Thesis Summary

Summary of the Thesis

Chapter 7

Agriculture has a large global influence, with numerous studies focusing on the applications of diverse plants. The agricultural business market has shrunk, and more than 400 global firms are now actively involved in nanotechnology research and development, a figure that is predicted to grow to thousands in the following decade. The toxic impact of Nanoparticles (NPs) has been disclosed, limiting plant growth, elongation of shoot and root, biomass production, photosynthetic processes, and chlorophyll contents, as well as elevated actions of catalase and peroxidase enzymes in treated plant roots. The phytotoxicity profile of NPs relates to NM shape, surface or size-mediated generation of stress or stimulation, and chemical composition-dependent chemical toxicity of Nanomaterials (NMs). The development of a safe-by-design work based on nanotechnology is dependent on the regulation of healthcare, safety, and environmental concerns throughout the manufacturing, handling, preservation, assimilation, and disposal of nanomaterials. Because of the potential risks of nanomaterials, most developed countries have actively participated in International Organisations (OECD Working Party on Manufactured Nanomaterials (OECD WPMN), International Organisation for Standardisation / Technical Committee 229 (ISO/TC 229), Registration, Evaluation, Authorization, and Restriction of Chemicals Annex (REACH Annex), and so on. Green synthesis of alloy nanomaterials is a sustainable method of producing nanostructures utilizing plant extracts as reducing and stabilizing agents. This eco-friendly process has many advantages over traditional chemical synthesis, including cost-effectiveness, environmental friendliness, and the use of renewable resources. The green synthesis method begins with the selection of suitable plant species that contain bioactive chemicals with reducing and capping characteristics. Plant extracts derived from leaves stems, or roots contain phytochemicals such as polyphenols, flavonoids, and terpenoids, which work as natural reducing agents to convert metal ions into nanoparticles. These phytochemicals not only aid in the reduction of metal ions, but they also help to stabilize the resultant nanomaterials, avoiding agglomeration and promising uniform distribution.

The first objective of the research was to identify, collect, and select the best possible antioxidant-rich plant species locally available, for which nanomaterial synthesis has not been reported yet. This study also aimed to sustainably use plant leaves as only pruned leaves were collected for examination. Rigorous literature review and after the most popular ethnobotanical traditional knowledge, five plant species i.e., *Peltophorum innerme* Roxb., *Polygonum microcephalum* Wall. ex D. Don., *Chrysalidocarpus lutescens* H. Wendl., *Crinum asiaticum* L., and *Aquilaria malaccensis* Lam., were selected for the study. Plant leaves were collected, washed, and air dried for 3 months at room temperature during autumn season followed by the grinding of leaves which made smooth powder. It was labeled and stored for further use. Out of these five plant species, only the aqueous leaf extracts of *Polygonum microcephalum* Wall. ex D. Don., *Chrysalidocarpus lutescens* H. Wendl., were further chosen for nanomaterial synthesis, as these two plants showed exceptional antioxidant quality and quantity compared to *Peltophorum innerme* Roxb., *Crinum asiaticum* L., *and Aquilaria malaccensis* Lam. Both qualitative phytochemical analysis and quantitative analysis data provided much-needed insights about the total antioxidant capacity, total phenolic content, and ferric reducing power assay of the plant leaf extracts. However, out of the above-mentioned two species, final selection was made based on DPPH free radical assay (IC_{50}) value. Plant leaf extract mediated green synthesis of Zn Monometallic, Zn-Cu Bimetallic, and Zn-Cu-Fe-Mn Quadrimetallic Nanoparticles (MNP, BNP, QMNP) were synthesized with different permutations combinations of Aqueous Leaf Extracts (ALE) of *C.lutescens* , pH range (2,5,7) while keeping other parameters constant. Zinc, copper, iron, and manganese were selected as alloying elements because they are abundant and biocompatible. These metals are cheap and serve critical roles in a variety of biological activities, including enzymatic reactions, redox signalling, and antioxidant defense mechanisms. The green synthesis process usually entails mixing metal precursors with plant extracts under precise reaction conditions, such as temperature, pH, and reaction time. Metal ions are reduced by transferring electrons from bioactive components in the plant extract, resulting in the nucleation and development of alloy nanoparticles. The reaction kinetics, particle size, and morphology can all be adjusted by changing the reaction parameters, allowing for exact tuning of nanoparticle features. As such, phase I of the study concluded with the green synthesis of Zn-based nanoparticles.

Even though the green synthesis of 3 types of nanoparticles was optimized following the standard, temperature, precursor raw materials, and SPR peaks from UV-Vis spectroscopy. But reaction yield (%) was later added to reduce waste and to follow sustainable goals. After synthesis, the alloy nanoparticles are characterized with a variety of analytical techniques, including Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), X-ray diffraction (XRD), and Fourier-transform infrared spectroscopy (FTIR). These approaches reveal information about the size, shape, crystallinity, and chemical composition of the nanoparticles, validating their successful synthesis and structural features. Zinc (Zn) is required for enzyme activity, protein synthesis, and glucose metabolism in chickpeas. It's essential for chlorophyll synthesis, photosynthesis, and overall plant development. Zinc deficiency can cause stunted growth, reduced yield, and disease susceptibility in chickpea plants. Chickpeas require another critical element, copper (Cu), for enzyme activation, photosynthesis, and nitrogen metabolism. Copper deficiency can cause chlorosis, diminished seed set, and poor root development in chickpea plants. It also contributes significantly to the development of lignin, which offers structural support to plants. Manganese (Mn) is required for photosynthesis, enzyme activity, and the oxidation of carbohydrates and nitrogen in chickpea plants. It's essential for chlorophyll production and detoxifying reactive oxygen species. Manganese deficiency can induce chlorosis, leaf swirling, and reduced seed output in chickpea plants. Zinc, copper, iron, and manganese, in addition to their roles in plant metabolism, improve the nutritional value of chickpeas. These micronutrients are vital to human health and play crucial roles in a variety of physiological functions, including immunological function, energy metabolism, and antioxidant defense. As such, phase-II of the study concluded with the optimization and characterization of green synthesis of Zn-based nanoparticles.

The next target was to see the potential applications of synthesized materials in a plant-microbe-soil environment. The application first began with bacterial compatibility assay with some concentrations (500-2000 $\mu g\ ml^{-1}$) of MNP, BNP and QMNP against soil beneficial and pathogenic bacteria. As no significant zone of inhibition was observed, it was decided to further apply the materials in seed germination followed by hydroponic cultivation at lab scale. Germination assay followed by examination of oxidative stress enzymes and metal uptake detection in hydroponically grown *Cicer arietinum* seeds concluded that at certain doses MNP, BNP and QMNP performs the best compared to conventional inorganic salts. Next, soil experiments were conducted in pot culture to observe the dose-dependent response of metal uptake in plants and soil nutrient dynamics and soil organic carbon. Total bacterial and fungal count was also considered for LD50 dose establishment. Nanomaterials at the microbe-soil-plant interface can affect microbial diversity, abundance, and activity. Low concentrations of nanomaterials can promote microbial activity and improve nutrient cycle mechanisms, resulting in increased soil fertility and plant development. However, excessive dosages of nanomaterials can suppress microbial growth, destabilise soil microbial communities, and impede soil activities like organic matter breakdown and nutrient cycling. Nanomaterials can influence plant development and physiology by modifying nutrient availability, water retention, and root shape. Nanomaterials at low dosages may improve plant nutrient uptake, oxidative tolerance, and root growth. Understanding these linkages is critical for analysing the environmental hazards and benefits of nanomaterial applications in agricultural and environmental management, which will eventually contribute to the development of sustainable and environmentally friendly nanotechnology. As such, phase-III of the study concluded with the dose establishment of green synthesis of Zn-based nanoparticles.