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Influence of the Mineral Potassium Permanganate on the Biochemical Constituents in the Fat Body and Haemolymph of the Silkworm, *Bombyx mori* L.

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Oral supplementation with potassium permanganate (30, 50 and 100 µg) to fifth instar larvae of the $CSR_2 \times CSR_4$ race of the silkworm, *B. mori* resulted in a significant increase in the glycogen content of the fat body and haemolymph trehalose. The protein content of the fat body is also significantly increased in all the potassium permanganate treated groups where as that of the haemolymph is significantly increased only in the 30 µg fed group. The total lipids content of the fat body increased significantly in all the potassium permanganate treated groups. This indicates that the potassium permanganate may stimulate metabolic activity, there by influencing the biochemical contents in the fat body and haemolymph of the silkworm, *B. mori*.

Key words: Potassium permanganate, Fat body, Haemolymph, Glycogen, Trehalose, Protein, *Bombyx mori*

Introduction

The silkworm, *Bombyx mori*, is a monophagous and holometabolous insect and its growth, development and metabolism mainly dependent on its nutritional requirements and environmental conditions. The silkworm larva accumulates large quantity of fuel reserves in various tissues, and it is endowed with a unique biochemical adaptation to conserve nutritional resources available during the active larval stage. The carbohydrates and proteins are very essential for pupal and adult development and are obtained from the fat body and haemolymph stored during the last larval instar. Carbohydrates are stored in the fat body as glycogen, which is converted into simple sugar, trehalose in the fat body before it is released into the haemolymph for its utilization. Thakare *et al.* (1980) have reported that trehalose is a major haemolymph sugar in the last nymphal instar of the dragonfly, *Orthetram chrysis*, and it is also reported that trehalose in many insects is maintained at a steady state through homeostatic regulation in all the stages of life-cycle (Wyatt, 1967) and the haemolymph serves as a storage tissue (Jungreis, 1980; Mullins, 1985).

Metals and their salts cations play a key role by acting as catalyst on structural components of large molecules with a specific function which are indispensable for life. It has been reported that magnesium is essential for complete activity of trehalose synthase (Murphy and Wyatt, 1965). Dasmahapatra *et al.* (1989) have reported that supplementation with cobalt, iodide, potassium, calcium chloride and potassium nitrate increases the protein, RNA and DNA content of the silkgland in the nistari race of *B. mori*. Therefore, it is evident that oral supplementation with metals and salts influences the economic parameters and biochemical constituents of the silkworm, *B. mori*. Hence, the present investigation was undertaken to find out the effect of potassium permanganate on the fat body glycogen, protein, and total lipids and haemolymph trehalose and protein of the silkworm, *B. mori*.

Materials and Methods

The eggs of the silkworm, *B. mori* were obtained from the Rayapur, Dharwad, Karnataka and reared in the laboratory by the improved method of rearing technique (Krishnaswami, 1978). The fifth instar larvae were divided into five experimental groups including control groups. Each

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group consisting of five replications of 20 silkworms each. The potassium permanganate was procured from Qualigens Fine Chemicals Ltd. Mumbai, India and was dissolved completely in lukewarm distilled water. The potassium permanganate was diluted to form 30, 50 and 100 µg/ml dilutions. The fresh mulberry leaves were soaked in these concentrations for 15 min and the leaves were dried and fed to the silkworm *ad libitum* at fifth stadium larvae. Amongst the four feeding per day, a feeding of treated leaves was followed by three feeding of untreated leaves. The carrier controls were fed with normal leaves. The treated, carrier control and normal control larvae were utilized for the estimation of glycogen, protein, total lipids from the fat body and protein and trehalose from the haemolymph. The silkworm larvae were dissected in *Bombyx saline* at pH 6.5 on the 6th day of the fifth stadium. The fat body was immediately collected and used for the glycogen (Sciefter *et al.*, 1950), protein (Lowry *et al.*, 1951), total lipids (Folch *et al.*, 1957) estimations. The haemolymph was collected by amputating one of the thorasic legs in a prechilled certrifuge tube and was used for the estimation of trehalose (Roe, 1955) and protein (Lowry *et al.*, 1951). Anthrone positive carbohydrate in the haemolymph is considered as trehalose.

Tissue preparation

The silkworms *B. mori* were dissected in *Bombyx saline* at pH 6.5 on 6th day of 5th instar. The fat body was immediately collected and used for the glycogen and protein estimation. The haemolymph was collected by amputating one of the larval thorasic legs in prechilled centrifuge tube. The haemolymph collected from 2 – 3 silkworms was used almost immediately for trehalose and protein estimation.

Glycogen estimation

Anthrone method of Sciefter *et al.* (1950) was used to determine the fat body glycogen. A known quantity of fat body was homogenized with 2 ml of 20% potassium hydroxide. The glycogen was precipitated by adding equal volume of 80% ethanol and the mixture was kept overnight at room temperature for digestion. It was then centrifuged at 3000 rpm for 15 min and the supernatant was discarded. The residue was dissolved in a known volume of distilled water. Glycogen content was estimated with a known aliquots in triplicate by the anthrone method. Glucose-D was used as the reference standard and the intensity of the colour was read on the UV spectrophotometer at 620 nm.

Trehalose estimation

The estimation of haemolymph trehalose was carried out according to the method of Roe (1955). Known quantity of haemolymph was collected in each test tube, and added 0.5 ml of 2% of sodium hydroxide to each test tube. After shaking, the tubes were kept in boiling water for 10 min and then the tubes were cooled in an ice box. Then 5 ml of anthrone reagent (0.05% anthrone in 70% sulphuric acid) was added to the tubes, and they were again kept in boiling water for 15 min for the development of colour. Then the tubes were cooled to room temperature. Then the colour intensity was read on the UV spectrophotometer at 620 nm. For the reference standard the trehalose (Sigma, USA) was used. Anthrone positive carbohydrate in the haemolymph is considered as trehalose.

Protein estimation

The method of Lowry *et al.* (1951) was used for the estimation of total protein in the fatbody and haemolymph. The tissue protein was precipitated by the addition of 1 ml of 30% trichloroacetic acid (TCA) solution followed by centrifugation at 3000 rpm for 30 min. It was repeated twice, then the precipitate was dissolved in 1 ml of 0.1 N sodium hydroxide. A known aliquot of this solution was then mixed with 5 ml of alkaline copper reagent (20% sodium carbonate prepared in 0.1 N sodium hydroxide containing sodium potassium tartarate and 1% copper sulphate). After 10 min 0.5 ml of Folin Ciocalteus reagent was added to the tubes and the tubes were shaken thoroughly. Then the tubes were kept for 20 min for colour development. The readings were taken on the UV spectrophotometer at 650 nm.

The total haemolymph protein estimation was also carried out. A known quantity of haemolymph was diluted with 0.5 ml of distilled water. A known aliquot of this solution was added with 5 ml of alkaline copper reagent. After 10 min 0.5 ml of Folin Ciocalteus reagent was added and were mixed thoroughly and kept for 20 min until the colour develops. The readings were taken on the UV spectrophotometer at 650 nm. For the reference standard Bovine Serum Albumen (BSA) (Fatty acid free) was used.

Extraction and estimation of lipids

The method of Folch *et al.* (1957) was used for the lipid estimation, using chloroform : methanol mixture (2 : 1 V/V). First, all the fat body was homogenized with appropriate volume of chloroform : methanol mixture (1 : 10). Then homogenate was quantitatively transferred to a 50 ml separating funnel and then added similar volume of chloroform. The two solvents were partitioned by the addition of 0.2 volume of water. After the funnel was shaken, the mixture was allowed to stand overnight. The lower chloroform layer containing lipids was drawn off.

The lipids sample was kept in a vacuum desiccator until constant weight was obtained.

The experiments were designed by the complete randomized block design (CRBD) method and the data colleted were subjected to the statistical analysis of variance (ANOVA) test to determine the significant difference between the treatment and control groups (Raghava Rao, 1983).

Results and Discussion

The requirement of minerals in various insects has been investigated, but the information regarding the indispensability of metal ions for proper nutrition or growth promotion of the insects are not adequately established. Metals are essential for the activity of several enzymes. The metals can directly bind to proteins or may attach through an organic ligand such as haem. It has been reported that mineral salts are essential for growth and develoment of the silkworm, *B. mori* (Dasmahapatra *et al.*, 1989; Nirwani and Kaliwal, 1996; Hugar *et al.*, 1998).

The results of the oral supplementation of potassium permanganate on biochemical constituents in the fat body and haemolymph of the silkworm, *B. mori* are presented in Table 1.

Effect of potassium permanganate on the fat body glycogen and haemolymph trehalose

The oral supplementation of each of the three concentrations of potassium permanganate significantly increased the fat body glycogen in all the treated groups. Similar increase in the fat body glycogen has been reported after supplementing the feed with potassium sulphate (Nirwani and Kaliwal, 1996) and zinc chloride in the bivoltine silkworm, *B. mori* (Hugar *et al.*, 1998). Accumulation of glycogen during the feeding period in *P. ricini* was shown to be due to the increased amylase activity and glycogenesis (Pant and Morris, 1969). The increase in the amylase activity of the midgut and the increased production of carbohydrates has been reported after supplementing the feed with mineral samples in the beetle, *Leptinotarsa decemlineata* (Izhevskiy, 1976). In the present study the increased fat body glycogen after supplementing the feed with potassium permanganate may possible be due to the stimulatory effect on the amylase activity of the midgut and glycogenesis resulting in an increased production of carbohydrates as suggested by Pant and Morris (1969), and Izhevskiy (1976).

The results of the present study indicate that haemolymph trehalose was significantly increased after supplementing the feed with lower concentration of potassium permanganate to the silkworm. It has been stated that the absolute amount of trehalose present in the fat body is directly related to the glycogen content of the tissue and trehalose production in insect fat body is influenced by a number of endogenous organic and inorganic factors and also has been reported that calcium ions enhance the production of trehalose by the fat body of the insect *Periplaneta americana* (Downer, 1979). It has also been stated that magnesium is essential for complete activation of trehalose synthesis (Murphy and Wyatt, 1965). The level of total sugar is changed by hydrolytic enzymes in the gut and haemolymph and is due to various inter-

Table 1. Effect of potassium permanganate on the biochemical constituents of the silkworm, *B. mori*

Treatment	Dose µg/ml	Fat body glycogen (µg/ml)	Haemolymph trehalose (µg/ml)	Fat body protein (µg/ml)	Haemolymph protein (µg/ml)	Fat body total lipids (µg/ml)
Potassium permanganate	30	2.833*(154)	720.37*(201)	16.499*(159)	8277.5*(265)	350*(104)
Potassium permanganate	50	3.333*(181)	456.75(127)	20.999*(175)	3757.5*(120)	380*(102)
Potassium permanganate	100	4.333*(236)	456.75(127)	24.999*(190)	2235.0*(171)	399.5*(108)
Carrier control	Distilled water	1.833(100)	357.00(100)	27.666(100)	3120.0(100)	370(100)
Normal control	-	1.166(63)	448.37*(153)	33.166(119)	4095.0(131)	340*(91)
		(S)	(S)	(S)	(S)	(S)
S.Em ±		0.887	71.996	3.001	2470.340	0.934
C. D. at 5%		1.811	156.952	6.112	5039.510	1.905

* - Significant increase/decrease at 5%.
S.Em ± - Standard error mean.
C. D. - Critical difference.
S - Significant.
Percentage increase/decrease over that of the carrier controls in parenthesis.

mediary metabolic pathways in phosphorylation according to Ito and Tanaka (1959). Therefore, in the present study the results suggest that the supplementation with potassium permanganate may have a role similar to that of calcium (Downer, 1979) and magnesium (Murphy and Wyatt, 1965) in activating the trehalose synthase activity of the fat body resulting in the increased production and release of trehalose into the haemolymph by the fat body. It has been stated that the fat body in insects is the main site for protein synthesis as well as the intermediating metabolism of amino acids (Wigglesworth, 1977). There are few reports on the effect of mineral salts on the protein content of the fat body in the silkworm, *B. mori*. Feeding of mulberry leaves supplemented with potassium iodide or cobalt chloride or calcium chloride increased silkgland protein in nistari race of *B. mori* (Dasmahapatra *et al.*, 1989). It has been reported that the feeding of potassium sulphate decreases the fat body protein and haemolymph protein of the silkworm, *B. mori* (Nirwani and Kaliwal, 1996) but zinc chloride significantly decreased the fat body protein and significantly increased the haemolymph protein (Hugar *et al.*, 1998).

The fat body protein and haemolymph protein in 30, 50 and 100 µg potassium permanganate fed groups are significantly increased. The increased protein content of the fat body and haemolymph might possibly be due to the stimulatory effect of the mineral salt at the given concentrations on the synthetic activity of the fat body and the increased haemolymph protein content might be due to the release of excess of protein by the fat body into the haemolymph and at the same time the weight of the silkgland is also significantly increased which also coincides with the subsequent increase in the cocoon weight and shell weight. The significant increase in the silkgland weight may possible suggest that the silkgland has not sequestered the protein from the haemolymph and fat bodies in excess of its requirement, hence, the accumulation of protein in the fat body and haemolymph. However, the protein content from the fat body and haemolymph might have been utilized for the sythesis of silk, which is evidenced by a significant increase in the cocoon weight, shell weight, filament length, filament weight and denier in all the fed groups (Goudar and Kaliwal, 2000).

Effect of potassium permanganate on total lipids content of the fat body

Lipids are important constituents of cuticle and help in acylation of glucose-6-phosphate during chitin synthesis (Wyatt, 1967). The lipid in the fat body is an energy reserves which can be mobilized rapidly during starvation, oogenesis, embryogenesis and moulting and is used to sustain continuous muscular activity (Gilbert and Chino, 1974). There are no reports on the effect of mineral salts on the fat body total lipids in the silkworm, *B. mori* except that feeding mulberry leaves supplemented with nickel chloride in the bivoltine silkworm, *B. mori* (Saha and Khan, 1995). The results of the present study indicate that the oral supplementation of each of the three concentrations of potassium permanganate significantly increased the total lipids of the fat body. From the present study it is inferred that potassium permanganate has stimulatory effect on the fat body synthetic activity. The significant increase in the total lipids may possibly suggest that the ovariole has not sequestered the lipids from the fat body.

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