ABSTRACT

Mulberry is a high biomass-producing, fast-growing, perennial plant belonging to the genus *Morus* under the family Moraceae. Mulberry leaves, especially those of the *Morus alba* L. (white mulberry), are agriculturally more important, serving as the only food source for the monophagous insect *Bombyx mori* (silkworm). It is estimated that almost 90% of global raw silk production depends upon mulberry silk, and in India, mulberry silk culture is performed mainly by moriculture (mulberry plant culture). Factually, India is the second largest producer of silk, and this silk industries have an immense contribution to its national economy. India's textiles and apparel industry holds economic importance, contributing 2.3% to the GDP, with a 12% share in exports and providing direct employment to 45 million people, along with 100 million in allied industries. Beyond its role in the sericulture industry, these plants have several medicinal properties, offering a range of health benefits, have strong food value, and serve as a component for phytoremediation purposes and landscaping practices.

Despite its numerous benefits, the cultivation area for mulberries is constraining both globally and in India. Due to rapid urbanization, expanding cultivable land is quite difficult, so improving the productivity of mulberry foliage can meet the growing demand for silk. In addition to limited land availability, the high necessity of water and scarcity of proper water resources chronically affect the mulberry plantations. Lack of adequate nutrient supply is another prime factor inhibiting the mulberry growth and development.

The cultivation of mulberry begins with the planting of saplings. In the case of mulberry, the preferred method for proliferation is through vegetative cuttings, as it proves to be the most efficient practice for the mass production of saplings. However, this process has shown significant delays in the succession of cuttings, the formation of roots, the

flourishing of buds, and subsequent development into leaves, eventually delaying the leaf harvesting time. Here, adequate water and nutritional elements were also found to be two important factors determining the success rate of this propagation process. Conventionally, chemical fertilizers were employed in the nutrient management process, but they have several adverse effects on the environment and human health. While biofertilizers show promise as an alternative, they are condition-specific and effective only in certain soil conditions. Recently, nanotechnology-based nano-fertilizers have emerged that are reported to play a potent role in both nutrient management and abiotic stress amelioration.

Concerning all these factors, in this study, iron, zinc, manganese, and copper nanoparticles (FeNP, ZnNP, MnNP and CuNP, respectively) were green synthesized using the aqueous decoction of pruning litter of tea plants [*Camellia sinensis* (L.) Kuntze] is a waste product of the tea industry and is widely available across North East and South Eastern India. During the synthesis, variation in the preparation process was followed to ensure minimal use of metallic precursor and plant extract and to prescribe an ideal synthesis condition. This involved mixing of 10 mM $FeCl_3$, 100 mM of $Zn(NO_3)_2$, 500 mM NaOH, 1 mM $KMnO_4$, and 500 mM of $Cu(NO_3)_2$ with 100 mg/mL extract of tea leaves while maintaining a volumetric ratio of 10:1 (metallic precursor: tea extract) for FeNP, CuNP and MnNP. Similarly, for ZnNP, the optimal ratio was 7:2:1 for zinc nitrate, NaOH, and tea extract, respectively. Notably, variations in light intensity, reaction time, temperature, and pH of the plant extract did not significantly affect nanoparticle synthesis, although extreme values should be avoided. All the synthesized nanomaterials showed a round to irregular morphology with a size range of 100 nm, as observed through instrumental characterization.

The nanoparticles formed under optimum conditions, were then applied to mulberry vegetative cuttings in different concentrations to determine the effectiveness of the applied nanoparticles in mulberry growth and propagation. The results demonstrated that FeNP and ZnNP applied at 10 mg/kg soil dosage and MnNP and CuNP applied at 5 mg/kg soil dosages were found to be optimum. These treatments significantly enhanced the sprouting percentage, survival percentage, and various phenotypic characteristics of the studied plants. Subsequently, phytotoxicity analysis through *Allium cepa* root tip bioassay confirmed that the optimum dosages for mulberry growth were safe and did not possess any potential cytotoxic and genotoxic effects.

Combining all the studied micronutrients is more efficient than the nanoparticles applied individually. This combined application effectively minimizes the adverse effects of a six-day watering gap, enhancing the viability of vegetative cuttings and facilitating subsequent plant growth as evaluated under drought conditions. The nutrient mobilization study indicated a substantial increase in soil micronutrient levels after nanoparticle application. Beyond the soil, this treatment significantly heightened the absorption, uptake, accumulation, and translocation of applied micronutrients within the plant system, as evidenced by bioaccumulation and translocation studies.

As the silkworm is the ultimate consumer of the mulberry leaves, a silkworm rearing practice was carried out by feeding the nano-micronutrients treated leaves to ensure the safety concerns of these nanoparticle treatments. Larvae supplemented with the leaves treated with a combination of all the essential micronutrients demonstrated enhanced ingestion and digestion, along with improved absorption and assimilation of nutrients, ultimately resulting in an increase in larval body weight. The findings from the current study also reveal that leaves treated with combined treatments do not induce larval mortality but instead positively impact enhancing silkworm growth parameters.

Though nanoparticles showed all the possibilities in plant nutrient management, bulk delivery of nutrients and nutrient leaching problems are the major concern. Further, there is still enough scope to improve the nutrient use efficiency (NUE) of these nano-fertilizers. Slow and controlled release nano-fertilizers (SNRF and CRNF) are one step ahead that can solve the mentioned problems more efficiently. These are the coated or composite nano-fertilizers that release nutrients in a controlled and regulated manner. In this study, we synthesized four different kinds of SRNFs, taking hydroxyapatite, chitosan, graphene oxide, and hydrogel as the matrix polymer. In all the SRNFs, Fe, Zn, Mn, and Cu are loaded, and the prepared SRNFs demonstrate efficient nutrient loading and encapsulation efficiency. The prepared SRNFs also demonstrated satisfactory nutrient holding capacity and showed slow release of nutrients over a prolonged period of time. Furthermore, as evidenced by morpho-biochemical analysis, all the synthesized SRNFs were very effective in improving mulberry growth, propagation, and internal health. However, among all, hydroxyapatite-based SRNFs had the highest efficacy and also it stood to be first in terms of NUE. This comprehensive approach aims to ensure precision in micronutrient management for mulberry. Regardless of the possible benefits of these nano-fertilizers, their incorporation into plant systems, the specific transportation mechanism, and the ultimate status of uptaken nanoparticles inside plant systems should be investigated.

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