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Impact of azadirachtin on the haemodynamics of *Cyrtacanthacris tatarica* L. (Acrididae: Orthoptera)Ψ

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ABSTRACT: Haemocoelic injection of azadirachtin (2, 4 and 6 µl/g body weight of the test insect) into the final instar female nymphs of *Cyrtacanthacris tatarica* L. increased the haemolymph volume significantly than the ethanol-injected individuals. Total haemocyte count (THC), computed from four recorded haemocytes (prohaemocytes, spherulocytes, granulocytes and plasmatocytes) decreased with increasing concentration of azadirachtin and the overall reduction ranged from 31 to 41%. A dose-dependent inverse relationship was obtained between the haemolymph volume and total haemocyte number. Bulging of cytoplasm, membrane breakdown and release of cytoplasmic materials were recorded in plasmotocytes, while granulocytes showed vacuoles in the cytoplasm and nucleus. However, no such deformities were observed in prohaemocytes and spherulocytes.

Azadirachtin, a natural plant pesticide isolated from the seeds of *Azadirachta indica* A. Juss (Meliaceae) (Butterworth and Morgan, 1968) acts as a potential antifeedant (Koul, 1993) and growth regulating agent (Schmutterer, 1990) in various insect groups. In recent years, considerable attention has been paid to study its impact on the behaviour (Fagoonee, 1981), food utilization efficiency (Barnby and Klocke, 1987), moulting inhibition (Sieber and Rembold, 1983), neuroendocrine regulation (Subrahmanyam, Muller and Rembold, 1989), hormonal imbalance (Pener *et al.*, 1989) and decrease in the fecundity (Steets and Schmutterer, 1975). However, knowledge about its impact on insect haemolymph and haemocyte is scanty. Quadri and Narasaiah (1978) reported changes in total haemocyte number and structure in *Periplaneta americana* L. when azadirachtin was applied topically. Rembold, Uhi, and Muller (1987) recorded a decrease in the total haemolymph protein titer in azadirachtin injected *Locusta migratoria* (R. & F.). Such quantitative decrease in the haemolymph protein was also studied in azadirachtin-treated *Schistocerca gregaria* (Forsk) during the gonadotropic cycle (Subrahmanyam and Rao, 1986; Annadurai and Rembold, 1993). However, the possible impact of this xenobiotic compound on the volume of the insect haemolymph has yet to be studied. Hence, the impact of azadirachtin on the haemocytes and haemolymph of cotton grasshopper, *Cyrtacanthacris tatarica* L. (Orthoptera: Acrididae), a polyphagous, bivoltine, sporadic pest of cotton, groundnut, *bendi*, red stem castor, *ragi* and pearlmillet (Peter, 1994), was investigated.

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MATERIALS AND METHODS

Laboratory culture of *C. tatarica* was maintained using the method of Peter and Ananthakrishnan (1993). Freshly moulted final instar female nymphs (±16 h) were separated from the stock culture and used as test individuals.

Source and mode of application of azadirachtin : Azadirachtin used in this study (1000 ppm-ethanol based) was obtained from SPIC Science Foundation, Southern Petrochemical Industries Corporation Limited, Madras (India). Haemocoelic injection of azadirachtin (2, 4 and 6 µl/gram body weight of the insect) to the test individual was done by following the method of Sieber and Rembold (1983). Control individuals were injected with an equal amount of ethanol. Both exprimental and control individuals were fed on fresh mature leaves of red stem castor, *Ricinus communis* L. (Euphorbeaceae). The trials were restricted for ten days since the insect moulted on the 12th day.

Haemodynamic studies : To collect haemolymph, the test insect was first anaesthetized with mild ether 10 days after the injection. It's antennae were cut at the base with a sharp blade. The body was inserted upside down in a centrifuge tube with its hind legs tied to the tube. Hand rotator was used at the rate of approximately 100-125 rpm for 2-3 minutes to collect the haemolymph. The collected haemolymph was measured using micropipette. Staining of haemocyte and total haemocyte count (THC) were made as described by Arnold and Hinks (1979), and Bahadur (1993), respectively.

Statistical analysis : The data were subjected to Student's t- test using the package Epistat, Tray L Gustatson. Linear regresion and correlation coefficient were made using Lotus Freelance taking the percentage haemolymph volume on x-axis and the total haemocyte count on the y-axis.

RESULTS AND DISCUSSION

Volume of haemolymph : Haemolymph volume increased in a dose-dependent manner ($p=0.01$; 't' test) in azadirachtin-injected insects as compared to the control individuals. The rate of increase was 26 to 62% when the insect was injected with 2 to 6 µl of azadirachtin/g insect body weight (Table 1). The colour of the haemolymph in control

Table 1. Total haemolymph volume (THV) and total haemocyte count (THC) in azadirachtin-injected *C. tatarica*

Dose of azadirachtin	THV (µl)	THC/mm^3
Control	455 ± 32.59^{a}	96030 ± 2656^{a}
2 µl	576 ± 60.14^{b}	66457 ± 2369^{b}
4 µl	$679 \pm 45.96^{c,e}$	61818 ± 2452^{c}
6 µl	$740 \pm 65.19^{d,e}$	53319 ± 2074^{d}

n = 5 ± S.D.

Means with same letters are not significant at p = 0.1

Means with different letters are significant at p = 0.01

insects was light greenish. In azadirachtin-treated individuals, it became turbid and colourless. Naqvi (1987) reported such colour changes with abdominal bulging when *Callosobruchus analis* F. was treated with the extracts of winter *neem* leaves.

In general, body water loss is reported in insects either due to diuretic hormone which may enhance fluid loss or due to antidiuretic hormone which may retard it (Spring, Morgan and Hazelton, 1988). Both the hormones are released from corpus cardiacum, and may either singly, or in combination, act on the single cell type that comprises the secretory regions of the Malpighian tubule (Hazelton, Parker, and Spring, 1988). Interestingly, azadirachtin inhibits the release of neurosecretory materials from corpus cardiacum (Subrahmanyam *et al.*, 1989) and binds with the excretory cells of the Malpighian tubules (Paranagama *et al.*, 1993). Therefore, increase in haemolymph volume must be due to a retardation in fluid loss through excretion. Such accumulation might have changed the colour of the haemolymph through dilution.

Total haemocyte count : In all the doses tested, total haemocyte count decreased and it was progressive with increasing concentration of azadirachtin. The overall reduction ranged from 31-41% in the treated individuals. These findings, in a broad sense agree with those of Quadri and Narasaiah (1978) where the final instar female nymphs of *P. americana* showed 20-25% decrease subsequent to topical application of azadirachtin. An inverse relationship between the total haemocyte count and haemolymph volume in *C. tatarica* (Fig. 1) (r = -0.94) can plausibly be attributed to change in blood volume (Bahadur, 1993) and cellular disruption (Saxena and Tikku, 1990).

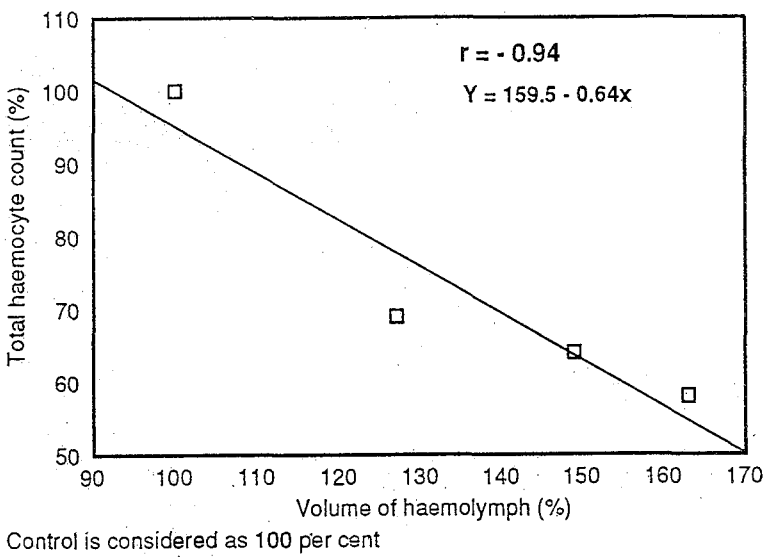

Fig. 1. Regression analysis between haemolymph and haemocyte population.

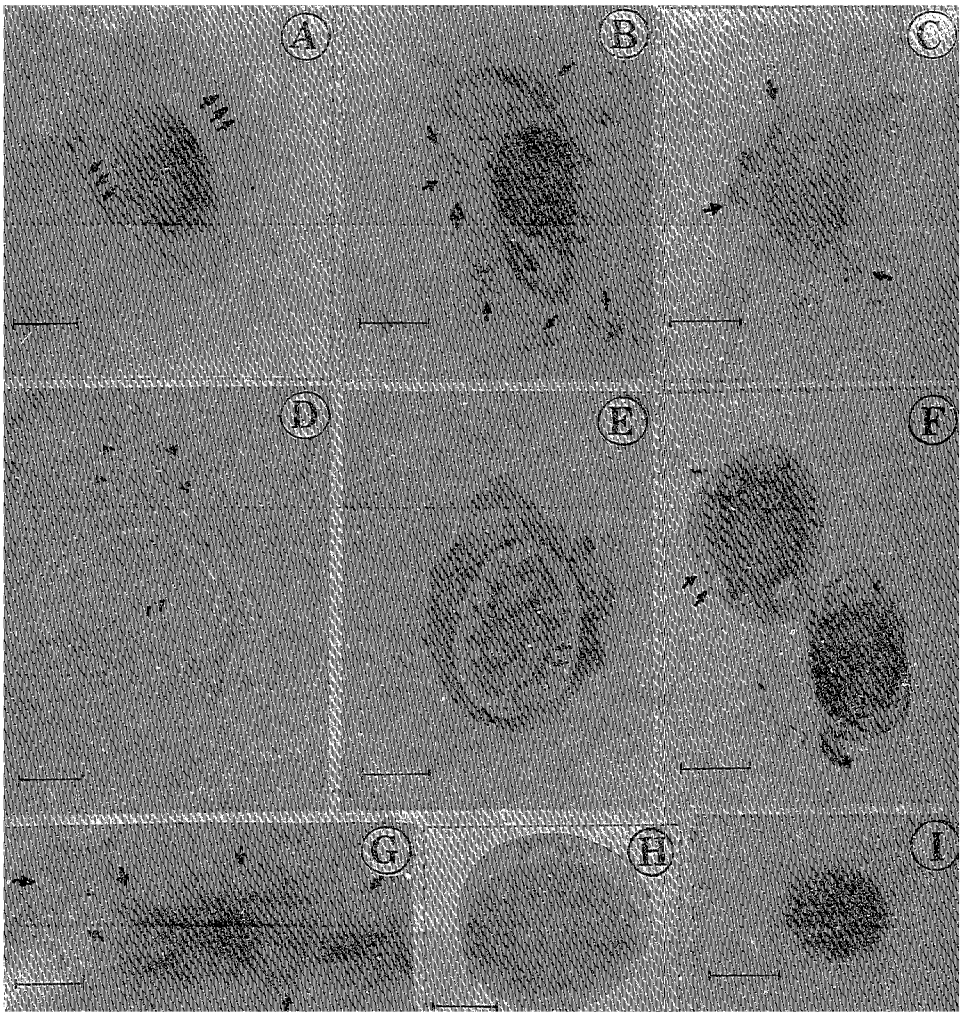

Fig. 2. Impact of azadirachtin on the haemocytes : A—Arrow marks show the bulging of the plasmatocyte. B—Plasmatocytes disruption ; release of cytoplasm due to disruption. C & F—Fully ruptured plasmatocytes with cytoplasmic release. D—Release of cytoplasmic content. E & F—Granulocytes showing nuclear and cytoplasmic vacuoles. H—Undamaged spherulocyte. I—Undamaged prohaemocyte (|——| = 25μ).

Structural deformities in the haemocytes : Four types of haemocytes, *viz.*, prohaemocytes, plasmatocytes, spherulocytes and granulocytes were recorded in the haemolymph of the ethanol as well as azadirachtin-injected *C. tatarica*. Treatment of azadirachtin resulted in abnormalities in the structure of plasmatocytes and granulocytes. Plasmatocytes changed their contour, lysed or became fragile and were on the verge of collapse (Fig. 2).

Formation of vacuole in the nucleus and cytoplasm were the prominent changes noticed in the granulocytes. These observations corroborate the findings of Quadri and Narasaiah (1978) in *P. americana* and Maheswari and Sehgal (1977) in *Dysdercus koenigii*

when they were treated with azadirachtin or with certain chemosterilant such as hempa and thiohempa, respectively. However, no structural deformities were recorded in the prohaemocytes and spherulocytes. Azadirachtin, thus, has significant impact on the haemocyte number and structure in addition to its effect on haemolymph volume.

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