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EFFECT OF ZINC CHLORIDE AND ZINC SULPHATE ON THE SILKWORM, *BOMBYX MORI* GROWTH TISSUE PROTEINS AND ECONOMIC PARAMETERS OF SERICULTURE

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ABSTRACT

The relative impact of zinc chloride ($ZnCl_2$) and zinc sulphate ($ZnSO_4$) on larval growth, tissue proteins and economic parameters of sericulture was studied in *Bombyx mori* by feeding its larvae with zinc- enriched mulberry leaves. In general, the two zinc salts showed differential effects on all parameters examined. $ZnCl_2$ caused 357% increase in the larval body weight and 2900% increase in silk gland weight, while $ZnSO_4$ showed an increase of 329% in body weight and 1900% in gland weight. Significantly, $ZnCl_2$ elevated the protein levels in silk gland and haemolymph by 526% and 265% respectively, but $ZnSO_4$ reduced them by 356% and 181% respectively in silk gland and haemolymph. Both the zinc salts reduced fat body and muscle protein levels by 48 to 84% during the study period. Both $ZnCl_2$ and $ZnSO_4$ elevated cocoon weight by ~11 and 14%, and shell weight by ~17 and 9% respectively, but reduced floss weight by 50% each. While $ZnCl_2$ increased shell proteins by ~22% and floss protein by ~23%, $ZnSO_4$ reduced them by ~23% and 9% respectively. $ZnCl_2$ raised the shell-cocoon ratio by ~50% and floss-shell ratio by 16%, $ZnSO_4$ showed no effect on the former, but increased the latter by 17%. The $ZnCl_2$ caused a 9% decline in floss-silk ratio, while $ZnSO_4$ caused 2% decrease in this parameter. While $ZnCl_2$ caused 5%, 3% and 29% increase respectively in silk weight, raw silk percentage and denier, ZnSO4 showed marginal positive trends in these parameters. While, $ZnCl_2$ reduced the renditta by 4%, $ZnSO_4$ increased it by 2.5%. The study indicates that the nutritive value of $ZnCl_2$ was higher than that of $ZnSO_4$ and hence it could be tried as a potential modulator of silk production.

INTRODUCTION

Improving the quantity and quality of silk produced by the silk worm, *Bombyx mori*, using exogenous nutrients and minerals has been practiced as a traditional scientific method in the sericulture industry (Magadum *et al*., 1992; Qader *et al*., 1993; Chamundeswari and Radhakrishnaiah, 1994; Willott and Trans, 2002; Goyal *et al*., 2003). This is often done by feeding the silkworm larvae with nutrient-fortified mulberry leaves and observing the changes in growth development, metamorphosis, metabolism and in the silk yield (Kumararaj *et al*., 1972; Ahmed *et al*., 1998; Rahmathulla, 2002). For instance, the supplementation of mulberry diet with vitamin B derivatives enhanced disease resistance, body weight and silk yield in silkworm (Ito, 1978; Das and Medda, 1998), while, another vitamin, ascorbic acid enhanced the larval survival rate (Ito and Nimimura, 1966a, 1966b). Of late, the impact studies of minerals on silkworm biochemistry and metabolism have been widely reported. For example, the synergetic effects of potassium and magnesium chlorides on the biochemical constituents such as the glycogen, total lipids, trehalose, and total protein content of the silkworm has been analyzed with a positive impact on metabolism (Bhattacharya and Kaliwal, 2005a). One of the exogenous modulators that attracted the attention of investigators is the zinc, a micronutrient carried through the diet and bio-accumulates in the tissues of silkworm. It plays an important role in larval growth and development by stimulating metabolism through enhanced enzyme activities, hormonal mediation, replication, transcription and neuronal activity (Wright, 1984; Neto *et al*., 1995). Though the impact of zinc on larval growth and metamorphosis has been extensively studied, its influence on silk yield and other economic factors has not been examined so far. In view of the positive impact of zinc on silkworm growth and metamorphosis, it is imperative to assess its role in silk production. In the current study, we made a comparative analysis of the impact of two zinc salts, viz, zinc chloride ($ZnCl_2$) and zinc sulphate ($ZnSO_4$) on silkworm growth, protein profiles and economic parameters of sericulture.

MATERIALS AND METHODS

The present investigation was carried out on the Pure Mysore x CSR_2 hybrid strain of *Bombyx mori*, reared under standard environmental conditions of 28°C, 85% RH, as per the method given by Krishnaswami (1986). After hatching, the worms were reared on M_5 variety of mulberry leaves with 5 feeds per day at 6 AM, 10AM, 02 PM, 06 PM and 10 PM, under normal 12h light and 12h dark conditions. The experimental design was divided into three phases, namely zinc feeding pattern, assay of proteins and analysis of economic parameters of sericulture.

Zinc feeding pattern

After the third moult, the fourth instar larvae were divided into four batches of 100 larvae each. The first batch was given normal feedings 5 times a day and treated as the control. The second and third batch larvae were fed with zinc-fortified mulberry leaves at its minimum effective dose as determined by us in our previous study (Kavitha *et al*., 2011). Accordingly, the second batch larvae were fed with mulberry leaves soaked in 1μg $ZnCl_2$, dissolved in 100mL of distilled water, while the third batch larvae were fed with mulberry leaves soaked in 1μg $ZnSO_4$ solution. In each case, mulberry leaves were dipped in $ZnCl_2$ /$ZnSO_4$ solution separately, dried under cool dry weather conditions and fed to the larvae of fourth and fifth instars, once in a day at 06 PM, while continuing the normal pattern feeding at other timings.

Assay of proteins

Tissues such as the silk gland, fat body and muscle were isolated by mid–dorsal dissection of fifth instar larvae in the Silkworm Ringer (Yamaoka *et al*., 1971) and the haemolymph was extracted by cutting the telson and prolegs of the silkworm larvae. The total protein content of the tissues was estimated in 1% homogenates of tissues and 1:9 diluted haemolymph (1:9 haemolymph and water) by the method of (Lowry *et al*., 1951) and expressed in mg protein / gm wet weight of tissue or mg/mL of haemolymph. Similarly, the total protein content of the shell and floss of the cocoon was estimated in 1 % homogenates in distilled water as per the method given by Lowry *et al*. (1951). Since the silk cocoon is not soluble in distilled water, it was first soaked in diluted sodium hydroxide (NaOH) solution before homogenized in distilled water. The amount of proteins present in the sample was computed using a standard prepared from bovine serum albumin, and the values were expressed as mg/g wet weight of the shell or floss.

Analyses of economic parameters

Some important economic parameters of the sericulture industry, such as the larval weight, silk gland weight, gland–body ratio, cocoon weight, cocoon-shell weight, cocoon - shell ratio, renditta, raw silk–larval body ratio, raw silk percentage, filament weight, denier (filament size), floss-shell ratio were analyzed separately in each batch as per the methods given by (Bohidar *et al*., 2007 and Rahmathulla *et al*., 2007; Sailaja and Sivaprasad, 2010a; Chakrabarthy and Kaliwal, 2011).

RESULTS AND DISCUSSION

The growth patterns of the larval body and tissue specific changes in the levels of total proteins under the impact of two zinc salts ($ZnCl_2$ and $ZnSO_4$) were analyzed in two ways. Firstly, its immediate impact (day-to-day effect) as reflected in the form of deviations from the control value on each day of experimentation, and secondly, its overall impact as recorded in the form of instar-end changes in protein levels, wherein the day-1 value of fifth instar is taken as the control and that of the day-7 as the experimental (Table 1 and Fig. 1).

Effect of zinc on the growth of silk gland and larval body

Since, the silk gland-body ratio is an important economic indicator of silk production by the silkworm; the higher the ratio, the greater would be the output and vice versa (Sailaja and Sivaprasad, 2010a, 2010b). Higher gland-body ratios could be achieved by enriching the silkworm diet (mulberry leaves) with a variety of exogenous modulators, more particularly with zinc salts. The day-to-day changes and instar-end changes in the growth patterns of the larval body and the silk gland, together with the gland-body ratio were measured under the influence of $ZnCl_2$ and $ZnSO_4$, and presented in

Table 1: Growth of silkworm, *Bombyx mori* larvae in terms of body weight (g), silk gland weight (g) and silk gland-body ratio during fifth instar development, under the impact of $ZnCl_2$ and $ZnSO_4$. Each value is a mean, ± standard deviation of four individual observations

Days of V Instar		Control Body	Silk gland	Gland-Body ratio	$ZnCl_2$ treated Body	Silk gland	Gland-Body ratio	$ZnSO_4$ treated Body	Silk gland	Gland-Body ratio
	Mean	0.7	0.03	4.6	0.6	0.03	4.5	0.6	0.02	4.7
1	PC	-	-	-	-	-	-	-	-	-
	SD	±0.015*	±0.002*	±0.05*	±0.006*	±0.002*	±0.006*	±0.010*	±0.002*	±0.01*
2	Mean	1.2	0.1	4.9	1.2	0.1	5	1	0.04	4.3
	PC	71.4	100	6.5	87.1	107.1	11.1	74.9	83.3	4.9
	SD	±0.013*	±0.02*	±0.06*	±0.040*	±0.002*	±0.05*	±0.028*	±0.002*	±0.006*
3	Mean	1.6	0.1	7.5	1.4	0.1	8	1.2	0.1	6.6
	PC	30.1	93.3	53.1	20.7	93.1	60	20.2	86.4	53.5
	SD	±0.03*	±0.002*	±0.13*	±0.029*	±0.002*	±0.06*	±0.030*	±0.002*	±0.006*
4	Mean	2.1	0.2	10.7	2	0.3	12.7	1.7	0.2	9.9
	PC	31.3	89.7	42.7	40.7	123.2	58.8	37.9	107.3	50
	SD	±0.05*	±0.02*	±0.05*	±0.010*	±0.002*	±0.006*	±0.033*	±0.002*	±0.008*
5	Mean	2.3	0.4	15.4	2.3	0.4	15.5	2.1	0.3	16
	PC	9.5	59.1	43.5	18.3	44	22	23.4	98.8	61.8
	SD	±0.05*	±0.02*	±0.06*	±0.071*	±0.016*	±0.025*	±0.050*	±0.002*	±0.005*
6	Mean	2.8	0.6	22.1	2.9	0.6	21.4	2.6	0.6	21.2
	PC	21.7	76	43.8	24.5	72.2	38.1	23.7	63.3	32.3
	SD	±0.03*	±0.002*	±0.014*	±0.060*	±0.016*	±0.006*	±0.038*	±0.002*	±0.006*
7	Mean	3	0.7	24.4	3.2	0.9	28	3	0.6	21.7
	PC	7.1	18.5	10.3	11	50	31	14.2	17	2.5
	SD	±0.03*	±0.02*	±0.006*	±0.088*	±0.016*	±0.06*	±0.079*	±0.003*	±0.006*

* Statistically significant. ** Statistically not significant

Table 1. In the control batch (*i.e.*, the larvae fed with normal mulberry leaves), the body weight increased by ~329% (from 0.7 g to 3.0 g), the silk gland weight by ~2233% (from 0.03 to 0.7g) and the gland-body ratio by ~430% (from 4.6 to 24.4) during fifth instar development. The two zinc salts showed significantly varying proportions of changes in the body growth, silk gland growth and gland-body ratio. When the larvae were fed with $ZnCl_2$-treated mulberry leaves the larval body weight increased by 357% (from 0.7 g to 3.2g), gland weight by 2900% (from 0.03 g to 0.94 g) and the gland-body ratio by ~509% (from 4.6 to 28.0) at the end of fifth instar. At the same time, when the larvae were fed with $ZnSO_4$, the larval body weight increased by ~329 (from 0.7 to 3.0 g), gland weight by 1900% (from 0.03 to 0.6g) and the gland-body ratio by ~372% (from 4.6 to 21.7). The study indicates that $ZnCl_2$ has more promising effect on larval growth and the gland-body ratio compared to $ZnSO_4$, which in fact has an inhibitory effect on these two important parameters (Table 1). Obviously a mineral that could elevate gland weight and cause higher gland body- ratio has more nutritive and economic values than that causes low gland weight and lower gland-body ratio. Many earlier nutritional studies on silkworm revealed that mineral salts, particularly those in chloride form (eg. Magnesium chloride, calcium chloride, potassium chloride and cobalt chloride) showed positive growth trends with reference to the growth of silk gland and larval body (Chakraborti and Medda, 1978; Bhattacharya and Kaliwal, 2005a, 2005b, 2005c). Our study, while replicating the positive role of mineral nutrition in the silkworm growth as a whole, further demonstrates that the chloride form of zinc salt (*i.e.*, $ZnCl_2$) appears to have more stimulating effect on the growth of silk gland and larval body than its sulphate form (*i. e.,* $ZnSO_4$) and with this effect it shifts the balance towards higher gland-body ratio during fifth instar development.

Table 2: Economic parameters of the silkworm, *Bombyx mori*, under the impact of $ZnCl_2$ and $ZnSO_4$, Each value is a mean, ± standard deviation of four individual observations

Parameter		Control	Experimental $ZnCl_2$ treated	$ZnSO_4$ treated
	Mean	86.5	80	86
No. of green cocoons in one 1.kg weight	P.C	-	-7.5	-0.5
	S.D	±1.3	±0.8*	±0.8**
	Mean	1.3	1.4	1.4
Weight of single cocoon (g)	P.C	-	7.7	7.7
	S.D	±0.01	±0.01*	±0.01*
	Mean	0.2	0.3	0.3
Weight of single shell (g)	P.C	-	50	50
	S.D	±0.01	±0.01*	±0.01*
	Mean	0.02	0.03	0.03
Weight of single floss (g)	P.C	-	50	50
	S.D	±0.00	±0.01*	±0.01*
	Mean	42.4	51.5	32.5
Shell protein (mg /g)	P.C	-	21.5	-23.3
	S.D	±2.7	±0.01*	±0.00*
	Mean	4.41	5.4	4
Floss protein (mg/g)	P.C	-	22.7	-9.1
	S.D	±0.4	±0.9*	±0.1*
	Mean	0.2	0.3	0.2
Shell-cocoon ratio	P.C	-	50	0
	S.D	±0.00	±0.00*	±0.00*
	Mean	8.7	10.1	10.2
Floss- shell ratio	P.C	-	16	17.1
	S.D	0.3	1.6**	0.2**
	Mean	0.2	0.2	0.2
Silk -Shell ratio	P.C	-	0	0
	S.D	±0.00	±0.00*	±0.00*
	Mean	20.5	18.6	20
Floss-Silk ratio	P.C	-	-9.3	-2.4
	S.D	±0.05	±0.05*	±0.06*
	Mean	18.7	19.7	18.9
Raw silk weight (g)	P.C	-	5.3	1.1
	S.D	±0.01	±0.01*	±0.01*
	Mean	12.7	13.1	12.3
Raw silk percentage	P.C	-	3.1	-3.1
	S.D	±0.0	±0.01*	±0.0*
	Mean	7.9	7.6	8.1
Renditta	P.C	-	-3.8	2.5
	S.D	±0.01	±0.01*	±0.01*
	Mean	14	18.1	15.6
Denier (d)	P.C	-	29.3	11.4
	S.D	±0.5	±0.9*	±1.4*

Effect of zinc salts on tissue proteins

Silk gland proteins

Silk gland is the prime site of synthesis of two silk proteins; fibroin and sericin and over 90 other proteins (Jin *et al*., 2004; Zhang *et al*., 2006; Hou *et al*., 2007a). Notwithstanding some minor discrepancies in day-to-day effects, $ZnCl_2$ showed positive influence on silk gland protein profiles while $ZnSO_4$ has an opposite effect (Fig. 1A). Clearly, $ZnCl_2$ caused an elevation in the silk gland protein levels by about 9% on day-1and day-2, ~5% on day-3, ~2% on day-4, ~26% on day-7 while on the other two days (days 5 and 6) the protein levels declined marginally. The day-to-day elevatory effects of $ZnCl_2$ on protein profiles have resulted in an overall increase of ~526% at the end of fifth instar. At the same time $ZnSO_4$ caused a decrease in their levels by ~9% on day-1, ~6% on day-3, ~10 % on day-5, ~13% on day-6 and by ~15% on day-7. This negative impact culminated in an overall decrease of ~356% in the silk gland protein levels in the $ZnSO_4$-treated larvae. As reported earlier (Chakraborti and Medda, 1978; Bhattacharya and Kaliwal, 2005a, b, c), chloride salts of potassium, cobalt, calcium and magnesium could bring about silk gland growth by enhancing rate of protein synthesis in silk gland cells, much like $ZnCl_2$ as reported in the present study. The $ZnCl_2$-stimulated growth rate in the silk gland is accompanied by increased accumulation of silk proteins during fifth instar development and that this salt does so, by shifting the gland-body ratio towards positive side. Hence, it is suggested that mineral salts in chloride form could be effectively supplemented with the silkworm diet so as to enhance its nutritive value and to boost silk production.

Haemolymph proteins

The haemolymph of *B. mori* is the chief circulating fluid and transport medium for about 298 proteins (Lix *et al.,* 2006), involved in larval growth, ecdysis, metamorphosis, silk production, apoptosis, chitin and haemocyte formation, growth of salivary glands and reproduction (Lix *et al*., 2006; Chai *et al*., 2008; Nakahara *et al*., 2009).The effect of two Zinc salts on haemolymph proteins is more or less similar to that of the silk gland. Obviously, $ZnCl_2$ showed a positive impact on haemolymph proteins while $ZnSO_4$ had a negative impact (Fig. 1B). The positive effect of $ZnCl_2$ was recorded on the second, third, fourth, fifth, sixth and seventh days of fifth instar, during which the haemolymph proteins were elevated by about 1% to 19%. On the other hand, the negative impact of $ZnSO_4$ on haemolymph proteins was more significant (~20% to 18% decrease) during the same period. The differential effects of two zinc salts have culminated in an overall increase of ~265% in haemolymph proteins under the influence of $ZnCl_2$ and a total decline of ~181% under the impact of $ZnSO_4$.

Fat body proteins

Similar to that of the mammalian liver and adipose tissue, the

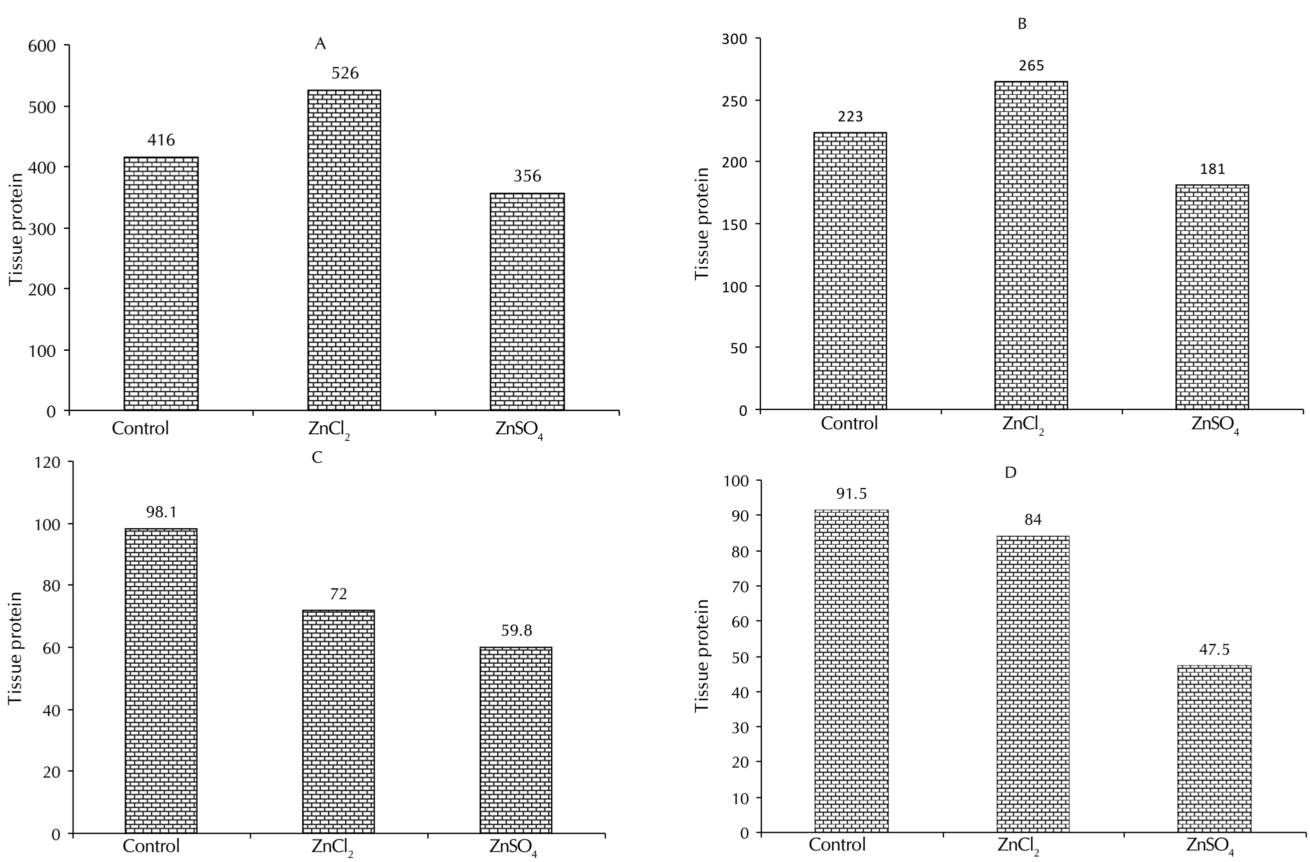

Figure 1: Tissue protein profiles of the silkworm, *Bombyx mori* under the influence of ZnCl2 and $ZnSO_4$. The total protein values, expressed in mg/g wet weight of tissues or mg/mL of haemolymph represent the mean ± standard deviation of four individual observations, each comprising tissues from 10 to 20 larvae. ($p < 0.001$) A: Silk gland; B: Haemolymph; C: Fat body; D: Muscle

silkworm fat body synthesizes and stores over 177 proteins that are involved in its growth and metabolism (Scott *et al*., 2004; Hou *et al*., 2007 b). Barring a marginal increase in the levels of fat body proteins on day-1, both zinc salts ($ZnCl_2$ and $ZnSO_4$) caused significant decline (~ 5 to 50%) in their levels throughout the fifth instar development. The overall downfall in the fat body protein levels was about 72% under the influence of $ZnCl_2$ and 60 % under $ZnSO_4$ (Fig .1C). Obviously, both the zinc salts have an inhibitory effect on the fat body metabolism and this result from unavoidable release of fat body enzymes and proteins into the haemolymph as evidenced by the elevation of haemolymph proteins on one hand and the disruption of protein synthesis in the fat body cells on the other.

Muscle proteins

The skeletal muscle of silk worm is known to synthesize and store about 258 proteins (Zhang *et al*., 2007; Sivaprasad and Sailaja, 2011), that play a vital role in larval locomotion and body movements apart from larval growth and development (David and Anantha Krishnan, 2006; Sivaprasad and Murali Mohan., 2009a, b). Despite some initial elevatory effects (~ 17 to 36% increase), both $ZnCl_2$ and $ZnSo_4$ showed an inhibitory effect on muscle proteins. The day-to-day inhibitions ranged from ~10 to 81% from day-3 to day-7 during fifth instar development (Fig. 1D). The overall inhibition at the end of fifth instar was ~84% under $ZnCl_2$ and ~48% under $ZnSo_4$, a trend that probably reflects the stimulatory influence of zinc salts on intracellular proteolysis in muscle and fat body.

Effect of zinc salts on economic traits of sericulture

The productivity and profitability of the sericulture industry depend on the quantity of silk proteins produced in the silk gland and the quality of silk extracted from the cocoons. The effect of $ZnCl_2$ and $ZnSO_4$ on the economic parameters of sericulture was analyzed separately and presented in (Table 2). In all, 14 economic parameters such as the number of green cocoons in one 1kg weight, cocoon weight, shell weight, floss weight, shell protein, floss protein, shell-cocoon ratio, floss-shell ratio, silk-shell ratio, floss-silk ratio, raw silk weight, raw silk percentage, renditta and denier were analyzed after feeding the silkworm larvae with the mulberry leaves enriched with $ZnCl_2$ and $ZnSO_4$. The positive impact of $ZnCl_2$ observed on the silk gland and haemolymph protein profiles has been carried through the economic parameters of sericulture. $ZnCl_2$ decreased number of cocoons required for one kilogram weight by 7.5%, while $ZnSO_4$ caused a decrease of only 0.5%. Thus, $ZnCl_2$ yields 7% more productivity in cocoons than $ZnSO_4$. Both zinc salts showed an elevatory effect on cocoon weight (~11 and 14%) and shell weight (~17 and 9%) and inhibitory effect on floss weight (50% each). The protein content of the shell, which includes the core fibroin, has been elevated by ~22% under the influence of $ZnCl_2$, but decreased by ~23% under $ZnSO_4$ treatment. On the other hand the floss protein, which chiefly comprises sericin, has recorded an increase of ~ 23% under $ZnCl_2$ treatment but decreased by ~9% under $ZnSO_4$ treatment. The shell-cocoon ratio recorded a rise of ~50% under $ZnCl_2$ treatment, but not affected by $ZnSO_4$ treatment. The floss- shell ratio recorded an increase of about 16 - 17% under the influence of $ZnCl_2$ and $ZnSO_4$, but the silk-shell ratio has not been affected. Another parameter; the floss-silk ratio, which is a measure of relative proportions of sericin and fibroin contents of silk, has been declined by ~9% under $ZnCl_2$ treatment and only by ~2% under $ZnSO_4$ treatment. The raw silk weight increased by ~5%, under $ZnCl_2$ treatment and marginally under $ZnSO_4$ treatment, while the raw silk percentage increased by ~3% under the influence of $ZnCl_2$, but declined by same proportion under the influence of $ZnSO_4$. Renditta, the number of cocoons required for the production of 1Kg of raw silk, declined by ~4% under $ZnCl_2$ but increased by ~2.5% under $ZnSO_4$ treatment. At the same time, the denier which is the measure of silk texture and thickness of the silk fiber marginally increased by ~29% under $ZnCl_2$ and ~11% under $ZnSO_4$. The positive impact of mineral nutrition on the silkworm economic traits such as cocoon weight, shell weight, fecundity, larval duration, effective rearing rate, silk filament length and weight, denier, cocoon-shell ratio has been well documented with reference to nutritional role of several mineral salts such as potassium iodide and cobalt chloride (Chakraborti and Medda, 1978), copper sulphate, nickel chloride and potassium iodide (Magadum, 1987; Narasimhamurthy and Govindappa, 1988), ferrous and magnesium sulphates (Nirwani and Kaliwal, 1995), magnesium chloride, potassium chloride and potassium permanganate (Bhattacharya and Kaliwal, 2005b, 2006), potassium nitrate and nickel chloride (Hugar and Kaliwal, 1997; Goudar and Kaliwal, 2000), potassium permanganate, potassium and magnesium chlorides (Bhattacharya and Kaliwal, 2005c, 2005d) potassium bromide and nickel sulphate (Kochi and Kaliwal, 2005) and potassium and magnesium carbonates (Chakrabarty and Kaliwal, 2011). Further, Chamundeswari and Radhakrishnaiah (1994) and Kavitha *et al*. (2009) have demonstrated the elevatory effect of zinc salts on silk parameters such as the filament length and its weight. Our study substantiates the positive role of zinc salts (particularly $ZnCl_2$) on economic traits of sericulture. Further, it demonstrates that the positive impact of $ZnCl_2$ reflects at two levels. Firstly, it stimulates silk protein synthesis in the silk gland and enhances silk output, as reflected in higher shell-cocoon ratios, silk- body ratio, raw silk percentage, denier and renditta and secondly and it lowers the floss-shell ratio by decreasing the floss protein synthesis, which is removed as wastage at the time of silk reeling.

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REFERENCES

Ahmed, C. A. and Chandrakala, M. 1998. Food and water utilization patterns under restricted feeding duration in *Bombyx mori* of Pure Mysore race. *J. Exp. Zool. India*. 129-34.

Bhattacharya, A. and Kaliwal, B. B. 2005a. Synergetic effects of potassium and magnesium chloride on biochemical contents of the silkworm, *Bombyx mori* L. *Caspian. J. Env Sci*. **3**: 1-7.

Bhattacharya, A. and Kaliwal, B. B. 2005b. Influence of the mineral potassium permanganate on the economic parameters of the silkworm, *Bombyx mori* L. *National Conference on Sericulture for*

Global Competitiveness. 334-336.

Bhattacharya, A. and Kaliwal, B. B. 2005c. The biochemical effects of potassium chloride on the silkworm, *Bombyx mori L. Insect Science*. **12**: 95-100.

Bhattacharya, A. and Kaliwal, B. B. 2006. Fortification of potassium and magnesium chloride and synergetic effect on economic traits of the silkworm, *Bombyx mori* L. *Casp. J. Environ. Sci.* (communicated).

Bhattacharya, A. and Kaliwal, B. B. 2005d. Fortification of mulberry leaves with mineral magnesium chloride ($MgCl_2$) on biochemical contents of the silkworm, *Bombyx mori* L.*The Phillippine Agricultural Scientist*. **38**: 337-340.

Bohidar, K., Sahoo, B. S. and Singh, D. K. 2007. Effect of different varieties of mulberry leaves on economic parameters of the silkworm *B. mori* L. under Orissa climate. *Bull. Ind. Acad. Seri.* **11:** 60-64.

Chai, S. S., Rhee, W. J. and Park, T. H. 2008. Inhibition of human cell apoptosis by silkworm haemolymph. *Biotech. Prog*. **18:** 874-878.

Chakrabarty, S. and Kaliwal, B. B. 2011. Supplementation with Potassium Carbonate, Magnesium carbonate and their synergetic effects on the economic traits of the silkworm, *Bombyx mori* L. *World J. Science and Technology*. **1**: 10-23.

Chakraborti, M. K. and Medda, A. K. 1978. Effect of cobalt chloride on silkworm (*Bombyx mori* L.) *Nistari Race. Sci. and Cult*. **44**: 406-408.

Chamundeswari, P. and Radhakrishnaiah, K. 1994. Effect of zinc and nickel on the larval and cocoon characters of the silkworm *B.mori* L. *Sericologia*. **34:** 327-330.

Das, S. and Medda, A. 1988.Effect of cyanocobalamine on protein and nucleic acid contents of ovary of silkworm, *Bombyx mori* L. during larval, pupal and adult stages of development. *Insect Sci. Appl*. **9**: 641-646.

David, B. V. and Ananthakrishnan, T. N. 2006. General and Applied Entomology. New Delhi: Tata McGRaw-Hill Publishing Company Ltd. pp. 89-91.

Goudar, K. S. and Kaliwal, B. B. 2000. Effect of potassium nitrate supplementation on economic parameters of the silkworm, *B.mori* L. *Int J. Indust. Entomol*. **1:** 47-52.

Goyal, S. K., Tenguria, R. K. and Saxena, R. C. 2003. Nutritional management of *Morus alba* for good quality cocoon production. *Inter. J. Chemical Sciences.* **1:** 440-445.

Hou, Y., Xia, Q., Zhao, P., Zou, Y., Liu, H.,Guan, J., Gong, J. and Xiang, Z. 2007a. Studies on middle and posterior silk glands of silkworm (*Bombyx mori*) using two-dimensional electrophoresis and mass spectrometry. *Insect Biochem. Mol. Biol*. **37:** 486-496.

Hou, Y., Zhao, P., Liu, H. L., Zou, Y., Guan, J. and Xia, Q. Y. 2007b. Proteomics analysis of fat body from silkworm *(Bombyx mori). Sheng. Wu Gong. Cheng. Xue Bao.* **23:** 867-872**.**

Hugar, I. I. and Kaliwal, B. B. 1997. Effect of nickel chloride supplementation on some economic traits of the bivoltine silkworm, *B. mori* L. *Bul Sericult. Res*. **8:** 23-27.

Ito, T. 1978. Silkworm Nutrition. In the silkworm an important laboratory tool. (Ed),Y Tazima (Tokyo: Kodansha Ltd.). pp. 121- 157.

Ito, T. and Niminura, M. 1966a. Nutrition of silkworm *Bombyx mori*. Its specific requirement and its nutrition in relation the mineral nutrition of its host plant mulberry, *Morus Indica* L. *Indian J. Exptl.Bio*. **4:** 31-36.

Ito, T. and Nirminura, M. 1966b. Nutrition of silkworm *Bombyx mori.* XII Nutritive effects of minerals *Bul. Sericult. Expt Sta*. **20:** 373.

Jin, Y. X., Chen, Y. Y., Xu, M. K. and Jiang, Y. H. 2004. Studies on middle silk gland proteins of cocoon colour sex-limited silkworm (*Bombyx mori*) using two-dimensional polyacrylamide gel electrophoresis. *J. Biosci.* **29:** 45-49.

Kavitha, S., Sivaprasad, S. and Yellama, K. 2009. The effect of zinc and potassium on the silk gland protein profiles and economic parameters of the cocoon in the silkworm, *Bombyx mori*. *Proceedings of XXII Annual Conference of National Environmental Science Academy*. **2**: 135-143.

Kavitha, S., Sivaprasad, S. and Yellama, K. 2011. Determination of minimum effective dose of zinc chloride for modulation of metabolism and silk production in the silkworm, *Bombyx mori. The Bioscan***. 6:** 147-152.

Kochi, S. C. and Kaliwal, B. B. 2005. Synergetic effect of minerals mixture of potassium bromide and nickel sulphate on the economic traits of CSR_2, CSR_4 and CSR_2 x CSR_4 cross breed races of the silkworm, *Bombyx mori* L. *Int. J. Indust. Entomol*. **10:** 107-117.

Krishnaswami, S. 1986. New technology of silkworm rearing. Central Sericultural Research and Training Institute, Mysore, India.

Kumararaj, S., Vijayaraghavan, S. and Krishnaswami, S. 1972. Studies on Fortification of mulberry leaves for feeding silkworms. *India J. Seric*. **11:** 68-72.

Lix, H., Wu, X. F., Liu, J. M., Li, G. L. and Miao., Y. G. 2006. Proteomic analysis of the silkworm (*Bombyx mori L)* haemolymph during developmental stage. *J. Proteome Res.* **5:** 2809-2814.

Lowry, O. H., Rosenbrough, N. J., Farra, L. and Randall, R. J. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem*. **1933:** 265-275.

Magadum, S. B., Holli, M. A. and Magadum, V. B. 1992. Effect of the feeding copper sulphate on the economic parameters of the polyvoltine silkworm *Bombyx mori* L. *Sericologia*. **32:** 395-399.

Magadum, S. B. 1987. Studies on the effect of some chemicals and hormones on the gonads and the life cycle of the silkworm, *B. mori* L. Ph. D thesis, Karnataka University, Dharwad, India.

Nakahara, Y., Shimura, S., Ueno, C., Kanamari, Y., Mita, K., Kiuchi, M. and Kamimura, M. 2009. Purification and characterisation of silkworm haemocytes by flow cytometry. *Dev. Comp. Immunol*. **33:** 439 – 448.

Narasimhamurthy, C. V. and Govindappa, S. 1988. Effect of cobalt on silkworm growth and cocoon crop performance. *Indian J. Seric*. **27**: 45-47.

Neto, J. B., Stefan, V., Mendonca, B. B., Bloise, W., Ana Valeria, B. and Castro, M. D. 1995. The essential role of zinc in growth. *Nutrition Research*. **15:** 335-358.

Nirwani, R. B. and Kaliwal, B. B. 1995. Effect of ferrous sulphate and magnesium sulphate supplementation on some commercial characters of silkworm *B.mori. Bull. Ser. Res*. **6:** 21-27.

Qader, M. A., Sarker, A. A., Rab, M. A. and Barman, A. C. 1993. Effects of Potassium iodide salt and iodized salt on the larval growth and cocoon characters of the silkworm, *B.mori* L. Breed BSR-832, *Sericologia*. **33:** 495-598.

Rahmathulla, V. K. 2007. Effect of VAM fungi and bacterial biofertilizers on mulberry leaf quality and silkworm cocoon characters under semiarid conditions: *Caspian J. Env. Sci*. **5:** 111-117.

Rahmathulla, V. K., Suresh, H. M., Mathur, V. B. and Geethadevi, R. G. 2002. Feed conversion efficiency of Elite bivoltine CSR hybrids silkworm *Bombyx mori* L. reared under different environmental condtions. *Sericologia*. **42:** 197-203.

Sailaja, B. and Sivaprasad, S. 2010a. Photoperiodic modulation of circadian rhythms in the Silk gland protein profiles of *Bombyx mori* and its influence on the silk productivity and quality. *J. Appl. and Nat. Science*. **2:** 48-56.

Sailaja, B. and Sivaprasad, S. 2010b. Photoperiodic modulation of circadian protein rhythm in the silk gland of *Bombyx mori* during fourth instar development. *The Bioscan*. **5:** 177-183.

Scott, R. C., Schuldiner, O. and Neufeld, T. P. 2004. Role and regulation of starvation – induced autophagy in the *Drosophyla* fat body. *Dev. Cell*. **7:** 167-178.

Sivaprasad, S. and Murali Mohan, P. 2009a. Neuromuscular systems in the fifth instar larva of the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae): I-Cephalothoracic musculature and its innervation. *J. Appl. and Nat. Sci.* **1:** 201-209.

Sivaprasad, S. and Murali Mohan, P. 2009b. Neuromuscular systems in the fifth instar larva of the silkworm, *Bombyx mori*. (Lepidoptera: Bombycidae): II- Abdominal musculature and its innervation. *J. Appl. and Nat. Sci*. **1:** 210-226.

Sivaprasad, S. and Sailaja, B. 2011. Photoperiod-modulated instar-specific clock-shifting in the circadian protein and amino acid rhythms in the larval segmental muscle of *Bombyx mori*. *J. Appl. and Nat. Science*. **3(2):** 176-188.

Willott, E. and Trans, H. Q. 2002. Zinc and *Manduca Sexta* hemocyte functions. *J. Insect Sci*. **2:** 6.

Wright, M. D. 1984. Zinc: effect and interaction with other cations in the cortex of the rat. *Brain Research*. **311:** 343-347.

Yamaoka, K., Hoshino, M. and Hirai, T. 1971. Role of sensory hairs on the anal papillae in oviposition behaviour of *Bombyx mori*. *J. Insect Physiol*. **47:** 2327–2336.

Zhang, P. B., Aso, Y. K., Jikuya, H., Kusakabe, T., Lee, J. M., Kawaguchi, Y., Yamamoto, K., Banno, Y. and Fujii, H. 2007. Proteomic profiling of the silkworm skeletal muscle proteins during larval-pupal metamorphosis. *J. Proteome Res*. **6:** 2295-2303.

Zhang, P. B., Aso, Y. K., Yamamoto, B. Y., Wang, Y.Q., Tsuchida, K.Y., Kawaguchi and Fujii, H. 2006. Proteome analysis of silk gland proteins from the silkworm, *B. mori*. *Proteomics*. **6:** 2586-2599.

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