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Effect of Insecticides on *Achaea janata* Linn. I. Haemolymph Volume, Body Moisture and Cations

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A comparative study on the effect of four different insecticides on haemolymph constituents of *Achaea janata* was carried out. pp′-DDT advanced pupation at least by 12 hr whereas heptachlor delayed it by the same period. Malathion and carbaryl, however, did not affect pupation. Insecticidal treatment, in general, resulted in reduction in haemolymph volume. Decrease in haemolymph volume due to DDT treatment led to decrease in body moisture. However, in case of heptachlor and malathion, decrease in haemolymph volume led to increase in body moisture. In carbaryl, increase or decrease in haemolymph volume led to corresponding increase or decrease in body moisture. All insecticides, in general, increased the concentration of Na^+, K^+ and Ca^{++} during early stages of poisoning and decreased at late stages of poisoning; whereas magnesium showed reduction during the same period. The significance of these changes with reference to the action of insecticides at cellular level was discussed.

Key Words: *Achaea janata*, **Insecticides, Haemolymph, Biochemical changes**

Introduction

Under the effect of insecticides, insects loose body water, show decrease in weight and develop various abnormalities (Mansingh 1965, Wall 1970, Banerjee et al. 1977, Edney 1968, Samaranayaka 1977, Thompson and Sikorowski 1982). Samaranayaka (1977) studied the effect of lindane on haemolymph cations of *Schistocerca gregaria* adults while Kulkarni and Mehrotra (1973) reported the effect of aldrin and Sumithion on some of the haemolymph constituents of the same insect. However, there is hardly any information on the effect of insecticides on holometabolous insects. Moreover, there is no report on a comparative study of the effects of insecticides belonging to different groups on a particular stage of any insect.

Ramdev and Rao (1979a and b) not only worked out the nutritional profile of *Achaea janata* but also studied the haemolymph

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constituents of 5th instar larvae (Ramdev & Rao 1984a) including characterisation of haemolymph amino acids (Ramdev & Rao 1984b). The present work is aimed to make a comparative study of the effect of pp'-DDT, heptachlor, malathion and carbaryl on haemolymph volume, body moisture and haemolymph cations of the 5th instar larvae of the same insect. Such a study will not only enable us to understand as to the type of changes brought about by insecticides of different mode of actions but also bring about how each insecticide differs from the other in these changes.

Materials and Methods

The insect culture was maintained in the laboratory at 27°±1°C according to Ramdev and Rao (1979a). For present studies, 36 hr old 5th instar larvae were used.

Application and Toxicity of Insecticides

Four insecticides were used in the present study: (1) pure pp'-DDT [1, 1, 1-trichloro-2, 2-bis (p-chlorophenyl) ethane], a widely used chlorinated hydrocarbon against many insect pests; (2) heptachlor (1, 4, 5, 6, 7, 10, 10 heptachloro-4, 7, 8, 9 tetrahydro-4, 7-methylene indane) of 72% purity as a representative of cyclodiene group; (3) malathion (95%) [0, 0-dimethyl S-(1,2-dicarboethoxy) ethyl phosphorodithioate], an organophosphorus compound with a very low mammalian toxicity; and (4) carbaryl (99%) (1-Napthyl N-methyl carbamate) representing carbamate group with high insecticidal toxicity.

Dilutions of insecticides were prepared in analytical grade acetone and the required doses were topically applied on the dorsal surface of the mesothoracic region using electrically operated microapplicator (Model M, Instrumentation Specialities Company, Inc. USA). After treatment, insects were allowed to feed on castor leaves and mortality counts were taken up to 60 hr after treatment. Larvae treated with the same amount of acetone served as controls. Both treated and control insects were kept at 27°±1°C during experimental period. The mortality data obtained were subjected to probit analysis as outlined by Finney (1952) to calculate the median lethal dose (LD_{50}) of each insecticide. LD_{50} dose of respective insecticides was used for the study of insecticidal effects. This dose facilitated observations for a 60 hr period after treatment and thus covered the entire larval period of this instar. Larvae which died during the experimental period were discarded.

Sampling Time

Samples of haemolymph from both treated and control larvae were collected at 0. 5, 6, 12, 24, 36, 48 and 60 hr after treatment.

Collection of Haemolymph

Method of collection and processing of haemolymph for its constituents have been given elsewhere (Ramdev & Rao 1984a).

Haemolymph Volume

Haemolymph volume of the larvae at different intervals was determined by absorption method (Richardson et al. 1931).

Body Moisture

Accurate wet and dry weights (up to 0.1 mg) of the larvae were determined on an electrical analytical balance. For dry weight determination, the larvae were dried at 80°C to a constant weight. Difference between the wet and dry weights gave the body moisture content which was then transformed into per cent body moisture.

Cations

Four cations namely, Na, K, Ca and Mg were determined by atomic absorption spectroscopy using atomic absorption spectrophotometer (Varian Tectron, Model AA-120, Melbourne). The plasma samples were processed immediately after collection according to Willis (1960a,b,c).

Any increase or decrease in concentration or amount of a constituent is expressed in relation to its corresponding control at that point of observation, unless stated otherwise.

Results

Toxicological data of the insecticides are presented in table 1. The median lethal doses for pp'–DDT, heptachlor, malathion and carbaryl were 175.66, 204.25, 81.95 and 17.46 μg/g body weight respectively.

General Observations

Larvae treated with pp'-DDT pupated at least 12 hr earlier than control larvae, whereas in heptachlor pupation was delayed by at least 12 hr (table 2). Data presented in table 2 clearly show that 91% of DDT treated larvae pupated earlier and 90.5% of heptachlor treated larvae pupated later as compared to control. Malathion and carvaryl did not affect pupation. In case of heptachlor, malathion and carbaryl sampling of blood was done only up to 60 hr after the treatment i.e., up to the normal period of pupation whereas in DDT treatment it had to be stopped at 48 hr due to early pupation.

Haemolymph Volume

The data on haemolymph volume are presented in table 3. In general, with all the treatments there was a reduction in haemo-

Table 1 *Toxicity of insecticides to 36 hr old 5th instar larvae*

Insecticide	Regression equation	Fiducial limit	Hetero-geneity $[\lambda^2_{(4)}]$	LD_{50} (μg/g)
pp'–DDT	$Y=2.8280\times-0.7210$	0.0753 ± 2.0230	4.150	175.66
Heptachlor	$Y=4.4826\times-4.3621$	0.0521 ± 2.0885	1.340	204.25
Malathion	$Y=3.2444\times-0.4893$	0.0575 ± 1.6919	3.918	81.95
Carbaryl	$Y=6.4415\times-8.0128$	0.0300 ± 2.0202	1.018	17.46

Table 2 *Effect of insecticides on larval development*

Insecticide	No. of larvae surviving	No. of larvae pupated	Per cent of larvae pupated	
pp'–DDT	22	20	90.9	Pupated 12 hr earlier than control
Heptachlor	21	19	90.5	Pupated 12 hr later than control
Malathion	18	17	94.4	Normal pupation
Carbaryl	17	16	94.1	–do–
Control	35	35	100.0	–do–

Table 3 *Effect of insecticides on haemolymph volume* (μl)

Treatment	Hr after treatment						
	0.5	6	12	24	36	48	60
pp'-DDT	310.0	335.5	339.0	437.0	290.0	436.0	—
Heptachlor	366.5	284.0	329.0	302.5	303.0	244.5	283.0
Malathion	349.0	341.5	438.5	319.0	466.5	309.0	293.5
Carbaryl	284.0	177.5	415.5	410.5	433.0	433.0	349.0
Control	399.5	403.5	398.5	401.5	449.0	461.0	398.0
CD	40.49	47.88	44.93	31.97	45.42	32.24	29.29

lymph volume. DDT treatment caused reduction over the control throughout the period of poisoning except at 24 hr. There was a gradual increase in volume during first 24 hr after treatment whereas in control it remained more or less constant. During 24 to 36 hr, it showed an increase in control in comparison to a sharp decrease in treatment. Maximum reduction (35.4%) in haemolymph volume was observed at 36 hr after treatment. At 36 hr, the values for treated and control insects were 290 ± 10.72 and $449.0 \pm 9.12 \mu l$ respectively. With heptachlor the difference in haemolymph volume between treatment and control was significant at all stages of poisoning except at early stage of poisoning (0.5 hr). Maximum reduction (47%) in volume was observed at 48 hr after treatment. The haemolymph volume of treated and control insects at 48 hr was 244.5 ± 7.80 and 461.0 ± 13.11 μl respectively. In case of malathion treatment there was reduction in haemolymph volume except at 12 and 36 hr. The maximum reduction of 32.97% was at 48 hr after the treatment. With carbaryl a reduction of 28.9% was observed within 30 min of poisoning which further decreased to 55.6% at the end of 6 hr and this was the maximum for experimental period. The haemolymph volume of treated and control insects at 6 hr was 177.5 ± 16.06 and 403.5 ± 22.4 μl respectively. After 6 hr, treated insects recovered from the insecticidal effect as there was a restoration of haemolymph volume. The difference in volume at 12, 24, 36 and 48 hr between treatment and control was found to be statistically not significant.

Body Moisture

Data on body moisture are presented in table 4. In DDT treatment, the moisture content remained lower throughout experimental period except for slight increase at 24 hr. The difference in moisture content was significant at 0.5, 6, 24, and 36 hr. In case of heptachlor, except for a decrease (6.47%) at 36 hr, the moisture content in treatment remained higher throughout. No significant difference between treatment and control was observed at 0.5 hr.

Malathion treatment resulted in increase in body moisture during first 24 hr. At 36 hr there was a significant reduction in body moisture of the treated larvae in contrast to an increase at 60 hr over the control. The maximum fluctuation in body moisture content in both treatment and control was only about 10%.

Carbaryl caused significant reduction in body moisture at 6 and 36 hr in contrast to a

Table 4 *Effect of insecticides on body moisture* (%)

Treatment	Hr after treatment						
	0.5	6	12	24	36	48	60
pp'-DDT	79.13 (62.84)	80.65 (63.91)	78.69 (62.51)	79.21 (62.87)	77.23 (61.51)	74.81 (59.87)	—
Heptachlor	83.94 (66.36)	83.44 (65.99)	82.58 (65.31)	79.04 (62.76)	74.36 (59.58)	77.89 (61.97)	74.47 (59.67)
Malathion	84.24 (66.63)	82.22 (65.08)	81.70 (64.70)	80.70 (63.97)	73.39 (58.95)	75.72 (60.49)	74.73 (59.60)
Carbaryl	82.98 (65.66)	79.16 (62.85)	80.61 (63.88)	81.77 (64.74)	74.50 (59.68)	74.75 (59.85)	73.74 (59.07)
Control	83.26 (65.86)	81.99 (64.91)	78.97 (62.74)	77.84 (61.90)	79.50 (63.13)	75.45 (60.29)	73.00 (58.97)
CD	0.95	1.14	1.19	1.13	1.69	1.02	0.62

Figures in the parentheses are arcsin transformation

significant increase at 12, 24 and 60 hr. Maximum increase of 5.05% over the control was observed at 24 hr. During 24–36 hr of experimental period, moisture content in control increased from 77.84 to 79.5% whereas in treatment it decreased from 81.77 to 74.5%.

Cations

Data on haemolymph cations are presented in figures 1 to 3.

Na/K: With DDT poisoning sodium-potassium ratio showed an increasing trend up to 12 hr. This increase was two-fold over control at 12 hr. However, during the last three stages of poisoning it was lower to control, the least being at 48 hr. It may be pointed out that increase or decrease in Na/K ratio in treatment was mostly due to increase or decrease in concentration of sodium rather than potassium.

Heptachlor treatment, in general, resulted in higher Na/K ratio throughout as compared to control except at 6 hr. The ratio was the highest (73.6% over control) in

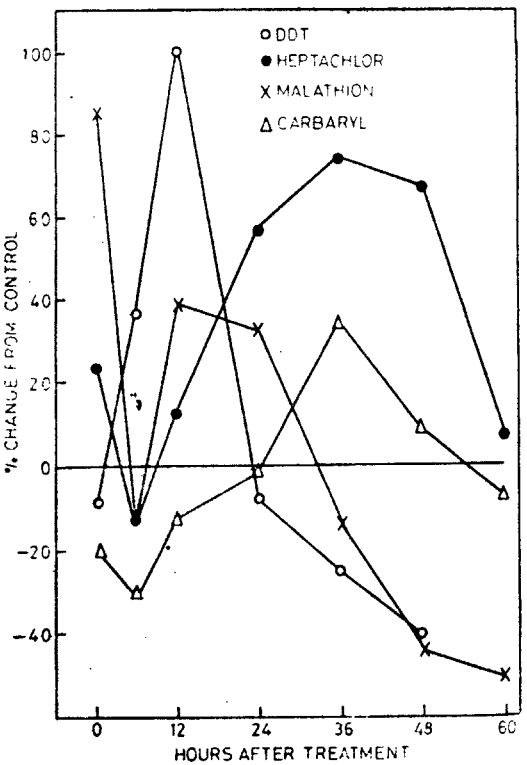

Figure 1 Effect of insecticides on sodium-potassium ratio

treatment at 36 hr while it was lower to control (13%) at 6 hr Higher ratio at 36 hr in treatment was due to more increase in Na concentration (95.1%) rather than small increase in K (12%).

Malathion, in general, caused drastic variation in Na-K ratio from one stage of poisoning to another. Except at 6 hr, Na-K ratio was higher in treatment up to 24 hr and subsequently it decreased till the end, the maximum reduction (51%) being at 60 hr.

Carbaryl decreased Na-K ratio throughout the period of poisoning except at 36 and 48 hr. At 36 hr it increased by 34.36% over the control. Though carbaryl affected the ratio, this change was not due to sodium alone.

Calcium: In general, DDT treatment increased calcium concentration. The concentration of calcium at 36 hr was the highest in the treatment for the experimental period. However, it is interesting to note that calcium concentration of both treatment and control at 24 hr was more or less the same.

Heptachlor caused an increase in Ca^{++} at the early stage of poisoning (0.5 hr). This was followed by a decrease as compared to control at 6 and 12 hr after treatment. At 24 hr, Ca^{++} concentration was similar to that of control while at 48 hr it was higher by 47%. However, at 36 and 60 hr of poisoning it was about 19% lower to control. Thus, the maximum effect of heptachlor on haemolymph calcium occurred during last 24 hr of poisoning.

Effect of malathion on haemolymph calcium was observed only from 24 hr and was significantly higher from this stage till the end. The increase at 48 hr was more than 2 fold over control.

In case of carbaryl, cyclic changes were observed after the treatment. There was a decrease in concentration initially (0.5 hr) followed by slight increase at 6 hr and then a considerable decrease at 12 hr. These cyclic changes continued up to 60 hr. Maximum increase by 67% and decrease by 19.8% in

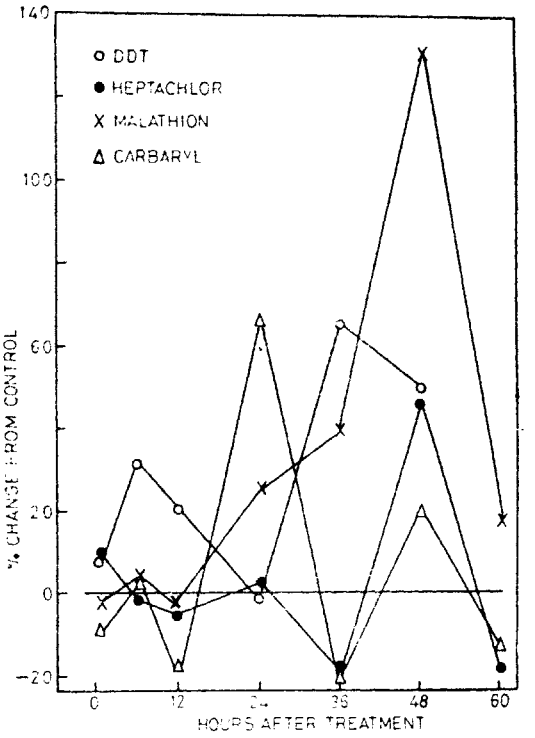

Figure 2 Effect of insecticides on calcium ion

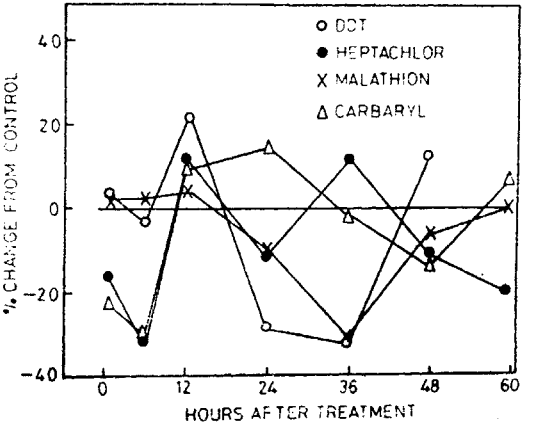

Figure 3 Effect of insecticides on magnesium ion

treatment over control occurred at 24 and 36 hr respectively.

Magnesium: DDT caused increased magnesium levels over control at 0.5, 12 and 48 hr, the most significant being at 12 hr. Significantly lower levels of magnesium were observed at 6, 24 and 36 hr, the least being at 36 hr.

Heptachlor treatment, in general, decreased magnesium concentration. However, the concentration in treatment at 12 and 36 hr was found higher to control. Maximum reduction in magnesium concentration occurred in treatment at 6 hr which was about 31%. Whereas malathion treatment resulted in increase of magnesium content during early phase of poisoning i.e., up to 12 hr. From 24 hr onward there was reduction over the control, the maximum (30.78%) being at 36 hr. During the last stage of poisoning (60 hr) magnesium concentration was restored and was the same in both control and treatment.

Like calcium, magnesium also showed cyclic changes after carbaryl treatment. It decreased Mg^{++} concentration significantly during first 6 hr. Thereafter it showed significant increase at 12 and 24 hr. At 36 and 48 hr it was lower to control. Finally at the last stage (60 hr) it increased by about 7% over control.

Discussion

The development of 5th instar *Achaea janata* under insecticidal treatment showed three distinct patterns:

(*i*) Advancement in pupation—DDT;
(*ii*) Postponement in pupation—Heptachlor; and
(*iii*) Normal pupation—malathion and carbaryl.

It has been suggested that insecticides affect neuroendocrine system in insects (Roan & Hopkins 1961). Early pupation with DDT treatment could be due to either decrease in JH titre or earlier release of MH. Since brain hormone controls the activity of both corpora allata and prothoracic gland, it is likely that these changes were brought about by the action of the insecticide on the neurosecretory cells of the brain itself. In this regard, the effect of heptachlor on pupation was the opposite of DDT. Obviously malathion and carbaryl do not appear to interfere in the development of this insect at the doses studied since the surviving larvae pupated along with the untreated larvae. But, the results on carbamate are not in agreement with those reported on 6th instar larvae of *Choristoneura occidentalis* treated with sublethal dose of a carbamate, lannate (Smith 1971) in which postponement of pupation was observed. These differences may be due to difference in species and the doses used.

Haemolymph Volume and Body Moisture

Insecticidal treatment causes water loss in insects (Roan & Hopkins 1961, Mehrotra & Sethi 1966, Samaranayaka 1977). In the present studies treatment with any one of the four insecticides resulted in reduction of haemolymph volume. This reduction in early stages of poisoning could be due to loss of water through transpiration by spiracles as pointed out by Buck and Keister (1949) in DDT-treated *Phormia regina* and by Mansingh (1965) in malathion-treated *Blatella germanica*. Further it could also be due to regurgitation as reported by Jochum (1956) in parathion poisoned *Leptinotarsa decemlineata* and *Bombyx mori*. Indeed regurgitation was observed by us in the early stages of poisoning.

If the haemolymph volume is considered along with body moisture content certain interesting patterns emerge out. In case of DDT, in general, the reduction in haemolymph volume coincided with the reduction in body moisture. In case of carbaryl, also, increase in body moisture content coincided with increase in haemolymph volume and decrease with a decrease in haemolymph

volume. But in case of haptachlor and malathion the body moisture content increased when there was reduction in haemolymph volume.

The reduction in haemolymph volume after DDT treatment could be due to loss of water to outside the body either through excretion or spirocles. But with heptachlor and malathion, the reduction in haemolymph volume could be due to altered distribution of water among haemocoel, tissue and lumen of gut (Samaranayaka 1977) because the body moisture content of the tissues remained high at a time when haemolymph volume was low. Therefore, it appears logical to infer that reduction in haemolymph volume could not be due to loss of water through excretion or spirocles alone. The conditions in carbaryl treatment could have resulted either due to loss of greater moisture both from haemolymph and tissues (low volume and low body moisture) to outside the body or greater absorption of water from the food and its retention in the body (high volume and high body moisture). Since carbaryl treated insects, in the early stages of poisoning were found to be literally wet with water, it can safely be inferred that the water loss was also through general body surface. Interestingly there was recovery of blood volume in treated insects after 12 hr. The recovery could have been brought about by a number of factors like absorption of water from food, outflow of tissue water into haemocoel or also due to minimal loss of water through excretion probably due to secretion of antidiuretic hormone. Secretion of such a hormone under water stress is known to occur in insects (Wall 1967, Cazal and Girardie 1968). Further it had been shown that insecticides affect excretion (Patton et al. 1959).

Reduction in volume at later stages of poisoning could definitely be not due to regurgitation as this phenomenon was confined to early stages of poisoning and this loss was only due to transpiration through spiracles and inter-segmental membranes. It may be that DDT alters the ability of a larva to resist desiccation as shown in *Popillia japonica* (Ludwig 1946).

Cations

In general, treatment with insecticides increased the concentration of Na^+, K^+ and Ca^{++} in the early stages and decreased at late stages of poisoning whereas Mg^{++} concentration was found to decrease most of the time. Increased potassium concentration in lindane treated *Schistocerca gregaria*, similar to the present observations, was reported earlier (Samaranayaka 1977).

In connection with the varied effects of insecticides on cations, the views expressed by Pichon (1970) are of great interest. He pointed out that in an animal ionic concentration at a given moment is the result of two antagonistic mechanisms namely absorption of the ions from food and drink and their active excretion by the malpighian tubules. His earlier studies (Pichon 1963) showed that in insects under starvation and dehydration, the K^+ concentration increased first and then decreased. Relatively large increase in K^+ concentration followed by a slow decrease with time was attributed to lysis of K^+ rich tissue coupled with a decrease in the active excretion by the Malpighian tubules and a reduction of the blood volume in the starving insect. Since the larvae treated with insecticides, in general, showed decreased food intake and haemolymph volumes it could be that the factors enumerated by Pichon (1963, 1970), may be equally relevant for the changes observed in ionic concentration at different periods of poisoning.

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