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Minerals as exogenous modulators in enhancing silk productivity of silkworm, *Bombyx mori* L.

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Abstract
Experiments were conducted by supplementing minerals *viz.,* zinc sulphate, magnesium sulphate and potassium chloride with mulberry leaves to improve the silk yield of *B. mori*. *Per os* administration of minerals to silkworm larvae during third, fourth and fifth instars significantly enhanced the larval, cocoon and silk reeling related parameters. Among the different minerals studied, it was found that the treatment with Zinc sulphate @ 100 ppm + Magnesium sulphate @ 200 ppm + Potassium chloride @ 100 ppm recorded significantly higher mature larval weight (4.45 g), ERR (97.16%), cocoon weight (2.30 g), shell weight (0.55 g), pupal weight (1.76 g) and shell ratio (23.19%), filament length (1458 m), filament weight (0.431 g) and silk productivity (6.35 cg/day). The proteins in silk gland (62.70 mg/g) and haemolymph (46.00 mg/g) were also found be higher in the above treatment apart from fibroin (410 mg/shell) and sericin (102.0 mg/shell) contents in cocoons.

Keywords: Minerals, exogenous modulators, silk productivity, *Bombyx mori* L.

Introduction
Sericulture is one of the promising agro-based industries in India, playing an important role in rural development because of its unique characteristics of being labour intensive, having short gestation period and capacity of developing into a family level enterprise requiring limited skill. It provides gainful occupation for more than eight million people in our country either directly or indirectly.

As sericulture is an industry to change the mulberry leaf into silk very efficiently, nutrition plays an indispensible role in improving the growth development and survival of the silkworm. *Bombyx mori* derives its entire nutritional requirement from mulberry leaves due to its monophagous nature and can complete the life cycle successfully on mulberry leaves (Vlaic *et al*., 2004) [28]. Legay (1958) [17] stated that silk production is dependent on the larval nutrition and nutritive value of mulberry leaves which plays a very effective role in producing good quality cocoons. Seki and Oshikane (1959) [23] observed better growth and development of silkworm larvae as well as good quality cocoons when fed on nutritionally enriched leaves. Though the nutrients are balanced in mulberry leaves, the quantity available is not sufficient for robust larval growth and development (Ito, 1978) [15].

The cocoon production mainly depends on the nutrition and health status of silkworm larvae. The supplementation of various minerals was important to increase the cocoon production and quality. The supplementation of various minerals *viz.,* potassium iodide, cobalt chloride and calcium chloride (Dasmahapatra *et al.,* 1989) [9], calcium chloride (Subburathinam *et al.,* 1990) [26], phosphorus (Subburathinam and Sulochanachetty, 1991) [27], nitrogen (Zaman *et al*, 1996) [29], iron (Rai *et al.,* 2002) [22], potassium permanganate (Bhattacharya and Kaliwal, 2004) [5] and zinc chloride (Sivaprasad *et al.,* 2012) [24] has enhanced the biological parameters and cocoon productivity. But, the studies on the effects of mineral combination on silkworm are very limited. With this background, the experiments were undertaken to assess the impacts of minerals and their combination on growth of silkworm and silk productivity.

2. Materials and methods
Experiments were conducted by supplementing minerals *viz.,* zinc sulphate, magnesium sulphate and potassium chloride with mulberry leaves individually and in combination to

improve the silk yield of *B. mori*. The methodology followed and materials used for the study are detailed below.

i) Disinfection of rearing house

Before commencement of rearing, the rearing house and appliances were thoroughly washed and disinfected with 2.5 per cent chlorine dioxide in 0.5 per cent slaked lime solution at 2 lit/m^2 floor area. After disinfection, the rearing house was kept closed in air tight condition for 24 hours and then opened to ward off the smell of chlorine completely (Dandin and Giridhar, 2014) [8].

ii) Silkworm rearing

Rearing of bivoltine Double Hybrid silkworm [(CSR 6 × CSR 26) × (CSR 2 × CSR 27)] was carried out using leaves from V1 mulberry variety as standard method advocated by Krishnaswami *et al.* (1973) [16]. The larvae were fed three times a day and bed cleaning was done as per schedule. As age of the larvae advanced, the required spacing was provided. As prophylactic measure, the bed disinfectant was applied @ 5g/sq.ft bed area to prevent diseases after bed cleaning and 30 minutes before feeding (Baig and Pradip Kumar, 1987) [3].

iii) Preparation of stock solution and application of treatment

A stock solution of 500 ppm was prepared by dissolving 500 mg of mineral in one litre distilled water and from the stock solution, different concentrations *viz.*, 10, 25, 50,100 and 200 ppm were prepared by serial dilution

Weighed quantities of fresh mulberry leaves were separately sprayed with aqueous solution of the respective minerals by using a hand automiser. Two larval batches *viz.*, one with water spray and another with untreated mulberry leaves (Control) were also maintained. The treated leaves were shade dried and fed twice during each third and fourth instars (first and third days) and thrice during fifth instar (first, third and fifth days). Each treatment was replicated thrice with 50 larvae per replication. The experiments were conducted in Completely Randomized Design (CRD).

iv) Estimation of protein

For the analysis of protein content, the haemolymph of silkworm was collected in microtubes, centrifuged at 14000 rpm and after removing the supernatant kept at –20 ºC for analysis (Etebari *et al.*, 2007) [11]. The silk gland was macerated with pestle and mortar in phosphate buffer (pH 7.0) and the supernatant was collected after centrifuging the content at 5000 rpm at 4□ C. The estimation of protein content was carried out by adopting standard procedure (Bradford, 1976) [7].

Sericin and fibroin contents in cocoon were analyzed by taking individual cocoon in a weighed crucible, to which 20 ml of 5 per cent NaOH was added and allowed to remain soaked for 12 hours. The sericin was removed by washing with boiling distilled water twice, leaving behind the fibroin. Then the crucible containing fibroin was oven dried at 90□ C for 24 hours. The percentage of fibroin and sericin was calculated according to Radha and Muthukrishnan (1981) [21].

The different observations on mature larval weight, fifth instar larval duration, effective rate of rearing, cocoon weight, pupal weight, shell weight, shell ratio, silk filament length, silk filament weight, denier and silk productivity were recorded following standard procedure (Gokul, 2015) [12].

v) Statistical analysis

Statistical analysis of data was done using methods suggested by Panse and Sukhatme (1957) [20] and means were compared with Duncun□s Multiple Range Test (Duncun, 1955) [10].

3. Results and discussion

Per os administration of minerals and their combination to silkworm larvae during third, fourth and fifth instars significantly enhanced the larval, cocoon and silk reeling related parameters of *B. mori*.

i) Larval parameters

Among the different treatments studied, it was found that the treatment with Zinc sulphate @ 100 ppm + Magnesium sulphate @ 200 ppm + Potassium chloride @ 100 ppm recorded significantly higher fifth instar larval weight (4.45 g) and ERR (97.16%). This treatment was found to be statistically superior over all other treatments and was followed by Zinc sulphate @ 100 ppm and Potassium chloride @ 100 ppm (4.38 g and 95.10%, respectively). The least fifth instar larval weight (3.85 g) and ERR (89.60%) was registered in control. Here, the minerals enhanced the mature larval weight and ERR by 15.58 and 8.43 per cent, respectively (Table 1).

This result is in agreement with the findings of Ashfaq *et al.* (1998) [1] and Hugar and Kaliwal (2002), who reported the increased larval weight and ERR by supplementation of zinc chloride, calcium chloride and potassium chloride to the fifth instar. Dasmahapatra *et al.* (1989) [9] also reported the highest larval weight and ERR by supplementing mineral mixture.

The fifth instar larval duration was significantly reduced to 162.31 hrs due to the application of Zinc sulphate @ 100 ppm + Magnesium sulphate @ 200 ppm + Potassium chloride @ 100 ppm from 170.31 hrs in control (Table 1). This results fall in line with Balamani *et al.* (1995) [4], Hugar and Kaliwal (1999) [13] and Ashfaq *et al.* (2010) [2] who reported decreased fifth instar larval duration by supplementing minerals at higher concentration.

ii) Cocoon parameters

Among the three minerals studied, the treatment with Zinc sulphate @ 100 ppm + Magnesium sulphate @ 200 ppm + Potassium chloride @ 100 ppm registered maximum cocoon weight (2.30 g), shell weight (0.55 g), pupal weight (1.76 g) and shell ratio (23.19%) which was found to be statistically superior over all other treatments. This was followed by Zinc sulphate @ 100 ppm & Potassium chloride @ 100 ppm combination which recorded the value of 2.25 g, 0.52 g, 1.73 g, and 23.11% respectively for cocoon weight, shell weight, pupal weight and shell ratio, whereas, the minimum value of 2.00 g, 0.42 g, 1.56 g and 21.00 g, respectively was registered in control (Table 2).

Bhattacharya and Kaliwal (2005) [6] observed an increase in cocoon weight due to supplementation of calcium, potassium and zinc salts in the chloride form. Magudam (1987) [18] and Ashfaq *et al.* (2010) [2], reported that supplementation of potassium and zinc chloride at higher concentration has increased the shell weight in the fifth instar. Ashfaq *et al.* (2010) [2] and Sivaprasad *et al.* (2012) [24] reported that zinc chloride application at higher concentration either individually or in combination increased the shell ratio. These findings are in agreement with the present observations.

Table 1: Effect of minerals on larval parameters of *B. mori*

Treatment	V instar larval duration (h)	V instar larval weight (g)	ERR (%)
Zinc sulphate @ 100 ppm	165.49^{bc}	4.24^{c}	92.70^{cd} (75.15)
Magnesium sulphate @ 200 ppm	167.01^{cd}	4.10^{d}	91.33^{e} (73.12)
Potassium chloride @ 100 ppm	166.16^{bcd}	4.21^{c}	92.24^{c} (74.32)
Zinc sulphate @ 100 ppm + Magnesium sulphate @ 200 ppm	164.46^{abc}	4.33^{bc}	94.83^{b} (78.04)
Magnesium sulphate @ 200 ppm + Potassium chloride @ 100 ppm	165.46^{bc}	4.30^{c}	93.50^{bc} (76.10)
Zinc sulphate @ 100 ppm + Potassium chloride @ 200 ppm	164.01^{ab}	4.38^{b}	95.10^{b} (80.48)
Zinc sulphate @ 100 ppm + Magnesium sulphate @ 200 ppm + Potassium chloride @ 100 ppm	162.31^{a}	4.45^{a}	97.16^{a} (82.15)
Water spray	169.01^{de}	3.88^{e}	90.50^{e} (73.10)
Control	170.31^{e}	3.85^{e}	89.60^{e} (71.58)
S.Ed	1.31	0.03	0.86
CD (0.05)	2.64	0.05	1.75

Values are mean of three replications and pooled mean of two rearing.
Means followed by common letters are not significantly different at 5% level by DMRT (P=0.05)

iii) Silk gland and haemolymph proteins

Results of the studies conducted to assess the protein level in silk gland and haemolymph showed that larval batch applied with Zinc sulphate @ 100 ppm + Magnesium sulphate @ 200 ppm + Potassium chloride @ 100 ppm registered maximum silk gland and haemolymph proteins of 62.70 mg/g and 46.00 mg/g, respectively which was found to be statistically superior over all other treatments tested (Table 3).

In case of silk gland protein, the next best treatments were Zinc sulphate @ 100 ppm + Potassium chloride @ 100 ppm and Zinc sulphate @ 100 ppm + Magnesium sulphate @ 200 ppm with the protein content of 59.00 mg/g and 58.02 mg/g, respectively. These treatments were found to be on par among them and statistically differed from all other larval batches (Table 3). The present result on increase in silk gland protein content fall in parallel with findings of Bhattacharya and Kaliwal (2004) [5] and Sivaprasad *et al.* (2012) [24] who reported significant increase in silk gland protein content due to supplementation of potassium and zinc chloride either in individual or in combination when compared with untreated control.

In case of haemolymph protein, the next best treatment was Zinc sulphate @ 100 ppm & Magnesium sulphate @ 200 ppm (43.00 mg/ml) combination which was followed by Zinc sulphate @ 100 ppm & Potassium chloride @ 100 ppm (42.25 mg/ml) combination. The minimum was recorded in control (29.50 mg/ml) (Table 3). High level of haemolymph protein in the present study indicated that the minerals like potassium chloride and zinc chloride are rapidly incorporated into the silk gland leaving low titre value in the haemolymph. This was supported by Bhattacharya and Kaliwal (2005) [6] and Spurgeon *et al.* (2000) [25].

Table 2: Effect of minerals on cocoon parameters of *B. mori*

Treatment	Cocoon weight (g)	Shell weight (g)	Pupal weight (g)	Shell ratio (%)
Zinc sulphate @ 100 ppm	2.10^{d}	0.47^{cd}	1.63^{bc}	22.38^{cd} (28.81)
Magnesium sulphate @ 200 ppm	2.03^{d}	0.45^{cde}	1.58^{c}	22.17^{d} (27.52)
Potassium chloride @ 100 ppm	2.05^{d}	0.46^{cde}	1.59^{bc}	22.43^{bcd} (28.46)
Zinc sulphate @ 100 ppm + Magnesium sulphate @ 200 ppm	2.17^{bc}	0.50^{b}	1.67^{bc}	23.04^{bc} (29.01)
Magnesium sulphate @ 200 ppm + Potassium chloride @ 100 ppm	2.16^{bc}	0.49^{bc}	1.67^{bc}	22.68^{bcd} (28.87)
Zinc sulphate @ 100 ppm + Potassium chloride @ 200 ppm	2.25^{b}	0.52^{ab}	1.73^{ab}	23.11^{b} (29.21)
Zinc sulphate @ 100 ppm + Magnesium sulphate @ 200 ppm + Potassium chloride @ 100 ppm	2.30^{a}	0.55^{a}	1.76^{a}	23.91^{a} (29.40)
Water spray	2.01^{d}	0.43^{de}	1.58^{c}	21.39^{e} (27.08)
Control	2.00^{d}	0.42^{e}	1.56^{c}	21.00^{e} (26.94)
S.Ed	0.04	0.02	0.03	0.33
CD (0.05)	0.10	0.04	0.08	0.68

Values are mean of three replications and pooled mean of two rearing.
Means followed by common letters are not significantly different at 5% level by DMRT (P=0.05)

iv) Fibroin and sericin content

Application of minerals to the silkworm larvae significantly improved the fibroin content in cocoon. Among all the treatments, the mineral combination with Zinc sulphate @ 100 ppm, Magnesium sulphate @ 200 ppm & Potassium chloride @ 100 ppm recorded maximum fibroin content of 410 mg/shell which was found to be superior over all other treatments. This was followed by Zinc sulphate @ 100 ppm + Potassium chloride @ 100 ppm (370 mg/shell) and Zinc sulphate @ 100 ppm + Magnesium sulphate @ 200 ppm (360 mg/shell). The minimum fibroin content of 190 mg/shell was recorded in control (Table 4) The present study corroborates with the findings of Dasmahapatra *et al.* (1989) [9] and Sivaprasad *et al.* (2012) [24], who reported that supplementation of minerals in individual or in combined form increase the fibroin content of the shell.

The mineral also had positive effect on the sericin content in cocoon. Among all the minerals tested, Zinc sulphate @ 100 ppm + Magnesium sulphate @ 200 ppm + Potassium chloride @ 100 ppm registered significantly higher sericin content (102.00 mg/shell) and was followed by Zinc sulphate @ 100 ppm (81.50 mg/shell) and Potassium chloride @ 100 ppm (79.25 mg/shell). The minimum sericin of 51.30 mg/shell was recorded in control (Table 4). It is supported by Subburathinam *et al.* (1990) [26] who reported that

supplementation of minerals led to increase in sericin secretion in the middle region of silk gland.

Table 3: Effect of minerals on protein content of silk gland and haemolymph of *B. mori*

Treatment	Protein content	
	Silk gland (mg/g)	Haemolymph (mg/ml)
Zinc sulphate @ 100 ppm	53.00^{cd}	38.02^{e}
Magnesium sulphate @ 200 ppm	52.03^{d}	36.50^{e}
Potassium chloride @ 100 ppm	54.00^{cd}	38.50^{de}
Zinc sulphate @ 100 ppm + Magnesium sulphate @ 200 ppm	58.02^{b}	43.00^{b}
Magnesium sulphate @ 200 ppm + Potassium chloride @ 100 ppm	56.50^{bc}	40.50^{cd}
Zinc sulphate @ 100 ppm + Potassium chloride @ 200 ppm	59.00^{b}	42.25^{bc}
Zinc sulphate @ 100 ppm + Magnesium sulphate @ 200 ppm + Potassium chloride @ 100 ppm	62.70^{a}	46.00^{a}
Water spray	35.50^{e}	31.00^{f}
Control	32.50^{e}	29.50^{f}
S.Ed	1.63	1.20
CD (0.05)	3.50	2.50

Values are mean of three replications and pooled mean of two rearing.
Means followed by common letters are not significantly different at 5% level by DMRT (P=0.05)

v) Silk productivity and reeling parameters

The *per os* administration of minerals to silkworm significantly enhanced silk productivity and reeling related traits of silkworm. Among the various treatments, Zinc sulphate @100 ppm + Magnesium sulphate @ 200 ppm + Potassium chloride @ 100 ppm recorded maximum silk productivity (6.35 cg/day), filament length (1458 m) and filament weight (0.431 g) which was found to be superior over all the treatments studied. This was followed by Zinc sulphate @100 ppm + Magnesium sulphate @ 200 ppm and Zinc sulphate @100 ppm + Potassium chloride @ 100 ppm (Table 5). The present study synchronizes with the earlier findings of Balamani *et al.* (1995) [4], Hugar and Kaliwal (1999) [13] and Ashfaq *et al.* (2010) [2], who reported that supplementation of zinc chloride led to high silk productivity and increased the filament weight. Bhattacharya and Kaliwal (2004) [5], and Sivaprasad *et al.* (2012) [24] reported that supplementation of zinc chloride and potassium chloride increased the filament length and weight. Tsuneyama and Tanaka (2001) and Kavitha *et al.* (2012) [24], reported that zinc chloride application either individually or in combination increased the silk filament weight. The above findings are also fall in line with the present observations.

Thin denier is a desirable character for the quality silk fibre. The supplementation of minerals also altered the thickness of filament. The minimum denier of 2.49 was registered in Magnesium sulphate @ 200 ppm + Potassium chloride @ 100 ppm which was found to be on par with the treatment, Zinc sulphate @100 ppm + Magnesium sulphate @ 200 ppm + Potassium chloride @ 100 ppm (2.60). On the contrary, the maximum denier was registered in control (2.80) (Table 5). This is supported by Subburathinam *et al.* (1990) [26] and Maqbool (1991) [19] who reported that supplementation of calcium resulted in decreased filament weight leading to fine denier.

Table 4: Effect of minerals on sericin and fibroin content in cocoon of *B. mori*

Treatment	Fibroin (mg/shell)	Sericin (mg/shell)
Zinc sulphate @ 100 ppm	330de	81.50^{b}
Magnesium sulphate @ 200 ppm	310^{e}	72.43^{c}
Potassium chloride @ 100 ppm	340^{cd}	79.25^{b}
Zinc sulphate @ 100 ppm + Magnesium sulphate @ 200 ppm	360^{bc}	61.08^{d}
Magnesium sulphate @ 200 ppm + Potassium chloride @ 100 ppm	350^{bcd}	60.76^{d}
Zinc sulphate @ 100 ppm + Potassium chloride @ 200 ppm	370^{b}	69.10^{c}
Zinc sulphate @ 100 ppm + Magnesium sulphate @ 200 ppm + Potassium chloride @ 100 ppm	410^{a}	102.00^{a}
Water spray	240^{f}	53.05^{e}
Control	190^{g}	51.30^{e}
S.Ed	10.00	3.15
CD (0.05)	21.00	6.80

Values are mean of three replications and pooled mean of two rearing.
Means followed by common letters are not significantly different at 5% level by DMRT (P=0.05)

Table 5: Effect of minerals on silk productivity and reeling parameters of *B. mori*

Treatment	Silk productivity (cg/day)	Filament length (m)	Filament weight (g)	Denier
Zinc sulphate @ 100 ppm	5.43^{cde}	1288^{d}	0.379^{cde}	2.64^{cd}
Magnesium sulphate @ 200 ppm	5.15^{e}	1272^{d}	0.361^{e}	2.55^{ab}
Potassium chloride @ 100 ppm	5.25^{de}	1226^{e}	0.370^{de}	2.66^{cd}
Zinc sulphate @ 100 ppm + Magnesium sulphate @ 200 ppm	5.90^{ab}	1407^{b}	0.408^{b}	2.60^{bc}
Magnesium sulphate @ 200 ppm + Potassium chloride @ 100 ppm	5.55^{bcd}	1393^{b}	0.385^{cd}	2.49^{a}
Zinc sulphate @ 100 ppm + Potassium chloride @ 200 ppm	5.68^{bc}	1330^{c}	0.396^{bc}	2.68^{cd}
Zinc sulphate @100 ppm + Magnesium sulphate @ 200 ppm + Potassium chloride @ 100 ppm	6.35^{a}	1458^{a}	0.431^{a}	2.60^{bc}
Water spray	4.56^{f}	1042^{f}	0.325^{f}	2.71^{de}
Control	4.20^{f}	1024^{f}	0.306^{f}	2.80^{e}
S.Ed	0.17	10.31	0.01	0.04
CD (0.05)	0.37	20.65	0.02	0.09

Values are mean of three replications and pooled mean of two rearing.
Means followed by common letters are not significantly different at 5% level by DMRT (P=0.05)

4. Conclusion

It is crystal clear form the present study that *per os* application of mineral combination having zinc sulphate @ 100 ppm, magnesium sulphate @ 200 ppm and potassium chloride @ 100 ppm through mulberry leaves to silkworm larvae significantly enhances biological, protein, cocoon and silk related traits.

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