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EFFECT OF DICHLOROVAS ON TREHALOSE CONTENT IN HAEMOLYMPH AND FAT BODY OF THE SILKWORM, *BOMBYX MORI* L

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ABSTRACT

Trehalose levels in haemolymph and fat body of V Instar larvae of silkworm, *Bombyx mori* (PM x NB_4 D_2) were studied on sixth day of treatment with dichlorovas (Nuvon) at different ambient temperatures. Compared to controls, trehalose content increased in haemolymph but it decreased in fat body at both lethal and sub lethal doses of dichlorovas. The level of increase (61.19 %) in haemolymph and reduction (44.48 %) in fat body was found to be higher at lethal intoxication.

INTRODUCTION

Carbohydrates in insects mainly include glycogen, glucose and trehalose. Among these, trehalose is the characteristic sugar of insects and is maintained at steady state levels through homeostatic regulation at all stages of the life cycle of silkworm (Wyatt 1967). Saito (1963) reported that the fat body of silkworm is the main site for the synthesis of trehalose. The mechanism for regulation of haemolymph trehalose was demonstrated at the enzyme level consisting of activation by magnesium and feedback inhibition by trehalose (Murphy and Wyatt 1965).

Apart from the trehalose , 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 insecticidal stress.

The principal carbohydrate utilized by the cell is mainly in the form of glucose (Harper et al. 1979). Its 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.

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 3 different ambient temperatures viz. optimum (24°C), low (20°C) and high (28°C).

MATERIALS AND METHODS

The larvae of the cross breed silkworm PM x 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_{50} values were determined according to Finney (1971). One fifth of the LD_{50} values were taken as sub - lethal doses for further studies.

The V instar larvae were kept in incubators maintained at different temperatures like optimum (24°C), Lower (20°C) and higher (28°C). Their haemolymph and fat body were collected separately. The quantity of total carbohydrates, glycogen and glucose in haemolymph and fat body of untreated and treated larvae was estimated on the sixth day as per method described by Carol et al. (1956). Trehalose content was determined by substraction of glucose plus glycogen values from the total carbohydrates as per the method of Schmidt and Platzer (1980) followed by Venkata Rami Reddy et al. (1992). The statistical analysis (Standard Deviation & Level of Significance) of the data was carried out as per the methods suggested by Fischer and Yates (1963).

RESULTS AND DISCUSSION

LD_{50} 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 is more toxic to silkworm larvae at higher temperature. The data of total carbohydrate content of haemolymph (mg/100 ml) and fat body (mg/ g net weight) of the silkworm are presented in Table 1, of glycogen content in Table 2, of glucose content in Table 3 and of trehalose content in Table 4.

Compared with controls, trehalose content increased in haemolymph at both lethal and sub lethal doses of dichlorovas whereas it decreased in the fat body and alterations in content was found to be higher at lethal intoxication (Table 4). Further, maximum increase in trehalose content of haemolymph at lethal and sub-lethal doses was 67.58% and 23.69% respectively at higher temperature. Similarly maximum reduction of trehalose content in the fat body at lethal dose and sub – lethal doses were 58.18 % and 40.63 % respectively.

Carbohydrate metabolism gained importance in the physiology of an animal because of readily available energy reserves. The principal carbohydrate utilized by the cell is mainly in the form of glucose (Harper et al. 1979). As the glucose is energy yielding precursor, its role in compensatory mechanism of silkworms can be expected during insecticidal stress. In the present study, trehalose content de-creasing in the fat body however, 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 fat body as suggested by

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	Haemolymph	Fat body	Haemolymph	Fat body
Control	227.81 ± 18.26	17.56 ± 1.62	241.91 ± 22.60	20.24 ± 2.34	255.35 ± 24.76	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↑)	11.31 ± 1.06 P<0.01 (23.72↓)

Each value is a mean of six replicates; ± Standard Deviation; P: Level of Significance (<0.05)
Data in parenthesis indicate percentage increase / decrease relative to controls

Table 2. Effect of dichlorovas on the glycogen 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	Haemolymph	Fat body	Haemolymph	Fat body
Control	13.82± 1.28	10.26± 1.82	14.18± 2.08	11.69± 1.98	10.16± 1.98	9.06± 1.72
Lethal	4.88± 0.57	6.99 ± 1.02	5.40 ± 1.42	7.04 ± 0.86	3.60± 0.64	5.80± 0.75
	P<0.001	P<0.01	P<0.001	P<0.001	P<0.001	P<0.01
	(64.68%↓)	(31.87%↓)	(61.91%↓)	(39.77 %↓)	(64.56% ↓)	(35.98%↓)
	10.26 ± 0.86	7.56± 1.68	11.03± 1.85	8.40± 1.78	7.46± 1.24	6.28± 1.62
Sub-lethal	P<0.01	P0.05	P<0.05	P<0.05	PO.05	P<0.05
	(25.75%↓)	(26.31%↓)	(22.24%↓)	(28.14%↓)	(26.57%↓)	(30.68%↓)

Each value is a mean of six estimations; ± Standard Deviation; P : Level of Significance
Data in parenthesis indicate percentage increase / decrease relative to controls

Table 3. Effect of Dichlorovas on the glucose content in haemolymph (mg/100ml) and fat body (mg/g tissue) V Instar of Silkworm, *Bombyx mori*

Dose	Low Temperature (20°C)		Optimum Temperature (24°C)		High Temperature (28°C)	
	Haemolymph	Fat body	Haemolymph	Fat body	Haemolymph	Fat body
Control	31.99±3.082	0.7640±0.062	32.57 ± 4.026	0.8175 ±0.092	34.67±4.621	0.6980±0.0564
	40.69±3.990	0.4559±0.038	47.34±5.856	0.5110±0.042	55.20±6.026	0.3155±0.042
Lethal	P<0.01	P<0.01	P<0.001	P<0.001	P< 0.001	P<0.001
	(27.19% ↑)	(40.44% ↓)	(45.34% ↑)	(37.45% ↓)	(59.21% ↑)	(54.87% ↓)
	36.80±3.680	0.5775±0.046	39.74±4.830	0.6945±0.071	44.80±5.164	0.4860±0.046
Sub lethal	P < 0.05	P< 0.001	P<0.05	P<0.001	P<0.01	P<0.001
	(13.07% ↑)	(24.47% ↓)	(22.01% ↑)	(15.05% ↓)	(29.21% ↑)	(30.37% ↓)

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

Table 4. Effect of Dichlorovas on the trehalose content in haemolymph (mg/100ml) and fat body (mg/g tissue) V Instar of Silkworm, *Bombyx mori*

Dose	Low Temperature (20°C)		Optimum Temperature (24°C)		High Temperature (28°C)	
	Haemolymph	Fat body	Haemolymph	Fat body	Haemolymph	Fat body
Control	182.0±16.20	6.54± 0.72	195.16± 15.2	7.73±0.42	210.52±22.5	5.07±0.56
	298.68±26.52	3.99±0.46	321.64±29.42	4.23±0.32	352.80±38.26	2.12±0.19
Lethal	P<0.001	P<0.001	P<0.001	P < 0.001	P<0.001	P<0.001
	(64.10% ↑)	(38.90 % ↓)	(64.80% ↑)	(45.27 % ↓)	(67.58% ↑)	(58.18% ↓)
	218.41±19.8	4.98±0.58	221.65±20.86	6.96±0.51	260.40 ±29.32	3.01±0.28
Sub-lethal	P< 0.01	P < 0.01	P < 0.05	P < 0.05	P < 0.05	P < 0.001
	(20.00% ↑)	(23.85 % ↓)	(13.57% ↑)	(9.96 % ↓)	(23.69% ↑)	(40.63% ↓)

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

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 Sridhara (1973). Thus, the glucose which is formed in fat body by glycogenolysis might have transformed to trehalose and transported into the haemolymph. This may be one of the reasons for the increase of trehalose content in haemolymph. The increase of trehalose content in haemolymph may be to meet the energy demand under dichlorovas stress.

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

REFERENCES

Bhosale, S.H., V.B. Yadwad and V.L. Kallapur. 1988. Residual toxic effect of Ekalux EC-25 on the biochemical constituents of the fat body of 5th instar *Bombyx mori.* Indian Journal of Sericulture 27 (2):73-77.

Burchfield, H.P., N.D. Hilchey and E.E. Storrb. 1952. An objective method for insecticide bioassay based on photo-migration of mosquito larvae. Contribution Boyce Thomson Institute 17:60.

Caroll, N.V., R.W. Longley and J.H. Roe. 1956. Glycogen determination in liver and muscle by use of anthrone reagent. Journal of Biological Chemistry *220:* 583-593.

Chitra, C. and S. Sridhara.1973. Transport of sugars by the fat body of the silkworm, *Bombyx mori.* Journal of Insect Physiology19:2053-2061.

Duke, T.W. and P. Dumas. 1974. Implications of Pesticide Residues in the Costal Environment. Pollution and Physiological of Marine Organism. W.B. Vernberg, Academic Press, New York.

Finney, D.J. 1971. Probit Analysis. 3rd edition, London and New York, Cambridge University Press. pp. 333

Fischer, S.R.A and F. Yates. 1963. Statistical Tables for Biological, Agricultural and Medical Research. 6th edition, Oliver and Boyd, London, 39, P-98

Harper, H.A. ,V.M. Rodwell and P.A. Mayer. 1979. In: Review of Physiological Chemistry. 17th edition, Longe Medical Publications, Maruzer Company Limited, California.

Hohnke, L. and B.T. Scheer. 1971. Carbohydrate metabolism in Crustaceans. M. Florikin and B.T. Scheer (Eds.), Chemical Zoology .vol. V. Academic Press, New York, pp. 147-164.

Mayes, P.A. 1977. In: Review of Physiological Chemistry. 16th edition, Harper, H.H., Rodwell, V.W., and Myres (Eds.), 4p, Longe Medical Publications, California

Murphy, T.A and G.R. Wyatt. 1965. The enzymes of glycogen and trehalose synthesis in silkworm fat body. Journal of Biological Chemistry 240: 1500 – 1508.

Precht Christopherson, J., H. Hensal and W. Lancher (Eds.). 1973. Temperature and Life. Heidelberg, Springer Verleg.

Saito, S. 1963. Trehalose in the body fluid of the silkworm, *Bombyx mori* (L). Journal of Insect Physiology 9:506 - 519.

Schmidt. S .P. and E.G Platzer. 1980. Changes in body tissue and haemolymph composition of *Culex pipiens* in response to infection *Romanomermis culicivorax*. Journal of Invertebrate Pathology 36: 240 – 254.

Silva, G.M., W.P. Doyle and C.H. Wang. 1959. Glucose catabolism in the DOT treated American Cockroach, *Peiplaneta americana* L. *Arquivos protugueses.* Bioguimica 3 : 298 - 305.

Turner, L.V. and K.L. Manchester. 1972. Effects of denervation on the glycogen content and on the activities of enzymes of glucose and glycogen metabolism in rat diaphragm muscle. Biochemical Journal. 789-801.

Ullal.S.R. and M.N. Narasimhanna. 1987. Environmental Conditions for rearing . In: Hand Book of Practical Sericulture, 3 rd edition, Central Silk Board, Bangalore. pp.95.

Venkata Rami Reddy, K.V., K.V. Benchamin and O. K. Ramadevi. 1992. Metabolic profiles of the haemolymph and fat body of the silkworm, *Bombyx mori* in response to parasitization by the Uzi fly, *Exorista sorbillans* (Diptera : Tachinidae) during the final instar. Sericologia 32 (2): 1-7.

Wyatt, G.R. 1967. The biochemistry of sugars and polysaccharides in insects. Advance in Insect Physiology 4:287 – 360.