

RESPONSE OF *SCHISTOCERCA GREGARIA* (FORSK.) TO AZADIRACHTIN EXTRACTED FROM NEEM TREE SEEDS (*AZADIRACHTA INDICA* A JUSS.)

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Abstract

The antifeedant action of Azadirachtin as well as its biological effects against the nymphal and adult stages of the desert locust, *Schistocerca gregaria* were studied.

Low concentrations of this compound (20, 2 and 1ppm) exhibited strong antifeedant action against the 4th and 5th instars and the adult stage of this insect. These concentrations induced 100, 60 and 40% feeding reduction against the 4th instar ; 100, 75, and 72.5% feeding reduction against the 5th instar ; and 100, 50 and 18.75 % feeding reduction against the adult stage respectively.

Using injection technique, azadirachtin had a hormone- like effect against the last nymphal instars such as expressed by high failure in ec-dysis, prolongation in the 4th and 5th stadium and permanent nymphs.

INTRODUCTION

Neem extracts contain many compounds , their combined activities include antifeedant properties, insect growth regulator (IGR) activities , oviposition deterrent effects, and effects on insect fecundity (Schmutterer, 1990).

Many years ago, the antifeedant properties of the neem tree *Azadirachta indica* A. Juss, were first reported in field experiments with the adult desert locust (Pradhan and Jotwani, 1968). The primary antifeedant component of neem seeds is

azadirachtin a tri-terpenoid that was first isolated by Butterworth and Morgan (1971), and characterized by Nakanishi (1975).

Champagne *et al.*, (1989) reported that the desert locust *S. gregaria* is the most sensitive insect to the antifeedant action of azadirachtin. Moreover, the neem tree leaf extracts exhibited high antifeedant activity against the same pest (El-Gammal *et al.*, 1988). Champagne *et al.*, (1989) added that the migratory grasshopper, *Melanoplus sanguinipes* was undeterred by feeding on cabbage leaf discs treated with up to 500 ppm azadirachtin; however, azadirachtin consumed by 5th instar nymphs had a clear moult-disrupting effect that was dose dependent.

Working on the whitefly *Bemisia tabaci*, Cudriet *et al.*, (1985) believed that azadirachtin acted as an antiecdysteroid which may affect the neuroendocrine control of ecdysteroids.

The present work is an attempt to evaluate more the biological response of *S. gregaria* to azadirachtin.

MATERIALS AND METHODS

Evaluation of the antifeedant response of *S. gregaria* to azadirachtin

A powder formulation of the neem tree seed extract azadirachtin 20% was used in this study. Its antifeedant properties were tested against the last nymphal instars and the adult stage of *S. gregaria* at the concentrations 20, 2 and 1 ppm using the disc dipping technique of Butterworth and Morgan (1971). Discs of filter paper Whatmann no.1 (5.5cm in diameter) were impregnated with the previous concentrations dissolved in water. The control discs were dipped in 0.25 M sucrose solution. The treated and untreated discs were allowed to dry for 24h then sprayed with sucrose solution and weighed before being introduced to a pair of 4th, 5th instar nymphs and adults. The discs were fixed in a vertical position by the sand in cylindrical glass containers in which the starved insects for 24h were confined for eight hours. The discs were weighed after that time to calculate the feeding reduction percentages relative to their initial weight.

Determination of the biological action of azadirachtin on *S. gregaria*

Three doses of 2.0, 1.0 and 0.5 μg azadirachtin per insect were injected by Burkard-Hand Microapplicator to newly moulted 4th instar nymphs through the intersegmental membrane between the first and the second sternum.

The doses were dissolved in 4 μl of locust saline solution and the untreated insects received 4 μl saline solution only. Treatment was replicated three times with the dose 2.0 μg and 4 times with the dose 1.0 and 0.5 μg using 10 insects per each replicate.

After treatment the treated and untreated insects were transferred to a wooden cage (30x30x30cm in size, 10 insects per cage) and all the cages were incubated in a constant temperature room of $32 \pm 2^\circ\text{C}$, 60% R.H. and 12:12 L/D.

Mortality percentages and ecdysis to the next instar were daily observed, mortality was calculated relatively to the whole treated number of the 4th instar, while the 4th, and the 5th instar durations were estimated by Dempster's equation (1957).

RESULTS AND DISCUSSION

Antifeeding action of azadirachtin

Table 1 shows that the high concentration of azadirachtin (20.0 ppm) completely inhibited the feeding activity of the 4th and the 5th instar nymphs as well as the adult stage (100% feeding reduction). The effect of the second concentration (2.0 ppm) was moderate as it induced 60.0, 75.0 and 50.0 % feeding reduction against 4th, 5th instar nymphs and adult stage, respectively. The lowest concentration 1 ppm produced 40.0, 72.5 and 18.75% feeding reduction for the same insect stages, respectively. These results are in agreement with those of Narayan *et al.*, (1980) who tested the cold aqueous alcohol and acetone extracts of neem leaves against the adult stage of *S. gregaria*. The extracts afforded 99.28 and 98.57% protection, respectively when used at the rate of 1%. Jotwani and Srivastava (1981) stated that the main action of neem (*Azadirachta indica*) is an antifeedant

Table 1. Antifeeding action of Azadirachtin against *Schistocerca gregaria*.

Concentration ppm	4th instar nymphs		5th instar nymphs		Immature adults	
	Wt. Consumed part of wafers (g)	Feeding reduction %	Wt. Consumed part of wafers (g)	Feeding reduction %	Wt. Consumed part of wafers (g)	Feeding reduction %
20.0	0.00	100.00	0.00	100.00	0.00	100.00
2.0	0.02	60.00	0.030	75.00	0.08	20.00
1.0	0.03	40.00	0.033	75.50	0.13	18.75
Control	0.05	---	0.120	---	0.16	---

against arthropods on growing plants.

The biological action of azadirachtin

Table 2 indicates that the initial kill of the three doses against the 4th instar nymphs were 53.3, 42.5 and 37.5% for the doses 2, 1 and 0.5 respectively. Mortality percentages for the last nymphal instar were 90.0, 85.0 and 50.0 % for the doses 2.0, 1.0 and 0.5 μ g/ 4th instar nymphs, respectively. This showed that mortality was dose dependent.

Table 2. Effect of azadirachtin on some biological aspects of *Schistocerca gregaria*.

Doses in μ g/nymph	No. treated 4th instar nymphs	4th instar nymph		5th instar nymph		Total Mortality %	Perfect adult %
		Mortality %	Failure to 5th instar %	No. resulting nymphs	Failure to adult stage %		
2.0	30.0	53.3 (16)	13.3 (4)	10.0	23.3 (7)	90.0 (27)	10(3)
1.0	40.0	42.5 (17)	0.0 (-)	23.0	42.5 (17)	85.0 (34)	15(6)
0.5	40.0	37.5 (15)	0.0 (-)	25.0	12.5* (5)	50.0 (20)	50(20)
Control	20.0	---	---	20.0	---	---	100(20)

* Duration of these nymphs was prolonged as permanent 5th instar nymphs then eventually died after one month.

- Figures in parentheses indicate the number of insects for each indicated group.

The same table reveals that the high dose (2.0ug/nymph) was the only dose which induced 13.3 % failure in ecdysis to the 5th instar, whereas the first two doses (2.0 and 1.0 ug) induced 23.3 and 42.5% failure in ecdysis to the adult stage, respectively . In case of the dose 0.5ug, the resulting 5th instar nymphs couldn't moult and survived as permanent 5th instar nymphs for about one month then eventually died.

As shown in Table 3, the duration of the treated 4th instar nymphs and the resulting 5th instar nymphs were prolonged by azadirachtin treatment . The durations reported for the treated 4th instar were 11.33, and 12.20 days for the doses 2.0, 1.0, 0.5ug /4th instar, respectively compared with 6.4 days for the control. Similarly, the durations of the 5th stadium were prolonged in comparison to the untreated control. The life span of each instar was also prolonged (Table 3) .

Jotwani and Srivastava (1981) stated that a slight initial kill was observed , a matter that could be attributed to the stomach or contact poison of neem . Failure in ecdysis was attributed to disruption of growth or moulting .

The prolongation in the 4th and 5th stadium and the resulting permanent 5th instar nymphs in the present study might be due to the effect of azadirachtin as an antiecdysteroid (Coudriet *et. al.*, 1985; Schumtterer, 1990).

Table 3. The duration of the last nymphal instars of *Schistocerca gregaria* as affected by azadirachtin.

Doses in /ug nymph	No. treated 4th instar nymphs	4th stadium in days	5th stadium in days	The life span of the last instars
2.0	30.0	11.33	18.25	29.58
1.5	40.0	13.86	15.25	29.11
0.5	40.0	12.20	12.00	24.20
Control	20.0	6.40	8.40	14.80

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استجابة الجراد الصحراوي «شيسستوسيركا جريجاريا» لمستخلص بذور شجرة النيم Azadirachtin

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تم في هذه الدراسة اختبار صفة منع التغذية لمستخلص بذور شجرة النيم وكذلك تأثيراته البيولوجية على حوريات الجراد الصحراوي.

واتضح من الدراسة أن التركيزات المستخدمة رغم أنها منخفضة نسبياً (٢٠، ٣٠، ١ جزء في المليون) فلقد أدت إلى منع تغذية حوريات العمرين الرابع والخامس وكذلك الحشرة الكاملة بنسب عالية تراوحت بين ١٠٠٪ إلى ١٨٠،٧٥٪

ويحقن حوريات العمر الرابع بهذا المستخلص بتركيزات ٢، ١، ٥، ٥٠ ميكروجرام لكل حورية نتجت نسب مختلفة من الموت في الطور الحوري الرابع والخامس تراوحت بين ٥٣٪ إلى ١٠٠٪، وكذلك ظهرت نسب فشل في الانسلاخ إلى العمر الحوري الخامس نتيجة المعاملة بالجرعة العالية، وزادت بعد ذلك نسب الفشل في انسلاخ العمر الحوري الخامس إلى الحشرة الكاملة، وعموماً فلقد تراوحت نسب الموت الكلية بما فيها الفشل والموت بين ٩٠٪ إلى ٥٠٪ مما يعطي لهذا المركب قيمة تطبيقية في الحقل ضد هذه الآفة.