INTRODUCTION

Sericulture is recognized as a crucial agro-based cottage industry that is pivotal in fostering economic growth and supporting the livelihoods of numerous individuals involved in its production and processing. More than 30 countries across the globe are currently involved in sericulture; among those, India ranks second in raw silk production (Vikaspedia 2023). From the perspective of India and Bengal, almost 84-90% of total silk production depends on mulberry plants (Karthik and Rathinamoorthy 2017).

Mulberry belongs to the genus *Morus*, under the family Moraceae, and is a deciduous, woody, perennial plant (Acharya et al. 2022; Wani et al. 2017). Leaves of this plant, particularly of the white mulberry (*Morus alba* L.), are agriculturally important as these are the only source of food for the monophagous insect *Bombyx mori* (silkworm larvae), the producer of raw silk. Besides sericulture, the mulberry plant holds immense economic significance due to its versatile nature and is often referred to as the 'Kalpa Vriksha' in India due to its multipurpose utilities (Jan et al. 2021). Despite numerous benefits, the mulberry cultivation area is being constrained day by day. Given the impracticality of expanding cultivable land at this stage, the only viable solution to fulfill the increasing demand for silk is to enhance the growth and productivity of mulberry plants.

The propagation of mulberry trees can be accomplished through seed germination and vegetative propagation, grafting, and tissue culture (Saraçoğlu et al. 2016). Among all, propagation through stem cutting (softwood cutting) is the simplest and most cost-effective vegetative proliferation practice used for the mass production of saplings (Hawramee et al. 2019). However, this multiplication process demonstrates a major delay in the flourishing of the buds, the appearance of leaves, and sometimes in root succession, eventually delaying the harvesting time. In vegetative propagation, root establishment and succession of cuttings depend on various factors; among these, water availability playing a critical role in promoting adventitious root growth, as maintaining a positive water balance is necessary for the development of roots (Loach 1988).

Besides ensuring water availability, proper nutrient management, including macro and micronutrients, is essential for establishing roots, growth, and leaf production (Abshahi et al. 2022; Lu et al. 2003). While micronutrients like iron, zinc, copper, and manganese are needed in small quantities, maintaining their optimal balance is critical for the development and quality of leaf production (Noor-Ul-Din 2012; Hänsch and Mendel 2009).

A study conducted by ICAR-Indian Institute of Soil Science in Bhopal between 2011-2017, analyzing soil samples from across Indian districts, revealed deficiencies of 4.2% for Cu, 7.1% for Mn, 12.8% for Fe, and 36.5% for Zn (Shukla et al. 2018). Another study focusing on mulberry-growing fields in India's North Eastern Region (NER) reported a 36% deficiency for Zn and 10% for Mn (Ray et al. 2018). Multi-micronutrient deficiency in mulberry, as highlighted by Geetha et al. (2017), can lead to up to a fifty percent loss in yield. Zinc deficiency in mulberry plants results explicitly in growth retardation, reduced leaf area expansion, alterations in the shoot-to-root ratio, and adverse effects on biochemical and enzymatic parameters, potentially causing an up to 80% reduction in mulberry yield (Tewari et al. 2008).

Conventional chemical-based fertilizers are commonly employed to address nutrient deficiencies; however, their prolonged use can result in soil mineral imbalance, loss of soil fertility and texture, and enduring impacts on the ecosystem (Elemike et al. 2019).

While biofertilizers can potentially improve soil fertility and crop production to some extent (Bhardwaj et al. 2014), their large-scale production poses challenges, and their efficacy can be influenced by pH and temperature conditions.

Recently, nanotechnology-based 'nano-fertilizer' has emerged as a promising innovation with significant potential to revolutionize the agricultural sector (Bala et al. 2023). Nano-fertilizers are composed of nano-scale particles with a diameter ranging from 1 to 100 nm in at least one dimension (Du et al. 2019) and exhibit a high surface-to-volume ratio, leading to increased reactivity. This enhanced reactivity makes them highly efficient at adsorbing essential compounds and nutrients crucial for plant growth and metabolism (Morteza et al. 2013). Nano fertilizers have demonstrated the capability to enhance plant growth, promote seed germination, improve soil quality, and manage pathogen attacks and pesticide degradation (El-Saadony et al. 2022). The key advantages of nano fertilizers over traditional chemical fertilizers include proficient nutrient management through improved nutrient use efficiency (NUE), enhanced crop quality and yield, improved soil fertility, and reduced frequency of fertilizer application, thereby lowering environmental protection costs (Zulfiqar et al. 2019).

Among micronutrients, Fe, Zn, Mn, Cu, Ni, and Co exhibit cationic properties, while Mo, B, and Cl are anionic (Washington State University 2023). The conversion of these anionic micronutrients into nano-form is challenging due to their non-metallic or transitional metallic nature. Moreover, as soils carry a negative charge, anionic micronutrients can freely move in soil water and are readily taken up by plants (Nadeem and Farooq 2019). In contrast, cationic micronutrients are tightly bound to soil particles, often remaining in a plant-unavailable form (Goldy 2013; Shuman 1991). Notably, cobalt (Co) is considered non-essential, and deficiencies of nickel (Ni) are rarely reported in the

Indian sub-continent (Singh and Patra 2020. Therefore, our study primarily focuses on synthesizing essential cationic nano-micronutrients, specifically Fe, Zn, Mn, and Cu.

In the present study, iron, zinc, manganese, and copper nanoparticles were synthesized using tea plant pruning litter via a green synthesis method. Optimal synthesis conditions were determined systematically by varying the major defining parameters such as metallic precursor salt concentration, plant extract concentration, reaction time, pH, and temperature. The nanoparticles prepared under optimized conditions were then characterized by a series of instrumental analyses.

Following the successful synthesis of micronutrient nanoparticles, the bio-efficacy of the prepared nanoparticles was estimated through mulberry growth and propagation study. To achieve this, a pot experiment was carried out, where iron (FeNP), zinc (ZnNP), manganese (MnNP), and copper nanoparticles (CuNP) were applied to mulberry cuttings at various concentrations, and their growth performance was subsequently assessed. The cytotoxic and genotoxic effects of the studied nanoparticles and the applied dosages were also evaluated through *Allium cepa* root tip bio-assay to determine whether the optimized dosage was safe. In the subsequent phase of the study, a combined dosage of all four micronutrients was applied to mulberry cuttings under different irrigation regimes to determine the role of applied nano-micronutrients in effective vegetative propagation using minimum water utilization or in the area affected by drought stress. Furthermore, the absorption, uptake, translocation, and accumulation of these micronutrient nanoparticles in various parts of the treated plants were evaluated using atomic absorption spectroscopy.

Given that silkworms are voracious feeders and the ultimate consumers of mulberry leaves, evaluating the impact of nanoparticle-treated leaves on silkworm health is a paramount concern. Concerning this, a silkworm rearing practice was conducted by feeding nano-treated leaves, and afterward, several growth and cocoon parameters were evaluated. Moreover, the mortality rate was considered a benchmark to assess any potential toxicity these nano-treatments imposed.

Despite the numerous benefits, nano-fertilizers have limitations in addressing all fertilization-related challenges, including issues such as leaching and bulk nutrient delivery. Improving the nutrient use efficiency (NUE) of these fertilizers also remains an area of significant importance. Slow and controlled release nano-fertilizers (SRNF and CRNF) are one step ahead of the normal nanoparticles and can solve the mentioned problems more efficiently. These are the coated or composite nano-fertilizers that release nutrients in a controlled and regulated manner, thereby reducing the chances of leaching and bulk delivery of nutritional elements (Nongbet et al. 2022). In this study, we synthesized four types of SRNFs using chitosan, hydroxyapatite, graphene oxide, and hydrogel as the matrix polymer or carrier molecule, and inside each SRNF, all the essential cationic nano-micronutrients (FeNP, ZnNP, MnNP, and CuNP) were incorporated. The prepared SRNFs were finally applied to mulberry cuttings for growth attributes study and nutrient use efficiency analysis. This comprehensive approach aims to ensure precision in micronutrient management for mulberry.

The following objectives were undertaken to fulfill the aims and objectives of the present work:

1. Green synthesis of essential cationic nano-micronutrients (iron, copper, zinc, and manganese), its characterization, and preparation process variation.

2. Application of these nano-micronutrients on mulberry vegetative propagation by determining optimum dose.

3. Assessment of morphological characteristics of treated mulberry cuttings.

4. Evaluation of biochemical content and antioxidant enzyme activity of mulberry cuttings after treatments.

5. Measurement of oxidative stress of mulberry after nano-fertilizer application under different irrigation regimes.

6. Analysis of micronutrient mobilization in plant and soil after application of nanoparticles.

7. Determining the impact of feeding mulberry leaves treated with essential cationic nano-micronutrients on larval and cocoon parameters of silkworms.

8. Analysis of isozyme patterns and protein profile by NATIVE and SDS gel electrophoresis in treated mulberry leaves.

9. Standardization of sustained release of nano-micronutrients through conjugate formation.