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M Younus Wani
Temperate Sericulture Research Institute, Mirgund, SKUAST-Kashmir, J&K, India

RA Rather
Division of Environmental Sciences, SKUAST-Kashmir, J&K, India

M Bashir
Temperate Sericulture Research Institute, Mirgund, SKUAST-Kashmir, J&K, India

S Shafi
Temperate Sericulture Research Institute, Mirgund, SKUAST-Kashmir, J&K, India

S Rani
Temperate Sericulture Research Institute, Mirgund, SKUAST-Kashmir, J&K, India

Correspondence
M Younus Wani
Temperate Sericulture Research Institute, Mirgund, SKUAST-Kashmir, J&K, India

Effect of zinc on the larval growth and quality cocoon parameters of silkworm (*Bombyx mori* L.): A review

M Younus Wani, RA Rather, M Bashir, S Shafi and S Rani

Abstract
Silkworm is an economic insect producing valuable silk. All insects require a variety of minerals and trace elements as micronutrients. However, caterpillars are known to require appreciable amounts of potassium (8000-9000 ppm DW), phosphate (2000- 6000 ppm), and magnesium (1000 ppm) (Muniandy, 2001). The role of mineral nutrition, more particularly that of Zinc, needs to be ascertained as it is known to play a vital role in the synthesis of lipids, proteins and carbohydrates and in reducing the duration of larval and pupal stages (Bhattacharya and Kaliwal, 2005). Such studies provide substantial evidences for practical application of Zinc and other microelements for qualitative and quantitative improvements in silk production. The cocoon spinning activity is an important phase in the silkworm which produces the cocoons, the final product of the insect. This activity which lasts for 5 to 6 days requires continuous function of the nervous system and the muscular system where the Cholinergic and Glutametargic neurotransmitters play key roles. 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. Zinc fortified mulberry leaves increases the fecundity of silkmoths after feeding to silkworms and also quality parameters of cocoons like higher shell cocoon ratio, silk-body ratio, raw silk percentage, denier and renditta and secondly it lowers the floss-shell ratio by decreasing the floss protein synthesis.

Keywords: Effect of zinc, quality cocoon parameters, *Bombyx mori* L.

Introduction
Sericulture is both an art and science of raising silkworms for silk production. India's traditional and culture bound domestic market and an amazing diversity of silk garments that reflect 'geographic specificity' gave India the status of being the second largest producer of raw silk after china. Nutrition plays an important role in improving the growth and development of the silkworm, *Bombyx mori* L. and the silk production is dependent on the larval nutrition and nutritive value of mulberry leaves and finally in producing good quality cocoons. Previous studies have been focused mainly at measuring the usefulness of the leaves or their specific components and the results so far obtained on the silkworm nutrition (Hanson *et al*., 2004) [15]. Fortification of mulberry leaves with supplementary nutrient (Muniandy *et al*., 2001) [23] and feeding silkworms is a useful modern technique to increase economic value of cocoon. Hence, it is the concern of every researcher to use the best ingredients to launch the premium silk products and also other Seri-byproducts in the market for the consumers. All insects require a variety of minerals and trace elements as micronutrients. Mineral nutrition has been neglected compared with other nutrient requirements, and the quantitative requirements for insects are largely unknown. However, caterpillars are known to require appreciable amounts of potassium (8000-9000 ppm DW), phosphate (2000- 6000 ppm), and magnesium (1000 ppm) (Muniandy, 2001) [23]. The minerals play a crucial role in osmotic pressure regulation of the intra- and extra-cellular liquids and participate as co-factors in different enzyme systems. Islam *et al.* (2004) [16] studied the effect of nickel chloride supplementation on silkworm growth. The nickel chloride dissolved in water at a rate of 100, 200, 400, 800 and 1600 ppm. 3 replicates were carried out for each concentration, each group being consisted of 100 larvae. The treated leaves were administered in the 4th and 5th age. At the end of the experiment, the most important increase of the larvae weight was recorded in the group that received nickel chloride 800 ppm, the lowest growth being recorded at a concentration of 1600 ppm nickel chloride.

The cocoon weight increased as the concentration of nickel chloride increased; the use of nickel chloride at a dose of 1600 ppm reduces the weight of the cocoon, as this concentration is having an inhibitory effect on this species. Bhattacharya *et al*. (2005) [2] studied the effect of potassium chloride on silkworm larvae (*Bombyx mori* L.). After supplementation with 50, 100 and 150 ppm potassium chloride solution, the following parameters were recorded: glycogen and total protein from fat bodies and glycogen and total lipids from haemolymph. An increase in total protein, total lipids and glycogen from haemolymph and fat bodies was recorded. The administration of organic Selenium, Zinc nitrate and potassium nitrate to the silkworm larvae, in doses of 100, 200 and 400 ppm. The organic selenium positively influenced the body weight gain of the larvae with increasing dosage (100-400 ppm); the same effect was reported in the case of the other minerals. The weight of the crude cocoon, the silk increment and the total protein content of the haemolymph increased; the silk thread had a greater length and resistance, having a higher content of fibroin as a result of mineral supplements administered. The larvae fed with mulberry leaf supplemented with minerals, had a significant increase of the final body weight compared to the control group, at the end of the 5th age. Also, an increased content of total protein was recorded in all groups that received mineral treatments (Etebari and Fazilati, 2003) [13]. The minerals administered to silkworms determined an increase of the length and weight of the larvae, an improved food consumption and food utilization (Khan *et al*., 2010) [18]. Mulberry silkworm, *Bombyx mori* L. (Lepidoptera, Bombycidae), in particular is a very sensitive and choosy for its food habit. Silkworms requires certain essential sugars, proteins, amino acids, fatty acids, vitamins and micro nutrients for its normal growth, survival and for growth, survival and for growth of silk gland and higher production of good quality silk. Silkworm nutrition is considered as the major area of research from the scientific point of view. It has been suggested that the supplementation with minerals play a vital role in the larval development and cocoon characters**.** It has been reported that magnesium, calcium, phosphorous, potassium, iron, manganese and zinc are essential salt elements required by *B. mori*. Oral supplementation with copper sulphate, nickel chloride and potassium iodide in different concentrations to polyvoltine pure Mysore breed of the Silkworm, *B. mori* L., cupric chloride, zinc chloride, manganese and copper sulphates to eri silkworm increases the economic parameters and larval weight respectively. Feeding with calcium, magnesium sulphate, iron, potassium iodide and iodized salt, zinc, nickel, manganese and ferrous sulphate to silkworm have been shown to increase the commercial characters and decrease the larval duration. Mihai *et al*. (2011) [22] aimed to evaluate the influence of Se and Zinc, administered as food supplements for silkworm (*Bombyx mori* L.) on the larvae weight, the weight of the serigene gland and on some reproduction indices (prolificacy and fecundity). The research has been carried out on 9 groups of silkworms one control group (G1) and 8 experimental groups (4 groups that received zinc and 4 groups that received Selenium), consisting of 50 larvae/group. Zinc was administered to 4 experimental groups, in doses of 17, 34, 68 and 136 mg kg-1 larvae (G2-G5). Selenium was administered to the other 4 experimental groups, in doses of 4, 8, 16 ad 32 mg kg-1 body weight (G6-G9). The minerals were mixed with water to form a solution and the solution was pulverized on the mulberry leaf. The larvae were weighted in the 1st, 4th and 7th day from the 5th age. The serigene glands were collected in the 7th day of the same age and the reproduction parameters were analyzed a few days after the eggs were laid down by the females. The use of Zinc determined a very significant increase of the larvae weight, to group G5 followed by groups G4, G3 and G2 compared to the control group. The use of Zinc positively influenced the mass of the serigene glands, the number of eggs laid, and the number of fertilized eggs laid by the female silkworms. The use of organic Selenium determined an increased larvae weight, the differences being very significant compared to the control group. Selenium influenced the weight of the serigene gland, the number of eggs laid and the number of fertilized eggs. Zinc is an essential element both for plants and animals, but if present in high concentration in plants and animals it can also be toxic. Hence, Toxicity evaluation of zinc will be useful in final evaluation of designing, safe level (or) tolerable level of zinc to the animals. It helps in making programme for Sericulture rearing. Lethal dose (LD50) is common measure of toxicity that causes death in 50 percent of the test population LD50 is expressed as the dose in milligram (mg) of ten per killogram (kg) of body weight. The silkworm larvae must be separated from chemical environment through the membrane lithe integument, the epithelium of the intestine and epithelium of the respiratory tract. The II and III instar larvae are sensitive to chemical substances even at lower doses. Hence, the toxicity studies are to be carried out in IV and V instars of silkworm larvae. Many reports and now available on toxicity studies on various insects including *B. mori* (Kuwana *et al*., 1967 and Venkat Reddy *et al*., 1989) [35]. LD50 values are an important parameter to evaluate the toxicity level and also to determine the sublethal doses, the present toxicity studies begin with 48 hrs. LD50 of zinc CSR2 x CSR4 bivoltine silkworm race. Varaprasad and Rao, (2017) [33] carried out an experiment for determination of Zinc LD50 to CSR2XCSR4 race of silkworm by dose response curves. The lethal dose of zinc alone is not sufficient in assessing various responses of the CSR2XCSR4 race of silkworm to the trace element Zinc. For CSR2 X CSR4 race of silkworm both lethal and sub lethal doses of zinc has negative impact and sub-sub lethal dose has positive on growth and productivity of silkworm.

A detailed study conducted by Ashfaq *et al*. (2010) [1] demonstrated that the 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. An excellent review by Rajabi *et al*. (2006) [27] 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. The quantitative requirements of minerals and trace elements as micronutrients for insects are largely unknown. Even though many basic biological functions depend on sodium, calcium, and chloride ions, their specific requirement has been difficult (Dunphy *et al*., 2002) [12]. Because of cofactor functions, iron (Nichol *et al*., 2002) [25] and zinc are considered as having almost universal importance to insects in general. Zinc is the only heavy metal that shows amphoteric properties and is freely soluble in water and alkalis to form zincates. It has multiple biological functions such as, an integral constituent of over 20 different enzymes including DNA and RNA polymerases; confer protection on cells against the actions of a number of toxins and helps in structural and functional maturation of neurons and also in homeostasis under different conditions (Willot and Tran, 2002) [36]. Since 1930s, Zinc has been widely recognized as an important constituent of animal diet and is widely distributed in all animal tissues. In this direction, the role of mineral nutrition, more particularly that of Zinc, needs to be ascertained as it is known to play a vital role in the synthesis of lipids, proteins and carbohydrates and in reducing the duration of larval and pupal stages (Bhattacharya and Medda, 1981). Such studies provide substantial evidences for practical application of Zinc and other microelements for qualitative and quantitative improvements in silk production. Further, no studies are available on the modulatory role of Zinc on neurotransmitters and the associated enzymes of neural function. Varaprasad and Rao, (2017) [33] suggested that Oxygen passes through the tracheal system from the spiracles to the tissues and ultimately must reach the mitochondrial in order to play a part in oxidative processes. There are two district phases in the transport of gases, one through the tracheal system, known as air-tube diffusion, and one through the tissues in solution in the cytoplasm, known as air-tube diffusion, and one through the tissues in the cytoplasm, known as tissue diffusion. The results indicated that the oxygen up take is decreased significantly in silkworm treated with lethal and sub lethal dose. However, in sub-sub lethal dose, the oxygen consumption rate increases significantly. Zinc acts as a suppressor of oxygen consumption whereas at sub sub-lethal dose of zinc acts as an inducer of oxygen consumption. The skeletal muscle of silk worm is known to synthesize and store about 258 proteins (Zhang *et al*., 2007; Sivaprasad and Sailaja, 2011) [31], 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) [11, 29, 30]. Despite some initial elevatory effects (~ 17 to 36% increase), both $ZnCl_2$ and ZnSo4 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. The overall inhibition at the end of fifth instar was ~84% under $ZnCl_2$ and ~48% under ZnSo4, a trend that probably reflects the stimulatory influence of zinc salts on intracellular proteolysis in muscle and fat body. Structural protein levels in the silk gland, haemolymph, fat body and muscle tissues of silk worms treated with different doses of Zinc chloride showed an increasing trend from day 1 to day 7 in all selected tissues and the effect is more pronounced on 7th day at 2µg/ml l. However, at 20µg/ml Zinc chloride, the structural proteins contents in the selected tissues were dropped down to lower levels in all tissues. Similarly, the haemolymph of *B. mori* is the chief circulating fluid and flowing reservoir of about 241 to 298 proteins (Lix *et al.,* 2006) that promote larval growth, metamorphosis, silk production, apoptosis, chitin and haemocyte formation, growth of salivary glands, reproductive organs and ecdysis. Though, the impact of higher doses of zinc on the haemolymph proteins is negative, it exerted significantly positive role at lower doses viz.2µg. The insect fat body plays a key role in the metabolism similar to that of the mammalian liver and adipose tissues. In *B. mori,* it synthesizes and stores over 177 proteins that constitute nine glycolysis related proteins, several metabolically related proteins like diacylglycerol binding protein, triacylglycerol lipase, putative hydrolases, defense proteins etc. From the detailed analysis on the protein profiles of the fat body, it was obvious that the impact of $ZnCl_2$ was opposite to that of silk gland and haemolymph (Nirvani and Kaliwal, 1996). The overall effect of $ZnCl_2$ on muscle proteins was similar to that of the fat body, with higher doses showing less impact and lower doses eliciting a more positive effect. From these observations, it was suggested that the metabolism in this tissue could be positively modulated. Varaprasad and Rao, (2017) [34] exposed the silkworm at lethal, sub-lethal and sub-sub lethal doses of zinc. The results indicated that the rate of heart beat decreased significantly at the lethal dose. (12.26 mg/kg b. w) and sub lethal dose. (2.45 mg/Kg b.w). However, the sub- sub lethal dose of Zinc enhanced slightly over control. It indicates that the metabolic activity of silkworm was increased at a very low level of Zinc ions. The decrease in the lethal and sub- sub lethal dose is due to zinc stimulation. On the whole it can be concluded that the zinc ions might have a role on cardio acceleratory peptides (CAPS).

The rate of heart rate in Vth instar of CSR2 X CSR4 race of silkworm Bombyx mori on exposure to Zinc (Varaprasad *et al*., 2017) [33].

S. No	Dose	No. of pulse/minute
1	Control	46
2	Lethal	35
3	Sub- lethal	38
4	Sub- Sub lethal	52

Lakshmi Devi and Yellamma (2013) [21] investigated various economic parameters of the silkworm cocoon, when fed on mulberry leaves fortified with selected trace element Zinc, vitamin, Pyridoxine and hormone, Methoprene. 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. Cumulatively, the findings of the present study were observed to evaluate the modulatory role of Zinc, Pyridoxine and Methoprene with particular reference to the quality and quantity of the silk. The Mixed dose of Zinc, Pyridoxine and Methoprene on selected days significantly elevated all the selected economic parameters of the cocoons.

Effect of zinc salts on economic traits of sericulture

Sahu *et al*. (2015) [28] aimed to evaluate the growth and antioxidant defense protection of the silkworm (*Antheraea mylitta*) larvae (IInd instar), when fed on *Terminalia arjuna* leaves supplemented with a trace element, Zinc, for 10 days Experimental animals, comprising control (group I; supplemented with distilled water) and the experimental groups (groups II, III and IV) were supplemented with Zinc, sprayed on leaves in doses of 68, 136, 272 mg/kg larval body weight, respectively. Antioxidant defense capacity in the whole-body tissue of *A. mylitta* was found to be modulated with higher activity of Superoxide dismutase and Glutathione S- transferase in the experimental groups. Same trend was observed for total glutathione, reduced glutathione and ascorbic acid content. Results suggest that Zinc plays an important role in augmenting the growth and antioxidant protection of the larvae of *A. mylitta*, which may improve the larval fitness, quality and quantity of silk production. Chamundeswari and Radhakrishnaiah (1994) [10] and Kavitha *et al*. (2009) [17] 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. 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. The effect of $ZnCl_2$ and $ZnSO_4$ on the economic parameters of cocoons was analyzed. In all the 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 ZnSO4. 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) [9], copper sulphate, nickel chloride and potassium iodide (Narasimhamurthy and Govindappa, 1988) [24], ferrous and magnesium sulphates (Nirwani and Kaliwal, 1995) [26], magnesium chloride, potassium chloride and potassium permanganate (Bhattacharya and Kaliwal, 2005b, 2006) [3, 6], potassium nitrate and nickel chloride (Goudar and Kaliwal, 2000) [14], potassium permanganate, potassium and magnesium chlorides (Bhattacharya and Kaliwal, 2005c, 2005d) [4, 5] potassium bromide and nickel sulphate (Kochi and Kaliwal, 2005) [19] and potassium and magnesium carbonates (Chakrabarty and Kaliwal, 2011) [8]. Zhangji *et al*. (1997) [38] suggested that mulberry leaves that were sprayed with different concentrations of zinc sulfate solution and then were fed to the different aged silkworms were conducted. The results showed that the leaves that treated with 0.2% zinc sulfate significantly increased the body weight and the total amount of blood amino acid of silkworm. The whole cocoon weight, cocoon shell weight and cocoon shell rate of the silkworm fed 0.2% of zinc sulfate treated leaves were significantly higher than those of the control. The total number of the spawned eggs and the normal eggs increased significantly. Sridhara and Bhat, (1966) [32] reported that trace elements zinc, copper, manganese, molybdenum and cobalt have been shown to have varying effects on growth and trace element composition of the silkworm. Results indicate the important role of manganese in the normal metabolism of the insect. Cobalt has been shown to exert a very favourable effect on growth and silk yield.

Summary and Conclusions

The role of mineral nutrition, more particularly that of Zinc, needs to be ascertained as it has a number of roles in the synthesis of lipids, proteins and carbohydrates and in reducing the duration of larval and pupal stages. Such studies provide evidences for practical application of Zinc and other microelements for qualitative and quantitative improvements in silk production. For the cocoon spinning activity there is involvement of nervous system and the muscular system where the Cholinergic and Glutametargic neurotransmitters play key roles. 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. Zinc fortified mulberry leaves increases the fecundity after feeding to silkworms. Zinc increases the silk output as reflected from higher shell cocoon ratio, silk-body ratio, raw silk percentage, denier and renditta and secondly 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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