Effects of Ethanolic Bouhinia Extraction on Fourth Instar Nymph of *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae)

Osama Mohamed El-Ansary[1]; Hanan Mohamed Ramadan[1]; Gehan Aly Mohamed[2] and Waffaa Mohamed Gabra[3]

[1]Alexandria University, Faculty of Agriculture, Applied Entomology Dept.
[2]Locust and Grasshoppers Research Dept., Plant Protection Research institute, Al-Dokki, Egypt
[3]Minstry of Agriculture, Burg Al-Arab Station

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ABSTRACT

Effect of the *Bouhinia purpurea* ethanolic extract on the desert locust *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae) 4th nymphal instar was evaluated under laboratory conditions. The studied parameters when the topical application was applied were: percentages of nymphal mortality, inhibition of the next nymphal instar and adult formation. Effects on the reproductive system of the survived males and females were observed. The tested concentrations were 50, 100, 500, 1000, 5000 and 7500 ppm. by using ethyl alcohol as a solvent. The nymphal mortality percentages were ranged from 6.66 to 40%. 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 *Bohinia* extract, it was proved to be moderate deterrent for the desert locust.

Keywords: *Schistocerca gregaria, Bouhinia purpurea* ethanolic extraction, Physiological effects.

INTRODUCTION

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

Botanical insecticides are generally pest-specific and are relatively harmless to non-target organisms including man. They are also biodegradable and harmless to the environment (Rembold, 1997). Furthermore, unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise an array of chemical compounds which act concertedly on both behavioural and physiological processes. Thus the chances of pests developing resistance to such substances are less likely (Saxena *et al.*, 1992). FAO (1989 & 2004) recommended appropriate applied researches to control desert locust. One of them was testing of environmental friendly products, such as IGRs, mycopesticides and other products. On the other hand, Macedo *et al.* (2007) recorded insecticidal activity of *Bouhinia monandra* leaf lectin extract against some stored grain pests.

The aim of the present work was to evaluate the effects of the ethanolic extraction of *Bouhinia purpurea* on the desert locust *Schistocerca gregaria* under laboratory conditions.

MATERIALS AND METHODS

Insect rearing:

S. gregaria used in this study was obtained from the culture reared in the laboratory of Locust and Grasshoppers Research Department, Plant Protection Research Institute, Dokki, Giza, Egypt. *S. gregaria* was reared under the laboratory conditions of 30 ± 5 °C, 60 ± 5% RH and 12:12 hours of (L.D.) Newly hatched nymphs and 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.

Application of Bouhinia crude extract:

The *B. purpurea* Leguminasae (Fabaceae) flower crude extract, contains flavones (high percent), coumarin and kenon was provided by Shimaa Eslam, Faculty of Pharmacy, Alexandria University. It was dissolved in ethyl alcohol and used in dosages of 50, 100, 500, 1000, 5000 and 7500ppm for the topical application method. The tested dosages were applied on the neck of newly molted 4th instar nymph, at the rate of 1 microliter/nymph. Fifteen nymphs were used for each concentration in 3 replicates, each one with 5 nymphs. Control was set up using the solvent only.

Feeding assay with no-choice test technique was used against newly molting 4th instar nymph of

according to Morimoto *et al.* (2006). Fresh leaves of clover were prepared by dipping in 50, 100, 500, 1000, 5000 and 7500 ppm concentrations for 30 seconds. Three replicates were carried out for each concentration, each replicate with 5 nymphs.

Criteria studied for the tested material :

Measurements of percentages of nymphal mortality, inhibition of 5th instar nymph and adult formation and abnormalities in nymphs and adults followed all the tested concentrations by topical application or by feeding assay. Dissection of the reproductive system of the survived male and female adults in different treatments was carried out under binocular microscope to observe any effect on the reproductive system. The ovaries and testes of normal and abnormal adults were removed and photographed.

Measurment of chitin formation of the body wall:

This experiment was conducted on the newly molted 4th instar nymphs of the desert locust that fed for an interval of 186 hours, on fresh clover leaves in the control or on treated leaves with 100, 500 and 1000 ppm *Bouhinia* extract according to Hughes *et al.* (1989) method. After feeding, the nymph were weighed , anaesthetized by chilling and 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 maintain overnight at room temperature .The remaining chitin from each nymph was washed thoroughly with cold water. The body walls were oven dried overnight at 80°C and weighed individually. By this way, the ratio of chitin dry weight to the nymphal fresh weight could be determined for individual nymph, as follow:

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

Food consumption and utilization :

Effect of the 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 0.50 gm clover leaves treated with dipping in different concentrations of the crude extract (in ppm) for a period (30 sec.) and in ethyl alcohol solvent for the check. All nymphs, feces and unconsumed food were weighed every 24 hours through 24, 48 and 72 hours feeding period. The nutritional indices were namely the relative consumption rate (RCR), relative growth rate (RGR) (Miller & Miller, 1988), efficiency of conversion of ingested food (ECI), consumption rate (CI) according (Klein & Kogan,1974) and efficiency of conversion of digested food (ECD). Approximate digestibilities (AD) were calculated according to the formulae of Waldbauer (1968) and Farrar *et al.*, (1989). To measure the activity of different crude extracts, feeding-deterrence index which was suggested and calculated by Isman *et al.*, (1990) was used.

Statistical analysis of data was fulfilled using F test according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

A. Effect of *Bouhinia purpurea* extraction on mortality, inhibition of 5th nymphal instar and adults formation of *S. gregaria*:

Figure (1) shows different effects of the *B. purpurea* extract on the mortality, and the inhibition of nymphal and adult formation of the 4th instar nymph of *S. gregaria* treated with topical application. The tested concentrations were 50, 100, 500, 1000, 5000 and 7500 ppm, while ethyl alcohol was used only in the check. Percentages of 4th nymphal mortatity were 6.66, 10, 13.33, 26.59, 26.66 and 40% for the tested dosages, in respect, while there was no mortality in the check. Inhibition of 5th nymphal instar formations was, 6.66, 20, 20, 26, 33.33 and 40%, in respect. It was obtained when there was no inhibition in the check. Also, when the values of inhibition of adult formation percentages were calculated, they scored 20, 40, 60, 73.34, 80 and 86.67%, in respect, for the tested concentrations, while it was zero % in the check. Ghoneim *et al.* (2009) assessed both methanolic, petrolium ether and n-butanolic extract of *Fogonia bugueri* against the penultimate and last instar nymphs of *S. gregaria*. After treatment of the penultimate instar nymphs, a dose-dependent trend of mortality could be observed for the methanolic extract. El-Gammal *et al.* (2008) studied the growth rate and metamorphosis of the *S. gregaria* 4th instar nymph feeding on maize leaves and treated with different extracts (methanol, acetone and exthoxy-ethanol) from leaves and roots of Bermuda grass (*Cynodon dactylon* L.). Grass leaves extracts caused 8.35 % mortality and 91.7 5 malformation of 5th instar nymphs. The other 60.05 molted to weak and small 5th instar nymphs. Feeding of 5th instar nymphs on the treated leaves of maize with methanol, acetone, ethoxyethanol leaves and roots of Bermuda grass resulted in different percentages of mortality ranged from 11.1 to 90%. The highest mortality percent was obtained by ethoxyethanol leaves extract (90.0%). The lowest one resulted after feeding on methanol leaves extract which induced the highest percent of adult (86.7%) followed by methanol and acetone extracts (60.0%). The emerging adults showed intermediate features of the 5th instar and the adult stage.

B. Effect of *Bouhinia purpurea* extract on abnormality of nymphs and adults

Figure (2) illustrates the effect of *Bouhinia* extract on malformation of the desert locust nymphs. The nymphs became distorted in the thoracic region for all concentrations (Figure 2-B). Ecdysis liquid was confined in swelling protuberance on the dorsal side of thorax, (Figure 2-C- H).

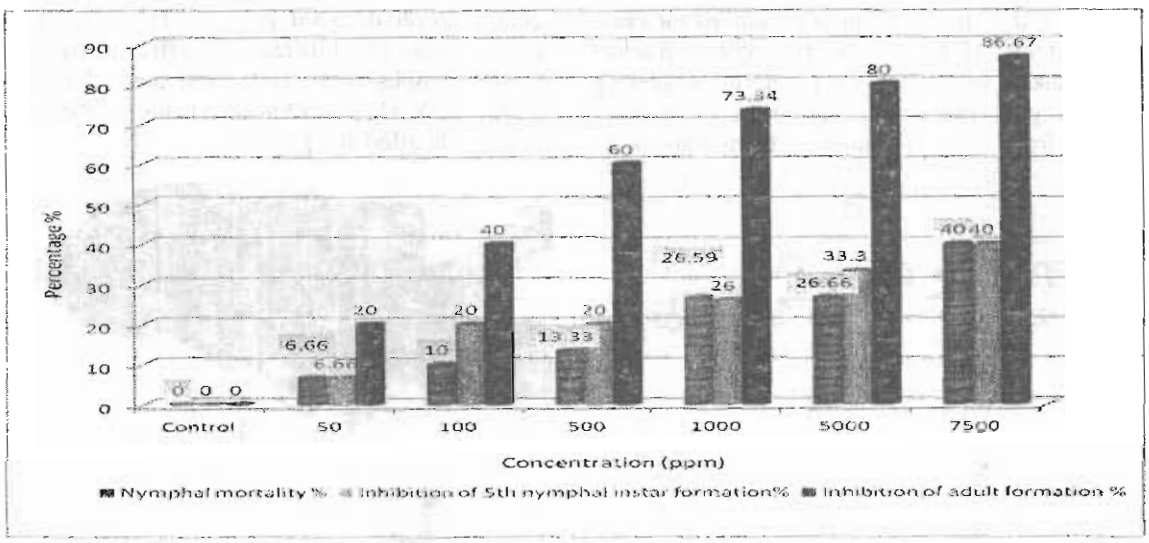

Figure 1: Effects of the *Bauhinia purpurea* extract on mortality, inhibition of 5th nymphal instar and adult formation of the 4th nymphal instar of desert locust treated with topical application under laboratory conditions.

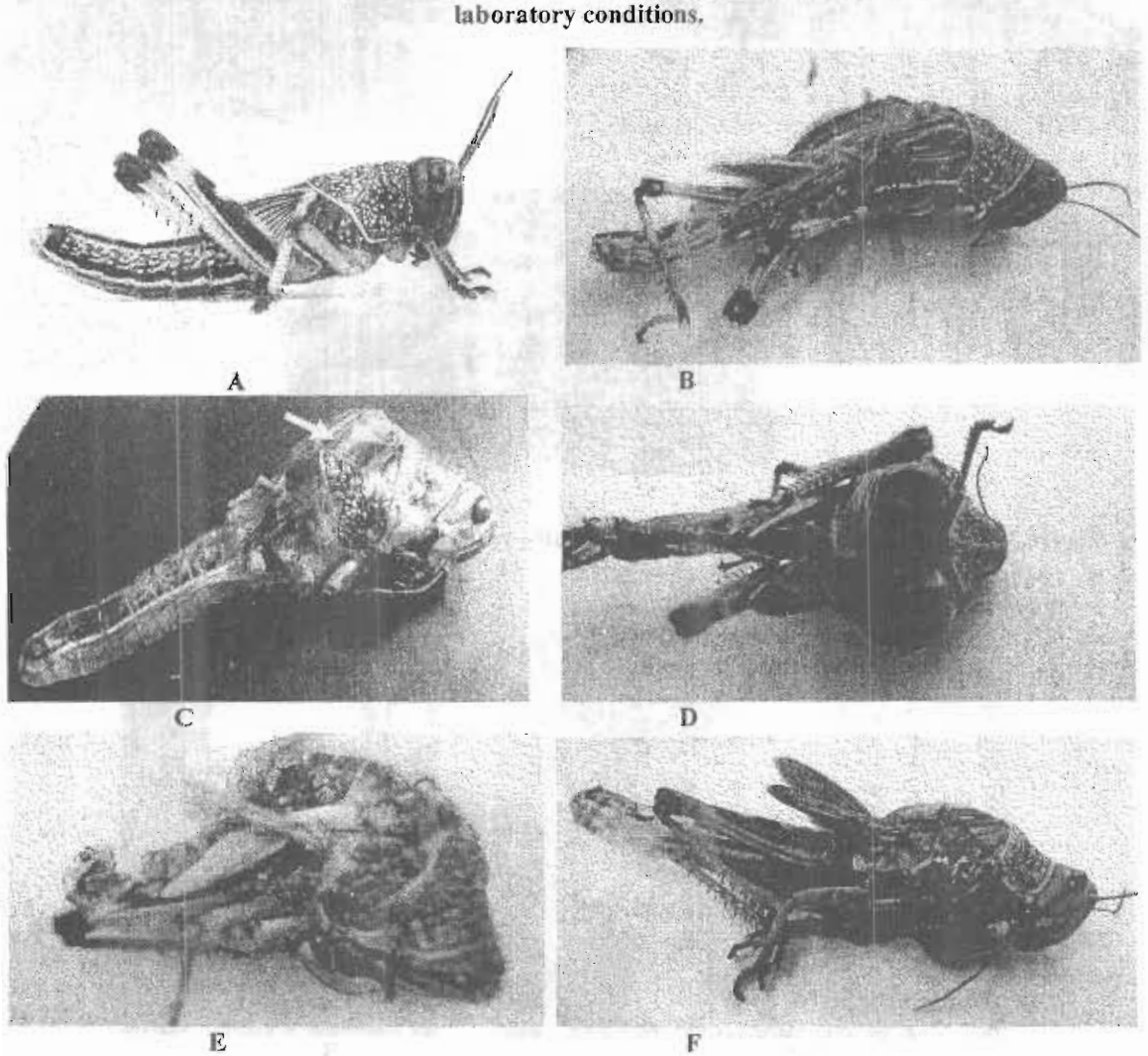

Figure 2: A- normal 5th instar nymph; B – H abnormal 5th instar nymphs.

The deformed 5th nymphal instar continued for three months. Figures (3 B - E) illustrate deformed adult with shrunken wings and died before completing ecdysis. Figure (4) shows the testes of a male resulted from the treatment of nymph with concentration of 5000 ppm as the testes became loose. Figure (5) illustrates the effect of treatments of the nymphs with 100, 5000 ppm of *Bouhinia* extract (B& C, respectively) on the ovaries which became dwarfed and pygmy.

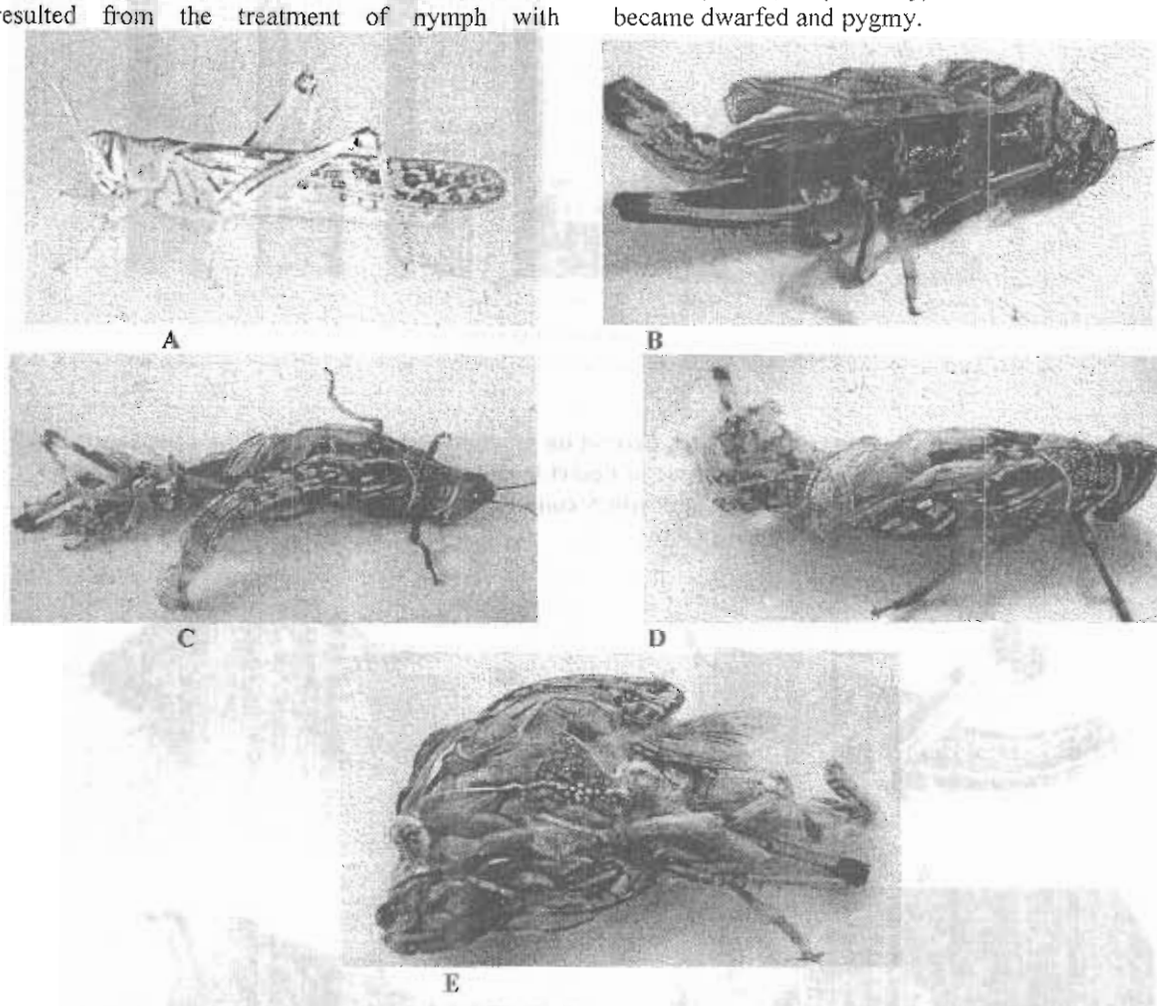

Figure 3: A- Normal adult B- E Deformed adults, with shrunken wings and failed to ecdysis.

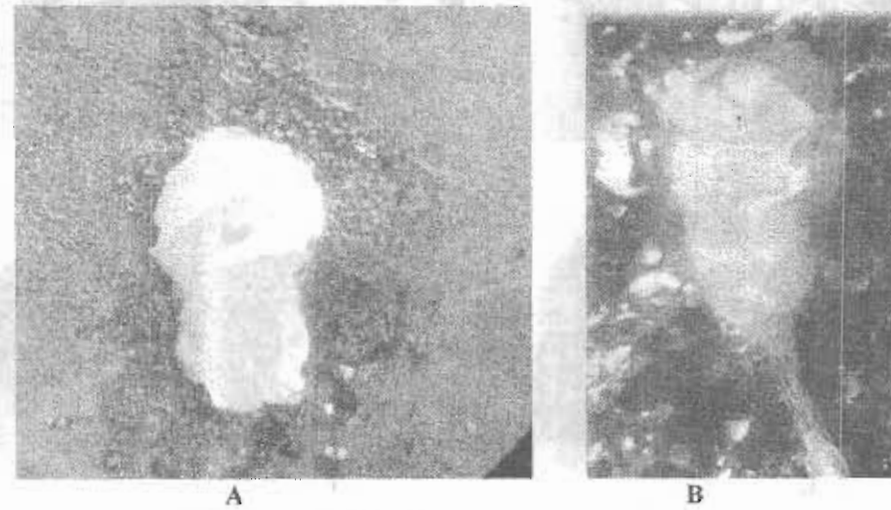

Figure 4: A- normal testis, B-Deformed testis

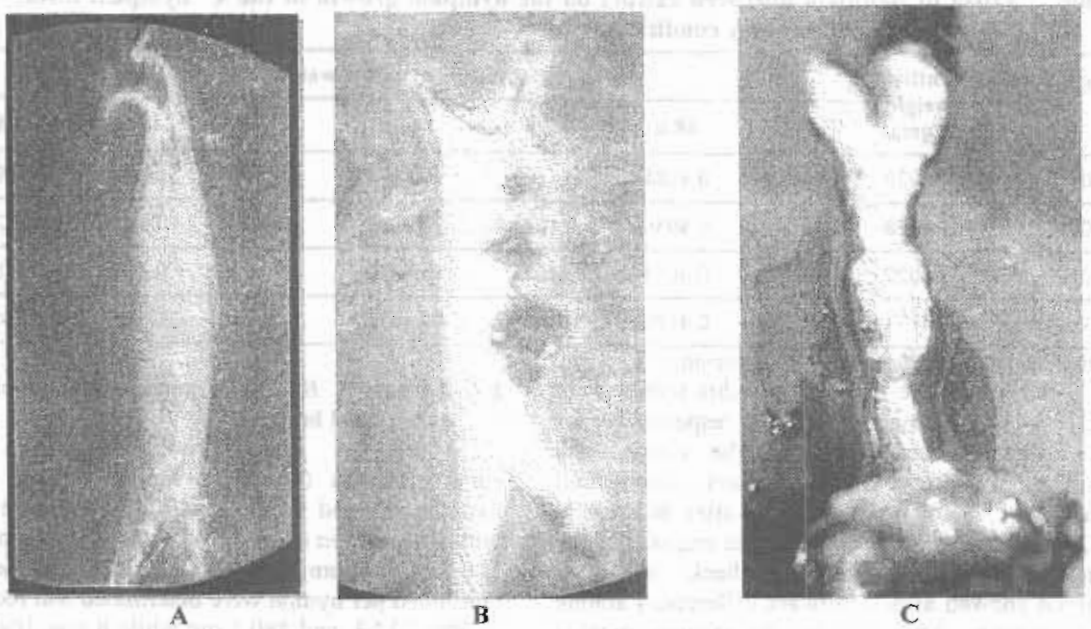

Figure 5: A normal adult, B & C dwarfed and pygmy ovary

C-. Effect of *Bouhinia purpurea* extract on chitin deposition of the body wall.

Measurements of chitin deposition of the body of the locust 4th instar nymph are presented in Table (1). The nymphal fresh weights after removing its viscera scored 0.25, 20, 0.17 gm, and 0.34 gm. Also, the chitin dry weights recorded were 2.67, 1.71, 1.26 and 3.75 mg for the used concentrations, and the check, respectively. Statistical analysis indicated that there were significant differences among concentrations and also, between them and the control, either in the nymphal fresh weights or chitin dry weights. The chitin formation ratio (mg/g) recorded 10.75, 8.57, 7.43 and 11.27 mg/g, in respect, for the tested concentrations and the check. The statistical analysis proved that the chitin formation ratios were significantly differed between each of 500, 1000 ppm concentrations and the check. These results were in accordance with those of Hughes *et al* (1989) who worked on the inhibition of growth and development of tobacco hornworm and El-Sabrout (2009) who worked on the body wall of *Spodoptera littoralis* larvae and. Also, Abo El-Hamed (2005) proved that the nomolt retarded slightly the chitin formation in the 4th instar larvae in the pink bollworm.

D-. Effect of *Bouhinia purpurea* extract on nymphal weight.

Data in Table (2) illustrate the effect of *B. purpurea* extract on the nymphal weight of desert locust through the seven days of the 4th instar nymph. The tested concentrations were 100, 500 and 1000 ppm. The nymphal initial weights were 0.402, 0.403, and 0.403 gm for the tested concentrations and 0.403 in the check, in respect. After 24 hr, the nymphal weights scored 0.408, 0.405, 0.402 and 0.413 gm, respectively, for the tested concentrations and the check. Statistical analysis proved that there were significant differences between them. After 48 hr the significant differences were detected in the nymphal weight, except between the weights resulted from treatment with the concentrations of 500 and 1000 ppm. The nymphal weights after 48 hr were 0.415, 0.409, 0.406 and 0.423 gm, in respect, for tested concentratons and for the check.

Table 1: Assay of the chitin of body wall of the desert locust nymph when fed on clover leaves treated with *Bauhinia* extracts under laboratory conditions.

Concentration	Nymphal fresh weight (g)	Chitin dry weight (mg)	Chitin formation ratio (mg/g)
100	$0.25^{a} \pm 0.01$	$2.67^{a} \pm 0.12$	$10.75^{a} \pm 0.45$
500	$0.20^{b} \pm 0.01$	$1.71^{b} \pm 0.09$	$8.57^{b} \pm 0.57$
1000	$0.17^{c} \pm 0.01$	$1.26^{c} \pm 0.08$	$7.43^{b} \pm 0.56$
Control	$0.34^{d} \pm 0.01$	$3.75^{d} \pm 0.05$	$11.27^{a} \pm 0.39$
F (p)	72.154*	150.466*	13.139*

Table 2: Effect of *Bauhinia purpurea* extract on the nymphal growth of the 4th nymphal instar of the desert locust under laboratory conditions.

Concen. (ppm)	Initial weight (gm)	Average nymphal weight						
		24 h	48 h	72 h	96 h	120 h	144 h	168 h
100	0.4026	0.4051	0.4084	o.4109	0.4175	0.4223	0.4454	0.4978
500	0.4028	0.4039	0.4056	0.4073	0.4086	0.4126	0.426	0.4274
1000	0.4027	0.4030	0.4035	0.4042	0.4053	0.4074	0.4089	0.4153
Control	0.4031	0.4076	0.4126	0.4325	0.4641	0.4982	0.5267	0.5441

Each treatment was represented by 10 nymphs

After 72 hr, the nymphal weights scored were 0.423, 0.418, 0.409 and 0.445 gm, respectively, for the tested concentratons and the check, and significant diferences were found among all treatments . The nymphal weights after 96 hr were 0.436, 0.421, 0.411 and 0.464 gm, in respect, for the tested concentratons and the check. Statistical analysis showed also significant differences among all treatments. After 120 hr, the average nymhal weights were 0.436, 0.421, 0.411 and 0.464 gm, in respect, for the three tested concentrations and the check. Statistical analysis showed significant differences among all treatments and it was clear that the effect of *B.purpurea* extract depended on the concentration. After 144 hr, the nymphal weights recorded were 00.463, 0.426, 0.415 and 0.527, in respect, for tested concentrations and the check. After 168 hr, the previous trend was detected also and nymphal weight recorded 0.544 gm in the check and then decreased gradually in descending order to 0.498, 0.428 and 0.416 gm for the concentrations of 100, 500, and 1000 ppm, respectively.

E-. Effect of *Bouhinia purpurea* extract on nutritional indices:

Nutritional indices were calculated and summarized in (Table 3) for the desert locust nymphs allowed to feed on clover leaves treated with *B. purpurea* extract. The concentrations of 100, 500 & 1000 ppm were tested. Weights of the food consumed per nymph were determined and recorded as 986, 712.3, and 480.3 mg while it was 1049.4 in the check. On the other hand, the change in the body weights (AB) was also calculated and recorded 95, 24.8 and 12.6 mg, while it was 139.3 mg in the check. It is clear that the weight of the food consumed and the change in the body weight were decreased according to the concentration. The calculation of BaT values had also the same previous trend. The check scored value of 664.23, while the 100 ppm concentration recorded 630.42, 500 ppm recorded 581 and 1000 ppm recorded 572.6.

Table 3: Calculation of the nutritional indices of the desert locust nymphs allowed to feed on clover leaves treated with *Bauhinia purpurea* extract under laboratory conditions.

Conc. ppm	I	Δ B	Bat	F	RCR	RGR	ECI	ECD	AD	CI	FDI
100	986	128.8	142.06	276	6.94	18.4	13.06	18.14	72.00	140.86	6.43
500	712.3	83.7	135.56	242	5.25	11.96	11.75	17.80	66.03	101.76	47.32
1000	480.3	52.6	131.14	219.3	3.66	7.51	10.95	20.15	54.34	68.61	54.23
Control	1049.4	139.3	144.14	290.8	7.28	19.9	13.27	18.36	72.29	149.9	-

I = Weight of the food consumed = consumed food/ No. of nymph
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
(while C is the consumption of control discs, and T is the consumption of treated discs)

The weights of the feces produced per nymph during the feeding period (F) were 267, 187.2 and 119.3 mg, while it was 290.8 mg in the check When the relative consumption rate (RCR) for each treatment was calculated the values scored were 1.58, 1.56, 1.23 and 0.84 for the check and the three tested concentrations. When the relative growth rate (RGR) value, which equal the change in the body weight divided on the feeding period, was calculated , the values recorded were 0.209, 0.151, 0.043 and 0.022 , in respect , for the check and the three tested concentrations. Efficiency of conversion of ingested food (ECI) values recorded the percentages of 13.28, 9.63, 3.48 and 2.62 %for the check and the three tested concentrations, in respect. Efficiency of conversion of digested good (ECD) values was calculated for each treatment and scored 18.36, 13.38, 5.27 and 4.83, in respect, for the check and the three tested concentrations. Consumption rate (CI) values were also calculated and attained 149.9, 140.86, 101.76 and 68.16, in respect, for the check and the three tested concentrations. To complete the studying of nutritional indices, the approximate digestability values (AD) were calculated and reached 72.29, 72.00, 73.03 and 75.16, in respect, for the check and the three tested concentrations. Finally, the feeding deterrence index (FDI) for the *B. purpurea* extract against the desert locust 4th instar nymph were determined and was 6.04 for the 100 ppm concentration which means no feeding deterrence, while the concentration of 500 ppm proved to be a weak feeding deterrente (FDI=32.12) and the concentration of 1000 ppm caused moderate feeding deterrence (FDI= 54.23) according to Isman *et al.* (1990).

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الملخص العربى

تأثيرات مستخلص نبات البوهينيا (خف الجمل)على حوريات العمر الرابع للجراد الصحراوى *Schistocerca gregaria*

اسامة الانصارى[١]، حنان رمضان[١]، جيهان على محمد [٢] وفاء جبره[٣]

[١]كلية الزراعة جامعة الاسكندرية قسم علم الحشرات التطبيقى

[٢]مركز البحوث الزراعية – قسم بحوث الجراد الدقى الجيزة

[٣]محطة برج العرب –وزارة الزراعة

تم فى هذه التجربة دراسة المستخلص الايثانولى لنبات البوهينيا *Bouhinia purpurea* (خف الجمل) على حوريات العمر الرابع للجراد الصحراوى*Schistocerca gregaria* بالتركيزات التالية: ٥٠ ، ١٠٠، ٥٠٠، ١٠٠٠، ٥٠٠٠ و ٧٥٠٠ جزء فى المليون تحت الظروف المعملية. تراوحت النسبة المئوية لموت حوريات العمر الرابع بين ٦,٦٦ و ٤٠% فى حين كانت النسبة المئوية لتثبيط تكوين حوريات العمر الخامس تراوحت بين٦,٦٦ – ٤٠% وكذلك النسبة المئوية لتثبيط تكوين الحشرات الكاملة فقد تراوحت بين ٢٠– ٨٦,٦٧%.

بدراسة تأثير المستخلص على تكوين الكيتين للتركيزات ١٠٠، ٥٠٠، ١٠٠٠ جزء فى المليون بطريقة التغذية وجد ان هناك تناقص فى نسبة تكوين الكيتين تراوحت مابين ٧,٤٣– ١٠,٧٥ملجم/ جم بالمقارنة بالكنترول (١١,٢٧ملجم/ جم). و عند دراسة تاثيرها على وزن العمر الحورى الرابع بعد سبعة ايام من المعاملة وجد تناقص فى وزن الحوريات يتراوح بين ٠,٤١٥ – ٠,٤٩٨ جم/ حورية بالمقارنة بالكنترول (٠,٥٤٤جم حورية). بدراسة معدل استهلاك الغذاء وتقدير معامل منع التغذية فقد وجد ان هذا المستخلص مانع التغذية بدرجة متوسطة حيث تراوحت قدرته بين ٦,٤٣ و ٥٤,٢٣ %.

وكما ادت المستخلص بالتركيزات السابقة لحدوث تشوه لكل من حوريات العمر الخامس والحشرات الكاملة وكذلك للخصية والمبيض والقنوات التناسلية فى الحشرات الكاملة للجراد.