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Effect of azadirachtin on the hemolymph protein of the adult desert locust, *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae)

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ABSTRACT

Azadirachtin is a botanical insecticide affects a variety of biological processes of insects. Effect of azadirachtin on the hemolymph protein of 7 days old adult locust, *Schistocerca gregaria* was studied in this work. Through intersegmental membrane between the third and fourth abdominal tergite, *Schistocerca gregaria* was injected with the three chosen doses of azadirachtin (7.5 – 15 – 30 µg/g body weight). After 24 h post injection, the hemolymph protein of the treated and control (untreated) insects were fractionated by SDS polyacrylamide gel electrophoresis (PAGE). The results revealed that, total amount percentage of protein patterns between treated and untreated hemolymph generally decreased due to treatment course and there are three protein bands appeared (18- 45 and -72 kDa) due to treatment course with three doses of azadirachtin. Azadirachtin might interfere with hemolymph protein in desert locust, *Schistocerca gregaria*.

Introduction

Azadirachtin obtained from the neem tree (*Azadirachta indica*). It was considered as one of the most important bioinsecticide in the world. It was less harmful to environment and human and biodegradable by sunlight. It was more advantageous than synthetic insecticide, which has extreme persistent and bioaccumulation. Azadirachtin can be recommended for many integrated pest management (IPM) [1,2]. Azadirachtin has an adverse effect on more than 400 insect species. It has antifeedant effect [1,3,4] has affected pest growth by disruption of hormones that control moulting and metamorphosis [5], it has repellence effect [6], oviposition deterrence [7] , direct toxicity [8], feeding deterrence [9] and cause anatomical abnormalities for several insect pests [10].

Desert locust, *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae) is considered as one of the most dangerous insect pests. It occurs in Northern Africa, Peninsula and Southwest Asia [11]. Locusts begin as a solitary phase which has no economic importance but due to food competition it changes from solitary to gregarious phase which has large economic importance [12,13].

In the present study we attempt to study the effect of azadirachtin on hemolymph protein profile of adult locust, *Schistocerca gregaria* as a step in control adult locusts by neem.

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Materials and methods

1- Insect rearing

Crowded (gregarious) adults of *Schistocerca gregaria* (*S. gregaria*) were collected from Cairo governorate, Egypt. The colony were reared in wooden cages for three generations at 30 ± 2°C and relative humidity (70-80%) under a 12:12 h light: dark regime. The insect were fed on *Sesbania aegyptiaca*, which is freshly supplied daily. Suitable pots for oviposition were put in the cages, examined daily to remove pots that contain eggs into another cage. After three generations, 7 days old adult locusts were used for the experiments. The age of locusts was 7 days after final ecdysis because this represents the peak of protein synthesis according to [14].

2- Extraction and treatment with azadirachtin

Azadirachtin were obtained from neem seeds [15] and purified chromatographically according to [16] by HPLC to get final concentration of azadirachtin (1 mg/ml). Azadirachtin was dissolved in 10% ethanol in selected concentrations to comprise the desired doses (7.5, 15 and 30 µg/g body weight) according to [4]. Each of these doses was injected, through the dorsolateral area of the intersegmental membrane between the third and fourth abdominal tergite into the adult of *S. gregaria*.

Control insects were injected with the same doses of 10% ethanol/g body weight by 25 µl syringe.

After 24 h post injection the hemolymph samples were collected from the base of the hind coxa of the insect by

5 µl micropipette.
Hemocytes were removed by centrifugation at 12,000 r.p.m. for 15 min., the supernatant (plasma) was removed from hemocytes pellet into another tube and stored at -18^{o}C until use.

3- Protein electrophoresis

Hemolymph proteins were fractionatd by SDS polyacrylamide gel electrophoresis (PAGE) as described by [17] modification method. The hemolymph samples were prepared by mixing the sample with β-mercaptoethanol, glycerol, SDS and bromophenol blue and heating the mixture in boiling water bath for 4 min to denature the proteins. The gel was run at a constant voltage 100 V for 4 h at room temperature. The electrophoretic protein profiles of hemolymph for both control and treated adults were done along with wide range protein marker by disc electrophoresis on 10% polyacrylamide gel after 24 h post injection. The gel was scanned with gel documentation system by using a scanner (Scan tack, Sport Technology) and then, the bands were analyzed by using software: Gel-Pro Analyzer, version 3.1 for windows 95/NT, from Media Cybernetics (1993-1997) U.S.A.

Results

Hemolymph proteins of 7 days old adult locust, *S. gregaria* control and treated with three doses of azadirachtin ((7.5, 15 and 30 µg/g body weight), were analyzed after 24h of treatment. Changes in the hemolymph proteins of treated and control adults are presented in **Fig. 1** as well as in **Tables 1** and **2**. Characterization of hemolymph proteins detected a total 10 protein bands in control pattern and 11 protein bands in treated patterns with 7.5 µg/g body weight azadirachtin, 8 protein bands in hemolymph treated with dose 15 µ/g body weight of azadirachtin, and 10 protein bands in hemolymph treated with 30 µg/g body weight of azadirachtin. The molecular weight of total protein bands in treated and control samples were ranged from (11-140 kDa).

A change could be detected in hemolymph protein patterns of treated with 7.5 µg/g body weight when compared with control, where 3 protein bands were disappeared due to treatment with azadirachtin (11- 16.6 and 86.8 kDa).

On other side, 4 new protein bands were specific to treatment with azadirachtin (18.5, 45, 72 and 120 kDa). Seven protein bands were common between treated and control hemolymph (15, 23.6, 25.4, 58, 65.4, 108.5 and 140 kDa). Three of them were increased in their amount percentage due to treatment (58- 108.5 and 140 kDa) by 2.8, 16.6 and 1.1 folds, respectively. The other four protein bands (15, 23.6, 25.9 and 65.4 kDa) were decreased in their amount percentage by 2.3, 2.6, 2 and 3.2 folds, respectively.

Comparing control hemolymph protein patterns and the treatment with 15 µg/g body weight azadirachtin, it was observed a disappearance of 4 protein bands (15, 25.4, 86.8 and 140 kDa) and appearance of 3 new protein bands due to treatment (18, 45 and 72 kDa) with protein amount percentage (1.2, 7.9 and 7.3 %), respectively. Five common protein bands were detected between treated and control (11, 16.6, 23.6, 16.6 and 11 kDa) all of them were decreased in their amount percentage by (1.3, 5.8, 1.1 and 2.7 folds), respectively except 58 kDa was increased by 3 folds.

Injection with 30 µg/g body weight azadirachtin showed a disappearance of 4 protein bands (15, 23.6, 86.8 and 140 kDa) and appearance of new 4 new protein bands (18, 45, 72 and 140 kDa) with amount percentage (2.5, 9.7, 10.6 and 4.3 %), respectively, as compared to control. Six common protein bands were detected between treated and control (11, 16.6, 25.4, 58, 65.4 and 108.5 kDa), 4 of them (11, 16.6, 25.4 and 65.4 kDa) were decreased in their amount percentage by 5.1, 24.6, 2.6 and 1.1folds , respectively except 58 and 108.5 kDa which was increased in amount percentage by 3.1 and 8.7 folds, respectively.

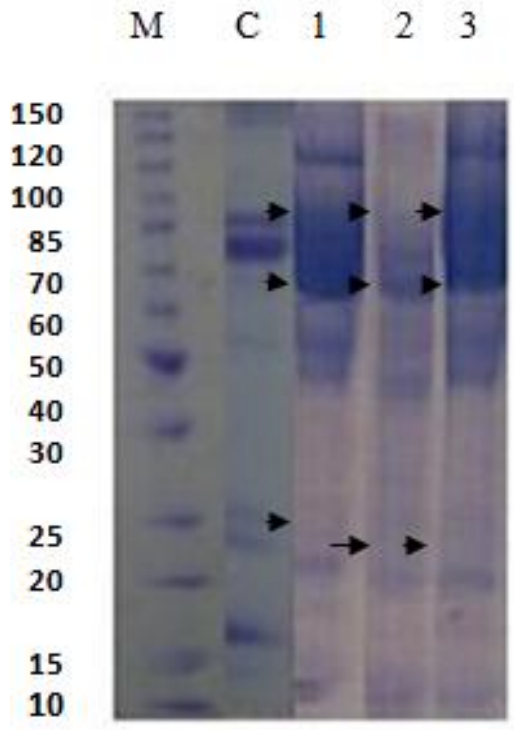

Fig (1): Photograph illustration of electrophoretic hemolymph protein patterns of treated and control adults of *S. gregaria.* M= wide range protein marker (150, 120, 100, 85, 70, 60, 50, 40, 30, 25, and 15 kDa). Lane C= control. Lanes 1, 2 and 3 = treated hemolymph protein patterns with (7.5, 15 and 30 µg/g body weight of azadirachtin), respectively.

Discussion

Proteins play an important role in the cell such as hormonal regulation. They act as structural element as carbohydrates and lipids [18,19]. In addition, protein synthesis is necessary for maintenance of body growth and reproduction [20]. Azadirachtin affected pest growth by disruption of hormones that control moulting and metamorphosis [3,4].

The results reported in this paper of the protein expression in 7 days old adult *S. gregaria* with and without treatment with azadirachtin**,** showed that when adult locusts injected with three doses of azadirachtin (7.5, 15 and 30 µg/g body weight), the results were shown that in low dose 7.5 µg/g body weight, the total number of protein bands were slightly increased from 10 protein bands for control to 11 protein bands in treated pattern. By increasing dose of azadirachtin to 15 µg/g body weight, number of protein bands were decreased from 10 to 8 due to treatment and not changed by increasing the injected azadirachtin dose to 30 µg/g body weight. Generally the protein expression in treated adult *S. gregaria* was decreased due to azadirachtin treatment. This result was agreed with the results of [20,21], they shown that protein expression can be lowered by exposing insects to diet containing azadirachtin or injecting larvae with it. In addition by treatment with (7.5, 15 and 30 µg/g body weight) of azadirachtin. 3, 4 and 4 protein bands were disappeared, and 3 new protein bands were appeared in hemolymph of adult stage (18, 45 and 72 kDa). This inhibition in hemolymph protein of *S. gregaria* may be due to the destructive effect of neem on cerebral neurosecretory in the brain which are responsible for protein secretion. It may be also due to insufficient stimulation of neurosecretory activity which leads to reduction in protein amount in hemolymph that required for oocytes development according to [22]. In addition,[23] stated that reduction in hemolymph proteins in adult *S. gregaria* treated with consult (Chitin-synthesis inhibitor, Hexaflumuron) may reflect the reduction in the capacity of the protein synthesis, and this decrease in hemolymph proteins which resulted in the inhibition of oogenesis and ovarian development lead to sterility of adult insect.

From the previous results it was concluded that azadirachtin may affect on adult *S. gregaria* protein profile, which agreed with [21]**,** they suggested that azadirachtin interfered with protein synthesis in desert locust. Azadirachtin has a potential to be used as bio-pesticides in insect pest management [24]**.**

Table 1: Hemolymph protein monitoring of treated and control adults *S.gregaria* expressed as molecular weight. M= wide range protein marker (150, 120, 100, 85, 70, 60, 50, 40, 30, 25 and 15 kDa). Lane C= control. Lanes 1, 2 and 3 = treated hemolymph protein patterns with (7.5, 15 and 30 µg/g body weight of azadirachtin), respectively.

Lanes:	**Marker**	**Lane C**	**Lane 1**	**Lane 2**	**Lane 3**
Rows	**(mol.w.)**	**(mol.w.)**	**(mol.w.)**	**(mol.w.)**	**(mol.w.)**
1	150	140	140		
2	120		120		120
3	100	108.57	108.57		108.56
4	85	86.875			
5	70		72	72	72
6		65.455	65.445	65.44	65.273
7	60				
8		58	58	58	58
9	50				
10			45	45	45
11	40				
12					
13	30				
14					
15	25	25.455	25.409		25.4
16		23.6	23.667	23.6	
17	20				
18			18.571	18	18.095
19		16.667		16.6	16.605
20	15	15	15		
21	10	11		11	11.1

Table 2: Amount percentage (amount %) and relative fragmentation of hemolymph protein fractions of treated and control adults *S.gregaria.* M= wide range protein marker (150, 120, 100, 85, 70, 60, 50, 40, 30, 25 and 15 kDa). Lane C= control. Lanes 1, 2 and 3 = treated hemolymph protein patterns with (7.5, 15 and 30 µg/g body weight of azadirachtin), respectively.

Lanes:	**Marker**	**Lane C**	**Lane 1**	**Lane 2**	**Lane 3**
Rows	**Amount %**	**Amount %**	**Amount %**	**Amount %**	**Amount %**
r1	1.0797	2.6875	3.145		
r2	1.8834		5.5564		4.3564
r3	1.7361	1.4565	9.3562		12.483
r4	1.7192	1.1042			
r5	2.896		11.185	7.3107	10.653
r6		24.968	7.6281	8.943	20.941
r7	3.1986				
r8		4.8695	13.539	14.578	15.149
r9	4.0487				
r10			7.054	7.9215	9.7226
r11	11.613				
r12					
r13	6.229				
r14					
r15	6.626	3.7006	1.8996		1.4747
r16		5.1355	1.9293	4.358	
r17	9.2321				
r18			0.59991	1.2684	2.53
r19		12.339		2.1149	0.56544
r20	13.318	4.2239	1.8741		
r21	10.5	6.2412		4.6883	1.2716
Sum	74.08	81.831	73.88	75.172	74.981
In Lane	100	100	100	100	100

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