

BIOCHEMICAL STUDIES ON THE DESERT LOCUST, SCHISTOCERCA GREGARIA AFTER POISONING WITH THE INSECTICIDE BANCOL

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Abstract

To evaluate the initial and bio-residual efficacy of Bancol on *Schistocerca gregaria* (Froskal), the last nymphal stadium were fed with 100 ppm on the 2nd-3rd day after moulting to analysis fat body and haemolymph on day 6. Two days before eclosion and on day 5 after final ecdysis a basic metabolism that affected Bancol treatment which are generated in the central nervous system was established.

Derived a cessation of some metabolism in nymphs such as carbohydrates, protein, lipid and cholesterol. This is due to decrease of triglycerides reaching 56.82% and 80.28% for alkaline phosphatase. Reflectional, decreased the amount of food intake by 53.26% and pellets 49.32% besides 45.55% mortality in 5th instar. The change is due to interference with the central nervous system rather than to the altered feeding behaviour. Thus, the mortality of adult was 100% at 5th day old in spite of increase of metabolism in fat body (site of synthesis), but did no uptake to haemolymph. This is due to decrease of alkaline phosphatase reaching 85.03% and the enzyme of transaminase it was 77.93%. Consequently, it could be recommended to use the Bancol in frame of an integrated pest management system.

Key Words: *Schistocerca gregaria*, Bancol, feeding, metabolism.

INTRODUCTION

Schistocerca gregaria are considered the world's most destructive insect pest. Therefore, after moulting to adults, huge swarms may engage in long-distance flights, invade farmland and destroy the crops over vast areas (Showler, 1995).

Increasing concern about pesticides accumulation in the environment, stimulated search for natural compounds that could replace synthetic insecticides in insect pest control. Bancol extract was used as a tool, able to produce neural discharges of ventilatory pattern upon action on sensitive target-site (central nervous system) was related to metabolic processes via respiration. The enzymes can be routinely used as reliable biochemical markers for metabolic rate monitoring such as alkaline phosphatase, triglycerides and transamination (glutamic-oxaloacetic transaminase (GOT) and pyruvic transaminase (GPT) (Schneider *et al.*, 1995).

Finally, the best evidence for the chemical disturbance in CNS of *S. gregaria* comes from studies on its mechanism of action and hence biochemical response (Bus-

tami and Hustert, 2000). So, nerve-excitatory were performed by Bancol in view of the potential use of this extract as botanical pesticides. Thus, a major goal of our recent examination was to clarify the role of Bancol extraction on endogenous ventilatory patterns that produces cellular metabolism

MATERIALS AND METHODS

Maintenance of insects: *S. gregaria* were raised under laboratory conditions. The insects were kept crowded in cages containing a few hundred specimens. The temperature was kept at 32°C and a light/dark cycle of 16/8 h was used. Insects were fed once a day, in the morning, with fresh lettuce leaves (Veelaert *et al.*, 1997).

Natural compound tested: Bancol 50% WP, extracted from navy larvae of genus *Lumbries* which contain 50% benultap (AI) as wettable powder. It is an antifeedant with the action on the central nervous system (CNS), produced by Takeda Chemical Industries Ltd, Japan.

Bioassays: To standardize the state of insect hunger prior to the assay, insects were deprived of food overnight for 16 h. The experiments were done the next morning. Bioassays were undertaken on instar nymphs 2-3 days in the fifth stadium that had been fed for 24 h. These nymphs were placed individually into clear plastic observation cages (13x10x8 cm). The feeding activity of the compound was assessed by applying aliquots (100 µl) of known concentration of a test compound onto glass-fibre discs (Whatman 2.1 cm dia.) as described by Blaney and Simmonds (1990). The discs were made palatable by the addition of 100 µl of 100 mM sucrose solution. Discs were designated as either control (sucrose alone) or treatment (sucrose plus test compound). The discs were pinned to corks, so that the edge of the disc was free for the locust to feed on. There were 10 replicates per species for each concentration in each bioassay. The amount consumed after 24 h was determined by drying the discs and re-weighing them. The feeding activity index $[(C - T)/(C + T) \times 100]$ was calculated where C and T represent the amount eaten of the control and treatment discs, respectively. The index identified both phagostimulants (- values) and antifeedant (+ values) and the duration was obtained using Dempester equation (1957). Haemolymph was collected from the arthroal membrane of the hind leg of the locust after being pierced with a sterile needle. The haemolymph was collected using a 10 µl Eppendorf pipetman over ice to prevent coagulation. Samples were then centrifuged and stored until use at -20°C.

Homogenization was carried out to adult fat body at 5 day old and nymph prior ecdysed at 5000 rpm for 10 min. at 0°C and the supernatants were used directly for assays.

Colourimetric determination of GOT and GPT enzymes activities were assayed us-

ing the method of Reitman and Frankel (1957), alkaline phosphatase activity revised assay according to Belfield and Goldberg (1971), triglycerides (Fossati, 1982), total protein by Biuret colourimetric method (Henry *et al.*, 1974), total lipids (Knight *et al.*, 1972), cholesterol (Roeschlau *et al.*, 1974) and carbohydrates according to Trinder mod. (1969).

Statistical analysis : the data obtained were statistically analyzed using student's t-test between the mean of treated and non - treated groups.

RESULTS AND DISCUSSION

The effect of Bancol extract on *S. gregaria* showed 2 different phases: the 1st taking place during the nymphal instar after the oral administration of 5th instar nymphs and was characterized by a significant decrease in fat body active enzymes and metabolic rate contents, but simultaneously increase in haemolymph active enzymes due to continuous ventilation pattern. Subsequently, a 2nd phase appeared characterized by a significant increase in fat body active enzymes and metabolic rate contents and at the same time decrease in haemolymph active enzymes for a period of adult instar due to discontinuous ventilations pattern (DV). Biochemical assays as shown in Table 1 revealed that the level of enzymes activities in the fat body for male and female nymphs exhibited a significantly lower level, mainly, triglycerides (TG) 56.82% and Acpase 80.28% for females. A similar trend was found in fat body metabolic contents, Fig. 1. As for carbohydrate, 69.12% and 93.42% depression for female and male, respectively; also, for protein, 82.52% and 78.40 % for female and male, respectively; for lipid; 82.16% for female lipid and 75.55% for male cholesterol. In contrast with haemolymph enzymes activities, Table 1 especially alkaline phosphatase (Acpase), 135.14% increased for male, 91.86% increased for GPT females and 92.98% for GOT females. Hence, the vigorous ventilation (gulping), apparently increases the *catabolic* rate for energy production which was in agreement with Alaoui *et al.* (1994), who found that haemolymph peptide extracts from locusts previously poisoned with the pyrethroid insecticide deltamethrin showed hypertrehalosemic activity. In the adult fat body, the aforementioned data, Table 1 significantly revealed that higher level of enzymes especially Acpase reaching 118.90% and 108.95% for male and female, respectively and TG increasing to 540.0% and 51.44% for male and female, respectively. Accompanied with the pattern of changes in carbohydrate reserves, Fig.1 increasing reached to 265.87% for male, 227.96% for female lipid and for cholesterol reaching to 612.33 and 108.75% with male and female, respectively. But, in contrast with haemolymph enzymes a steady decrease in its activity, Table1, such as Acpase it was 85.03 and 41.24% for male and female, respectively and female GPT it was 77.93% indicated that one typical ventilatory pattern of resting or quiescent insects has been termed chewing ventilation, resulting in stressed ventilation and blocked transformation of me-

tabolism material from its site of synthesis (fat bodies), finally death the adult within 5 day old.

The present findings shed some light on the nature of that recorded by Bustami and Hustert (2000) who suggested that the dominance of intrinsic rhythmogenesis of ventilation in the metathoracic ganglion of locust are generated in the central nervous system (CNS), target of Bancol potency appear to play pivotal roles in reference to metabolism con-sumption processes. Through the data of this study, it is clearly evident that for the first time the amount of food intake (antifeedant index) decreased by 53.26% in locust fifth instar nymphs treated with 100 ppm of Bancol and the amount of pellet per individual is correlated with food consumption; being 49.32% reduction. Recent studies reconfirmed with that found by Peter *et al.* (2000) proposed that sulfakinins, neuropeptide present in corpus cardiacum of *S. gregaria* significantly inhibit food uptake in fifth instar nymphs by decreasing the sensitivity of the taste receptors.

Mortality as effective parameter in Table 2., It was 46.67% and 44.44% for female and male 5th instar nymphs, respectively and the reduction in duration was 8.69 ± 0.19 and 7.63 ± 0.19 days compare with control, it was 10.33 ± 0.46 and 10.25 ± 0.77 days for female and male, respectively.

Curiously, Bancol is considered to be a new promising approach for suppressing the population of certain pests. Therefore, it seems prudent to develop alternative bio-rational strategies that could be drawn upon to counter periodic population outbursts.

Table 1. Changes in the enzymatic activities of nymphs and adults of *S. gregaria* after treatment with Bancol extract.

Instar	Fat body contents						Haemolymph contents							
	AcP (U/100 ml)		GPT (U/ml)		GOT (U/ml)		T.G (mg/ml)		AcP (U/100 ml)		GPT (U/ml)		GOT (U/ml)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Nymphs	6.53 ***	108.80***	2.80*	7.10	3.20***	5.00**	125.29*	111.22***	69.53***	40.78	11.20*	16.50***	8.20**	0.40***
Control	2.78	21.46	3.9	7.51	6.20	8.50	120.97	257.51	29.57	38.91	6.70	8.59	4.49	5.70
Adults	13.90**	14.94**	3.65*	3.40	6.60***	6.42	209.46***	133.86***	47.57***	92.93***	4.80	1.60***	5.15	5.51
Control	6.35	7.15	2.8	2.73	4.80	4.30	34.29	88.39	25.71	158.16	5.75	7.25	6.50	6.80

*: Significant at P<0.05; **: Significant at P<0.01; ***: Significant at P<0.001
 Figures without asterisks are not significant

Table (2): Biological activities of 5th instar nymphs of *S. gregaria* after treatment with Bancol.

Status	Mortality (%)		Duration (days)	
	Male	Female	Male	Female
Treatment	44.44	46.67	7.63±0.19	8.69±0.19
Control	-	-	10.33±0.46	10.25±0.77

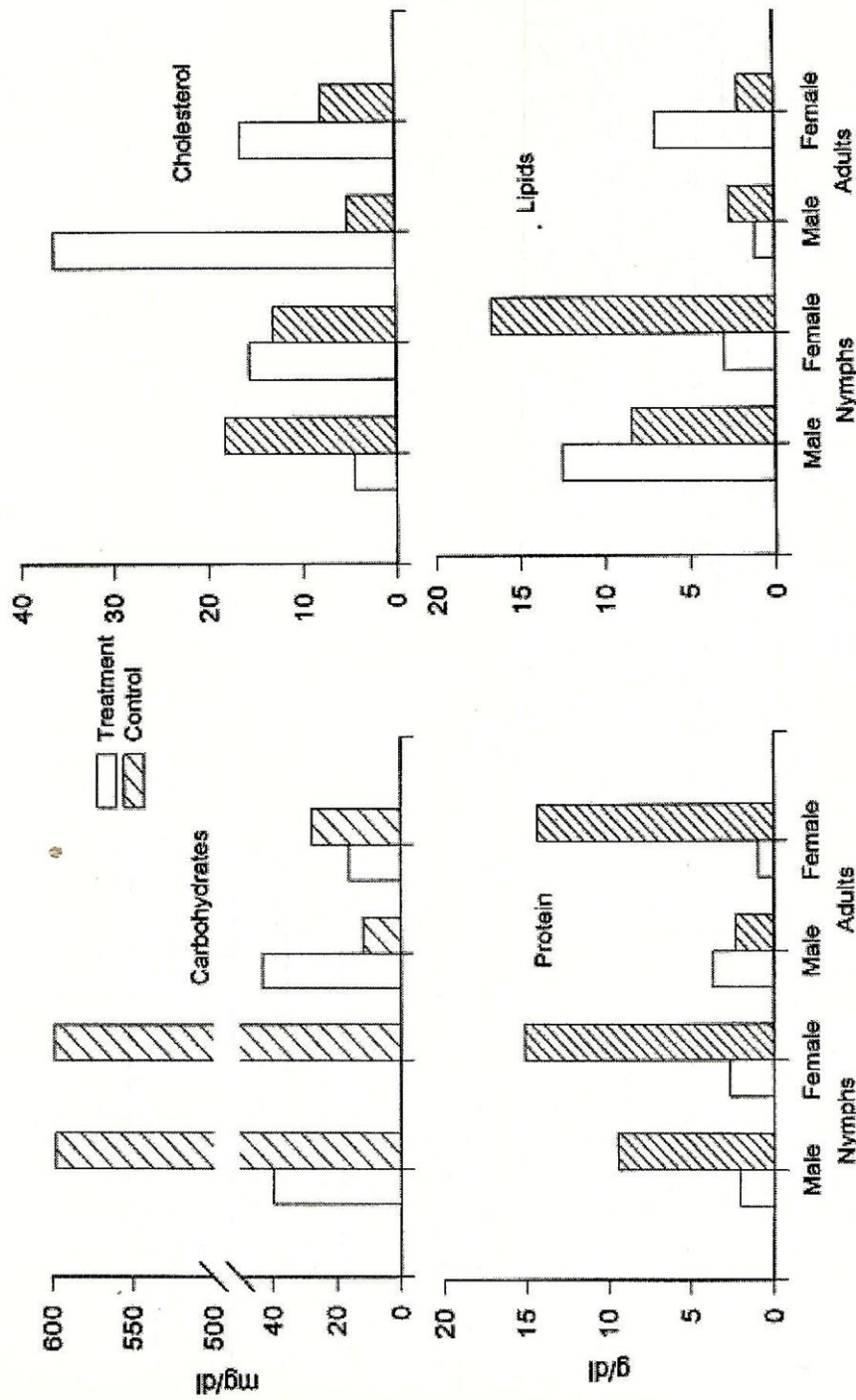


Fig.1. Determination of metabolic rate in 5th instar nymphs and adults fat body of *S. gregaria* after treatment with Bancol extract

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دراسات بيوكيميائية على الجراد الصحراوي سثتوسيركا جريجاريا بعد التسمم بمستخلص مبيد البانكول

إبراهيم على أحمد عبدالكريم

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استهدفت الدراسة إلقاء الضوء على التأثيرات البيوكيميائية للمستخلص الطبيعي بانكول ضد حوريات العمر الخامس للجراد الصحراوي كأداة لإثارة الجهاز العصبى فى المعمل باستخدام أقراص للتغذية مضاف إليها مادة سكرية بتركيز ١٠٠ جزء فى المليون حيث تم قياس نشاط بعض الإنزيمات ونواتج التمثيل الغذائى فى الدم ومكان تصنيعها بالأجسام الدهنية فى كل من الحوريات قبل الإنسلاخ مباشرة أو فى اليوم الخامس للحشرة الكاملة.

أظهرت النتائج زيادة فى عمليات الهدم وانطلاق الطاقة للحوريات رغم حدوث نقص فى كمية الغذاء وصل إلى ٥٢,٢٦% مما تسبب فى حدوث الموت بنسبة ٤٥,٥٥%، بينما حدث العكس مع العمر اليافع مما أدى إلى حدوث الوفاة بنسبة ١٠٠% تقريبا فى اليوم الخامس، حيث أدى النقص فى تركيز التراى جلسريد إلى ٥٦,٨٢% والألكالين فوسفاتيز إلى ٨٠,٢٨% بالحوريات إلى نقص فى معدل التمثيل وزيادة فى الهدم وصلت إلى ٩٢,٤٢% للكربوهيدرات و ٨٢,٥٢% للبروتين و ٨٢,١٦% لليبيدات، وأعقب ذلك حدوث الموت للحشرة الكاملة رغم زيادة نواتج التمثيل بالأجسام الدهنية لعدم انتقال نواتج التمثيل من الأجسام الدهنية إلى الدم لارتباط ذلك بعملية التنفس وليس التمثيل الغذائى أو التغذية، حيث وصل الإنخفاض لإنزيم الألكالين فوسفاتيز إلى ٨٥,٠٢% والإنزيم الناقل للأحماض الأمينية إلى ٧٧,٩٢% فى دم الحشرات الكاملة.