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Research Paper

Effect of biologically synthesized copper oxide nanoparticles on metabolism and antioxidant activity to the crop plants *Solanum lycopersicum* and *Brassica oleracea* var. botrytis

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ABSTRACT

Study on the ecological effect of metal oxide nanomaterials (NMs) has quickly amplified over the precedent years because it is assumed that these NMs will sooner or later be released into the environment. The present study deals with biologically oriented process for the green synthesis of copper oxide nanoparticles (CuO NPs) by using *Morus alba* leaf extract as reducing agent. Powder X-ray diffraction (XRD) and transmission electron microscope (TEM) analysis revealed the monoclinic phase and 20–40 nm size respectively. The presence of reducing and capping agents revealed by Fourier transform infrared (FTIR) spectroscopy. The seedlings of *Brassica oleracea* var. botrytis and *Solanum lycopersicum* were exposed to 10, 50, 100, and 500 mg L^{-1} concentrations of CuO NPs in the sand medium. Bioaccumulation of Cu was also investigated by atomic absorption spectroscopy (AAS). Plant exposure to 100 and 500 mg L^{-1} of CuO NPs has resulted in significant reduction of total chlorophyll and sugar content in the two test plants while 10 mg L^{-1} of NPs slightly increased the pigment and sugar content in tomato plants only. Augmentation of lipid peroxidation, electrolyte leakage, and antioxidant enzyme activity was observed in a dose dependent manner upon plants exposure to CuO NPs. Deposition of lignin in roots of both plants treated with the highest concentration of CuO NPs was observed. Histochemical analysis of leaves of treated plant with nitroblue tetrazolium and 3′3′ diaminobenzidine showed a concentration dependent increase in superoxide and hydrogen peroxide formation in leaves. The green synthesis of CuO NPs was carried out by using *Morus alba* leaf extract. Accumulation of NPs more actively by tomato plants as compared to cauliflower was possibly due to the difference in root morphology. The histochemical visualization highlights the spatial organization of oxidant biochemistry occurring in response to metal stress.

1. Introduction

During the past few decades, there has been a rapid growth of research in the field of nanotechnology. Nanotechnology is an interdisciplinary science associated with deliberately manufactured materials with at least one dimension less than 100 nm (Raliya et al., 2015). Novel properties *i.e.* electrical, optical, catalytic, mechanical and electromagnetic which come forth at the nanoscale have facilitated the manufacture of nano-enabled products, which are being consumed in several sectors like electronics, medical diagnostics, therapeutics, agriculture, and clothing (Singh et al., 2015a). Industrial manufacturers have produced a range of engineered nanoparticles (ENPs). ENPs are used widely in a broad spectrum of industrial and commercial applications. The enormous use of ENPs can lead to their acquaintance into the environment, and the impacts of their unique physical and chemical properties on the ecosystem are becoming a major concern (Ghodake et al., 2011). Among different metal oxide NPs, copper oxide nanoparticles (CuO NPs) are used in a wide range of applications such as in electronics, air and liquid filtration, ceramics, wood preservation, textiles, bioactive coatings, skin products, films, lubricant oils and in inks (White et al., 2006). Copper (Cu) is an active transition metal involved in many redox processes in animal and plant cells. In plants, copper is a component of regulatory proteins, a cofactor of phenol oxidases, ascorbate oxidase, superoxide dismutase (SOD) and participates in electron transport in the respiratory and photosynthetic chains (Yruela., 2005; Nekrasova et al., 2011). The action of CuO NPs on plant cells has not been studied satisfactorily. The existing results did not provide clear information about the effect of CuO NPs on plant cells. However, it is known that nanopowders are successfully used as micro fertilizers and pesticides (Selivanov and Zorin, 2001; Raikova et al., 2006). It is also reported that exposure of CuO NPs has resulted in their uptake, bioaccumulation, and translocation in mung bean and wheat plants

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(Lee et al., 2008). Plants need only traces of copper, therefore, the increased concentrations of CuO NPs exert noxious effect by penetrating directly into the cell, apparently by causing oxidative impairment to cell structures and molecules (Nekrasova et al., 2011). Hence, the extensive use of CuO NPs has raised alarms over their prospective toxic effects on the ecosystem, human and plant health due to their release from different products into the environment (Chen et al., 2012). Nekrasova and Maleva (2007) reported that free Cu ions can affect the activity of enzymic proteins by binding unspecifically to thiol groups, which results in the impairment of their secondary structure. It has been reported that CuO NPs have more toxicity on *Lemna minor* as compared with that of bulk CuO (Song et al., 2016). The authors have also suggested that Cu^{2+} released by CuO NPs in the culture media was the reason for this toxicity which leads to growth inhibition. Moreover, Hossain et al. (2015) reported that CuO NPs treatment in rice seedling led to an increase in activity of antioxidant enzymes and elevated MDA concentration. The CuO NPs modulated photosynthetic performance and antioxidative defense system demonstrated the restriction in root and shoot growth with reduced photosynthetic performance index in *Hordeum vulgare* (Shaw et al., 2014). CuO NPs mediated DNA damage and plant growth restriction were also reported in *Raphanus sativus*, *Lolium rigidum* and *Lolium perenne* (Atha et al., 2012). The augment in the production of nanomaterials (NMs) and their application in different fields certainly lead to an additional ecological impact on the environment which directs the relevance of studies on the ability of NPs to accumulate in plants and animals (Bogatikov, 2003).

There are various approaches like chemical and physical methods available for the synthesis of NPs. The biosynthetic or green methods play a very significant role in nanotechnology especially dealing with the biological application as these are cost-effective, eco-friendly and have negligible contamination (Hussain et al., 2016). Biological molecules can undergo highly controlled hierarchical assemblies which make them favorable for the eco-friendly synthesis of metal NPs (Upendra et al., 2009). The synthesis of CuO NPs has not been as widely explored as many other metals due to the easily oxidizable nature of Cu, which is enhanced in nanoscale structures (Sampath et al., 2014).

In comparison with other transition metal oxides such as Fe_2O_3, TiO_2, and ZnO, many reports have not described the synthesis approaches adopted for CuO NMs along with the introduction of their related applications. Therefore, the present study stands significant, as it deals with the green synthesis of CuO NPs employing *Morus alba,* commonly known as white mulberry, plant extract and testing their phytotoxicity against *Solanum lycopersicum* (tomato) and *Brassica oleracea* var. botrytis (cauliflower) because of their importance as crop plants. Leaves of mulberry are the only food for the silkworm (*Bombyx mori*) and plant has wide adaptability to environmental condition. It can be grown under wide range of climatic conditions ranging from temperate to the tropical regions in India. Medical and antimicrobial activities of this plant are well known from the past several decades. In the extracts of mulberry fruit, a total of 14 hydroxycinnamic acid esters, 13 flavonol glycosides, and 14 anthocyanins have been identified which clearly portray its property as potent superoxide anion radical (O^{2-}) scavenger and reducing agent (Natic et al., 2015). Biosynthesis of gold (Au) NPs via leaf of mulberry has been suggested as possible eco-friendly alternatives to chemical and physical methods (Adavallan et al., 2015).

The aim of this study was to synthesize CuO NPs via novel biological method using plant extract of *M. alba* and analyze specific features of the accumulation of CuO NPs by the test plants. The present study investigates the impact of CuO NPs on germination and seedling growth of test plants in petri plate bioassay while uptake of Cu, biophysical growth viz. fresh and dry weight, shoot and root length. The biochemical parameters viz. pigment and sugar content, nitrate reductase activity, lipid peroxidation, electrolyte leakage and antioxidant enzyme activities and histochemical like *in vivo* detection of O^2, hydrogen peroxide (H_2O_2) and lignin deposition responses in both the test plants were studied in sand culture. To the best of our knowledge, this is the first report which provided *M. alba* mediated synthesis of CuO NPs and detailed assessment of their effect on growth, metabolism and antioxidant activity in cauliflower and tomato plants.

2. Materials and methods

2.1. Biological synthesis of copper oxide nanoparticles

Leaves of *M. alba* were collected, washed and shade dried for 10 days at room temperature for the biological synthesis of CuO NPs. Leaves (25 g) were chopped and allowed to boil for 5 min at 80 °C with 100 mL of double distilled water (DDW) and then cooled down to room temperature. The resulting solution was filtered via Whatman No. 1 filter paper and then stored at 4 °C as a stock for the synthesis of CuO NPs.

For the further synthesis process, 1% (w/v) of CuO NPs was prepared by dissolving 1 g of copper acetate monohydrate Cu $(CH_3COO)_2{\cdot}H_2O$ in 100 mL of DDW and it was stirred for 20 min. To the above solution, 10 mL of the *M. alba* leaves extract was added drop by drop. The formation of the particles can be seen within 1 h. The color of the solution turned from blue to black after some time and a black suspension formed simultaneously. The reaction was carried out under stirring and boiling for 3 h. Further, the mixture was cooled to room temperature and centrifuged at 10,000 rpm for 10 min. The obtained wet precipitate was washed thrice with ethanol and DDW to eliminate the impurity. The entire process was repeated to get a sufficient amount of NPs. The collected CuO NPs were allowed to dry and formed black precipitate was ground for further characterization.

2.2. Characterization of copper oxide nanoparticles

First round characterization of the CuO NPs was carried out using UV–visible spectroscopy by Lambda 35 Perkin Elmer spectrophotometer in the wavelength range of 200–500 nm operated at a resolution of 1 nm. The X-ray diffraction (XRD) pattern of the CuO nanopowder was recorded on a Rigaku d'max 2200 diffractometer with Cu K_α radiation (K = 1.5406 Å). The data of the samples were recorded and plotted in the "Origin 8.1". Particle size distributions of the NPs were measured with a Nanotrac wave W3372 particle size analyzer (PSA). Fourier transform infrared radiation (FTIR) transmittance spectra of synthesized CuO NPs were recorded by locating the pellet in the lane of the IR beam of IR spectrometer (FTIR Spectrum RX-1, Perkin Elmer) with the detector at 4000–500 cm. The shape and size of the CuO NPs were resoluted using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). SEM studies were done by JEOLJXA-8230. TEM analysis was done by using TECNAI 200 kV instrument. For TEM analysis nanopowder was diluted with DDW and dispersed by the ultrasonic bath. After that, one drop was placed on a carbon-coated copper grid to make a thin film and kept in the grid box sequentially for TEM analysis.

2.3. Application of nanoparticles on seed germination using petriplate bioassay

The synthesized CuO NPs were suspended directly in DDW and dispersed by ultrasonic vibration (100 W, 40 kHz) for 30 min. Small magnetic bars were put in the suspension for stirring before use to avoid aggregation of the particles. Different concentrations viz. 0, 10, 50, 100 and 500 mg L^{-1} of CuO NPs were prepared for experiment on the germination and seedling growth.

Seeds of two plant species *i.e.* cauliflower and tomato were purchased from local certified seed agency at Allahabad, India. The average germination rates of both plants were around 70–75% as shown by initial study. Seeds were kept in a dry and dark condition under room temperature. To avoid surface contamination, seeds were