

AZADIRACTIN EFFECTS ON *SCHISTOCERCA GREGARIA* FORSKAL DURING OVARIAN DEVELOPMENT

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ABSTRACT

The effect of azadirachtin (2 $\mu\text{g/g}$ body weight) on the fresh weight of body and ovary, haemolymph proteins and amino acids and activity of the median neurosecretory cells of the desert locust, *Schistocerca gregaria* was studied for 12 days after treatment. Though there was a marginal increase in body weight, ovarian development was completely inhibited. Haemolymph proteins decreased both quantitatively and qualitatively. It delayed the synthesis and release of neurosecretion from the A-type median neurosecretory cells of brain thereby affecting the ovarian development.

INTRODUCTION

AZADIRACTIN, a tetranotriterpenoid from the neem tree (*Azadirachta indica* A. juss) is a strong antifeedant and growth disruptor to several insect species and thus a potential candidate for use in plant protection¹⁻⁵. There is now sufficient evidence to show that these two effects are independent^{6,7}. Growth disruption by azadirachtin has been shown to be primarily due to its effects on neuroendocrine centres leading to changes in pool size of terminal morphogenetic hormones^{8,9}. Of particular interest is that a single dose of injected azadirachtin (< 10 $\mu\text{g}/\text{female}$) inhibits ovarian development in *Locusta migratoria*⁸, but how exactly this is brought about is yet to be fully understood. Since ovarian development and associated metabolic changes are under endocrine control¹⁰⁻¹², it would be worthwhile to study azadirachtin action from this angle. We have already demonstrated the feeding inhibition and growth regulatory effects of azadirachtin on final instar *Schistocerca gregaria*¹³. This study aims at following the action of azadirachtin during the vitellogenic phase of *S. gregaria*. We present data on its effects on haemolymph constituents, ovarian growth and median neurosecretory cells (MNC) of the brain.

MATERIALS AND METHODS

Gregarious phase of the desert locust, *S. gregaria* was maintained on maize and cauliflower leaves supplemented with wheat bran and yeast according to Mehrotra and Rao¹⁴. Batches of freshly moulted female adults were separated and 24 hr-old adults were injected (4 $\mu\text{l}/\text{adult}$) with azadirachtin diluted in 70%

alcohol at a dose of 2 $\mu\text{g/g}$ body weight. (Azadirachtin was a generous gift from Prof. H. Rembold, Max-Planck Institute for Biochemistry, Munchen, West Germany.) The adults were anaesthetized with CO_2 before injecting azadirachtin with a 10 μl Hamilton syringe. Since the first ovarian cycle starts about 7 days after adult emergence in *S. gregaria*, haemolymph was collected during injection (0 day) and also on 8th and 12th day after treatment. Haemolymph was collected through a puncture at the base of a metathoracic leg into prechilled Eppendorf tubes containing reduced glutathione. Clear plasma was separated by centrifugation at 5000 rpm for 10 min and stored at -5°C till used for assay. Fresh weights of the adults and ovary were recorded and the brain tissue was fixed in aqueous Bouins' fluid.

Protein and free amino acid contents of haemolymph samples were estimated according to Lowry *et al*¹⁵ and Rosen¹⁶, respectively. Deproteinized plasma with 70% alcohol, was used to estimate free amino acids. Proteins were separated by electrophoresis on 7% polyacrylamide gels¹⁷ and scanned on Beckmann 36 spectrophotometer.

The secretory activity of the MNC was studied by *in situ* staining with paraldehyde fuchsin according to Dogra and Tandan¹⁸.

RESULTS AND DISCUSSION

The data on the effect of azadirachtin on body and ovary weights and haemolymph proteins and amino acids are presented in table 1.

Body and ovary weight

There was no increase in body weight of treated

Table 1 Effect of azadirachtin (2 µg/g) on body and ovary weights and haemolymph protein and amino acid levels of adult female *S. gregaria*

Treatment	Days after treatment	Fresh weight (g)	Fresh weight of ovary (mg)	Haemolymph constituents	
				Proteins (mg/ml)	Free amino acids (µm/ml)
Control	0	1.400 (0.189)	10.500 (1.224)	40.847 (6.525)	147.20 (41.50)
	8	3.427 (0.356)	14.500 (0.577)	6.213 (0.899)	49.53 (7.72)
	12	2.757 (0.171)	317.000 (57.500)	75.465 (4.720)	66.50 (6.38)
Azadirachtin	8	1.467 (0.203)	8.375 (3.092)	17.953 (0.795)	149.05 (19.44)
	12	2.230 (0.577)	13.750 (4.110)	9.387 (0.825)	82.25 (4.78)

n = 4; Figures in parenthesis represent standard deviation.

adults within the first 8 days, whereas control adults gained about 2 g during the same period. In the subsequent 4 days, the body weight of treated females increased by 763 mg in contrast to a loss of 632 mg in control.

Similar to body weight, no substantial increase in the wet weight of the ovary of the treated females was observed during the 12-day experimental period. However, ovary weight of the untreated female increased marginally during the first 8 days and substantially by 300 mg during the subsequent 4 days.

When ovary weight is considered with the body weight it appears that somatic growth precedes initiation of the ovarian cycle. The results suggest that maturation of oocyte, as indicated by the ovary weight, in control started one week after the adult emergence. This was also marked by the change in body colour from pink to light yellowish brown. Though body weight of azadirachtin-treated adults increased marginally, there was no increase in weight of the ovary suggesting complete inhibition of the ovarian development. Indeed, the body colour also remained pinkish in treated females as in freshly moulted adults. Moreover, the marginal increase in body weight could be due to greater retention of water as has been pointed out by Rao and Subrahmanyam¹³ in 5th instar hoppers of the same insect under azadirachtin stress.

Haemolymph constituents

Protein concentration of the haemolymph was initially high. In control, it decreased within 8 days and

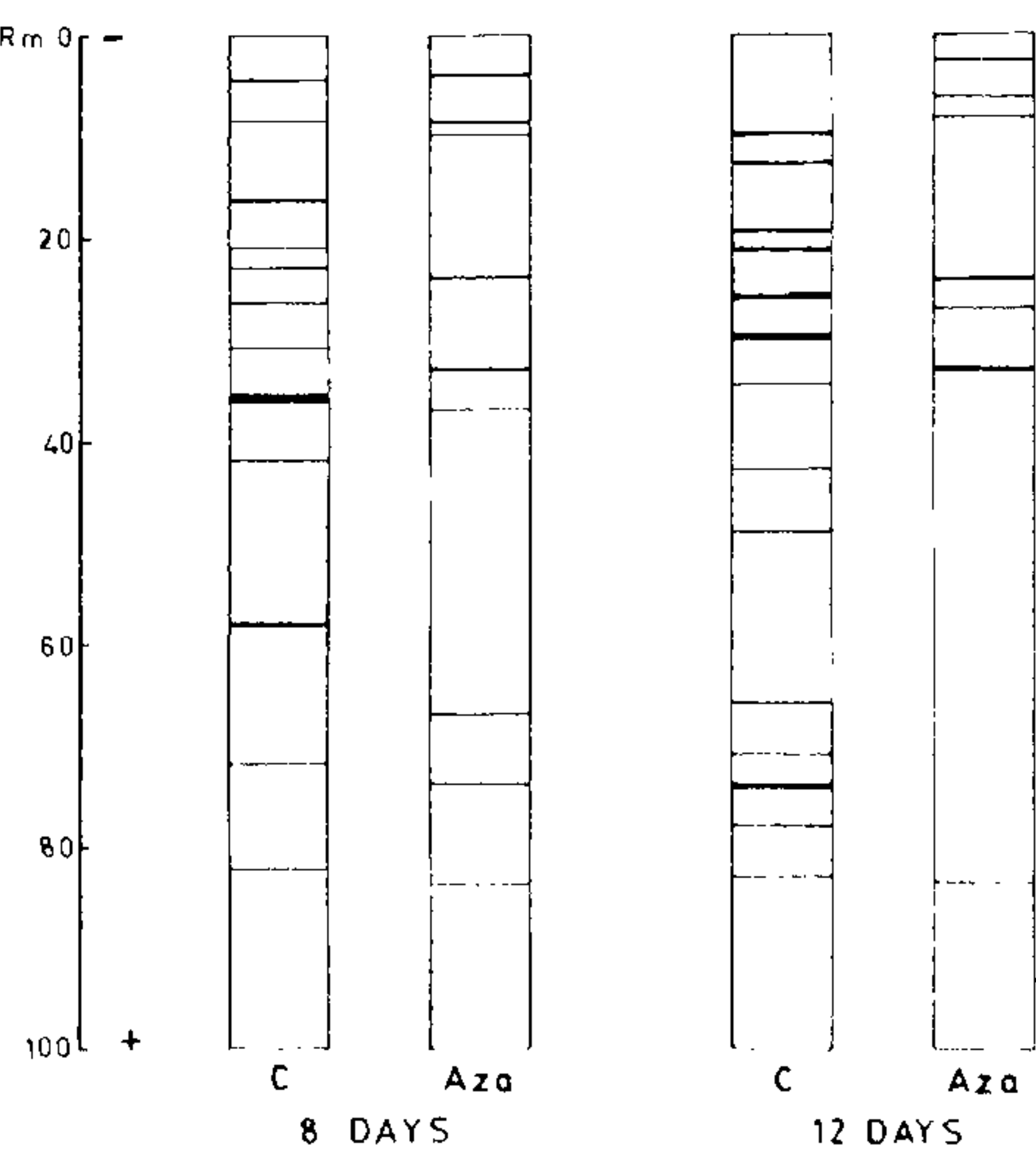
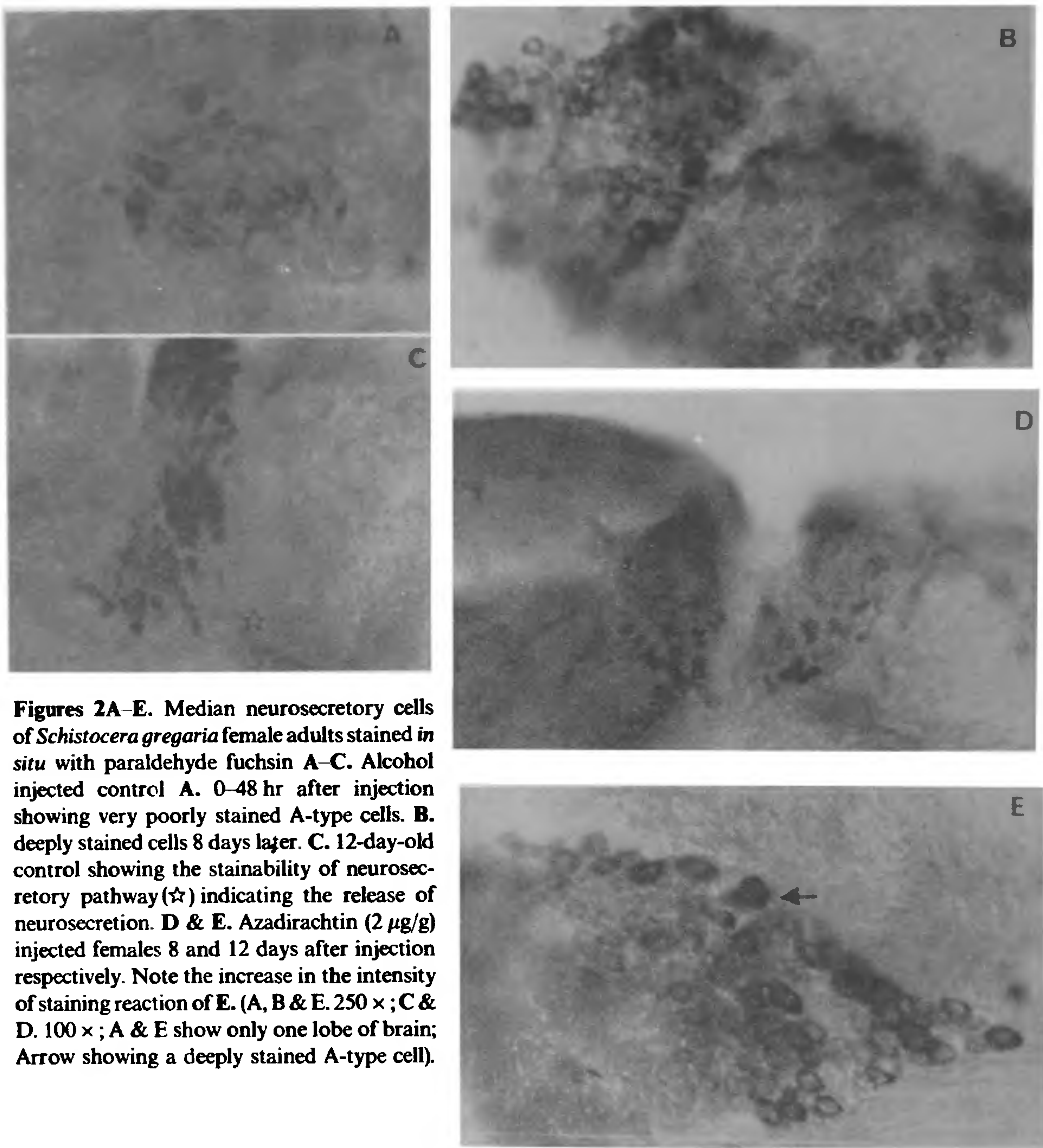


Figure 1. Electrophoretic pattern of haemolymph proteins of adult female *S. gregaria* 8 and 12 days after injection of alcohol (C) and azadirachtin (2 µg/g) (Aza). *R_m* = Relative mobility of protein fractions on 7% acrylamide gel.

then increased in the subsequent 4 days. In contrast, azadirachtin-treated females showed a continuous decrease in protein concentration. In treated females



Figures 2A–E. Median neurosecretory cells of *Schistocera gregaria* female adults stained *in situ* with paraldehyde fuchsin A–C. Alcohol injected control A. 0–48 hr after injection showing very poorly stained A-type cells. B. deeply stained cells 8 days later. C. 12-day-old control showing the stainability of neurosecretory pathway (☆) indicating the release of neurosecretion. D & E. Azadirachtin (2 µg/g) injected females 8 and 12 days after injection respectively. Note the increase in the intensity of staining reaction of E. (A, B & E. 250 × ; C & D. 100 × ; A & E show only one lobe of brain; Arrow showing a deeply stained A-type cell).

there was no change in amino acid pool size during the first 8 days. However, in the subsequent 4 days it was reduced considerably.

A comparison of haemolymph electrophoretic pattern of proteins of 8- and 12-day-old control and treated adults (figure 1) suggests a decrease in the total number of bands in treated females in contrast to

increase in control. On the 8th day the treatment showed 9 protein bands as compared to 12 in control. The number of bands in treatment decreased to 7 on 12th day in contrast to increase in control to 14. Besides, the R_m values of control and treatments differed considerably.

The changes in haemolymph proteins and amino

acid levels, when considered with changes in body and ovary weights, show certain interesting facts. The decline in protein and amino acid levels in control during the first 8 days coincides with a considerable increase in body weight and a marginal increase in ovarian weight and thus haemolymph proteins and amino acids during this period were mostly utilized for tissue or somatic growth. However, in the subsequent 4-day period the ovary weight increased 22-fold in control which coincides with increase in protein and amino acid level and a decrease in body weight. Probably most of the proteins and amino acids were utilized for ovarian growth and the increase in number of protein bands in control during this period also suggests specific protein requirement of the developing ovary. The changes in haemolymph protein and amino acid levels, body and ovarian weight in azadirachtin-treated females show its inhibitory effect both on somatic growth and ovarian development. The decrease in number of protein bands over the period also suggests inhibition of the synthesis of the specific proteins required for maturation of ovary. These results also get support from the observations of Hill¹⁹, who demonstrated that yolk deposition in the oocyte begins only when the haemolymph protein concentration reaches its minimum, as observed in untreated adults in our study. The inhibitory effect of azadirachtin on development of oocytes in *S. gregaria* is similar to that of inhibition of oocyte development in *L. migratoria* upto 15 days after azadirachtin treatment⁸.

Neurosecretory activity

In situ staining of the MNC of pars intercerebralis of the brain with paraldehyde fuchsin facilitated the identification of A-type cells in *S. gregaria* (figure 2). The number of A-type cells staining deep purple during the experimental period is presented in table 2.

Hundreds of A-type cells were stained in each lobe and to express the activity, only the number of cells whose cytoplasm were completely filled with stainable neurosecretion was considered. Not only the number of cells loaded with neurosecretion increased but the general staining reaction was also intense in 8-day-old control as compared to 0-48 hr and 12-day-old adult females. This observation suggests the release of neurosecretion between 8 and 12 days in adult females. Classical studies by Highnam and co-workers²⁰⁻²² demonstrated that MNC exhibit cyclical changes and that maturation of the first batch of oocytes was preceded by release of A-type neurosecretory product between 4 and 14 days leading to slight storage in the

Table 2 Effect of azadirachtin on MNC of adult females of *S. gregaria*

Age of the adults	A-type cells per lobe	
	Control	Azadirachtin
0-48 hr	3	-
8 days	26	7
12 days	12	23

pars intercerebralis. The increase in ovary weight by 22 fold between 8th and 12th day in our study suggests that maturation of ovary started after the release of neurosecretion. In the azadirachtin-treated group, on the other hand, a progressive build-up of neurosecretion in the A-type cells was noticed with increase in the number of cells loaded with neurosecretion and the intensity of staining. Such a situation corresponds to inhibition in release as shown by Highnam and Mordue²². The poor ovarian growth in treated females even 12 days after treatment could be due to inhibition of the release of neurosecretion.

In *S. gregaria*, ovarian development and maturation are under hormonal control. The gonadotropic effect of juvenile hormone on locusts²³⁻²⁵ has already been demonstrated. It is known that secretions from MNC (brain hormone) control the production of juvenile hormone. Inhibitory effect of azadirachtin on imaginal growth and ovary development in *S. gregaria* appears to be mediated through blockade of the neurosecretory release as in *L. migratoria*⁹. We have demonstrated that azadirachtin-treated female hoppers reach catabolic state wherein haemolymph protein loss was conspicuous. The present study clearly shows the inhibitory effect of azadirachtin on ovarian development and haemolymph protein. Since azadirachtin affected haemolymph protein levels in female 5th instar hoppers¹³ and adults, a detailed study of neuroendocrine system along with protein turnover under azadirachtin stress would lead to a better understanding of its action.

ACKNOWLEDGEMENTS

The authors are grateful to Dr S. K. Bhatia, for his interest and to Mrs. S. Singh for technical assistance.

20 December 1985

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ANNOUNCEMENT

NATIONAL SEMINAR ON ENVIRONMENTAL POLLUTION CONTROL AND MONITORING

Central Scientific Instruments Organization (CSIO) is organizing a national seminar on Environmental Pollution Control Monitoring during October 21–23, 1986. The Seminar aims to focus attention on the present day national problems in the area of environmental pollution and how best to solve these problems. During the seminar it is also proposed to discuss the basic physical and chemical processes affecting the behaviour of pollutants in the environment (both air

and water). Another important subject included is the theme of environmental monitoring techniques and instrumentation. The full papers accepted for presentation will be published in the form of proceedings of the Seminar.

For details please contact Dr. V. S. Bhatnagar, Chairman, Environmental Monitoring Instruments Division, P. B. 76 (GPO), CSIO Sector 30, Chandigarh 160 020.