INSECT REGULATORY EFFECT OF MARGOSAN A NATURAL AZADIRACHTIN – CONTAINING PREPARATION ON THE DESERT LOCUST, *SCHISTOCERCA GREGARIA* (FORSK.) (ORTHOPT., ACRIDIDAE)

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Abstract

The morphogenetic and metabolic effects of neem kernel extract, azadirachtin of the neem tree *Azadirachta indica* were investigated as indicators for its insect regulatory action in *Schistocerca gregaria*. Three doses of Azadirachtin (10,20 and 40 µg/nymph) were injected into one day old 4th instar nymphs. The injected doses had resulted in prolongation in the duration of the last instars and a positive correlation between failure in ecdysis to 5^{th} instar and adult stage. These morphogenetic features induced by azadirachtin in 4^{th} and 5^{th} instar nymphs were considered as Juvenilizing effect of this compound .

The same doses were injected into one and 5 day-old 5^{th} instar nymphs to disclose the metabolic effects of azadirachtin on their haemolymph main metabolites . This had resulted in high level of proteins and carbohydrates and low levels of lipids and cholesterol this reflecting the juvenilizing action of azadirachtin in *S.gregaria* .

It was concluded that azadirachtin plays the same insect regulatory role of Juvenile hormone in delaying or prohibiting metamorphosis, increasing levels of proteins and carbohydrates and suppressing lipids and cholesterol in gregarious nymphs of *S.gregaria*.

INTRODUCTION

Azadirachtin is the most effective tetranortriterpenoid present in the seed Kernel extracts of the neem tree, *Azadirachta indica*. This compound was proven to be an insect growth regulator, in *Locusta migratoria* (Schmutterer,1990); insect antifeedant and biologically active compound in *Schistocerca gregaria* (El-Gammal,1994) .

There are possible direct effects of azadirachtin on tissues such as, muscles, gut epithelia and cells undergoing mitosis (Nasiruddin and Mordue, 1994) . Moreover, tests from injected *S.gregaria* with azadirachtin showed significant reduction in widths, lengths and volumes compared to the control insects (Linton *et al*, 1997). In the cockroach, *Periplaneta americana* azadirachtin decreased ingestion of food that resulted in

retardation of nymphs growth and finally reduction of adults reproductivity (Richter *et al.*,1997) .

The present study is an attempt to investigate azadirachtin regulatory effects on some morphogenetic and metabolic aspects in *S.gregaria* .

MATERIALS AND METHODS

The experimental nymphs of *S. gregaria* (Forsk.) were segregated from the gregarious stock colony of locust and Grasshoppers Research Department, Plant Protection Res. Institute, Agric. Res. Center, Ministry of Agric ., Dokki, Cairo, Egypt. The segregated nymphs were reared and handled under the crowded conditions of hunter-Jones (1961). Newly moulted 4^{th} instar nymphs were maintained at a temperature of 30±2°C, RH 60% and L:D,12:12. The insects were daily fed with fresh leaves of clover.

The powder formulation, margosan (20% azadirachtin, Fig.1.) was kindly provided by Rohm and Haas. This compound was disolved in saline solution and injected into one day-old 4^{th} instar nymphs with three doses of 40,20 and 10 µg/nymph to study its morphogenetic effect .

To evaluate its metabolic effect, the same doses were injected into one and 5 day-old 5^{th} instar nymphs . Treatments were carried out with Hamilton micro syring model (NCH-701).

The treated 4^{th} instar nymphs were daily observed to evaluate failure in ecdysis to 5^{th} instar and adult stage. Mortality percentages were also recorded. These criteria were calculated relatively to the whole number of the treated 4^{th} instar nymphs. Fourth and Fifth instar durations were estimated by Dembester equation (1957) .

Treated 5^{th} instar nymphs were subject to evaluation the metabolic effect of azadirachtin on their haemolymph main metabolities during growth and adult cuticle deposition in the last instar (Taha and El-Gammal,1990) .

Haemolymph main metabolites were quantitatively determined as total carbohydrates, proteins, lipids and cholesterol in treated 5^{th} instar nymphs compared to the control. The determination was carried out for treated and untreated 8 and 9 day old-

5^{th} instar nymphs. Three pools for each dose were used and each pool consisted of 8 nymphs. Each tissue pool was divided into four equal samples, each was assayed for one metabolite. Protein content was determined according to the method described by Gornall *et al.* (1949).Total carbohydrate was estimated by the method of Trinder (1969). Total lipids were measured by the modified method of knight *et al.* (1972). Cholesterol was determined by the enzymatic colourimetric method of Richmond (1973).

RESULTS AND DISCUSSION

A. Morphogenetic effect of azadirachtin : The morphogenetic effect of azadirachtin was investigated after injection of three doses of this compound to one day-old 4^{th} instar nymphs of *S. gregaria* .

Table1 indicates a prolongation in the duration of treated 4^{th} instar nymphs and the resulting 5^{th} nymphal instar compared to the untreated control. This prolongation seems to be dose dependent since it was 11.54,12.68 and 13.23 days for the doses, 10,20,and 40 $\mu g/4^{th}$ instar nymph, respectively compared with 6.24 days for the untreated control. The induced prolongation in the stadium of the resulting 5^{th} instar nymphs as well as the life span of the last two instars showed a similar trend. The stadium of the resulting 5^{th} instar nymphs were 12.89,16.54,18.22 and 8.44 for the doses 10,20,40 and untreated control nymphs , respectively

Table 1 shows that percentages of failure in ecdysis to 5^{th} instar are correlated positively with the injected doses to one day old 5^{th} instar nymphs . These Percentages were 0.0, 5.0 , 9.33 for the doses 10 , 20 , and 40 mg / nymph , respectlvely

Mortality percentages during ecdysis to 5^{th} instar nymphs showed the same correlation, they were 35.00,48.33 and 50.07 for the same dose , respectively .

During the last ecdysis to the adult stage, mortality percentages were however lower. These results are in harmony with those of Banby and Klocke (1990) who stated that growth regulatory effects of azadirachtin in insects are thought to result from ablockage release of morphogenetic peptides , resulting In alteration in ecdysteroid and Juvenlle hormone titre . This interference with the hormonal system is also responsible

for defects, disturbance of moult and delay of *locusta* development (Mordue *et al.* 1986).

B. Metabolic effect of azadirachtin during metamorphosis: To disclose the metabolic effect of azadirachtin on *S. gregaria* during metamorphoses, three doses of 10.20 and 40 µg of azadirachtin were injected into one and 5 day - old 5^{th} instar nymphs .The rsulting 5^{th} instar nymphs were subjected to chemical analysis.

Table 2 indicates that, injection of 10,20 and 40 µg of azadirachtin into one day-old 5^{th} instar female nymphs significantly increased their haemolymph protein concentration during day 9 of their stadium. Protein concentrations in the haemolymph of the treated female nymphs were, 6.58 and 7.52 mg/100 ml for each doses, respectively compared to 4.79 in the control. The corresponding values for males were 6.90 , 6.17and 6.27 mg in comparison with 4.86 mg in the control.

Injection of the same doses into 5 day-old 5^{th} instar female and male nymphs significantly decreased haemolymph protein concentrations ,these concentrations were 3.92 , 3.99 and 3.77 mg in females nymph compared to 5.01 mg in the control and were 2.87 , 3.70 and 3.21 mg in males compared to 5.03 mg in the control.

The same treatments to one and 5 day-old 5^{th} instar female and male nymphs increased significantly the carbohydrate contents. For the resulting 5^{th} instar female nymphs, carbohydrate concentration were 58,38,67.20, and 57.42 mg compared with 39.25 mg in the control. The corresponding values for males were 60.2,52.39 and 54.67 mg compared with 33.84 mg in the control, Table 2.

The injection of these doses into 5day- old 5^{th} instar female and male nymphs resulted in reduction in carbohydrate contents. These were, 38.78 , 31.68 and 30.73 mg for treated female nymphs compared to 39.56 mg in the control. The cencentrations of carbohydrates in the haemolymph of the treated 5^{th} instar male nymph were,28.12, 23.22 and 25.26 mg compared with 34.25 mg in the control, Table 2.

It seams that, the metabolic effects of azadirachtin leads to a juvenilizing pattern in *S,gregaria* , especially when application is carried out during the first day of the last nymphal instar, Table 1. Similar findings were also observed in the desert locust which have been observed by Nicol and Schmutterer (1991) .They showed that , the

application of neem oil to gregarious hoppers of *S.gregaria* had changed their cuticle colours from typical yellow and black to the green solitary type of solitarious nymphs which containing high juvenile hormone in their haemolymph (Schmutterer and Freres, 1990) . So, the high-level of proteins and carbohydrates in azadirachtin treated nymphs indicates juvenilizing effects of azadirachtin in the desert locust. El-Gammal (1979) showed that, the solitary phase of *S.gregaria* having high titre of Jh (Uvarov , 1966) was characterized by high levels of proteins and carbohydrates in their haemolymph .

Table 2 indicates that, treatment with azadirachtin in day 5 of the last instar reduced the concentration of protein and carbohydrate in their haemolymph. This reduction occurred as the result of azadirachtin application on that last instar nymphs in which the epidermal cells were more sensitive to ecdyson (Taha and El-Gammal 1990) . So, this had led to failure in ecdysis to adults due to the lack of proteins needed for their formation (Bernays and woothead, 1984). Sieber and Rembold (1983), found that injection of azadirachtin in *locusta* nymphs reduced their food intake , weight gain and ecdysteroid peak hence resulting in faillure of ecdysis .

Table 3, shows that injection of azadirachtin with 10,20 and 40 µ/ nymph into one and 5 day - old 5^{th} instar male and female nymphs decreased total lipids and cholesterol in their haemolymph .

Lipid concentrations were,311.45 , 308.24 and 269.01 µg /100ml haemolymph in treated one day old female nymphs , for each dose, respectively compared to 331.46 in the control. The corresponding concentration for one day-old male nymphs were , 458.41 , 353.74 and 343.80 mg /100 ml , respectively compared to 495.03 in the control . The application of azadirachtin with same doses to 5 days-old 5^{th} instar female nymphs decreased contents to 305.95 , 216.52 and 215.86 mg, respectively in comparison with 332.27 mg haemolymph in the control . Lipid concentrations in the treated 5 day- old 5^{th} instar male nymphs were , 327.021, 295.32 and 279.14 mg, respectvely compared to 494.47 mg in the control.

The effect of azadirachtin on haemolymph cholesterol contents of the treated one day- old 5^{th} instar female nymphs is shown in Table3 ,in which the concentrations were , 24.88 , 22.96 and 22.94 mg, respectively compared to 40.39 mg in the un-

treated control . These concentrations were , 32.20 , 30.21 and 25.mg in treated one day -old 5^{th} instar male nymphs , respectively compared to 41.49 mg in the control nymphs .

Table 3 shows that , treated 5 day- old 5^{th} instar female nymphs with 10., 20 and 40 µg azadirachtin induced significant reduction in cholesterol . The concentrations were , 27.89 , 17.69 and 11.41 mg, respectively in comparison with 40.14 mg in the control .

The corresponding valus for 5 day-old 5^{th} instar male nymphs were 18.59 , 17.26 and 15.02 mg for each dose , respestively compared to 40.99 mg in the control.

In general , Treatment with azadirachtin induced Juvenilizing effects on treated 5^{th} instar nymphs of *S.gregaria*. El – Gammal (1979) found that , administration of Juvenile hormone (JH) to the 4^{th} instar nymphs of *S.gregaria* resulted in a green colour in their cuticle and a reduction in their haemolymph content of lipids . Sieber and Rembold (1983) stated that , ecdysteroid was affected in favour of JH in the haemolymph of azadirachtin treated nymphs of *locusta migratoria* , by the conversion of cholestrol into 2–deaxyecdysone and related ecdysteroids. It could be concluded that azadirachtin plays an insect regulatory role in *S.gregaria* since it induced morphogenetic effect in the solitary phase as shown in tables, 1, 2 and 3 . It is therefore evident that azadirachtin plays the same regulatory role of JH in elevating proteins and carbohydrates and suppressing lipids and cholestrol in gregarious nymphs of *S.gregaria* .

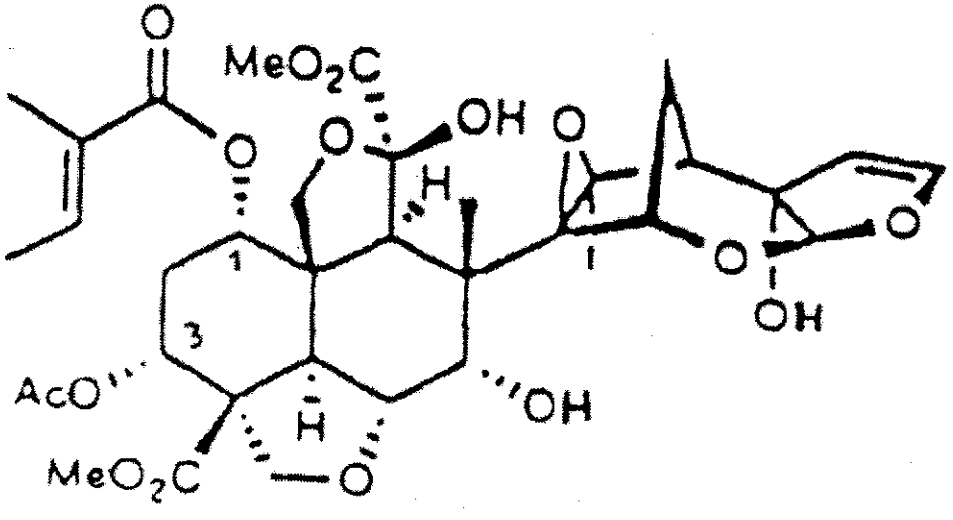

Fig. 1. Structure of Azadirachtin (2) detigloyl-(6-[2,4 dinitrophenylamino[hexanoyl])-22,23-dihydroazadirachtin (After, Simmonds *et al.*, 1995)

Table 1. Effects of seed kernel extract (azadirachtin) of Neem Tree , *Azadirachta indica* on some biological aspects of *Schisocerca gregaria.*

No. treated 4th instar *	Doses µg/ nymph	4th instar mymphs ** stadium in days	%failure to 5th instar	%mortality	5th instar nymphs ** %resulting 5th instar	stadium in days	%mortality	Life span of the last instar	Adult stage ** %
45	Control	6.24	-	-	-	8.44	-	14.68	100.00(45)
60	10	11.54	0.00(-)	35.00(21)	65.00(39)	12.89	12.82(5)	24.43	56.67(43)
60	20	12.68	5.00(3)	48.33(29)	46.67(28)	16.54	28.57(8)	29.22	33.33(20)
75	40	13.23	9.33(7)	50.07(38)	45.33(43)	18.22	29.41(10)	31.25	32.00(24)

* The three doses of azadirachtin were injected into 1- day old 4th instar nymphs.

** The figures in paranthesis indicate the number of the resulting nymphs of each indicated instar and adult stage.

Table 2. Effect of azadirachtin on haemolymph protein and carbohydrate levels in treated one and 5 day old 5th instar nymphs of *Schistocerca gregaria*.

Dose (µg)	Protein content (mg/100ml)* +s.d. Treatment during				Carbohydrate content (mg/100ml)* +s.d. Treatment during			
	The 1st day		The 5th day		The 1st day		The 5th day	
	Female	male	Female	male	Female	male	Female	male
control	4.79±0.13c	4.86±0.07d	5.01±0.10a	5.03±0.17a	39.25±0.09d	33.84±0.07d	39.56±0.40d	34.25±0.52d
10	6.58±0.08b	6.90±0.09a	3.92±0.13b	58.35±0.08b	58.35±0.08b	60.2±0.12a	38.78±0.40b	20.12±1.05a
20	7.52±0.06a	6.17±0.13b	3.70±0.37b	3.70±0.37b	67.20±0.07a	52.39±0.06b	31.68±0.47a	23.22±0.89c
40	6.65±0.07b	6.27±0.07b	3.21±0.26c	3.21±0.26c	57.42±0.36c	54.67±0.28c	30.73±0.56c	25.26±0.56b
L.S.D 05	0.17	0.15	0.31	0.47	0.37	0.3	0.88	1.48

• The chemical analysis was carried out on day 8 during the last day of the normal duration of the last stadium and on day 9 for the treated nymphs.

Table 3. Effect of azadirachtin on haemolymph lipid and cholesterol levels in treated one and 5 day old 5th instar nymphs of *Schistocerca gregaria.*

Dose (µg)	Lipid content (mg/100ml)* +s.d. Treatment during				Cholesterol content (mg/100ml)* +s.d. Treatment during			
	The 1st day		The 5th day		The 1st day		The 5th day	
	Female	male	Female	male	Female	male	Female	male
control	331.46±062a	495.03±0.27a	332.27±0.70a	495.47±0.75a	40.39±0.66a	41.49±0.92a	40.14±0.28a	40.99±0.25a
10	311.45±1.21b	458.41±0.63b	305.95±0.60b	327.21±1.03b	24.88±0.6444b	32.20±0.93b	27.89±0.33b	18.59±0.38b
20	308.24±0.89c	353.74±1.05c	216.52±0.65c	295.32±0.63c	22.96±0.25c	30.21±0.86c	170.69±0.36c	17.26±0.32c
40	269.01±0.55d	343.80±1.54d	215.86±0.92d	279.14±0.17d	22.94±0.55c	25.69±0.54d	11.41±0.41d	15.02±0.11d
L.S.D 05	1.61	1.87	1.37	1.35	1.03	1.56	0.65	0.53

* The chmical analysis was carried out on day 8 during the last day of the normal duration of the last stadium and on day 9 for the treated nymphs.

The analyses of variance was carried out by Microsoft Excel.

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التأثير المنظم للنمو الحشرى للمركب الطبيعى المحتوى على الأزاداراختن (المرجوزان) ، فى الجراد الصحراوى شيستوسيركا جريجاريا (مستقيمات الأجنحة - الجراديات)

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تم دراسة التأثيرات المورفولوجية والأيضيه كمؤشرات على التأثير المنظم للنمو الحشرى لمستخلص شجرة النيم ، الأزادارختن ، فى الجراد الصحراوى وذلك بحقن ثلاثة جرعات منه (١٠ ، ٢٠ ، ٤٠ ميكروجرام / حورية) فى حوريات العمر الرابع فى اليوم الأول من العمر ، أدى ذلك إلى زيادة فى العمرين الرابع والخامس عن الحشرات الطبيعية غير المعاملة بالإضافة إلى ظهور علاقة موجبة بين الجرعات المستخدمة ونسب الفشل فى الإنسلاخ إلى العمر الخامس والحشرات الكاملة . هذه المظاهر المورفولوجية تعتبر من مظاهر إرتفاع هرمون الحداثة (Juvenile hormone-JH) فى دم حوريات الجراد الصحراوى المعاملة بهذا المركب نتيجة لتدخل الأزادراختن فى النظام الهرمونى للحوريات المعاملة وهذا التدخل هو المسئول عن القصور والفشل الذى حدث فى الأنسلاخ والتأخير فى النمو .

ومن جهة أخرى ، تم حقن هذه الجرعات فى حوريات العمر الخامس خلال اليوم الأول واليوم الخامس من عمرها لكشف التأثيرات الأيضية للأزادارختن على المواد الحيوية الأساسية فى دم هذه الحوريات. أدت هذه المعاملة إلى إرتفاع فى مستوى البروتين والكربوهيدرات وإنخفاض مستوى كل من الدهون والكوليسترول ، وهذا يعتبر من التأثيرات المعروفة لهرمون الحداثة (JH) فى حوريات الجراد الصحراوى .

من النتائج السابقة يتضح أن المستخلص الطبيعى لشجرة النيم (الأزادارختن) قد لعب نفس الدور الذى يقوم به هرمون الحداثة (JH) إذا زاد مستواه فى حوريات الجراد عن المستوى الطبيعى مما يؤدى إلى فشل فى إنسلاخها وتأخير فى نموها وزيادة فى بروتينات وسكريات الدم وإنخفاض فى مستويات الدهون والكوليسترول فى الدم مما يؤكد التأثير المنظم الحشرى لهذا المركب فى الجراد الصحراوى .