

# EFFECT OF DICHLOROVAS ON TOTAL CARBOHYDRATE CONTENT IN HAEMOLYMPH AND FAT BODY OF THE SILK WORM, BOMBYX MORI L

## G. Md. Ameen\* and Md. Basha Mohideen

Department of Zoology, PVKK Degree & P.G.College, Anantapur – 515001 (A.P)

\*Corresponding Author

#### ABSTRACT

Quantitative levels of total Carbohydrates in haemolymph and fatbody of silkworm, Bombyx mori. (PM x NB<sub>4</sub> D<sub>2</sub>) treated with dichlorovas (Nuvon) were studied on sixth day of V Instar at different ambient temperatures. Compared to controls, the total carbohydrates content increased in haemolymph and decreased in fat body at both lethal and sub lethal doses of dichlorovas and the level of increase was found to be higher (61.19%) in haemolymph and the level of decrease was found to be higher (44.48%) in fat body at lethal intoxication.

#### INTRODUCTION

Total Carbohydrates of insects consists of Glycogen, Glucose and Trehalose. Among these Glycogen is a polysaccharide known as animal starch which is considered as the primary precursor for energy yielding metabolic processes in animal tissue (Mayes 1977). It is a readily available source of hexose units for glycolysis to yield energy (Mayes 1977). The maintenance of glycogen reserves is an essential feature of normal organismal metabolisam (Turner and Manchester 1972). Glycogen phosphorylase is the enzyme concerned with the breakdown of glycogen in hepatopancreas and muscle of crustaceans (Hohnke and Scheer 1971). Bhosale et al. (1988) reported alterations in glycogen content in fat body of silkworm, *Bombyx mori* under inseticidal stress.

Apart from the glycogen, principal carbohydrate utilized by the cell is mainly in the form of glucose (Harper et al. 1979). In general level in the animals is maintained through active absorption from the digested food stuffs, but also formed from glycogen through glycogenolysis and from amino acids and glycerol through gluconeogenesis under stress conditions (Silva et al. 1959). Chitra and Sridhara (1973) explained the role of glucose in the intermediate metabolism of silkworm.

Trehalose, the characteristic sugar of insects is maintained at steady state levels through homeostatic regulation at all stages of the life cycle of silkworm (Wyat 1967). Saito (1963) revealed that the fat body of silkworm is to be the main site for the synthesis of trehalose. The mechanism for regulation of haemolymph trehalose was demonstrated at the enzyme

level consisting of an activation by magnesium and feedback inhibition by trehalose (Murphy and Wyatt 1965).

The organo-phosphorous insecticides are highly soluble in water and are relatively less persistent in the environment than the organochlorides (Duke and Dumas 1974). However, studies of the effect of organophosphorous insecticides on silkworm are highly conspicuous by their absence. Dichlorovas is one of the most widely used organophosphorous insecticides on different plantations including mulberry.

Temperature is one of the most important environmental factors with a tremendous influence on the metabolism, activity and distribution of animals (Precht et al. 1973). Among various environmental factors which influence the commercial silkworm cocoon production, the most important is the atmospheric temperature especially in tropical countries like India. Therefore, an attempt was made in this investigation to study the effect of dichlorovas on total carbohydrate content in both haemolymph and fatbody of V instar silkworm at different ambient temperatures like optimum (24°C), Lower (20°C) and higher (28°C).

### MATERIALS AND METHODS

The larvae of the cross breed silkworm  $PM \times NB_4 D_2$  were used as test insects in the present investigation were obtained from the Government Grainage, Anantapur. Two large groups (200 larvae) were maintained for control and treatment separately. Commercial grade dichlorovas (Nuvon, Ciba-Geigy) was diluted in acetone as there was no significant effect on silkworm larvae when treated with acetone.

Therefore, acetone is least toxic over the other solvents as also suggested by Burchfield et al. (1952). 1.3 ml of (76%) dichlorovas was dissolved in 2 ml of acetone and was made upto 1000 ml with distilled water to prepare the stock solution of 1000 ppm. A batch of 50 larvae was taken and 0.5 ml of 10 different concentrations of dichlorovas ranging 0.1 to 0.55 ppm topically applied by micro pippette and mortality rate was observed. LD<sub>50</sub> values were determined according to Finney (1971). One fifth of the LD<sub>50</sub> values were taken as sub - lethal doses for further studies.

The V instar larvae of the cross breed silkworm were kept in incubator to maintain the different ambient temperatures like optimum (24°C), Lower (20°C) and higher (28°C). Haemolymph and Fatbody were collected separately from the larvae of the Silkworm. The quantity of glycogen in haemolymph and fatbody of untreated and treated larvae was estimated on the sixth day as per method described by Caroll et al. (1956). The statistical analysis of the data was carried out as per the methods suggested by Fischer and Yates (1963).

#### RESULTS AND DISCUSSION

The LD<sub>50</sub> values at higher, optimum and lower temperatures for V instar were found to be 0.25, 0.29, and 0.43 µg/mg body weight respectively. This indicates that dichlorovas was found to be more toxic to silkworm larvae at higher temperature. The data on the total carbohydrates content in haemolymph (mg/100 ml) and the fat body (mg/g net weight) of the silkworm are presented in Table 1. Compared to controls, the total carbohydrates content increased in haemolymph and decreased in fat body at both lethal and sub - lethal doses of dichlorovas and level of increase was found to be higher at lethal intoxication in haemolymph and the level of decrease was found to be higher at lethal intoxication in fat body. Further, maximum increase of total carbohydrates in haemolymph is 61.19 % at lethal dose & 22.83 % at sub - lethal dose at higher temperatures. In the same way the maximum decrease of total carbohydrates in fat body is 44.48 % at lethal dose & 23.72 % at sub – lethal dose at higher temperature.

The Carbohydrate metabolism gained importance in the Physiology of an animal, because of readily available energy reserves. Carbohydrates are most rapidly oxidized. The principal carbohydrate utilized by the cell is mainly in the form of glucose (Harper et al. 1979). As the glucose is energy vielding precursor, its role in compensatory mechanism of silkworms can be expected during insecticidal stress. In the present study, total carbohydrate content is de-creased in the fat body, and increased in the haemolymph in V instar silkworms when exposed to the lethal and sub lethal doses of dichlorovas. This is due to the breakdown of glycogen by glycogenolysis in the fatbody and the synthesis of glucose from amino acids and glycerol by gluconeogenesis as suggested by Silva et al. (1959) in American cockroach, Periplanata americana. Transport of sugars by the fat body of the silkworm, Bombyx mori was explained by Chitra and Sndhara (1973). Thus, the glucose which is formed in fatbody by glycogenolysis might have transported into the

Table 1. Effect of Dichlorovas on the Total Carbohydrate content in haemolymph (mg/100ml) and fat body (mg/g tissue) of V instar of Silkworm, Bombyx mori

Dose	Low Temperature (20°C)		Optimum Temperature (24°C)		High Temperature (28°C)	
	Haemolymph	Fat body	Hæmolymph	Fat body	Haemolymph	Fat body
Control	$\begin{array}{c} 227.81 \\ \pm 18.26 \end{array}$	$17.56 \pm 1.62$	$241.91 \pm 22.60$	$20.24 \pm 2.34$	$\begin{array}{c} 255.35 \\ \pm 24.76 \end{array}$	14.83 ± 1.66
Lethal	344.25 ± 32.82 P<0.001 (51.11?)	11.43 ± 1.23 P<0.001 (34.88?)	374.39 ± 31.21 P<0.001 (54.76?)	12.78 ± 1.42 P<0.01 (36.85?)	411.60 ± 40.26 P<0.001 (61.19?)	8.23 ± 0.98 P<0.01 (44.48?)
Sub - lethal	260.27 ± 23.56 P<0.05 (14.24?)	14.15 ± 1.46 P<0.01 (19.39?)	281.42 ± 25.42 P<0.05 (16.33?)	15.14 ± 1.88 P<0.01 (25.19?)	313.66 ± 28.04 P<0.01 (22.83?)	$ \begin{array}{c} 11.31 \\ \pm 1.06 \\ P < 0.01 \\ (23.72?) \end{array} $

Each value is a mean of six estimations; ± Standard Deviation; P: Level of Significance (<0.05)

Data in parenthesis indicate percentage INCREASE / DECREASE relative to controls

haemolymph. This may be one of the reasons for the increase of total carbohydrate content in haemolymph. The increase of total carbohydrate content in haemolymph may be to meet the energy demands under dichlorovas stress.

Besides insecticidal stress, environmental temperature exerts a profound in-fluence on the metabolism. With the rise in ambient temperature, the metabolic activity of the silkworm is accelerated while it is slackened when the temperature goes down (Ullal and Narasimhanna 1987). In the present investigation, it is observed that the total carbohydrate content is increased with both the lethal and sublethal doses of dichlorovas at higher temperature of 28°Cwhen compared to the lower temperature of 20°C.

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