Toxicity Assessment of Calotropis procera and Zygophyllum simplex Leaf Extracts on the

Desert Locust Schistocerca gregaria (Orthoptera acrididae)

Abstract: The present work was conducted to study the effect of *Calotropis procera* and *Zygophyllum simplex* extracts on the 4th and 5th nymphal instars of the desert locust *Schistocerca gregaria*. The leaf extract of *C. procera* was significantly more effective on the 4th and 5th nymphal instars than *Z. simplex* extract. The two plant extracts caused feeding inhibition with slightly increased nymphal duration. On the other hand, adult longevity was decreased and the preoviposition period of females was increased, particularly by increasing concentration of *C. procera* and *Z. simplex* extracts. Also these extracts affected different morphogenic abnormalities in the adult stages as will as nymphal instars.

تقييم سمية مستخلصات أوراق نباتات العشار و القرمل على الجراد الصحراوي محمد على ربيع عبدالله

المستخلص. تمت هذه الدراسة لمعرفة تأثير مستخلصات أوراق نبات العشار (Zygophzyllum simple) ونبات القرمل (Calotropis procera) على كل ست حوريات الطور الرابع والخامس، وكذلك الأطوار البالغة للجراد المصحراوي (Shistocera gregaria) وقد وجد أن مستخلص أوراق نبات العشار كان أكثر فاعلية في التأثير على حوريات الطور الرابع والخامس من مستخلص نبات القرمل. أيضاً وجد أن كلاً من المستخلصين أدى إلى زيادة مدة بقاء الحوريات مع توقفها عن التغذية. من ناحية أخرى فقد نقص طول عمر الحشرة البالغة وكذلك زادت فترة ما قبل وضع البيض بالنسبة للإناث بعد المعاملة بالمستخلصين، وخاصة عند الجرعات العالية. كما أن هذه المستخلصات أدت إلى ظهور تشوهات شكلية لكل من الحشرات البالغة والحوريات.

Introduction

Locusts have caused problems for agriculture and man's well-being ever since the first records were kept. The mobile swarms of locusts frequently cause severe damage to the food of humans and animals. Grasshoppers and locusts as major pests have attracted several scientests (Chapman and Robertson, 1958; Uvarov, 1977 and Majeed, 1978). Morphogenic abnormalities of the locust were studied by El-Gammal (1983) and Fridman *et al.* (1984), in relation to the effect of precoccene II on the newly moulted 4th nymphal instar of *S. gregaria.*

The challenge now is to develop a new generation of effective natural pesticides, such as plant extracts. Those extracts may play roles as control agents, which may reduce environmental pollution, lower costs and provide greater effectiveness than other chemical control agents. The two medically important wild plants, *Calotropis procera* and *Zygophyllum simplex* are commonly found in the sandy warm parts of Saudi Arabia, particulary in the western low lands of Abha and south of Riyadh (Betty, 1977; Migahid, 1978; Abulfatih, 1984; Zahran *et al.* 1985 and Alwadi and Abulfatih, 1996).

Many authors have studied the effects of plant extracts on grasshoppers and locusts. Rao and Mehrotra (1978) used extracts of *C. gigantea* against *Locusta migratoria*. Rao (1982) studied the antifeedant properties of *C. gigantea* on *S. gregaria*. Langewald and Schmutterer (1992); Nasseh and Freres (1991) and Wilps *et al.* (1990), studied the effect of neem products on desert locust *S. gregaria*

Materials and Methods

1- Insect colony:

The susceptible strain of *Schistocerca gregaria* was obtained from the Ministry of Agriculture and used throughout the present investigation. Obtained insects were reared in cages 45 x 45 x 60 cm. Each

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cage was supplied every day with fresh clover and the egg-pods were checked daily to isolate into empty cages until hatching. The stock colony was reared according to the method of Ewen *et al.* (1984) with some modifications.

2- Preparation of the plant extracts:

Plant samples were collected from warm sandy desert regions in Abha, and identified by a specialist (Alwadi) in the Biology Department, Faculty of Science, King Khalid University.

The plant leaves were dried, then ground to a fine powder and extracted consecutively by using water as a solvent (250 ml of distiled water added to 50 gm of plant powder; i.e, each 100 ml was added to 20 gm of powder), then filtered over anhydrous sodium sulphate to remove the solvent. Five concentrations, 5, 15, 25, 35 and 45% respectively, were selected of each extract after preliminary tests. The crude extracts were kept in a freezer until bioassay.

3- Bioassay and statistical analysis:

The 4th and 5th nymphal instars and the adult stages of *Schistocerca gregaria* were fed on the clover leaves treated with *Calotropis procera* and *Zygophyllum simplex* extracts. Clover leaves were treated by dipping in each extract for 5 minutes and then left in the air to evaporate the solvent. The newly ecdysed 4th and 5th nymphal instars were divided into groups (5 in each). Each group received the treated clover with different concentrations (5, 15, 25, 35, and 45%) of each extract. Treatment was replicated three times, and another three replicates without treatment served as controls.

Data obtained was recorded after 48 hours and statistically analyzed by using T-distribution, refined by Bessel correction (Morney, 1956) for the test significance of difference between means.

Results and Discussion

I-Effect of Calotropis procera leaf extract on 4th and 5th nymphal instars of Shistocerca gregaria

From data recorded in Table (1) and Fig. (1) it was observed that treatment of newly ecdysed one day old 4th and 5th nymphal instars of *S. gregaria* with *C. procera* extracts caused different mortality percentages. A dose-dependent trend of mortality could be easily observed (8, 12, 22, 30 and 51% in case of the 4th nymphal instar and 11, 17, 32, 45 and 66% in case of the 5th instar nymph at concentrations of 5, 15, 25, 35 and 45% respectively). Such a trend was also observed for the mortality of the moulted 5th from the treated 4th nymphal instar (6, 9, 14, 26

and 40% at concentrations of 5, 15, 25, 35 and 45%, respectively) with *C. procera* extracts.

The results in Table (1) were assessed and no significant effect was found due to the action of C. procera extracts on the 4th nymphal durations at the concentrations of 5, 15 and 25%. On the other hand, 5th instar nymphs developing from treated 4th instars had prolonged durations without feeding (P<0.05) in the case of treatment with the concentrations of 15, 25, 35, and 45% (9.4 \pm 1.2, 9.8 \pm 1.3, 10.6 \pm 0.8 and 10.9 ± 1.1 days respectively vs. 8.5 ± 1.8 days in the controls). The lower concentration (5%) had no significant effect (P>0.5) on the 5th nymphal duration (which resulted from treated 4th instars). Meanwhile, after feeding of 5th nymphal instars on the clover treated with all the concentrations of C. procera extracts, a remarkably significant (P<0.05) prolongation of duration was recorded (Table 1). Both 4th and 5th nymphal instars of S. gregaria stopped their feeding after treatment with C. procera extracts before changing to the next stages and/or died.

The treatments of 4th and 5th nymphal instars with *C. procera* extracts resulted in various degrees of nympal and adult malformations. The majority of such malformations appeared as deformed nymphs, adults and nymphal adult intermediates, which increased with increasing concentrations. On the other hand, the percentage of 5th nymphal deformations were more than the 4th nymphal ones. Such deformation was 3, 5, 7 and 9% in case of 4th instar nymphs and 4, 12, 23, 28 and 34% of the 5th instar nymphs after feeding on clover treated with 5, 15, 25, 35 and 45% of *C. procera* extract respectively.

The trend of adult deformations (10, 17, 26, 31, and 0.0%) which resulted from treated 4th instars at concentrations of 5, 15, 25, 35 and 45% respectively, were more than the deformations of the treated 5th instar nymphs (3, 8, 11, 17 and 0.0% at the same previous concentrations, respectively vs. 00.0% of adult deformation of the controls at both stages). Contrary to this trend, the percentage of normal adults decreased with increasing concentration of C. procera extract. This decrease was more in the 4th nymphal instars than 5th nymphal instars. Moreover, no adult was produced after treatment of 4th and 5th nymphal instars with the higher concentration (45%) (Table 1).

	Treated 4 th nymphal instar						% Treated 5th nymphal instar					%
							Normal	mal				
%	%		Dura	Duration		%	Adult	% Duration %			Adult	
Conc.	Mortality		±s.d		Deformation			Mortality ±s.d Deformation				
	4 th	5 th	4 th	5 th	4 th	Adult		5 th	5 th	5 th	Adult	
5	8	6	8.2 ± 0.3 NS	$8.8 \pm 0.7 \text{NS}$	3	10	73	11	9.3 ± 1.2*	4	3	82
15	12	9	8.4 ± 0.2 NS	9.4 ± 1.2*	5	17	57	17	9.5 ± 0.6*	12	8.0	63
25	22	14	8.7 ± 0.4 NS	9.8 ± 1.3*	7	26	31	32	10.1 ± 1.1*	23	11	34
35	30	26	$9.0 \pm 0.3*$	10.6 ± 0.8 *	9	31	4	45	$10.8 \pm 0.7*$	28	17	10
45	51	40	$9.3 \pm 0.2*$	10.9 ± 1.1*	9	0	0	66	11.1 ± 1.1*	34	0	0
Control	0	0	8.1 ± 1.6	8.5 ± 1.8	0	0	100	0	8.5 ± 1.8	0	0	100

Table (1): Effect of *Calotropis procera* extract on 4th and 5th nymphal instars of *Schistocerca gregaria*.

NS: Not significant, P> 0.05 * : Significant, P< 0.05

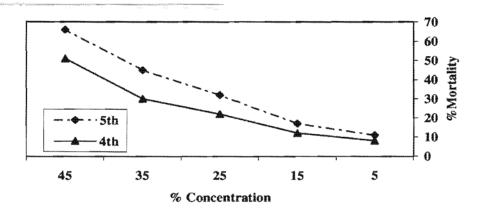


Fig. 1 Effect of Calotropis procera extract on % mortality of 4th and 5th nymphal instars of Schistocera gregaria

II- Effect of Zygophyllum simplex extract on 4th and 5th nymphal instars of Schistocerca gregaria

The data presented in Table (2) and Fig. (2) show that the percentages of mortality of 4th and 5th nymphal instars of *S. gregaria* were increased with increasing concentrations of *Z. simplex* extract (0.1, 6, 10, 14 and 16% of 4th insatr nymphs and 11, 19, 23, 29, and 31% of the treated 5th instars at concentrations of 5, 15, 25, 35 and 45%, respectively). Although the treated 5th nymphal instars were more sensitive than the 4th instars, the 5th nymphal instar resulting from the treated 4th instars were in reverse course; i.e least sensitive to the mortality percent. On the other hand, the two lowest concentrations (5 and 15%) caused no mortalities of the 5th nymphal instars resulting from the treated 4th nymphs.

As shown in Table (2), no significant effect was found due to the action of *Z. simplex* extract on the developmental duration of treated 4th nymphal instars. On the other hand, both the treated 5th instar nymphs and the ones resulting from treated 4th nymphal instars had significant (P<0.05) prolonged duration at the concentration of 25 and 35% (10.7 and 11.1 days in case of the 5th instars resulting from treated 4th instars and 10.6 and 12.4 days in the case of treated 5th nymphal instars, respectively).

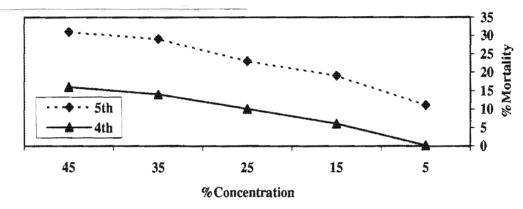
According to the results represented in Table (2), it was observed that the percentages of nymphal and adult deformations which were produced from the treated 4th and 5th nymphal instars were increased by increasing concentrations of *Z. simplex*. In addition, these deformations were more in the case of treated 5th nymphal instars than of treated 4th ones. The percentage of normal adults which was obtained

	Treated 4th nymphal instar						%	Treated 5th nymphal instar				%
							Normal					Normal
%	% Mortality		Duration			%		% Duration		n %		Adult
Conc.			±s.d		Deformation			Mortality ±s.d D		Deforr	nation	
	4 ^{1h}	5 th	4 th	5 th	4 th	Adult	word))	5 th	5 th	5 th	Adult	
5	0.1	0	8.3 ± 0.4 NS	8.9 ± 0.6Ns	45	45	54	11	9.0 ± 1.6 NS	0	41	60
15	6	0	8.4 ± 0.8 NS	9.6 ± 1.4 NS	53	53	40	19	9.1 ± 0.5 NS	126	20	35
25	10	2	8.7 ± 0.5 NS	10.7 ± 1.2 *	64	64	20	23	10.6 ± 1.1*	30	33	14
35	14	4	8.8 ± 0.6 NS	11.1 ± 0.7*	69	69	7	29	12.4 ± 1.2*	35	36	0
45	16	6	8.5 ± 0.6 NS	8.8 ± 1.5 NS	70	70	0	31	$8.8 \pm 1.2 \text{NS}$	39	30	0

Table (2): Effect of Zygophyllum simplex extract on 4th and 5th nymphal instars of Schistocerca gregaria.



 8.5 ± 1.8



100

Fig. 2 Effect of Zygophyllum simplex extract on % mortality of 4th and 5th nymphal instars of Schistocera gregaria

from treated 4th and 5th nymphal insatrs of *S. gregaria* were decreased by increasing concentrations of *Z. simplex* extracts. Moreover no adults were obtained after treatment of 5th nymphal instars with the concentration of 35%, while at concentrations of 45%, no adults resulted after treatment of both 4th and 5th instar nymphs.

0

Control

0

 8.1 ± 1.6

As shown by the data in Tables 1 and 2, nymphal mortality was increased as affected by *C. procera* extract more than *Z. simplex* extract. Also 5th instar nymphs were more affected in various degrees of lethality by *C. procera* and *Z. simplex* extracts than the treated 4th ones. The killing power may suggest that the application of these plant extracts on nymphal instars of *S. gregaria* induced the inhibition of nymphal development by hormonal unbalance. The mortality potency of *C. procera* and *Z. simplex* extracts during the present study is not the same as that caused by the conventional insecticides because no initial killing could be observed. Sehnal (1983) suggested that death was not directly related to unbalanced hormonal activity

of juvenoids, but to secondary factors such as suffocation, bleeding and desiccation due to exuvation, imperfect starvation leading morphological defects, and failure of vital mechanisms. Similar results were obtained by Chandra and Thapar (1985), who concluded that Nerium indicum extract acted as a deterrent and toxin to S. gregaria and Cotte et al. (1988), who found that azadirachtin and other plant secondary compounds (nicotine, quinine, salicin, umbelliferone, sinigrin, and isothiocyanine) had toxic effects on S. gregaria. Our results were in agreement with the results obtained by Nicol and Schmutterer (1991) and Doumbia (1994). They found that Azadirachtin and neem tree extracts caused moult inhibition and mortality as effective parameters against S. gregaria.

 8.5 ± 1.8

0

0

100

Data in Tables (1 & 2) show different degrees of delayed development and slightly prolonged nymphal duration by using the two extracts against 5th instar nymphs rather than 4th instar nymphs. The prolongation of nymphal duration in *S. gregaria*

may be interpreted as being due to the altering of prothoracicotropic hormone (PTTH), which governs metamorphosis. According to the present results, *C. procera* and *Z. simplex* extracts may accelerate PTTH release. Similar results due to the effect of plant extracts and juvenoids against *S. gregaria* have been reported by several researchers: Novak (1969); El-Gammal *et al.* (1988); and Ghoneim and Ismail (1995).

III-Influence of *C. procera* and *Z. simplex* leaf extracts on adult longevity and female oviposition of *Shistocerca gregaria*

As shown in Table (3), male and female longevity was significantly (p<0.05) shortened by increasing concentration of both extracts. The decrease was slightly more in the case of females treated with *C. procera* extract (100.4, 92.4, 75.3, 52.4 and 33.6 days vs. 132 days in controls at concentrations of 5, 15, 25, 35 and 45%, respectively) than males (115.4, 96.6, 84.4, 68.3 and 44.6 days vs. 141.6 days in controls treated with the same extract at the same concentrations). In the case of *Z. simplex* extract, female longevity was lower (113.3, 98.4, 82.6, 67.4 and 41.8 days at

concentrations of 5, 15, 25, 35, and 45%, respectively) than male longevity (125.6, 112.4, 96.6, 84.5, and 68.6 days at the same concentrations). From the data presented in Table (3), a significant (p<0.05) increase in the female preoviposition period at concentrations of 15 and 25% of C. procera and Z. simplex extracts was observed. The prolongation of preoviposition periods was more when the female was treated with C. procera (38.1, 48.6 and 56.7 days at concentrations of 5, 15 and 25%, respectively) than Z. simplex extract (37.3, 44.4 and 48.3 days at the same concentrations). The higher concentrations of 35 and 45% of both extracts inhibited female oviposition. These extracts could be considered as insect growth regulators (IGRs), potent prolongators of female oviposition and growth inhibitors, in addition to having a lethal effect on the desert locust, S. gregaria (Peleg, 1988; Yokoyama and Miller, 1991)

The same pattern took place with eggs laid by treated females, where the mean numbers of egg pods/female and number of eggs/female/day were significantly (p<0.05) decreased with increasing concentrations of *C. procera* and *Z. simplex* extracts. The higher decrease (2.0 egg pods/female

Table (3): Influence of *C. procera and Z. simplex* extracts on adult longevity and the female oviposition of *Schistocerca gregaria.*

	Longevity/d	ay ± S.d	Female	Female Fecundity				
% conc.			Preovipostion	Mean No. of	Mean No. of			
C. procera			Period/day	egg-pod/female	egg-/female/day			
***************************************	Male	Female	± S.d	± S.d.	± S.d.			
5	115.4 ± 4.3*	100.4 ± 5.3*	$38.1 \pm 2.1*$	3.4 ± 1.1*	2.1 ± 0.8*			
15	96.6 ± 3.2*	92.4 ± 4.4*	48.6 ± 1.8 *	2.1 ± 0.6*	1.2 ± 0.3 *			
25	84.4 ±5.2*	75.3 ± 6.5*	56.7 ± 2.4*	2.0 ± 0.4 *	1.0 ± 0.2*			
35	68.3 ±4.4*	52.4 ± 4.4*	0.00	00.0	0.00			
45	44.6 ± 6.1*	33.6 ± 5.2*	0.00	0.00	0.00			
Z. simplex								
5	125.6 ± 6.1*	113.3 ± 4.4*	37.3 ± 1.2 NS	4.6 ± 1.2*	2.2 ± 1.1*			
15	112.4 ± 4.4*	98.4 ± 5.5*	44.4 ± 2.6*	3.4 ± 1.1*	2.2 ± 0.6*			
25	96.6 ± 3.3*	82.6 ± 4.3*	48.3 ± 1.9*	2.6 ± 0.7*	1.7 ± 1.1*			
35	84.5 ± 5.1*	67.4 ± 4.1*	00.0	00.0	0.00			
45	68.6 ± 4.6*	41.8 ± 3.2*	0.00	00.0	0.00			
Control	141.6 ± 4.6*	132 ± 5.2	36.4 ± 2.2	6.2 ± 1.1	3.4 ± 1.2			

NS: Not significant, P> 0.05

* : Significant, P< 0.05

and 1.0 egg/female/day) was obtained after treatment of females with C. procera extract at the concentration of 25% while the lower decrease (4.6 egg pods/female and 2.2 eggs/female/day) occurred at the lower concentration, 5%, of Z. simplex extract. These figures can be compared to control of 6.2 egg pods/female eggs/female/day). Moreover, no eggs were produced at the higher concentrations of 35 and 45% of both extracts. When Azadirachta indica extract was offered directly to the abdomen of S. gregaria, few egg-pods were laid and the reduction of oviposition was dose-dependent (Brajendra et al. 1988 a). This result was in disagreement with the findings of Brajendra et al. (1988 b), who reported there was no significant difference in the number of eggs laid in sand treated with neem cake vs. controls.

IV- Effect of *C. procera* and *Z. simplex* leaf extracts on nymphs and adult morphogensis of *Schistocerca gregaria*

a- Nymphal instars:

As shown in Plate (1A), of the treated 4th nymphal instars, the nymphal-adult intermediates appeared with abnormal legs. They could not walk or eat and perished in a few days. This form of juvenilizing effect of the plant extract was recorded after treatment of the 4th (1A) and 5th (1B) instar nymphs. The supreme action of these extracts were exhibited when they were applied at concentrations of 25 and 35%.

b- Adult stage:

When nymphs of *S. gregaria* were treated with the plant extracts of *C. procera* and *Z. simplex*, various degrees of the malformed adults resulted. The majority of such malformations appeared as severely or slightly curled wings, and in a few cases antennal or leg deformities were observed (see Plate 1C).

Morphogenic abnormalities induced by plant extracts on different insects were reported by many authors: El-Gammal et al. (1988) and Omyma (1999) on S. gregaria; and Shalaby et al. (1988) and Youssef et al. (1990) on Musca domestica. Deformed adults of Muscina stabulans appeared after topical application of pyriproxyfen onto larvae or pupae (Nassar, 1995). These morphogenic disorders of nymph and adult may be interpreted, as Sehnal (1983) suggested, as being related to the fact that juvenoids do not interfere with the function and

growth of insect cells but prevent their imaginal differentiation. Generally, the hormonal imbalance in *S. gregaria* stages caused by the action of plant extract treatments may be considered to explicate the appearance of malformations.



A Normal 4th nymph



1A Treated 4th nymph



B Normal 5th nymph



1B Treated 5th nymph



C Normal adult



1C Treated adult

Plate 1

Finally, in view of the obtained results in the present investigation, plant extracts of C. procera and Z. simplex could be considered as a potent inhibitor of growth and metamorphosis of the desert locust, S. gregaria, acting as JH mimic, thereby intervening in the metamorphosis developmental program. Plant extracts attract the attention of entomologists because most of the botanical extracts are non toxic to warm-blooded animals and show no, or moderate, side effects on natural enemies of insect pests (Schmutterer, 1985). Plant extracts can be suggested as a possibly promising agent in integrated pest management programs for the control of S. gregaria.

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