A SPECIES DEPENDENT STUDY OF THE EFFECT OF CuO NANOPARTICLE PRODUCED BY CHEMICAL AND GREEN SYNTHESIS ON MICROGREEN PLANTS

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Abstract
Global challenges such as population growth, increasing urbanisation, climate change, and limited access to important plant nutrients have all contributed to the development of nanotechnology as a new innovation and its application in agriculture. Nanoparticles can be synthesized through chemical, physical or biological synthesis methods. However, the applied approach can significantly affect their chemical properties, reactivity and biological activity [1]. In recent years, the use of green synthesis methods has received increasing attention due to their ease of characterization, lower toxicity and favorable production costs. In our work, we examined and compared the species-dependent effects of CuO nanoparticles produced by both chemical and green synthesis on three different microgreen plants. Seed-priming was used for seed treatment and the biomass, pigment, and protein contents of one-week-old plants were investigated.

Introduction

Nanotechnology in agriculture is a potential way to improve sustainable crop production. The use of nanoparticles in crop production is already known to increase germination rates, germination speed and growth. It has positive effects on chlorophyll content, increases cell elongation and enhances nutrient uptake [2]. Furthermore, the use of nanoparticles also plays a role in plant pathogen control, thereby reducing the use of chemical products and contributing to the further development of sustainable crop production [3]. Soilless and nanotechnology-based crop production, including the cultivation of microgreens, could be a revolutionary innovation in traditional farming, which is increasingly important due to the increasing population growth and the reduction of cultivable land with urbanisation [4]. Microgreens are young and immature plants that have been recently introduced as a new category of vegetables, adapting their production at the micro-scale. Microgreens are typically harvested between 10-14 days after their first leaves appeared. Their rapid growth and space-efficient nature make them especially appealing for modern agriculture [5].

The right amount of copper is essential for the proper functioning and development of plants. Copper (Cu) is involved in a number of morphological, physiological and biochemical processes and functions as a cofactor for many enzymes. It plays an essential role in respiration, photosynthesis and the electron transport chain. However, in excessive amounts, it negatively affects plant growth and productivity and has negative effects on mineral nutrition, chlorophyll biosynthesis and antioxidant enzyme activities [6].

Related to this, the subject of these work was to synthesize copper-oxide (CuO) nanoparticles, using chemical and green methods, and then to determine the resulting particles physical and chemical properties. As their effects on microgreen plants have not been well investigated so far, our aim was to characterize the concentration- and species-dependence of chemical and green synthesized NP effects on plants; therefore, experiments were carried out in three agriculturally important plant species, *Brassica rapa cymosa, Lepidium sativum and Eruca sativa*.

Experimental

NP synthesis and characterization

CuO nanoparticles were synthesized through a precipitation method. Initially, copper nitrate ($Cu(NO_3)2{\cdot}3H_2O$) was dissolved in 100 mL of deionized water to achieve a 0.2 M concentration. Subsequently, 25% ammonia solution was added drop by drop under vigorous stirring until the pH reached 12. The resulting black precipitate was washed with deionized water until the pH reached 7. Following this, the washed precipitate was dried at 60°C overnight to remove any remaining solvent and then calcined at 450 °C for 2 hours. Finally, the obtained powder was ground and stored at room temperature for further use.

A similar procedure was employed for the green synthesis, with the exception that Virginia creeper (VC) extracts were used instead of ammonia solution. The leaf extracts were prepared by boiling the dry leaves in 100 mL of deionized water at 80 °C for 80 minutes. The extracts were then vacuum-filtered, and the filtrate served as both a reducing agent and a stabilizer for the as-synthesized CuO NPs. "Green" VC-CuO materials were synthesized by adding the plant extracts to the 0.2 M aqueous copper solution in a 1:1 volume ratio at room temperature, with continuous stirring for 24 hours.

Characterization of CuO NPs:

The morphological characteristics of CuO NPs were analyzed using transmission electron microscopy (TEM) with a FEI Tecnai G2 20× microscope at an acceleration voltage of 200 kV. To verify the crystal structure and phase of the nanoparticles, X-ray powder diffraction (XRD) was performed. The XRD scans were conducted with a Rigaku MiniFlex II powder diffractometer, utilizing Cu Kα radiation and a scanning rate of 2° min^{-1} within the 10°–80° 2θ range.

Seed nano-priming

For the treatment of seeds with nanoparticles, seed nano-priming was used. To compare, a CuO NP's prepared chemically and from wild grapes were used to prepare a stock solution at a concentration of 100 mg/l and then dilutions were performed. A nanoparticle suspension of 100 mg/l was prepared as a treatment solution at dilution levels of 10, 25, 50 and 75 mg/l. Seeds soaked in purified water were used as controls. The nano-priming time was 6 h for broccoli and 1 h for arugula and cress due to the hydrate coating on the seeds. After nano-priming, broccoli seeds were dried at room temperature for 24 hours and stored at 4°C until use.

Microgreen cultivation

Broccoli (*Brassica rapa cymosa*), cress *(Lepidium sativum)* and arugula *(Eruca sativa)* microgreen plants were grown on coconut fibre under hydroponic conditions. The plants were provided with a tomato nutrient solution containing: 1 M $Ca(NO_3)_2$; 1 M $MgSO_4$ x 7 H_2O; 0.1 M KH_2PO_4; 0.1 M KCl; 0.1 M Na_2HPO_4 x 2 H2O; 0.01 M Fe-EDTA and micro-elements. Microgreens were cultivated under a controllable lighting fixture, under LED light containing excess blue light for 7 days. The illumination treatment of all three microgreen species provided the same total photon flux density (TPFD) of 190.3 μmol/m^2/s.

Biomass measurement
In each tray, 20 seeds were planted on coconut fibre and then grown for 7 days. After harvesting the plants fresh weight of the tray-grown plants was measured.

Figure 1: Preparation and processing of plant samples

Determination of protein content by Bradford Protein Assay
Biochemical parameters and content values are determined as shown in the first figure (Figure 1). The shoot part of the plants is harvested at 7, 10 or 14 days of age, depending on the experiment, the plant sample is homogenized in buffer and absorbance is measured at the appropriate wavelength.
A 200 mg plant sample was homogenized in a pH 7, 50 mM mixed phosphate buffer (KH_2PO_4, Na_2HPO_4). The mixed plant sample was collected in an Eppendorf tube and centrifuged for 10 minutes at 4 °C and 12000 rpm. The supernatant (enzyme extract) of the centrifuged samples was pipetted into another Eppendorf tube. Using Bradford's method, the following reaction mixture was prepared: 900 µl of purified water, 5 µl of enzyme extract and 100 µl of Bradford's reagent. After mixing the samples, the optical density of the samples was determined at 595 nm.

Determination of pigment content
20 mg of plant sample was rubbed in 95% ethanol. The wetted plant samples were centrifuged for 10 min at 4 °C and 12 000 rpm. 200 µl of the supernatant was pipetted three times per sample into 96-well plates and the absorbance was measured at 664, 648, 470, 534, 643 and 661 nm. We measured the amount of chlorophyll a, chlorophyll b and total chlorophyll and determined the ratio of chlorophyll a to chlorophyll b.

Results and discussion
XRD measurements were used to determine the crystal structure and the chemical composition of the synthesized nanoparticles.. The characteristic peaks located at 2θ = 32.53°, 35.52°, 38.87°, 48.74°, and 68.24° are assigned to (110), (002), (200) , (-202), and (220) plane orientation of monoclinic structure of CuO (JCPDS 892531). According to TEM images, the average size of this VC-CuO sample was around 30 nm, and polydispersity was observed as in case of the chemical sample.
The treatment with nanoparticles can increase the yield/biomass. For this reason, we conducted an investigation to determine whether two types of nanoparticle treatments resulted in differences in biomass mass between species. The results showed a significant difference in arugula after seed nano-priming treatment with CuO nanoparticle concentration of 10 mg/l. (Figure 2, 3).

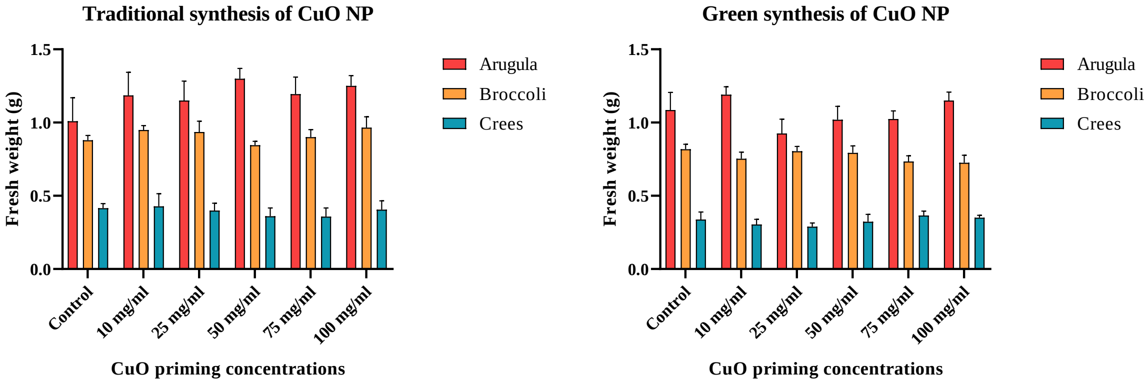

Figure 2: Changes in the biomass of three different plant species after differently synthesized nanoparticle treatments

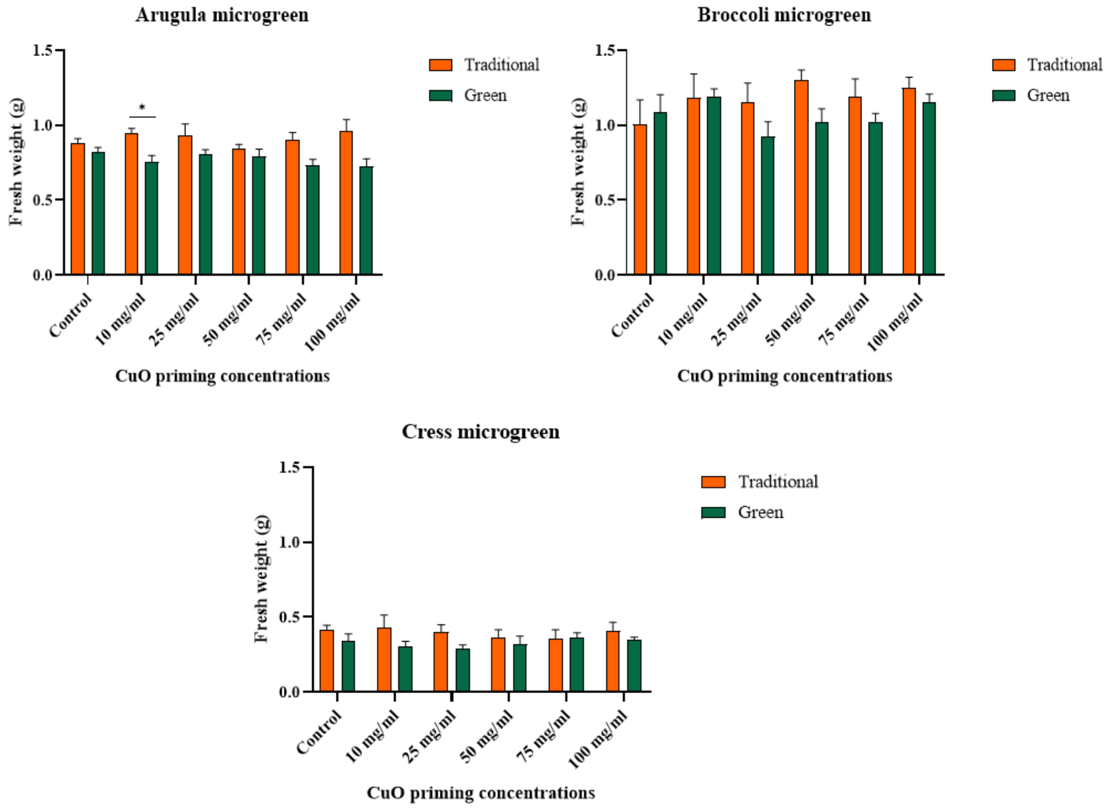

Figure 3: Effect of chemically and green synthesized CuO treatments on biomass by plant species

However, the biomass of the treated plants also showed us that the LED illumination with an excess of blue light had a positive effect on broccoli growth, while the cress did not grow well under the chosen illumination.

Conclusion

Based on the measurements, we successfully synthesized CuO nanoparticles with using plant waste extract of VC. The properties of the obtained nanoparticles were similar to the conventional chemically synthesized nanoparticles. Subsequently, the particles were introduced into microgreens to investigate the concentration- and species-dependent effects of both chemically and green-synthesized nanoparticles on these plants. Our observations revealed differences in the biomass of all three plant species. This finding provides a rationale reason for conducting further experiments to explore the impact of differently synthesized nanoparticles on other parameters, including vitamin C content, antioxidant enzymes activity and the nutritional values of these plants.

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PRELIMINARY STUDIES ON ELECTROCHEMICAL BEHAVIOUR OF SULPHITE ON STAINLESS STEEL IN NEUTRAL MEDIA

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Abstract

SO_2 emissions are widely converted to sulphite through the flue gas desulphurization process, in which SO_2 is scrubbed and then chemically absorbed as sulphite (SO_3^{2-}) in alkaline solutions [1]. Furthermore, the oxidation of SO_3^{2-} ions can produce additional benefits, such as generation of an energy carrier like hydrogen [2]. Sulphite electrooxidation occurs in both acidic and alkaline media, according to the reaction (1) and (2), respectively [3]:

$$SO_3^{2-} + H_2O \rightarrow SO_4^{2-} + 2H^+ + 2e^- \qquad (1)$$

$$SO_3^{2-} + 2HO^- \rightarrow SO_4^{2-} + H_2O + 2e^- \qquad (2)$$

Several studies regarding the sulphite electrooxidation were performed using noble metals such as platinum [4] and gold [5] due to their good catalytic activity [6], but the high price of these materials is a major drawback for their widespread use, therefore the present paper targets low-cost electrodes such as AISI 420 and Incoloy 800.

In this paper, the anodic oxidation of the sulphite ions on AISI 420 and Incoloy 800 electrodes in neutral solution (1 mol L^{-1} Na_2SO_4) was studied to determine the relationship between the kinetic parameters and the sulphite concentration added in the electrolyte (10^{-3}, 10-2, 10-1, 0.5 and 1 mol L^{-1}). Due to their electrochemical stability in aqueous solutions, in acidic and neutral electrolytes, stainless steal electrodes can be a practical alternative as anode material. Also, their tendency to passivation can be an advantage both due to the high corrosion resistance and the catalytic effict on the anodic oxidation of sulphite [7].

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