Effects of *Artemisia* Extract on The Fourth Instar of *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae)

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ABSTRACT

Different parameters of *Artemiaia monosperma* petroleum ether extract were studied on treated desert locust *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae) 4th nymphal instar. The studied parameters when the topical application was applied were: percentages of nymphal mortality, inhibition of the next nymphal instar and adult formation, and the effects on the reproductive system of the survived males and females. The tested concentrations were 50, 100, 500, 1000 and 5000 ppm. by using ethyl alcohol as a solvent. The nymphal mortality percentages were zero for 50 ppm and the control, while they ranged from 13.33 to 26.66 % for the higher concentrations. The inhibition of nymphs and adult formation was concentration dependent. Drastic malformation was observed in the treated nymphs and adults resulted from the treated nymphs either externally or in the reproductive system of the survived males and females. Also, food consumption, food deterrent effects and chitin deposition of body wall were estimated by using no choice feeding method. The results of the chitin deposition of the body wall indicated that the treated nymphs were less than the check. Studies of some nutritional indices showed that food consumption and growth rate decreased with the increase of the tested concentrations. By calculating the feeding deterrent effect of the *Artimisia* extract, it was proved to be moderate deterrent for the desert locust.

Keywords: Schistocerca gregaria, Artemesia extract, Physiological effects.

INTRODUCTION

The desert locust *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae: Cryrtacanthacridinae) is a serious and major pest of any green plant all over the world. Locusts and their control have attracted great attention especially in the context of eco-toxicological studies and development of environmentally safe control alternatives. Plant products have proved to be suitable candidates that fit reasonably well in locust management programs (Abdalla 2004).

Recently, interest in the development of natural insecticides has increased to avoid using synthetic insecticides which cause much of environmental pollution, development of insecticide resistance, insecticide-induced resurgence of insect pest and adverse effect on non-target organisms (Abdellaoui *et al.,* 2009). Plants as sources of active chemical compounds used in combating insects were and still are the subject of interest of many workers all over the world (El Zoghby,1975). During recent years, some plants attracted global attention and their extracted components have been formulated as botanical pesticides for plant protection. *Artemisia* species are widely used as medical plants in folk medicine. According to Shakarami *et al.,* (2004) and Negahban, *et al.,* (2004) these species may possess insecticidal, repellent or anti-feedent properties.

The aim of this study was to evaluate the effect of petroleum ether extract of *Artemisia monosperma* L. on the desert locust *S. gregaria.*

MATERIALS AND METHODS

Insect rearing:

S. gregaria (Forskal) used in this study originated from the culture reared in laboratory of Locust and Grasshoppers Research Department, Plant Protection Research Institute, Cairo, Dokki, Egypt. *S. gregaria* was reared under laboratory conditions of 30 ± 5 °C, 60 ± 5% RH and 12:12 hours of (L.D.) The newly hatched nymphs and the adults were fed on clover (Berseem) plant, *Trifolium alexandranium*, and dry wheat bran mixed with powdered milk and fortified with 5% yeast powder in Petri dishes as a source of vitamin B_1.

***Artemisia* crude extract:**

The aerial parts of *Artemisia monosperma* (Fam.: Compositae) were extracted by petorlium ether by Dr .Maha Aboulela, Faculty of Pharmacy, University of Alexandria. The plant extract contains coumarins, hydroxy coumarins and some flavonides(Kim *et al.,* 2004). It is dissolved in ethyl alcohol and used in dosages of 50, 100, 500, 1000 and 5000 ppm by the topical application method. The tested dosages were applied on the neck membrane of the newly molted 4th instar nymph at the rate of 1 microliter per nymph. Fifteen nymphs

were used for each concentration in 3 replicates. Controls were set up using the solvent only.

Feeding assay with the no-choice test technique was used against the newly molted 4th instar nymph of the desert locust according to Morimoto *et al.* (2006). Clover fresh leaves were prepared by dipping in 50, 100, 500, 1000 and 5000 ppm concentrations. Three replicates were carried out for each concentration each replicate with 5 nymphs.

Parameters studied for the tested material:

Measurments followed for all the tested concentrations by topical application or by feeding assay were: percentages of nymphal mortality, inhibition of nymphal and adult formation, abnormality of nymphs and adults. Dissection of the reproductive system of the survived males and females adults in different treatments and control were carried out under a binocular microscope to observe if they are affected with the treatments.

Measurment of chitin deposition of the body wall:

This experiment was conducted on the newly molted 4th instar nymph of the desert locust where nymphs fed for the interval of 186 hours on fresh leaves for the control and on treated leaves with 100, 500 and 1000 ppm. of *Artemisia* extract according to Hughes *et al.*, (1989). All concentrations were dissolved in ethanol 95% and the check was treated only by ethanol 95%. After feeding, the nymph weighed, anaesthetized by chilling, tissues were removed .After rinsing under water, the body wall of each nymph was placed in 3 ml of 10% (w/v) potassium hydroxide (KOH) at 100°C for 4 hours, then allowed to stand overnight at room temperature. The remaining chitin from each nymph was washed thoroughly with cold water and oven dried overnight at 80 °C, then weighed. In this way, the ratio chitin dry weight to the nymphal fresh weight could be determined for the individual nymph , as follows:

Ratio of chitin formation= chitin dry weight / nymphal fresh weight

Food consumption and utilization:

The effect of tested material on food consumption and utilization by the newly molted 4th instar nymph of the desert locust was investigated. The nymphs were reared on clover leaves. They were given known weights of fresh leaves (0.50 gm.) treated by dipping in different concentrations (ppm) of the crude extract and ethyl alcohol solvent for the control for a period of 30 sec. All nymphs faeces and unconsumed food were weighted every 24 hours through 24, 48 and 72 hours feeding period. The measured nutritional indices were namely the relative consumption rate (RCR) and relative growth rate (RGR), they were calculated according to Miller and Miller, (1988). Efficiency of conversion of ingested food (ECI), consumption rate (CI) according to Klein & Kogan, (1974), efficiency of conversion of digested food (ECD) and the approximate digestibilities (AD) were calculated according to the formulae of Waldbauer (1968) and Farrar *et al.*, (1989). To measure the activity of the different concentrations of *Artimisia* extract, feeding-deterrence index Isman *et al.*, (1990) was used.

RESULTS AND DISCUSSION

Effect of *A. monosperma* extract were studies on the following parameters:

A- Mortality and inhibition of nymph and adult formation:

Figure (1) illustrated the different effects of *A.monosperma* extract on the mortality, development and successful adult formation of the 4th instar nymph of the desert locust *S. gregaria* treated with topical application. The dosages of 50, 100, 500, 1000 and 5000 ppm caused nymphal mortalities of 0, 13.33, 20, 26.66 and 26.66 % while it was 0% in the check. When the inhibition percentages of the 5th instar nymphs were calculated, they were 13.33, 20, 26.66,40 and 53.33%, respectively, while it was 0% in the check.

The inhibition of adult formation percentages were 26.66, 40, 53.34, 80 and 88.6%, in respect, for the previous tested concentrations, while the check recorded zero% inhibition.

In other words, the dosage of 50ppm did not cause nymphal mortality and equals the effect of control. The other dosages displayed concentration dependent effects, while there was no difference between 1000 and 5000 ppm. Concerning with the inhibition of nymphs and adult formation percentages they have the same trend. These data gave the impression that *Artemesia* extract has the same manner of the insect growth regulators as illustrated by El -Zoghby (1991 and 1992 a&b), who detected the effect of some plant materials on the larval development in *Spodoptera littoralis* and the activity of these materials as insect growth regulators.

B- Chitin body wall formation:

Data presented in Table (1) showed that the average nymphal fresh weight after removing its viscera was 0.29, 0.27 and 0.25 gm for the used successive concentrations, respectively, while the check recorded 0.33gm. When the chitin dry weight was calculated in mg., the weights of 2.72, 1.99, 1.52 and 3.60 mg were obtained, in respect, for the used concentrations and the check. Finally, the chitin formation ratios (mg/g) were calculated and recorded 9.38, 7.73, 6.61 and 10.91 mg/g, in respect for the previous concentrations and in the check. The obtained data proved that the chitin formation ratio decreased in *S. gregaria* nymph in relation to the increasing *Artemisia* extract concentrations. These results emphasized that the *Artemisia* extract could be considered as a nymphal growth inhibitor and also, an inhibitor of chitin synthesis.

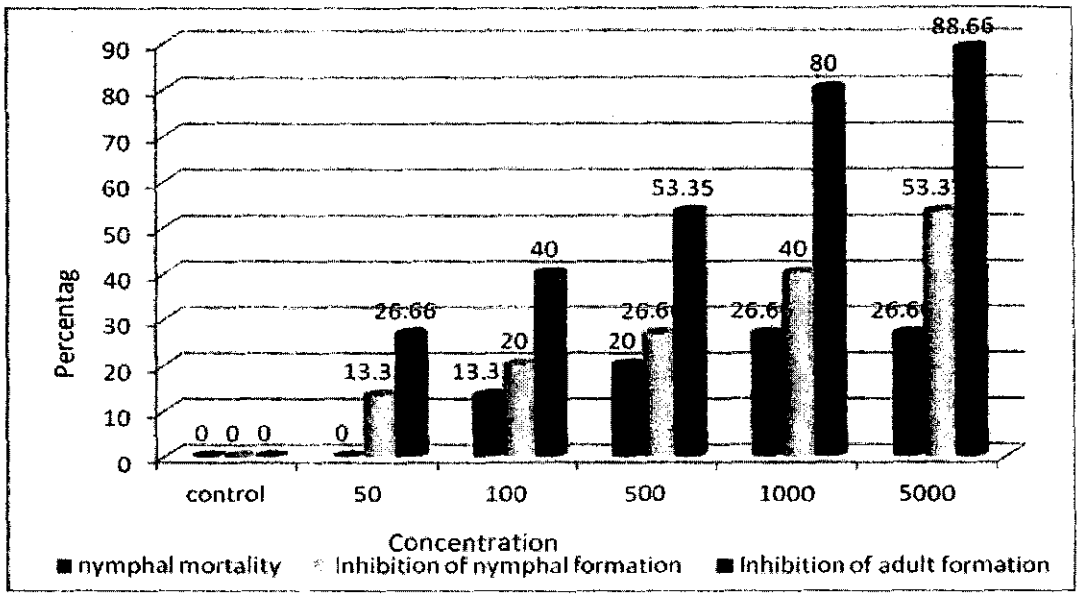

Figure 1: Different effects of *A. monosperma* petroleum ether extract on the mortality, development and the inhibition adult formation of the 4th instar nymph of the desert locust treated with topical application.

Table 1: Effect of *A. monosperma* extract on chitin formation of the desert locust nymphs

Concentration	Average of nymphal fresh weight after removing its viscera (g)	Average of chitin dry weight (mg)	Chitin formation ratio (mg/g)
100	$0.29^{a} \pm 0.1$	$2.72^{a} \pm 0.07$	$9.38^{a} \pm 0.38$
500	$0.27^{b} \pm 0.01$	$1.99^{b} \pm 0.17$	$7.73^{b} \pm 0.64$
1000	$0.25^{b} \pm 0.0$	$1.52^{c} \pm 0.14$	$6.61^{b} \pm 0.56$
Control	$0.33^{c} \pm 0.4$	$3.60^{d} \pm 0.21$	$10.91^{a} \pm 0.62$
F	19.505*	29.712*	13.185*

Data are expressed as (mean ± SE)
F: F test (ANOVA)
Different superscripts within each column indicate statistical significant differences between groups at 0.05
*: Statistically significant at $p \leq 0.05$

These interpretations are in accordance with the findings of Bakr *et al.* (2008) who reported that the growth of Lufenuron treated nymphs of *S. gregaria* was profoundly inhibited because there weight gain was drastically reduced. Such reducing action of Lufenuron was dose- dependent after treatment of newly moulted 5th instar nymphs. On the contrary, Hughes *et al.* (1989) found that this ratio did not differ between treated larvae of the tobacco hornworm and control when cytomozine was used.

C- Malformation of nymphs and adults:

Abnormal nymphs were resulted from the treatments of all concentrations of *Artemesia* extract. They were distorted in the thoracic region with wings folded and failed in ecdysis. Some have a swelling protuberance on the dorsal side of thorax filled with ecdysis liquid. Also, the adults have distorted wings and legs. For all high concentrations (500, 1000 and 5000 ppm.) the adults stopped egg laying as a result of dryness of the end of the abdomen. When the adults obtained from treatments and dissected, the ovaries were found enlarged in some cases and pigmy in other cases, and abnormal oviducts were found especially at the dosage of 5000 ppm, abnormal testes were observed with the treatment of 5000 ppm.

In the same trend of investigations, Rembold (1997) found that *Melia volkensii* crude powder caused retarded growth and increased the rate of malformation of the desert locust in different doses. Also, El- Sayed and El-Otaibi, (2006) investigated the adult grasshopper, *Poecilocerus brotnius* which fed on three host foliages *Calotropis procera*, *Zygophylum simplex*, and *Pulicaria crispa*. The development of adult females was shorter when fed on *C. procera* than those fed on other host foliages.

D- Nymphal growth rate:

Data of Table (2) showed the difference between the initial weight and the daily gained weight, it could be noticed that weight increase in the treatments was very low when compared with that of the control in the first two days.

Table 2: The effect of *A. monosperma* extract on the nymphal weight of desert locust nymphs

Concentration (ppm)	Initial weight (gm)	Average nymphal weight through 7 days						
		24 hr	48 hr	72 hr	96 hr	120 hr	144 hr	168 hr
100	$0.443^{a}\pm0.002$	$0.450^{a}\pm0.001$	$0.463^{a}\pm0.001$	$0.471^{a}\pm0.002$	$0.486^{a}\pm0.003$	$0.490^{a}\pm0.004$	$0.497^{a}\pm0.003$	$0.496^{a}\pm0.002$
500	$0.433^{b}\pm0.001$	$0.440^{b}\pm0.001$	$0.447^{b}\pm0.001$	$0.452^{a}\pm0.005$	$0.468^{b}\pm0.006$	$0.476^{b}\pm0.001$	$0.474^{b}\pm0.001$	$0.480^{b}\pm0.004$
1000	$0.420^{c}\pm0.005$	$0.426^{c}\pm0.001$	$0.432^{c}\pm0.002$	$0.428^{a}\pm0.002$	$0.426^{c}\pm0.002$	$0.416^{c}\pm0.002$	$0.410^{c}\pm0.002$	$0.410^{c}\pm0.003$
Control	$0.456^{d}\pm0.001$	$0.478^{d}\pm0.001$	$0.489^{d}\pm0.003$	$0.498^{a}\pm0.049$	$0.523^{d}\pm0.002$	$0.545^{d}\pm0.003$	$0.561^{d}\pm0.003$	$0.574^{d}\pm0.002$
F	70.260^{*}	1392.750^{*}	475.400^{*}	1.864	367.089^{*}	1123.0^{*}	2293.262^{*}	1647.515^{*}

Data are expressed as (mean ± SE) F: F test (ANOVA)

Different superscripts within each column indicate statistical significant differences between groups at 0.05*: Statistically significant at $p \leq 0.05$

Table 3: The calculation of the nutritional indices of the desert locust nymphs allowed to feed on clover leaves treated with *Artemisia monosperma*

Conc. ppm	I	Δ B	BaT	F	RCR	RGR	EC1	ECD	AD	CI	FDI%
100	914.2	59	130.71	247	6.99	8.43	6.45	8.84	72.98	130.6	12.95
500	770	43.1	127.86	134.3	6.02	6.16	5.60	6.78	82.56	110	26.66
1000	630	11.2	123.39	103	5.11	1.60	1.78	2.13	83.65	90	40
Control	1050	141.3	143.04	291.2	7.44	20.19	13.46	18.62	72.26	150	-----

I = Weight of the food consumed = consumed food/ No. of nymphs
Δ B = Change in body weight = final weight – initial weight/ no of nymph
BaT = (initial weight + final weight/ no. of nymphs) × feeding period
F = Weight of the feces produced during the feeding period/ no of nymph
RCR = Relative consumption rate = I/ BaT
RGR = Relative growth rate = ΔB/BaT
ECI = Efficiency of conversion of ingested food = ΔB/I × 100
ECD = Efficiency of conversion of digested food = ΔB/ (I – F) × 100
AD = Approximate digestibility = (I – F) / 100
CI = Consumption rate = I/feeding period
FDI= the feeding deterrence index = [(C-T)/C] x 100

At the third day (72 h.), no significant differences appeared between the weight of the treatments or between them and the control. At the fourth day, the treated nymphs with the highest concentrations (1000 ppm) started to lose weight until the end of the nymphal period. For the less concentrations, the average nymphal weight after 168 h. decreased when the applied concentrations increased. While the check increased from 0.456 gm. to 0.574 after the same period.

E- Nutritional indices:

The obtained data were summarized in Table (3). The weights of food consumed per nymph (I) were 914.2, 770 and 630 mg. for the tested concentrations, respectively, while it recorded 1050 mg. in the check. Therefore, the change in the nymphal body weight (ΔB) decreased when the concentration increased. The relative consumption rate (RCR) values were calculated according to the formulae followed Table 3. They were 7.44 in the check while and 6.99, 6.02 and 5.11 for the tested concentrations, respectively. The relative growth rate (RGR) was calculated for each treatment and gave the values of 8.43, 6.16 and 1.6 for the tested concentrations , in respect, while it was 20.19 in the check. On the other hand, the efficiency of conversion of ingested food (ECI) values gave the values of 13.46, 6.45, 5.60 and 1.78 ,in respect for the check and the tested concentrations, respectively. Another nutritional index which was the efficiency of conversion of digested food (ECD) values scored 8.84, 6.78 and 2.13 for the previous tested concentrations, in respect, while the check scored a value of 18.62. Also the consumption rate (CI) values were determined giving values of 130, 110 and 90 for the tested concentrations, in respect, while the check scored 150. The approximate digestibility (AD) recorded values of 72.26, 72.98, 82.56 and 83.65 for the check and the tested concentrations, in respect .Finally, the feeding deterrence index gave the values of 12.95, 26.66 and 40.00 for the previous tested concentrations ,in respect .According to Isman *et al.* (1990), the feeding deterrence index was concedered: no feeding deterrence when FDI %> 20 % and moderate feeding deterrance when FDI>50% and strong feeding deterrence when FDI> 70%. The obtained results emphasized that the value of FDI at the concentration of 1000 ppm gave a value of 40.00%which means that the *Artemisia* extract was considered a moderate feeding deterrence at the concentration of 1000 ppm. The obtained data were in accordance with those of Kabaru and Gichia (2001) who found antifeedant activity of the extracts derivated from different parts of the mangrove tree *Rizophora mucronata* against the desert locust. Also, Zypman *et al.* (2001) detected antifeedent activity of different types of *Azadirachta indica* on the desert locust nymphs through the no-choice feeding bioassay.

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الملخص العربى

التأثيرات المختلفة لمستخلص نبات الارتيميزيا على حوريات العمر الرابع للجراد الصحراوى

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تم فى هذه التجربة دراسة المستخلص البترولى لنبات الارتيميزيا مونوسبرما على حوريات العمر الرابع للجراد الصحراوى بخمس تركيزات وهى ٥٠، ١٠٠، ٥٠٠، ١٠٠٠ و ٥٠٠٠ جزء فى المليون. التركيز ٥٠ جزء فى المليون لم يعطى أى نسبة موت فى الحوريات كما فى الكنترول بينما تراوحت هذه النسبة المئوية بين ١٣,٣٣ و٢٦,٦٦ % للتركيزات الاعلى فى حين كانت النسبة المئوية لتثبيط تكوين حوريات العمر الخامس وكذلك النسبة المئوية لتثبيط تكوين الحشرات الكاملة تتراوح بين١٣,٣١ – ٥٣,٣٣ و٢٦,٦٦ – ٨٨,٦٦% على الترتيب. وعند دراسة تأثير هذا المستخلص البترولى على تكوين الكيتين للتركيزات ١٠٠، ٥٠٠، ١٠٠٠ جزء فى المليون بطريقة التغذية وجد ان هناك تناقص فى تكوين الكيتين يتراوح مابين ٦,٠٥ – ٩,٤٤ ملجم/جم بالمقارنة بالكنترول (١٠,٦١ ملجم/ جم). وعند دراسة تاثيرها على وزن حوريات العمر الرابع بعد سبعة ايام من المعاملة وجد تناقص فى وزن الحوريات يتراوح بين ٠,٤١٠ – ٠,٤٩٥ جم/حورية بالمقارنة بالكنترول(٠,٥٧٤جم حورية).بدراسة معدل استهلاك الغذاء وعند تقدير التأثير المعوق للتغذية للتركيزات المختلفة فقد وجد ان هذا المستخلص يصنف على انه متوسط فى المنع للتغذية عند تركيز ١٠٠٠ جزء فى المليون حيث سجل ٤٠%. و قد ادى استخدام هذا المستخلص بتركيزاته المختلفة لحدوث تشوهات لكل من حوريات العمر الخامس والحشرات الكاملة وكذلك للخصية والمبيض والقنوات التناسلية.