Int. J. Indust. Entomol. Vol. 3, No. 1, 2001, pp. 7~11

International Journal of *Industrial Entomology*

Effect of 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Naphthoxyacetic Acid (NOA) on the Biochemical Changes in the Fat Body and Haemolymph of the Silkworm, *Bombix mori* L.

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(Received 2 May 2001; Accepted 25 June 2001)

The effect of topical application with 200, 400 and 600 μg/ml 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Naphthoxyacetic Acid (NOA) to the fifth stadium larvae of the silkworm, *B. mori* on fat body glycogen, protein, total lipids, phospholipids, neutral lipids and haemolymph trehalose and protein has been studied. The fat body glycogen significantly increased in all the groups treated with 2,4-D whereas there is no significantly change in fat body glycogen in all the groups treated with NOA. However, the haemolymph trehalose was significantly increased in all 2,4-D and NOA treated groups except in the groups treated with 400 and 600 μg/ml 2,4-D where the increase was not significant. The fat body protein did not show any significant change in all groups treated with 2,4-D and NOA except in the groups treated with 200 μg/ml 2,4-D where the fat body protein was significantly increased. The total lipids, phospholipids and neutral lipids of the fat body increased significantly in all the groups treated with 2,4-D and NOA when compared with those of the corresponding parameters of the carrier control.

Key words : 2,4-Dichlorophenoxyacetic Acid (2,4-D), Naphthoxyacetic Acid (NOA), Silkworm, Protein, Fat body, Haemolymph, Glycogen, Trehalose

Introduction

The silkworm is entirely dependent on mulberry leaves as a food source and protein content of the leaves plays an important role in the silk production. There is an evidence, that the plant growth regulators may act through their effects on the insect neuroendocrine system or perhaps directly on insect cells (Osborne *et al.*, 1968). It has been reported that the plant growth regulators mimics the moulting hormone ecdysone and restricts the insect growth of *Drosophila hydei* (Alonso, 1971) Since, ABA, GA_3 and JH III are biochemically similar terpenoid compounds derived from mevalonate. De Man *et al.* (1981) have suggested that dietary plant growth regulators may regulate insect growth and reproduction directly by altering the rate of DNA synthesis and/or the rate of synthesis of the insect moulting hormone. There are number of reports on the supplementation of various plant growth regulators that affect the physiological processes, growth and development in different insects (Edwards, 1966; Osborne *et al.*, 1968; Guerra, 1970; Backer and Raussaux, 1980; Neumann, 1982; Chrominiske *et al.*, 1982; Bur, 1985).

It has been reported that the dietary supplementation with paraaminobenzoic acid (PABA) affects protein profile in the haemolymph and silkgland (Pramodkumari, 1990). Rup *et al.* (1997) have reported that quantitative changes in the protein, lipid and carbohydrate contents under the influence of GA_3 in *Zaprionus paravittigel.* Hugar (1997) have reported that the topical application with BAP and IAA increases the fat body glycogen and protein and haemolymph protein whereas the haemolymph trehalose decreases in the silkworm, *B. mori.*

It has been reported that supplementation with various plant growth regulators affect the physiological processes, growth and development in different insects (Backer and Roussaux, 1980; Edwards, 1966; Osborne *et al.*, 1968; Guerra, 1970; Neumann, 1982; Chrominiske *et al.*, 1982; Bur, 1985) and economic parameters of the silkworm *B. mori* (Krishnaswami *et al.*, 1978; Kamada and Ito, 1984; Magadum and Hooli 1991a, b). Pramodkumari (1990) has

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reported that dietary supplementation with paraaminobenzoic acid (PABA) affects protein profile in the haemolymph and silkgland. However, the reports on the effect of plant growth regulators viz., 2,4-dichlorophenoxyacetic acid (2,4-D) and naphthoxyacetic acid (NOA) on biochemical parameters of the silkworm, *B. mori* are lacking. In the present study, therefore, the effect of topical application with 2,4-D and NOA in different concentrations on fat body glycogen, haemolymph trehalose fat body and haemolymph protein, fat body total lipids, phospholipids and neutral lipids and were studied.

Materials and Methods

The disease free layings (DFLS) of the silkworm, *B. mori* were obtained from the grainage centre Rayapur, Dharwad, Karnataka and reared in the laboratory by the improved method of rearing technique (Krishnaswami, 1978). The fifth instar larvae were divided into eight experimental groups including control groups to treat respectively. Each group consisting of five replications of 20 worms each. The hormones, 2,4-D and NOA were procured from M/S HI media chemicals company Pvt. Ltd. India and were dissolved separately in small quantity of distilled water. The 2,4-D and NOA were diluted to form 200, 400 and 600 µg/ml solutions by adding distilled water. The topical application was made on dorsal side of the larvae. Each larva in its group was topically applied with one of the three concentrations of 2,4-D and NOA on alternate day in the V stadium up to the spinning stage. In each application 5 ml of solution was used to treat 100 larvae. The larvae of carrier control was topically applied with distilled water and served as carrier control for 2,4-D and NOA treated groups, while the normal controls did not receive any treatment. The treated carrier control and normal control larvae were utilized for the estimation of glycogen, protein, total lipids, phospholipids and neutral 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 (Shiefter *et al.*, 1950), protein (Lowry *et al.*, 1951), total lipids, phospholipids and neutral lipids (Folch *et al.*, 1957) estimations. The haemolymph was collected by amputing

Table 1. Effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on the biochemical constituents of the silkworm, *B. mori*

Treatment	Dose µg/ml	Fat body glycogen µg/mg	Haemolymph trehalose µg/ml	Fat body protein µg/mg	Haemolymph protein µg/ml	Fat body		
						total lipids µg/100 mg	phospholipids µg/100 mg	neutral lipids µg/100 mg
2,4-Dichlorophenoxyacetic acid	200	29.833*	720.37*	51.166*	3487.5	283.3*	116.6*	166.6*
		(596)	(201)	(162)	(116)	(111)	(112)	(111)
2,4-Dichlorophenoxyacetic acid	400	14.583*	456.75	19.999	2302.5	283.3*	116.6*	166.6*
		(291)	(127)	(63)	(76)	(111)	(112)	(111)
2,4-Dichlorophenoxyacetic acid	600	23.666*	456.75	21.833	2437.5	296.6*	120.0*	176.6*
		(473)	(127)	(69)	(81)	(117)	(116)	(117)
Carrier control	Distilled water	4.999	357.00	31.499	3000.0	253.3	103.3	150.0
		(100)	(100)	(100)	(100)	(100)	(100)	(100)
Normal control	-	4.666	548.37*	32.999	4050.0*	240.0	100.0	140.0
		(93)	(153)	(104)	(135)	(94)	(96)	(93)
		S	S	S	S	S	S	S
S.Em±		2.220	71.996	6.045	350.41	5.715	2.309	4.898
CD at 5%		4.841	156.952	13.178	763.91	13.202	5.334	11.316

* - Significant increase/decrease at 5%
** - Angular transformed values
S - Significant
S.Em± - Standard error mean
CD - Criticle difference
Percentage increase/decrease over that of the carrier control in parenthesis.

one of the thoracic legs in a prechilled centrifuse tube and was used for the estimation of trehalose (Roe, 1955) and protein (Lowry, 1951). Anthrone positive carbohydrate in the haemolymph is considered as trehalose. The experiments were designed by the complete randomised block design (CRBD) method and the data collected were subjected to the statistical analysis of variance (ANOVA) test to determine the significant difference between the treatment and control groups (Raghav Rao, 1983).

Results and Discussion

The late age larval stage is the most active feeding stage during which the larva accumulates large quantity of fuel reserves in various tissues and is endowed with unique biochemical adaptations to conserve nutritional resources available during active larval stage of the silkworm, *B. mori*. The plant growth regulators affect the economic parameters in silkworms; it is likely that they may also affect the synthesis, storage and release of those food substances in and from the fat body to the haemolymph to meet the developmental needs of the insects.

Effect of 2,4-dichlorophenoxyacetic acid (2,4-D) and naphthoxyacetic acid (NOA) on the fat body glycogen and haemolymph trehalose

The results of the present study showed that the fat body glycogen significantly increased in all the groups treated with 2,4-D (Table 1). Similar results have been obtained in the bivoltine silkworm, *B. mori* after the treatment with BAP and IAA (Hugar, 1997). The increase in the fat body glycogen might be due to the stimulatory effect of these plant growth regulators on glycogenesis even though glycogenesis was reported to increase during feeding period (Pant and Morris, 1969). However, fat body glycogen is not significantly changed in all the groups treated with NOA (Table 2).

There was a significant increase in the haemolymph trehalose in all the groups treated with 2,4-D and NOA except in 400 and 600 μg 2,4-D treated groups where the increase was not significant (Table 1 and 2). The significant increase in the haemolymph trehalose might possibly be due to the conversion of glycogen into trehalose and its subsequent release into the haemolymph by the fat body.

From the present study, it may be inferred that the

Table 2. Effect of naphthoxyacetic acid (NOA) on the biochemical constituents of the silkworm, *B. mori*

Treatment	Dose μg/ml	Fat body glycogen μg/mg	Haemolymph trehalose μg/ml	Fat body protein μg/mg	Haemolymph protein μg/ml	Fat body		
						total lipids μg/100 mg	phospholipids μg/100 mg	neutral lipids μg/100 mg
Naphthoxyacetic acid	200	11.22	378*	8.44	3190	total lipids μg/100 mg	phospholipids μg/100 mg	neutral lipids μg/100 mg
		(80)	(120)	(80)	(77)	(102)	(103)	(102)
Naphthoxyacetic acid	400	12.99	399*	8.44	4470	379.6*	170.0*	210.0*
		(92)	(126)	(80)	(108)	(105)	(106)	(105)
Naphthoxyacetic acid	600	13.88	455*	11.32	4660	420.0*	196.4*	233.1*
		(99)	(144)	(108)	(113)	(116)	(122)	(116)
Carrier control	Distilled water	13.99	315	10.44	4110	359.9	160.3	200.0
		(100)	(100)	(100)	(100)	(100)	(100)	(100)
Normal control	-	12.99	294	10.88	2100*	359.3	160.3	199.6
		(92)	(93)	(104)	(51)	(99)	(100)	(99)
		NS	S	NS	S	S	S	S
S.Em±		2.508	25.165	2.873	467.782	1.063	0.756	1.189
CD at 5%		5.795	58.133	6.637	1080.576	2.456	1.748	2.746

* - Significant increase/decrease at 5%
** - Angular transformed values
S - Significant
NS - Non significant
S.Em± - Standard error mean
CD - Criticle difference
Percentage increase/decrease over that of the carrier control in parenthesis.

increased fat body glycogen and haemolymph trehalose in all the groups treated with 2,4-D and NOA may be utilized as additional sources of fuel or energy required during the pupal and adult transformation. However, the mechanism of action of these plant growth regulators on the fat body synthetic activity and haemolymph trehalose is not known. Hence, further investigation is essential in this regard.

Effect of 2,4-dichlorophenoxyacetic acid (2,4-D) and naphthoxyacetic acid (NOA) on the fat body protein and haemolymph protein

Fat body of an insect is regarded as liver of vertebrate in carrying out intermediary metabolism as well as protein synthesis and its storage (Wigglesworth, 1977). Therefore, fat body is an important organ of the insects, which plays an important role in anabolic as well as catabolic activities of insects.

The protein content of the fat body in the groups treated with 200 μg 2,4-D was increased significantly whereas that of the haemolymph, though increase was not significant (Table 1). The unchanged haemolymph protein possibly suggests that its protein was utilized by the silk gland. The increased fat body protein may possibly suggest that they may be utilized by the silk gland after the commencement of spinning activity.

The results of the present study showed a decrease in the fat body protein in the groups treated with 200 and 400 μg and haemolymph protein in the groups treated with 200 μg NOA. However, the fat body protein increased in the groups treated with 600 μg and haemolymph protein increased in the group treated with 400 and 600 μg NOA (Table 2). The decrease in the fat body protein in the groups treated with 200 and 400 μg and haemolymph protein in the groups treated with 200 μg NOA suggests that it may be the haemolymph protein that is utilized for the growth of the silk gland at this stage.

Effect of 2,4-dichlorophenoxyacetic acid (2,4-D) and naphthoxyacetic acid (NOA) on the total lipids, phospholipids and neutral lipids

In the present study, the fat body total lipids, phospholipids and neutral lipids are increased in all the groups treated with 2,4-D and NOA (Table 1 and 2). The increased total lipids, phospholipids and neutral lipids of the fat body might possibly be due to the stimulatory effect of 2,4-D and NOA at a given concentration on the synthetic activity of the fat body.

Guerra (1970) citing the references of Harper (1963) has quoted, that the metabolic processes taking place within the living organism are merely the reflection of the chemical composition of the body. Since, the concentration of the most chemical substances in the body fluids varies within rather narrow limits, significant changes in the normal metabolism, which are detrimental to insect development and/or reproduction, could be produced by inducing an excess or a deficiency of essential metabolites. The sensitive and efficient regulation of the rates of metabolic processes controlling life is made possible by several known mechanisms. These are the nervous system, hormones, the stimulation or inhibition of enzyme activity, feed back inhibition and the induction or suppression of enzyme synthesis whether the increase/decrease in the fat body glycogen, haemolymph trehalose, fat body protein and haemolymph protein, fat body total lipids, phospholiphds and neutral lipids after treatement with all these plant growth regulators is due to their influences on nervous system or hormones or the stimulation or inhibition of enzyme activity or the induction or suppression of enzyme synthesis not known. Hence further investigation on mechanisms of plant growth regulators on the silkworm is necessary.

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