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Effect of Dimethyl Sulfoxide on Economic Traits and the Change of Some Metabolic Sustances of Bivoltine Silkworm, *Bombyx Mori* L.

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Abstract

Oral application of dimethyl sulfoxide in different concentration (1, 3 and 5 percent) to silkworm, *Bombyx mori* at the fourth and the fifth instar has significantly increased the commercial characters such as larval and silkgland weights, female and male cocoon weights, their shell weights and egg production. However, larval duration, cocooning and hatching percentages did not showed any significant change, as compared with that of the corresponding parameters of the carrier control. Similarly the glycogen and protein contents of the fat body and trehalose and protein contents of the haemolymph were significantly increased in 1 and 3 percent, whereas the increase of them in 5 percent concentration did not takes place significantly, except haemolymph trehalose where it was found to be significant, as compared with that of carrier control.

Key words : *Bombyx mori*, dimethyl sulfoxide, commercial characters, fat body, haemolymph, glycogen, protein, trehalose.

INTRODUCTION

The use of solvents as carriers for injecting various compounds of both organic and inorganic origin has been in practice since long (Tazima, 1978). There are few reports showing the effect of some solvents on the physiological process of the animals. Apart from alcohol and benzene, there are a number of solvents which are used as carriers for pesticides, vertebrate and invertebrate hormones and other chemical which are not water soluble. McDonald *et al.* (1974) have stated that in the field of insect neuromuscular pharmacology, the study of simple narcoties, such as alcohols and organic solvents, has been neglected. This is particularly unfortunate, since they are often used as solvents for drugs. Thus, the effect of the drug-solvent-saline solution mainly might be due to the action of the drug but the solvent also have some effect on the tissues. Hence, the statement of McDonald *et al.* (1974) holds good even today for the study of silkworms, since the silkworm is subjected to various chemical treatments along with various solvents. Dimethyl sulfoxide is one solvent that is used as carrier for the juvenile hormone analogue, methoprene, to study the effect of this analogue on the silkworm (Bosquet *et al.* (1989b). Miller *et al.* (1995) have applied this solvent along with Sodium N-methyl dithiocarbamate (a fumigant) to the pine trees, on which the southern pine beetle was a pest and found that the brood production is adversely affected by this beetle. They have stated that dimethyl sulfoxide is a penetrant carrier that enhances absorption. The present investigation is, therefore, an attempt to study the effect of the solvent, dimethyl sulfoxide on the commercial characters, glycogen and protein contents of the fat body and trehalose and protein contents of the haemolymph of the silkworm, *B. mori*.

MATERIALS AND METHODS

The biovlotine race (NB_4D_2) of silkworm larvae were reared according to the improved method of rearing techniques (Krishnaswami, 1978 b, c). The fourth instar larvae were randomly selected and

grouped into five experimental groups. Each group consisted of five replications and of 20 worms in each replication.

The solvent, dimethyl sulfoxide procured from E-merck (India) Ltd., Bombay, was diluted in three different concentrations viz., 1, 3, and 5 percent solutions. The fresh mulberry leaves (175 gms) were dipped for 10 minutes in each concentration of the solvent and then dried at room temperature and fed *ad Libitum* to silkworms. One of the four normal feeds per day was substituted with treated leaves. The treated leaves were fed to the fourth and the fifth instars on alternate days. The carrier controls were fed once with distilled water treated leaves alternating with three feeds of untreated leaves. The normal controls were fed four times a day with the normal leaves.

The larval, cocoon and post cocoon parameters were recorded separately. The larval parmeters such as larval and silkgland weights were recorded before commencement of the spinning activity in the last larval instar. The larval duration was recorded from the day of hatching till the completion of the spinning activity. The cocoon parameters, such as female and male cocoon weights and their shell weights were recorded on the 5th day after the completion of spinning activity. The cocoon shell ratio was calculated. The egg productivity was recorded in the adult after mating.

The cocooning, moth emergence and hatching percentages were also calculated by the formulas shown in the tables. Each mean value, a record of 10 observations except larval duration wherein each mean value was the observation of 5 readings shown in tables 1, 2 and 3. The experiments were conducted twice to conclude the results.

1. Preparation of tissue

The fifth instar larvae were used for the estimation on the sixth day. The larvae were dissected in *Bombyx* saline at the pH 6.5. Immediately the fat body was collected and used for the estimation of glycogen by anthrone method (Sciefter *et al.*, 1950) and protein (Lowry *et al.*, 1951). The haemolymph collected in free chilled centrifuge tube was used for the estimation of trehalose (Roe *et al.*, 1955) and protein (Lowry *et al.*, 1951). Anthrone positive carbohydrate in the haemolymph is considered as trehalose.

The data collected were subjected to analysis of variance test to find out the significance between the parameters of the untreated and treated groups (Snedecor and Cochron, 1967). The percent values for cocooing, female and male cocoon shell ratio and hatching percenttage were transformed into sine angular values for statistical analysis. The percent index was calculated for each parameter of the experimental groups over that of the corresponding parameters of the controls.

RESULTS AND DISCUSSION

The data on the effect of dimethyl sulfoxide on the commercial characters, glycogen and protein contents of the fat body and trehalose and protein contents of haemolymph of the silkworm, are summarized in tables 1 and 2 and figs. 1 and 2. As far as the authors are aware this is the first report on the effect of dimethyl sulfoxide on the silkworm, *B. mori*.

1. Economic parameters

1) Larval and silkgland weights

The larval and silkgland weights are increased significantly in all the treated groups except for silkgland weight in 5 percent treated group (Table 1). A maximum increase in larval weight (20%) and silk-g land weight (32%) was observed in 1 and 3 percent treated groups, respectively. Increase in larval weight has also been shown in acetone fed eri silkworm, *Philosamia ricini* (Padaki, 1991). Radhakrishna and Delvi (1992) have stated that feeding of acetone treated leaves had considerable effect on food utilization in silkworm. The increase in the larval weight in the present study might possibly be due to the effect of dimethyl sulfoxide in terms of increase of food utilization as mentioned above, whereas the increase in silkgland weight might possibly be due to the availability of additional raw materials due to the increase food utilization as well.

2) Larval duration

The larval duration did not show any significant

Table 1. *Effect of dimethyl sulfoxide on larval and post-cocoon parameters of the silkworm, B. mori*

Treatment	Concent ration (%)	Larval weight (gm)	Silkgland weight (gm)	Larval duration (hrs)	Cocooning (%)	Egg produ ctivity (n)	Hatching (%)
Dimethyl Sulfoxide	1	3.607*	1.268*	632	92.54 72.74**	666*	93.58 73.49**
		(12)	(128)	(99)	(105)	(108)	(101)
Dimethyl Sulfoxide	3	3.597*	1.303*	633	92.29 74.24**	692*	92.08 73.65*
		(120)	(132)	(99)	(100)	(112)	(99)
Dimethyl Sulfoxide	5	3.442*	1.076	633	90.01 73.98**	658	92.26 72.96**
		(115)	(109)	(99)	(99)	(106)	(99)
Carrier Control	Distilled Water	2.989	0.986	634	90.51 71.56**	616	92.44 74.01**
		(100)	(100)	(100)	(100)	(100)	(100)
Normal Control	-	3.005	0.991	633	90.69 72.04**	593	93.45 75.23**
		(100)	(100)	(99)	(100)	(96)	(101)
F-test	-	S	S	N.S	N.S	S	N.S
S.Em±	-	0.098	0.046	2.61	1.08	22.16	2.45
C.D.at 5%	-	0.196	0.092	5.33	2.22	44.79	5.01

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

$$\% \text{ cocooning} = \frac{\text{Number of cocoon formed}}{\text{Total number of larvae tested}} \times 100$$

$$\% \text{ hatching} = \frac{\text{Total number of eggs hatched}}{\text{Total number of eggs laid}} \times 100$$

change in all the treated groups, as compared with that of carrier control. It could be suggesting that the concentration fed to the larvae, may not have any effect on the hormonal level, juvenile hormone and moulting hormone, which control moulting and metamorphosis in insects.

3) Cocooning percentage

The solvent had no significant effect on survival percentage, thereby indicating that the concentrations fed to the silkworm larvae had no toxic effect on them, although dimethyl sulfoxide, at less than 30% concentration, is reported to be less toxic to animals and plants (Miller *et al.*, 1955).

4) Cocoon weight, shell weight and shell ratio

The female and male cocoon weights and their shell weights were significantly increased in 1 and 3 percent treated groups. Male cocoon shell weight was also increased in 5 percent treated group. A maximum increase in female (26%) and male (20%) cocoon shell weights was observed in 3 percent treated group. The results suggest that the used concentrations might be used to increase the female and the male cocoon shell weights, thereby increaseing the silk yield. It is not known exactly how the solvent act on the silkgland to increase the silk synthesis. However, there was no significant change in

the female and male cocoon shell ratios.

5) Egg productivity and egg hatching percentage

The egg productivity was increased significantly in 1 and 3 percent treated groups, whereas the increase was not significant in 5 percent treated group. A maximum increase (12%) was observed in 3 percent treated group. However, it is not known how the solvent stimulates the reproductive potential in silkworm. The egg hatching percentage has not shown any significant change. The results, therefore, suggest that used concentrations had no adverse effect on the reproduction and egg hatching of the silkworm, *B. mori*.

2. Biochemical parameters

1) Fat body glycogen and haemolymph trehalose contents

Oral application of dimethyl sulfoxide to silkworm larvae has significantly increased the fat body, glycogen and haemolymph trehalose contents in all the treated groups. However, the increase of glycogen content in 5 percent treated group was not significant. A maximum increase of glycogen and trehalose contents were observed in 1 and 3 percent treated groups, respectively (Fig. 1). Similarly it has been reported in eri silkworm fed with acetone treated leaves has significantly increased the fat body glycogen and haemolymph trehalose (Padki, 1991). The increased glycogen and trehalose may serve as additional source of energy during pupa to adult transformation.

2) Protein contents of fat body and haemolymph

The protein content of fat body and haemolymph had significantly increased in 1 and 3 percent treated groups, whereas the increase in 5 percent treated group was not significant. A maximum increase in protein content was observed in 1 percent treated group (Fig. 2). The increase in the protein content of the fat body and haemolymph coincides with the simultaneous increase in the silkgland weight, female and male cocoon shell weights (Table 1 and 2). The simultaneous increase in the silkgland weight may possibly 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 took place.

Guerra (1970) citing the reference of Harper (1963) has quoted that the metabolic process taking place with the living organism are merely the reflection of the chemical composition of the body. The chemical composition of the body fluids varies in limits, changes in the normal metabolism are detrimental to insect development and/or reproduction could be produced by inducing an excess or a deficiency of essential metabolites. The regulation of the rate of metabolic processes controlled by several mechanism like nervous system, hormones, the stimulation or inhibition of enzyme activity, feedback inhibition and the induction or suppression of enzyme synthesis. Whether the increase/decrease in the fat body

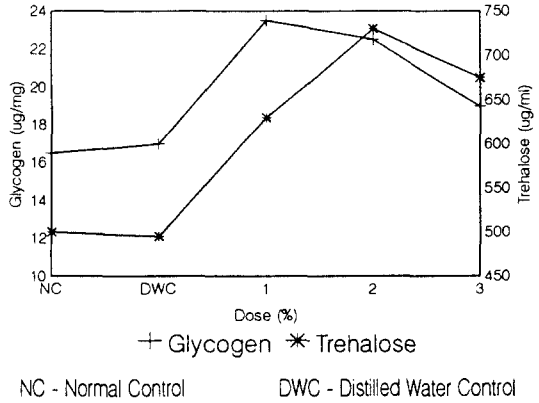

Fig. 1. *Effect of dimethyl sulfoxide on the fat body, glycogen and haemolymph trehalose content of the silkworm, B. mori.*

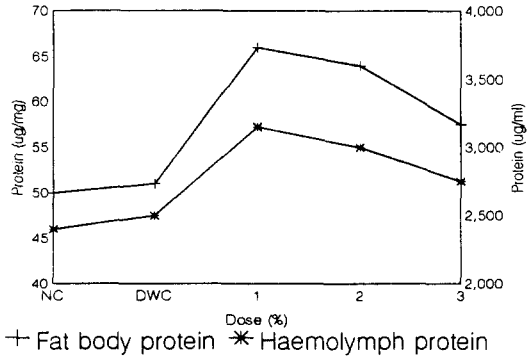

Fig. 2. *Effect of dimethyl sulfoxide on the protein content of fat body and haemolymph of the silkworm, B. mori.*

Table 2. *Effect of dimethyl sulfoxide on cocoon parameters of the silkworm, B. mori*

Treatment	Concent ration (%)	Female cocoon weight (gms)	Female cocoon shell weight (gms)	Female cocoon shell ratio (%)	Male cocoon weight (gms)	Male cocoon shell weight (gms)	Male cocoon shell ratio (%)
Dimethyl Sulfoxide	1	1.878*	0.357*	18.87	1.613*	0.331*	30.62
				25.86**			26.95**
		(111)	(126)	(100)	(118)	(114)	(97)
Dimethyl Sulfoxide	3	1.980*	0.357*	18.08	1.549*	0.347*	22.46
				25.10**			28.23**
		(117)	(126)	(96)	(113)	(120)	(106)
Dimethyl Sulfoxide	5	1.826	0.307	18.32	1.463	0.332*	22.39
				25.28**			28.19**
		(108)	(108)	(97)	(107)	(115)	(100)
Normal Control	-	1.715	0.305	18.00	1.330	0.295	22.31
				25.13**			28.14**
		(101)	(107)	(95)	(97)	(102)	(105)
F-test	-	S	S	N.S	S	S	N.S
S.Em±	-	0.072	0.072	0.69	0.052	0.010	0.88
C.D.at 5%	-	0.139	0.025	1.38	0.105	0.020	1.75

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

$$\text{Cocoon shell ratio} = \frac{\text{Shell weight}}{\text{Cocoon weight}} \times 100$$

glycogen, haemolymph trehalose contents and protein content of the fat body and haemolymph after feeding with the solvent dimethyl sulfoxide may be due to its influence on nervous system, hormones, the stimulation or inhibition of enzyme activity on the induction or suppression of enzyme synthesis is not known. Hence, further investigation is essential in this regard.

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적 요

4령과 5령 누에에 1%, 3%, 5%의 dimethyl sulfoxide를 첨식시키면 누에의 실용형질인 잠채중, 견사선 무게, 암 · 수 견중 및 견충중과 산란량이 현저히 증가했다. 그러나 경과일수, 족중생존율과 부화율은 대조와 큰 차이를 보이지 않았다. 한편 dimethyl sulfoxide 1.3%구에서는 누에의 血液內 蛋白質과 트리할로스량 및 지방체의 단백질 함량과 글리코겐량은 대조에 비해 현저히 증가한 반면 5%구에서는 혈액 트리할로스량을 제외하고는 차이가 없었다.

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