Available online at www.ijpab.com

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **1 (5):** 11-18 (2013)
Research Article

International Journal of Pure & Applied Bioscience

The Promontory role of trace element and nutrients on Morphometric Traits in the silkworm, *Bombyx mori* (L)

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ABSTRACT

The present study has been aimed at examining the stimulatory effect of Zinc, Pyridoxine, Methoprene and Mixed dose on Morphometric Traits of silkworm such as Total body weight, Silk Gland weight and Gland-Body Ratio of V-instar larvae. The experimental worms were divided in to four groups and fed with mulberry leaves soaked in the selected compounds i.e. Zinc chloride, Pyridoxine, Methoprene and with Mixed dose (Zn+B6+H). The Control group of silkworm larvae was fed with normal mulberry leaves. All groups of silkworm larvae were fed four times in a day throughout the 5th instar larval period. Both Control and Experimental silkworm larvae were weighed daily and sacrificed on selected days to collect the Silk Glands from silkworm larvae and the weight of Silk Glands were also recorded daily. Cumulatively, the findings of the present study finally suggest that Mixed dose induced the Morphometric Traits, creating the conditions that are highly congenial for Silk Gland growth and silk production.

Key words: *Morphometric Traits, Silk Gland, Pyridoxine, Methoprene.*

INTRODUCTION

Morphometric data is an important source of information to understand many biological phenomena. The use of morphological measurements has been widespread in studies of phylogenetic relationships [1], evolution [2], reconstruction of history and structure of past populations [3], body condition [4], growth [5], life histories [6], animal behaviour [7]. Morphometrics [8] refer to the quantitative analysis of form, a concept that encompasses size and shape. Morphometric analyses are commonly performed on organisms and are useful in analyzing the impact of mutants on shape, developmental changes in form, co-variances between ecological factors and shape, as well as estimating quantitative-genetic parameters of shape. Morphometrics can be used to quantify a trait of evolutionary significance and by detecting changes in the shape, deduce something of their ontogeny, function or evolutionary relationships. A major objective of morphometric is to statistically test hypotheses about the factors that affect shape.

Fortification of food with certain vitamins successfully tried as a prophylactic measure in silkworm [9]. Supplementation with vitamin B increased the resistance against poor environmental conditions and increased body weight in silkworm [10]. Riboflavin enhances the silk production and reduced the uric acid excretion and choline and its derivatives sprayed on mulberry leaf and feeding to silkworm enhanced the fiber yield [11]. Ascorbic acid is reported to enhance the larval survival rate Nutritional background of the larval stage is significantly influenced by the status of the resulting larva, pupae, adult and silk fiber [12].

In view of these earlier reports, the present study, the Morphometric traits in different experimental groups of v-instar silk worm larvae were assessed. The Morphometric traits were recorded for all groups of silk worm larvae on selected days viz., 1st, 3rd, 5th and 7th days.

RESULTS

(Table: 1; Figs: 1 to 3)

The total body weights and silk gland weights of control and experimental groups of silk worm larvae were recorded using a digital balance at selected time periods. The results revealed that the control silk worm larvae showed a gradual increase in their body weights from day-1 to day7. When compared to the control ones, the mixed dose treated larvae at all selected groups altered significantly the morphological traits of the silkworm such as the body weight, silk gland weight and silk gland-body ratio at all selected time periods whereas the Zinc, Pyridoxine and Methoprene treated larvae gained significantly less weight with respect to the Mixed dose. But when compared to the control all the four experimental groups gained significant weights from day-1 to day-7 during the 5th instar larval stage, as shown in the table -1.

Control silkworms:

As seen from the data, it was obvious that both the weight of the worm body and the silk gland recorded continuous increase from first day to seventh day. There was approximately a fivefold gain in the weight of the body and the silk gland by seventh day.

Experimental silkworms Treated with Zinc:

After treatment with 2μg/ml of Zinc chloride, significant increase in both the total body weight and the weight of the silk gland were recorded in the silk worms from day 1 to day 7 against the control worms of the corresponding days. As in controls, the gain in the weight of Total body and Silk gland was approximately 5 folds. Further, on any given day either total body weight or silk gland weight was 2 times more than in controls.

Experimental silkworms Treated with Pyridoxine:

A similar trend in the weight gain of total body and silk gland was recorded in Pyridoxine treated silkworms. However, it was slightly higher when compared to 2μg Zinc treated silk worms on all selected days during the 5^{th} instar larval stage.

Experimental silkworms Treated with Methoprene:

Similar to the results obtained on silkworms treated with Zinc chloride and Pyridoxine, Methoprene (2μg) also, induced a significant, increase in the weight of the total body and silk gland compared with controls. One interesting observation was that the weight gain in silkworms treated with Methoprene is much higher than that of Pyridoxine and Zn treatment.

Experimental silkworms Treated with Mixed dose:

As in the case of Zinc chloride, Pyridoxine and Methoprene treated worms, Mixed dose also caused continuous increase in the weight of the total body and the silk gland from day 1 to day 7. When compared with these groups, there was much increase in the weight of the body and the silk gland of silkworms treated with Mixed dose on any day throughout the 5^{th} instar larval period.

A phenomenal gain in the morphometric traits of the silkworm was noticed in all experimental groups. The body weight, the silk gland weight and the gland-body ratio showed significantly increasing trend in all the experimental groups, in the following order.

Control < E1 (Zn) < E2 (B6) < E3 (H) < E4 (Zinc + Pyridoxine + Methoprene)

i. e. highest elevation was recorded in silkworms treated with Mixed dose.

Table -1: Morphometric changes in the Total Body Weight and Silk Gland and their ratios in control and different groups of 5th instar Silkworms

DAY OF V INSTAR		CONTROL			E1 (Zn)			E2 (Vit.B6)			E3 (H)			E4 (Zn + E6 +H)		
		BOBY Wt	SG-Wt	SG-BODY RATIO	BOBY Wt	SG-Wt	SG-BODY RATIO	BOBY Wt	SG-Wt	SG-BODY RATIO	BOBY Wt	SG-Wt	SG-BODY RATIO	BOBY Wt	SG-Wt	SG-BODY RATIO
1	**Mean**	2.86	0.06	2.09	4.64	0.18	3.9	4.72	0.19	4.02	5.01	0.28	5.58	5.43	0.51	9.40
	PC	-	-	-	(62.24)	(200.0)	(86.60)	(65.03)	(216.0)	(92.34)	(75.17)	(366)	(166)	(89.86)	(750)	(349)
	SD	±0.01	±0.01	±0.1	±0.01	±0.01	±0.1	±0.01	±0.01	±0.02	±0.01	±0.01	±0.1	±0.01	±0.01	±0.1
3	**Mean**	5.18	0.21	4.05	9.01	0.68	7.5	10.01	0.87	8.70	10.72	0.91	8.58	11.23	1.25	11.13
	PC	-	-	-	(73.94)	(223)	(85.18)	(93.24)	(314)	(114)	(106)	(333)	(111)	(116)	(495)	(174)
	SD	±0.01*	±0.01*	±0.1*	±0.01*	±0.01*	±0.1*	±0.01*	±0.01*	±0.1*	±0.01*	±0.01*	±0.1*	±0.01*	±0.01*	±0.1*
5	**Mean**	9.61	1.02	10.61	15.97	2.50	16.3	16.43	2.55	15.52	18.05	3.02	16.73	18.75	3.25	17.33
	PC	-	-	-	(65.45)	(145)	(53.63)	(70.96)	(150)	(46.28)	(87.82)	(196)	(57.6)	(95.1)	(218)	(63.3)
	SD	±0.01*	±0.01*	±0.1*	±0.01*	±0.01*	±0.1*	±0.01*	±0.01*	±0.1*	±0.01*	±0.01*	±0.1*	±0.01*	±0.01*	±0.1*
7	**Mean**	14.12	2.45	17.35	18.25	4.08	22.4	19.24	4.28	22.24	21.72	5.01	23.07	22.21	5.43	24.45
	PC	-	-	-	(29.25)	(66.53)	(29.11)	(36.26)	(74.69)	(28.18)	(53.82)	(104)	(32.9)	(57.29)	(121)	(40.9)
	SD	±0.01*	±0.01*	±0.1*	±0.01*	±0.01*	±0.1*	±0.01*	±0.01*	±0.1*	±0.01*	±0.01*	±0.1*	±0.01*	±0.01*	±0.1*

Values are Mean ± SEM of four observations each from tissues pooled from 4 silkworms

Values in parentheses are percent change from control

Values are significantly different from control at $p < 0.01$

Fig-1: Changes in the growth of larval body and silk gland of Control and different Experimental groups of Silkworm

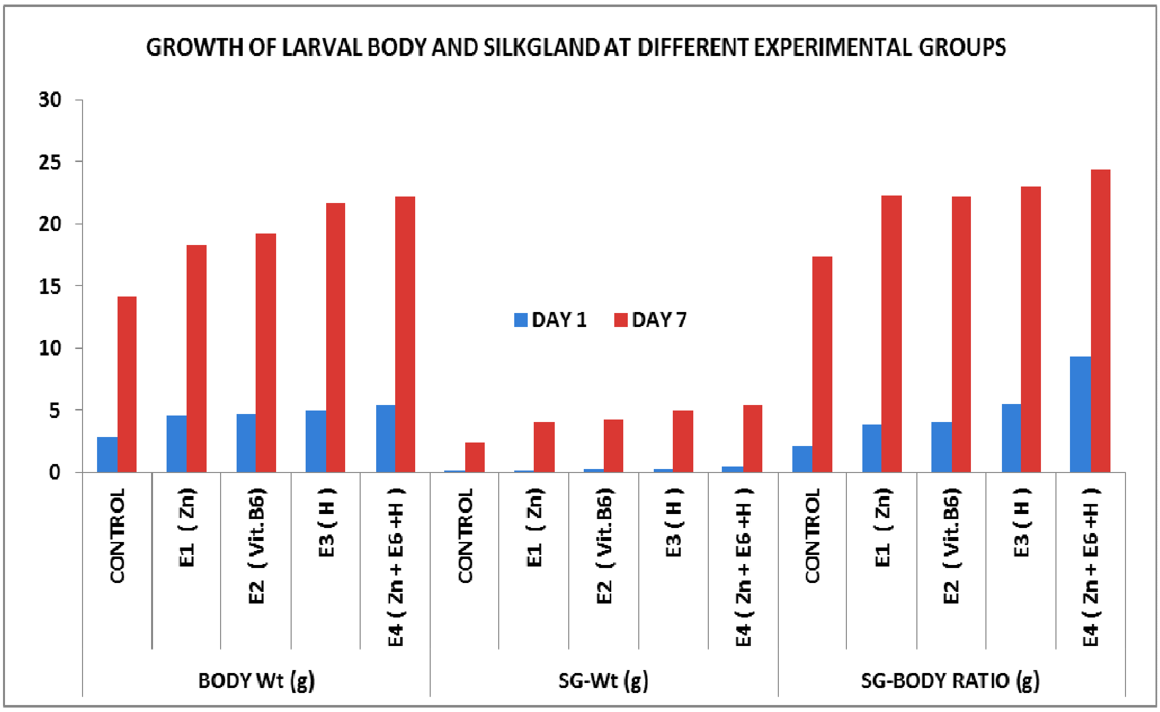


Fig-2: picture showing variation in the body size of 5th instar silkworm lavae from different experimental groups.

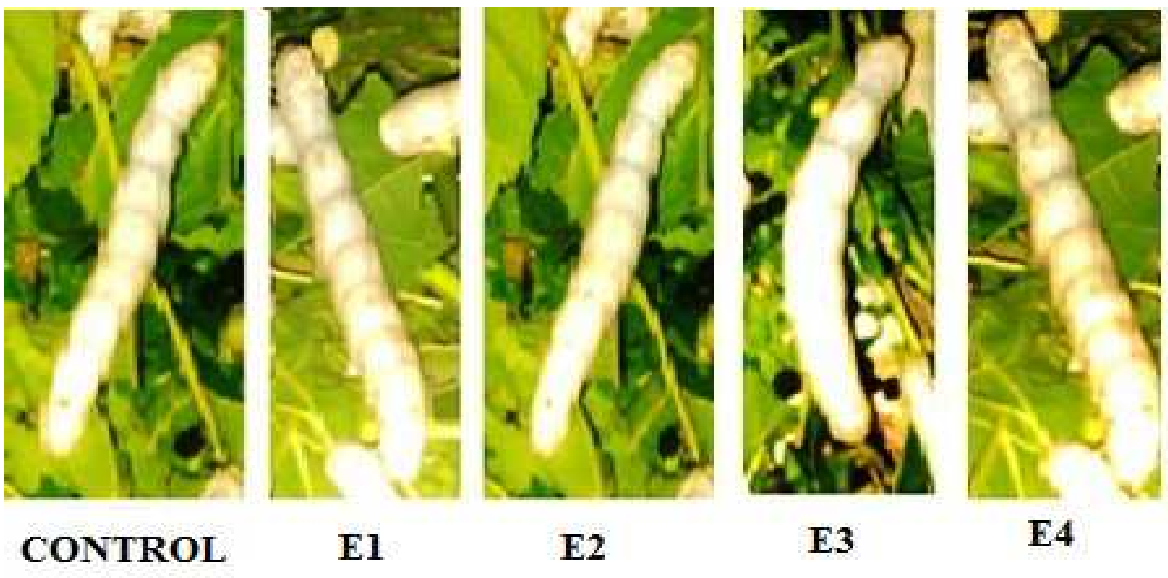
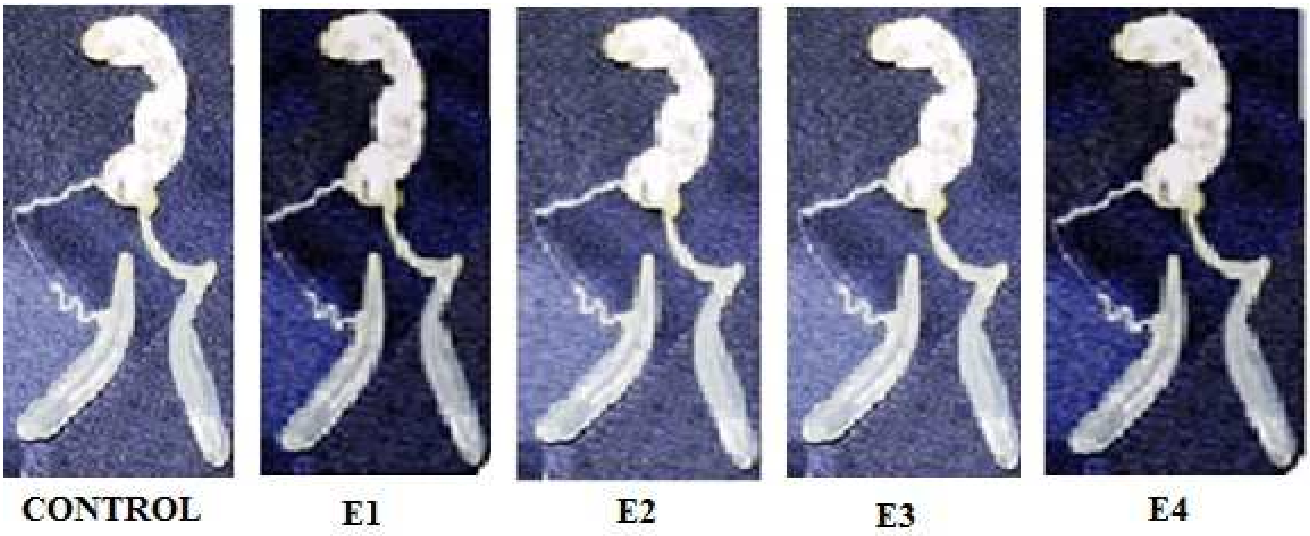


Fig-3: picture showing differences in the size of silk glands from different experimental groups of silk worms



E1 – Zinc **E3 – Methoprene**

E2 – Pyridoxine **E4 – Mixed Dose**

DISCUSSION

Observations on the morphometric traits of the silkworms under different exposure conditions revealed that the fourth experimental group which received the Mixed dose (Zinc + Pyridoxine + Methoprene) treatment exhibited a profound promontory effect on all morphometric traits compared with other experimental groups which received individual doses of these compounds.

The role of nutrients, minerals and a variety of exogenous modulators on the growth and metabolism of silkworm and its impact on the economic parameters of sericulture has been well documented. For instance, the modulatory effect of zinc chloride, nickel chloride [13], ascorbic acid [14], magnesium sulphate, ferrous chloride [15], potassium sulphate [16], folic acid [12], soya bean protein [17], thyroxin [18], on the silkworm metabolism, immune response and silk production has been extensively studied. The ultimate target in sericulture industry is in trough many ways such as replacement of mulberry leaves with leaves of other plants, more easily available around the year, formulations of pastes containing dried mulberry leaf powder, and finally, having fresh mulberry leaves sprayed with or immersed in various additives, such as minerals, vitamins, hormones etc.

Research regarding silkworm nutrition experienced a real progress in recent years because of the development and introduction of artificial diets, which lead to the achievement of important benefits in the textile industry. Silkworm requires certain essential sugars, proteins, amino acids, fatty acid, vitamins and micronutrients for its growth and higher production of good quality of silk. Low productivity is mainly due to low mulberry yield and poor quality of leaf [19]**.** It has been reported that enrichment of mulberry leaves with supplementary compounds enhances the silk productivity**.** Magnesium, calcium, phosphorus, iron, manganese and zinc are the essential salts required by silkworms. Fortification of mulberry leaves with mineral supplementation is reported to play an important role in the larval development and cocoon characters [20, 21]. In silkworms, all developmental stages are controlled over by the neurosecretory system which in turn is regulated by the Central Nervous System. Of all the larval stages, the 5th instar larval stage in the silkworm assumes lot of significance since it is in this stage the silk gland develops rapidly and all the biochemical constituents such as Glycogen, Trehalose, Proteins and Lipids reach their peak levels. Since, the larval stage is the only feeding stage in silkworm development, intake of balanced diet is very essential for silk production. By supplementing the diet with minerals, vitamins and trace elements, the various functions of the hormonal system, neuromuscular system, reproductive system etc., can be modulated effectively.

Insects, like a vertebrate, require a variety of minerals for their growth and development. It has been shown that the mulberry silkworm, *B. mori* requires calcium, iron, magnesium, manganese, phosphorus, potassium and zinc for its growth and development. It has been reported that feeding of mulberry leaves soaked in minerals increases cocoon quality and significantly increases the economic parameters of the silkworm, *B. mori* [22] .The results of the present study showed that the larval weight was significantly increased in all selected experimental groups. Phosphate of lime, magnesium, phosphosphate of iron, natrum phos and phosphate of soda in equal composition [22] and such supportive results are also reported by [20, 21]. In the present study, significant increase in the larval weight in selected experimental groups may be due to the stimulatory effect of Zinc, Pyridoxine, Methoprene or might possibly be due to increased midgut enzyme activity. The results of the present study, therefore, suggest that Zinc, Pyridoxine, Methoprene and Mixed dose have a stimulatory effect on the larval growth of the silkworm, silk gland weight of *B. mori*.

In the present research work, a phenomenal increase in the morphometric traits of the silkworm during the 5th instar larval stage on treatment with selected doses of Zinc, Pyridoxine, Methoprene and Mixed dose revealed that, Zinc exerted significant elevation in the morphometric traits such as total body weight, silk gland weight and silk gland-body ratio were gradually increased in silkworms when treated with Zinc. When compared to this, Pyridoxine caused slight increase in all morphometric traits. The same increasing trend was observed in the case of Methoprene treated silkworm also. The Mixed dose treatment has more pronounced on 7th day when compared to other selected groups and days.

However, a detailed study conducted by [23] , demonstrated that entry of Zinc into the food chain of *Bombyx mori* from mulberry plants irrigated using Zinc containing synthetic effluents caused accumulation of higher concentration of Zinc in larval body, eventually leading to increased mortality of the silkworms. Though, investigations of Zinc on invertebrate are sparse, the requirements of minerals in various insects have been investigated thoroughly. Zinc is essential at low concentration for the activity of several enzymes. Zinc is involved in diverse cellular processes, including catalysis and gene expression and has been implicated as an inhibitor of apoptosis and of oxidative stress. Zinc becomes phytotoxic if it exceeds a maximum soil concentration of 400 mg/kg. Zinc is a good Lewis acid, making it a useful catalytic agent in hydroxylation and other enzymatic reactions. The metal also has a flexible coordination geometry, which allows proteins using it to rapidly shift conformations to perform biological reactions. Two examples of Zinc-containing enzymes are carbonic anhydrase and carboxypeptidase, which are vital to the processes of carbon dioxide (CO2) regulation and digestion of proteins, respectively.

A phenomenal increase in the morphometric traits of the silkworm during the 5th instar larval stage on treatment with Pyridoxine elevated slight increase in the morphometric traits such as the body weight, silk gland weight and silk gland-body ratio. Body weight, silk gland weight and silk gland-body ratio were gradually increased in silkworms when treated with Pyridoxine when compared to Zinc treated group.

Similarly, the vitamin B complex is traditionally made up of 10 members that differ in their biological actions, although many participate in energy production from carbohydrates and fats. The optimal levels of essential vitamins such as biotin, choline, pyridoxine, panthotinate, inositol, riboflavin, thiamine, nicotinic acid have been determined by several research findings. An excellent review by [19] reported that enrichment of mulberry leaves with various vitamins have different effects on economic traits and biological parameters of the silkworm and trace metals even essential ones, are toxic when present above threshold levels. Pyridoxine is necessary for the proper functioning of over 60 enzymes that participate in amino acid metabolism. It also participates in carbohydrate and fat metabolism. Without pyridoxine or its derivatives no larva reached the third instar under aseptic condition. Pyridoxine is important in protein metabolism. **Banerjee and Khan**[24]**,** observed that Pyridoxine enhances the oviposition of the silk worm.

From the results of the present study, it was obvious that a phenomenal increase in the morphometric traits of the silkworm during the 5th instar larval stage on treatment with Methoprene elevated significantly. The changes in the morphometric traits such as the body weight of the silk worm, silk gland and silk gland-body ratio were estimated in the 5th stage silkworm treated with Methoprene for 7 days. Body weight, silk gland weight and silk gland-body ratio were gradually increased in silkworms when treated with Methoprene when compared to Zinc, Pyridoxine treated groups.

Similarly, juvenile hormone analogues (JHA) are known to prolong the larval life in insects and these have been long utilized for the improvement of silk production in the silk worm *B. mori* (L). In the last two or three decades, a number of newer JHA compounds have been developed: and many investigators have tried to study the effect of JHA compounds have on various hybrids of silk worms to elucidate the contribution of varied JHA formulations in increasing the yield. JH is secreted during the fourth ecdysis and then disappears from the haemolymph during early days of the fifth instar. Following this, JH titer increases gradually from day 5 until pupation [25].

The results of the present study indicated that the increase in the silk gland weight at lower concentration of Zinc, Pyridoxine, Methoprene and Mixed dose have stimulatory effect of silk gland which might possibly be due to their stimulatory effect on protein synthetic activity of the silk gland. Zinc elevated the body weight, silk gland weight as well as body-silk gland ratio. In Pyridoxine treated group, significant elevation was noticed. When compared to these groups, more elevation was recorded in the Methoprene treated group. In the Mixed dose treated group the morphometric traits such as body weight, silk gland weight as well as body-silk gland ratio were more pronounced on 7th day, when compared to other experimental groups thus indicating the cumulative effect of these compounds on morphometric traits.

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