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Effect of 2,4-Dichlorophenoxyacetic Acid (2,4-D) on the Economic Parameters of the Silkworm *Bombyx mori* L.

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The effect of topical application with 200, 400 and 600 μg/ml 2, 4-dichlorophenoxyacetic acid (2, 4-D) on the fifth larval stadium of the silkworm, *B. mori*, was analyzed. Larvae treated during fifth larval stadium enhanced larval, cocoon and adult parameters. The larval period was significantly decreased with increase in silk gland weight at 400 and 600 μg/ml, male cocoon weight and shell weight in all the treated groups and filament length and weight at 200 μg/ml treated group. Length of the ovariole, eggs per ovariole and hatching percentage increased significantly in all the treated groups when compared with those of the carrier control. This suggests that the plant growth regulator 2, 4-D in addition to affecting silk production also affect reproductive performance.

Key words : 2,4-Dichlorophenoxy acetic acid, Silkworm, Economic parameters

Introduction

It has been reported that the plant growth regulators may influence appetite, nutrition and absorption of plant material in the phytophagous insects and thereby influence physiology, development and reproduction of insects (De Man *et al.,* 1981; Neumann, 1982). It has been reported that injection, dietary supplementation or topical application of plant growth regulators, gibberlic acid (GA_3) and indole-3-acetic acid (IAA) affect development, longevity, fertility, egg productivity, egg viability and emergence percentage in different species of insects (Ellis and Carlisle, 1965; Guerra, 1970; Salama and Sharaby, 1972; Carlise *et al.,* 1979; Castro and Rossetto, 1979; Neumann, 1980; Thakur and Mann, 1982; Thakur and Ashok Kumar, 1984; Bur, 1985). Recent studies have shown that the feeding/topical application of IAA, GA3 to IV and V instar resulted in a significant change in economic parameters of the silkworm, *B. mori* (kamada and Ito, 1984; Magadum and Hooli, 1989, 1991; Hugar and Kaliwal, 1997). The results indicate that plant growth regulators treatment influences favourable changes in the economic parameters of the silkworm, *B. mori.* Therefore, in the present investigation an attempt has been made to study the effect of a plant growth regulator 2,4-dichlorophenoxyacetic acid on various economic parameters of the silkworm, *B. mori.*

Materials and Methods

The disease free layings (DFLS) of multivoltine cross breed (PM × NB_{18}) silkworm were obtained from Grainage Center Rayapur, Dharwad, Karnataka and reared in the laboratory by improved methods of silkworm rearing techniques (Krishnaswami, 1978). The larvae were maintained on fresh mulberry leaves (K2). The fifth instar larvae were divided into five experimental groups including controls and every group consists of uniformly weighed larvae in five replications of 20 worms. The 2,4-dichlorophenoxyacetic acid procured from M/s. Hi-media Laboratories ptv Ltd. It was dissolved in small quantity of distilled water and diluted to form 200, 400, and 600 μg/ml by adding distilled water. Each larva was topically applied with one of the three doses of 2,4-dichlorophenoxyacetic acid at alternate day. In each application 5 ml of solutions was used to treat 100 larvae. The larval, cocoon and adult parameters were recorded separately. The larval and silk gland weights were recorded before commencement of spinning. The larval duration was recorded from the day of hatching till the completion of

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spinning. The cocoon parameters such as female and male cocoon weight and their shell weights were recorded on the 5th day after the completion of the spinning activity. The filament length was recorded with epprovette by reeling a single cocoon. The reeled silk was dried in hot air oven and weight was taken in the electrical balance. The cocoon shell ratio and denier of the filament was calculated. The fecundity was recorded in the adult after mating. The cocooning, moth emergence and hatching were also calculated by the formulas shown in the tables. Each mean value, a record of 10 worms is shown in Table 1, 2 and 3.

The data collected were subjected to analysis of variance tests to find out the significance between the treated and control group (Raghawa Rao, 1983). The percent emergence and hatching percentage were transformed to sine angular values for statistical analysis. The percent index was calculated for each parameter of the experimental groups over those of the corresponding parameters of the carrier control.

Results and Discussion

The effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on larval, cocoon and adult parameters are summarized in Table 1,2 and 3.

Larval weight

The results of the present study have shown that the larval weight was increased in all the groups treated with 2,4-D but the increase is not significant when compared with the corresponding parameters of the carrier controls (Table 1). The results obtained in the present study are in agreement with those reported for the Japanese and Indian multivoltine race of the silkworm, *B. mori* after the treatment with IAA and GA_3 (Kamada and Ito, 1984; Magadum and Hooli, 1989, 1991) and IAA in bivoltine silkworm, *B. mori* (Hugar and Kaliwal, 1997).

The increase in the larval weight obtained in the present study might possibly be due to the effect of these plant growth regulators on appetite, absorption of plant material

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

Treatment	Dose µg/ml	Larval weight (g)	Silk gland weight (g)	Larval duration (h)	Cocooning percentage (%)
2,4-dichlorophenoxyacetic acid	200	2.764	0.684	623.8*	98.2 82.29**
		(104)	(102)	(93)	(99)
2,4-dichlorophenoxyacetic acid	400	2.780	0.732*	635.6*	98.2 82.29**
		(105)	(109)	(95)	(99)
2,4-dichlorophenoxyacetic acid	600	2.678	0.754*	648.0*	98.6 83.20**
		(101)	(112)	(97)	(100)
Carrier control	Distilled water	2.642	0.668	668.0	98.4 82.73**
		(100)	(100)	(100)	(100)
Normal control	--	2.770	0.624	668.4	98.4 82.73**
		(104)	(93)	(100)	(100)
		NS	S	S	NS
S.Em±		0.078	0.028	1.623	0.419
CD at 5%		0.154	0.055	3.441	0.889

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

$$\text{Cocooning percentage} = \frac{\text{No. of cocoon formed}}{\text{Total no. of larvae at kept}} \times 100$$

Table 2. Effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on the cocoon parameters of the silkworm, *B. mori*

Treatment	Dose µg/ml	Female cocoon weight (g)	Female cocoon shell weight (g)	Female cocoon shell ratio (%)	Male cocoon weight (g)	Male cocoon shell weight (g)	Male cocoon shell ratio (%)	Filament length (mts)	Filament weight (g)	Denier
2,4-dichlorophenoxyacetic acid	200	1.435	0.214	14.91 22.71**	1.296*	0.212*	16.35 23.81**	744.37*	0.216*	2.612
		(103)	(102)	(98)	(113)	(110)	(97)	(114)	(110)	(96)
2,4-dichlorophenoxyacetic acid	400	1.690*	0.241*	14.28 22.14**	1.367*	0.217*	15.87 23.42**	610.08	0.206	3.044
		(122)	(115)	(94)	(119)	(113)	(94)	(93)	(105)	(112)
2,4-dichlorophenoxyacetic acid	600	1.587*	0.238*	14.99 22.71**	1.606*	0.227*	14.12* 22.06**	616.83	0.213	3.107
		(115)	(114)	(99)	(140)	(118)	(83)	(94)	(108)	(114)
Carrier control	Distilled water	1.380	0.208	15.07 22.79**	1.143	0.192	16.82 24.20**	650.04	0.196	2.713
		(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
Normal control	-	1.408	0.213	15.12 22.87**	1.261	0.179*	14.19* 22.06**	755.08*	0.200	2.384
		(102)	(102)	(100)	(110)	(93)	(84)	(116)	(102)	(87)
		S	S	NS	S	S	S	S	S	NS
S.Em±		0.073	0.009	0.676	0.055	0.007	0.459	30.346	0.009	0.298
CD at 5%		0.143	0.017	1.325	0.108	0.014	0.900	70.099	0.020	0.690

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

$$\text{Female/male cocooning shell ratio} = \frac{\text{Cocoon shell weight (g)}}{\text{Cocoon weight (g)}} \times 100$$

$$\text{Denier} = \frac{\text{Singile cocoon filament weight (g)}}{\text{Singile cocoon filament length (mt)}} \times 9000$$

or by altering the DNA synthesis or the rate of insect moulting hormone synthesis. Since, the plant growth regulators may influence appetite, nutrition, absorption of plant material, DNA synthesis and rate of synthesis of the insect moulting hormone (Neumann, 1982). However, this view needs to be confirmed by further investigation.

Silk gland weight

The silk gland weight was significantly increased in all the groups treated with 2,4-dichlorophenoxyacetic acid except in 100 µg 2,4-dichlorophenoxyacetic acid treated group where the increased silk gland weight is not significant. Similar results have been reported after the topical application with IAA and GA_3 to the silkworm, *B. mori* (Kamada and Ito, 1984; Magadum and Hooli, 1989, 1991; Hugar and Kaliwal, 1997). The increased silk gland weight obtained in the present study might be due to the increased DNA synthesis in the silk gland or due to the general growth-stimulating effect of 2,4-dichlorophenoxyacetic acid. Since, the plant growth regulators are reported to alter the DNA synthesis in insects (Neumann, 1982).

Larval duration

There was a significant decrease in the larval duration in all the groups treated with 2,4-dichlorophenoxyacetic acid. Similar results have been reported after the topical application with IAA and GA_3 to the silkworm, *B. mori* (Magadum and Hooli, 1989, 1991). The decreased larval duration observed in the present study might possibly be due to the increased synthesis of moulting hormone. Since the treatment with plant growth regulators is reported to alter the rate of synthesis of the insect moulting hormone (Neumann, 1982).

Cocooning percentage

Topical application with all the three concentrations of

Table 3. Effect of 2,4-Dichlorophenoxyacetic acid (2,4-D) on the adult parameters of the silkworm, *B. mori*

Treatment	Dose μg/ml	Moth emergence percentage (%)	Fecundity (No.)	Hatching percentage (%)
2,4-dichlorophenoxyacetic acid	200	98.6 83.20**	566.4	94.4* 76.31**
		(100)	(93)	(105)
2,4-dichlorophenoxyacetic acid	400	98.6 83.20**	618.0	94.4* 76.31**
		(100)	(102)	(105)
2,4-dichlorophenoxyacetic acid	600	98.6 83.20**	624.6	94.6* 76.56**
		(100)	(103)	(105)
Carrier control	Distilled water	98.4 82.73**	604.0	89.6 71.19**
		(100)	(100)	(100)
Normal control	--	98.6 83.20**	608.8	90.0 71.56**
		(100)	(100)	(100)
		NS	NS	S
S.Em±		0.260	36.684	0.360
CD at 5%		0.552	77.770	0.764

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

$$\text{Moth emergence percentage} = \frac{\text{No. of moth emerged}}{\text{Total no of eggs kept}} \times 100$$

$$\text{Hatching percentage} = \frac{\text{Total no. of eggs hatched}}{\text{Total no. of eggs laid}} \times 100$$

2,4-dichlorophenoxyacetic acid had no effect on the cocooning percentage there by indicating that the used concentrations has not adversely affected the survival rate of the larvae.

Cocoon weight, shell weight and shell ratio

The female cocoon weight and its shell weight were significantly increased in 400 and 600 μg 2,4-dichlorophenoxyacetic acid treated groups. In male, the cocoon weight and its shell weight was increased significantly in all the treated groups when compared with the corresponding parameters of the carrier controls (Table 2). The results of the present study suggest that the 400 and 600 μg treated groups yields heavier cocoons and cocoon shells on both sexes and increased in the female and male cocoon shell weights are preceded by the increase in silk gland weight. There results are comparable to the treatment with IAA and GA_3 in multivoltine races (Magadum and Hooli, 1998, 1991) and IAA in bivoltine race (Hugar and Kaliwal, 1997) Similar increase in these parameters was also reported after treatment with IAA in bivoltine silkworm, *B. mori* (Hugar and Kaliwal, 1997).

The significant increase/ decrease in cocoon shell ratio is given in Table 2.

Filament length, weight and denier

The filament length and weight increased significantly in 200 μg 2,4-dichlorophenoxyacetic acid treated groups when compared with the corresponding parameters of the carrier controls (Table 2). Similar results have been reported after the topical application with IAA to the bivoltine silkworm, *B. mori* (Hugar and Kaliwal, 1997).

Moth emergence percentage

There was no significant change in the moth emergence percentage in all the groups treated with 2,4-dichlorophenoxyacetic acid when compared with the corresponding parameters of the carrier controls (Table 3). This indicates that these concentrations of 2,4-dichlorophenoxyacetic acid has no toxic effects on the cocoon crop.

Fecundity

There was no significant change in the fecundity in all the groups treated with 2,4-dichlorophenoxyacetic acid when compared with the carrier controls (Table 3). However there was a significant increased in fecundity in the multivoltine pure Mysore breed silkworm, *B. mori* after the treatment with IAA, GA_3 and IAA respectively (Magadum and Hooli, 1989, 1991; Hugar and Kaliwal, 1997). Hence further investigation is essentiol in this regard.

Hatching percentage

The hatching percentage was increased significantly in all the groups treated with 2,4-dichlorophenoxyacetic acid. The increased hatching percentage was similar to those reported in the bivoltine silkworm, *B. mori* with IAA (Hugar and Kaliwal, 1997). The results of the present study suggest that 2,4-dichlorophenoxyacetic acid to the larvae have not adversely affected the hatchability.

Consideration of action of the plant growth regulators

The silkworm is entirely dependent on mulberry leaves as a food source and plant growth regulators play an important role in the silkworm growth and reproduction. It is important to note that the compounds belonging to all five classes of plant growth regulators have shown to alter insect growth as well as reproduction (Neumann, 1982). Indications for such an influence of the plant growth regulator GA_3 have been reported in locusts (Carlisle *et al.*, 1963) and honey bees (Nation and Robinson, 1966). But a paradoxial thing is that the results due to the effect of these plant growth regulators on insect growth and reproduction do not reveal similar effects in all the insects. The precise mechanism of action of plant growth regulators is still obscure although the effect of plant growth regulators (either added to the diet, injected or topically applied) has been investigated in insects. Pantiz (1967) has found a specific effect of GA_3 upon the activity of the genome, expressed by puffs in the polytene chromosomes of larvae of midge *Acricotopus indicus*. The specific changes in the pattern of puffs, which are thought to reflect gene activity, indicated that GA_3 interferred with normal development (Baudisch and Panitz, 1968). Plant growth regulators mimic the moulting hormones and restrict the insect growth of *Dorsophila hydei* (Alonso, 1971), serves as biochemical signals to regulate insect growth, DNA synthesis and reproduction of *Aulocare ellitti* (Neumann, 1980) and ABA, GA_3 and JH III are biochemically similar compounds derived from mevalonate (Neumann, 1982). De Man *et al.* (1981) have suggest 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 insect moulting hormone. Of course, it is difficult to draw clear conclusion from these data, but it seems of great interest to mention here in each case the results agree the four working hypothesis of the different authors viz.,

1. Plant growth regulators have specific effect on the insect growth probably as a result of plant growth regulator action upon the activity of the genome (Pantiz, 1967) and thought to reflect gene activity, interfered with normal development (Baudisch and Panitz, 1968).
2. Plant growth regulator restricts the insect growth since, plant growth regulator mimics the moulting hormones and restricted the insect growth of *Drosophila hydei* (Alonso, 1971).
3. Plant growth regulators have specific effect on insect growth, DNA synthesis and reproduction probably plant growth regulators serve as biochemical signals since, ABA, GA_3 and JH III are biochemically similar compounds derived from mevalonate (Neumann, 1980, 1982).
4. Plant growth regulators regulate insect growth and reproduction probably by altering the rate of DNA synthesis and/or the rate of synthesis of insect moulting hormone (De Man *et al.,* 1981).

In our study, it is interesting to note that the plant growth regulators, 2,4-dichlorophenoxyacetic acid enhance the silk yield like larval weight, silk gland weight, cocoon weight, cocoon shell weight, filament length, filament weight and denier. However, larval duration was significantly decreased in the groups treated with 2,4-dichlorophenoxyacetic acid. Additional studies using other races of silkworm and variety of exercise paradigms will be necessary to determine the physiological significance and generalisability of the present results.

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