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Effect of Copper Oxide Nanoparticle-Fortified Mulberry Leaves on Protein Profiles and Economic Traits of Bivoltine Silkworm *Bombyx mori* ($FC_1 \times FC_2$)

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***Abstract*: The silkworm Bombyx mori L. is a lepidopteran insect used as laboratory tool for various experiments. Being a domesticated insect, it has been reared mainly for the production of silk. The successful production of the silk not only governed by dynamic environmental factors but also affected by quality of mulberry leaf provided to silkworm. Hence, dietary nutrients are the most important factor which determine quality and quantity of silk production. An experiment was carried out to know the impact of mulberry leaf fortified with copper oxide nanoparticle at varied concentrations viz., 1%, 1.5%, and 2% on protein contents as well as commercial parameters in the FC1 silkworm hybrid. The larvae reared on copper oxide at 1.5% concentration registered higher protein contents in the haemolymph, fat body and silk gland over absolute and distilled water control. However, protein contents were relatively higher in the fat body when compared to haemolymph in all the treatments. A similar trend was also observed at 1.5% concentration for commercial parameters such as larval weight, cocoon weight, shell weight, pupal weight, shell ratio, filament length, filament weight, denier, renditta and raw silk percentage.**

***Keywords*: silkworm, copper oxide nanoparticle, FTIR analysis, infrared spectrum, Protein Profile, economic parameters.**

1. Introduction

Nanotechnology is rapidly advancing, offering new ways to improve nutrition and transform agriculture. It is gaining significant attention, focusing on tiny particles called nanoparticles, which are typically 1-100 nanometers in size and can be made from carbon, metals, metal oxides, or organic materials. Nanoparticles are generally defined as particulate matter with at least one dimension less than 100 nm. This places them in a similar size range as ultrafine particles (airborne particulates) and categorizes them as a subset of colloidal particles. In 2008, the International Organization for Standardization (ISO) defined a nanoparticle as a discrete nano-object where all three Cartesian dimensions are less than 100 nm. Components of nanoparticles include silica, Fe, CuO, titanium dioxide, cerium oxide, aluminium oxide, gold, Zn Cd/ZnS core-shell, P/ZnS core-shell, and Mn/Zn quantum dots. The size, composition, concentration, and chemistry of nanoparticles greatly influence the effectiveness of nano fertilizers in promoting plant growth.

Nutrient release occurs when these nano-fertilizers react with water in the soil. Nanoparticles such as metal oxides, AgO, MgO, CuO, and TiO2 are inorganic nanomaterials, whereas lipids, polymers, and CNTs are organic nanomaterials. Biodegradable, natural, and agriculturally safe carriers like chitosan are called polymeric NPs. Due to its polymeric cationic properties and ability to interact with negatively charged molecules or polymers, chitosan is a promising carrier for agrochemicals. Different types of nanomaterials, including copper, zinc, titanium, magnesium, gold, and silver nanoparticles, have emerged with effective antimicrobial activity against viruses, bacteria, and other eukaryotic microorganisms. Some nanomaterials exhibit antiviral, antibacterial, and antifungal properties and have an excellent capacity to combat pathogen-related diseases. Nanoparticles display unique physical, chemical, and biological characteristics compared to their larger-scale counterparts. This phenomenon results from their relatively larger surface area-to-volume ratio, increased reactivity or stability in chemical pro cesses, enhanced mechanical strength, and other factors. Elia (2017). These properties of nanoparticles have led to various applications, including medicine, engineering, catalysis, and environmental remediation. The incorporation of nanomaterials in sericulture is novel; therefore, it is essential to understand and harness their effects on mulberry silkworms and silk productivity. This review aims to elucidate the impact of nanoparticles on the growth and development of mulberry plants and silkworms. It comprehensively examines relevant data and explores how nanoparticles influence larval growth, cocoon production, and disease resistance in silkworms. Moreover, it investigates potential opportunities within the fascinating field of nanomaterials in sericulture.

The application of nanotechnology in some areas of science is still new, and evaluating its effects on tissues and organs of model organisms is important. Silk glands are present in the larval stage of the silkworm and are responsible for storing and

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synthesizing silk proteins (i.e., sericin and fibroin). Chemical residues from fungicides and pesticides applied to crops may contaminate mulberry leaves and cause damage or death to vital tissues and organs when ingested by the silkworm. Higher concentrations and sub-lethal doses of some nanomaterials lead to increased mortality, poor cocoon quality, and lower body weight compared to control groups. Feeding silkworms with nanomaterials such as carbon nanotubes (CNTs), titanium dioxide, copper, and graphene has been reported to enhance the mechanical properties and secondary structures of silkworm silk.

Copper oxide nanoparticles (CuO NPs) have shown potential in enhancing mulberry silkworm rearing. When applied to mulberry leaves, CuO NPs can improve silkworm growth, cocoon characteristics and silk production. Specifically, lower concentrations of CuO NPs, as a foliar spray can lead to increased larval weight, cocoon weight, shell weight, filament length and finer denier. However, higher concentrations may have adverse effects.

2. Material & Methods

The disease-free laying's of bivoltine double hybrid - FC_1XFC_2 were procured from National Silkworm Seed Organisation (NSSO), Mananadavadi road, Mysuru. The Copper oxide nanomaterial was procured from Adichunchanagiri Institute, Bengaluru.

A. Administration of (CuO) nanoparticles to Silkworm Larvae

The larvae of the bivoltine double hybrid FC_1XFC_2 silkworm were reared following standard rearing procedure (Giridhar and Dandin, 2010). After the 5th moult, the healthy silkworm larvae having equal weight were divided into five experimental groups as T1 to T5.

Where in T1- control, T2- absolute control, T3- 1% CuO-NPs, T4 – 1.5% CuO-NPs, and T5 – 2% CuO-NPs, treatments. Each treatment group consists of three replications and 30 larvae were used in each replication. On the second and fourth day of the fifth instar, each group of silkworm larvae received 10 mg, 15 mg, and 20 mg of treated leaves. Normal leaves were fed to the absolute control larvae, while distilled water-smeared mulberry leaves were fed to the control batch.

B. Collection of Haemolymphs from Silkworm Larvae

The haemolymph was collected in precooled vials containing a few crystals of thiourea (to prevent oxidation of haemolymph) by cutting the first larval prolegs of treated and control batches separately. The haemolymph was centrifuged at 3000 rpm for 10 min's at 4°C and the supernatant was used for qualitative and quantitative protein estimation (Takai and Tamashiro, 1975).

C. Quantitative Estimation of Protein

Haemolymph protein was estimated following Lowry's method (Lowry, *et al*,1951) using crystalline bovine serum albumin (BSA) as standard. To 0.1 ml of the haemolymph sample, 0.9 ml of distilled water was added followed by addition of 5 ml of protein reagent. The tubes were kept for 15 min's at room temperature. Then 0.5 ml of Folin's reagent was added and the tubes were allowed to stand for 30 min's. the spectrophotometer absorbance at 660 nm was recorded. The results were exposed in µg of protein per µl of haemolymph, silk glands fatbody.

D. FTIR Analysis of CuO Nanoparticles

The infrared spectrum of CuO nanoparticles shows characteristic absorption bands that can be attributed to Cu–O stretching vibrations and surface hydroxyl groups. Key points observed: The main transmittance decreases in the low wavenumber region (400–600 cm^1), which is typical for Cu–O lattice vibrations. - Broad features in the 3200–3600 cm^1 range (if present) are usually linked to O–H stretching vibrations due to adsorbed water or surface hydroxyl groups. - Bands in the 1400–1600 cm^1 region may correspond to bending vibrations of hydroxyl groups or residual nitrate/carbonate impurities. Overall, the spectrum confirms the formation of CuO nanoparticles with expected vibrational features. The sharp and well-defined peaks in the low-frequency region reinforce the crystalline nature of the oxide.

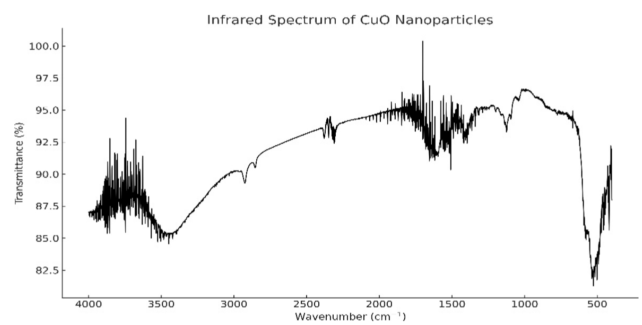

Fig. 1.

3. Results of Selected Biochemical Traits

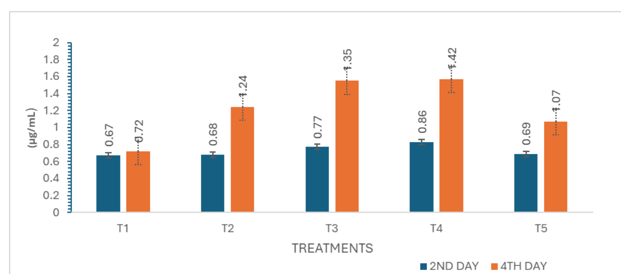

Fig. 2. Influence of mulberry leaves supplemented with copper oxide at varied concentrations on haemolymph protein of bivoltine double hybrid FC_1XFC_2

The silkworm larvae fed on mulberry leaves fortified with copper oxide NPs recorded a notable difference in respect of total protein content in haemolymph of silkworm larvae with maximum being in double hybrids FC_1XFC_2 in day wise treatment like (5th instar 2nd day, 5th instar 4th day respectively) i.e.,1.5% treatment CuO (0.86, 1.42 µg/mL) The minimum was recorded at 2% treatment CuO (0.69, 1.07 µg/mL) as against to absolute control (0.67, 0.72 µg/mL) and distilled water control, (0.68, 1.24 µg/mL) respectively.

The silkworm larvae fed on mulberry leaves fortified with copper oxide NPs recorded a notable difference in respect of total protein content in the fat body of the silkworm larvae with maximum being in double hybrid FC_1XFC_2 in day-wise treatment, like (5th instar 2nd day, 5th instar 4th day respectively) i.e., 1.5% treatment CuO (2.62, 2.01µg/mL). Minimum was recorded at 2% treatment CuO (1.62, 1.53 µg/mL) as against to absolute control, (1.51, 0.95 µg/mL) and distilled water control, (1.59, 1.27 µg/mL) respectively.

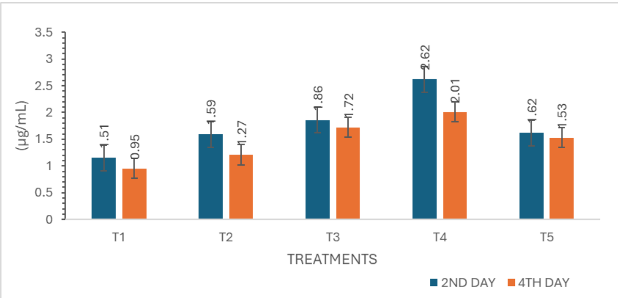

Fig. 3. Influence of mulberry leaves supplemented with copper oxide at varied concentrations on fat body protein of bivoltine double hybrid FC_1XFC_2

The silkworm larvae fed on mulberry leaves fortified with copper oxide recorded a notable difference in respect of total protein content in silk glands of the silkworm larvae with maximum being in double hybrid FC_1XFC_2 in day wise treatment like (5th instar 2nd day, 5th instar 4th day respectively) i.e.,1.5% treatment CuO (1.80, 2.73 µg/mL). The minimum was recorded at 2% CuO (1.59, 1.66 µg/mL) as against to absolute control, (1.29, 1.24 µg/mL) and distilled water control, (1.29, 1.67 µg/mL) respectively.

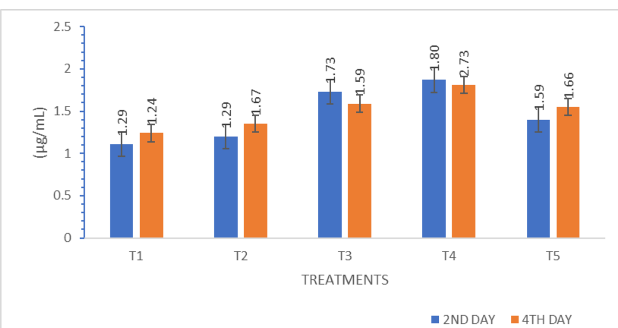

Fig. 3. Influence of mulberry leaves supplemented with copper oxide at varied concentrations on silk gland protein of bivoltine double hybrid FC_1XFC_2

A. Economic Parameters of the Silkworm, Bombyx Mori for Control and Treated Batches

1) Larval weight (g)

The larval weight was found significantly increased in all the treatments of FC_1XFC_2 when compared to the control it was found highest in the treated with 1.5% of copper oxide in all day treatments i.e., CuO (22.45±0.02). The minimum was recorded at 2% treatment CuO (22.35±0.02) as against to absolute control, (21.15±0.02) and followed by distilled water control, (21.33±1.01) respectively.

2) Cocoon Weight (g)

The cocoon weight was found to significantly increase in all the treatments of FC_1 XFC_2 when compared to the control. It was found highest in the treated with 1.5% of copper oxide in all day treatments i.e., CuO (11.12±0.01g) The minimum was recorded at 2% treatment CuO (10.06±0.02g) as against to absolute control, (10.06±0.02g) and distilled water control, (10.15±0.02g) respectively.

3) Shell Weight (g)

The shell weight was found to have significantly increased in all the treatment batches of FC_1 XFC_2 when compared to the control batches. It was found highest in the batches treated with 1.5% of copper oxide in all day treatments like (5th instar 2nd day, 5th instar 4th day respectively) i.e., CuO (2.35±0.02g). The minimum was recorded at 2% treatment CuO (2.06±0.02 g) and as against to absolute control, (2.02±0.01g) and distilled water control, (2.15±0.02 g), respectively.

4) Shell Percentage (%)

The shell percentage was found significantly increased in all the treatment batches of FC_1 XFC_2 when compared to the control batches. It was found highest in the batches treated with 1.5% of copper oxide in all day treatments like (5th instar 2nd day, 5th instar 4th day respectively) i.e., CuO (22.14±0.214 %) and the minimum was recorded at 2% treatment CuO (20.52±0.123%) and as against to absolute control, (20.12±0.213%) and distilled water control, (20.37 ±0.123 %) respectively.

5) Filament Length (m)

The filament length was found significantly increased in all the treatment batches of FC_1 XFC_2 when compared to the control batches. It was found highest in the batches treated with 1.5% of copper oxide in all day treatments like (5th instar 2nd day, 5th instar 4th day respectively) i.e., CuO (1336±1 m). The minimum was recorded at 2% treatment CuO (1195±2 m) as against to absolute control, (1185±1 m) and distilled water control, (1154±2.08 m) respectively.

6) Silk Weight (g)

The silk weight was found significantly increased in all the treatment batches of FC_1 XFC_2 when compared to the control batches. It was found highest in the batches treated with 1.5% of copper oxide in all day treatments like (5th instar 2nd day, 5th instar 4th day respectively) i.e., CuO (2.35±0.02 g). The minimum was recorded at 2% treatment CuO (2.06±0.02 g) as against to absolute control, (2.02±0.01g) and distilled water control, (2.15±0.02g) respectively.

7) Denier (d)

The denier was found significantly increased in all the

Table 1

Influence of mulberry leaves supplemented with copper oxide at different concentrations on commercial parameters of FC1 silkworm

Concentration	Larval weight (g)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Filament length (m)	Filament weight (g)	Denier	Renditta
1%	22.39±0.02	10.66±0.02	2.14±0.02	21.16±0.214	1246±1	2.34±0.025	2 .33±0.02	4.71±0.253
1.5%	22.45±0.02	11.12±0.01	2.35±0.02	22.14±0.214	1336±1	2.37±0.02	2.57±0.015	4.62±0.037
2%	22.35±0.02	10.06±0.02	2.06±0.02	20.52±0.123	1195±2	2.11±0.02	2.24±0.015	4.77±0.029
Control Distilled water	21.33±1.01	10.15±0.02	2.15±0.02	20.37±0.123	1154±2.08	2.20±0.015	2.05±0.02	4.92±0.046
Absolute Control	21.15±0.02	10.06±0.02	2.02±0.01	20.12±0.123	1185±1	1.76±0.01	1.79±0.035	5.72±0.337

treatment batches of FC_1 XFC_2 when compared to the control batches. It was found highest in the batches treated with 1.5% of copper oxide in all day treatments like (5th instar 2nd day, 5th instar 4th day respectively) i.e., CuO (2.57±0.015). The minimum was recorded at 2% treatment CuO (2.24±0.015) as against to absolute control, (1.76±0.01) and distilled water control, (2.05±0.02) respectively.

8) Renditta (Kg)

The renditta was found significantly increased in all the treatment batches of FC_1 XFC_2 when compared to the control batches. It was found highest in the batches treated with 1.5% of copper oxide in all day treatments like (5th instar 2nd day, 5th instar 4th day respectively) i.e., CuO (4.62 ±0.037 Kg). The minimum was recorded at 2% treatment CuO (4.77±0.029 Kg) as against to absolute control, (5.72 ± 0.337 Kg) and distilled water control, (4.92±0.046Kg) respectively.

4. Discussion

The findings of our study on the effects of copper oxide (CuO) NPs enrichment in mulberry leaves on the growth and silk production of bivoltine hybrid silkworms (*Bombyx mori* L) are in stark contrast to those reported by a cheng, *et al*., (2021) study and Muhammad, *et al*., (2022). While these studies found that exposure to and CuO nanoparticles had detrimental effects on the silkworms, including reduced larval weight, survival rate and cocoon yield. Our study demonstrated significant improvements in key economic traits when silkworms were fed with 1.5% CuO enriched mulberry leaves compared to 1% and 2% concentration.

The disparity between our findings and those of previous studies may be attributed to differences in the form and concentration of CuO and used. While previous studies used nanoparticles, our study used a specific concentration of CuO enrichment in mulberry leaves. This suggests that the effects of CuO on the silkworms may be dependent on the form and concentration used.

5. Conclusion

A comparative study was conducted to assess the effects of fortifying mulberry leaves with copper oxide (CuO) on the growth and silk-producing performance of the bivoltine double hybrids the silkworm, *Bombyx mori* (FC_1× FC_2). Among all tested concentrations, 1.5% fortification showed significant improvements in key economic traits including larval weight, cocoon weight, shell weight, filament length, silk weight, renditta and denier.

In addition to better physical traits, the silkworms fed with fortified leaves showed an increase in total protein content, with copper oxide treatments producing higher protein levels than copper oxide. This protein enhancement is closely linked to improved larval growth and silk output.

The study concludes that 1.5% CuO fortification in the silkworm feed effectively boosts both quantitative and qualitative aspects of cocoon production. This method is recommended for sericultural farmers as a low-cost, high-impact strategy to enhance silk yield and overall productivity.

References

[1] R. Belal and A. Gad, "Copper oxide nanoparticles induce oxidative stress, genotoxicity, and apoptosis in the haemocytes of *Bombyx mori* larvae," *Scientific Reports*, vol. 13, no. 1, Art. no. 3520, 2023.

[2] L. Cheng, H. Huang, S. Chen, W. Wang, F. Dai, and H. Zhao, "Characterization of silkworm larvae growth and properties of silk fibres after direct feeding of copper or silver nanoparticles," *Materials and Design*, vol. 129, pp. 125–134, 2017.

[3] D. Das, P. Paul, and P. Mandal, "Prolonging mulberry leaf longevity with silver-doped copper oxide nanoparticles: A postharvest approach," *Discover Plants*, vol. 2, Art. no. 47, 2025.

[4] A. Eskin, Ş. Öztürk, and M. Körükçü, "Determination of the acute toxic effects of zinc oxide nanoparticles (ZnO NPs) in total hemocyte counts of *Galleria mellonella* (Lepidoptera: Pyralidae) with two different methods," *Ecotoxicology*, vol. 28, pp. 801–808, 2019.

[5] S. S. Fometu, G. Wu, L. Ma, and J. S. Davids, "A review on the biological effects of nanomaterials on silkworm (*Bombyx mori*)," *Beilstein Journal of Nanotechnology*, vol. 12, pp. 190–202, 2021.

[6] M. Y. Ruiz-Aguilar, L. A. Aguirre-Uribe, S. N. Ramírez-Barrón, Y. E. Castro-del Ángel, and A. Hernández Juárez, "Insecticidal efficacy of zinc oxide and silicon dioxide nanoparticles against larvae of *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae)," 2025.

[7] A. H. Mir, A. Qamar, and I. Qadir, "Accumulation and trafficking of zinc oxide nanoparticles in an invertebrate model, *Bombyx mori*, with insights on their effects on immunocompetent cells," *Scientific Reports*, vol. 10, Art. no. 1617, 2020.

[8] H. Mirzaei and M. Darroudi, "Zinc oxide nanoparticles: Biological synthesis and biomedical applications," *Ceramics International*, vol. 43, no. 1, pp. 907–914, 2017.

[9] A. Muhammad, J. He, T. Yu, C. Sun, D. Shi, Y. Jiang, Y. Xianyu, and Y. Shao, "Dietary exposure of copper and zinc oxide nanoparticles affect the fitness, enzyme activity, and microbial community of the model insect, silkworm *Bombyx mori*," *Science of the Total Environment*, vol. 813, Art. no. 152608, 2022.

[10] S. Mumtaz, S. Ali, and H. M. Tahir, "Biological applications of biogenic silk fibroin–chitosan blend zinc oxide nanoparticles," *Polymer Bulletin*, vol. 81, pp. 2933–2956, 2024.

[11] A. Sirelkhatim, S. Mahmud, and A. Seeni, "Review on zinc oxide nanoparticles: Antibacterial activity and toxicity mechanism," *Nano-Micro Letters*, vol. 7, pp. 219–242, 2015.

[12] O. Bulut and K. Biswas, "Effect of feed supplementation with biosynthesized silver nanoparticles using leaf extract of *Morus indica* L. on *Bombyx mori* L. (Lepidoptera: Bombycidae)," *Scientific Reports*, vol. 9, Art. no. 14839, 2019.

[13] T. Bayzid, M. Khalid, M. S. Akand, M. A. Alam, M. M. Karim, S. M. N. Alam, and M. Gazi, "Crystallographic analysis of silk sericin-stabilized zinc oxide nanoparticles with enhanced antimicrobial and photocatalytic properties," *Inorganic Chemistry Communications*, 2025.

[14] Y. Xu, W. Wang, L. Ma, X. Cui, I. Lynch, and G. Wu, "Acute toxicity of zinc oxide nanoparticles to silkworm (*Bombyx mori* L.)," *Chemosphere*, vol. 259, Art. no. 127481, 2020.

[15] L. Zhou, X. Chen, Z. Shao, P. Zhou, D. P. Knight, and F. Vollrath, "Copper in the silk formation process of *Bombyx mori*," *FEBS Letters*, vol. 554, no. 3, pp. 337–341, 2003.

[16] H. Kalita, M. Pathak, K. S. Sahoo, H. Sikha, P. Dutta, K. Ningthoujam, and R. K. Patidar, "Effect of nanoparticles on morpho-histology of eri silkworm, *Samia cynthia ricini* (Boisduval) (Lepidoptera: Saturnidae)," 2024.