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LABORATORY EVALUATION OF NEEM AGAINST THE FIFTH NYMPHAL INSTAR OF *Schistocerca gregaria* (FORSKAL)

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ABSTRACT

Laboratory studied were carried out in an attempt to disclose the effect of antifeedant (Neem) at different concentrations on one day old of the 5th nymphal instar of the desert locust, *Schistocerca gregaria* (Forskal) by feeding technique. Results showed that Neem caused 42% mortality after 12 days from treatment of LC_{50} values. Neem decreased the THC after 2 and 4 days while significant decreased after 6 days compared to control. The free amino acids were non significant between Neem and control after 2 days while significant decreased after 4 and 6 days from treatment. The trehalase was non significant between Neem and control after 2 days while increased after 4 and 6 days from treatment.

Keywords: *Schistocerca gregaria*, Azaderachtin, Total haemocyte count, Free amino acids, Trehalase.

INRODUCTION

The desert locust, *Schistocerca gregaria* (Forskal) (Acrididae: Orthoptera) is one of the most important insect pests, because of its polyphagous nature, attacks on a wide range of plants including agricultural crops. The desert locust is an international pest. Efforts have been made to control this cosmopolitan insect through the International Locust Control Organization of the Food and Agriculture Organization (FAO). The bioinsecticides used in this connection are likely to cause an extensive damage to the haemolymph by which toxic chemicals are conveyed from the site of absorption to the organs, where they are metabolized and excreted (Welling & Paterson, 1985).

The present work aims to study the effect of Neem against the 5th nymphal instar of *S. gregaria* under laboratory conditions. The biochemical effects of Neem on the mortality percentages, total haemocyte count, free amino acids and trehalase.

MATERIALS AND METHODS

1. Experimental insects:

The stock colony of the desert locust, *S. gregaria* was maintained for several years at the Locust Research Division, Plant Protection Research Institute, Agricultural Research Center, Dokki, Cairo. The insects were reared

and handled according to (Hunter-Jones, 1961). Fresh clover leaves *Alexandranium trifolum* Linnaeus were used for feeding the insects in winter and the leaves of leguminous plant, *Sesbania aegyptiaca* Webster, were introduced during summer.

2. Chemical under tests:

Neem:

Neem Force 0.15 % EC (Azaderachtin) ([22,23-3h$_2$] dihydroazadirachtin).

3- Bioassays studies and treatment of experimental insects:

Both sexes of one-day old of the 5th nymphal of *S. gregaria* during synthesis and deposition of the newly adult cuticle (Taha and El-Gammal 1990) were treated by feeding technique with Neem as the following: leaves of *A. trifolum* were dipped in 25, 50 and 100 ppm of Neem for two minutes intervals. Then leaves were air dried before being offered to the nymphs for feeding while the control used was offered leaves treated with distilled water. Three replicates of 20 nymphs were subjected to each of the treated leaves.

After feeding for 24 hr on the treated leaves, the alive nymphs were transferred onto untreated leaves and left to feed for 24 hr, after that mortality counts were recorded.

4- Collection of haemolymph:

Since heat fixation technique proved by many authors to be an excellent one in preventing blood coagulation and preserving the form of haemocytes as they had in circulating blood, this technique was followed as described by (Amin, 1998).

5- Total Haemocyte count:

According to (Wintrobe, 1974).

6- Free amino acids determination:

According to Lee and Takabashi (1966).

7- Determination of Trehalase activity:

According to Ishaaya and Swiriski (1976)

8- Calculations and data analysis:

a) The percentage of nymphal mortality was corrected according to Abbot's formula (Abbot, 1925).

$$\text{Corrected mortality} = \frac{X-Y}{100-Y} \times 100$$

Where: X = % mortality in treatment.

Y= % mortality in control.

9- Statistical analysis:

All experiments were conducted in five replicates. Data are presented as means ± SD. Data were subjected to analysis of variance (ANOVA), and Duncon's multiple range test to differentiate between the means at $P<0.05$, using SAS program (SAS, 1988).

LC_{25}, LC_{50} values and slope of regression lines were calculated by using (Lpd line) a soft ware for calculating and drawing toxicity lines according to Finney Method (1971).

RESULTS AND DISCUSSION

1. Effects of Neem on *S. gregaria* by feeding technique:

Effects of Neem on mortality percentage of *S. gregaria*:

Results in Table (1) showed the effect of four concentrations of Neem against 5th nymphal instar of the desert locust, *S. gregaria*.

Data cleared that the percentages of nymphal mortality of the 5th nymphal instar of *S. gregaria* were 24, 53, 91 and 98% after 12 days old treatment with 375, 750, 1500 and 3000 ppm of Neem, respectively comparing with control.

The present results in this concern agreed to (Saxena and Khan, 1985); they observed to high mortality rates of the brown plant hopper *Nilaparvata lugens* Stal. (Homoptera) were caused by higher concentrations of Azt. Some products of *A. indica* exhibited a larvicidal activity in the horn fly *Haematobia irritans*, stable fly *Stomoxys calcitrans* and house fly *Musca domestica* (Miller and Chamberlain, 1989). The oils pressed from seeds of *Azadirachtin indica* achieved mortality rates of 65-100 % in *S. gregaria* (Schmutterer and Freres, 1990; Nicol and Schmutterer, 1991). Also, high mortality of *S. gregaria*, red locust *Nomadacris septemfasciata* and variegated grasshopper *Zonocerus variegates* was caused by the Neem oil (Schmutterer *et al*., 1993).

Table (1): Biological effects of Neem on *S. gregaria* by feeding technique:

Days	375 ppm	750 ppm	1500 ppm	3000 ppm	Control
1st	0	0	0	1	0
2nd	0	0	0	2	0
3rd	3	0	0	3	0
4th	5	1	0	4	0
5th	3	7	10	19	0
6th	5	14	20	28	0
7th	10	20	28	39	0
8th	12	30	45	47	0
9th	15	39	58	62	0
10th	18	45	64	73	0
11th	21	50	79	89	0
12th	24	53	91	98	0

The data represented in Table (2) and Fig. (1) showed that LC_{25}, LC_{50} and LC_{90} of Neem were 326, 589 and 1806 respectively, and slope values= 2.6.

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

LC	Con. ppm	Lower limit ppm	Upper limit ppm
25	326.3648	164.6631	437.577
50	588.769	367.4418	891.0526
90	1806.4377	1406.6525	4131.3478

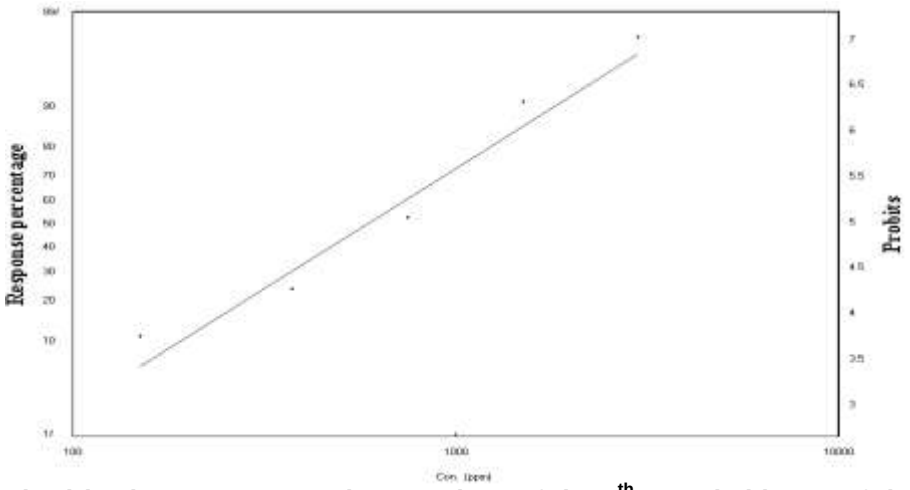

Fig. (1): The LC_{25}, LC_{50} and LC_{90} values of the 5th nymphal instar of the *S. gregaria* treated by Neem.

2. Biochemical effects of Neem on *S. gregaria* by feeding technique:

The effects of bioinsecticides, Neem with LC_{50} values 589 ppm, after 2, 4 and 6 days after treatment, while control were treated with distilled water.

Determination of total haemocyte count:

The total haemocyte count (THC) were recognized, in Table (3) showed the fluctuation of the calculated mean number of THC in the haemolymph of the 5th nymphal instar of *S. gregaria* as a result of bioinsecticides treatments. The THC were clearly affected by the tested bioinsecticides. Neem showed highly significant decreased the THC 10670, 1246 and 1115 cells per cm^3 after 2, 4 and 6 days of treatment compared to control 3240, 2706 and 2210 cells per cm^3.

Similarly (Halouane *et al*., 2001) noted reductions in the haemocyte's numbers after infection of adult of migratory locust *L. migratoria* by *Beauvaria bassiana*. Xia *et al*. (2000) have recorded a decline in the total haemocyte count and a marked reduction in the proportion of plasmatocytes and coagulocytes after inoculation of mature adult males of the desert locust infected with *Metarhizium anisopliae* var. *acridium*.

The present result in this concern agreed to the results of (Gad and Abdel-Mageed, 2006); they observed that Spinosad and Proclaim decreased

the THC. Starvation effect has also been reported to increase the THC in *Prodenia eridania* (Rosenberger and Jones, 1960). The main active constituent of Neem gold is azadirachtin, a tetranortriterpenoid which is a strong antifeedent and also acts as a potent growth inhibitor at microgram levels (Schmutterer, 1990). Ayyangar and Rao (1990) recorded a steep fall in THC/ mm^3 (percentage reduction 64) after 72 hr of injection of azadirachtin to the last instar larvae of *S. litura* Fab., whereas reduction in THC was observed to be 49.95% after 72 hr of oral feeding of 1500 ppm of Neem gold. In *Periplaneta americana* (Qadri and Narsaiah, 1978), injection of azadirachtin to the last instar nymphs results in 20-25 % decrease in haemocytes after 24 hr of treatment. These findings clearly bring forth the efficacy of the botanical in drastically lowering down the counts. The initial triggering in THC after 24 hr of treatment was due to the starvation effect. The decrease in THC was observed after 48 to 72 hr due to the biological effects induced by Neem gold. The THC came down from 27, 894 to 14, 528 at 1500 ppm of Neem gold after 72 hr of treatment (Sharma *et al*., 2003).

Table (3): The effect of Neem on THC of the 5^{th} nymphal instar of *S. gregaria*, (the number of cells per cm^3).

	Neem	Control	LSD
THC 2 days	1670 d	3240 b	287.7
THC 4 days	1246 d	2706 b	245.3
THC 6 days	1115 d	2210 b	229.8

F: Measurement of distance between individual distributions.
Means with the same letter are not significantly different.
Means with the same letter are not significantly different and a=**, b=*** ,c=**, d=***

Determination of Free Amino Acids:

Data in table (4) show that, the free amino acids non significant between Neem (5.69) and control (5.61) µg alanine per gm body weight after 2 days while significant decreased after 4 and 6 days 5.7 and 9.76 µg alanine per gm body weight from treatment.

This result agrees with those obtained by Bakr (1986) on *M. domestica* after feeding the two days old larvae 1 ppm of Dimilin, BAY SIR 8514 and Altosid. These IGRs increased of the majority of the free amino acids. The feeding deterrence caused by azadirachtin was manifested by a severely reduced food intake resulting in diminished weight gain compared with controls. This is in general agreement with previous observations (Mordue and Blackwell, 1993). Trumm and Dorn (2000) reported that the reduction of food intake after the azadirachtin treatment was accompanied and probably caused by a prolonged retention of food in the crop and a strongly retarded passage through the midgut. The effects of azadirachtin on gut physiology have been mostly related to efficiency of diet conversion and inhibition of digestive enzymes (Koul *et al*., 1996). Timmins and Reynolds (1992) showed that Azadirachtin reduced growth of *M. sexta* larvae due to impaired protein digestion by inhibition of trypsin synthesis and/or secretion by midgut cells. Other hypothesis puts forward to explain this phenomenon: reduction in the haemolymph nutrient content, i.e. proteins, lipids and

carbohydrates. The growth rate of insects is generally more closely correlated with nutrient content in the leaves. Also malformations of nymphs are seen and nymphs developed abnormal. Before nymphs moved slowly and had no appetite. (Mordue and Blackwell, 1993) attributed the direct toxicity and rapid mortality to the combined activities of antifeedancy and insect growth regulatory (IGR) effects.

Table (4):The effect of Neem on free amino acids of the 5th instar nymphs *S. gregaria*, (µg alanine per gm body weight).

	Neem	Control	LSD
Trehalase 2 days	439 c	430 c	37.0
Trehalase 4 days	441 c	351 d	34.1
Trehalase 6 days	399 c	306 d	10.7

F:Measurement of distance between individual distributions.
Means with the same letter are not significantly different and a=**, b=*** ,c=**, d=***

Determination of Trehalase activity:

Data presented in Table (5) cleared that, the trehalase non significant between Neem (439) µg glucose released /min / gm fresh weight and control (430) after 2 days while significant increased 441 and 399 µg glucose released /min / gm fresh weight after 4 and 6 days from treatment.

This results agree with (Mohamad, 2009) observed increasing consumption of trehalose by the *M. anisopliae* var. *acridum* to obtain the sole source of carbon after 24 and 48 hrs from infection. Also (Tanani *et al*. 2012) showed that the treated of newly molted last 5th instar of the *S. gregaria* was by through fresh plant IGR Tebufenozide caused statistically significant increased to trehalase after 4 days.

Table (5): The effect of Neem on trehalase activity of the 5th nymphal instar of *S. gregaria* (µg glucose released /min / gm fresh weight).

	Neem	Control	LSD
Free Amino Acids 2 days	5.69 b	5.51 b	0.8
Free Amino Acids 4 days	5.7 b	9.07 a	0.69
Free Amino Acids 6 days	9.76 c	13.23 a	0.68

F:Measurement of distance between individual distributions.
Means with the same letter are not significantly different and a=**, b=*** ,c=**, d=***

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تقييم معملى للنيم ضد حوريات العمر الخامس للجراد الصحراوى

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أجريت هذه الدراسة المعملية للكشف عن تأثير التركيزات المختلفة من مانع التغذية (النيم Neem) على العمر الحورى الخامس للجراد الصحراوى باستخدام تكنيك التغذية.

وتوضـح النتـائج أن النـيم سـبب 42 % مـوت باسـتخدام (LC_{50}) , أظهـرت المعاملـة باستخدام النيم انخفاض معنوى فى العدد الكلى لخلايا الدم THC فى جميع المعـاملات بعد 2 , 4 و 6 أيام مقارنة بالكنترول.

كما أوضحت النتائج أن المعاملة بالنيم لم تعطى تأثيرا معنويا على الأحماض الأمينية الحرة بعد يومين في حين انخفضت بنسة كبيرة بعد 4 و 6 أيام من المعاملة مقارنة بالكنترول.

بينت النتائج تأثير النيم على نشاط انزيم Trehalase ولكن كان غير معنويا بعد يومين في حين أدى إلى زيادة معنوية بعد 4 و 6 أيام من المعاملة.

قام بتحكيم البحث

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