control versus 12.2±2.2, 15.6±2.6 and 17.2±3.5 cells, respectively. The number of (Oe) and (Sph) were also decreased from 11.2+ 2.2 cells in the control to 5.1±1.2, 6±1.4 and 7±1.4 cells and from 7±1.2 in the control to 3±1.1, 4.2±1.2 and 5±1.7 cells after 24 hrs of the treatment.

Proclaim and spinosad, decreased the number of all haemocyte types. Proclaim at 101.58 ppm and spinosad at 425.58 ppm decreased the number of (Gr) from 21.8±2.1 cells in the control to 12.3±2.6 and 7.6±2.2, respectively and also decreased the number of (Pl) from 17±2.5 cells in the control to 12.3±2.1 and 7.6±1.4 cells after 48hrs of the treatment. A reduce in the number of both (Oe) and (Sph) characterized the treatment locust compared to the control (Fig., 2).

The present result in this concern agreed to the results of Gad and Abdel-Megeed (2006), they observed that spinosad and proclaim decreased the total and differential haemocyte counts also effect and damage on the larval DNA in the *Spodoptera littoralis*. Verma, (1992) studied the changes in total haemolymph protein of last instar larvae of *S. littoralis* by treatment with a carbamate. It was found that for all stages and ages at application, haemolymph proteins decline, 24 hrs after treatment, but later some changes followed as a near pattern with the lower dose, while for the higher dose there is a sharp rise.

These results supported those obtained by Arnold and Hinks, 1983 when they noted a high mitotic and a rapid

Table (1) Comparative toxicity index of the selected insecticides against 4th instar nymphs *S. gregaria*.

	Marshal		chlorozan		Deltamethrin		Spinosad		Proclaim	
	24 hrs	24 hrs	24 hrs	48 hrs	24 hrs	48 hrs	48 hrs	72 hrs	48 hrs	72 hrs
LC_{95}	159.71	159.71	784.02	482.87	1960.22	396.46	5625	140.45	803.81	485.85
LC_{50}	44.29	44.29	171.16	48.98	410 .05	33.35	425.58	47.44	101.58	23.34
LC_5	12.28	12.28	37.37	4.97	3.65	1.73	7.33	16.03	12.84	1.12
Slope	2.95	2.95	2.49	1.66	0.61	1.53	0.93	3.49	1.831	1.25
Chi square	15.46	15.46	5.53	10.04	1.97	7.41	1.58	19.23	7.91	8.41
Variance of slope	$4.68\ 10^{-2}$	$4.68\ 10^{-2}$	$3.385\ 10^{-2}$	$2.257\ 10^{-2}$	$9.286\ 10^{-3}$	$1.458\ 10^{-2}$	1.5	$1.789\ 10^{-2}$	$1.635\ 10^{-2}$	$1.286\ 10^{-2}$
P	0.133	0.133	0.2375	0.03975	0.74102	0.11583	0.81174	$6.6996\ 10^{-5}$	$9.504\ 10^{-2}$	$7.7738\ 10^{-2}$

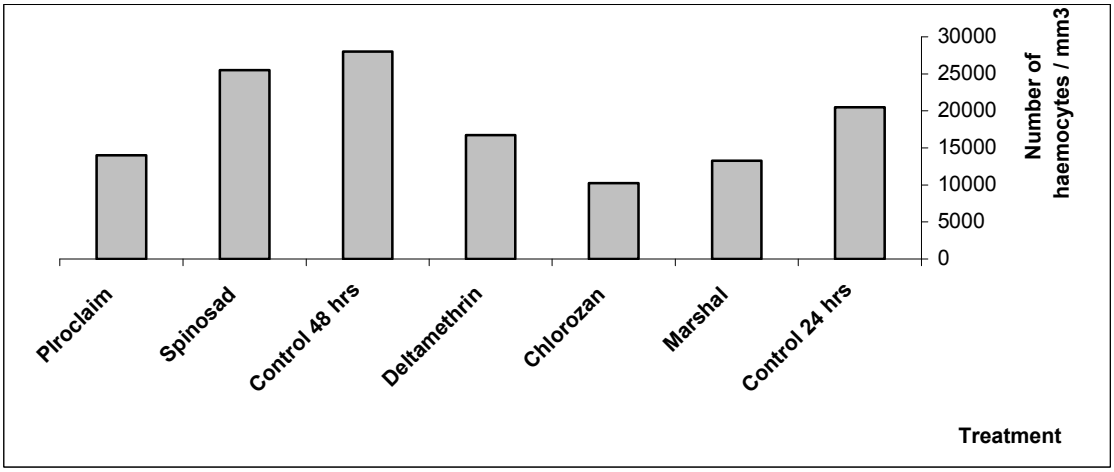

Figure (1): Effect of five insecticides on total haemocyte count of the 4th instar nymphs *S. gregaria*.

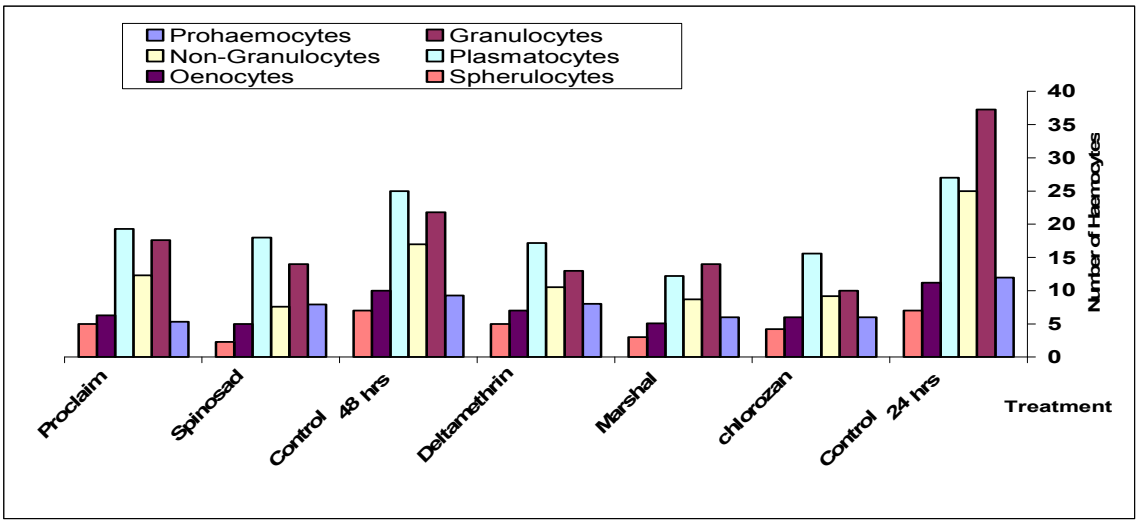

Figure (2): Effect of five insecticides on different haemocyte count of the 4th instar nymphs *S. gregaria*.

turnover of spherule cells, possibly as a mechanism of releasing products of their metabolism into the haemolymph. In addition, spherule cells were noted to play a role in recreation of some haemolymph proteins (Akai and Sato, 1973). Verma (1992) studied the changes in total haemolymph protein of last instar larvae of *S. littoralis* by treatment with a carbamate.

There is a real need/responsibility to make the best use of these new tools as soon as possible. For a variety of reasons, including the continuing rapid consolidation of the agrochemical industry worldwide, the future replacement of any of these new tools is increasingly problematic. In addition to new modes of action, and in some cases directly attributable to a novel mode of action, many of the new insect control agents also possess very favorable mammalian and environmental profiles, as well as high levels of selectivity towards a variety of beneficial insects that should be maximized.

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