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Effect of Diflubenzuron on Some Haemolymph Constituents of *Achaea janata* Linn.

B SUBRAHMANYAM and P J RAO
Division of Entomology, Indian Agricultural Research Institute
New Delhi 110012

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Effect of oral feeding of diflubenzuron (1 and 5 ppm) on proteins, free amino acids, trehalose and cations of the haemolymph of the last instar *Achaea janata* L. larvae was studied. The protein concentration in treatments remained throughout more or less similar to that of control but amino acid concentration was higher at 96 hr post-treatment. Unlike in control there was no rapid initial increase in trehalose concentration in treatments. Na^+ concentration decreased two fold in treatments whereas K^+, Ca^{++} and Mg^{++} levels were unaffected.

Key Words : *Achaea janata*, **Diflubenzuron, Haemolymph**

Introduction

Diflubenzuron belongs to benzoylphenylurea group of compounds that offer great promise in insect pest control. It exhibits contact (Ascher & Nemmy 1976) ovicidal and sterilizing activity (Thompson & Sikorowski 1982, Pickens & De Milo 1977). The juvenomimetic activity of this compound has also been reported by Subrahmanyam et al. (1980) against *Achaea janata*. It is known that diflubenzuron inhibits chitin synthesis in the cuticle through inhibition of polymerisation enzyme—chitin synthetase—leading to accumulation of the precursor, uridine diphospho-N-acetyl-glucosamine (Van Eck 1979, Post et al. 1974). Recently De Loach et. al. (1981) demonstrated that it also affects DNA synthesis in the imaginal histoblasts of stable-fly. However, the exact mechanism how chitin synthesis is inhibited is yet to be elucidated.

Excepting the work of Baumler and Salama (1976) on *Porthetria dispar*, there is no information on the effect of diflubenzuron on haemolymph constituents which reflect the metabolic state of an insect. Information on this aspect is important as moulting is a complex process involving various constituents of haemolymph and chitin synthesis inhibitors, in general, affect moulting process. The present study is in continuation of our work on effects of diflubenzuron on *Achaea janata* (Subrahmanyam et al. 1980) and aims to provide information on the effect of this compound on haemolymph proteins, amino acids, trehalose and cations of the last instar larvae of the same insect. The information on haemolymph trehalose and cation changes under diflubenzuron stress forms the first report on this aspect.

Materials and Methods

Achaea janata was reared on castor leaves at 25 ± 2°C and 70% RH according to Ramdev and Rao (1979). The development of the larvae was followed critically to separate out the fourth stage

larvae of the same age for experimental purpose. Late fourth instar larvae that would moult within the next 12 ± 2 hr were fed for 2 hr on castor leaves treated with 1 or 5 ppm of diflubenzuron (Dimilin ® 25 WP) suspension according to the method described already (Subrahmanyam et al. 1980). Larvae fed on castor leaves treated with distilled water served as control.

Haemolymph samples were collected from the test larvae into small glass tubes kept in ice by carefully puncturing one of the prolegs. A few crystals of reduced glutathione were added to the samples to prevent melanization and then centrifuged at 3500 rpm for 10 min to separate clear plasma. The plasma was stored at 5°C till used for estimation of its constituents. Total proteins were estimated by the Folin-phenol method using bovine serum albumen as standard (Lowry et al. 1951). Estimations of free amino acids and trehalose were carried out after deproteinizing the plasma with three volumes of 90% ethanol followed by centrifugation in cold. Total free amino acids were estimated according to Rosen (1957) using leucine as standard. Trehalose was assayed by the anthrone reaction method (Wyatt & Kalf 1957) and anthrone reagent was prepared according to Mokrasch (1954). The plasma samples for determination of Na^+, K^+, Ca^{++} and Mg^{++} were processed immediately after collection according to Willis (1960*a*, b, c) and estimated by atomic absorption spectroscopy using atomic absorption spectrophotometer (Varian Techtron Model AA-120).

Estimations of protein, trehalose and free amino acid were made from the haemolymph of individual larvae whereas cations were estimated from pooled samples.

Results

The data on proteins, free amino acids and trehalose are presented in table 1 and cations in table 2.

Proteins: Protein concentration of the control larvae increased from 27.60 mg/ml at 0 hr to 96.88 mg/ml at 96 hr and within the first 36 hr the increase was more than two fold. In 1 ppm treatment there was no marked deviation in protein concentration. However, in 5 ppm treatment the total protein concentration decreased to 68.22 mg/ml as compared to 82.93 mg/ml in control at 60 hr.

Free amino acids: Free amino acid concentration in the control larvae increased up to first 36 hr and thereafter decreased gradually during the next 24 hr but sharply decreased to 110.4μM/ml in control prior to prepupal stage (96 hr). In 1 ppm treatment, the amino acid concentration was more or less similar to that of control up to the first 60 hr and subsequently no sharp decrease was observed as in control. However, in 5 ppm treatment the values were relatively lower throughout as compared to control.

Trehalose: Trehalose concentration in control increased by more than two-fold during the first 36 hr (from 4.76 mg/ml at 0 hr to 11.37 at 36 hr) and thereafter it was maintained at a slightly lower concentration till 96 hr. In 1 ppm treatment the trehalose concentration increased from an initial 7.23 mg/ml to 11.04 mg/ml during the same period. Unlike in control, there was no initial sharp increase in trehalose concentration in treated larvae.

Cations: Out of the two monovalent cations, Na^+ concentration fluctuated markedly while that of K^+ remained at a fairly constant level (≃ 30 meq/l). In control, increase in Na^+ concentration altered with a decrease at 24 hr interval. In 1 ppm treatment Na^+ level followed a similar pattern up to 60 hr but subsequently in the next 24 hr it decreased in contrast to increase in control. Interestingly at 60 hr, a similar condition in Na^+ concentration was observed in 5 ppm treatment. At 84 hr Na^+ concentration of 1 ppm was just half that of control. In both the treatments and control the concentrations of Ca^{++} and Mg^{++} were maintained almost at a constant level excepting at 36 hr.

Discussion

Proteins: Generally haemolymph protein concentration of an instar increases with age and decreases prior to pupation (Ramdev & Rao 1984, Green & Dahlman 1973). We observed similar increase up to 96 hr of experimental period. However, the initial increase was sharp. The observations were confined to 96 hr as all control larvae entered prepupal condition by this time. Diflubenzuron at the concentrations studied did not significantly influence the haemolymph protein concentration. Hunter & Vincent (1974) showed earlier that diflubenzuron did not alter the deposition of proteins into the cuticle of adult locust. Similarly, no significant change in the incorporation of lysine into free amino acid/lipoprotein fraction of bollweevils was observed by Mitlin et al. (1977). Since most of the cuticular and tissue proteins are derived from metabolic pool of haemolymph (Tobe & Laughton 1969), unaltered protein concentration would suggest that in general diflubenzuron has no significant effect on protein metabolism.

Table 1 *Effect of diflubenzuron on haemolymph proteins, free amino acids and trehalose of* A. janata

Constituent	Treatment	Hours after treatment			
		0	36	60	96
Protein	Control	27.60 ± 1.33(10)	58.87 ± 8.16(10)	82.93 ± 5.85 (6)	96.88 ± 8.53(10)
(mg/ml)	1 ppm	29.11 ± 1.40(11)	46.60 ± 2.85(10)	78.43 ± 3.61 (8)	84.01 ± 6.43(10)
± SD	5 ppm	33.07 ± 1.52(10)	59.77 ± 4.44(10)	68.22 ± 3.34(10)	—
Free amino-	Control	151.82 ± 1.57 (4)	189.09 ± 0.51(4)	174.09 ± 0.46(4)	110.46 ± 7.43 (4)
acid (μM/ml)	1 ppm	135.91 ± 9.90 (4)	192.73 ± 3.34(4)	181.14 ± 8.27(4)	172.42 ± 12.73(3)
± SD	5 ppm	133.64 ± 10.16(4)	176.36 ± 4.50(4)	152.50 ± 4.18(4)	—
Trehalose	Control	4.76 ± 0.51(3)	11.37 ± 0.12(3)	8.44 ± 0.68(3)	9.74 ± 1.11(4)
(mg/ml)	1 ppm	7.23 ± 1.51(3)	8.29 ± 2.33(3)	10.88 ± 0.41(3)	11.04 (3)
± SD	5 ppm	4.76 ± 1.34(3)	7.57 ± 0.67(3)	6.67 ± 0.92(3)	—

Figures in parentheses denote the number of samples used for assay
—, Test larvae died due to treatment
SD, Standard deviation

Table 2 *Effect of diflubenzuron on haemolymph cations of* A. janata

Treatment	Hrs after treatment	Ions (meq/l)			
		Na +	K +	Ca + +	Mg + +
Control	12	5.54	30.69	19.95	84.35
	36	12.39	30.69	59.35	121.38
	60	8.15	30.05	23.44	59.60
	84	12.39	29.41	24.41	76.12
1 ppm	12	4.78	—	23.44	65.83
	36	10.44	31.98	33.91	92.57
	60	9.24	26.86	18.95	59.66
	84	6.30	30.70	18.45	59.66
5 ppm	12	7.83	34.54	18.95	72.00
	36	9.35	31.97	31.92	76.12
	60	4.78	28.14	20.45	65.83

—, Not sampled

Amino acids: Haemolymph amino acids are derived as the hydrolytic products of proteins from food or fat body (Woodring et al. 1977). A high concentration of haemolymph amino acids during the first 60 hr suggests that they are mainly derived from food as it is the most active feeding stage of the instar. Decrease in amino acid concentration at 96 hr can be explained on the basis that the larvae preparing for a prepupal stage show cessation or decrease in feeding as has already been suggested for this insect (Nath & Srivastava 1981). Although, the difference in amino acid concentration between treatments and control was only marginal up to 60 hr, the maintenance of amino acid concentration in 1 ppm treatment at 96 hr in contrast to a sharp decrease in control was due to maintenance of feeding in the treatment.

Trehalose: Trehalose level increases generally in the active feeding stage and gradually decreases suggesting its utilization for chitin synthesis and also as an energy source (Hill & Goldsworthy 1968). Absence of rapid increase in haemolymph trehalose level after diflubenzuron treatment could either be due to poor utilization of food or greater utilization of trehalose for energy. Maintenance of moderately high level of trehalose in treatments as compared to control at late larval stage may be primarily due to adverse effect of diflubenzuron on chitin synthesis for which trehalose is the main carbohydrate source.

Cations: A two-fold decrease in Na^+ concentration of 1 and 5 ppm treatments was observed at 84 and 60 hr as compared to their respective controls. Similarly, Thomson and Sikorowski (1982) reported a seven-fold decrease in blood Na^+ concentration in adults of *Anthonomus grandis* fed on diet containing 100 ppm diflubenzuron followed by irradiation. Na^+ concentration in the haemolymph is a result of equilibrium among processes of absorption from food, excretion by Malpighian tubules, rectal reabsorption and hypothetical tissue storage (Pichon 1970). It is apparent that such a balance is disturbed due to diflubenzuron treatment. It is well known that decrease in Na^+ concentration effectively depresses the action potential and finally brings about a blockage in the nerve impulse (Yamasaki & Narahashi 1959). However, it was demonstrated in *Periplaneta americana* that despite large variation in haemolymph Na^+ concentration, its activity remains relatively constant indicating that effective Na^+ concentration is regulated within relatively close limits (Treherne et al. 1975). It can be argued that in *A. janata* larvae, the alternating increase and decrease pattern of Na^+ is an indication of ionic homeostasis and diflubenzuron affected this phenomenon.

The treated larvae in the present study maintained a fairly constant K^+ concentration. A similar observation was made in *A. grandis* adults that were fed on diflubenzuron treated diet and subjected to subsequent irradiation. In our study, we found no appreciable change in Ca^{++} and Mg^{++} concentration in treatments in contrast to four-fold increase in Mg^{++} and corresponding decrease in Ca^{++} reported in *A. grandis* due to feeding of diflubenzuron followed by irradiation (Thompson & Sikorowski 1982). Since adults of *A. grandis* were also subjected to irradiation, it is difficult to attribute the changes to diflubenzuron alone.

Salient findings from the study are that diflubenzuron (i) did not affect, in general, protein metabolism, (ii) maintained higher levels of free amino acid and trehalose at late larval stage; and (iii) excepting a two-fold increase in Na^+ concentration other cations, in general, were unaffected.

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