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Effect of nano micronutrients supplementation to mulberry for growth, yield and quality parameters

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Abstract
A field experiment was conducted in a well established mulberry garden with V-1 mulberry to study the effect of nano micronutrients on mulberry for growth, yield and quality parameters at Sericulture unit, Department of Agricultural Entomology, College of Agriculture, UAS, Raichur, Karnataka during 2018-19. The experimental plot was laid in randomized block design with thirteen treatments with three replications. Nano micronutrients were supplemented through foliar spray and the results indicated that nano ZnO + nano Cu each @ 500 ppm resulted in significant superiority for growth, yield and quality parameters of mulberry 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 compared to chemical spray of micronutrients to mulberry.

Keywords: Nano micronutrients, supplementation, mulberry, nutrition

Introduction
Morus, a genus of flowering plants in the family Moraceae, comprises 10–16 species of deciduous trees commonly known as mulberries growing wild and under cultivation in many temperate regions of the world. Mulberry (*Morus alba* L.) is a perennial, deep rooted, fast growing and high biomass producing foliage plant. Mulberry leaves, particularly those of the white mulberry are ecologically important as the sole food source of the silkworm (*Bombyx mori* L., named after the mulberry genus *Morus*). Mulberry leaf is a major economic component in sericulture since the quality and quantity of leaf produced per unit area would have a direct bearing on cocoon harvest. Mulberry leaf quality plays a predominant role in healthy and robust growth of silkworm, *Bombyx mori* L. Nanoparticles are smaller in size and have larger surface area, so foliar supplementation of nanomicronutrients can result in rapid absorption and utilization to meet the bulk of the nutrient requirement of mulberry and in turn of mulberry silkworms.Significant increased yields have been observed due to foliar application of nano particles as fertilizers. The studies on nanomicronutrients supplementation in mulberry is very limited and has been attempted to investigate the effect of nanomicronutrients on mulberry as well as silkworms for growth and yield parameters. Several workers have reported the improved nutritive parameters like increased moisture, protein, sugars and chlorophyll contents in mulberry through foliar application of micronutrients. Mulberry as a foliage crop responds well to timely application of foliar sprays (Geetha *et al*., 2016) [6]. Foliar spray of Zn as ($ZnSO_4$) increased the moisture content in mulberry leaves and helped to retain the leaf freshness for longer periods (Lokanath and Shivshankar 1981) [6]. Cu NPs might enhance photosynthesis and /or increase synthesis of antioxidants in plant. Micronutrients sprayed leaves would get the required nutrients directly and enhances the photosynthetic efficiency. Further, plants grow well and give higher foliage yields with superior quality. Nanomicronutrients supplementation might be an economical option as the requirement of quantity of micronutrients would be significantly lower because of vast surface area and its nano size. Hence, nanomicronutrients might penetrate more efficiently into the leaf and involve in metabolic activities for betterment of health, quality and yield.

Material and Methods

A field experiment was conducted in a well established mulberry garden with V-1 mulberry to study the effect of nano micronutrients supplementation to mulberry for growth, yield and quality parameters at Sericulture unit, Department of Agricultural Entomology, College of Agriculture, Raichur, Karnataka during 2018-19. The experimental plot was laid in randomized block design with thirteen treatments with three replications. The treatments and treatment combinations used in the experiment was present (Table 1). Mulberry was grown as per standard package with application of both recommended organic and chemical fertilizers. The micronutrients were supplied through foliar application on 25^{th} and 35^{th} day after pruning. The standard methods have been employed for analysis of biometric parameters and were presented hereunder.

Leaf yield, shoot yield and plant biomass was calculated by harvesting the mulberry shoots from each of 5 labeled plants per replication separately at 65^{th} day after pruning and weighed separately and recorded as biomass per plant. The leaves were harvested from shoots of these five plants independently and separately replication wise and weighed and averaged as leaf yield per plant. The remaining shoots were also weighed separately and independently and averaged as shoot yield per plant.

The total shoot length per plant was recorded by measuring the length of each shoot in a plant from one foot above ground to the base of top most fully opened leaf and were added and recorded.

Total number of leaves per plant from five labeled plants were harvested and counted and arrived at mean number of leaves per plant.

Hundred leaves per plant were harvested from shoots (top, middle and top portion) replication wise and weighted on a sensitive balance and recorded as 100 leaves weight.

Leaf area was determined by using CI-202 portable laser leaf area meter. Moisture percentage in leaf was estimated through gravimetric method (A.O.A.C., 1980) [1].

Results

Effect foliar spray of nano Zinc and nano Copper on mulberry for growth parameters

The growth parameters of mulberry such as total shoot length, total number of leaves and leaf area per plant were significantly influenced by the foliar application of green nano micronutrients (Table 1). Significantly higher shoot length (202.33 cm) was observed in treatment combination of nano ZnO + nano Cu @ 500 ppm each (T_{11}) and was found to be on par with treatment combination of nano ZnO + nano Cu @ 1000 ppm each (T_{12}) (199.40 cm) followed by nano ZnO @ 500 ppm (186.07 cm). Significantly shortest shoot length was recorded in control (143.00 cm). The order of superiority for the rest of the treatments was found to be $T_{10} > T_4 > T_1 > T_8 > T_6 > T_5 > T_7$ (159.87 to 173.80 cm) during season I.

During season II, significantly longer shoot length (197.00 cm) was observed in treatment combination of nano ZnO + nano Cu @ 500 ppm each (T_{11}) which was found to be on par with nano ZnO + nano Cu each @ 1000 ppm (T_{12}) (189.87 cm) and nano ZnO @ 500 ppm (T_3) (184.60 cm) which was on par with Cu @ 500 ppm (142.20 cm). Significantly shortest shoot length was found in control (141.27 cm) and the order of superiority for the rest of the treatments was found to be $T_2 > T_7 > T_4 > T_8 > T_6 > T_1 > T_{10} > T_9$ (159.07 to 168.80 cm) (Table 1).

Similarly in pooled data, significantly higher shoot length (199.67 cm) was observed in treatment combination of nano ZnO + nano Cu @ 500 ppm each (T_{11}) which was found to be on par with nano ZnO + nano Cu @ 1000 ppm each (T_{12}) (194.63 cm) followed by nano ZnO @ 500 ppm (T_3) (185.33 cm). Significantly shortest shoot length was recorded in control (T_{13}) (142.13 cm). The order of superiority for the rest of the treatments was found to be $T_4 > T_{10} > T_2 > T_1 > T_8 > T_7 > T_6 > T_9 > T_5$ (151.20 to 170.43 cm).

The total number of leaves per plant was significantly highest in nano ZnO + nano Cu @ 500 ppm each (T_{11}) (328.73) followed by nano ZnO + nano Cu @ 1000 ppm each (T_{12}) (309.53), nano ZnO @ 1000 ppm (T_4) (278.80) and nano ZnO @ 500 ppm (T_3) (263.13). Significantly lowest number of leaves per plant was observed in control treatment (T13) (216.40) which was on par with nano Cu @ 1000 ppm (220.67), Zn + Cu @ 1000 ppm each (222.87) and Cu @ 1000 ppm (224.87). The order of superiority for the rest of the treatments was $T_2 > T_5$ and $T_7 > T_9 > T_1$ (220.67 to 234.07) during season I (Table 1).

During season II, maximum number of leaves per plant was observed in nano ZnO + nano Cu @ 500 ppm each (T_{11}) (325.13) followed by nano ZnO @ 500 ppm (T_3) (291.87) and nano ZnO + nano Cu @ 1000 ppm each (T_{12}) (284.73) which were on par with each other. Significantly lowest number of leaves per plant was observed in control (T_{13}) (215.00). The order of superiority for the rest of the treatments was $T_4 > T_2 > T_1 > T_7 > T_5 > T_6 > T_9 > T_{10} > T_8$ (223.87 to 258.27) (Table 1).

Similarly in pooled data, maximum number of leaves per plant was observed in nano ZnO + nano Cu @ 500 ppm each (T_{11}) (326.93) followed by nano ZnO + nano Cu @ 1000 ppm each (T_{12}) (297.13) and nano ZnO @ 500 ppm (T_3) (277.05). Significantly lowest number of leaves per plant was observed in control treatment (T_{13}) (215.70) which was on par with nano Cu @ 1000 ppm (222.27) and Zn + Cu @ 1000 ppm (226.03). The order of superiority for the rest of the treatments was $T_4 > T_2 > T_1 > T_7 > T_5 > T_6 > T_9$ (233.53 to 268.53) (Table 1).

Significantly maximum leaf area per plant was observed in nano ZnO + nano Cu @ 500 ppm each (T_{11}) (267.13 cm^2) which was on par with nano ZnO + nano Cu @ 1000 ppm each (T_{12}) (259.36 cm^2) followed by nano ZnO @ 500 ppm (T_3) (238.66 cm^2) and nano ZnO @ 1000 ppm (T_4) (231.12 cm^2) and the leaf area per plant was minimum in control (154.76 cm^2).The order of superiority for the rest of the treatments was $T_1 > T_2 > T_6 > T_7 > T_5 > T_9 > T_8 > T_{10}$ (179.21 to 213.00 cm^2) during season I(Table 1).

During season II, maximum leaf area per plant was noticed in nano ZnO + nano Cu @ 500 ppm each (T_{11}) (266.41 cm^2) and was found to be on par with nano ZnO + nano Cu each @ 1000 ppm (T_{12}) (256.64 cm^2) followed by nano ZnO @ 500 ppm (T3) (238.25 cm^2) and nano ZnO @ 1000 ppm (T4) (235.79 cm^2). Significantly lowest leaf area was recorded in control (T_{13}) (154.33). The order of superiority for the rest of the treatments was found to be $T_2 > T_7 > T_1 > T_6 > T_8 > T_5 > T_9 > T_{10}$ (176.23 to 209.73 cm^2) (Table 1).

Similarly, in pooled data maximum leaf area per plant obtained was nano ZnO + nano Cu @ 500 ppm each (T_{11}) (266.77 cm^2) and was found to be on par with nano ZnO + nano Cu @ 1000 ppm each (T_{12}) (258.00 cm^2) followed by nano ZnO @ 500 ppm (T3) (238.45 cm^2) and nano ZnO @ 1000 ppm (T4) (233.46 cm^2). The minimum leaf area per plant was recorded in control (T_{13}) (154.54 cm^2). The order of superiority for the rest of the treatments were T1 > T_2 > T_6 > T_7 > T_8 >T_5 > T_9 > T_{10} (177.72 to 210.49 cm^2) (Table 1).

The application of micronutrients yielded better growth and development in mulberry compared to control. The data in the Table 1 clearly indicated foliar application of nano ZnO and Cu either alone or in combination performed better than Zn and Cu either alone or in combination. Nano ZnO and nano Cu @ 500 ppm exhibited superiority over nano ZnO and nano Cu @ 1000 ppm.

Effect of foliar spray of Zinc and Copper to mulberry for leaf and shoot yield and biomass

The yield parameters of mulberry such as shoot yield (g/plant), leaf yield (g/plant) and total biomass (g/plant) was significantly influenced by the foliar application of nanomicronutrients and were presented in Table 2. Significantly highest leaf yield was found in treatment combination of nano ZnO + nano Cu @ 500 ppm each (T_{11}) (1010.47 g/plant) and was found to be on par with nano ZnO + nano Cu @ 1000 ppm each (T_{12}) (994.07 g/plant) followed by ZnO @ 500 ppm (T_3) (982.67 g/plant). Leaf yield was significantly lowest in control treatment control (T_{13}) (751.87 g/plant). The order of superiority for the rest of the treatments was found to be T_4 > T_1 > T_2 > T_6 > T_5 >T_8 > T_{10} > T_7 > T_9 (815.87 to 969.13 g/plant) during season I.

In season II, significantly higher leaf yield was found in nano ZnO + nano Cu @ 500 ppm each (T_{11}) (990.g/plant) and was found to be on par with nano ZnO + nano Cu each @ 1000 ppm (T_{12}) (981.13 g/plant) and nano Zn @ 500 ppm (T_3) (978.13 g/plant). Significantly lowest leaf yield was noticed in control (758.00 g/plant) and the order of superiority for the rest of the treatments was found to be T_4 > T_1 > T_2 > T_5 > T_6 >T_{10} > T_8 > T_7 > T_9 (811.13 to 966.07 g/plant) (Table 2).

Similarly in pooled data, significantly higher leaf yield was observed in nano ZnO + nano Cu @ 500 ppm each (T_{11}) (1000.47 g/plant) and was on par with nano ZnO + nano Cu each @ 1000 ppm (T_{12}) (987.13 g/plant) followed by nano ZnO @ 500 ppm (T_3) (980.40 g/plant). Significantly lowest leaf yield was recorded in control (T_{13}) (754.93 g/plant) and the order of superiority for the rest of the treatments was T_4 > T_1 > T_2 > T_6 > T_5 >T_8 > T_{10} > T_7 > T_9 (813.50 to 967.60 g/plant) (Table 2).

Combined application of nano micronutrients, nano ZnO + nano Cu each @ 500 ppm (T_{11}) had given higher shoot yield (895.13 and 891.47 g/plant) and was found to be on par with nano ZnO + nano Cu each @ 1000 ppm (T_{12}) (837.07 and 863.60 g/plant) followed by nano ZnO @ 500 ppm (823.60 and 804.40 g/plant) during first and season II respectively. Significantly lowest shoot yield was noted in control (T13) (685.67 and 622.47 g/plant). The order of superiority for the rest of the treatments was T_1 > T_2 > T_4 > T_6 > T_7 >T_{10} > T_9 > T_8 > T_5 (740.40 to 796.93 g/plant) during season I and the order of superiority for the rest of the treatments during season II was T_1 > T_4 > T_6 > T_5 > T_2 >T_9 > T_8 > T_{10} > T_7 (707.27 to 785.67 g/plant) (Table 2).

Similarly in pooled data, significantly higher shoot yield was recorded in nano ZnO + nano Cu each @ 500 ppm (T_{11}) (893.30 g/plant) followed by nano ZnO + nano Cu each @ 1000 ppm (T_{12}) (850.33 g / plant) and nano ZnO @ 500 ppm (T_3) (814.20 g/plant). Whereas, the significantly lowest shoot yield was noticed in control (T_{13}) (654.07 g/plant) and the order of superiority for the rest of the treatments was T_1 > T_4 > T_2 > T_6 > T_9 >T_{10} > T_8 > T_5 > T_7 (736.53 to 791.30 g/plant) (Table 2).

With respect to total biomass significantly higher biomass was found in combined application of nanomicronutrients ZnO + Cu each @ 500 ppm (T_{11}) (1905.60, 1841.73 and 1873.67 g/plant) followed by nano ZnO + nano Cu each @ 1000 ppm (T_{12}) (1806.27, 1785.93 and 1796.10 g/plant) and nano ZnO @ 500 ppm (1790.27, 1755.80 and 1773.03 g / plant) and significantly lowest biomass was observed in control (T_{13}) (1393.20, 1380.47and 1386.83 g/plant).The order of superiority for the rest of the treatments was found to be T_4 > T_1 > T_2 > T_{10} > T_6 >T_5 > T_7 > T_8 > T_9 (1574.47 to 1753.73 g/plant), T_4 > T_6 > T_1 > T_2 > T_5 >T_8 > T_{10} > T_9 > T_7 (1525.13 to 1748.60 g/plant) and T_4 > T_1 > T_6 > T_2 > T_{10} >T_5 > T_8 > T_9 > T_7 (1555.73 to 1751.17 g/plant) in season I, season II and pooled data, respectively (Table 2).

Effect of foliar spray of nano Zinc and nano Copper on mulberry for 100 leaf weight and moisture content of leaves

The data pertaining to fresh leaf weight (g/100 leaves), dry leaf weight (g/100 leaves) and moisture content of mulberry leaves revealed that there was significantly increased values by the foliar application green nanomicronutrients and are presented in Table 3. Combined application of nano ZnO and nano Cu @ 500 ppm significantly had higher fresh leaf weight (342.76, 333.93 and 338.35 g/100 leaves) followed by nano ZnO and nano Cu @ 1000 ppm each (293.96, 297.08 and 295.52 g/100 leaves) and nano ZnO @ 500 ppm treatment (264.34, 276.97 and 270.66 g/100 leaves) respectively. Significantly lower fresh leaf weight was observed in control (181.29, 184.54 and 182.91 g/100 leaves) which was on par with Cu @ 1000 ppm (T_6) (206.67, 200.57 and 203.62 g/100 leaves) for season I, season II and pooled data, respectively. The order of superiority for the rest of the treatments was found to be T_4 > T_1 > T_5 > T_2 > T_9 >T_7 > T_{10} > T_8 (211.39 to 248 g/100 leaves); T_4 > T_2 > T_1 > T_5 > T_7 >T_{10} > T_8 > T_9 (202.16 to 267.51 g/100 leaves) and T_4 > T_2 > T_1 > T_5 >T_7 > T_9 > T_{10} > T_8 (209.17 to 257.96 g/100 leaves) for season I, season II and pooled data, respectively in order (Table 3).

Dry leaf weight was significantly higher (74.43, 88.82 and 83.13 g/100 leaves) in combination of nano ZnO and Cu @ 500 ppm each, followed by same combination treatment with 1000 ppm each (72.59, 80.51 and 76.55 g/100 leaves) and nano ZnO @ 500 ppm treatment (70.94, 64.75 and 67.85 g/100 leaves) for season I, season II and pooled data, respectively and significantly lowest dry leaf weight was recorded in control (49.97, 50.18 and 50.07 g/100 leaves) which was on par with Zn + Cu @ 500 ppm (T9) (51.28, 53.58 and 52.43 g/100 leaves), Cu @ 500 ppm (T_5) (52.86, 52.19 and 52.53 g/100 leaves), nano Cu @ 1000 ppm (T_8) (53.50,54.72 and 54.11 g/100 leaves), nano Cu @ 500 ppm (T_7) (53.71, 55.58 and 54.65 g/100 leaves) and Cu @ 1000 ppm (T_6) (55.15, 53.79 and 54.47 g/100 leaves) for season I, season II and pooled data respectively. The order of superiority for the rest of the treatments was T_4 > T_1 > T_2 > T_{10} > (56.27 to 65.06 g/100 leaves); (56.87 to 64.61 g/100 leaves) and (56.87 to 64.83 g/100 leaves) for season I, season II and pooled data, respectively (Table 3).

Combined application of nanomicronutrients nano ZnO + nano Cu @ 500 ppm each (T_{11}) gave higher moisture (77.38, 76.66 and 77.02 %) and was found to be on par with nano ZnO + nano Cu @ 1000 ppm each (T_{12}) (77.02, 76.57 and 76.80 %) and nano ZnO @ 500 ppm (T_3) (76.93, 75.70 and 76.32 %) for season I, season II and pooled data, respectively. The lowest moisture content was recorded in control (T_{13}) (69.85, 70.66 and 70.25 %) and the order of superiority for the rest of the treatments was $T_9 > T_7 > T_8 > T_2 > T_1 > T_{10} > T_4 > T_6 > T_5$ (73.04 to 75.28 %); $T_7 > T_1 > T_8 > T_4 > T_9 > T_2 > T_5 > T_6 > T_{10}$ (72.57 to 75.30 %) and $T_7 > T_9 > T_8 > T_1 > T_2 > T_4 > T_{10} > T_6 > T_5$ (72.94 to 75.24 %) which were on par with each other for season I, season II and pooled data, respectively (Table 3).

Effect of foliar supplementation of Zinc and Copper to mulberry for leaf Chlorophyll content

The chlorophyll a, chlorophyll b and total chlorophyll content of mulberry leaves was influenced by the foliar application of green nanomicronutrients and were presented in Table 3 (Fig.2). Significantly higher chlorophyll 'a' (0.97, 0.95 and 0.96 mg/g of fresh weight) was found in combined application of nano ZnO + nano Cu @ 500 ppm each (T_{11}) followed by nano ZnO + nano Cu @ 1000 ppm each (T_{12}) (0.92, 0.91 and 0.91 mg/g of fresh weight), nano ZnO @ 500 ppm (T_3) (0.89, 0.88 and 0.88 mg/g of fresh weight) during season I, season II and pooled data, respectively. Significantly lowest chlorophyll 'a' content was found in control (T_{13}) (0.40 and 0.48 mg/g of fresh weight) during season I and pooled whereas, nano Cu @ 1000 ppm (T_8) recorded significantly lowest chlorophyll 'a' content (0.52 mg/g of fresh weight) during season II. The order of superiority for the rest of the treatments was $T_4 > T_1 > T_5 > T_9 > T_2 > T_6$ and $T_7 > T_{10} > T_8$ (0.51 to 0.84 mg/g fresh weight); $T_4 > T_1 > T_5 > T_9 > T_2 > T_{10} > T_6$ and $T_7 > T_{10} > T_{13}$ (0.55 to 0.83 mg/g fresh weight) and $T_4 > T_1 > T_5 > T_9 > T_2 > T_{10} > T_6$ and $T_7 > T_{10} > T_8$ (0.52 to 0.84 mg/g fresh weight) in season I, season II and pooled data, respectively (Table 4).

Significantly higher chlorophyll 'b' content was found in combined application of nano ZnO + nano Cu @ 500 ppm each (T_{11}) (1.79 mg/g of fresh weight) followed by nano ZnO + nano Cu @ 1000 ppm each (T_{12}) (1.69 mg/g of fresh weight) and nano ZnO @ 500 ppm (T_3) (1.65 mg/g of fresh weight) and the lowest chlorophyll 'b' content was observed in control (T_{13}) (0.72 mg/g of fresh weight). The order of superiority for the rest of the treatments was $T_4 > T_2 > T_1 > T_9 > T_5 > T_{10} > T_6 > T_7 > T_8$ (0.80 to 1.51 mg/g fresh weight) during season I.

In season II, significantly higher chlorophyll 'b' content was noticed in combined application of nano ZnO + nano Cu @ 500 ppm each (T_{11}) (1.80 mg/g of fresh weight) which was on par with nano ZnO + nano Cu @ 1000 ppm each (T_{12}) (1.73 mg/g of fresh weight) followed by nano ZnO @ 500 ppm (T_3) (1.63 mg/g of fresh weight). Whereas, significantly lowest chlorophyll 'b' content was found in control (T_{13}) (0.75 mg/g of fresh weight) which was on par with nano Cu @ 1000 ppm (T_8) (0.83 mg/g of fresh weight) and the order of superiority for the rest of the treatments were $T_4 > T_1$ and $T_2 > T_9 > T_5 > T_{10} > T_7 > T_6$ (0.92 to 1.45 mg/g fresh weight) (Table 4).

Similarly in pooled data higher chlorophyll 'b' content was observed in combined application of nano ZnO + nano Cu @ 500 ppm each (T_{11}) (1.80 mg/g of fresh weight) followed by nano ZnO + nano Cu @ 1000 ppm each (T_{12}) (1.71 mg/g of fresh weight) and nano ZnO @ 500 ppm (T_3) (1.63 mg/g of fresh weight). The lowest chlorophyll 'b' content was noticed in control treatment (T_{13}) (0.73 mg/g of fresh weight) and the order of superiority for the rest of the treatments was $T_4 > T_2 > T_1 > T_9 > T_5 > T_{10} > T_7 > T_6 > T_8$ (0.82 to 1.48 mg/g fresh weight) (Table 4).

With respect to total chlorophyll content highest value was found in combined application of nano ZnO + nano Cu @ 500 ppm each (T_{11}) (2.76, 2.75 and 2.76 mg/g of fresh weight) followed by nano ZnO + nano Cu @ 1000 ppm each (T_{12}) (2.61, 2.64 and 2.62 mg/g of fresh weight) and nano ZnO @ 500 ppm (2.54, 2.49 and 2.51 mg/g of fresh weight). Significantly lowest total chlorophyll content was found in control (T_{13}) (1.12, 1.30 and 1.21 mg/g of fresh weight) for season I, second II and pooled data, respectively. The order of superiority for the rest of the treatments was $T_4 > T_1 > T_2 > T_9 > T_5 > T_6 > T_7 > T_{10} > T_8$ (1.32 to 2.35 mg/g fresh weight); $T_4 > T_1 > T_2 > T_5 > T_9 > T_{10} > T_7 > T_6 > T_8$ (1.35 to 2.28 mg/g fresh weight) and $T_4 > T_1 > T_2 > T_5 > T_9 > T_{10} > T_7 > T_6 > T_8$ (1.33 to 2.32 mg/g fresh weight) in season I, season II and pooled data, respectively (Table 4).

Discussion

The results of the current study clearly demonstrated that there was significant increased shoot length observed in the foliar application of micronutrients ($ZnSO_4$) might be due to the involvement of Zn in chlorophyll formation, which might have helped to influence physiological activity of plants *viz.*, meristematic activity in apical tissue, expansion of cell and formation of cell wall, cell division and differentiation leading to higher shoot length, number of shoots per plant and leaf area and this in turn enhanced the growth and yield parameters in mulberry as reported by Lokanath and Shivashankar (1981) [6]; Prasannakumar *et al.* (2001) [9]; Bose *et al.* (1994) [2] and Misra *et al.* (1995) [7]. Further, the increase in number of leaves could be due to increased shoots per plant and consequently in more number of internodes. The increased number of internodes gave rise to more number of leaves and such similar kind of findings was observed by Rashmi *et al.* (2006). Also increased shoot length can be ascribed to higher precursor activity of Zinc in auxin production (Kobayashi and Mizutani 1970) [4]. Cu NPs at 0.05ppm dose increased root and shoot length of Rapeseed plant to 38.83 and 21.72 per cent respectively over control. This increment in root and shoot length probably might be due to enhanced photosynthesis and / or increased synthesis of antioxidants in plant (Vishwakarma and Anil, 2017). Further, *Vigna radiate* and *Cicer arietinum* absorbed more of ZnO NPs and promoted the root and shoot growth resulting higher biomass (Pramod *et al.*, 2011) [8]. Furthermore, Jyothi and Hebsur (2017) [4] reported ZnO NPs improved growth, leaf area, and leaf dry weight of cereals. Hence, nanoparticles micronutrient group performed better than the chemical micronutrient group due to their nano properties and higher specific surface area. In mulberry also there might be increased leaf, root and shoot elongation due to foliar spray of combination of nano ZnO and nano Cu micronutrients.

Table 1: Effect of foliar supplementation of Zinc and Copper on mulberry shoot length, number of leaves and leaf area

Treatment	Total shoot length (cm)			Total number of leaves / plant			Leaf area (cm^2)		
	Season I	Season II	Pooled	Season I	Season II	Pooled	Season I	Season II	Pooled
T_1: Zn @ 500ppm	170.80^{cd}	164.00^{c}	167.40^{c}	234.07^{e}	245.40^{cd}	239.73^{e}	213.00^{c}	207.98^{c}	210.49^{c}
T_2: Zn @ 1000ppm	166.53^{d}	168.80^{bc}	167.67^{c}	251.53^{d}	249.80^{cd}	250.67^{d}	211.17^{c}	209.73^{c}	210.45^{cd}
T_3 : Nano Zn @ 500ppm	186.07^{b}	184.60^{a}	185.33^{b}	263.13^{c}	291.87^{b}	277.50^{c}	238.66^{b}	238.25^{b}	238.45^{b}
T_4: Nano Zn @ 1000ppm	173.20^{c}	167.67^{bc}	170.43^{c}	278.80^{c}	258.27^{c}	268.53^{cd}	231.12^{bc}	235.79^{b}	233.46^{bc}
T_5: Cu @ 500ppm	160.20^{e}	142.20^{d}	151.20^{d}	234.60^{e}	235.67^{de}	235.13^{ef}	187.66^{de}	184.33^{de}	186.00^{de}

T_6: Cu @ 1000ppm	164.07^{d}	164.07^{c}	164.07^{cd}	224.87^{f}	235.13^{de}	230.00^{ef}	207.92^{cd}	206.45^{c}	207.19^{cd}
T_7: Nano Cu @ 500ppm	159.87^{e}	168.60^{bc}	164.23^{cd}	234.60^{e}	242.27^{d}	238.43^{e}	200.35^{cd}	208.55^{c}	204.45^{cd}
T_8: Nano Cu @ 1000ppm	164.47^{d}	165.80^{c}	165.13^{cd}	220.67^{f}	223.87^{f}	222.27^{g}	187.36^{de}	192.56^{d}	189.96^{d}
T_9: Zn + Cu @ 500ppm each	160.53^{e}	159.07^{c}	159.80^{cd}	234.53^{e}	232.53^{de}	233.53^{ef}	187.38^{d}	183.96^{de}	185.67^{de}
T_{10}: Zn + Cu @ 1000ppm each	173.80^{c}	162.60^{c}	168.20^{c}	222.87^{f}	229.20^{de}	226.03^{g}	179.21^{de}	176.23^{e}	177.72^{e}
T_{11}: Nano Zn+Cu @ 500ppm each	202.33^{a}	197.00^{a}	199.67^{a}	328.73^{a}	325.13^{a}	326.93^{a}	267.13^{a}	266.41^{a}	266.77^{a}
T_{12} :Nano Zn+Cu @ 1000ppm each	199.40^{a}	189.87^{a}	194.63^{a}	309.53^{b}	284.73^{bc}	297.13^{b}	259.36^{a}	256.64^{a}	258.00^{a}
T_{13} : Control	143.00^{f}	141.27^{d}	142.13^{e}	216.40^{f}	215.00^{g}	215.70^{g}	154.76 ^{e}	154.33 ^{f}	154.54^{f}
CV (%)	4.93	5.43	4.54	2.54	3.29	2.50	3.17	2.98	2.52
S.Em ±	4.87	5.25	4.44	3.67	4.78	3.62	3.84	3.61	3.04

Figures in the column followed by same letters are not-significant at p=0.05 by DMRT

Table 2: Effect of foliar supplementation of Zinc and Copper on mulberry leaf yield, shoot yield and biomass

Treatment	Leaf yield (g/plant)			Shoot yield (g/plant)			Total biomass (g/plant)		
	Season I	Season II	Pooled	Season I	Season II	Pooled	Season I	Season II	Pooled
T_1: Zn @ 500ppm	951.93^{d}	946.00^{c}	948.97^{e}	796.93^{bc}	785.67^{bc}	791.30^{bc}	1748.87^{c}	1731.67^{cd}	1740.27^{cd}
T_2: Zn @ 1000ppm	928.87^{e}	925.40^{d}	927.13^{d}	786.87^{bc}	744.60^{d}	765.73^{bcd}	1715.73^{cd}	1670.00^{d}	1692.87^{d}
T_3 : Nano Zn @ 500ppm	982.67^{b}	978.13^{a}	980.40^{b}	823.60^{b}	804.80^{b}	814.20^{bc}	1790.27^{bc}	1755.80^{bc}	1773.03^{bc}
T_4: Nano Zn @ 1000ppm	969.13^{c}	966.07^{b}	967.60b^{c}	784.60^{bc}	782.53^{bc}	783.57^{bc}	1753.73^{c}	1748.60^{c}	1751.17^{c}
T_5: Cu @ 500ppm	869.27^{f}	866.87^{e}	868.07^{e}	740.40^{d}	748.73^{cd}	744.57^{e}	1609.67^{ef}	1615.60^{e}	1612.63^{e}
T_6: Cu @ 1000ppm	869.60^{f}	864.33^{e}	866.97^{e}	773.40^{bc}	754.53^{d}	763.97^{bcd}	1643.00^{def}	1745.00^{bc}	1694.00^{d}
T_7: Nano Cu @ 500ppm	820.53^{g}	817.87^{f}	819.20^{f}	765.80^{c}	707.27^{e}	736.53^{e}	1586.33^{f}	1525.13^{f}	1555.73^{efg}
T_8: Nano Cu @ 1000ppm	832.27^{g}	822.87^{f}	827.57^{f}	753.53^{c}	743.53^{d}	748.53^{bcd}	1585.80^{f}	1566.40^{f}	1576.10^{ef}
T_9: Zn + Cu @ 500ppm each	815.87^{g}	811.13^{f}	813.50^{f}	758.60^{c}	744.33^{d}	751.47^{bcd}	1574.47^{f}	1555.47^{f}	1564.97^{fg}
T_{10}: Zn + Cu @ 1000ppm each	825.13^{g}	823.33^{f}	824.23^{f}	760.53^{c}	738.80^{d}	749.67^{bcd}	1670.87^{d}	1562.13^{f}	1616.50^{e}
T_{11}: Nano Zn + Cu @ 500ppm each	1010.47^{a}	990.47^{a}	1000.47^{a}	895.13^{a}	891.47^{a}	893.30^{a}	1905.60^{a}	1841.73^{a}	1873.67^{a}
T_{12} :Nano Zn+Cu @ 1000ppm each	994.07^{a}	981.13^{a}	987.60^{a}	837.07^{a}	863.60^{a}	850.33^{b}	1806.27^{b}	1785.93^{b}	1796.10^{b}
T_{13} : Control	751.87^{h}	758.00^{g}	754.93^{g}	685.67^{e}	622.47^{f}	654.07^{f}	1393.20^{g}	1380.47^{g}	1386.83^{g}
CV (%)	1.49	1.53	1.48	4.86	2.33	2.78	2.55	1.58	1.65
S.Em ±	7.68	7.84	7.60	21.93	10.27	12.42	24.65	15.07	15.90

Figures in the column followed by same letters are not-significant at p=0.05 by DMRT

Table 3: Effect of foliar supplementation of Zinc and Copper on mulberry leaf weight and moisture content of leaves

Treatment	100 fresh leaf weight (g)			100 dry leaf weight (g)			Moisture (%)		
	Season I	Season II	Pooled	Season I	Season II	Pooled	Season I	Season II	Pooled
T_1: Zn @ 500ppm	231.95^{cde}	231.27^{de}	231.61^{de}	60.05^{d}	60.60^{d}	60.33^{e}	73.97 (59.32)^{c}	73.68 (59.19)^{b}	73.82 (59.23)^{bc}
T_2: Zn @ 1000ppm	229.67^{cde}	241.37^{d}	235.52^{d}	58.88^{de}	64.62^{c}	61.75^{d}	74.04 (59.37)^{b}	73.02 (58.71)^{bc}	73.53 (59.04)^{bc}
T_3 : Nano Zn @ 500ppm	264.34^{c}	276.97^{c}	270.66^{c}	70.94^{bc}	64.75^{c}	67.85^{c}	76.93 (61.29)^{a}	75.70 (60.47)^{a}	76.32 (60.88)^{a}
T_4: Nano Zn @ 1000ppm	248.41^{cd}	267.51^{cd}	257.96^{cd}	65.06^{c}	64.61^{c}	64.83^{cd}	73.63 (59.10)^{c}	73.34 (58.91)^{bc}	73.48 (59.00)^{bc}
T_5: Cu @ 500ppm	231.43^{cde}	226.94^{de}	229.18^{de}	52.86^{e}	52.19^{e}	52.53^{g}	73.04 (58.72)^{c}	72.84 (58.59)^{bc}	72.94 (58.65)^{bc}
T_6: Cu @ 1000ppm	206.67^{g}	200.57^{fgh}	203.62^{g}	55.15^{e}	53.79^{e}	54.47^{g}	73.20 (58.82)^{c}	72.71 (58.51)^{bc}	72.95 (58.66)^{bc}
T_7: Nano Cu @ 500ppm	218.69^{cdef}	225.75^{de}	222.22^{de}	53.71^{e}	55.58^{e}	54.65^{g}	75.19 (60.13)^{b}	75.30 (60.20)^{a}	75.24 (60.16)^{b}
T_8: Nano Cu @ 1000ppm	211.39^{cdef}	206.95^{f}	209.17^{def}	53.50^{e}	54.72^{e}	54.11^{g}	74.28 (59.53)^{c}	73.45 (58.98)^{bc}	73.87 (59.26)^{bc}
T_9: Zn + Cu @500ppm each	225.20^{cdef}	202.16^{fg}	213.68^{def}	51.28^{e}	53.58^{e}	52.43^{g}	75.28 (60.19)^{b}	73.27 (58.87)^{bc}	74.28 (59.53)^{b}
T_{10}: Zn + Cu @1000ppm each	215.59^{cdef}	209.40^{def}	212.49^{def}	56.27^{de}	57.47^{d}	56.87^{f}	73.90 (59.28)^{c}	72.57 (58.42)^{d}	73.24 (58.85)^{bc}
T_{11}: Nano Zn+Cu @ 500ppm each	342.76^{a}	333.93^{a}	338.35^{a}	77.43^{a}	88.82^{a}	83.13^{a}	77.38 (61.60)^{a}	76.66 (61.11)^{a}	77.02 (61.36)^{a}
T_{12} :Nano Zn+Cu @ 1000ppm each	293.96^{b}	297.08^{b}	295.52^{b}	72.59^{b}	80.51^{b}	76.55^{b}	77.02 (61.36)^{a}	76.57 (61.05)^{a}	76.80 (61.21)^{a}
T_{13} : Control	181.29^{g}	184.54^{fgh}	182.91^{g}	49.97^{e}	50.18^{e}	50.07^{g}	69.85 (56.70)^{d}	70.66 (58.47)^{e}	70.25(57.58)^{d}
CV (%)	4.94	4.17	3.51	3.81	5.24	3.67	1.59	1.39	1.29
S.Em ±	6.80	5.75	4.83	1.31	1.86	1.29	0.68	0.59	0.54

Figures in parenthesis are arcsine transformed values

Figures in the column followed by same letters are not-significant at p=0.05 by DMRT

Table 4: Effect of foliar supplementation of Zinc and Copper on mulberry for leaf Chlorophyll content

Treatment	Chlorophyll 'a' (mg/g of fresh wt.)			Chlorophyll 'b' (mg/g of fresh wt.)			Total Chlorophyll (mg/g of fresh wt.)		
	Season I	Season II	Pooled	Season I	Season II	Pooled	Season I	Season II	Pooled
T_1: Zn @500ppm	0.79^{e}	0.78^{d}	0.79^{e}	1.31^{e}	1.36^{ef}	1.34^{ef}	2.10^{e}	2.14^{e}	2.12^{e}
T_2: Zn @1000ppm	0.64^{gh}	0.65^{g}	0.64^{g}	1.38^{d}	1.36^{ef}	1.37^{e}	2.01^{f}	2.01^{f}	2.01^{ef}
T_3 : Nano Zn @ 500ppm	0.89^{c}	0.88^{b}	0.88^{c}	1.65^{bc}	1.61^{b}	1.63^{c}	2.54^{c}	2.49^{c}	2.51^{c}
T_4: Nano Zn @ 1000ppm	0.84^{d}	0.83^{c}	0.84^{d}	1.51^{c}	1.45^{c}	1.48^{d}	2.35^{d}	2.28^{d}	2.32^{d}
T_5: Cu @ 500ppm	0.71^{f}	0.70^{e}	0.71^{f}	1.18^{f}	1.16^{d}	1.17^{fg}	1.89^{g}	1.86^{g}	1.88^{g}
T_6: Cu @ 1000ppm	0.60^{h}	0.60^{hi}	0.60^{h}	0.96^{gh}	0.92^{e}	0.94^{h}	1.56^{h}	1.52^{h}	1.54^{ij}
T_7: Nano Cu @ 500ppm	0.60^{h}	0.60^{hi}	0.60^{h}	0.95^{gh}	0.95^{e}	0.95^{hi}	1.55^{hi}	1.55^{hi}	1.55^{i}
T_8: Nano Cu @ 1000ppm	0.51^{j}	0.52^{k}	0.52^{j}	0.80^{h}	0.83^{g}	0.82^{i}	1.32^{i}	1.35^{j}	1.33^{j}
T_9: Zn + Cu @500ppm each	0.65^{g}	0.66^{f}	0.65^{gh}	1.25^{ef}	1.17^{f}	1.21^{f}	1.90^{g}	1.83^{f}	1.87^{g}
T_{10}: Zn + Cu @1000ppm each	0.55^{i}	0.61^{h}	0.58^{hi}	0.99^{g}	1.09^{f}	1.04^{g}	1.54^{hi}	1.70^{g}	1.62^{h}

T_{11}: Nano Zn+Cu @ 500ppm each	0.97^{a}	0.95^{a}	0.96^{a}	1.79^{a}	1.80^{a}	1.80^{a}	2.76^{a}	2.75^{a}	2.76^{a}
T_{12} :Nano Zn+Cu @ 1000ppm each	0.92^{b}	0.91^{b}	0.91^{b}	1.69^{b}	1.73^{a}	1.71^{b}	2.61^{b}	2.64^{b}	2.62^{b}
T_{13} : Control	0.40^{k}	0.55^{j}	0.48^{k}	0.72^{i}	0.75^{g}	0.73^{j}	1.12^{j}	1.30^{k}	1.21^{k}
CV	2.03	2.84	1.84	3.04	4.17	2.92	1.73	2.63	1.61
S. Em. (±)	0.01	0.01	0.01	0.02	0.03	0.02	0.02	0.03	0.02

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 indicated that nano ZnO + nano Cu each @ 500 ppm resulted in significant superiority for growth, yield and quality parameters of mulberry followed by nano ZnO + nano Cu each @ 1000 ppm and nano ZnO @ 500 ppm. The nano size of micronutrients and its unique property of weight/volume more surface area, the nano micronutrients might penetrate more efficiently and effectively when applied through foliar means when compared to chemical micronutrients and this might have given significantly higher values for growth parameters of mulberry compared to control and chemical micronutrients.

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