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Effect of nano micronutrients on mulberry silkworm, *Bombyx mori* L. for larval and cocoon traits

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Abstract
An experiment was conducted in a well established mulberry garden with V-1 mulberry grown as per package of practices to know the effect of nano micronutrients on silkworms for grainage and silk technological parameters. The experiment was laid out in completely randomized block design with 13 treatments replicated thrice at Sericulture unit, Department of Agricultural Entomology, College of Agriculture, UAS, Raichur, Karnataka during 2018-19. Nano micronutrients were sprayed to mulberry as per treatment details to mulberry on 25th and 35th day after pruning. Mulberry leaves were harvested from 45th day after pruning and fed to silkworms replication and treatment wise from hatching till spinning stage as per standard package. The results indicated that nano ZnO + nano Cu each @ 500 ppm resulted in significant superiority for full grown larval weight, total larval duration, effective rearing rate, fifth instar larval duration, silk productivity and cocoon parameters. Therefore, the nano size of micronutrients and its unique property of more surface area, the nano might penetrate more efficiently and effectively when applied through foliar compared to foliar spray of chemical micronutrients.

Keywords: Nano micronutrients; *Bombyx mori;* Zn and Cu nutrition; larval and cocoon traits

Introduction
The mulberry silkworm, *Bombyx mori* L., is domesticated insect from Bombycidae. The silkworm is an economically important insect and a primary producer of silk. Mulberry silkworm prefers white mulberry leaves, though they may eat other mulberry species. Domesticated silkworms are completely dependent on humans for their survival, as a result of millennia of selective breeding. Sericulture is the practice of rearing silkworms for the production of raw silk, has been under way for at least 5,000 years in China, from where it has spread to India, Korea, Japan and West. Silkworms were unlikely to have been domestically bred before the Neolithic Age. Before then, the tools to manufacture quantities of silk thread had not been developed. Mulberry leaf is a major economic component in sericulture since the quality and quantity of leaf produced per unit area have a direct bearing on cocoon harvest. Mulberry leaf quality plays a predominant role in healthy growth of silkworm, *Bombyx mori* L. and also in improving larval, cocoon, grainage and silk technological traits (Bose and Majumder, 1996) [3]. The silkworm economic traits are greatly influenced by the nutritional status of mulberry leaves fed to silkworms (Krishnaswami *et al*., 1971) [13]. Foliar supplementation of micronutrients has improved the yield and quality of mulberry leaves (Geetha *et al*., 2016) [9]. Presence of all micronutrients in the leaves may improve the plant health, quality and yield and subsequently the healthy growth of silkworms and resulting in increased cocoon yield with quality.

Nanoparticles are smaller in size and have larger surface area, so foliar supplementation of nano micronutrients can result in rapid absorption and utilization to meet the bulk of the nutrient requirement. Nano fertilizers more particularly micronutrient mixtures have received more attention presently. Mulberry as a foliage crop responds well to timely application of nutrients through foliar sprays (Geetha *et al*., 2016) [9]. Further, Bose *et al*. (1995) [4] reported that the increased nutritive status and concentration of micronutrients in mulberry leaves might have stimulated the metabolic activities in silkworm resulting in reduction of larval duration. The increased nutritional status and concentration of micronutrients in mulberry leaves when supplemented through nano micronutrients *viz*., nano ZnO + nano Cu @ 500 ppm (T_{11}) might have stimulated the metabolic activities in silkworm resulting in better growth and development and subsequently good quality cocoon production. Bose *et al*. (1995) [4] and Shankar *et al*. (1990) [17] also reported that feeding of micronutrients treated leaves showed significantly higher mature larval weight, cocoon weight, shell weight, pupal weight and lower melting percentage of pupa compared to control. Therefore the quality of leaves largely determines the performance of silkworms during their development and spinning of quality cocoons. In view of above, the present study was undertaken on effect of nano micronutrients on mulberry silkworm, *Bombyx mori* L. for larval and cocoon traits.

Material and Methods

An experiment was conducted in a well established mulberry garden with V-1 mulberry variety grown as per package of practices to know the effect of nano micronutrients on silkworms for grainage and silk technological parameters. The experiment was laid out in completely randomized block design with 13 treatments replicated thrice at Sericulture unit, Department of Agricultural Entomology, College of Agriculture, UAS, Raichur, Karnataka during 2018-19. Nano micronutrients were sprayed to mulberry as per treatment details to mulberry on 25th and 35th day after pruning. Mulberry leaves were harvested from 45th day after pruning and fed to silkworms replication and treatment wise from hatching till spinning stage as per standard package with 200 larvae per replication (Dandin *et al*., 2003) [6]. Observations were made on full grown larval weight, total larval duration, effective rearing rate, fifth instar larval duration, silk productivity, cocoon weight, shell weight and shell ratio. The parameters *viz*., effective rearing rate, silk productivity and shell weight were calculated. The data were transformed wherever necessary and analyzed statistically as suggested by Gomez and Gomez (1984) [10]. Duncan's multiple range test (DMRT) was applied for comparing the treatment means.

Results

Effect of nano Zinc and nano Copper nutrition to mulberry silkworm, *Bombyx mori* L. for full grown larval weight, total larval duration and effective rearing rate

Significant variations were observed in full grown larval weight (g/10), total larval duration (h) and effective rearing rate (%) due to the application effect of green nanomicronutrient to mulberry and are presented in Table 1. Maximum larval weight was (43.42 g/10 larvae) in the treatment combination of nano ZnO + nano Cu each @ 500 ppm (T_{11}) followed by nano ZnO + nano Cu each @ 1000 ppm (T_{12}) (41.58 g/10 larvae) and nano ZnO @ 500 ppm (T_3) (40.61 g/10 larvae) and larval weight was significantly minimum in control (T_{13}) (34.71 g/10 larvae). The order of superiority for the rest of the treatments was $T_4 > T_6 > T_7 > T_1 > T_5 > T_2 > T_8 > T_{10} > T_9$ (36.73 to 39.18 g/10 larvae) in first rearing (Table 1).

During second rearing, significantly maximum full grown larval weight (37.95 g/10 larvae) and was observed in nano ZnO + nano Cu each @ 500 ppm (T_{11}) which was on par with nano ZnO + nano Cu each @ 1000 ppm (T_{12}) (37.59 g/10 larvae), nano ZnO @ 500 ppm (T_3) (37.55 g/10 larvae), Zn @ 1000 ppm (T2) (37.50 g/10 larvae), nano ZnO @ 1000 ppm (T_4) (36.92 g/10 larvae), Cu @ 1000 ppm (T_6) (36.77 g/10 larvae) and Zn + Cu @ 1000 ppm each (T_{10}) (36.07 g/10 larvae). Larval weight was significantly minimum in control (T_{13}) (28.58 g/10 larvae). The order of superiority for the rest of the treatments was $T_8 > T_1 > T_7 > T_9 > T_5$ (33.97 to 35.11 g/10 larvae) (Table 1).

Similarly in pooled data significantly maximum larval weight was recorded in nano ZnO + nano Cu each @ 500 ppm (T_{11}) (40.68 g/10 larvae) which was on par with nano ZnO + nano Cu each @ 1000 ppm (T_{12}) (39.58 g/10 larvae) followed by nano Zn @ 500 ppm (T_3) (39.08 g/10 larvae), T_4 (38.05 g/10 larvae) and T_6 (37.92 g/10 larvae). The significantly lowest larval weight was recorded in control (T_{13}) (31.65 g/10 larvae). The order of superiority for the rest of the treatments was $T_2 > T_1 > T_7 > T_{10} > T_8 > T_9$ (35.50 to 37.54 g/10 larvae) (Table 1).

Significantly shorter total larval duration was noticed in nano ZnO + nano Cu each @ 500 ppm (T_{11}) (588.66 h) followed by nano ZnO + nano Cu @ 1000 ppm each (T_{12}) (597.80 h), nano Zn @ 500 ppm (T_3) (598.59 h), nano Zn @ 1000 ppm (600.21), nano Cu @ 500 ppm (T_7) (600.49 h), Zn + Cu each @ 500 ppm (T_9) (601.53h), Zn + Cu each @ 1000 ppm (T_{10}) (602.88 h), nano ZnO @ 500 ppm (600.21 h) and nano Cu @ 1000 ppm (604.26 h) which were on par with each other. The larval duration was significantly longer in control (T_{13}) (628.12 h) followed by Zn @ 100 ppm (T_2) (612.26 h) and treatments Zn @ 500 ppm (T_1) (607.86 h) and Cu @ 1000 ppm (T_6) (605.21 h) were on par with each other (Table 1).

During second rearing significantly shorter larval duration was noticed in nano ZnO + nano Cu each @ 500 ppm (T_{11}) (588.66 h) followed by nano ZnO + nano Cu each @ 1000 ppm (T_{12}) (576.76 h), nano Zn @ 500 ppm (T_3) (593.22 h), nano Zn @ 1000 ppm (T_4) (595.35 h), Cu @ 500 ppm (T_5) (597.80 h), nano Cu @ 500 ppm (T_7) (600.49 h), Zn + Cu each @ 500 ppm (T_9) (601.53 h), Zn + Cu each @ 1000 ppm (T_{10}) (602.88 h), nano Cu @1000 ppm (T_8) (604.26 h) and Cu @ 1000 ppm and (T_6) (605.21 h). The larval duration was significantly longer in control (T_{13}) (620.81 h) followed by Zn @ 100 ppm (T_2) (612.26 h) and Zn @ 500 ppm (T_1) (607.86 h) (Table 1).

Similarly in pooled data the shorter larval duration was observed in nano ZnO + nano Cu each @ 500 ppm (T_{11}) (581.61 h) followed by nano ZnO + nano Cu each @ 1000 ppm (T_{12}) (588.76 h), nano ZnO @ 500 ppm (596.72 h), nano ZnO @ 1000 ppm (T_4) (596.97 h), Cu @ 500 ppm (T_5) (597.80 h) and nano Cu @ 500 ppm (600.49 h) and the longer larval duration was observed in control (T_{13}) (624.46 h) followed by Zn @ 1000 ppm (T_2) (612.26h), Zn @ 500 ppm (T_1) (607.86 h), Cu @ 1000 ppm (T_6) (605.21h) and nano Cu @ 1000 ppm (T_8) (604.20 h) (Table 1).

Effective rearing rate was maximum in nano ZnO + nano Cu each @ 500 ppm (T_{11}) (95.17 %) followed by nano ZnO + nano Cu each @ 1000 ppm (T_{12}) (93.03 %) and nano ZnO @ 500 ppm (T_3) (91.67 %). The minimum effective rate of rearing was recorded in control (73.97 %). The order of superiority for the rest of the treatments was $T_4 > T_1 > T_5 > T_6 > T_8 > T_2 > T_9 > T_{10} > T_7$ (82.07 to 90.03 %) during first rearing (Table 1).

During second rearing, effective rate of rearing was found maximum in nano ZnO + nano Cu each @ 500 ppm (T_{11}) (92.80 %) which was on par with nano ZnO + nano Cu each @ 1000 ppm (T_{12}) (91.70 %) followed by nano ZnO @ 500 ppm (T_3) (89.73 %) and nano ZnO @ 1000 ppm (T_4) (88.43 %) and significantly minimum effective rate of rearing was recorded in control (T_{13}) (74.23 %).The order of superiority for the rest of the treatments was $T_{10} > T_6 > T_5 > T_1 > T_9 > T_2 > T_8 > T_7$ (80.00 to 83.73%) (Table 1).

Similarly in pooled data combined application of nano ZnO + nano Cu each @ 500 ppm (T11) recorded maximum effective rate of rearing (93.98 %) which was on par with nano ZnO + nano Cu each @ 1000 ppm (T_{12}) (92.37 %) followed by nano ZnO @ 500 ppm (T3) (90.70 %). whereas, significantly lowest effective rate of rearing was recorded in control (T13) (74.10 %). The order of superiority for the rest of the treatments was $T_4 > T_1 > T_5 > T_6 > T_8 > T_9 > T_2 > T_{10} > T_7$ (81.03 to 89.23%) (Table 1).

Effect of nano Zinc and nano Copper nutrition to mulberry silkworm, *Bombyx mori* L. for fifth instar larval duration and silk productivity

The fifth instar larval duration (h) and silk productivity (cg/day) data was influenced by the foliar application of nanomicronutrients and were presented in Table 2. Significantly shorter fifth instar larval duration was noted in nano ZnO + nano Cu each @ 500 ppm (T_{11}) (167.87 and 192.70 h), which was on par with nano ZnO + nano Cu each @ 1000 ppm (T_{12}) (169.09 and 196.94 h) and nano Zn @ 500 ppm (T_3) (171.22 and 199.17h). The fifth instar larval duration was significantly longer in control (T_{13}) (188.13 and 234.11h) which was on par with Zn @ 500 ppm (T_1) (185.65 and 223.44 h) and Zn @ 1000 ppm (T_2) (185.92 and 220.85 h). The order of superiority for the rest of the treatments was $T_8 > T_9 > T_7 > T_6 > T_{10} > T_5 > T_4$ (174.46 to 182.67 h) and $T_8 > T_9$ and $T_{10} > T_7 > T_6 > T_5 > T_4$ (202.98 to 214.70 h) during first and second rearing, respectively in order (Table 2).

Similarly, in pooled data the shorter fifth instar larval duration was observed in nano ZnO + nano Cu @ 500 ppm each (T_{11}) (180.29 h) followed by nano ZnO + nano Cu @ 1000 ppm each (T_{12}) (183.02 h), nano ZnO @ 500 ppm (T_3) (185.19 h). Whereas, longer fifth instar larval duration was observed in control (211.12 h) followed by Zn @ 500 ppm (204.54 h) and Zn @ 1000 ppm (203.38 h) and the order of superiority for the rest of the treatments was $T_8 > T_9 > T_{10} > T_7 > T_6 > T_5 > T_4$ (188.72 to 198.67 h) (Table 2).

Silk productivity largely influenced by the combined application of nano ZnO + nano Cu @ 500 ppm each (T_{11}) (0.57, 0.67 and 0.62 cg/day) followed by nano ZnO + nano Cu @ 1000 ppm each (T_{12}) (0.53, 0.64 and 0.58 cg/day) and nano ZnO @ 500 ppm (T_3) (0.51, 0.55 and 0.53 cg/day). Significantly lowest silk productivity was observed in control (0.33, 0.40 and 0.37 cg/day). The order of superiority for the rest of the treatments was $T_4 > T_6 > T_5 > T_8 > T_7 > T_{10} > T_9 > T_2 > T_1$ (0.37 to 0.48 cg/day). $T_4 > T_5 > T_6 > T_8$ and $T_{10} > T_9 > T_7 > T_2 > T_1$ (0.43 to 0.54 cg/day) and $T_4 > T_5$ and $T_6 > T_8$ and $T_{10} > T_7 > T_9 > T_2 > T_1$ (0.40 to 0.51 cg/day) in season I, season II and pooled data, respectively on order (Table 2).

Effect of nano Zinc and nano Copper nutrition to mulberry silkworm, *Bombyx mori* L. for cocoon parameters

The data pertaining to cocoon characters such as cocoon weight, shell weight and shell ratio was influenced by foliar application of micronutrients and were presented in Table 3. The weight of the cocoon was significantly higher (19.89, 19.40 and 19.64 g/10 cocoon) in treatment combination of nano ZnO + nano Cu each @ 500 ppm (T_{11}), which was on par with nano ZnO + nano Cu @ 1000 ppm each (T_{12}) (19.39, 19.26 and 19.32 g/10 cocoon) followed by nano ZnO @ 500 ppm (T_3) (18.20, 17.70 and 17.95 g/10 cocoon). Significantly lowest cocoon weight was recorded in control (15.33, 15.18 and 15.26 g/10 cocoon). The order of superiority for the rest of the treatments was $T_4 > T_5 > T_8 > T_{10} > T_1 > T_9 > T_6 > T_2 > T_7$ (15.86 to 17.73 g/10 cocoon); $T_4 > T_5 > T_7 > T_9 > T_8 > T_{10} > T_1 > T_6 > T_2$ (14.90 to 17.10 g/10 cocoon) and $T_4 > T_5 > T_8 > T_{10} > T_9 > T_1 > T_7 > T_6 > T_2$ (15.44 to 17.40 g/10 cocoon) in season I, season II and pooled data, respectively (Table 3).

Maximum shell weight (4.59 g/10 shell) was obtained from nano ZnO + nano Cu each @ 500 ppm (T_{11}) which was on par with nano ZnO + nano Cu each @ 1000 ppm (T_{12}) (4.38 g/10 shell) followed by nano ZnO @ 500 ppm (T_3) (4.20 g/10 shell). The minimum shell weight was recorded in control (3.23 g/10 shell) which was on par with Zn @ 500 ppm (3.47 g/10 shell). The order of superiority for the rest of the treatments was $T_4 > T_6 > T_5 > T_8 > T_{10} > T_7 > T_9 > T_2$ (3.59 to 4.07 g/10 shell) during first rearing (Table 3).

During second rearing and pooled data maximum shell weight was recorded in nano ZnO + nano Cu each @ 500 ppm (T_{11}) (4.71 and 4.65 g/10 shell) followed by nano ZnO + nano Cu each @ 1000 ppm (T_{12}) (4.48 and 4.43 g/10 shell) and nano ZnO @ 500 ppm (T_3) (3.95 and 4.07 g/10 shell). Significantly minimum shell weight was recorded in control (3.16 and 3.19 g/10 shell) which was on par with Zn @ 500 ppm (3.30 and 3.38 g/10 shell). The order of superiority for the rest of the treatments was $T_4 > T_{10} > T_8 > T_5 > T_6 > T_9 > T_2 > T_7$ (3.41 to 3.94 g/10 shell) and $T_4 > T_6 > T_{75}$ and $T_8 > T_{10} > T_7 > T_9 > T_2$ (3.51 to 4.00 g/10 shell), respectively (Table 3).

Higher shell ratio was recorded in Cu @ 1000 ppm (T_6) (24.72) during season I of rearing which was on par with nano Cu @ 500 ppm (T_7) (23.52). The minimum shell ratio was found in Zn @ 500 ppm (T_1) (0.207) and was on par with control (T_{13}) (0.211). The order of superiority for the rest of the treatments was T_3 and $T_{11} > T_4 > T_5$, T_8 and $T_{12} > T_{10} > T_2$ (22.52 to 23.12) and were on par with each other(Table 3).

During season II of rearing highest shell ratio was obtained from nano ZnO + nano Cu each @ 500 ppm (T_{11}) (24.26) which was on par with Cu @ 1000 ppm (T_6) (23.37), nano ZnO + nano Cu each @ 1000 ppm (T_{12}) (23.27), Zn @ 1000 ppm (T_2) (23.06) and nano ZnO @ 1000 ppm (T_4) (23.02) and the minimum shell ration was recorded in control (T_{13}) (20.84). The order of superiority for the rest of the treatments was $T_{10} > T_8 > T_5 > T_1 > T_7$ (20.77 to 22.80) and were on par with each other (Table 3).

Similar trend of results was observed in pooled data, maximum shell ratio was found Cu @ 1000 ppm (T_6) (24.05), which was on par with nano ZnO + nano Cu each @ 500 ppm (T_{11}) (23.67), nano ZnO @ 1000 ppm (T_4) (23.00), nano ZnO + nano Cu each @ 1000 ppm (T_{12}) (22.92), Zn @ 1000 ppm (T_2) and nano ZnO @ 500 ppm (T_3) with same value 22.79. The minimum shell ratio was recorded in control (T_{13}) (20.96). The order of superiority for the rest of the treatments was T10 > T8 > T_5 and $T_7 > T_9 > T_1$ (20.96 to 22.67) (Table 3).

Discussion

The increased nutritional status and concentration of micronutrients in mulberry leaves obtained due to combined supplementation of nano ZnO + nano Cu @ 500 ppm (T_{11}) might have stimulated the metabolic activities in silkworm resulting in better growth and development subsequently silk production. The result was in conformity with the micronutrients role in silkworm growth and development were reported by Ito and Nimimura (1966) [12] and Horie *et al*. (1967) [11] where they indicated that micronutrients accelerated the growth of larvae.). Zinc plays a vital role in the synthesis of lipids, protein and carbohydrates and in reducing the duration of larval (Bhattacharya and Medda, 1981) [2]. Bajapeyi *et al*. (1991) [1] and Qader *et al*. (1993) [15] also reported an improvement in morphometric traits of silkworm and reduction in larval duration with micronutrients supplementation to silkworm. Zhangji *et al*. (1997) [19] suggested that mulberry leaves that treated with 0.2 % Zinc sulfate significantly increased the body weight and the total amount of blood amino acid of silkworm. The Cu could play a role in the natural spinning process in silkworms.

Micronutrients are useful in enhancing the energetic efficiency of the worm, as they are co factors in the activity of many enzymes. This increase in energy levels could be utilized for protein synthesis and silk production in silkworms (Chamundeshwari and Radhakrishnaiah, 1994) [5]. Silk production is dependent on the larval nutrition and nutritive value of mulberry leaves and finally in producing good quality cocoon (Etebari and Matindoost, 2005) [8]. Mulberry leaf quality has high correlation with leaf bio chemical constituents and the production efficiency of the cocoon shell, *i.e*. the cocoon shell weight relative to the total amount of mulberry leaves consumed by the silkworm (Machii and Katagiri, 1991) [14]. Further, Subburathinam et al.1990 [18]; Devi and Yellamma (2013) [7] and Rao *et al*. (1998) [16] also reported an improvement in growth parameters of silkworm when mulberry leaves were sprayed with magnesium sulphate, Zinc sulphate ferrous ammonium sulphate, Copper sulphate and sodium sulphate were fed to silkworm. Furthermore, Bose *et al*. (1995) [4] and Shankar *et al*. (1990) [17] also reported that feeding of micronutrients treated leaves showed significantly higher mature larval weight, cocoon weight, shell weight, pupal weight and lower melting percentage of pupa compared to control. Therefore the quality of leaves largely determines the performance of silkworms during their development and spinning of quality cocoons. Zhangji *et al*. (1997) [19] reported that mulberry leaves that were sprayed with 0.2% Zinc sulfate significantly increased the total number of eggs and the normal eggs increased significantly.

Table 1: Effect of Zinc and Copper nutrition on mulberry silkworm, *Bombyx mori* L. for full grown larval weight, effective rearing rate and total larval duration

Treatment	Full grown larval weight (g/10)			Total larval duration (h)			Effective rearing rate (%)		
	Season I	Season II	Pooled	Season I	Season II	Pooled	Season I	Season II	Pooled
T_1: Zn @500ppm	38.27^{cd}	35.07^{ab}	36.67^{bc}	607.86^{c}	607.86^{bc}	607.86^{bc}	88.00 (69.73)^{e}	82.77 (65.47)^{d}	85.38 (67.52)^{d}
T_2: Zn @1000ppm	37.57^{d}	37.50^{a}	37.54^{bc}	612.26^{b}	612.26^{b}	612.26^{b}	84.07 (66.48)^{fg}	80.37 (63.70)^{e}	82.22 (65.06)^{efg}
T_3 : Nano Zn @ 500ppm	40.61^{bc}	37.55^{a}	39.08^{b}	598.59^{d}	593.22^{def}	596.72^{de}	91.67 (73.22)^{b}	89.73 (71.31)^{a}	90.70 (72.24)^{b}
T_4: Nano Zn @ 1000ppm	39.18^{c}	36.92^{a}	38.05^{b}	600.21^{d}	595.35^{de}	596.97^{de}	90.03 (71.59)^{c}	88.43 (70.11)^{a}	89.23 (70.84)^{c}
T_5: Cu @ 500ppm	38.02^{cd}	33.97^{abc}	36.00^{bcd}	600.77^{d}	597.80^{de}	597.80^{de}	85.10 (67.29)^{f}	82.90 (65.57)^{d}	84.00 (66.42)^{e}
T_6: Cu @ 1000ppm	39.07^{cd}	36.77^{a}	37.92^{b}	605.21^{cd}	605.21^{bcd}	605.21^{bcd}	84.87 (67.11)^{fg}	83.00 (65.65)^{d}	83.93 (66.37)^{e}
T_7: Nano Cu @ 500ppm	38.53^{cd}	34.66^{ab}	36.59^{bc}	600.49^{d}	600.49^{de}	600.49^{de}	82.07 (64.95)^{g}	80.00 (63.43)^{e}	81.03 (64.18)^{h}
T_8: Nano Cu @ 1000ppm	37.43^{d}	35.11^{ab}	36.27^{d}	604.26^{d}	604.26^{cd}	604.26^{bcd}	84.47 (66.79)^{fg}	80.27 (63.63)^{e}	82.37 (65.17)^{efg}
T_9: Zn + Cu @500ppm each	36.73^{e}	34.27^{ab}	35.50^{d}	601.53^{d}	601.53^{cd}	601.53^{d}	84.03 (66.45)^{fg}	82.47 (65.25)^{c}	83.25 (65.84)^{ef}
T_{10}: Zn + Cu @1000ppm each	36.76^{e}	36.07^{a}	36.42^{bc}	602.88^{d}	602.88^{cd}	602.88^{d}	80.23 (63.60)^{h}	83.73 (66.21)^{b}	81.98 (64.88)^{efg}
T_{11}: Nano Zn+Cu @ 500ppm each	43.42^{a}	37.95^{a}	40.68^{a}	588.66^{e}	574.56^{fg}	581.61^{f}	95.17 (77.30)^{a}	92.80 (74.44)^{a}	93.98 (75.80)^{a}
T_{12} :Nano Zn+Cu @ 1000ppm each	41.58^{b}	37.59^{a}	39.58^{a}	597.80^{d}	576.76^{f}	588.76^{e}	93.03 (74.69)^{b}	91.70 (73.26)^{a}	92.37 (73.96)^{a}
T_{13} : Control	34.71^{f}	28.58^{d}	31.65^{e}	628.12^{a}	620.81^{a}	624.46^{a}	73.97 (59.32)^{i}	74.23 (59.49)^{f}	74.10 (59.41)^{i}
CV (%)	1.83	4.58	2.40	0.71	0.69	0.67	1.09	2.05	1.08
S.Em $\pm$	0.41	0.94	0.51	2.47	2.39	2.33	0.54	1.00	0.53

Figures in parenthesis are arcsine transformed values
Figures in the column followed by same letters are not-significant at p=0.01 by DMRT

Table 2: Effect of Zinc and Copper nutrition on mulberry silkworm, *Bombyx mori* L. for fifth instar larval duration and silk productivity

Treatment	5th instar larval duration (h)			Silk productivity (cg day^{-1})		
	Season I	Season II	Pooled	Season I	Season II	Pooled
T_1: Zn @500ppm	185.65^{a}	223.44^{b}	204.54b^{c}	0.37^{g}	0.43^{defg}	0.40^{f}
T_2: Zn @1000ppm	185.92^{a}	220.85^{c}	203.38^{b}	0.39^{f}	0.44^{defg}	0.42^{f}
T_3 : Nano Zn @ 500ppm	171.22^{e}	199.17^{def}	185.19^{def}	0.51b^{c}	0.55^{c}	0.53^{c}
T_4: Nano Zn @ 1000ppm	174.46^{d}	202.98^{de}	188.72^{de}	0.48^{d}	0.54^{cd}	0.51^{cd}
T_5: Cu @ 500ppm	177.30^{c}	205.90^{de}	191.60^{de}	0.45^{de}	0.49^{d}	0.47^{d}
T_6: Cu @ 1000ppm	182.02^{b}	209.97^{de}	195.99^{cd}	0.46^{de}	0.47^{de}	0.47^{d}
T_7: Nano Cu @ 500ppm	182.29^{b}	210.24^{de}	196.26^{cd}	0.42^{def}	0.45^{def}	0.44^{def}
T_8: Nano Cu @ 1000ppm	182.67^{b}	214.70^{cd}	198.69^{cd}	0.43^{def}	0.48^{de}	0.45^{de}
T_9: Zn + Cu @500ppm each	182.32^{b}	213.28^{cd}	197.80^{cd}	0.41^{fg}	0.46^{def}	0.43^{def}
T_{10}: Zn + Cu @1000ppm each	181.92^{b}	213.28^{cd}	197.60^{cd}	0.42^{def}	0.48^{de}	0.45^{de}
T_{11}: Nano Zn+Cu @ 500ppm each	167.87^{e}	192.70^{def}	180.29^{h}	0.57^{a}	0.67^{a}	0.62^{a}
T_{12} :Nano Zn+Cu @ 1000ppm each	169.09^{e}	196.94^{def}	183.02^{g}	0.53^{b}	0.64^{b}	0.58^{b}
T_{13} : Control	188.13^{a}	234.11^{a}	211.12^{a}	0.33^{h}	0.40^{g}	0.37^{g}
CV(%)	1.34	1.97	1.34	2.97	7.39	4.37
S.Em $\pm$	1.39	2.40	1.51	0.01	0.01	0.01

Figures in parenthesis are arcsine transformed values
Figures in the column followed by same letters are not-significant at p=0.01 by DMRT

Table 3: Effect of Zinc and Copper nutrition on mulberry silkworm, *Bombyx mori* L. for cocoon parameters

Treatment	Cocoon weight (g/10)			Shell weight (g/10)			Shell ratio (%)		
	Season I	Season II	Pooled	Season I	Season II	Pooled	Season I	Season II	Pooled
T_1: Zn @500ppm	16.78^{cd}	15.60^{f}	16.19^{def}	3.47^{f}	3.30^{g}	3.38^{g}	20.73 (27.08)^{c}	21.18 (27.40)^{bc}	20.96 (27.25)^{d}
T_2: Zn @1000ppm	15.98^{cde}	14.90^{fg}	15.44^{g}	3.59^{cde}	3.42^{f}	3.51^{def}	22.52 (28.33)^{b}	23.06 (28.70)^{a}	22.79 (28.51)^{a}
T_3 : Nano Zn @ 500ppm	18.20^{b}	17.70^{b}	17.95^{b}	4.20^{b}	3.95^{c}	4.07^{c}	23.12 (28.74)^{b}	22.31 (28.19)^{b}	22.72 (28.47)^{b}
T_4: Nano Zn @ 1000ppm	17.73^{bc}	17.10^{d}	17.42^{c}	4.07^{c}	3.94^{cd}	4.00^{cd}	22.97 (28.64)^{b}	23.02 (28.67)^{a}	23.00 (28.66)^{a}
T_5: Cu @ 500ppm	17.05^{c}	16.80^{de}	16.93^{d}	3.86^{cd}	3.63^{de}	3.74^{de}	22.65 (28.42)^{b}	21.60 (27.69)^{b}	22.12 (28.06)^{b}

T_6: Cu @ 1000ppm	16.25^{cde}	15.39^{g}	15.82^{g}	4.02^{c}	3.59^{de}	3.80^{d}	$24.72\ (29.81)^{a}$	$23.37\ (28.91)^{a}$	$24.05\ (29.37)^{a}$
T_7: Nano Cu @ 500ppm	15.86^{e}	16.41^{e}	16.14^{def}	3.72^{cde}	3.41^{f}	3.56^{def}	$23.52\ (29.01)^{a}$	$20.77\ (27.11)^{bc}$	$22.14\ (28.07)^{b}$
T_8: Nano Cu @ 1000ppm	16.90^{cd}	16.16^{ef}	16.53^{de}	3.82^{cd}	3.66^{de}	3.74^{de}	$22.63\ (28.41)^{b}$	$22.65\ (28.42)^{b}$	$22.64\ (28.41)^{b}$
T_9: Zn + Cu @500ppm each	16.60^{cd}	16.22^{ef}	16.41^{def}	3.61^{cde}	3.50^{de}	3.55^{def}	$21.80\ (27.83)^{b}$	$21.62\ (27.71)^{b}$	$21.71\ (27.77)^{c}$
T_{10}: Zn + Cu @1000ppm each	16.83^{cd}	16.13^{ef}	16.48^{de}	3.76^{cde}	3.67^{d}	3.71^{de}	$22.37\ (28.23)^{b}$	$22.80\ (28.52)^{b}$	$22.59\ (28.38)^{b}$
T_{11}: Nano Zn+Cu @ 500ppm each	19.89^{a}	19.40^{a}	19.64^{a}	4.59^{a}	4.71^{a}	4.65^{a}	$23.09\ (28.72)^{b}$	$24.26\ (29.51)^{a}$	$23.67\ (29.11)^{a}$
T_{12} :Nano Zn+Cu @ 1000ppm each	19.39^{a}	19.26^{a}	19.32^{a}	4.38^{a}	4.48^{b}	4.43^{b}	$22.58\ (28.37)^{b}$	$23.27\ (28.84)^{a}$	$22.92\ (28.60)^{a}$
T_{13} : Control	15.33^{f}	15.18^{h}	15.26^{h}	3.23^{f}	3.16^{g}	3.19^{g}	$21.09\ (27.34)^{c}$	$20.84\ (27.16)^{c}$	$20.96\ (27.25)^{d}$
CV (%)	1.85	2.17	1.66	3.45	2.66	2.80	3.38	3.45	3.37
S.Em ±	0.22	0.18	0.16	0.08	0.06	0.06	0.51	0.47	0.44

Figures in parenthesis are arcsine transformed values

Figures in the column followed by same letters are not-significant at p=0.01 by DMRT

Conclusion

The studies on nano micronutrients supplementation to mulberry silkworms indicated that nano ZnO + nano Cu each @ 500 ppm resulted in significant superiority for yield and quality parameters of mulberry and subsequently when fed to silkworms, silkworms exhibited improved performance in respect of full grown larval weight, total larval duration, effective rearing rate, fifth instar larval duration, silk productivity and cocoon parameters followed by nano ZnO + nano Cu each @ 1000 ppm and nano ZnO @ 500 ppm. The nano size of micronutrients and its unique property of more surface area, the nano micronutrients might penetrate more efficiently and effectively when applied through foliar when compared to foliar spray of chemical micronutrients.

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